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CONTAMINATION OF EUROPEAN OTTERS (*LUTRA LUTRA*) BY PCB CONGENERS AND ORGANOCHLORINATED PESTICIDES IN THE WETLANDS OF WESTERN FRANCE

by

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ABSTRACT

During the past fourty years, otter (*Lutra lutra*) populations have severely declined over much of Europe. One of the main regression factors appears to be pollution by organochlorine xenobiotics, especially PCBs. The present study took place in France, in the wetlands of the Atlantic border, where otter populations are considered to be stable. Muscle, pericaudal fat, and liver of 33 otters were analysed for 22 PCB congeners and organochlorine pesticides. The mean concentrations of organochlorinated pesticides in samples are relatively low (from 0 to 5.71 mg kg^{-1} lipid weight). The PCB concentrations are higher (mean in muscular tissue: 26.19 mg kg^{-1} lipid weight), but still remain low compared to similar samples from other countries. The relation between PCB concentration and physiological variables is discussed. Congener-specific analysis shows an important contamination due to highly chlorinated congeners and a poor contamination due to low chlorinated congeners. Potential hazards due to organochlorinated compounds are also discussed.

KEY WORDS: otter, PCBs, organochlorinated pesticides.

INTRODUCTION

During the last decades, European otter (*Lutra lutra*) populations have declined sharply in the main part of northern, western, and central Europe (MASON, 1989). Although many regression factors have adversely affected populations, such as habitat destruction, anthropic activities, and accidents (ROSOUX & LIBOIS, 1994), the strong decline of otters seems to have partly been caused by pollutants, especially PCBs (MACDONALD & MASON, 1994).

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In France, otter populations are extinct in the northern and eastern parts of the country but the species is still thriving on the Atlantic coast and in the Massif Central, where a population expansion has even been recorded (BOUCHARDY, 1986; FONDERFLICK *et al.*, 1995; ROSOUX *et al.*, 1995). In the Atlantic wetlands area (Fig. 1), populations are present from the river Loire to the river Gironde (ROSOUX *et al.*, 1995). In the northern part of this area, the department of the Loire-Atlantique, the species seems to be declining (LODE, 1993), while in the Marais Poitevin otter is still present in every wet habitat (ROSOUX & LIBOIS, 1994).

In France, few studies have attempted to determine the contamination level of otter populations. Therefore, the aim of the present study is to measure the concentrations of PCBs and organochlorinated pesticides in tissues of otters from the Marais de l'Ouest, in order to evaluate their possible impact on the populations in this area.

MATERIALS AND METHODS

Between 1987 and 1994, 33 otters found dead, mainly from road casualties, in the wetlands of western France (Fig. 1) were collected. Each specimen was frozen at $-30°C$ and autopsied, and samples of muscle, liver and pericaudal fat were removed for ecotoxicological analysis.

PCBs and organochlorinated pesticides were extracted according to a slightly modified E.P.A. 608 method as previously described by HUGLA *et al.* (1995). Aliquots of 2 g of each sample (32 muscles, 23 livers and 32 pericaudal fats) were prepared according to this method. The extracts in hexane-acetone (1/1; v/v) were evaporated to dryness under a gentle stream of nitrogen and placed for one hour in a dessicator, in order to weigh the lipids extracted, with a precision of 1×10^{-5} g. Three ml of n-hexan were then added to the extract. The acid and Florisil clean-up procedures and the chromatographic separation of the organochlorine in the extracts by means of High Resolution Gas Chromatography were also carried out according to THOME *et al.* (1987) and HUGLA *et al.* (1995).

PCB congeners and organochlorinated pesticides were identified according to their retention times. 25 PCB congeners, from di- to nonachlorinated congeners (I.U.P.A.C. nos. 8, 18, 28, 44, 52, 70, 77, 95, 101, 110, 118, 126, 128, 138, 149, 153, 156, 169, 170, 180, 183, 187, 194, 195, 206) were determined. The organochlorinated pesticides identified were α-HCH, β-HCH, and γ-HCH (or lindane), HCB, heptachlor, heptachlor epoxyde, aldrin, endrin, dieldrin, pp'DDE, op'DDD, pp'DDD and pp'DDT. In order to quantify endrin and dieldrin, which are destroyed during the acid clean-up step, 6 samples of liver, muscle and fat were analysed without performing this step.

The height of peaks was calculated by an integrator (LDC Milton Roy), and xenobiotic concentrations were calculated according to the peak heights in standards. PCB 209 and op'DDE were used as internal standards. Individual congener

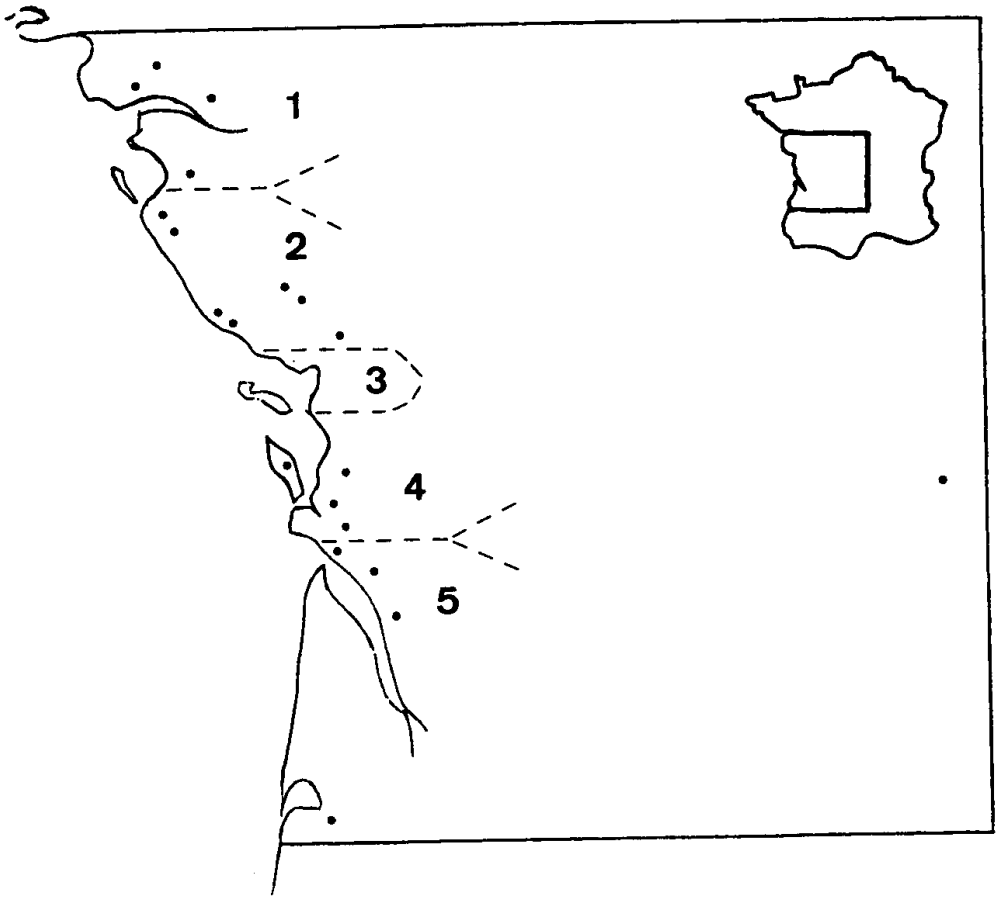

Fig. 1. Map of the study area showing the places where otter carcasses were found. The area is divided into five geographic subareas: 1. Loire; 2. Bocage Vendéen; 3. Marais Poitevin (13 individuals); 4. Marais de Charente; 5. Gironde.

values were then compared to the congener concentrations in various mixtures of Aroclor 1242, 1254 and 1260 by means of a statistical index of similarity developped by DE ALENCASTRO *et al.* (1985) and modified by HUGLA *et al.* (1995), taking into account the relative importance of each congener in the mixture. Total PCB concentration in the extract was then expressed in terms of the 'most suitable' composition of PCB commercial mixtures, considering the relative importance of the 25 congeners identified in these mixtures. In order to compare the PCB patterns of the different samples without considering the total PCB concentration, concentrations of each PCB congener present in each sample were expressed relative to a persistent congener (I.U.P.A.C. 153), according to the method proposed by DUINKER *et al.* (1991).

The K body condition index of individuals, taking into account the length and weight of individuals, was calculated according to a method developped by KRUUK (1995).

RESULTS AND DISCUSSION

Organochlorinated pesticides

The contamination levels of organochlorinated pesticides in the 3 tissues analysed are presented in Table I. The concentrations measured are very low (from 0 to 5.71 mg kg^{-1} lipid weight). The metabolites of pp'DDT (especially pp'DDE), lindane and its metabolites, and dieldrin were usually detected. The other pesticides were detected at extremely low concentrations in some individuals, or were never found. In pericaudal fat and muscle, the most abundant pesticide was pp'DDE, while in liver samples the pp'DDD concentration prevailed. Moreover, pp'DDT has never been detected in liver, while it was regularly found in both other tissues. This could be related to the well-known metabolisation of such compounds in liver, leading to a biotransformation of this pesticide into its metabolites. As a matter of fact, high concentrations of DDT metabolites (especially pp'DDE) and poor concentrations of DDT in the samples reveal not only the important use of this pesticide in the past decades, but also its biological degradation into metabolites.

The contamination level by organochlorinated pesticides in the otters from the wetlands of western France are among the lowest recorded in European populations. Since organochlorinated pesticides are considered to be poorly toxic to the otter (MASON, 1989; SMIT *et al.*, 1994), one can think that the concentrations measured in this work are insufficient to involve a significant negative impact over these populations.

TABLE I

Concentrations of organochlorinated pesticides in otter tissues from the wetlands of western France (mg kg^{-1} lipid weight) (mean ± SD).

Pesticides	Muscle (n = 32)	Liver (n = 23)	Fat (n = 32)
HCB	0.08 ± 0.07	0.32 ± 0.27	0.07 ± 0.04
α-HCH	0.01 ± 0.02	0.002 ± 0.006	0.003 ± 0.005
β-HCH	0.18 ± 0.25	0.10 ± 0.16	0.10 ± 0.17
γ-HCH	0.18 ± 0.46	0.02 ± 0.03	0.10 ± 0.11
Heptachlor	0.003 ± 0.016	–	–
Heptachlor epoxyd	0.003 ± 0.010	0.004 ± 0.017	0.01 ± 0.01
Aldrin	0.02 ± 0.07	–	–
Endrin	–	–	–
Dieldrin	0.75 ± 1.45	1.57 ± 2.56	0.12 ± 0.18
pp'DDE	2.17 ± 2.22	3.63 ± 3.40	1.23 ± 1.27
op'DDD	–	–	0.004 ± 0.016
pp'DDD	0.149 ± 0.19	5.71 ± 6.00	0.05 ± 0.06
pp'DDT	0.09 ± 0.42	–	0.11 ± 0.26

–: not detected.

Polychlorinated biphenyls (PCBs)

The PCB congener patterns in the 3 tissues analysed were compared to mixtures composed of Aroclor 1242, 1254, and 1260. For each tissue, the statistical index of similarity was the highest for a mixture composed of 100% Aroclor 1260, thus indicating a strong bioaccumulation of highly chlorinated congeners (likely the hexa- and hepta-CBs).

The mean contamination levels of liver, muscle, and adipose tissues by PCB congeners, and the mean total PCB concentrations are presented in Table II.

Most otters show a low PCB content, only a few outliners reaching higher values. The contamination levels of liver and muscle are not significantly different (One-Way Anova, $p > 0.05$), but differ significantly from fat content (One-Way Anova, $p < 0.05$). Moreover, the PCB concentrations in the different tissues are significantly correlated (least squares regression test). The relationships between the PCB concentrations in the 3 tissues

TABLE II

Total PCB and individual congener concentrations in otter tissues from the wetlands of western France (mg kg^{-1} lipid weight) (mean ± SD). Congeners 18, 44, 70, 77, 95, 126 and 169 have never been detected.

Congener	Muscle (n = 32)	Liver (n = 23)	Fat (n = 32)
8	0.05 ± 0.22	–	–
28	0.03 ± 0.17	–	–
52	0.05 ± 0.17	–	–
101	0.42 ± 1.41	0.10 ± 0.23	0.03 ± 0.09
110	0.03 ± 0.12	–	–
118	2.90 ± 3.65	3.85 ± 5.99	1.31 ± 1.38
128	0.58 ± 0.69	0.82 ± 0.80	0.29 ± 0.30
138	6.25 ± 5.33	10.95 ± 8.88	2.83 ± 2.07
149	0.11 ± 0.32	0.08 ± 0.26	–
153	7.54 ± 5.80	6.62 ± 4.32	3.59 ± 2.43
156	0.56 ± 0.56	0.90 ± 1.01	0.31 ± 0.24
170	1.65 ± 1.23	2.79 ± 2.79	0.95 ± 0.73
180	3.65 ± 2.79	3.64 ± 3.21	1.86 ± 1.48
183	0.51 ± 0.40	0.47 ± 0.34	0.24 ± 0.20
187	0.87 ± 0.75	3.69 ± 3.39	0.38 ± 0.34
194	0.75 ± 0.64	0.64 ± 0.62	0.44 ± 0.34
195	0.13 ± 0.19	0.56 ± 0.67	0.14 ± 0.11
206	0.16 ± 0.16	0.29 ± 0.20	0.08 ± 0.07
Total PCBs	26.19 ± 21.74	37.85 ± 32.91	12.45 ± 8.94

–: not detected.

are:

– fat (y) vs liver (x):	$y = 0.233x + 5.210$	$r = 0.824$	$p < 0.001$
– fat (y) vs muscle (x):	$y = 0.332x + 4.025$	$r = 0.822$	$p < 0.001$
– liver (y) vs muscle (x):	$y = 1.363x - 0.245$	$r = 0.789$	$p < 0.001$

No clear relationship could be found between the PCB contamination level of tissues and sex (Two-Way Anova, $p > 0.05$), body length or weight (Two-Way Anova, $p > 0.05$), estimated age (Two-Way Anova, $p > 0.05$) and K body condition index of individuals (Two-Way Anova, $p > 0.05$). Similarly, no significant difference of the mean PCB concentration in the muscular tissue has been found between five subsamples of otters grouped according to their geographic origin (Loire, Bocage vendéen, Marais Poitevin, Marais de Charente, and Gironde, see Fig. 1) (Two-Way Anova, $p > 0.05$).

Our results are in a good agreement with the total PCB concentrations previously measured in otters from the same region (Table III), except in the case of liver, where more variable values have been detected. Comparison with the PCB values obtained in other countries shows that the otter population from the wetlands of western France is contaminated in a similar way as other stable populations (rewiewed by SMIT *et al.*, 1994).

The effect of PCB contamination has never been experimentally tested in otters directly but 'safe' and 'toxic' threshold concentrations have been deduced from contamination experiments on the American mink (*Mustela vison*) (reviewed by LEONARDS *et al.*, 1994). The mean PCB concentration in otter tissues from our study area is below the value of 50 mg total PCBs kg^{-1} lipid weight considered by JENSEN *et al.* (1977) as sufficient to reduce the reproduction rate of individuals, and thereby cause a population decline (MASON, 1989). However, 5 individuals show a higher PCB concentration than this value. In a more recent study, MASON (1996) considered a concentration of 30 mg total PCBs kg^{-1} lipid weight as a limit value, and 10 mg total PCBs kg^{-1} lipid weight as a 'safe' level. The mean PCB concentrations in all tissues analysed here are well above this safe level, and the mean liver PCB contamination level is even above 30 mg kg^{-1}, contrary to what is observed in muscle.

TABLE III

Total PCB concentrations in otter tissues from the wetlands of western France (mg kg^{-1} lipid weight) (mean ± SD) (MENANTEAU, personal communication).

Muscle (n = 8)	Liver (n = 11)	Pericaudal fat (n = 11)
22.36 ± 20.18	135.51 ± 148.85	13.22 ± 12.01

The different proposed limit values give rise to contradictory interpretations of the risks due to PCB concentrations measured in the otter populations of the wetlands of western France. Threshold levels should be carefully employed only as comparative tools and not as absolute indicators of the decline or of the stability of any wild otter population. Moreover, one must not ignore the influence of the animals' physiological condition on the toxic effects of PCBs: for example, an animal with a high fat content will accumulate a high PCB burden in the adipose tissue, but fat mobilization will lead to a redistribution of PCBs to other organs, where they will exert negative effects (MASON, 1989). Finally, the possible behavioural effects of a PCB contamination, such as those reported by MASON & O'SULLIVAN (1992), could induce a rise of the mortality level due to, for example, road casualties, which is the principal cause of death in our study area (ROSOUX & TOURNEBIZE, 1995).

Total PCB concentrations give global information about the contamination of animals, but recent studies showed the importance of considering the different congeners in the samples (MASON & RATFORD, 1994; BROEKHUIZEN & DE RUITER-DIJKMAN, 1988), since great differences have been observed in the congener toxicity (KIHLSTRÖM *et al.*, 1992). The mean congener pattern of the analysed tissues is shown in Fig. 2. First, one must note that the congener pattern in the same tissue is quite variable from one specimen to another. The main characteristics are quite similar in the 3 tissues, as highly chlorinated congeners predominate, contrary to those containing from 1 to 5 chlorine atoms. However, differences can be observed between the analysed tissues. In muscle and fat, the most abundant congeners are in a decreasing order 153 > 138 > 180 > 118 > 170, while in liver the order is 138 > 153 > 187 > 180 > 118. Such variability of the congener pattern of otter individuals has already been observed in other studies (for a review, see SMIT *et al.*, 1994). Despite this variability each population which has been analysed for a congener-specific study showed a PCB pattern similar to that observed in the present study (MASON & RATFORD, 1994; SMIT *et al.*, 1994).

CONCLUSIONS

All the examined otters were contaminated by organochlorinated pesticides and by PCBs, the concentrations being highly variable from one individual to another. The organochlorinated pesticide levels are very low and seem insufficient to explain the decline of these otter populations.

The PCB congener-specific analysis shows an important contamination due to highly chlorinated congeners and a low contamination due to less

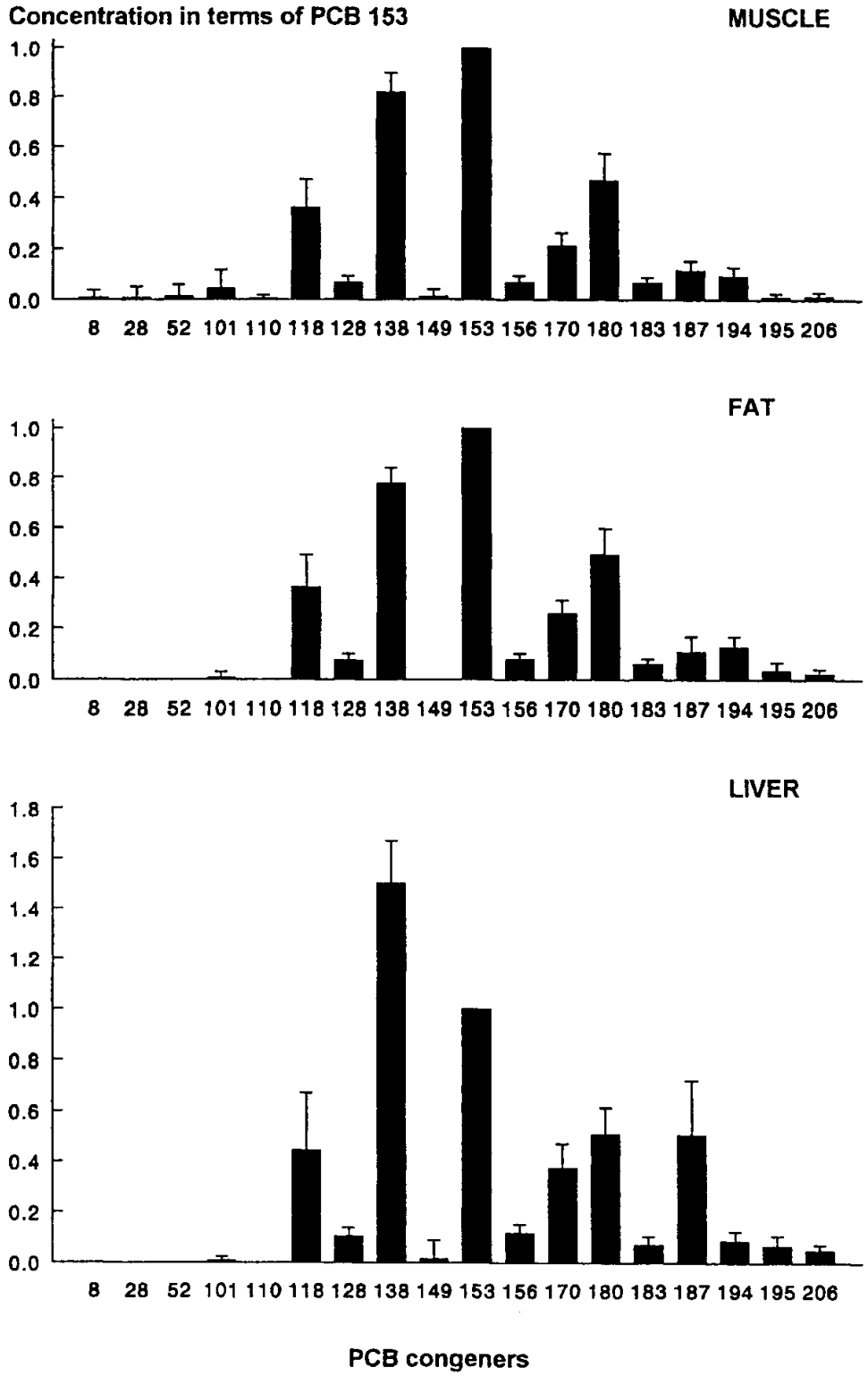

Fig. 2. Concentrations of individual PCB congeners in otter tissues from the wetlands of western France, expressed relative to PCB 153 (mean ± SD) (n = 32 muscles, 23 livers and 32 pericaudal fats). Congeners 18, 44, 70, 77, 95, 126 and 169 have never been detected.

chlorinated congeners. Small differences are present between the PCB patterns of muscle, fat and liver. Total PCB concentrations are quite high, but appear insufficient to induce directly a generalised decline of the populations in western France. However, due to poor information about the real toxicity of PCBs to the otter, on the one hand, and the possible increase of mean PCB concentrations in aquatic ecosystems in the near future through remobilization and atmospheric transport (TATEYA *et al.*, 1989) on the other hand, we find it difficult to evaluate the role of these compounds as a current threat to the extant populations. We think it is useful to continue to monitor the PCB concentrations in otters or in fish from this area since fish may provide reliable information about the organochlorine pollution levels of rivers (WEBER, 1990; MASON, 1995). PCBs however, are not the only problem faced by the otters. In the field, they are exposed to other micropollutants and we are not able to evaluate the effects of possible combinations, either synergistic or antagonistic, of several xenobiotics. Moreover, in several parts of the western wetlands, the suitable habitats are deteriorating. In the Marais Poitevin, the main prey of the otter, eel (*Anguilla anguilla*) (LIBOIS & ROSOUX, 1989) is at great risk due to overfishing (LEGAULT, 1987) and numerous weirs and flood-gates prevent the upstream migration of elvers (LEGAULT, 1987; TOURNEBIZE & ROSOUX, 1995).

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REFERENCES

BOUCHARDY, C., 1986. *La loutre*. Sang de la Terre, Paris.

BROEKHUIZEN, S. & E. M. DE RUITER-DIJKMAN, 1988. Otters *Lutra lutra* met PCBs: de zeehondjes van het zoete water? Lutra **31**: 68-78.

DE ALENCASTRO, L. F., V. PRELAZ & J. TARRADELLAS, 1985. An improved quantification method used to determine the origin of PCBs in wastewaters: the index of similarity. Intern. J. Anal. Chem. **22**: 183-201.

DUINKER, J. C., D. E. SCHULTZ & G. PETRICK, 1991. Analysis and interpretation of chlorobiphenyls: possibilities and problems. Chemosphere **23**: 1009-1028.

FONDERFLICK, J., J. DE KERMABON, R. LIBOIS, P. BAFFIE, R. ROSOUX & T. TOURNEBIZE, 1995. Evolution récente du statut de la loutre dans le Parc National des Cévennes. Cahiers d'Ethologie **15**: 233-238.

HUGLA, J. L., J. C. PHILIPPART, P. KREMERS, G. GOFFINET & J. P. THOME, 1995. PCB contamination of the common barbel, *Barbus barbus* (Pisces, Cyprinidae), in the river Meuse in relation to hepatic monooxygenase activity and ultrastructural liver change. Neth. J. Aquat. Ecol. **29**: 121-131.

JENSEN, S., J. E. KIHLSTRÖM, M. OLSSON, C. LUNDBERG & J. ÖRBERG, 1977. Effects of PCB and DDT on mink (*Mustela vison*) during the reproductive season. Ambio **6**: 239.

KIHLSTRÖM, J. E., M. OLSSON, S. JENSEN, A. JOHANSSON, J. AHLBOM & A. BERGMAN, 1992. Effects of PCB and different fractions of PCB on the reproduction of the mink (*Mustela vison*). Ambio **21**: 563-569.

KRUUK, H., 1995. *Wild Otters. Predation and Populations*. Oxford University Press, Oxford.

LEGAULT, A., 1987. *L'anguille dans le bassin de la Sèvre niortaise. Biologie, écologie, exploitation*. Parc Naturel Régional du Marais Poitevin & Ecole Nat. Sup. Agron., Rennes.

LEONARDS, P. E. G., M. D. SMIT, A. W. J. J. DE JONG & B. VAN HATTUM, 1994. *Evaluation of dose-response relationships for the effects of PCBs on the reproduction of mink (Mustela vison)*. Dutch Otter Station Foundation, Amsterdam.

LIBOIS, R. M. & R. ROSOUX, 1989. Ecologie de la loutre (*Lutra lutra*) dans le Marais Poitevin. I. Etude de la consommation d'anguilles. Vie Milieu **39**: 191-197.

LODE, T., 1993. The decline of otter *Lutra lutra* populations in the region of the Pays de Loire, western France. Bull. Soc. Nat. Ouest France **11**: 69-76.

MACDONALD, S. M. & C. F. MASON, 1994. Status and conservation needs of the otter (*Lutra lutra*) in the western Palearctic. Council of Europe, Sauvegarde de la nature **67**: 1-54.

MASON, C. F., 1989. Water pollution and otter distribution: a review. Lutra **32**: 97-131.

MASON, C. F., 1995. Impact of pollution on the European otter. Cahiers d'Ethologie **15**: 307-320.

MASON, C. F. & W. M. O'SULLIVAN, 1992. Organochlorine pesticide residues and PCBs in otters (*Lutra lutra*) from Ireland. Bull. Environ. Contam. Toxicol. **48**: 387-393.

MASON, C. F. & J.R. RATFORD, 1994. PCB congeners in tissues of European otter (*Lutra lutra*). Bull. Environ. Contam. Toxicol. **53**: 548-554.

ROSOUX, R. & R. M. LIBOIS, 1994. Statut, écologie et devenir des populations de loutre d'Europe (*Lutra lutra*) en France. In: Groupe Loutre Luxembourgeois (Ed.): *La loutre au Luxembourg et dans les pays limitrophes*: 6-11. Graphic Press, Mamer.

ROSOUX, R., T. TOURNEBIZE, H. MAURIN & C. BOUCHARDY, 1995. Etude de la répartition de la loutre d'Europe (*Lutra lutra* L.) en France. Actualisation 1993. Cahiers d'Ethologie **15**: 195-206.

ROSOUX, R. & T. TOURNEBIZE, 1995. Analyse des causes de mortalité chez la loutre d'Europe (*Lutra lutra*) dans le Centre-Ouest atlantique (France). Cahiers d'Ethologie **15**: 337-350.

SMIT, M. D., P. E. G. LEONARDS, B. VAN HATTUM & A. W. J. J. DE JONGH, 1994. *PCBs in the European Otter (Lutra lutra) population*. Dutch Otter Station Foundation, Amsterdam.

TATEYA, S., S. TANABE & R. TATSUKAWA, 1989. PCBs on the globe: possible trend of future levels in the open ocean environment. In: N. W. SCHMIDTKE (Ed.): *Toxic contamination in Large Lakes, III: Sources, Fate and Controls of Toxic Contaminants*: 237-281. Lewis-Publisher.

THOME, J. P., M. H. DEBOUGE & M. LOUVET, 1987. Carnivorous insects as bioindicators of environmental contamination: organochlorine insecticide residues related to insect distribution in terrestrial ecosystems. Int. J. Environ. Anal. Chem. **30**: 219-232.

TOURNEBIZE, T. & R. ROSOUX, 1995. *Programme de restauration des voies d'échange pour les poissons migrateurs dans le Marais Poitevin.* Rapport Parc Naturel Régional du Marais Poitevin, La Ronde.

WEBER, D., 1990. La fin de la loutre en Suisse. OFEFP, Cahier de l'Environnement **128**: 72-101.