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Proceedings of the First Otter Toxicology Conference

Edited by J. W.H. Conroy, P. Yoxon and A. C. Gutleb

Isle of Skye
September 2000

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INTERNATIONAL OTTER SURVIVAL FUND
BROADFORD
ISLE OF SKYE IV49 9AQ
SCOTLAND

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Skye 2000

It is over ten years since a group of us thought there was a need to bring together those people throughout Europe who have been involved in otter toxicology. We felt it would be a good idea to get together and look at what we, as a group, were doing, and to decide some common approaches to techniques used, tissues sampled and how best to present data.

The gestation period for the meeting was long, but perhaps this was fortunate. In the intervening period, many new and innovate research projects have evolved and the role of pollutants has become integrated with other studies. For example recent work on diseases, the role of vitamin A, reintroductions etc.

The format of the conference allowed for several papers to 'set the scene' – an examination of the current status of the species in Europe; review the role of pollutants in the decline of the otter. Other papers presented new information including data from the 1970s in the UK (a critical period in the history of otter populations). Speakers examined the role of pollutants and diseases, as well as the potential of using DNA finger printing.

The aims of the meeting were to:

- Identify what future research might be required to determine pollutant impacts on otters;
- Determine common protocols for the collection, storage and analysis of otter tissues;
- Identify protocols for the post-mortem of otter carcasses;
- Try and develop a pan-European approach to the integration of pollutant research in otters.

These Proceedings are a result of the meeting. The papers show how far we have come in recent years regarding the role of otters and pollutants. It is clear that there are still many questions unanswered, however, we feel that this meeting made a start at addressing some of these.

It was agreed by all attending that the meeting was worthwhile and that we should consider a further meeting, also on Skye, in the not too distant future.

We are grateful to a number of people who helped make the conference possible. The financial support from Worldwide Fund For Nature, Skye and Lochalsh Enterprise and the Environment Agency ensured that we were able to invite a number of colleagues from eastern Europe and to ensure that the Proceedings are produced.

Can we also thank all who came to Skye; it was their conference and their input which made it a memorable event. We must also thank the staff at Sabhal Mor Ostaig who made us feel most welcome and looked after the inner needs of everyone! The young people from Skye and Lochalsh Feis who entertained everyone with their music and dance on the Saturday evening also deserve our thanks. And of course,

Janet Wildgoose, of IOSF, who painstakingly compiled these Proceedings from their different formats.

Finally to the 'gods of Skye', a big thank you for giving us beautiful weather and the opportunity to see wild otters swimming along the coasts most mornings. For some, this was the first time they had seen otters in the wild!

Jim Conroy, Paul & Grace Yoxon and Arno Gutleb
IOSF
Broadford
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THE STATUS OF THE EURASIAN OTTER (*LUTRA LUTRA*) IN EUROPE – A REVIEW

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1 INTRODUCTION

The Eurasian otter (*Lutra lutra*) has been described as having *one of the widest distributions of all Palearctic mammals* (CORBET, 1966). Its range originally extended from Portugal in the west to Japan in the east, and from Northern Europe and Asia, to the southern shores of the Mediterranean. In Asia, it is found as far south as Indonesia (FOSTER-TURLEY and SANTIAPILLAI, 1990).

Over the past 40 years there have been marked declines in the number of animals throughout much of the otter's range, particularly in Western Europe, and concern expressed for the survival of the species in several countries.

This paper reviews the current status in Europe, other reviews on the status of the species in other parts of its range can be found in CONROY, MELISCH and CHANIN (1998) and CONROY and CHANIN (in press).

2 THE BRITISH ISLES

The otter was once widespread throughout the British Isles, and this is reflected by its inclusion in many early natural history books, the fact that bounties were paid on them and that hunts were organised as a sport and as a means of control, with in some areas dramatic effects.

Populations appear to have been still relatively healthy in the early 1950s (STEPHENS, 1957), but shortly afterwards the situation changed. There was a serious decline in numbers, which started suddenly about 1957/58, and occurred simultaneously throughout much of England, Wales and the Scottish Borders. CHANIN and JEFFERIES (1979) reviewed the situation and concluded that the factor most likely to have been responsible for these events was the introduction in 1956 of the organochlorine groups of insecticides, in particular the dieldrin (see also JEFFERIES and HANSON, 2001).

Detailed monitoring programmes have shown that following the decline in otter numbers over large areas of the UK, there has been, since in the late 1970s, a slow expansion of the animals' ranges over the last 15 years (Table 1). These have centred on those otter strongholds, which survived the population crashes of the late 1950s. There are, however, still large areas, particularly of Central and Southern England, where the species remains absent, or is very rare; but even in some of these there are occasional reports of individual animals being seen, suggesting a continuing expansion of the species range.

Table 1 Results of the three national surveys of England, Wales and Scotland

Country	Year	No. of sites	No. positive	% positive	Reference
England	1977-79	2,940	170	5.8	Lenton *et al.* (1980)
	1984-86	3,188	286	9.0	Strachan *et al.* (1990)
	1991-93	3,188	706	22.2	Strachan & Jefferies (1996)
Wales	1977-78	1,030	210	20.4	Crawford *et al.* (1979)
	1984-85	1,097	421	38.4	Andrews *et al.* (1993)
	1991	1,102	579	52.5	Andrews & Crawford (1986)
Scotland	1977-79	4,636	3,385	73.0	Green & Green (1980)
	1984-85[1]	2,650	1,717	64.7	Green & Green (1987)
	1977-79	2,650	1,511	57.0	Green & Green (1987)[2]
	1991-94	3,706	3,245	88.0	Green & Green (1997)
	1977-79	2,538	1,726	68.0	Green & Green (1997)[2]

1 Only areas which showed sub-optimal distribution in 1977-79 were surveyed
2 Data for the sites that were surveyed in 1984-85

STRACHAN and JEFFERIES (1996) and JEFFERIES (1997) reviewed the findings of the three national otter surveys, and concluded that should the current rate

of recovery be maintained, a level of 75% occupation over all of Britain may be achieved by 2010, just over half a century following the population crash.

Shortly after the decline of the otter was reported in Britain, similar declines were recorded throughout much of Western Europe. (MACDONALD and MASON, 1990)

3 WESTERN EUROPE

Portugal, Spain, Andorra, France, Belgium, Netherlands, Luxembourg, Switzerland, Germany, Austria, Liechenstain

The otter is widespread and thriving throughout much of **Portugal,** with animals being found both on the coast and in freshwater habitats (TRINDADE, 1994; SANTOS-REIS, TRINDADE and BEJA, 1996). Since 1990, otters have been recorded as present in 171 10kmx10km UTM squares and at around 70% of the sites visited (RUIZ-OLMO *et al.,* 2001). The animals are most common in the north-east and south-west parts of the country and least common in the central area. Portugal could, therefore, hold one of the most important otter populations in Western Europe (TRINDADE, FARINHA and FLORENCIO, 1998). There is no evidence to suggest that the population is currently under threat, although SANTOS-REIS (1994) identified a new potential danger, periods without rain, resulting in many watercourses becoming dry in the summer. Also the damming of rivers has resulted in reduced water flow down stream (P.J. BEJA, *pers. comm.)* The species has been fully protected since 1974, but still subject to illegal hunting.

It was thought that there had been a marked decline in otter numbers in **Spain** since the mid 1960s (BLAS-ARITIO, 1978). In the early 1980s, however, it was still widely distributed in the west, but by the end of that decade was considered threatened in the east, and restricted in the Central Region (DELIBES, 1990).

A spraint survey of 3,966 sites throughout the country in 1984-85 found evidence of otters at just 1,327 (33.5%) (DELIBES, 1990). Signs of otters were most frequent in the north and north-west of the country: Galicia, Asturias, and in West Central Spain on the borders of Portugal, where there is also a healthy population (SANTOS-REIS, TRINDADE and BEJA, 1996). Fewest signs were found in the east, south-east and central part of the country, with Cataluña (3.1%) and Comunidad Valenciana (6.3%) being the lowest.

Recent surveys (1994 and 1996) have shown a recovery throughout much of the country. Of 4,198 sites visited, 2,082 had evidence of otters (49.6%) compared with 33.5% in 1984-85. Marked increases were found in five regions - Cataluña, Aragón, Asturias, Galicia and Western Andalucía, but declines were reported in others, including Navarra, the Basque country, Rioja and to the north of Castilla-León (RUIZ-OLMO and DELIBES, 1998). increases have also been reported from both the coastal and subalpine areas of the Pyrennes (RUIZ-OLMO, 1994).

There is, however, concern that damming of streams, particularly in the Mediterranean part of the country, will have an adverse effect on otter numbers (PEREZ and LACOMBA, 1991).

The otter was once found in **Andorra** (RUIZ-OLMO and GOSÁLBEZ, 1988), but is now thought to be extinct (RUIZ-OLMO *et al.,* 2001)

At the beginning of the 20th century, the otter was found in every region of **France** except Corsica, and remained common throughout the country until about1930 (ROSOUX *et al.,* 1996). Over the next two decades a decline occurred and since the 1950s the species has disappeared from 47 of the 95 French Departments. Otters are widespread in the area west of Brittany, south to the Pyrennes and in the Massif Central, but are absent from much of the north and east of the country (ROSOUX et *al.,* 1996).

There has been a recolonisation, in the area around the Massif Central, which started around 1984. In a survey carried out between 1989 and 1993, the otter was recorded as common in 11 Departments, all being around the Massif Central or in the western part of the country and it was sporadically seen in a further 16 Departments (ROSOUX *et al.,* 1996). The number of Departments without otters, however, continued to increase over the past 40 years, from 21 in the period 1930-1950 to 47 in the most recent survey (1989-1993). Recent surveys also confirm an expansion in Brittany which is now thought to contain about 25% of the country's otters (LAFONTAINE, 1993). More detailed information about the status of the species in certain parts of France can be found in GAUTIER, LIBOIS & ROSOUX (1996). The 1989-1993 survey recorded otters as occurring regularly in 192 10km x 10km UTM squares, and sporadically in a further 59 (from ROSOUX *et al.,* 1966). The species has been protected in France since 1972.

The otter was once common in **Belgium**. Such was the density of animals in Flanders that a campaign to eradicate the species was introduced in 1889, and over the next seven years more than 2,000 otters were destroyed (ANONYME, 1896). At the turn of the century around 300 individuals a year were being killed. During the 1960s numbers declined dramatically at most locations, and by the 1980s, only a few small relic populations were left (LIBOIS *et al.,* 1982; CRIEL, 1984, 1989). In the northern part of the country (Flanders), the species became extinct, although recently there have been occasional signs of animals. In the southern part of the country (Wallonie), a few animals continued to survive (LIBOIS *et al.,* 1982; METSU and VAN DEN BERGE, 1991; VAN DEN BERGE, 1998). OVERAL (1995) recorded that a small, but viable, population has existed, possibly for a number of years, on the Haute-Sûre, on the border between Luxembourg and Belgium. Between 1965 and 1969 game records showed otters to be present in 84 10km x 10km UTM squares.

Over the next 14 years evidence of otter presence dropped, and by 1984, the animal was recorded in only 50 squares (LIBOIS and HALLET, 1996).

The current state of the majority of Belgian rivers, heavily polluted and with few fish, means that they are considered unsuitable for otters (LIBOIS and HALLET, 1996). The species is fully protected in Belgium, and is listed in the Red Data Book (K. VAN DEN BERGE, *pers. comm.*).

The species is currently thought extinct in **Luxembourg.** Otters were, however, found throughout the country in the 19th century. Pressures from hunters and fishing interests convinced the legislators that the otter was *un pilland de poissons terrible*, and allowed otter hunting to take place. This lasted until 1955 and as a result the population was dramatically reduced. Although hunting ceased in the mid 1950s, and the species was protected in 1972, the population continued to decline (SCHMIDT and ADAM, 1992). The last otters were seen in 1995 in La Haute-Sûre Region (GROUPE LOUTRE LUXEMBOURG, 1997).

As early as 1940, the otter population in **the Netherlands** was thought to have reached an all time low of between 30 and 50 animals (BROUWER, 1940, 1942), and legislation was enacted to protect the species. By 1962, however, there had been a significant increase with an estimated 300 individuals distributed in five areas (VAN WIJNGAARDEN and VAN DE PEPPEL, 1970). As late as 1983, the species was considered to be widely distributed (VEEN, 1984). However, by 1988 otters were restricted to a few isolated areas (NOLET and MARTENS, 1989), and are thought to have become extinct shortly after this (WINTER, 1993). In recent years, signs of animals have been reported from some parts of the country (DULFER *et al.,* 1993).

In the early 1950s it was thought that there were 40-60 individuals in central and northern parts of **Switzerland** (KREBSER, 1959). The distribution of otters dropped from at least 50 localities in1960 to only one in 1989 (WEBER, 1990), this despite the reintroduction of eight animals in 1975 (WEBER, WEBER and MÜLLER, 1991). WEBER and WEBER (1991), however, suggested that as late as 1989, a small population, thought to be less than five animals, was living on the north-east shore of the Lake of Neuchâtel, but the species is now considered extinct.

In **Germany**, where the otter is fully protected by hunting law, the species is highly endangered in the old Federal Republic, with otters being rare or extinct in many of the federal states. Over 20 years ago, HODL-ROHN (1977) suggested that only one percent of the former otter population survived in both German Republics, however, she presented no information to support her statement, and only three years previously, RÖBEN (1974) had stated the population in the Federal German Republic (FDR) was nearly 500 animals. It is locally common in the former German

Democratic Republic, but even here, the distribution is becoming more restricted, possibly because of changes in land-use practices and the rapid increase in the volume of traffic in the former GDR following reunification in the late 1980s. The species is absent, for example, in the more lowland regions and along the Baltic coast (STUBBE, 1989; MACDONALD and MASON, 1990,1994; REUTHER, 1992; STUBBE *et al*., 1993). STUBBE and STUBBE (1994), however, reported that the species was now endangered and rare in the former GDR, with both populations and distribution area declining.

REUTHER (1980) examined the changing populations of otters in Lower Saxony over the past 100 years. Until about 1900, the species appeared to occur in relatively large numbers and was distributed fairly uniformly throughout the Province. Persecution, however, was taking its toll, e.g. between 1882 and 1913, bounties were paid for the bodies of over 8,000 otters in Hanover alone. By 1920, the species had become rare in the southern part of the province. This decline continued over the next 25 years. A second serious decline began in the 1950s and continued at least into the 1980s. Recent surveys in Lower-Saxony suggest that a recovery has taken place there in the past decade. Two hundred and twenty six sites were surveyed in 1991 and 1999. The number of positive sites increased from 2.2% in 1991 to 14.2% in 1999. In addition, there was a marked increase in the number of 10x10km UTM squares with evidence of otters, from 12.5% in 1991 to 43.8% in 1999 (REUTHER and ROY, 2001). REUTHER (1995) reported an east-west split in distribution, the species becoming rarer as one moved westwards.

The **Austrian** population is also expanding. In their account of the status of the otter in Austria, MACDONALD and MASON (1990) reported that the species was threatened. By 1997, A. KRANZ (*pers. comm.*) suggested otters were found in about 30% of the country. There are two main populations. The larger is found in the northern parts of Upper and Lower Austria and recent surveys have shown that this population is expanding southwards and has crossed the Danube (KRANZ, 1994). The smaller population, in the south-east of the country, expanded between 1986 and 1993-94; from 11.1% to 25.2% of the sites visited (SACKL, ILZER and KOLMANITSCH, 1996).

Both populations continue to expand (BODNER, 1994; GUTLEB, 1994) and there is some evidence that the two populations have made contact in the Northern Limestone Alps (A. KRANZ, *pers. comm.).*

A distribution map in GUTLEB (1994) shows definite evidence of otters in 39 10x10km UTM squares, with the possibility of otters in a further seven.

The otter in Austria is treated as a game species, but since 1947 has had all year round protection. It is listed as an endangered species in the Austrian Red Data Book (BAUER and SPITZENBERGER, 1994).

The otter is extinct in **Liechtenstein.**

4 SCANDANAVIA

Denmark, Norway, Sweden, Finland

The countries of Scandinavia have shown a slight increase in the range of the otter over the past few years. In **Denmark**, game bag statistics showed that otters had been killed all over the country with no apparent effect on the population (JENSEN, 1964). By 1967, however, concern was being expressed about the status of the species and it was granted full protection (SØGAARD and MADSEN, 1996). The otter population at the end of the 1970s was thought to be between 200 and 500 individuals (JENSEN, 1980). [This compares with a regular cull of around 200 animals per annum between 1941 and 1962 (JENSEN, 1964)] The critical state of the population was evident from surveys in the early 1980s when only 106 (9.2%) of 1,154 sites visited showed any presence of otters (MADSEN and NEILSEN, 1986), with the majority in Central and North-west Jutland. Six hundred and thirty three sites were visited in three surveys 1984-86; 1991 and 1995; the number of positive sites in each survey was 15.2%, 24.1% and 35.5% respectively (MADSEN and NEILSEN, 1986; HAMMERSHØJ *et al.,* 1996). Today the otter is still regarded as endangered, but MADSEN (1996) concludes that *after 10 years work, there are positive indications of a successful enhancement of the living conditions for otters in Denmark.*

The **Norwegian** populations are fragmented in the south, but large and widespread in the north, where it is widely distributed along the coast and inland in lower densities (HEGGBERGET, 1994). MYBERGET and FRØILAND (1972) showed that the species was already uncommon in the south, the decline continuing in many areas in the 1970s) (HEGGBERGET and MYBERGET, 1980). During the 1980s and 1990s the population in the north recovered to an estimated 10-15k individuals, while populations in the south of the country are considered fragmented, vulnerable and probably threatened (HEGGBERGET, 1996).

A survey of otters in 1989-1990 (CHRISTENSEN, 1995) indicated that the present distribution of otters in Norway is characterised by a metapopulation in the north (62^0N - $67\ ^0$30'N), where 85% of coastal sites surveyed had evidence of otters. The species had all but disappeared from along the south-eastern coasts $58\ ^0$N - $59^0$50'N where only 3% of 80 sites visited proved positive for otters. In the western provinces, between the northern and south-eastern areas, otters signs were found at 22.1% the

sites visited, there was an increase in the number of sites with evidence of otters from south to north. The northern region is considered to hold a viable otter population.

Based on two survey in the mid1960s and 1970s as well as information from game bags, it was concluded that that the otter population in **Sweden** was declining, a decline which probably began around 1950 (ERLINGE, 1971; ERLINGE and NILSSON, 1978; ERLINGE, 1980). The decline, at least in part of the country continued, and by 1997 it was estimated that there were only 500-1500 otters in the country, which was less than the annual otter harvest for around 1950 (ERLINGE and NILSSON, 1978).

Surveys in the 1980s showed only 5% of 2,000 sites visited in southern part of the country with evidence of otters (OLSSON and SANDEGREN, 1986), while in Northern Sweden otters were evident in slightly more, 10% of the sites visited (OLSSON in MACDONALD and MASON, 1994).

More recent research, based on the otter reintroduction programme in Central Sweden has shown an expansion of the otter population, (SJÖÅSEN and SANDEGREN, 1992; SJÖÅSEN, 1996). There is evidence that the reintroduced otters are now in contact with the northern population and signs of otters are being found in areas where there have been none reported for nearly 20 years (T. SJÖÅSEN, *pers. comm.*). The species is classified as vulnerable in the central and northern parts of Sweden and endangered in the south (T. SJÖÅSEN, *pers. comm.*).

Historically, otters in **Finland** were found throughout the country, including the coasts and on small offshore skerries. In the late 19th century hunting bags of 1,000 animals a year were reported, but by 1910, bags had fallen to around 100 otters per annum, and this decline in the numbers killed continued over the next 20 years (WIKMAN, 1996). The otter was first protected in 1938, but the subsequent increase in numbers resulted in trapping being legalised again 12 years later, after which bags of 100-200 animals were reported annually. The population declined and, despite protection being reintroduced in 1975, and MACDONALD and MASON (1990) reported that populations were becoming more fragmented in some areas and absent in others. KAUHALA (1996) confirmed otter numbers declined in the 1970s, but increased again in the 1980s, with a marked increase in distribution between 1981 and 1991.

In Finland, otters are currently thought to be widespread, but with a patchy distribution (SKARÉN and KUMPULAINEN, 1986; HAGNER-WAHLSTEN and STJERNBERG, 1991), and while they are rare in the southern part of the country and in coastal areas, good populations are found inland in eastern and central parts of the country (SKARÉN, 1990). WIKMAN (1996) suggested that there are currently in excess of 1,000 otters in the country.

The most recent surveys, using snow tracking suggest decreases in otter numbers throughout much of the country over the period 1989-1997 (HELLE *et al*., 1997). There were, however, some noticeable increases in parts of the country during the early part of the 1990s. For example, in Central Finland, from snow tracks, SULKAVA and STORRANK (1993) and SULKAVA (1994) estimated that the population had increased from 25 animals in 1985 to 35-40 in 1993.

The overall picture, therefore, appears confused, with increases in some areas, decreases in others and some marked fluctuations over the past ten years. The species is classified as "declining, in need of monitoring" (U. SKARÉN, *pers. comm*.)

5 EASTERN MEDITERRANEAN AND BALKANS

Italy, Greece, Cyprus, Albania, Slovenia, Croatia, Bosnia and Herzegovina, Federal Republic of Yugoslavia (Serbia and Montenegro), Former Yugoslav Republic of Macedonia

The **Italian** otter population is endangered and its survival depends upon the conservation of the populations living in the southern part of the country (PRIGIONI and FUMAGALLI, 1992). In the early 1970s the species range already appeared to be highly restricted (CAGNOLARO *et al*., 1975), and in the first national field survey conducted in 1984 found only 6.2% of nearly 1,300 sites visited with evidence of otters. Less than 100 individuals were thought to survive (CASSOLA, 1986). Some areas have been surveyed more recently with differing results. In the Sele-Calore river catchments, for example, the population appears to be stable, while some populations in Southern Tuscany and Northern Latium showed apparently dramatic decreases by late 1990, and may even be extinct (CASSOLA, 1994). The persistence of otters in several water bodies in Campania, Basilicata and Calabria was confirmed in 1994, when 45% of 35 sites visited had evidence of otters; the local density and demographic trends of the species in these regions remain unknown (REGGIANI *et al*., 1997).

Otters were found on 50 water bodies, mainly in Southern Italy, during the period 1984 to 1994, with the population fragmented into five main groups (PRIGIONI, 1997). Based on an estimate of 1.4 otter/10km of river, and the assumption that the species is distributed along 950km of watercourses, he calculated about 130 individuals in the country. In Italy, the species had been legally protected since 1977 and is included in the national red data book as critically endangered (AMORI *et al*., 1996).

The otter is thought to be widespread throughout much of **Greece**, but particularly in the north-east. The distribution is, however, fragmented in some parts of the

country, in particular the central area. A recent survey (1997) of the north-west part of the country found signs of otters at 63% of the 46 sites visited, suggesting the species is still relatively widespread (URBAN, 1998). In 1997, however, DELAKI *et al*. (1989) considered that otters had declined during the previous ten years. The species is still found along the north and east coasts of Corfu, but is absent from the west (URBAN, 1998). All year protection throughout the country was granted under the hunting laws in 1968.

Animals are still found along the north and east coasts of Corfu, but are absent from the west, where URBAN (1998) reported evidence of otters at 8 of 14 sites visited. On this island, the species is, however, considered endangered by tourist expansion and the traditional olive oil production (GRÉMILLET, 1993; URBAN, 1998).

The otter is not found on the island of **Cyprus** (SMIT and VAN WIJNGAARDEN, 1976).

Little is known about the distribution of otters in **Albania.** During the 1960s, the species was considered to be widespread in the country. In 1985, PRIGIONI, BOGLIANI and BARBIERI (1986) visited a small part of the country and found evidence of otters at nearly 55% of the sites examined. From this they concluded that the species was likely to be distributed throughout the country, but with some restriction of its range in the central area and coastal plain. URBAN (1998) reported that local fishermen persecute the species.

The situation in parts old **Yugoslavia** is difficult to determine because of the recent conflicts. MACDONALD and MASON (1990) reported that the species was found throughout much of the country, with the exception of the mountainous north-west area of the Adriatic coast. Inland, along the main rivers, the species was thought to be at a low density of extinct.

In Slovenia, the otter is considered rare. Enquiries in the 1980s (HÖNIGSFELD, 1985 *a* & *b*) indicated a decline throughout the country as well as a change in distribution over the previous decades. The main stronghold is in the north-east part of the country (Prekmurje), where the population is recorded as viable (HÖNIGSFELD, 1998). Of 74 sites surveyed in this area, between 1996 and 1998, 65 (88%) showed evidence of otters, while the sites with no evidence were considered as unsuitable for sprainting.

The species is listed as endangered in the Red List of Endangered Mammalia in Slovenia (KRYTSTUFEK, 1992). It has been fully protected since 1976, and is currently protected by the Order of the Government of the Republic of Slovenia under the Protection of Threatened Animal Species (1993).

Croatia - Rare along coastal strip, but relatively numerous in the northern part of the country (MACDONALD and MASON, 1994);

Bosnia and Herzegovina - Widespread and relatively numerous (MACDONALD and MASON, 1994).

Federal Republic of Yugoslavia (Serbia and Montenegro) - PAUNOVIĆ and MILKENOVIĆ (1994) concluded that the species was more widespread in these two republics than had previously been reported. Animals were found in most areas except for the central part of Serbia and West Central Montenegro. It is found along the coast, probably in small numbers up to 1,400m asl (PAUNOVIĆ and MILKENOVIĆ, 1996). The otter is currently protected (in Serbia since 1976 and Montenegro since 1982) as a 'natural rarity' under the hunting laws. This category has, however, become outdated, and hopefully the legislation will be revised, although it is expected that the otter will remain protected.

Former Yugoslav Republic of Macedonia - Widespread and relatively numerous in areas alongside Albanian border: rare elsewhere. M. PAUNOVIĆ (*pers. comm.*) confirms that the overall situation in the former Yugoslavia is of a general decline in numbers from east to west.

6 BALTIC REPUBLICS

Latvia, Lithuania, Estonia

In the Baltic Republics otters are widely distributed. Despite intensive hunting pressures, otters are widespread throughout **Latvia**, being found on most water courses (OZOLINŠ and RANTINŠ, 1992*a*), but with an uneven distribution. More dense populations are in the western and eastern parts of the country, with less dense populations in the north, north-east and on the coastal plain. ORNICANS (1994) detailed the changing otter population in Latvia this century, from around 500 individuals in 1914, numbers dropped to an all time low of 255 in 1947. This decline was associated with the rapid development of agriculture and land reclamation and persecution by fish and crayfish breeders (OZOLINŠ and PILÃTS, 1995). They increased to 2,370 in 1968, before declining again to 1,050 in 1982. Since then there has been a steady recovery, with the 1993 population being estimated at 4,000 animals. Between 1980 and 1987, the otter was included in the *Red Data Book of Latvia*, but was subsequently removed when it became clear from hunting returns that the species was numerous, It is thought that the successful re-establishment of the beaver in Latvia has benefited the otter (OZOLINŠ and RANTINŠ, 1995), the latter making use of the beaver lodges and fish ponds. OZOLINŠ and RANTINŠ (1992*b*) state that otters inhabit, at least for a short period, at least 50% of the Latvian lakes and over 80% of the rivers.

In **Lithuania**, the population is described as widespread, the species being found in all 44 regions of the country (MICKEVIČIUS, 1993). There was, however, evidence of a decline between 1969 and 1984, but since then the population has stabilised or increased. Based on a questionnaire survey it was estimated that there were 420 otters in the country in 1990 and 340 in 1991. According to K. BARANAUSKAS and E. MICKEVIČIUS (*pers. comm.*), the Annual State Wildlife Census suggested that there were 1,430 otters throughout the country in 1997, an increase of 130 from the previous year. These authors, however, feel that this number is rather low, and suggest a more realistic figure of between 3,000 and 12,000. A recent survey of 446 sections on 269 rivers found evidence of otters at 94% of the sites (BARANAUSKAS and MICKEVIČIUS, 1995). Beaver trapping is seen as a serious threat to the otter in this Baltic State, although even this danger has declined as the demand for beaver fur has diminished (BARANAUSKAS *et al.,* 1994). The species is classified in the *Red Data Book of Lithuania* (2nd edition, 1992) under Category 4, i.e. undetermined, insufficiently investigated.

At the beginning of the 20th century, the otter was considered widespread in **Estonia**, but according to LAANETU (1989), by the mid 1980s, the species was sparsely distributed throughout the country with an estimated population of only 600 individuals. Between 1920 and 1935, numbers dropped considerably, probably as a result of poaching (N. LAANETU, *pers. comm.*). Numbers slowly grew and, by the mid 1950s, the population was estimated to be 800-900 individuals, and had increased to over 2,000 by the 1960s, but numbers dropped by nearly 50% over the following eight years. This decline continued until about 1975, when it was thought that there were only 300-350 animals; since then numbers remained relatively stable for seven years after which there has been a slight increase with a count of around 550 in 1988. The species is now concentrated in eight districts which are not isolated (KILLI, 1991). The most recent estimates gave 1,400 -1,500 animals in 1993, but since then there have been a decline (N. LAANETU, *pers. comm.*). The species is currently protected, and is listed in the Red Book of Endangered Species.

7 EASTERN EUROPE

Czech and Slovak Republics, Poland, Hungary, Romania, Bulgaria, Belarus

There have been extensive surveys of the otter in the former Czechoslovakia, and these have continued in the newly formed **Czech** and **Slovak Republics**

In 1977/78, BARUŠ and ZEDJA (1981) identified 342 localities where otters were present in **Czechoslovakia**, and estimated that the minimum number to be 174 individuals. TOMAN (1992) reported the results of the most recent surveys using both snow tracking and spraints. He estimated 300-350 animals in the **Czech Republic** in three isolated populations - a small one in the north extending to the

German border, another, in the east, joining with Slovakia and a third, the main centre of otter activity, in the South Bohemian fish pond area, a population that extends into the Austrian Waldviertel. He suggested that about 25% of the country is occupied by otters, and that numbers have increased slightly over the past five years. In *The Atlas of Mammals of the Czech Republic*, three or four populations are identified, numbering 350-400 individuals (ANDĚRA and HANZAL, 1996).

In **Slovakia**, the status seems unclear, (KADLEČÍK, 1994) regarded the species to be seriously endangered, but the same author (KADLEČÍK, 1992) had earlier stated the species was still widely distributed, with the main population in the central and eastern parts of the country. URBAN (1992) reports a marked drop (70%) in otter numbers in part of Pol'ana, Slovakia over the previous quarter of a century, but showed that the population then remained stable in the area up to 1995 (URBAN, 1995). Following the most recent survey in 1994-1995, KADLEČÍK and URBAN (1997) concluded that there had been no major changes in otter distribution over the past 20 years, the species had, however, occupied new parts of the country.

In the Czech Republic the species has been protected since 1949, but in 1996 was listed in the new hunting laws, with a year-long open season. Thus the otter is currently subject to two conflicting laws, although the former still ensures the animals' protection (A. TOMAN, *pers comm*.). The species is listed as "endangered" in the *Red Data Book* (BARUŠ, 1989). In Slovakia, the otter is strictly protected under the Act on Native and Landscape Conservation, and is listed as "vulnerable" in the Red Data Book (ŠTOLLMANN, *et al*., 1997).

In **Poland**, BUCHALCZYK (1983) reported that the otter was still numerous, but several other reports published in the early 1980s indicated a decline of otters throughout the country (ROMANOWSKI, 1984; BIENIEK, 1988). The species was described as rare and endangered in the Polish Red Data Book (BIENIEK, 1992). Between 1991-1994, evidence of otters was found throughout Poland, with nearly 80% of over 2000 sites visited showing presence of the animal (BRZEZIÑSKI and ROMANOWSKI, 1997), this represents one of the highest percentages of positive sites of otters in continental Europe. Only two areas, Silesia and Central Poland had few signs of the species (BRZEZIÑSKI and ROMANOWSKI, 1994; BRZEZIÑSKI *et al.*, 1996). The last named authors reported increases throughout much of the country, including the capital Warsaw. They attributed the increase to a reduction in the effluent entering the water, and possibly the increase in beaver numbers. Summarising the national otter survey, BRZEZIÑSKI and ROMANOWSKI (1997) concluded that the species is widely distributed throughout Poland and should no longer be considered endangered in the country. More recent surveys in 1996 showed that the otter continues to expand its range, with positive signs of otters (54%) in an

area of Central Poland and the city of Warsaw that had no evidence of otters in survey of the early 1990s (ROMANOWSKI, GRUBER and BRZEZIÑSKI, 1997).

The **Hungarian** otter population is thought to be stable, but there has been a decline in the area east of the Danube (NECHAY, 1980). A large-scale questionnaire survey in 1995/96 showed that out of 464 10x10 km UTM squares, otters were living permanently in 333 (72%) (EGYETEMES LÉTEZÉS TERMÉSZETVÉDELMI EGYESÜLET KLUBJA, 1997), a slight drop from the 1987-88 survey, where the species was recorded in 86% of the sites visited (KEMENES, 1991). The population was reported as being stable, but growing. There was, however, concern about illegal killing (EGYETEMES LÉTEZÉS TERMÉSZETVÉDELMI EGYESÜLET KLUBJA, 1997). The species was given "strict" protection in 1978, but legal killing of individuals can be sanctioned after it has been established that they have been responsible for damage (RAKONCZAY, 1990; LANSKI and KÖROMENDI, 1996).

There are few data from **Romania.** Numbers declined from 2,050 in 1950 to 1,550 in 1991 (WEBER in MACDONALD and MASON, 1994). GEORGESCU (1994) reported that the species was found throughout the country, from sea level to the subalpine zone (1,700m asl) but that the population had declined from 2,180 in 1980 to 920 in 1993. IONESCU and IONESCU (1994) also reported a decline in population, in this case, between the delta of the Danube to the Carpathian Mountains, from *ca.* 3,200 animals in 1955 to *ca.* 1,700 in 1994. There are obvious discrepancies in the estimated number of otters, but the important feature is that they have all shown declines over the past 25-30 years, and the species is considered endangered.

According to SPIRIDONOV and MILEVA (1994), the **Bulgarian** population is considered to have been stable for the previous 15 years, and numbers 1,000 to 1,400 animals. They are widely distributed throughout the country from sea level to about 1,400m asl. MACODNALD and MASON (1994), however, report that numbers have declined since the 1950s, with increases in some areas. The species is currently considered "endangered" and has been protected since 1962 (ROMANOWSKI, 1991).

The otter in **Belarus is** widespread, and, since 1995 can only be hunted under license. Numbers were thought to have stabilised over the period 1984-1989, except for a slight decrease in numbers in the south-west of the country, and in areas of high human population (SIDOROVICH, 1991). The population was estimated as being nearly 12,000 individuals between 1984 and 1988; this had dropped to 7,000 by 1989-1991, the decline caused principally by large scale poaching (SIDOROVICH, 1991; SIDOROVICH and LAUZHEL, 1992). It is currently estimated that the

average density of otters on the country's rivers varies between 1.7 and 4 individuals per 10 km of river in protected areas to between 1.2 and 2.0 in exploited areas.

8 CIS RUSSIAN FEDERATION

The **CIS Russian Federation** extends from Eastern Europe through Asia to the Pacific Ocean. Within this area are many republics and provinces. Information from such a vast country is, as would be expected, patchy. For the sake of this review, we review the status of the species throughout the country and in its associated republics. The otter is distributed throughout the country with the exception of the tundra. It became extinct on the Kuril Islands at the beginning of the 20th century and more recently has disappeared from many waterways in the regions of Krasnodar and Kursk (BYTCHKOV and CHACHIN, 1994). In the European sector, the otter population is thought to have been stable over the past decade, but overall numbers have declined by 30-40% since the 1930s and 1940s when the population was thought to number 80,000-100,000. The species has been confirmed in the Zabaikalsky National Park, Lake Baikal, Siberia (KRANZ *et al*., 1995), from Sakhalin Island, Kargasoksky District (Tomskaya Region), Todzhinsky District (Tuvinskaya Autonomous Region) and the mid-reaches of the Pur River (Tyumenskaya Region), while the status in Northern Magadan district, Chukotka and Koryak is unknown (ZHOLNEROVSKAYA *et al*., 1994). Otters are rare in **Tajikistan** and **Uzbekistan** (ANON, 1983; ZHOLNEROVSKAYA *et al*., 1994; PERELADOVA, KREVER and WILLIMAS, 1997). They are also reported in **Turkmenistan (**MAROCHKINA, 1995), **Kazakhstan (**ANON, 1977) and the Far Eastern **Primorye Province** (R. MELISCH, *pers. comm.*).

On the basis of census returns, ROZHNOV and TUMANOC (1994) estimated the Russian population to be in the region 60,000 individuals in 1987 - 27,000 in the European sector; 3,500 in the region of the Urals and 30,000 in the Asian sector. There is a decrease in density from west to east. Between 1991 and 1995 numbers dropped from 60,400 to 52,600 animals, a decline of 13% over the five-year period. Declines were recorded in all but one of the 12 regions listed, with the greatest decline 17.5% in the Far East (BORISOV, 1996).

At present the species is protected in some, but not all of the republics, including Kazakhstan, Turkmenistan, Tajikistan, Uzbekistan, Kirghiztan. It is considered rare in both Tajikistan and Uzbekistan (PERELADOVA, KREVER and WILLIMAS, 1997).

Two sub-species *L. l. seistanica* and *l. l. meridionalis* were listed in the *Red Data Book of the USSR* (Vol. 1) published in 1984.

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10 REFERENCES

Amori, G., Anglici, F.M., Prigioni, C. & Vigna Taglianti, A. (1996). The mammal fauna of Italy: a review. *Hystrix,* **8**: 3-7.

Anděra, M. & Hanzal, V. (1996). *[Atlas of the Mammals of the Czech Republic A provisional version II.Carnivores (Carnivora).]* Praha. (In Czech with English summary.)

Andrews, E. & Crawford, A.K. (1986). *Otter Survey of Wales 1984-86.* The Vincent Wildlife Trust, London.

Andrews, E., Howell, P. & Johnson, K. (1993). *Otter Survey of Wales 1991.* The Vincent Wildlife Trust, London.

Anon (1977). Rare and endangered mammals and birds of Kazakhstan. *Alma-Ata, Nauka*, 80-83. (In Russian.)

Anon (1983). *Red Data Book of Uzbekistan SSR*. Volume 1. Vertebrates. Pp. 23-24. Tashkent. (In Russian.)

Anonyme (1896). La destruction des loutres en Belguigue. I Bulletin de pêche et pisciculture practigue, **9**.

Baranauskas, K. & Mickevičius, E. (1995). The new data on the otter in Lithuania. *Lutreola,* **5**: 11-14.

Baranauskas, K., Mickevičius, E., Macdonald, S.M. & Mason, C.F. (1994). Otter distribution in Lithuania. *Oryx,* **28**: 128-130.

Baruš, V. (1989). *Červená Kniha.Ohrožených a Vzácnych Druhů Rodtlin a živočichů ČSSR Volume 2.* Praha, SZN.

Baruš, V. & Zejda, J. (1981). The European otter (*Lutra lutra*) in the Czech Socialist Republic. *Acta Scientiarum Naturalium Academiae Scientiarum Bohemoslovacae Brno,* **15**: 1-41.

Bauer, K. & Spitzenberger, F. (1994). Rote liste der in Oesterreichs. Sâugetierarten (Mammalia). In: *Gepp Rote Listen gefaehrdter Tiere Oesterreichs*. Gruene Reihe des Bundesministeriums für Umwelt, Jugend & Familie. **2**: 35-39.

Bieniek, M. (1998). The otter, *Lutra lutra* (L.) in Poland's national parks. *Ochroan Przyrody*, **46**: 11-14. (In Polish with English summary.)

Bieniek, M. (1992). Otter *Lutra lutra* (Linné 1758). In: [*Polish Red Data Book of Animals]* (Edited by Glowacinski, Z.). Pp. 76-77. PWRiL, Warsaw. (In Polish with English summary.)

Blas-Aritio, L. (1978). Informe sobre la situación de la nutria en España. In: *Otters: Proceedings of the 1st. Working Meeting of the Otter Specialist Group, Paramaribo, Surinam, March 1977.* (Edited by Duplaix, N.). Pp. 140-142. IUCN, Morges, Switzerland.

Bodner, M. (1994). The European otter (*Lutra lutra L.*) in Austria. In: *Seminar on the Conservation of the European Otter (*Lutra lutra*), Leeuwarden, the Netherlands, 7-11 June 1994.* Pp. 98-103. Council of Europe, Strasbourg.

Borisov, B.P. (1996). [The otter (*Lutra lutra* L.)]. In*: [Resources of Major Species of Hunting Animals and Hunting Lands of Russia (1991-1995)]* (Edited by Lomanov, K.I.). Pp. 141-145. Izd-vo Tsnil Okhotdepartamenta Minsel'khozproda, Moscow. (In Russian.)

Brouwer, G.A. (1940). De uitreoiing van de visotteer (*Lutra lutra* L.) in Nederland aanstaande. *De Levende Natuur,* **45**: 18-25, 50-57, 84-91, 115-123.

Brouwer, G.A. (1942). De visotteer in de winter van 1940-1941. *De Levende Natuur,* **46**: 170-174, 183-187.

Brzeziñski, M. & Romanowski, J. (1994). Otter distribution in Poland. In: *Seminar on the Conservation on the European otter (*Lutra lutra*), Leeuwarden, the Netherlands,7-11 June 1994.* Pp. 69-70. Council of Europe, Strasbourg.

Brzeziñski, M. & Romanowski, J. (1997). Otter survey in Poland. In: *Proceedings 14th Mustelid Colloquium* (Edited by Toman, A & Hlavac, V). Pp. 78-81.

Brzeziñski, M., Romanowski, J., Cygan, J.P. & Pabin, P. (1996). Otter *Lutra lutra* distribution in Poland. *Acta Theriologica,* **41**: 113-126.

Buchalczyk, T. (1983) *Lutra lutra* (Linneaus, 1758). In: *Atlas Rozmieszczenia Ssakow w Polsce.* (Edited by Pucek, Z & Raczynski, J). **I**: 149-151; **II**: 143. PWN - Polish Scientific Publishers, Warsaw.

Bytchkov, V.A. & Chachin, G.V. (1994). The present condition of the river otter in Russia and problems of its conservation. In: *Seminar on the Conservation of the European Otter* (Lutra lutra*), Leeuwarden, the Netherlands, 7-11 June 1994.* Pp. 95-97. Council of Europe, Strasbourg.

Cagnolaro, L., Ruso, D., Spanesi, M. & Venturi, B. (1975). Inchiesta sulla distribuzione della lontra (*Lutra lutra*) in Italia e nei Cantoni Ticino e Grigioni (Svizzera) 1971-1973. *Laboratorio di Zoologia applicata alla caccia. Biologia della Selvaggina*, **63**: 1-120.

Cassola, F. (Editor). (1986) *La Lontra in Italia. Censimento, Distribuzione e Problemi di Conservatzione di una Specia Minacciata. [The otter in Italy Status, Distribution and Conservation of an Endangered Species.]* Rome: WWF. (Serie Atti e Studie, **5**, 135pp.) (In Italian with English summaries.)

Cassola, F. (1994). 5.2 Country Report: Italy. In: *Seminar on the Conservation of the European Otter (*Lutra lutra*), Leeuwarden, the Netherlands, 7-11 June 1994.* Pp. 215. Council of Europe, Strasbourg.

Chanin, P.R.F. & Jefferies, D.J. (1978). The decline of the otter *Lutra lutra* L. in Britain: an analysis of hunting records and discussion of causes. *Biological Journal of the Linnean Society,* **10**: 305-328.

Chapman, P.J. & Chapman, L.L. (1982). *Otter Survey of Ireland 1980-812.* The Vincent Wildlife Trust, London.

Christensen, H. (1995). *Determinants of Otter Lutra lutra Distribution in Norway.* Ph.D. Thesis, University of Trondheim, Trondheim.

Conroy, J.W.H. & Chanin, P.R.F. (in press). *The Distribution and Status of the Eurasian Otter (*Lutra lutra*) – a Review.* IOSF, Broadford, Skye.

Conroy, J.W.H., Melisch, R. & Chanin, P.R.F. (1998). The distribution and status of the Eurasian otter (*Lutra lutra*) in Asia – a review. *I.U.C.N. Otter Specialist Group Bulletin,* **15**: 15-30.

Corbet, G.H. (1966). *The Terrestrial Mammals of Western Europe*. Foulis, London.

Crawford, A., Evans, D., Jones, A. & McNulty, J. (1979). *Otter Survey of Wales 1977-78.* Society for the Promotion of Nature Conservation, Nettleham, Lincoln.

Criel, D. (1984). De situatie van de otter in Belguie: een vooronderzoek. *Lutra*, **27**: 36-41.

Criel, D. (1989). The situation of the otter in Flanders, Belgium. *I.U.C.N. Otter Specialist Group Bulletin*, **4**: 37-38.

Delaki, E-E., Kotzageogis, G., Ioannidou, V.G. & Stamopoulos, A. (1989). A study of otters in Lake Mikri, Prespa, Greece. *I.U.C.N. Otter Specialist Group Bulletin*, **3**: 12-16.

Delibes, M. (Editor). (1990). *La nutria (*Lutra lutra*) en España.* Serie Tècnica. ICONA, Madrid.

Dulfer, R., de Jongh, A., Smit, M. & Bavinck, K. (1993). The otter in the Netherlands. In: *Jahrestagung der Fischottergruppe Österrich, Bad Radkersburg.* (Edited by Gutleb, A.C.). Pp. 7-11. Fischottergruppe Österrich, Wien.

Egyetemes Létezés Természtvédelmi Egyesület Klubja (1997). *The National Survey of the Protected and Endangered Otter.* OSZK, Nyomda.

Erlinge, S. (1971). The situation of the otter population in Sweden. *Viltrevy*, **8**: 379-397.

Erlinge, S. (1980). Die situation des fischotters in Schweden. In: *Der Fischotter in Europa. Veerbreitung, Bedrohung, Erhaltung.* (Edited by Reuther, C. & Festetics, A.). Pp. 103-106. Oderhaus & Göttiningen, Selbstervlag.

Erlinge, S. & Nilsson, T. (1978). Nedslsende inventeringsresultat: Uttern fortsotter att minska- bara 1000-1500 uttrar kvar. *Svensk Jakt*, **3**: 254-156.

Foster-Turley, P. & Santiapillai, C. (1990). Action plan for Asian otters. In: *Otters: an Action Plan*

for their Conservation. (Edited by Foster-Turley, P., Macdonald, S.M. & Mason, C.F.). Pp. 52-63. IUCN, Gland, Switzerland.

Gautier, J.Y., Libois, R. & Rosoux, R. (Editors). (1996). *La Loutre et le Vison d'Europe.* (Proceedings of the XVIIth International Mammalogical Conference, Niort 23-25 October 1993.) *Cahiers d'Ethologie appliquée,* **15**, 1-433.

Grémillet, X. (1993). Field survey of *Lutra lutra* on Corfu Island (Greece). *I.U.C.N. Otter Specialist Bulletin,* **8**: 39-41.

Georgescu, M. (1994). The situation and status of the otter in Rumania. In: *Seminar on the Conservation of the European Otter (*Lutra lutra*), Leeuwarden, the Netherlands, 7-11 June 1994.* Pp 71-73. Council of Europe, Strasbourg.

Green, J. & Green, R. (1980). *Otter Survey of Scotland, 1977-1979.* The Vincent Wildlife Trust, London.

Green, J. & Green, R. (1987). *Otter Survey of Scotland, 1984-1985.* The Vincent Wildlife Trust, London.

Green, J. & Green, R. (1997). *Otter Survey of Scotland, 1991-1994.* The Vincent Wildlife Trust, London.

Groupe Loutre Luxembourg (1997). Evaluation de la qualité des cours d'eau au Luxembourg en tant qu'habitat pour la loutre. *Trav. Sci. Mus. hist. nat. Luxembourg,* **26**: 1-125.

Gutleb, A. (1994). Todesursachenforschung Fischotter. Grundlage für ein Schutzkonzept von *Lutra lutra L.* 1758 - Bericht für die Jahre 1990-1992. *Forschungsbericht Fischotter 2,* **11**: 75-81. WWF Österrich, Wien. (In German with English summary.)

Hagner-Wahlsten, N. & Stjernberg, T. (1991). The distribution of the otter (*Lutra lutra* L.) in Finland 1975 and 1985. In: *Proceedings of the Vth International Otter Colloquium.* (edited by Reuther, C. & Röchert, R.). *Habitat*, **6**, 37-43.

Hammershøj, M., Madsen, A.B., Bruun-Schmidt, I.Ø., Gaardmand, B., Jensen, A., Jensen, B., Jeppersen, J.L. & Laursen, J. T. (1996). *Overvågning af Odder* (Lutra lutra) *i Danmark 1996.[Otter (*Lutra lutra*) Survey of Denmark 1996.]* Danmarks Miljøundersøgelser, 40s. Faglig rapport fra DMU, nr. 172. (In Danish with English summary.)

Heggberget, T.M. (1994). Reproductive seasonality of the Eurasian otter (*Lutra lutra* L.) in Norway. In: *Seminar on the Conservation of the European Otter (*Lutra lutra*), Leeuwarden, the Netherlands, 7-11 June 1994.* Pp. 115-121. Council of Europe, Strasbourg.

Heggbeerget, T.M. (1996). En kunnskapsoversikt for eurasiatisk oter *Lutra lutra*; grunnlag for en forvaltningsplan. [An otter *Lutra lutra* report; basis for a management plan.] *NINA Oppdragsmelding*, **439**: 1-29. (In Norwegian with English summary.)

Heggberget, T.M. & Myrberget, S. (1980). Sex and age distribution in Eurasian otters (*Lutra lutra*) killed by human activity. In: *Der Fischotter in Europa. Veerbreitung, Bedrohung, Erhaltung.* (Edited by Reuther, C. & Festetics, A.). Pp. 93-101. Oderhaus & Göttiningen, Selbstervlag.

Helle, P., Wikman, M., Helle, E., Belkin, V., Bljudnik, L & Danilov, P. (1997). Riistakolmoiden talvilaskenta Soumessa ja Venajan Karjalan lumilinjalaskennat 1997. *Riistantutkimusken tiedote,* **147**, 1-25.

Hodl-Rohn, I. (1977). The status of the otter in the German Federal Republic. *Otter Trust Annual Report 1976,* 4pp.

Hönigsfeld, M. (1985*a*). Vidra virde in stevilkah. *Lovec*, **68**: 75-79.

Hönigsfeld, M. (1985*b*). Pravda za vidrino ko/ o. *Lovec*, **68**: 345-347.

Hönigsfeld, M. (1998). New threats to continuous viable otter populations in northeastern part of Slovenia. *Poster at the Seventh International Otter Colloquium (IUCN/SSC Otter Specialist Group), March 14-19, 1998, Trebon, Czech Republic.*

Ionescu, O. & Ionescu, G. (1994). The otter from the Danube Delta to the Carpathian mountains in Rumania In: *Seminar on the Conservation of the European Otter (*Lutra lutra*), Leeuwarden, the Netherlands, 7-11 June 1994.* Pp. 66-68. Council of Europe, Strasbourg.

Jefferies, D.J. (1997). The changing status of the otter in the British Isles as revealed by spraint surveys. In: *The Vincent Wildlife Trust Review of 1996.* Pp. 19-23. The Vincent Wildlife Trust, London.

Jefferies, D. J. & Hanson, H. M. (2001). The role of dieldrin in the decline of the otter in Britain: The analytical data. In: *Proceedings of the First Otter Toxicological Conference.* (Edited by Conroy, J.W.H., Yoxon, P. & Gutleb, A.C) . *Journal of the International Otter Survival Fund*, No. 1. Xx-xx. Skye, September 2000. International Otter Survival Fund, Broadford Skye.

Jensen, A. (1964). Odderen i Danmark. [The otter (*Lutra lutra* L.) in Denmark.] *Danske Vildtundersøgelser,* **11**: 1-48. (In Danish with English summary.)

Jensen, B. (1980). Die situation des fischotters in Dänemark. In: *Der Fischotter in Europa.*

Veerbreitung, Bedrohung, Erhaltung. (Edited by Reuther, C. & Festetics, A.). Pp. 115-116. Oderhaus & Göttiningen, Selbstervlag.

Kadlečík, J. (1992). Rozšírenie vydry Riečnej (*Lutra lutra L.,* 1758) na Slovensku - súčasný stav poznania. [Distribution of the Eurasian otter (*Lutra lutra L.,* 1758) in Slovakia - the present state of knowledge.] *Vydra,* **3**: 54-59. (In Slovak with English summary.)

Kadlečík, J. (1994). Research and protection of otter *Lutra lutra* and its habitat in Slovakia. *Council of Europe Environmental Series* **17**: 98-112.

Kadlečík, J. & Urban, P. (1997). [European otter (*Lutra lutra* L.) in Slovakia and its conservation.] *Folia venatoria,* No. 26-27: 111-128. (In Slovak with English summary.)

Kauhala, K. (1996) Distributional history of the American mink (*Mustela vison*) in Finland with special reference to trends in otter (*Lutra lutra*) populations. *Annales Zoologica Fennica,* **33**: 283-291.

Kemenes, I. (1991). Otter distribution, status and conservation problems in Hungary. *I.U.C.N. Otter Specialist Group Bulletin,* **6**: 20-23.

Kiili, J. (1991). Distribution and number of otters in Estonia. In: *Proceedings of the Vth International Otter Colloquium.* (Edited by Reuther, C. & Röchert, R.). *Habitat,* **6**, 45-50.

Kranz, A. (1994). Otters increasing – threats increasing. *I.U.C.N. Otter Specialist Group Bulletin*, **10**: 28-30.

Kranz, A., Knollseisen, M., Gutleb, A., Elmoros, M., Leonards, P. & Toman. A. (1995). Aspects of the ecology of otters (*Lutra lutra*) in the Zabaikalsky National Park, Siberia. *Lutreola,* **6**: 9-12.

Krebser, W. (1959). Di bestandsaufnature des fischotter (*Lutra lutra* L.) in der Schweiz. *Säugetierkunde Mitteilungen.* **7**: 67-75.

Krytstufek, B. (1992). [The Red List of Endangered Mammalia in Slovenia.] *Varstvo narave (Nature Conservation),* **17**: 19-27.

Laanetu, N. (1989). Zur ökologie des fischotters *Lutra lutra* (L., 1758) in Estland. In: *Populationsökologia marderartiger Säufgetiere*. (Edited by Stubbe, M.). *Wiss. Beitr.Univ. Halle,* **1**: 59-70.

Lafontaine, L. (1993). Distribution of *Lutra lutra* in Brittany and first preventive measures against road traffic. *I.U.C.N. Otter Specialist Group Bulletin,* **8**: 37-39.

Lanski, J. & Körmendi, S. (1996). Otter diet in relation to fish availability on a fish pond in Hungary. *Acta Theriologica*, **41**: 127-136.

Lenton, E.J., Chanin, P.R.F. & Jefferies, D.J. (1980). *Otter Survey of England, 1977-79.* Nature Conservancy Council, London.

Libois, R.M. & Hallet, C. (1996). Situation actuelle de la loutre, *Lutra lutra*, en Belgique et problématique de la conservation. In: *La Loutre et le Vison d'Europe.* (Edited by Gautier, J.Y., Libois, R. & Rosoux, R.) (Proceedings of the XVIIth International Mammalogical Conference, Niort, 23-25 October 1993.) *Cahiers d'Ethologie appliqué,* **15**: 157-168. (In French with English summary.)

Libois, R.M., Philippart, J.C., Rosoux. R. & Vraken, M. (1982). Quel avenir pour la loutres en Belgique? *Cahiers d'Ethologie appliquée,* **2**; 1-15.

Macdonald, S.M. & Mason, C.F. (1990). Action plan for European otters. In: *Otters; an Action Plan for their Conservation.* (Edited by Foster-Turley, P., Macdonald, S.M. & Mason, C.F.). Pp. 29-40. IUCN, Gland, Switzerland.

Macdonald, S.M. & Mason, C.F. (1994). *Status and Conservation needs of the Otter (Lutra lutra) in the Western Palaearctic.* Council of Europe, Strasbourg.

Madsen, A.B. (1996). *Odderens Lutra lutra Økologoo og forvaltning i Danmark. [The Ecology and Conservation on the Otter Lutra lutra in Denmark.]* Ph.D. thesis University of Århus, Århus. (In Danish with English summary.)

Madsen, A.B. & Neilsen, C.E. (1986). Odderens (*Lutra lutra* L.) forekomst i Danmark 1984-1986. [Otter (*Lutra lutra* L.) survey of Denmark 1984-1986.] *Flora og Fauna*, **92**: 60-62. (In Danish with English summary.)

Marochkina, V.V. (1995). Materials on carnivorous mammals of Eastern Turkmenistan. *Lutreola,* **5**: 20-23.

Mason, C.F. & Macdonald, S.M. (1986). *Otters: Ecology and Conservation.* Cambridge University Press, Cambridge.

Metsu, I. & van den Berge, K. (1991). The distribution of the otter in Flanders - Belgium. In: *Proceedings of the Vth International Otter Colloquium.* (Edited by Reuther, C. and Röchert, R.). *Habitat,* **6**, 51-52.

Mickevičius, E. (1993). The otter in Lithuania. *I.U.C.N. Otter Specialist Group Bulletin,* **8**: 29-31.

Myrberget, S. & Frøiland, q. (1972). Oteren i Norge omkring 1970. *Fauna,* **25**, 149-159.

Nechay, G. (1980). Die situation des fischotters in Ungarn. In: *Der Fischotter in Europa. Veerbreitung, Bedrohung, Erhaltung.* (Edited by Reuther, C. & Festetics, A.). Pp. 215-221. Oderhaus & Göttiningen, Selbstervlag.

Nolet, B.A. & Martens, V. (1989). De achteruitgang van de otter in Nederland. *De Levende Natuur,* **90**: 34-37.

Olsson, M. & Sandegren, F. (1986). Projekt utter. *Fauna och Flora*, **81**: 157-159. (In Swedish with English summary.)

Ornicans, A. (1994). Legal status of the otter in Latvia. In: *Seminar on the Conservation of the European otter (Lutra lutra), Leeuwarden, the Netherlands, 7-11 June 1994.* Pp. 80-83. Council of Europe, Strasbourg.

Overal, B. (1995). Présence de la loutre dans la Haute-Sûre. *Les Naturalistes belges*, **76**: 315-322.

Ozolinš, J. & Pilāts, V. (1995.) Distribution and status of small and medium sized carnivores in Latvia. *Annales Zoologica Fennici*, **32**: 21-29.

Ozolinš, J. & Rantinš, M. (1992*a).* The distribution and habitat conditions of the otter (*Lutra lutra*) in Latvia. *Proceedings of the first Baltic Theriological Conference, Tartu*

Ozolinš, J. & Rantinš, M. (1992*b*). Einige voraussetzungen zur heutugen bestandsentwicklung und verbeitung des fischotters *Lutra lutra* (L.) in Lettland. [Some preconditions for the present development of the otter *Lutra lutra* (L.), number and distribution in Latvia.] *Semiaquatische Säugetiere,* Wiss. Beitr. Univ. Halle 1992: 365-384. (In German with English summary.) **Ozolinš, J. & Rantinš, M.** (1995). The data on otter *Lutra lutra* population provided by beaver trapping in Latvia. *Ecologija*, 1995, No. 2: 64-68.

Paunovic´, M. & Milenkovic´, M. (1994). Distribution and status of otter *Lutra lutra* L., 1758 (*Mustelidae, Carnivora*) in Serbia and Montenegro (Yugoslavia). *68 Jahrest der Deutschen Gesellschaft für Säugetierkunde, Kurzfassungen, 34, Wien.*

Paunovic´, M. & Milenkovic´, M. (1988). The current status and distribution of the otter *Lutra lutra* L., 1758 in Serbia and Montenegro. *I.U.C.N. Otter Specialist Group Bulletin,***13**: 71-76.

Pereladova, O., Krever, V. & Williams, M. (1997). *Biodiversity Conservation in Central Asia – analysis of modern situation and project portfolio*. WWF Russian Programme Office, Almata, Ashgabad, Bishkek, Dushanbe, Tashkent & Moscow.

Perez, J. & Lacomba, I. (1991). The influence of water demands on otter (*Lutra lutra*) distribution in Mediterranean Spain. In: *Proceedings of the Vth International Otter Colloquium.* (Edited by Reuther, C. and Röchert, R.). *Habitat,* **6**: 249-262.

Prigioni, C. (1997). *La lontra. Una vita silenziosa negli ambienti acquatici*. Eolagricole.

Prigioni, C. & Fumagalli, R. (1992). La Lontra: Status e conservazione in Italia. In: *La Lontra: Specie minnacciata in Italia. Le Ricerche in natura ed in Cattività Finalizzate all sua Conservazione.* Minestero dell'Agricoltura e dello Foreste. Callana, Verde, **89**: 1-27.

Prigioni. C., Bogliani, G. & Barbieri, F. (1986). The otter *Lutra lutra* in Albania. *Biological Conservation,* **36**: 375-383.

Rakonczay, Z. (Editor). (1990) *Vörös Könyv A Magyarországon Kipusztult és Veszélyeztetett Növény -és állatfajok*. Budapest, Akadémiai Kiadó. (In Hungarian with English summary.)

Reggiani, G., Ciucci, P., Boitani, L. & Rocca, F. (1997). In approccio muticriteri alla conservazione della lontra (*Lutra lutra*). *Ricerche di Biologia della Selvaggina*, **27**: 751-757.

Reuther, C. (1980). Der fischotter, *Lutra lutra* L. in Niedersachsen. *Naturschutz und Landschaftspflege in Niedersachsen.* **11**: 1-182. (In German with English summary.)

Reuther, C. (1992). Aktion Fiscotterschutz e.V. activities 1991-92. *I.U.C.N. Otter Specialist Group Bulletin,* **7**: 16-19.

Reuther, C. (1995). 'Otter 2000': an otter habitat network program for Germany – a first step for a European otter habitat network? In: *Proceedings of the VIth International Otter Colloquium* (Edited by Reuther, C. &Rowe-Rowe, D.). *Habitat,* **11**: 78-85.

Reuther, C. & Roy, A. (2001). Some results of the 1991 and 1999 otter (*Lutra lutra*) surveys in the River Ise catchment, Lower Saxony, Germany. *I.U.C.N. Otter Specialist Group Bulletin,* **18**: 28-40.

Röben, P. (1974). Zum vorkommen des otters, *Lutra lutra* (Linné, 1758), in der Bundersrepublik Deutschland. *Saugetierkundliche Mitteilungen,* **22**: 29-36. (In German with English summary.)

Romanowski, J. (1984). The occurrence of the otter *Lutra lutra* (Linneaus, 1758) in Poland in the light of inquiry by hunters. *Przeglad zoologiczny*, **28**: 397-406. (In Polish with English summary.)

Romanowski, J. (1991). Otters in Eastern Red Data Books. *I.U.C.N. Otter Specialist Group Bulletin,* **6**: 11-13.

Romanowski, J., Gruber, B. & Brzeziñski M. (1997). The recovering otter population from Central Poland. *I.U.C.N. Otter Specialist Group Bulletin,* **14**: 24-25.

Rosoux, R., Tournebize, T., Maurin, L. & Bouchardy, C. (1996). Étude de la répartition de la loutre d'Europe (*Lutra lutra*) en France. Actualisation 1993. In: *La Loutre et le Vison d'Europe.* (Edited by Gautier, J.Y., Libois, R. & Rosoux, R.) (Proceedings of the XVIIth International Mammalogical Conference, Niort, 23-25 October 1993.) *Cahiers d'Ethologie appliqué,* **15**: 195-206. (In French with English summary.)

Rozhnov, V. & Tumanov, I.L. (1994). The status of the river otter in Russia. In: *Seminar on the Conservation of the European Otter (Lutra lutra), Leeuwarden, the Netherlands, 7-11 June 1994.* Pp. 91-94. Council of Europe, Strasbourg.

Ruiz-Olmo, J. (1994). 5.4 Country Report: Spain. In: *Seminar on the Conservation of the European Otter (Lutra lutra), Leeuwarden, the Netherlands, 7-11 June 1994.* Pp. 218-219. Council of Europe, Strasbourg.

Ruiz-Olmo, J. & Delibes, M. (1998). *La Nutria en España ante el Horizonte del Año 2000.* SECEM, Grupo Nutria, Barcelona.

Ruiz-Olmo, J. & Gosálbez, J. (1988). Distribution of the otter (*Lutra lutra* L., 1758) in the NE of the Iberian Peninsula. *Publication of the Department of Zoology, Barcelona,* **14**: 1212-1232.

Ruiz-Olmo, J., Lafontaine, L., Prigioni, C., López-Martin, J.M. & Santos-Reis, M. (2001). Pollution and its effects on otter populations in south-western Europe. In: *Proceedings of the First Otter Toxicological Conference.* (Edited by Conroy, J.W.H., Yoxon, P. & Gutleb, A.C). *Journal of the International Otter Survival Fund*, No. 1. xx-xx. Skye, September 2000. International Otter Survival Fund, Broadford Skye.

Sackl, P., Ilzer, W. & Kolmanitsch, E. (1996). Historische und aktuelle verbreitung des fischotters in der Steiermark. *Forschungsbericht Fischotter 3 des Forschungsinstituts des WWF Österrich,* **14**: 4-25.

Santos Reis, M. (1994). 5.5 Country Report: Portugal. In: *Seminar on the Conservation of the European Otter (Lutra lutra), Leeuwarden, the Netherlands, 7-11 June 1994.* Pp. 220-221. Council of Europe, Strasbourg.

Santos Reis, M., Trindade, A. & Beja, P. (1996). Situation et état des recherches sur la loutre au Portugal. In: *La Loutre et le Vison d'Europe.* (Edited by Gautier, J.Y., Libois, R. & Rosoux, R.). (Proceedings of the XVIIth International Mammalogical Conference, Niort, 23-25 October 1993.) *Cahiers d'Ethologie appliqué,* **15**: 181-194. (In French with English summary.)

Schmidt, G. & Adam, S. (1992). La loutre au Luxembourg. *Bulletin de la Société des Natutralistes luxembourgeois*. **93**: 41-58. (In French with English summary.)

Sidorovich, V.E. (1991) Structure, reproductive status and dynamics of the otter population in Byelorussia. *Acta Theriologica,* **36**: 153-161.

Sidorovich, V.E. & Lauzhel, G.O. (1992). Numbers of otters and approach to population estimations in Byelorussia. *I.U.C.N. Otter Specialist Group Bulletin,* **7**: 13-16.

Sjöåsen, T. (1996). Survivorship of captive-bred and wild-caught reintroduced European otters in Sweden. *Biological Conservation,* **76**: 161-165.

Sjöåsen, T. & Sandegren, F. (1992). Reintroduction of Eurasian otters (*Lutra lutra*) in southern Sweden 1987-1992: a project in progress. In: *Otterschutz in Deutschland.* (Edited by Reuther, C.). *Habitat,* **7**: 143-146.

Skarén, U. (1990). Fish farming and otters in Finland. *I.U.C.N .Otter Specialist Group Bulletin,* **5**: 28-34.

Skarén, U. & Kumpulainen, J. (1986). Recovery of the otter *Lutra lutra* (L., 1758) population in North Savo, Central Finland, with analysis of environmental factors. *Lutra,* **29**: 117-140.

Smit, C.J. & van Wijngaarden, A. (1967). *Threatened Mammals in Europe*. Council of Europe, Strasbourg.

Søgaard,, B. & Madsen, A.B. (1996). Management plan for the otter (*Lutra lutra*) in Denmark. *I.U.C.N. Otter Specialist Group Bulletin,* **13**: 37-39.

Spiridonov, G. & Mileva, M. (1994). The status of the otter (*Lutra lutra*) in Bulgaria. In: *Seminar on the Conservation of the European Otter (Lutra lutra), Leeuwarden, the Netherlands, 7-11 June 1994.* Pp. 77-80. Council of Europe, Strasbourg.

Stephens, M.N. (1957). *The Otter Report.* Universities Federation for Animal Welfare, Potters Bar, London.

Štollmann, A., Urban, P., Kadlečík, J. & Uhrin, M. (1997). [Proposal of (Red) List of the Mammal

Fauna of the Slovak Republic.] *Ochrana prirody,* **15**: 201-217. (In Slovak with English summary.)

Strachan, R. & Jefferies, D.J. (1996). *Otter Survey of England 1991-1994.* The Vincent Wildlife Trust, London.

Strachan, R., Birks, J.D.S., Chanin, P.R.F. & Jefferies, D.J. (1990). *Otter Survey of England 1984-1986.* Nature Conservancy Council, London.

Stubbe, M. (1989). Verbreitung und ökologie des fischotters *Lutra lutra* (L. 1758) in der DDR. In: *Populationsökologie marderartiger säugetiere* (Edited by Stubbe, M.). *Wiss. Beitr. Univ. Halle* , **1**, 13-33.

Stubbe, M. & Stubbe, A. (1994). Säugetierarten und deren feld ekologische Erforschung in Ostlichen Deutschland [Mammals and field mammalogy in Eastern Germany] *Tiere im Konflikt* **3**: 1-52. Martin-Luthur-Universitat, Halle-Wittenberg (In German with English summary.)

Stubbe, M., Heidecke, D., Dolch, D., Teubner, J., Labes, R., Ansorge, H., Mau, H. & Blanke, D. (1993). Monitoring fischotter 1985-1991. *Tiere im Konflikt*, **1**: 11-59. (In German.)

Sulkava, R. (1994). The otter (*Lutra lutra*) in Central Finland. *I.U.C.N. Otter Specialist Group Bulletin,* **10**: 10-14.

Sulkava, R. & Storrank, B. (1993). Hur väl återspeglar bermarksinventeringar ett områdes verkliga utterstam? Erfarenheter från Kumo älvs källflöden 1990-91. [The use of spraints in evaluating the status of an otter (*Lutra lutra*) population - surveys on river Kokem Denjoki, Central Finland from 1990 to 1991.] *Memoranda Societas pro Fauna et Flora Fennica*, **69**: 65-76 (In Finnish with English summary.)

Toman, A. (1992). První výsledky akce Vydra. [First results of "Action Otter".] *Vydra,* **3**: 3-8. (In Czech with English summary.)

Trindade, A. (1994). Monitoring *Lutra lutra* habitats in Portugal: a conceptual plan. *I.U.C.N. Otter Specialist Group Bulletin,* **10**: 41-46.

Trindade, A., Farinha, N. & Florencio, E. (1998). *A Distribuiçao da Lontra* Lutra lutra *em Portugal. ituaçao em 1995*. Instituti da Conservaçao da Natureza. Ministério do Ambiente, Lisboa.

Urban, P. (1992). Verbreitund und schutz des fischotters *Lutra lutra* im landschaftsschutzgebeit Pol'ana, Mittels;pwakei. [Distribution and protection of the otter *Lutra lutra* in the Pol'ana protected landscape area, Central-Slovakia.] *Semiaquatische Säugetiere, (1992).* Wiss. Beitr. Univ. Halle, 1992, 385-392. (In German with English summary.)

Urban, P. (1995). Ďalšie sčícitanie vydry riečnej v oblasti Pol'any. [A further count of otter in the Pol'ana area.] *Vydra,* **6:** 68.

Urban, P. (1998). Eurasian otter (*Lutra lutra* L.) in the North – Western Greece – contribution to distribution, limiting factors and conservation measures. *Vydra*, **8**: 44-47**.**

van den Berge, K. (1998). The actual situation of mustelids in Flanders (Belgium). *De Lavende Natuur*, (in press).

van Wijngaarden, A. & van de Peppel, J. (1970). De otter, *Lutra lutra* (L.) in Nederland. *Lutra,* **12**: 3-72.

Veen, J. (1984). De verspreiding en enkele oecologische aspecten van de otter *Lutra lutra* in Nederland. *Lutra,* **27**: 25-35.

Weber, D. (1990). The end of the otter and of otter reintroduction plans in Switzerland. *I.U.C.N. Otter Specialist Group Bulletin*, **5**: 45-50.

Weber, J-M. & Weber, D. (1991). The otter (*Lutra lutra* L.) in western Switzerland. In: *Proceedings of the Vth International Otter Colloquium.* (Edited by Reuther, C. & Röchert, R.). *Habitat,* **6**, 53-55.

Weber D., Weber, J.-M. & Müller, H.-U. (1991). *Fischotter (Lutra lutra L.) in Schwarzwasser-sense gebeit; dokumentation eines gescheiterten*. [Otters in the Schwarz-wasser-Sense-catchment; documentation of an unsuccessful re-introduction project.] Mitteilingen der Naturforscheden Geseltschaft in Bern. Wiederein-bürgerungsversuches (In German with English summary.)

Wikman, M. (1996). Saukko. In: *Riistan jaljille*. (Edited by Linden, H., Hario, M. & Wikman, M.). Pp. 64-67. Riista- ja kalatalouden tutkimuslaitos, Edita. Helsinki.

Winter, L. (1993). *De otter in Limburg; het voorkomen van der otter (Lutra lutra L.) in Limburg en een voorstel voor een ecologische infrastructuur.* Maastricht, Natuurhistorisch Genootschap in Limburg; Leeuwarden, Stichting Otterstation Nederland; Arnhem, Rijkswaterstraat/RIZA.

Zholnerovskaya, E.I., Shvetsov, Y.G., Kalabin, S.L. & Lopatina, N.V. (1994). *Catalogue of Collections of the Zoological Museum of the Biological Institute, Siberian Branch, USSR Academy of Sciences. Mammals: To the consisting of the state cadaster of the animal wildlife*. Novosibirsk, Nauka Publishing, Siberian Branch 1989. (In *Lutreola* **4**: 23-32).

THE ROLE OF POLLUTANTS IN THE DECLINE OF THE OTTER

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1 Introduction

Populations of Eurasian otters (*Lutra lutra*) suffered serious declines during the past decades in many parts of Western Europe and in some areas the species has become extinct (for recent review see CONROY and CHANIN, 2001). Apart from factors such as habitat destruction, isolation of populations, mortality due to hunting, traffic accidents or drowning in fish fyke nets, the contamination of the diet and the otters themselves are considered as important causal factors with mercury, dieldrin and polychlorinated biphenyls (PCBs) named most frequently (MASON, 1989; KRUUK and CONROY, 1991; JEFFERIES, 1996; SMIT *et al*., 1998).

In this paper, a review on chemical properties, technical use, general toxic effects of mercury and PCBs and specific information on what is known in respect to otters will be given. For dieldrin such a review is given by JEFFERIES AND HANSON (2001).

2 COMPOUNDS AND THEIR EFFECTS ON OTTERS

2.1 Mercury

Mercury, a heavy metal, is naturally present in the environment at concentrations of up to 0.08mg/kg in most soils, with higher concentrations found near areas of volcanic activity. Some 800 metric tons are released every year due to natural erosion. Most is transported bound to particles into the oceans (GAVIS and FERGUSON, 1972). Human activities, both historical as well as the present industrial, agricultural and consumer uses, have produced and emitted mercury into the global environment for several centuries (KAISER and TÖDL, 1980). In addition, human influences, such as mining and acidic depositions due to sulphate emissions, increased the mobility for mercury, resulting in a potential increase in exposure of wildlife.

Mercury has been used for many applications including dyes and pesticides, production of chlorine, for laboratory purposes, as a catalytic agent in many chemical processes, as well as a drug and in dentistry. At a local level the use of elementary mercury for gold mining forms a specific threat to humans and wildlife (GREER, 1993). The use of elementary mercury for the process of amalgamation during gold mining forms the major input of mercury in South American countries (GUTLEB, SCHENCK, and STAIB, 1997).

Mercury is unique among the heavy metals in that it can exist in several physical and chemical forms. It is liquid at room temperature, and in the environment two

ionic states - mercury (I) and mercury (II) can be found. Both are water-soluble. They are therefore bioavailable, and can be converted into organomercury compounds, which are more toxic than inorganic forms. Elementary mercury is vaporised very easily and is thereby transported via the atmosphere. It returns to earth as a water-soluble form in precipitation, finding its way into lakes and oceans. Whereas this cycle mainly contributes to the long-range transport of mercury there is a second, more local, cycle that depends on the methylation of mercury into organic forms due to the biological activity of bacteria and algae. This is especially the case under tropical conditions such as low pH and low salinity, which favour the formation of dimethylmercury. This can be concentrated in fish and other aquatic organisms as a result of biomagnification and bioaccumulation (BOENING, 2000). In addition, average mercury concentrations in fish were found to increase steadily following the elimination of selenium-rich discharges of fly ash into a water-system (SOUTHWORTH, PETERSON and RYON, 2000). MASON, LAPORTE and ANDRES (2000) were able to show the importance of water chemistry in determining the bioaccumulation of mercury into insects and thereby into higher trophic levels. The passive uptake of methylmercury does not control bioaccumulation at the base of aquatic food webs and methylmercury concentrations at higher trophic levels reflect uptake at low trophic levels and other factors, such as diet and growth (WATRAS *et al*., 1998).

In adult mammals 1% to 3% of ingested inorganic mercury is resorbed in the gut. Juveniles, however, are especially threatened because 30% to 40% can be resorbed. Methylmercury is resorbed more or less completely from the diet (KOSTIAL *et al*., 1978). It is also the position in the food chain, which determines the risk of mercury exposure in wildlife. Carnivores tend to accumulate higher concentrations of mercury than herbivores, and fish-eating animals more than meat-eating animals (WREN, 1986).

The global cycling of released mercury has increased concentration even in remote areas. In mammals from the Canadian Arctic marine ecosystem increased levels of mercury in samples from the 1990s were found when compared with samples from the early 1980s, thus giving evidence that mercury levels continue to increase (MUIR *et al*., 1999). Although mercury is not used in the Arctic region, levels found in the traditional diet of Inuit are so high that dietary advice for Arctic people has been recommended (HANSEN, 2000). In addition, over the last 20 years there is evidence of increasing levels of mercury in animals living in Greenland (RIGET and DIETZ, 2000).

Methylmercury is more or less found exclusively in seafood and freshwater fish and is known to be a highly neurotoxic agent. In this form, mercury is readily transported across the placenta, in contrast to the inorganic form, which has a low transport into the foetus (KAJIWARA *et al*., 1996). The behavioural and cognitive changes associated with effects on the central nervous system and preclinic kidney changes are the symptoms which are of most concern today. The classical triad of symptoms, erethism, tremor and gingivitis are now rarely found outside humans living near gold-mining areas (DOLBEC *et al*., 2000).

Because of the high affinity between mercury and sulfhydril molecules, interaction between mercuric ions and the thiol groups of proteins, peptides and amino acids such as albumin, metallothionein, glutathione and cysteine occur. These mechanisms have all been shown to be involved in the proximal tubular uptake and accumulation, transport, and toxicity of mercuric ions in the kidney (ZALUPS, 2000). Mercury compounds often exerted clastogenic effects in eukaryotic cells,

acting as a spindle inhibitor, thereby causing c-mitosis and consequently aneuploidy and/or polyploidy (DE FLORA, BENNICELLI and BAGNASCO, 1994). Selenium has a protective effect against mercury intoxication. KOEMAN *et al*. (1973) reported on a 1:1 correlation between selenium and mercury in marine mammals, whereas metallothioneins appear to play a minor role in the binding and detoxification of mercury by marine mammals (DAS, DEBAKER and BOUQUEGNEAU, 2000). Among other effects mercury is also able to inhibit the coupling process of iodide in the thyroid gland resulting in changes of the thyroid homeostasis (NISHIDA *et al*., 1986), which is also influenced by several other xenobiotic such as PCBs (BROUWER *et al*., 1998).

2.2 Mercury and otters

Liver concentrations in otters have been reported from many countries such as Sweden (4.1 - 30.7µg g^{-1}, OLSSON, REUTERGÅRDH and SANDEGREN, 1981), Finland (0.05 - 31.0µg g^{-1}, SKARÉN, 1992), Orkney Islands (1.0 - 20.3µg g^{-1}, MASON and REYNOLDS, 1988), Spain (3.92 - 17.48µg g^{-1}, HERNANDEZ *et al*., 1985), and Ireland (0.15 - 17.03µg g^{-1}, MASON and O´SULLIVAN, 1993) and in general these levels are considered to represent the range of background levels.

Experimentally dosed Canadian river otters (*Lutra canadensis*) died with symptoms of mercurialism and mean total mercury levels of 33.4µg g^{-1} were found in their livers (O´CONNOR and NIELSEN, 1981). WREN (1985) reported on an otter (*Lutra canadensis*), which was found dead near to a river known to be severely mercury polluted. A concentration of 96µg g^{-1} mercury was found in the liver. The tracks indicated that the otter showed erratic behaviour such as travelling in circles, falling over and burrowing into the snow before dying. Two otters from the Shetland Islands were observed dying with similar symptoms and later liver concentrations higher than 30µg g^{-1} were analysed (KRUUK and CONROY, 1991). WOBESER and SWIFT (1976) reported on a mink (*Mustela vison*), which died due to mercury intoxication with a similar liver concentration. GUTLEB *et al*. (1998) reported a concentration of 55.6µg g^{-1} in the liver of an otter from the Czech Republic. Most animals of which data have been published are road victims and no observations on their behaviour prior to death are made. Therefore it cannot be excluded that negative effects of mercury occur at least on single individuals within otter populations as tissue concentrations were exceeding critical tissue concentrations.

2.3 Polyhalogenated aromatic hydrocarbons (PHAHs)

Over the past three decades intensive research on the toxic effects of polychlorinated dibenzo-p-dioxins (PCDDs), dibenzofurans (PCDFs) and polychlorinated biphenyls (PCBs) has taken place. The ubiquituos presence of members of these groups of persistent compounds has been widely documented (TATTON and RUZICKA, 1967; TANABE *et al*., 1983, 1987; TANABE and TATSUKAWA, 1986; VOOGT and BRINKMAN, 1989; KLEIVANE, SEVERINSEN and SKAARE, 2000). They belong to the large group of polyhalogenated aromatic hydrocarbons (PHAHs) and cause a broad range of toxic effects in different vertebrates, e.g. dermal-, hepato- and immunotoxicity, carcinogenic, teratogenic, neurobehavioural and endocrine effects as well as diverse biochemical responses especially the induction of several drug metabolising enzymes (SAFE, 1994; KIMBROUGH, 1995). A detailed description of the mechanisms involved in toxic effects of PHAHs, especially those related to

retinoid homeostasis is given by GUTLEB and MURK (2001).

2.4 PCBs and otters

The first suggestion that polychlorinated biphenyls (PCBs) played a major role in the decline of otters in Europe was made by SANDEGREN, OLSSON and REUTERGÅRDH (1980), OLSSON *et al.* (1981) and OLSEN and SANDEGREN (1991). This assumption was based on an observed correlation between the status of the population and the total concentration of PCBs in otter tissues. Low concentrations of PCBs were found in a thriving population in Norway and high concentrations in a threatened and declining population in Southern Sweden. This hypothesis was further supported by an extrapolation of toxicological data of PCBs for mink (JENSEN *et al*., 1977). Later, other authors (MASON, 1989; BROEKHUIZEN, 1989) have suggested similar relationships. Also, in the North American Great Lakes region, populations of Canadian river otters and American mink declined in areas (along the Great Lakes) where the diet of the mustelids (fish) is highly contaminated with PCBs (WREN, STOKES and FISCHER, 1986; WREN, 1991; GIESY *et al.*, 1994). However, high levels of total PCBs have been reported in a thriving otter population in Shetland (KRUUK and CONROY, 1991; KRUUK, CONROY and CARSS, 1993).

LEONARDS *et al*. (1996) conducted a study to investigate the concentrations and patterns of di-, mono- and non-*ortho* substituted CBs in autopsy material from different otter populations of Europe in relation to biological factors such as age, sex and reproductive status. The second aim of their study was to investigate if the health status of the otter was affected by contamination of CBs based on an extensive database for the Danish population. Experimental studies with several species have shown that CBs can affect the immune responses, and that immunosuppression is caused by non- and mono-*ortho* CBs, which are mediated by the *Ah*-receptor (DAVIS and SAFE, 1990).

The following questions will be answered:

- Are there regional differences in the PCB patterns in otters?
- Are there differences in the pattern of PCBs within a population?
- Are PCB concentrations declining in otters?
- Is there a relation of the health status of Danish otters in relation to the concentration of PCBs?
- Are PCBs now to be blamed for the decline of otters?

2.4.1 Are there regional differences in the PCB patterns in otters?

Principal component analysis was used to compare the CB patterns in otters from the Czech Republic, Denmark, Ireland, The Netherlands and the United Kingdom (LEONARDS *et al*., 1996). It was shown that the samples from the different countries could not be separated from each other. This indicates that the variation in CB pattern between otters is larger than the variation between countries.

In the same study it was shown that there are two clusters of CBs (LEONARDS *et al*., 1996). One contains the CBs (CB 28, CB 31, CB 44, CB 52, CB 77, CB 101 and CB 149), and the other cluster contains the persistent congeners. This indicates that the main separation factor in CB patterns between the otters is due to the differences

in the relative concentration of metabolisable and persistent CBs, and that no large differences can be observed in CB patterns between the countries. This implies that other factors such as age, sex, condition of the animal, reproduction status, the biological differences between animals and a concentration dependent metabolism (TANABE *et al*., 1987; WELLS *et al*., 1994; WELLS, MCKENZIE and ROSS, 1996; BOON *et al.*, 1997) are important to explain the differences in CB patterns between otters.

2.4.2 Are there differences in the pattern of PCBs within a population?

CB patterns of the metabolisable congeners (CB 28, CB 31, CB 44, CB 77, CB 101 and CB 149) between otters were not constant. This in contrast with the CB patterns of the persistent congeners, which are more or less constant between the otters. The ratios of the metabolisable CBs decrease with increasing concentration of the persistent congeners. A decreased ratio for the metabolisable congeners is observed with increasing concentration of CB 153. However, for the relative concentration of CB 180, which is a persistent congener, there is no relationship with the concentration of CB 153.

An increased induction of isozymes of cytochrome P450 due to an increased absolute concentration of CBs has been proposed as explanation for the concentration dependent metabolic process (LEONARDS *et al*., 1996). A consequence of this process is that otters exposed to high concentrations of CBs are not only exposed to "high" concentrations of the parent compound but also to relatively high concentrations of CB-metabolites. Hydroxylated CB-metabolites interfere with, for example, retinol and thyroid hormone homeostasis (BROUWER *et al*., 1994).

2.4.3 Is there a relation of the health status of Danish otters in relation to the concentration of PCBs?

From 145 Danish otters (MADSEN *et al.*, 1999), 43 were analysed for non-, mono- and di-*ortho* substituted CBs. Toxic equivalent concentrations (TEQ) were calculated by multiplying the toxic equivalent factor of SAFE (1994) for each non- or mono-*ortho* CB with the corresponding concentration. Furthermore, the sum of the individual TEQ concentrations was calculated (ΣTEQ).

For each exposure TEQ-group the percentage of diseases, which consists of bacterial diseases, viral infections, endoparasites and pathological changes was calculated (Table 1). In the first TEQ-group 17% of the otters had a disease. In the second group a higher percentage of disease (29%) was found. A further increase of the percentage of diseases to 33%, was found in the third group. In this group some otters had multiple diseases, which were less observed in both other groups. The number of diseases per sick-otter increased from one in group 1 to 2.25 in the highest TEQ-group. The diseases in the third group were a tumour in the intestine, hepatitis, gal stone, enlarged liver (10% of body weight), umbilical hernia and deformed uterus. The tendency of increased incidence of diseases with TEQ concentration however, is not significant (p>0.05), which is probably due to the low number of otters (n = 43).

These data suggest that an increased percentage of diseases are associated with increasing concentrations of PCBs, which may be caused by immunosuppression in the otter. This is further supported by a recently reported dose-effect relationship between vitamin A (retinol or retinylpalmitate) in the liver and the concentration of CBs on TEQ basis for otters (MURK *et al.,* 1996). Vitamin A plays an important role in resistance to microbial infections (HOF and WIRSING, 1979; NAUSS, MARK and

SUSKIND, 1979; SHENAI, CHYTIL and STAHLMAN, 1985). Retinol or retinylpalmitate levels in otter liver decreased sharply at TEQ concentrations higher than 4 to 5ng/g: lipid weight (MURK *et al*., 1998). This concentration corresponds very well with the TEQ range of enhanced percentage of diseases as reported in our study. The combination of a decreased level of retinol or retinylpalmitate in the liver and an increased percentage of diseases in otters supports the hypothesis that this is probably caused by PCBs. The presently found concentrations of CBs in wild otter population are high enough to cause severe adverse health effects for this animal.

The mean condition index of the otters from group 3 was significantly lower (p <0.05) than of the otters from group 1 or 2. This indicates that lower relative body weights were found in this group; 13% relative lower body weight than for otters without diseases. Experimental studies with mink have shown that at high levels of CB exposure the animals were eating less than the control groups. If this would also be the case for the otters of group 3 is not known. A higher mean relative liver weight (44% higher than in otters without diseases) was observed in group 3 compared with groups 1 and 2, however, this difference was not significant.

Table 1. For each TEQ-group (ng/g lipid weight) the number of otters with a disease (viral infection, bacterial disease, pathological deviations and/or endoparasites), number of diseases per sick-otter, percentage of otters with a disease, condition index (CI), liver somatic index and mean differences (%) of condition index and liver index compared to healthy otters are shown.

	TEQ Group 1	*TEQ* **GROUP 2**	*TEQ* **Group 3**
TEQ concentration (ng/g: lipid weight)	0-4	4-8	8-26
Number of otters in each group	24	7	12
Number of otters with diseases	4	2	4
Number of diseases Per sick otter	1	1.5	2.25
% diseases	17	29	33
CI (mean ± SD)	1.17 ± 0.14	1.22 ± 0.15	1.01* ± 0.19
Difference of CI compared to healthy otters	+ 1%	+ 5%	- 13%
LSI (mean ± SD)	0.037 ± 0.011	0.034 ± 0.011	0.052 ± 0.029
Difference of LSI compared To healthy otters	+ 3%	- 6%	+ 44%

LSI = Liver somatic index (relative liver weight to body weight);
***=mean CI of group 3 is significant lower than group 1 or 2.**

The mean condition index of the otters from group 3 was significantly lower (p <0.05) than of the otters from group 1 or 2. This indicates that lower relative body weights were found in this group; 13% relative lower body weight than for otters without diseases. Experimental studies with mink have shown that at high levels of CB exposure the animals were eating less than the control groups. If this would also be

the case for the otters of group 3 is not known. A higher mean relative liver weight (44% higher than in otters without diseases) was observed in group 3 compared with groups 1 and 2, however, this difference was not significant.

The main question is, whether an increased percentage of diseases has an adverse effect on the population. This will be the case if diseases influence the reproduction, growth or mortality rate in a population. At this moment the Danish population is stable and in some areas even expanding. During a survey in 1991 otter tracks were found in greater numbers than during a survey made in 1984-1986 (MADSEN and NIELSEN, 1986; MADSEN, 1991). In this period an annual decrease of 7% in the concentration of CBs in Danish otters was found (MASON and MADSEN, 1993). This suggests that the PCB exposure could have played a role in the population dynamics in the 1980s. But at this moment the increased frequency of diseases in the higher CB exposed otters does not seem to be a major factor in the Danish population dynamics.

Too limited information is currently available about the health status, population dynamics and CB levels of the different populations in Europe. More research in the field of detailed necropsies and the measurement of CBs on a congener specific basis (non- and mono-*ortho* substituted CBs) in otters is needed, to further clarify the relationship between diseases, CB contamination and population dynamics.

2.4.4 Are PCB concentrations declining in otters?

MASON (1998) found an annual decrease in PCB concentrations of about 8% in otters collected over the period 1983 to 1992 in England and Wales, which was later confirmed by the data of SIMPSON *et al.* (2000) for otters from South-West England. This rate is similar to the earlier observed annual decrease of 7% in otters from Denmark (MASON and MADSEN, 1993). ROOS *et al.* (2001) investigated time trends of PCBs in otters from Sweden collected in the years 1968 until 1999. For Northern Sweden they found an annual decrease of about 14% or an overall decrease during the study period of 70%. A similar, although smaller decrease of 50% (6% per annum), was observed in otters from Southern Sweden.

2.4.5 Are PCBs now to be blamed for the decline of otter populations observed in nearly all European countries?

This issue has raised a lot of discussion during recent years (KRUUK, 1997; MASON, 1997). The mechanisms of PCB toxicity have been well investigated during the last years (for detailed information see: BROUWER *et al.,* 1998; GUTLEB and MURK, 2001). For the Danish population a correlation was found between increasing PCB concentrations expressed as TCDD-equivalents, TEQs) and decreasing concentrations of Vitamin A (MURK *et al*., 1996). In the same sample a tendency of increasing frequency of diseases with increasing TEQ concentrations in the liver was found (LEONARDS *et al*., 1996).

In most areas where otters are scarce, or have decreased in numbers, high concentrations of PCBs have been found in otter tissues (MASON, 1989; SMIT *et al*., 1998). In thriving otter populations such as in Latvia (SJÖÅSEN *et al*., 1997) or Northern Norway (CHRISTENSEN and HEGGBERGET, 1995) PCB concentrations are low. The remarkable exception is the healthy otter population on Shetland with high mean PCB levels (KRUUK and CONROY, 1991; KRUUK, 1997). All data published for the Shetland otter population indicate a serious pollution of the islands, but the exact sample locations in respect to harbours etc. have never been published,

so that it remains unclear in how far the samples are representative for the overall situation on the Shetland Isles.

3 CONCLUSIONS

In otters from several countries, single individuals were found with mercury concentrations exceeding critical tissue concentrations so that it cannot be excluded that negative effects of mercury occur at least on an individual level.

Small differences in CB patterns between otters from the United Kingdom, Ireland, Czech Republic, The Netherlands, Sweden and Denmark were found. CB patterns of the metabolisable CBs differ between the otters; decreased ratios of metabolisable CBs with increasing concentrations of the persistent CBs were observed. Induction of cytochrome P450 enzymes offers the best explanation for this process.

Several studies on PCB concentrations in otter tissues showed annual decreases of about 6% to 14% during the last years, which is coincided by the observation that otter populations are expanding in range in these countries.

A tendency of an increased percentage of diseases in Danish otters was observed at liver concentrations higher than 4ng TEQ/g lipid weight. This increase correlates well with a reported dose-effect relationship between vitamin A and the TEQ concentration. The consequence of the increased percentage of disease for the population is not known, as too limited information about the population dynamics, and diseases in otter populations is available.

Further aspects of the role of PHAHs on vitamin A homeostasis and possible negative effects on otters are discussed by GUTLEB and MURK (2001; this volume).

4 REFERENCES

Boening, D. W. (2000). Ecological effects, transport, and fate of mercury: a general review. *Chemosphere,* **40:** 1335-1351.

Boon, J. P. J., van der Meer, J., Allchion, C. R., Law, J. R., Klungsøyr, J., Leonards, P. E. G., Spliid, H., Storr-Hansen, E., McKenzie, C. & Wells, D. E. (1997). Concentration dependent changes of PCB patterns in five species of fish-eating mammals in relation to uptake from food and biotransformation capacity. *Archives of Environmental Contamination and Toxicology,* **33:** 298-311.

Broekhuizen, S. (1989). Belasting van otters met zware metalen en PCBs. *De Levende Natuur,* **90**: 43-47.

Brouwer, A., Lans, M. C., de Haan, L. H. J., Murk, A. J. & Morse, D. C. (1994). Formation and toxicological aspects of phenolic metabolites of polychlorobiphenyls (PCBs) and related compounds. *Organohalogen Compounds,* **20**: 465-469.

Brouwer, A., Morse, D. C., Lans, M. C., Schuur, A. G., Murk, A. J., Klasson-Wehler, E., Bergman, Å. & Visser, T. J. (1998). Interactions of persistent environmental organhalogens with the thyroid hormone system: mechanisms and possible consequences for animal and human health. *Toxicology and Industrial Health,* **14**: 59-84.

Christensen, H., & Heggberget, T. M. (1995). *Polychlorinated Biphenyl (PCB) Residues and Reproductive Performance in Norwegian Coastal Otters* Lutra lutra *L*. Dr. scient. Thesis, Univ. Trondheim, Norway.

Das, K., Debaker, V. & Bouquegneau, J. M. (2000). Metallothioneins in marine mammals. *Cellular and Molecular Biology* **46**: 283-194.

Davis, D. & Safe, S. (1990). Immunosuppressive activities of polychlorinated dibenzofuran congeners: Quantitative structure-activity relationships and interactive effects. *Toxicology and Appliled Pharmacology*, **94**: 141-148.

De Flora, S., Bennicelli, C., & Bagnasco, M. (1994). Genotoxicity of mercury compounds. A review. *Mutation Research,* **317**: 57-79.

Dolbec, J., Mergler, D., Sousa Passos, C. J., Sousa de Morais, S. & Lebel, J. (2000). Methylmercury exposure affects motor performance of a riverine population of the Tapajos river, Brazilian Amazon. *International Archive of Occupational and Environmental Health,* **73**: 195-203.

Gavis, J. & Ferguson, J. F. (1972). The cycling of mercury through the environment. *Water Research*, **6**: 989-1008.

Giesy, J. P., Verbrugge, D. A., Othout, R. A., Bowerman, W. W., Mora, M. A., Jones, P. D., Newsted, J. L., Vandervoort, C., Heaton, S. N., Aulerich, R. J., Bursian, S. J., Ludwig, J. P., Dawson, G. A., Kubiak, T. J., Best, D. A. & Tillitt, D. E. (1994). Contaminants in fishes from Great Lakes - influences sections and above dams of 3 Michigan rivers. 2. Implications for health of mink. *Archives of Environmental Contamination and Toxicology,* **27** : 213-223.

Greer, J. (1993). The price of gold. *Ecologist,* **23**: 91 - 96.

Gutleb, A. C., Schenck, C. & Staib, E. (1997). Giant otter (*Pteronura brasiliensis*) at risk? Total mercury and methylmercury levels in fish from the Department Madre de Dios, Peru. *Ambio,* **26**: 511-514.

[G]utleb, A. C., Kranz, A., Nechay, G. & Toman, A. (1998). Heavy metal concentrations in livers and kidneys of the otter (*Lutra lutra*) from Central Europe. *Bulletin of Environmental Contamination and Toxicolology*, **60**: 273-279.

Gutleb, A. C. & Murk, A. J. (2001). Review of current knowledge of PHAH toxicity and vitamin homeostasis in the Eurasian otter (*Lutra lutra).* In: *Proceedings of the First Otter Toxicological Conference.* (Edited by Conroy, J. W. H., Yoxon, P. & Gutleb, A. C). *Journal of the International Otter Survival Fund*, No 1, Pp. Xx-xx. Skye, September 2000. International Otter Survival Fund, Broadford, Isle of Skye.

Hansen, J. C. (2000). Environmental contaminants and human health in the arctic. *Toxicological Letters* **112/113**: 119-125.

Hernandez, L. M., Gonzales, M. J., Rico, M. C., Fernandez, M. A. & Baluja, G. (1985). Presence and biomagnification of organochlorine pollutants and heavy metals in mammals of Doñana National Park (Spain) 1982-1983. *Journal of Environmental Science and Health*, **B20**: 633-650.

Hof, H. & Wirsing, C. H. (1979). Anti-infective properties of vitamin A. *Z. Ernährungsw.*, **18**: 221-232.

Jefferies, D. J. (1996). Decline and recovery of the otter – a personal account. *British Wildlife*, **7**: 353-364.

Jefferies, D. J. & Hanson, H. M. (2001). The role of dieldrin in the decline of the otter in Britain: The analytical data. In: *Proceedings of the First Otter Toxicological Conference.* (Edited by Conroy, J. W. H., Yoxon, P. & Gutleb, A. C). *Journal of the International Otter Survival Fund*, No 1, Pp. Xx-xx. Skye, September 2000. International Otter Survival Fund, Broadford, Isle of Skye.

Jensen, S., Johnels, A. G., Olsson, M. & Otterlind, G. (1977). Effects of PCB and DDT on mink (*Mustela vison*) during the reproductive season. *Ambio,* **6**: 239.

Kaiser, G. & Tödl, G. (1980). Mercury. In: *The Handbook of Environmental Compounds.* Vol. 3, Part A: Anthropogenic Compounds. (Edited by Hutzinger, O.). Pp. 1-58. Springer, Berlin.

Kajiwara, Y., Yasutake, A., Adachi, T. & Hirayama, K. (1996). Methylmercury transport across the placenta via neutral amino acid carrier. *Archives of Toxicology*, **7**: 310-314.

Kimbrough, R. D. (1995). Polychlorinated biphenyls (PCBs) and human health: an update. *Critical Reviews of Toxicology*, **25**: 133-163.

Kleivane, L., Severinsen, T. & Skaare, J. U. (2000). Biological transport and mammal to mammal transfer of organochlorines in Arctic fauna. *Marine Environmental Research*, **49**: 343-357.

Koeman, J. H., Peeters, W. H. M., Koudstaal-Hol, C. H. M., Tjioe, P. S. & de Goeij, J. J. M. (1973). Mercury - selenium correlations in marine mammals. *Nature,* **245**: 385-386.

Kostial, K., Jugo, S., Rabar, I. & Maljkovic, T. (1978). Influence of age on metal metabolism and toxicity. *Environmental Health Perspectives*, **25:** 81-86.

Kruuk, H. (1997). The significance of PCBs in otters: a reply. *I.U.C.N. Otter Specialist Group Bulletin*, **14**: 54-56.

Kruuk, H. & Conroy, J. W. H. (1991). Mortality of otters (*Lutra lutra*) in Shetland. *Journal of Applied Ecology*, **28**: 83-94.

Kruuk, H., Conroy, J. W. H. & Carss, D. N. (1993*). Otters, Eels and Contaminants*. (ITE Report to Scottish Natural Heritage). Institute of Terrestrial Ecology, Banchory.

Leonards, P. E. G., Elmeros, M., Cofino, W. P., Conroy, J. W. H., Gutleb, A. C., Mason, C. F, Murk, A. J., Smit, M., van Hattum, B. & Madsen, A. B. (1996). Toxic PCBs in European otter populations in relation to biological factors and health status. In: *Development of Otter-based Quality Objectives for PCBs.* (Edited by Smit, M. D., Leonards, P. E. G., Murk, A. J., de Jongh, A. W. J. J. & van Hattum, B.). Pp. 47-62. IVM 96/11.

Madsen, A. B. (1991). Otter (*Lutra lutra*) mortalities in fish traps and experience with using stopgrids in Denmark. In: Proceedings of the. Vth International Otter Colloquium, Hankensbuettel. (Edited by Reuther, C & Röchert, R.). *Habitat* **6**: 237-241.

Madsen, A. B. & Nielsen, C. E. (1986). Odderens (*Lutra lutra*) forekomst i Danmark 1984-1986. *Flora of Fauna,* **92**: 60-62.

Madsen, A. B., Dietz, H. H., Henriksen, P. & Clausen, B. (1999). Survey of Danish free living otters (*Lutra lutra*) - a consecutive collection and necropsy of dead bodies. *I.U.C.N. Otter Specialist Group Bulletin,* **16**: 65-75.

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

Mason, C. F. (1997). The significance of PCBs in otters at national and regional scale. *I.U.C.N. Otter Specialist Group Bulletin*, **14**: 3-12.

Mason, C. F. (1998). Decline in PCB levels in otters (*Lutra lutra). Chemosphere*, **36**: 1969-1971.

Mason, C. F. & Madsen, A. B. (1993). Organochlorine pesticide residues and PCBs in Danish otters (*Lutra lutra*). *The Science of the Total Environment*, **133**: 73-81.

Mason, C. F. & O´Sullivan, W. M. (1993). Heavy metals in the livers of otters, *Lutra lutra*, from Ireland. *Journal of Zoology, London*, **231**: 675-678.

Mason, C. F. & Reynolds, P. (1988). Organochlorine pesticide residues and metals in otters from the Orkney Islands. *Marine Pollution Bulletin*, **19**: 80-81.

Mason, R. P., Laporte, J. & Andres, S. (2000). Factors controlling the bioaccumulation of mercury, methylmercury, arsenic, selenium, and cadmium by freshwater invertebrates and fish. *Archives of Environmental Contamination and Toxicology,* **38**: 283-297.

Muir, D., Braune, B., de March, B., Norstrom, R., Wagemann, R., Lockhart, L., Hargrave, B., Bright, D., Addison, R., Payne, J. & Reimer, K. (1999). Spatial and temporal trends and effects of contaminants in the Canadian Arctic marine ecosystem. *The Science of the Total Environment,* **230**: 84-144.

Murk, A. J., Leonards, P. E. G., Luit, R., van Hattum, B. & Smit, M. D. (1996). Monitoring exposure and effects of polychlorinated hydrocarbons (PHAHs) in European otters (*Lutra lutra*). *Organohalogen Compounds*, **29**: 64-69.

Murk, A. J., Leonards, P. E. G., van Hattum, B., Luit, R., van der Weiden, M. & Smit, M. (1998). Application of biomarkers for exposure and effect of polyhalogenated aromatic hydrocarbons in naturally exposed European otters (*Lutra lutra*). *Environmental and Toxicological Pharmacology*. **6** : 91-102.

Nauss, K. M., Mark, D. A. & Suskind, R. M. (1979). The effect of vitamin A deficiency on the in-vitro cellular immuno response in rats. *Journal of Nutrition*, **109**: 1815-1823.

Nishida, M., Yamamoto, T., Yoshimura, Y. & Kawada, J. (1986). Subacute toxicity of methylmercuric chloride on mouse thyroid. *Journal of Pharmacobiological Dynamics* **9**: 331-338.

O´Connor, D. J. & Nielsen, S. W. (1981). Environmental survey of methylmercury levels in wild mink (*Mustela vison*) and otter (*Lutra canadensis*) from the North-Eastern United States and experimental pathology of methylmercurialism in the otter. In: *Proceedings of the World Furbearer Conference*. (Edited by Chapman, J. D, & Pursley, D.). Pp. 1728 – 1745. Frostburg, Maryland.

Olsson, M., Reutergårdh, L. & Sandegren, F. (1981). Var är uttern? *Sveriges Natur,* **6**: 234-240.

Olsson, M. & Sandegren, F. (1991). Is PCB partly responsible for the decline of the otter in Europe? In: Proceedings of the. Vth International Otter Colloquium, Hankensbuettel. (Edited by Reuther, C. & Röchert, R). *Habitat* **6**: 223-227.

Riget, F. & Dietz, R. (2000). Temporal trends of cadmium and mercury in Greenland marine biota. *The Science of the Total Environment,* **245**: 49-60.

Roos, A., Greyers, E., Olsson, M. & Sandegren, F. (2001). The otter (*Lutra lutra*) in Sweden – population trends in relation to ΣDDT and total PCB concentrations during 1968-99. *Environmental Pollution,* **111**: 457-469.

Safe, S. H. (1994). Polychlorinated biphenyls (PCBs): environmental impact, biochemical and toxic responses, and implications for risk assessment. *Critical Reviews of Toxicology*, **24**: 87-149.

Sandegren, F., Olsson, M., & Reutergårdh, L. (1980). Der Rückgang der Fischotterpopulation in Schweden. In: *Der Fischotter in Europa - Verbreitung, Bedrohung, Erhaltung* (Edited by Reuther, C., Festetics, A.). Pp. 107-113. Selbstverlag, Oderhaus und Göttingen.

Shenai, J. P., Chytil, F., & Stahlman, M. T. (1985). Vitamin A status of neonates with bronchopulmonary dysplasia. *Pediatric Research,* **19**: 185-188.

Simpson, V. R., Bain, M. S., Brown, R., Brown, B. F. & Lacey, R. F. (2000). A long term study of vitamin A and polychlorinated hydrocarbon levels in otter (*Lutra lutra*) in South West England. *Environmental Pollution,* **110**: 267-275.

Sjöåsen, T., Ozolins, J., Greyerz, E. & Olsson, M. (1997). The otter (*Lutra lutra*) situation in Latvia and Sweden related to PCB and DDT levels. *Ambio,* **26**: 196-201.

Skarén, U. (1992). Analysis of one hundred otters killed by accidents in central Finland. *I.U.C.N. Otter Specialist Group Bulletin*, **7**: 9-12.

Smit, M. D., Leonards, P. E. G., de Jongh, A. W. J. J. & van Hattum, B. G. M. (1998). Polychorinated biphenyls in the Eurasian otter (*Lutra lutra*). *Revues of Environmental Contamination and Toxicology*, **157**: 95-130.

Southworth, G. R, Peterson, M. J. & Ryon, M. G. (2000). Long-term increased bioaccumulation of mercury in largemouth bass follows reduction of waterborn selenium. *Chemosphere,* **41**: 1101-1105.

Tanabe, S. & Tatsukawa, R. (1986). Distribution and load of PCBs in the oceans. In: *PCBs and the Environment.* Vol. 1. (Edited by Waid, J.S.). Pp. 143-161, CRC Press, Boca Raton, Florida.

Tanabe, S., Mori, T., Tatsukawa, R. & Miyasaki, N. (1983). Global pollution of marine mammals by PCBs, DDTs and HCHs (BHCs). *Chemosphere*, **12**: 277-288.

Tanabe, S., Kannan, N., Subramanian, A., Watanabe, S. & Tatsukawa, R. (1987). Highly toxic coplanar PCBs: occurrence, source, persistency and toxic implications to wildlife and humans. *Archives of Environmental Contamination and Toxicology*, **47**: 147-163.

Tatton, J. O., & Ruzicka, J. H. A. (1967). Organochlorine pesticides in Antarctica. *Nature,* **215**: 346-348.

Voogt, P. de & Brinkman, U. A. Th. (1989). Production, properties and usage of polychlorinated biphenyls. In: *Halogenated Biphenyls, Terpenyls, Naphtalenes, Dibenzodioxins and related Products*. (Edited by Kimbrough, R., & Jensen, S.). Pp. 3-45. Elsevier Science Publishers, Amsterdam.

Watras, C. J., Back, R. C., Halvorsen, S., Hudson, R. J., Morrison, K. A. & Wente, S. P. (1998). Bioaccumulation of mercury in pelagic freshwater food webs. *The Science of the Total Environment,* **219**: 183-208.

Wells, D. E., McKenzie, C. & Ross, H. M., (1996). Chlorobiphenyl patterns in marine mammals from Northern European waters. *Scottish Fisheries Working Paper no. 1/96*. Scottish Office Agriculture, Environment and Fisheries Department, Marine Laboratory, Aberdeen, Scotland.

Wells, D. E., Campbell, L. A., Ross, H. M., Thompson, P. M. & Lockyer, C. H. (1994). Organochlorine residues in harbour porpoise and bottlenosed dolphins stranded on the coast of Scotland 1988-1991. *The Science of the Total Environment,* 151: 77-99.

Wobeser, G. A. & Swift, M. (1976). Mercury poisoning in a wild mink. *Journal of Wildlife Diseases,* **12**: 335-340.

Wren, C. D. (1985). Probable case of mercury poisoning in a wild otter, *Lutra canadensis*, in North-Western Ontario. *Canadian Field-Naturalist,* **99**: 112-114.

Wren, C. D. (1986). A review of metal accumulation and toxicity in wild mammals. I. Mercury. *Environmental Research*, **40**: 210-244.

Wren, C. D. (1991). Cause-effect linkages between chemicals and populations of mink (*Mustela vison*) and otter (*Lutra canadensis*) in the Great Lakes basin. *Journal of Toxicology and Environmental Health,* **33**: 549-585.

Wren, C. D., Stokes, P. M. & Fischer, K. L. (1986). Mercury levels in Ontario mink and otter relative to food levels and environmental acidification. *Canadian Journal of Zoology,* **64**: 2854-2859.

Zalups, R. K. (2000). Molecular interactions with mercury in the kidney. *Pharmacological Review*, **52**: 113-143.

DISEASES OF OTTERS IN BRITAIN

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ABSTRACT
Between 1988 and 2000 the author performed post mortem examinations on more than 230 otters from South West England. This paper reviews many of the pathological conditions seen, together with those reported elsewhere in Britain. Road traffic deaths were responsible for more than 80% of submissions. Approximately one sixth of all otters were suffering from bite wounds, apparently due to intraspecific aggression, and this was the second most common cause of death. The commonest infectious disease was adiaspiromycosis but in most cases the lesions were of little clinical significance. Tuberculosis has been recorded in British otters and, although suspected pulmonary lesions were seen in this study, all tests proved negative. Hepatic levels of polyhalogenated hydrocarbons decreased annually and this coincided with increased vitamin A levels. Biliary and adrenal hyperplasia were seen and the possible involvement of PCBs is discussed. Stress factors were considered responsible for adrenocortical nodular hyperplasia and for haemorrhagic gastritis, often with stomach ulcers. Convoluted, nodular uteri were seen in several otters but their significance was not established.

There is a tendency when referring to 'disease' in animals, for people to think only of those diseases caused by infectious agents, such as bacteria, viruses, fungi, parasites, etc. There are, of course, many other types of disease, for example toxic, metabolic, genetic and nutritional diseases. However, in Britain, as in most other countries, the diseases of otters (*Lutra lutra*) are poorly understood and this is principally because so few otters have been examined by veterinary pathologists (KEYMER, 1991). An important part of a veterinarian's training is to learn to recognise disease, and to distinguish one disease from another, - different diseases may produce clinical signs or pathological lesions that are similar. The work of the wildlife pathologist is made more difficult by the fact that we often do not have adequate knowledge of what is 'normal' for wild species. This is particularly important when trying to interpret the histopathological appearance of an organ.

In order to address these problems the author, with the support of the Environment Agency, has carried out post mortem examinations on more than 230 otters from South West England since 1988 (SIMPSON, 1997). This paper describes many of the pathological conditions seen and summarises those reported by earlier workers.

Over 80% of otters submitted for examination had died in road traffic accidents. This is slightly higher than the proportion (70%) killed by traffic in Southern Ireland (MASON and O'SULLIVAN, 1992) but markedly higher than the 42% recorded in Shetland (KRUUK and CONROY, 1991). The high proportion of road traffic deaths in South West England was possibly a consequence of the higher traffic density in this area. However, there was only one death in a fish trap, whereas in Shetland, as in some other countries, fish traps are a common cause of mortality. Approximately a

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sixth of all otters (16.6%) had suffered bite wounds, mainly to the head, feet and perineal area, and it is believed that most of these were due to intraspecific aggression. Some wounds became septic, mostly with a streptococcal infection, and bite wounds were the second most common cause of death (SIMPSON, 1997; SIMPSON and COXON, 2000). Fighting between adult males has been recorded previously, with anecdotal reports of males suffering fractures of the baculum and even castration as a result (STEPHENS, 1957). In the present study 22.6% of males had bite wounds and these frequently involved the anus and genitals. However, 12.6% of females had also been bitten and many had wounds to the vulva and anus. Bite wounds were seen in cubs and juveniles as well as adults. Dental disease, principally fractures, has become more common recently and often appears to be related to fighting.

The commonest infectious disease was adiaspiromycosis, caused by inhalation of spores of the fungus *Emmonsia* sp. Although the granulomatous lesions in the lungs can resemble those of tuberculosis they normally appear to be of little clinical significance. However, one juvenile which died naturally had very extensive lung pathology and was considered to have died from atypical adiaspiromycosis (SIMPSON and GAVIER-WIDEN, 2000). There is an historical record of tuberculosis (mycobacterial species not specified) in an otter in Cornwall (STEPHENS, 1957) and a recent report of a case in Scotland caused by *Mycobacterium avium* ssp. *avium* (PATTERSON, A., *pers. comm*.) However, although a small number of otters from South West England had pulmonary lesions suggestive of tuberculosis, all were negative on culture and no acid-fast organisms were seen in Ziehl-Neelsen stained sections. The lungs of the majority of the otters were examined histologically but none showed evidence of parasitic disease. *Angiostrongylus vasorum* infection has been reported in an otter in Denmark (MADSEN *et al*., 1999) and, as the parasite is commonly found in foxes (*Vulpes vulpes*) in Cornwall (SIMPSON, 1996), it is perhaps surprising that some cases were not seen in the present study.

Arteriosclerosis and arteritis were described in an aged otter from Norfolk which had lesions suggestive of Aleutian disease (WELLS, KEYMER and BARNETT, 1989). The animal also had a rounded apex to the heart and right ventricular dilation. Left ventricular hypertrophy was reported in an oiled otter in Shetland (BAKER *et al*., 1981). No specific cardiac or vascular lesions were seen in the otters from South West England. However, it was observed that otters have an unusually globular-shaped heart, associated with a thick walled left ventricle and a relatively thin walled right ventricle (SIMPSON, 1997). Some of the otters suffering from septic bite wounds had vegetative heart valve lesions and vascular thromboses, particularly in lungs.

No gross liver lesions were seen in the present study. Bacterial cultures were performed only where animals had died from non-traumatic causes. The predominant isolates were *Streptococcus* spp. and *Escherichia coli,* usually associated with septicaemia in animals suffering from bite wounds. *Staphylococcus lutrae* was also isolated on two occasions. This organism was first described from otters in Scotland (FOSTER *et al*., 1997) but its pathogenic significance is uncertain. KEYMER (1991) reported the isolation of *Yersinia pseudotuberculosis* from the liver of an otter found dead in Norfolk. However, the animal had no lesions typical of pseudotuberculosis and the cause of death was uncertain. In the otter with suspected Aleutian disease (WELLS, *et al.,* 1989), the liver was enlarged and there was a single cholelith (gall-stone) in the gall bladder. Neither choleliths nor Aleutian disease were seen in any of

the otters from South West England. WELLS *et al*. (1989) also described hepatic adenocarcinoma in their case and considered that it might be an extreme consequence of biliary hyperplasia. Polychlorinated biphenyls (PCBs) are known to cause biliary hyperplasia in various species and many otters in the present study appeared to show this to some degree. At the present time, however, it has not been possible to relate the degree of hyperplasia with the tissue concentrations of PCBs and other factors may be involved. Hepatic PCB and organochlorine pesticide (OC) concentrations in otters in South West England fell markedly during the ten year study period (SIMPSON *et al*., 2000) and similar declines in PCBs, though not OCs, have been reported for otters elsewhere in Britain (MASON, 1997). If biliary hyperplasia in otters is related to PCB levels, we might therefore expect the lesions to become progressively less marked in the future. In South West England it was also observed that, as the concentrations of PCBs and OC pesticides declined, the hepatic concentrations of vitamin A increased (SIMPSON *et al*., 2000). This is consistent with recent Dutch studies on Danish otters that have shown a strong negative relationship between the concentrations of certain PCB congeners and vitamin A (MURK *et al*., 1998).

Adrenocortical nodular hyperplasia has been described twice in British otters (KEYMER *et al.,* 1988; WELLS, *et al.*, 1989) and the authors suggested that this could be a consequence of age, stress or exposure to PCBs. The condition was commonly seen in the otters in the present study and the extreme cases were attributed to stress. Typically these were males that had died from severe bite wounds, but it was also present to a lesser degree in females that were in late pregnancy or lactating (SIMPSON, 1997). However, when these animals were excluded from the data set, there remained a strong positive correlation between adrenal weight and hepatic concentration of PCB congeners 138, 153 and 180 (SIMPSON, 1998). These results suggest that some PCB congeners may be influencing adrenal development and possibly function.

Renal calculi are common in captive otters and have been described occasionally in wild ones (STEPHENS, 1957; KEYMER, LEWIS and DON, 1981; WEBER, H., *pers. comm*.). However, only five out of 230 otters from South West England were affected. KEYMER *et al.* (1981) suggested that hypovitaminosis A might be one cause of renal calculi but the present author found no evidence of a relationship between vitamin A deficiency and renal calculi, in either wild or captive otters (SIMPSON, 1998, SIMPSON *et al*., 2000).

Evidence of reproductive failure can be difficult to obtain in a free-living carnivore but abortion due to foetal infection with *Plesiomonas shigelloides* has been reported in an otter from Scotland (WEBER and ROBERTS, 1989) and a novel *Brucella* sp. has also been isolated from an otter in Scotland (FOSTER *et al*., 1996). This latter organism was initially isolated from cetaceans and pinnipeds (ROSS *et al*., 1994) but the pathological significance of the organism in either marine mammals or otters is uncertain. KEYMER *et al*. (1988) reported a leiomyoma (smooth muscle tumour) of the uterus in an otter from Norfolk and there was a single case of pyometra in the South West otters (RIVERS and SIMPSON, unpublished data). Foetal resorption was suspected on several occasions where there was a pale orange placental scar in addition to normal blackish ones. Similar findings have been reported in Danish otters (ELMEROS and MADSEN, 1999). Apart from this there was little evidence of reproductive disease but in five females the uteri were nodular and highly convoluted. Single cases of what may be the same condition have been reported in Norway (HEGGBERGET, 1988) and in Denmark (ELMEROS and MADSEN, 1999).

Somewhat similar uterine changes have also been observed in seals and attributed to the effects of pollutants, such as PCBs (BERGMAN and OLSSON, 1985). However, although these changes in the otter uteri appear pathological, in each case a corpus luteum was present, and they may possibly represent a normal stage of uterine development in early pregnancy (SIMPSON, 1997). No convoluted uteri have been seen in otters in the last two years, but the number of pregnant females also appears to have decreased in this period. This merits further investigation.

There is little evidence of enteric infections in otters in Britain but KEYMER (1992) reported *Salmonella binza* infection in the gut of a free-living otter in Norfolk. Haemorrhagic gastro-enteropathy associated with ingestion of oil was considered to be the main cause of death in five otters following an oil spill in Shetland (BAKER *et al*., 1981). Several authors (KEYMER *et al*., 1988; KRUUK and CONROY, 1991) have observed blackish fluid in the stomach and intestines of otters in poor condition. Similar cases were seen in the present study, mostly in animals that had severe fight wounds, but also in orphaned cubs a few days after the start of attempted hand-rearing. In the case of two such cubs there was also a large ulcer in the mucosa of the stomach near the pylorus (SIMPSON, unpublished data). BAKER *et al*., (1981) observed very similar ulcers, plus blackish fluid in the lower intestine, of an oiled otter a few days after hospitalisation. The present author strongly suspects that these lesions are due to stress. An unusual post mortem finding in early 2000 was the presence of the remains of a cub, aged about three to four weeks, in the stomach of a male otter. This appears to be the first evidence of cannibalism in otters and was possibly a case of infanticide. The male had died in a road accident whilst fighting with another male and DNA analysis showed it was not the father of the cub (SIMPSON and COXON, 2000).

There have been a number of reports of otters showing neurological signs but, although tissues have been analysed for pollutants, rarely has a brain been made available for pathological examination. A notable exception was a cub from the Vincent Wildlife Trust rehabilitation centre in Scotland that was shown to have hydrocephalus (GREEN, R. *pers. comm*.). Two similar subsequent cases, one from Devon and the other from Scotland, had additional lesions suggestive of an in-utero viral infection. Further investigations are proceeding to try and establish the aetiology. Eyes were examined from many otters in South West England and the results will be reported elsewhere.

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REFERENCES

Baker, J. R., Jones, A. M., Jones, T. P. & Watson, H. C. (1981). Otter *Lutra lutra* mortality and marine oil pollution. *Biological Conservation,* **20**: 311-321.

Bergman, A., & Olsson, M. (1985). Pathology of Baltic grey seal and ringed seal females with special reference to adrenocortical hyperplasia: is environmental pollution the cause of a widely distributed disease syndrome? *Finnish Game Research*, **44**: 47-62.

Elmeros, M., & Madsen, A. B. (1999). On the reproductive biology of otters (*Lutra lutra*) from Denmark. *Zeitschrift für Saügetierkunde,* **64**: 193-200.

Foster, G., Jahans, K., Reid, R. J. & Ross, H. M. (1996). Isolation of *Brucella* species from cetaceans, seals and an otter. *Veterinary Record,* **138**: 583-586.

Foster, G., Ross, H. M., Hutson, R. A. & Collins, M. D. (1997). *Staphylococcus lutrae* sp. nov., a new coagulase-positive species isolated from otters. *International Journal of Systematic Bacteriology*, **47**: 724-726.

Heggberget, T. M. (1988). Reproduction in the female European otter in central and Northern Norway. *Journal of Mammalogy,* **69**: 164-167.

Keymer, I. (1991). Otters in East Anglia, Past, Present and Future. *Norfolk Naturalists Trust, 72 Cathedral Close, Norwich. 1.*

Keymer, I. (1992). Diseases of the otter (*Lutra lutra*). *Proceedings of the National Otter Conference, Cambridge, September 1992* (Edited by Moris, P.). Pp. 30-33. Mammal Society, London.

Keymer, I. F., Lewis, G. & Don. P. (1981). Sonderdruck aus Verhandlungsbericht des XXII Internationalen Symposiums uber die Erkrankungen der Zootier, Halle/Saale, 391-401.

Keymer, I., Wells, G., Mason, C. & Macdonald, S. (1988). Pathological changes and organochlorine residues in tissues of wild otters (*Lutra lutra*). *Veterinary Record,* **122**: 153-155.

Kruuk, H. & Conroy J. W. H. (1991). Mortality of otters (*Lutra lutra*) in Shetland. *Journal of Applied Ecology,* **28**: 83-94.

Madsen, A. B., Dietz, H. H., Henriksen, P. & Clausen, B. (1999). Survey of Danish free living otters *Lutra lutra* - a consecutive collection and necropsy of dead bodies. *I.U.C.N. Otter Specialist Group Bulletin,* **16**: 65-75.

Mason, C. (1997). The significance of PCBs in otters at national and regional scales. *I.U.C.N. Otter Specialist Group Bulletin*, **14**: 3-12.

Mason, C. F.& O'Sullivan, W. M. (1992). Organochlorine pesticide residues and PCBs in otters (*Lutra lutra*) from Ireland. *Bulletin of Environmental Contamination and Toxicology,* **48**: 387-393.

Murk, A. J., Leonards, P. E., van Hattum, B., Luit, R., van der Weiden, M. E. & Smit, M. (1998). Application of biomarkers for exposure and effects of polyhalogenated aromatic hydrocarbons in naturally exposed European otters (*Lutra lutra*). *Environmental Toxicology and Pharmacology,* **6**: 91-102.

Ross, H., Foster, G., Reid, R., Jahans, K. & Macmillan, A. (1994). *Brucella* species infection in sea-mammals. *Veterinary Record,* **134**: 359.

Simpson, V. R. (1996). *Angiostrongylus vasorum* infection in foxes (*Vulpes vulpes*) in Cornwall. *Veterinary Record***, 139**: 443-445.

Simpson, V. R. (1997). Health status of otters (*Lutra lutra*) in South West England based on post-mortem findings. *Veterinary Record,* **141**: 191-197.

Simpson, V. R. (1998). *A Post Mortem Study of Otters* (Lutra lutra*) found dead in South West England.* R&D Technical Report W148, Environment Agency, Bristol.

Simpson, V. R. & Coxon, K E. (2000). Intraspecific aggression, cannibalism and suspected infanticide in otters. *British Wildlife,* **11**: 423-426.

Simpson, V. R. & Gavier-Widen, D. (2000). Fatal adiaspiromycosis in a wild Eurasion otter (*Lutra lutra*). *Veterinary Record,* **147**: 239-241.

Simpson, V., Bain, M., Brown, R., Brown, B. & Lacey, R. (2000). A long-term study of vitamin A and polychlorinated hydrocarbon levels in otters (*Lutra lutra*) in South West England. *Environmental Pollution,* **110**: 267-275.

Stephens, M. (1957). *The Otter Report*. Universities Federation for Animal Welfare, Potters Bar, London.

Weber, J. M. & **Roberts, L.** (1989). A bacterial infection as a cause of abortion in the European otter, *Lutra lutra*. *Journal of Zoology, London,* **219**: 688-690.

Wells, G., Keymer, I., & Barnett, K. (1989). Suspected Aleutian disease in a wild otter (*Lutra lutra*). *Veterinary Record,* **125**: 232-235.

SURVEY OF DANISH FREE LIVING OTTERS *LUTRA LUTRA*. A CONSECUTIVE COLLECTION AND NECROPSY OF DEAD BODIES.

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ABSTRACT
During 1979-1993, 194 dead Danish otters Lutra lutra were received. Of these, 145 were necropsied and the cause of death, sex, age and body condition determined. Traffic mortality (45.4%) and drowning (32.5%) constituted the major cause of death. Shot gun lead pellets were detected in 5% of the otters. Inclusion bodies indicating distemper virus infection were found for the first time in a free living otter population. Angistrongylus vasorum larvae were found in the lungs of free living otters for the first time. No ectoparasites were found. Infectious agents were detected in 22.1% of the otters although only few individuals appeared to have died from infections. The age distribution was not significantly different between sexes. Body condition for otters which died violently in Denmark was comparable to findings in Shetland, where thriving populations exist. The results showed a considerable decrease in number of otters found drowned in fish traps coinciding with the introduction of stop grids in fish traps in 1986. The results suggest that the existing otter population in Denmark is healthy and in good condition but it cannot be excluded that the large number of otters killed by traffic threatens the continued expansion of the species.

1 INTRODUCTION

The Eurasian otter *Lutra lutra*, is a highly vulnerable mammal in Denmark as well as in much of Europe (MACDONALD and MASON, 1994). In 1996 a national survey (HAMMERSHØJ *et al.,* 1996) concluded that the species occured in the northern part of Jutland; in the counties of Nordjylland, Viborg, Ringkøbing, Århus, Ribe and Vejle. On Zealand, in the county of Vestsjælland, no signs were found in the national otter survey, but in a more detailed survey undertaken parallel to the national survey (LETH and BYRNAK, 1996), signs of otters were found at two sites (Figure 1).

It has been claimed that contaminants such as the organochlorine pesticide dieldrin, polychlorinated biphenyls (PCBs), and heavy metals, in particular mercury, have been responsible for the rapid decline in otter populations in Europe (MACDONALD and MASON, 1994). Decreasing otter population in Denmark was thought to be due mainly to river regulation, wetland destruction, drowning in fish

traps, and intensified traffic (MADSEN, 1991).

Otter carcasses have been collected annually in several European countries. In Germany e.g. more than 50 otters were found dead each year, but only a small number were necropsied (ZOGALL and REUTHER, 1992). Likewise only 24 of 113 dead otters collected in Shetland were necropsied (KRUUK and CONROY, 1991). In south-west England, 77 wild otters were examined postmortem (SIMPSON, 1997).

In this paper a comprehensive necropsy results of 145 carcasses submitted from a population of free living otters are evaluated to assess current threats to otters.

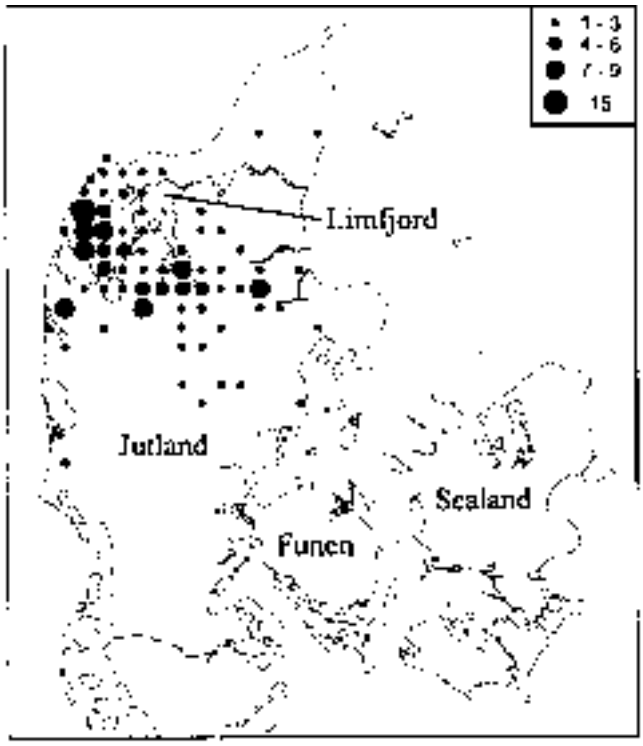

Figure 1. Geographical distribution of dead otters (N=193).

Figure 1. Geographical distribution of dead otters (N=193). the origin of one otter is unknown.

2 MATERIALS AND METHODS

Dead otters were received from hunters, motorists, anglers, forestmen etc. The otters were usually followed by written information about circumstantial evidence like killed on a road, died in a fish trap etc. Carcasses were frozen immediately upon arrival and stored at -18^0 C until necropsy was performed.

2.1 Necropsy

After thawing, the length (nose to tail) and weight were recorded. The animals were pelted followed by a routine necropsy procedure, including a search of the subcutis for lead pellets. Otters were aged as juveniles (less than about 5 months old) if tooth replacement was incomplete, as subadults (5-18 months) if the epiphyseal closure of humerus and femur at their proximal and distal ends was not complete or as adults (older than about 18 months). In males the length of the os penis was also used in

ageing (VAN BREE, JENSEN and KLEIJN, 1966). The craniums were cleaned from muscles etc. and the upper and lower jaw was inspected by a dentist.

2.2 Laboratory tests

Lungs and gut contents were examined for parasites, eggs and larvae from parasites using McMaster and modified Baerman techniques (HENRIKSEN, 1965; HENRIKSEN and KORSHOLM, 1984). Scrapings of epithelial lining from trachea, lungs, and urinary bladder from otters necropsied later than 1988 were examined for viral inclusion bodies using S3-staining and a routine immunohistochemical method to detect distemper virus. Bacteriological examinations (Aerobic cultures on blood agar), were performed on material from the digestive tract, lungs and kidneys.

The body condition (K) of otters was calculated using the equation $K = W/(a \times L^n)$ where W = weight (kg) and L = total length (m) according to LE CREN (1951). The constants were those calculated by KRUUK, CONROY and MOORHOUSE, (1987) viz. a = 5.02 for females and 5.87 for males; n = 2.33 for females and 2.39 for males.

3 RESULTS

Of the 194 otters received, 145 were necropsied and 52 X-rayed. For some of the animals complete data were not received. Therefore, the number of individuals in the various examinations is inconsistent (Table 1).

Table 1. Salient data and the number of animals included.

Type of data presented	Number of animals
Total received	194
Origin stated	193
Sex determined	192
Age determined	178
Length and weight determined	158
Necropsied	145
X - rayed	52

The geographical origins and densities of the otters are given in Figure 1. The vast majority came from the Limfjord area. One individual found in 1979 came from the island of Funen. Half of the otters were found in or close to marine habitats. The annual number of carcasses received varied from two in 1979 to 31 in 1993 (Figure 2). Major causes of death were identified as traffic mortality (88 = 45.4 %) and drowning (63 = 32.5 %).

No significant difference was found in age distribution between the two sexes, ($\chi^2 = 0.43$; d.f. = 2:n.s.) (Table 2). Considerably more males (113) than females (79) were received during the survey. The weight and length of adult males were

significantly higher than for adult females (weight - t = 9.60; d.f. = 65: $p < 0.001$, length - t = 20.35; d.f. = 67: $p < 0.001$). The condition index (K) of the otters had an overall mean value of 1.12, animals that died violently (traffic accidents and fish traps) had a value of 1.16 (Table 3).

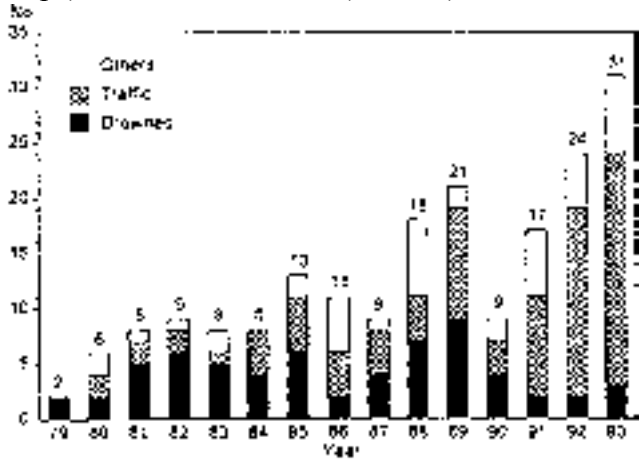

Figure 2. Annual number of dead otters and cause of death (N=194).

Figure 2. Annual number of dead otters and cause of death (N=194).

Table 2. Sex and age distribution of dead otters.

	Females	Males	Unknown	Total
Juvenile	8	12	-	20 (10.3%)
Subadult	30	48	-	78 (40.2%)
Adult	34	44	2	80 (41.2%)
Unknown	7	9	-	16 (8.3%)
Total	79	113 (58.2%)	2 (1.0%)	194 (100%)

Table 3. Weight and length of adult otters and calculated condition indices (K).

	X	s.d.	range	n
Weight (kg)				
Male	9.07	1.35	5.45-11.40	37
Female	6.02	1.17	3.36-7.60	30
Length (cm)				
Male	112.9	5.06	90.0-130.0	36
Female	103.0	3.17	95.5-110.0	33
Condition index (K)				
Non-violent	0.94	0.18		
Violent	1.16	0.16		30
Sum	1.12	0.18		154

The results of necropsy and the corresponding pathological findings are detailed in Table 4. No ectoparasites were found. Signs of endoparasites were found in only five individuals viz. two with one egg of *Ascaridae* per gram in the intestinal tract, one with one egg of *Strongylidae* per gram in the intestinal tract and one with *Angiostrongylus vasorum* larvae in the lungs. Two tapeworm *Cestedae* eggs per gram were found in the intestinal tract of one individual.

Table 4. Numbers and types of pathological findings recorded at necropsy of dead otters (N=145).

Pathological findings	Number of animals
Parodontal disease	11 (7.6%)
Endoparasites	5(3.4%)
- Ascaridae	2
- Strongylidae	1
- Angiostrongylus vasorum	1
- Cestedae	1
Viral infections	6 (4.1%)
- distemper virus	6
Bacterial diseases	7(4.8%)
- pneumonia	5
- peritonitis	1
- Streptococcus sp.	1
Kidneystone	3(2.1%)
Gallstone/enlarged gall bladder	2(1.4%)
Hepatitis	2(1.4%)
Hypertrophied suprarenal gland	2(1.4%)
Tumour in spleen/enlarged spleen	2(1.4%)
Tumour in the small intestine	1(0.7%)
Umbilical hernia	1(0.7%)
Blindness	1(0.7%)
Total	43(29.7%)

Inclusion bodies were found in six individuals, three females and three males of

different age. These otters were all collected in the Limfjord area. The six otters were not believed to have suffered from clinical distemper.

Due to often severe decomposition bacteriological examination could only be applied to eight otters. Pneumonia due to bacterial infection was found in five individuals, four females and one male, of which two were juveniles. One abandoned juvenile died from bacterial peritonitis two weeks after being taken into captivity. Local infection with *Streptococcus sp.* was recorded in one animal.

Kidney stones consisting of ammonium urate were found in three adults, two males and one female, and two otters had a gall bladder enlarged by gall stones. Two otters showed hypertrophy of the suprarenal glands. A small intestinal tumour possibly a leiomyoma (severe decomposition) and a minor umbilical hernia was seen in two otters, respectively. The eyes of one adult, male otter were completely opaque, probably causing total blindness.

Lead pellets were found in nine otters (5%) in numbers from one to five pellets except for one individual carrying 14 pellets. The lead pellets were generally found in the pelt or subcutaneously and none were found in or close to vital organs. Parodontal disease was detected in 11 otters indicating a relatively high proportion of diseased animals.

4 DISCUSSION

Based on condition (K) of violent death otters there was no significant difference between otters from Denmark (Table 3) and from Shetland (KRUUK and CONROY, 1991) ($K = 1.08 \mp 0.15$ s.d. $n = 49$), ($t = 2.99$; d.f. = 171: n.s.) where thriving populations exist. The results agree with condition indices estimated by the authors from Danish data collected by JENSEN (1964) ($K = 1.13 \mp 0.16$ s.d. $n = 81$).

The increase in the annual numbers of submitted otters during the survey period (Figure 2) might indicate an expanding population of otters (MADSEN, CHRISTENSEN and JACOBSEN, 1992) but a greater public awareness of otters cannot be excluded as the underlying cause of the increasing number of submissions.

The present results show that males achieve a larger overall size than females. MASON and MACDONALD (1986) classified animals weighing more than 4kg as adults. In our study adults were classified as individuals with fully developed growth. One female with pneumonia but no emaciation weighed as little as 3.36kg confirming that the weigth and length alone may not be used as an indicator of age.

No ectoparasites and only small numbers of endoparasites were found. This indicates that in the present situation the otter is not parasitized very often, probably due to their solitary living and the relative scarcity of the species. However, decaying before collecting the dead otters combined with freezing might have disintegrated some parasites and larvae.

Except for the larvae of *Angiostrongylus vasorum* all other endoparasites recorded have been described earlier to occur in otters (JEFFERIES, HANSON and HARRIS, 1990; SCHIERHORN *et al.,* 1991; WEBER, 1991). Otters forage on frogs which might act not only as paratenic but also as intermediate hosts for *A. vasorum* (BOLT *et al.,* 1993, 1995). None of the parasites recorded were considered to have influenced the health status of Danish otters.

Distemper virus in captive Eurasian otters was described by GEISEL (1979) and STEINHAGEN and NEBEL (1985). Our study is the first to record distemper virus

in a free living population of otters. The fact that the infected otters were collected from the Limfjord area in a period when distemper virus was present both in the common seal *Phoca vitulina* (BLIXENKRONE-MØLLER *et al.*, 1989) and in major outbreaks of distemper in farmed mink in this area indicates a wide range of host species for dis-temper virus. Negative findings in the remaining material may indicate a low propagatory rate of the virus in the population, but may also relate to the solitary life of otters and hence a low contact between animals.

Two cases of hepatitis probably causing severe health problems were seen. Pneumonic changes were found in five of 145 necropsied free living Danish otters. This corresponds to the findings of KRUUK and CONROY (1991) who found one case among 24 necropsied otters. Pneumonia has not hitherto been recorded in captive animals (ROGOSCHIK and BRANDES, 1991). One individual was recorded as blind in our study. WILLIAMS (1989) also reported blind otters from Britain during the period 1957-80.

Based on our study we would argue that only the two animals with hepatitis, and the five animals with pneumonia were likely to have died because of the diseases detected. In addition, one animal with peritonitis definitely died from this disease.

Since 1967, the Danish otters have been protected by law. During the period 1967-1982, fish farmers could be granted a special permission to kill otters at fish ponds but this exemption was terminated in 1982. However, this study shows that totally protected animals are still shot. To the less experienced hunter an otter may be mistaken for a free living mink of which more than 8,000 are shot annually in Denmark (ASFERG, 1999).

The level of PCBs in otters from Denmark (MASON and MADSEN, 1993) is at the same as found in 1988 among young common seals in the Limfjord area (STORR-HANSEN and SPLIID, 1993) and much lower than the 50mg/kg which causes reproductive failure among mink in laboratory studies and which is assumed by some to be a critical level for otters (KEYMER *et al.,* 1988; SMIT *et al.,* 1994).

It is seen (Figure 2) that the number of otters dying in fish traps has decreased. It is believed that this is the successful effect of a 1986 compulsory use of stop grids in fish traps for fishermen (MADSEN and SØGAARD, 1994). It should be noted that traffic mortality constitutes 45% of the total mortality (males as well as females, young as well as adults) indicating the need for preventive measures where roads are crossing rivers in Denmark.

In conclusion, our results suggest that the population of otters seems healthy and in good reproductive condition (ELMEROS and MADSEN, 1999), although traffic mortality may constitute a threat to the spread of the population.

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6 REFERENCES

Asferg, T. (1999). Nedlagt vildt i sæsonen 1997/98. *Vildtinformation* 99, Miljø- og Energiministeriet, Skov- og Naturstyrelsen, p. 21.

Blixenkrone-Møller, M., Svansson, V., Have, P., Bøtner, A. & Nielsen, J. (1989). Infection studies in mink with seal-derived morbillivirus. *Archives of Virology*, **106**: 165-170.

Bolt, G., Monrad, J., Frandsen, F., Henriksen, P. & Dietz, H. H. (1993) The common frog (*Rana temporaria*) as a potential paratenic and intermediate host for *Angiostrongylus vasorum*. *Parasitology Research*, **79**: 428-430.

Bolt, G., Monrad, J., Frandsen, F., Bindseil, E., Henriksen, P. & Dietz, H. H. (1995). Angiostrongylose hos hund.I. *Biologi og patologi. Dansk Veterinær Tidsskrift*, **78**: 387-390.

Elmeros, M. & Madsen, A. B. (in press). On the reproduction biology of otters (*Lutra lutra*) from Denmark. *Zeitschrift für Säugetierkunde*.

Geisel, O. ***(1979). Staupe bei Fischottern (*****Lutra lutra*****). Berliner und Münchener Tierärztliche Wochenschrift,*** *92: 304.*

Hammershøj, M., Madsen, A. B., Bruun-Schmidt, I. Ø., Gaardmand, B., Jensen, A., Jensen, B., Jeppesen, J. L. & Laursen, J. T. (1996). *Overvågning af odder (Lutra lutra) i Danmark 1996*. [Otter (*Lutra lutra*) Survey of Denmark 1996.]. Danmarks Miljøundersøgelser. 40 s. Faglig rapport fra DMU, nr. 172.

Henriksen, S. A. (1965). En forbedret teknik ved undersøgelse for lungeormelarver i faeces. *Nordisk Veterinær Medicin*, **17**:.446-454.

Henriksen, S. A. & Korsholm, H. (1984). Parasitologisk undersøgelse af fæcesprøver. *Dansk Veterinær Tidsskrift*, **67**: 1193-1196.

Jefferies, D. J., Hanson, H. M. & Harris, E. A. (1990). The prevalence of *Pseudoterranova decipiens* (Nematoda) and (*Corynosoma strumosum*) (Acanthocephala) in otters, *Lutra lutra* from coastal sites in Britain. *Journal of Zoology, London,* **221**: 316-321.

Jensen, A. (1964). Odderen i Danmark. *Danske Vildtundersøgelser* **11**. Vildtbiologisk Station, Kalø, Rønde, 48 pp.

Keymer, I. F., Wells, G. A. H, Mason, C. F. & Macdonald, S. M. (1988). Pathological changes and organochlorine residues in tissues of wild otters (*Lutra lutra*). *Veterinary Record,* **122**: 153-155.

Kruuk, H. & Conroy, J. W. H. (1991). Mortality of otters (*Lutra lutra*) in Shetland. *Journal of Applied Ecology*, **28**: 83-94.

Kruuk, H., Conroy, J. W. H. & Moorhouse, A. (1987) Seasonal reproduction, mortality and food of otters (*Lutra lutra* L.) in Shetland. *Symposium of the Zoological Society of London.* **58**: 263-278.

Le Cren, E. D. (1951). The length-weight relationship and seasonal cycle in gonad weight and condition in the perch *Perca fluviatilis*. *Journal of Animal Ecology*, **20**: 201-219.

Leth, P. & Byrnak, E. (1996). Odder (*Lutra lutra* L.) genfundet på Sjælland. *Flora og Fauna,* **101**: 41-46.

Macdonald, S. M. & Mason, C. F. (1994). *Status and Conservation needs of the Otter (Lutra lutra) in the Western Palaearctic*. Council of Europe Press, Strasbourg. 54 pp.

Madsen, A. B. (1991) Otter (*Lutra lutra*) mortalities in fish traps and experiences with using stop-grids in Denmark. In: *Proceedings of the Vth. International Otter Colloquium*. (Edited by Reuther, C. & Röchert, R.). *Habitat*, **6**: 237-241.

Madsen, A. B., Christensen, N. C. & Jacobsen, L. (1992). Odderens (*Lutra lutra* L.) forekomst i Danmark 1991 og udviklingen i bestanden 1986-1991. *Flora og Fauna*, **98**: 47-52.

Madsen, A. B. & Søgaard, B. (1994). Stop-grids for fish traps in Denmark. *I.U.C.N. Otter Specialist Group Bulletin*, **9**: 13-14.

Mason, C. F. & Macdonald, S. M. (1986). *Otters Ecology and Conservation*. Cambridge University Press, Cambridge, 236 pp.

Mason, C. F. & Madsen, A. B. (1993). Organochlorine pesticide residues and PCBs in Danish otters (*Lutra lutra*). *The Science of The Total Environment*, **133**: 73-81.

Rogoschik, B. & Brandes, B. (1991). Diseases among captive otters. In: *Proceedings of the Vth. International Otter Colloquium*. (Edited by Reuther, C. & Röchert, R.). *Habitat*, **6**: 309-315.

Schierhorn, K., Stubbe, M., Schuster, R. & Heidecke, D. (1991). Helminthofauna of the otter (*Lutra lutra* L., 1758) in East Germany. In: *Proceedings of the Vth. International Otter Colloquium*. (Edited by Reuther, C. & Röchert, R.). *Habitat*, **6**: 133-142.

Simpson, V. R. (1997). Health status of otters (*Lutra lutra*) in south-west England based on postmortem findings. *Veterinary Record*, **141**: 191-197.

Smit, M. D., Leonards, P. E. G., van Hattum, B. & de Jongh A. W. J. J. (1994). *PCBs in European Otter (Lutra lutra) Populations*. Report no. R-94/7 from Institute for Environmental Studies, Vrije Universiteit, Amsterdam. 77+44 pages.

Steinhagen, P. & Nebel, W. (1985). Staupe beim Steinmarder (*Martes foina*, Erxl.) in Schleswig-Holsten. *Deutsche tierärztliche Wochenschrift*, **92**: 176-181.

Storr-Hansen, E. & Spliid, H. (1993). Coplanar polychlorinated biphenyl congener levels and patterns and the identification of separate populations of harbor seals (*Phoca vitulina*) in Denmark. *Archives of Environmental Contamination and Toxicology*, **24**: 44-58.

van Bree, P. J. H., Jensen, B. & Kleijn, L. J. K. (1966). Skull dimensions and the length/weight relation of the baculum as age indications in the common otter, *Lutra lutra* (Linnaeus, 1758*). Danish Review of Game Biology*, **4**: 97-104.

Weber, J.-M. (1991). Gastrointestinal helminths of the otter, *Lutra lutra*, in Shetland. *Journal of Zoology, London,* **224**: 341-346.

Williams, J. (1989). Blindness in otters. *I.U.C.N. Otter Specialist Group Bulletin,* **4**: 29-30.

Zogall, A. & Reuther, C. (1992). Zur optimalen wissenschaftlichen verwertung tot aufgefundener Fischotter. In: *Otterschutz in Deutschland*. (Edited by Reuther, C.). *Habitat,* **7***:* 171-174.

OCULAR PATHOLOGY IN WILD OTTERS

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1 INTRODUCTION

Simpson and colleagues have previously reported their investigation of disease in wild otters presented predominantly as carcasses after road traffic accidents (SIMPSON, 1997). A small number of captive otters were also investigated after death by this group. An important part of that study was that tissues were stored both fixed for further histopathological study and frozen for measurement of levels of vitamin A and of pollutants such as the polychlorobiphenyl toxins. Among tissues collected were eyes, allowing study of pathological lesions and correlation with vitamin A and pollutant levels. The findings reported here concern eyes from the first 82 otters examined.

2 GROSS PATHOLOGICAL FINDINGS

Few eyes showed abnormalities on gross examination of the entire or hemitransected globe and measurements of globe diameter showed no correlation with intraocular pathology or with vitamin A and pollutant levels.

3 HISTOPATHOLOGICAL FINDINGS

The particularly striking finding of this study was that of retinal folding and rosetting characteristic of retinal dysplasia. This was seen in around 40% of the eyes. This finding of abnormalities in retinal development such as retinal folding and rosetting is complicated by concurrent artefactual changes. Retinal detachment occurs when eyes are fixed, this being particularly marked with the use of formalin as fixative. It is sometimes difficult to differentiate the lesions of true retinal dysplasia from those of artefactual detachment but the finding of rosettes is indicative of abnormal retinal development. Thus although around 15% of the eyes had detachments which could not be differentiated from artefactual change, a significant number had retinal folds or rosettes which could not have occurred artefactually. A number of eyes had changes that could have been artefactual but were most probably true retinal dysplasia.

Eyes in which rosettes, involving one or more retinal layers, were obvious, were deemed to be clearly dysplastic (Figure 1). Eyes with retinal folds alone were considered possibly dysplastic, especially where there was adhesion between arms of

the fold. Eyes with gross retinal detachment, which could have occurred as part of the pathological spectrum of dysplasia but was more likely to be fixation artefact, were considered to be artefactually damaged and not dysplastic.

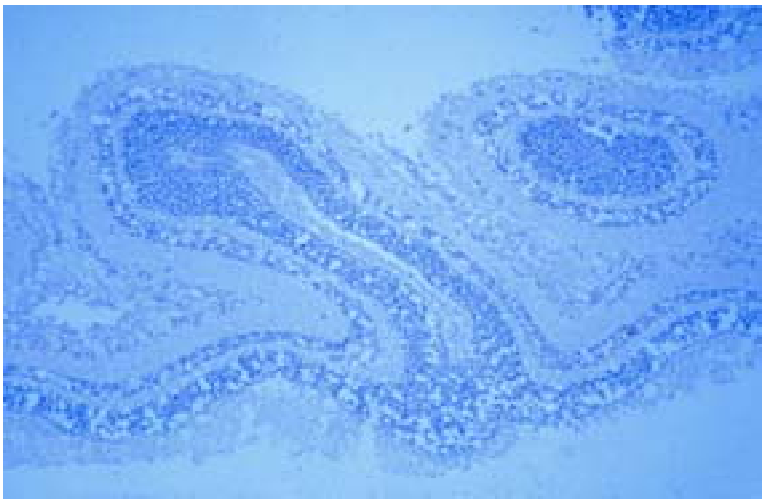

Figure 1

Given this categorisation, 32% of eyes had apparently artefactual posterior segment change. Twenty-five per cent were normal without significant retinal abnormality. Thirteen per cent had unmistakable dysplastic changes not complicated by any artefactual changes. A further 25% of eyes displayed potentially dysplastic lesions complicated by artefactual changes. Eleven per cent had other non-dysplastic pathology of the ocular surface, anterior or posterior segments. These included lesions such as lymphoid aggregates in the cornea and periocular multinucleate giant cell granulomas with protozoal cysts in periocular fat. A small number of eyes had degenerative changes in the lens and vitreal liquefaction while isolated cases had neurectodermal proliferation and choroidal thickening.

4 CORRELATION WITH VITAMIN A AND POLLUTANT LEVELS

Attempts to correlate retinal pathology with levels of various pollutants yielded significant results only in the case of dieldrin in which otters with dysplastic retinas had over three times the concentration of tissue dieldrin than in otters with normal eyes, this significant at $p = 0.028$. Comparison of liver vitamin A levels in animals with and without retinal changes also demonstrated statistically significant differences. Animals with retinal dysplasia had a statistically lower level of Vitamin A than those with normal retinas at $p = 0.023$.

5 DISCUSSION

The documentation of retinal dysplasia is the major finding in this study and one that may have significant implications as a sign of developmental abnormality in these otters. The discussion following will thus focus on this condition. Other abnormalities such as focal accumulations of leucocytes in the corneal stroma in some individuals and the finding of protozoal cysts in the extraocular muscles of one animal were seen in specific individuals and not as a finding over a number of otters in the

group. While these are interesting findings, discussion of these individual changes must take a secondary place in this preliminary report.

Retinal dysplasia literally means maldevelopment of the retina but has been used specifically to denote changes involving retinal rosettes, folds and gliosis since first described at the end of the last century (BERGHEIMER, 1894). In human ophthalmology the term has been used more specifically for babies with these retinal changes associated with anomalies of the central nervous system but also to cover retinal maldevelopment with numerous aetiologies (SILVERSTEIN, OSBURN and PRENDERGAST, 1971; LAHAV, ALBERT and WYLAND, 1973). In the veterinary sphere retinal dysplasia has been used to describe similar retinal changes inherited in a number of dog breeds, sometimes as multifocal vermiform fundus lesions (ASHTON, BARNETT and SACHS, 1968), sometimes as larger geographic areas of retinal detachment (BEDFORD, 1982; O'TOOLE *et al.*, 1983) and sometimes as complete retinal detachment (RUBIN, 1968; CARRIG *et al.*, 1977). Viral infections such as herpes virus in the dog (PERCY, 1971) and bluetongue virus in sheep (SILVERSTEIN *et al.*, 1971) can both give rise to dysplastic retinal lesions. These are probably caused by aberrant development after retinal inflammation. In both animal models and human babies, a number of other factors have given rise to retinal dysplasia including radiation (GORTHY, 1979), cytotoxic drugs (SHIMADA *et al.*, 1973; PERCY and DANYLCHUK, 1977) and other drugs such as LSD (CHAN, FISHMAN and EGHBERT, 1978). The ocular teratogenic influence of vitamin A deficiency has been reported to cause signs of retinal dysplasia (PALLUDAN, 1961; VAN DER LUGT and PROZESKY, 1989). Any microphthalmic eye with multiple congenital anomalies may be characterised by dysplastic areas of retina, as one of us has previously reported (WILLIAMS and BARNETT, 1993).

Thus the finding of dysplastic retinal lesions in these otters could be inherited, nutritionally or environmentally induced, or toxic. The first of these must be considered unlikely in out-bred wild animals, although given the substantial reduction in the size of the wild otter population in the UK over the last decades this cannot be excluded from consideration. The correlation of retinal pathology with hepatic levels of vitamin A suggests that this nutritional cause is important although, as will be discussed below, it is abnormalities in maternal vitamin A that is likely to be the important contributing factor in the development of retinal pathology.

The mechanism of retinal dysplastic change thus remains unclear. The hypothesis that retinal necrosis is the primary cause of dysplastic change has been supported by several models (SHIVELEY *et al.*, 1970) although not by others (O'TOOLE *et al.*, 1983) and not by the findings in the present study. O'Toole suggested that focal areas of dysplasia may be caused by developmental abnormalities in retinal or vitreal vasculature or anomalies of Muller cells in the locality of retinal change.

The possibility of retinal folding as an artefactual change caused by fixation must be considered in a study such as this one. SZCZECH, PURMALIS and CARLSON (1976) for example, reported artefactual retinal folding in rat foetuses produced by 70% alcohol fixation. Alcohol produces considerable tissue shrinkage and as there is only firm adhesion between retina and underlying choroids and sclera at the optic disc and ora serrata, retinal detachment and formation of large folds may occur with alcohol fixation in such cases. Globe fixation in 4% buffered formaldehyde can be seen to cause retinal detachment in adult eyes through osmotic effects (MARGO and LEE, 1995). While the 70% alcohol immersion, used in this study for post-fixation scleral hardening, is unlikely to have caused retinal artefacts, the use of formalin as an

initial fixative may well have caused a proportion of the total retinal detachments observed here. The retinal rosettes detected cannot, however, be explained as a fixation artefact and are thus taken as clear evidence of retinal dysplasia.

The most likely cause of the retinal dysplasia reported here, in these authors' opinion, is either hypovitaminosis A, given that otters with retinal lesions had a significantly lower level of vitamin A than those with normal retinas. While no obvious correlation could be made between levels of any one environmental pollutant and retinal pathology, there is a correlation between toxins such as dieldrin and vitamin A levels and thus these pollutants are likely to have had an indirect effect resulting in retinal pathology. One problem with the data as presented here is that the pathological effect of hypovitaminosis A on the retina occurs during the period of retinal development, that is to say before birth or as a cub suckling from the mother. Thus hepatic levels of vitamin A may not have much relevance to developmental defects such as the retinal dysplasia noted here. The statistical significance reported here does suggest that there is a meaningful relationship between vitamin levels and retinal pathology but does not, of course, prove a causative link between the two. Considerable further work is required to correlate disease severity with tissue levels of vitamin A and potentially teratogenic pollutants that may cause abnormal retinal development.

6 CONCLUSION

The finding of retinal dysplasia in these otters is surprising and probably points to an environmental toxin acting either directly by teratogenis or more likely through the agency of causing hypovitaminosis A. Considerable further work is required to substantial this and to rule out other possible explanations. These findings may signal that examination of retinal pathology in other wild species may show similar developmental abnormalities: the retinal may indeed be an exquisitely sensitive tissue to demonstrate toxic effects of pollutants.

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8 REFERENCES

Ashton, N., Barnett, K. C. & Sachs, D. D. (1968). Retinal dysplasia in the Sealyham terrier. *Journal of Pathology and Bacteriology,* **96**: 269-273.

Bedford, P. G. C. (1982). Multifocal retinal dysplasia in the rottweiler. *Veterinary Record,* **111**: 304-305.

Bergheimer, S. (1894). Ein betrag zur kenntniss der missbildungen des auges. *Archiven Augenheilkrangen,* **28**: 241-248.

Carrig, C. B., MacMillan, A., Brundage, S., Pool, R. R. & Morgan, J. P. (1977). Retinal dysplasia associated with skeletal abnormalities in Labrador retrievers. *Journal of the American Veterinary Medical Association,* **1570**: 49-57.

Chan, C. C., Fishman, M. & Eghbert, P. R. (1978). Multiple ocular anomalies associated with maternal LSD ingestion. *Archives of Ophthalmology,* **96**: 282-284.

Gorthy, W. C. (1979). Developmental ocular abnormalities in rats with X ray-induced cataract mutation. *Investigative Ophthalmology and Visual Science,* **18**: 939-946.

Lahav, M., Albert, D. M. & Wyland, S. (1973). Clinical and histopathological classification of retinal dysplasia. *American Journal of Ophthalmology,* **75**: 648-667.

Margo, C. E. & Lee, A. (1995). Fixation of whole eyes: the role of fixation osmolarity in the production of tissue artefact. *Graefes Archives of Clinical and Experimental Ophthalmology,* **233**: 366-370. 1995

O'Toole, D. O., Young, S., Severin, G. A. & Neumann, S. (1983). Retinal dysplasia of English springer spaniel dogs: light microscopy of the postnatal lesions. *Veterinary Pathology,* **20**: 298-311.

Palludan, B. (1961). The teratogenic effect of hypovitaminosis A in pigs. *Acta Veterinaria Scandinavica,* **2**: 32-59.

Percy, D. H. (1971). Lesions in puppies surviving infections with canine herpes virus. *Veterinary Pathology,* **8**: 37-44.

Percy, D. H. & Danylchuk, K. D. (1977). Experimental retinal dysplasia due to cytosine arabinoside. *Investigative Ophthalmology and Visual Science,* **16**: 353-363.

Rubin, L. F. (1968). Heredity of retinal dysplasia in Bedlington terriers. *Journal of the American Veterinary Medical Association* **152**: 260-262.

Shimada, M., Wakaizumui, S., Kasubuchi, Y., Kusunoki, T. & Nakamura, T. (1973). Cytosine arabinoside and rosette formation in mouse retina. *Nature,* **246**: 151-152.

Shiveley, J. N., Phemister, R. D., Epling, G. P. & Jensen, R. (1970). Pathogenesis of radiation-induced retinal dysplasia. *Investigative Ophthalmology and Visual Science,* **9**: 888-900.

Silverstein, A. M., Osburn, B. I. & Prendergast, R. A. (1971). The pathogenesis of retinal dysplasia. *American Journal of Ophthalmology,* **72**: 13-19.

Silverstein, A. M., Parshall, C. J., Osburn, B. I. & Prendergast, R. A. (1971). An experimental virus-induced retinal dysplasia in the foetal lamb. *American Journal of Ophthalmology*, **72**: 22-34.

Simpson, V. R. (1997). Health status of otters (*Lutra lutra*) in South-West England based on post-mortem findings. *Veterinary Record,* **141**: 191-197.

Szczech, G. M., Purmalis, B. P. & Carlson, R. G. (1976). Folded retinas in rat foetuses: artefacts produced by fixation in alcohol. *Toxicology and Applied Pharmacology,* **35**: 347-354.

Van der Lugt, J. J. & Prozetsky, L., (1989). The pathology of blindness in new-born calves caused by hypovitaminosis A. Ondesterport. *Journal of Veterinary Research*, **56**: 99-109.

Williams, D. L. & Barnett, K. C. (1993). Bilateral optic disc colobomas and microphthalmos in a thoroughbred horse. *Veterinary Record*, **132**: 101-103.

POLLUTION AND ITS EFFECTS ON OTTER POPULATIONS IN SOUTH-WESTERN EUROPE

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1 INTRODUCTION

The case of the otter (*Lutra lutra*) has been one of the clearest examples of effort to research and conserve an animal species in the 20th century. This resulted from the dramatic decline in numbers and restriction of range that occurred during the second half of the 20th century (Figure 1). During the 1970s and 1980s the disappearance of the species was confirmed in most of Western Europe (REUTHER, 1980; FOSTER-TURLEY, MACDONALD and MASON, 1990; MACDONALD and MASON, 1994).

The causes of this decline in most of these territories were: (1) direct persecution (MASON and MACDONALD, 1986), although the otter also disappeared in places where there was little persecution, (2) the transformation of its habitats (resting sites, breeding and sheltering places), and (3) also changes in the availability of the species upon which the otter fed (see summary in MASON and MACDONALD, 1986 and FOSTER-TURLEY, MACDONALD and MASON, 1990). However, this did not explain why this mustelid had also disappeared from catchments where the habitats had apparently not been modified and, in most cases, it neither included prey populations that had been affected such as those of our study areas. These include, for example, regions of both sides of a large area of the Pyrenean mountains, the Alps, the Sierra Nevada and coastal wetlands such as the Ebro and Po Deltas, the Camargue or Albufera of Valencia.

The otter was not the only animal to be drastically affected in those years. In Western Europe, some other predators also showed sudden declines and fell to their lowest distribution levels. The first indications of decline were found in species like the peregrine falcon (*Falco peregrinus*) (NEWTON, 1979). The most affected animals were, however, insectivorous and fish-eating species such as the osprey (*Pandion haliaetus*), and various species of *Ardeidae* (*Ardea purpurea,* for example). The otter's decline, as well as that of these other species, was considered to be the result of a group of cumulative or synergistically-acting causes.

Pollution and epizootic occurrences were suggested as possible causes of decline (OLSSON and SANDEGREN, 1983; MASON, 1989), as they were the latest and the most consistent with the simultaneous disappearance of otter populations and other species.

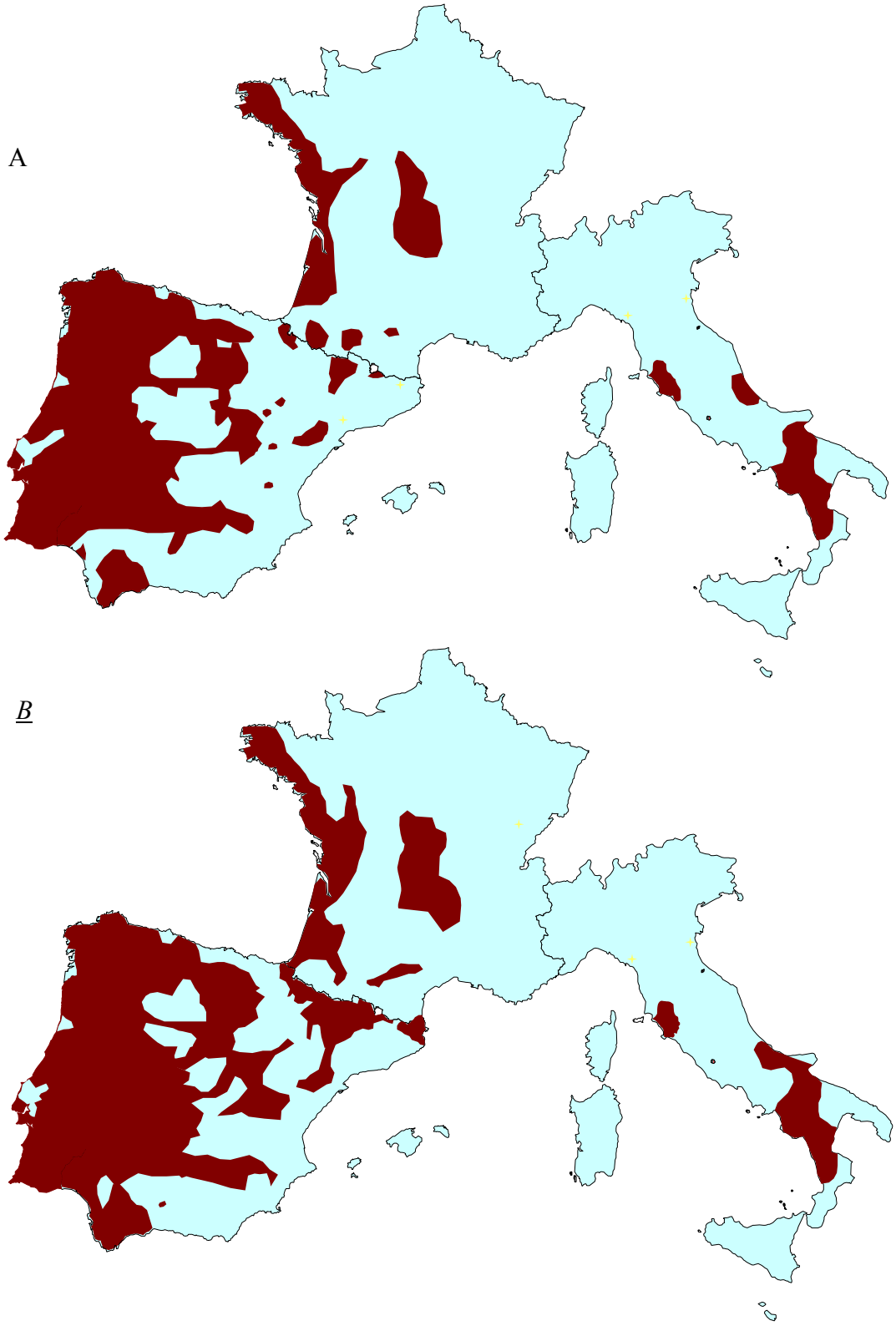


Figure 1. Distribution of the otter in SW Europe: a) 1980-85 and b) 1995-2000 (GREEN & GREEN, 1981; MACDONALD & MASON, 1982, 1983; ELLIOT, 1983; SANTOS-REIS, 1983; DELIBES & CALLEJO, 1983; BOUCHARDY, 1986; CASSOLA, 1986; DELIBES, 1990; ROSOUX *et al*., 1996; PRIGNIONI, 1997; RUIZ-OLMO & DELIBES, 1998; TRINIDADE *et al*., 1998).

In order to assess the impact of pollution and to explain the decline of the otter populations, as well as endowing managers with conservation measures, several research projects were begun in the 1980s. This paper is a synthesis of the current

knowledge on the effect of pollution on otter populations and their principal prey in South-west Europe. At the same time, an analysis of the effect that pollution dynamics and its control could have on the evolution in the distribution of this species was carried out.

2 STUDY AREA

The zone under consideration includes France, Italy, Portugal, Spain, Andorra, Monaco and San Marino. In the remaining countries of continental Western Europe (Netherlands, Belgium, Luxembourg, Switzerland and West Germany) otters disappeared, with only small populations surviving in the north of Germany (MACDONALD and MASON, 1994). The area covered in this paper is nearly 1,418,450km^2, and is bounded by the Atlantic Ocean and the Mediterranean Sea. This determines the climate notably, with a different climate in the Mediterranean area in the south (dry, especially in summer), in the Atlantic area to the west and north (wet). There is also a continental trend in the higher zones; the maximum altitudes of which are in the Alps (4,807m), the Pyrenees (3,404m) and the Sierra Nevada (3,478m). This determines a strong altitudinal, microclimatic and biogeographical gradient. The Atlantic and continental hydrographic networks are dense in watercourses with more or less permanent river flow. In the Mediterranean area, watercourses have a tendency to be of low density and of medium or low flow (or even dry), with minima in summer. Such rivers often show catastrophic flows, especially in autumn or spring, and have a great capacity for movement and cleaning of the river bed.

Temperatures are higher in Mediterranean rivers and lakes, especially during the summer. This means that great rates of metabolism could occur that in extreme cases determine the occurrence of anoxy situations.

All these differences could be important in pollution tolerance capacity or could mean its dilution, with the situation in Northern and Central Europe being different from that in Southern Europe, and also with differences in Southern Europe itself.

3 OTTER DISTRIBUTION 1980-85

Formally, the otter was widespread in most of aquatic European ecosystems up to the first half of the 20th century. Between these dates and the beginning of the 1980s a dramatic decline took place in Western Europe, and resulted in this species becoming extinct in some areas and/or restricted to small areas of previously occupied zones (MACDONALD and MASON, 1994). Otter surveys, based on the search for indirect signs of the species' presence (spraints [faeces] and tracks) (MASON and MACDONALD, 1986), allowed for an improvement in the precision and standardisation of the study of its distribution. Thereby, the distribution of the otter in our study areas has been described by several authors (Fig. 1a) (GREEN and GREEN, 1981; MACDONALD and MASON, 1982, 1983; ELLIOT, 1983; SANTOS-REIS, 1983; DELIBES and CALLEJO, 1983; BOUCHARDY, 1986; CASSOLA, 1986; DELIBES, 1990). The otter had disappeared from Andorra, where it was cited before (RUIZ-OLMO and GOSÁLBEZ, 1988), and other small states. In France there were only stable populations in the Departments on the Atlantic Ocean to the south of Normandy, including Normandy and the Massif Central, with isolated cores in the adjacent zones. In Italy, the otter had practically become extinct, having been reduced to some rivers in the south of the country (it was only found in 8.2% of the surveyed points in the southern half), and two rivers to the north of Rome (Fiora and Farma-Merse). There were also some isolated individuals in other zones near these. In

Portugal, the otter was distributed throughout the country (found at 70% of the survey sites). Finally, in Spain the otter was found in 40% of the surveyed sites in 1981 and in 33% in 1984-85. Otters were more abundant in the western half of the country, being absent from the more industrialised zones and the big cities and their surroundings and from the main intensive agriculture areas.

The process of decline has been well reported in various areas, mainly occurring between 1950 and 1985 (RUIZ-OLMO and GOSÁLBEZ, 1988; LODÉ, 1993; ROSOUX, TOURNEBIZE and MAURIN, 1996).

4 OTTER DISTRIBUTION AND POLLUTION: THE OTTER AS A BIOINDICATOR SPECIES

As causes of decline, various factors have been suggested that could affect *L. lutra*. These include: persecution; habitat changes; disturbance; decrease in food and water availability or illness, although in the last 15 years the possible role of pollution has grown in importance as a global factor that could explain this simultaneous decline, in a relatively short period of time (OLSSON and SANDEGREN, 1983; MASON and MACDONALD, 1986; MASON, 1989, 1997).

The effect of the pollution on the otter's distribution in our study areas has been demonstrated by several works (ADRIÁN, WILDEN and DELIBES, 1985; RUIZ-OLMO, 1985; LAFONTAINE, FORTUMEAU and MAINSANT, in press; RUIZ-OLMO, LÓPEZ MARTÍN and DELIBES, 1998). These authors found otters at significantly lower frequencies in many polluted areas.

For this reason, the otter has been suggested as a good bioindicator species of water quality and riparian habitat conservation, because of its sensitivity to pollution, to the transformation of its habitats and to the changes in the availability of prey species. RUIZ-OLMO *et al.* (1998) compared the distribution of the otter with those of the main orders of macroinvertebrates and the most-used indices: BMWP (Biological Monitoring Working Party) and ASPT (Average Score per Taxon) (HELLAWELL, 1978; ARMITAGE, 1983) in order to test if both behave in the same way (macroinvertebrates are commonly used as bioindicators of contamination and of quality of water; see VALENTINE, 1973; WESTMAN, 1978). According to these results, in northeast. Spain, otters act as a pollution bioindicator species. However, they found differences related to the biological, ecological and ethological characteristics between otter and macroinvertebrates. The use of the otter as a bioindicator thus needs revision. Invertebrates, otters and, surely, other species could be used as bioindicators in a complementary fashion.

5 LEVELS OF ORGANOCHLORINES AND HEAVY METALS IN OTTER TISSUES

What types of pollutants are therefore affecting otters?. A wide range of different pollutants could affect them directly or through their prey (MASON, 1989). Among these, organochlrine compounds and heavy metals should be highlighted (OLSSON and SANDEGREN, 1983; MASON, 1989, 1997; MASON and MACDONALD, 1986). MACDONALD (1991) linked pollutant distribution, in particular the polychlorinated biphynels (PCBs), to wind circulation (that transports micropollutants subsequently deposited by rain), the distribution of the main sources of pollution and the distribution of the otter. Otters have healthy populations in the territories next to the Atlantic Ocean or further south, away from these pollutant carrying winds. These tend to be located more to the west (Portugal, Galicia, Extremadura, Western France, Ireland, Scotland, Norway and an area of Denmark).

In Table 1 the levels of organochlorine compounds and heavy metals found in the muscular tissues of otters from France and Spain are shown. The levels of PCBs are the more elevated in all cases. However, high levels of some pesticides were found in Spain (mainly DDTs in the south, an average of nearly 80mg/kg: lipid weight) and also mercury in France (although the geometric mean is not very high, 4.71mg/kg), with a maximum of 41mg/kg, whereas the threshold of 30mg/kg has been proposed, beyond which neuronal damages are produced in mammals (GUTLEB, 1995). Locally, there are high levels of most of the analysed compounds (especially some heavy metals, oxychlordane or lindane), but these are not related to a possible decline of the species on a national/ international scale.

For these reasons, PCBs should have the greatest effects on otter survival from the studied regions, although locally other pollutants could have more influence and, in general, the effects should be added.

The arithmetic means of PCB levels (between 6.15 – 33.94mg/kg: lipid weight) are lower in three of the four studies than the threshold of 50 mg/kg, a level proposed as an upper limit for the presence and distribution of the otter (see MASON and MACDONALD, 1993). This is based on the results from JENSEN *et al*. (1977) for American mink (*Mustela vison*). They are also lower or similar to the 30mg/kg for the same species proposed by LEONARDS *et al.* (1994) and SMIT *et al*. (1996). The exception concerns data from our Spanish sample collected during the period 1981–1993 and from Doñana (1982–1983). The arithmetic mean of the whole country data (78.30mg/kg: lipid weight) exceeded both thresholds. However, the sample from the whole of Spain is very heterogeneous due to the geographical amplitude and difference in uses between zones; in the second case the sample is too small. In fact, RUIZ-OLMO, LÓPEZ-MARTÍN and DELIBES (1998) highlight important differences between the diverse regions (greater contamination in the southwest than in the north of the country), and even between river basins. In France, there are also differences between basins (LAFONTAINE, 1995). The presentation of results as arithmetic means could mask the real situation, since extreme values (of up to 1005mg/kg in South-west Spain) can affect the average values. For this reason LAFONTAINE (1995), following the arguments of NEWTON and WYLLIE (1992) and SMIT *et al*. (1994), used the geometric mean for his samples from France, while using both means as part of a pan-European comparative synthesis (see also Table 1). In Spain, only 28% of the otters analysed in the period 1981-1993 exceeded the threshold of 50mg/kg (RUIZ-OLMO, LÓPEZ-MARTÍN and DELIBES, 1998), being almost the same (29%) as in French otters from 1987-1995 (according to the sample studied by LAFONTAINE, 1995).

Table 1. Arithmetic ([a]) and geometric ([g])mean levels (mg/kg lipid basis in muscular tissues) of organochlorine compounds and heavy metals (mg/kg dry weight in liver) of otters from France and Spain (the range is given between brackets, except for France b: standard deviation). For Spain a (Doñana), levels are expressed as mg/kg wet weigh), and heavy metals were analysed in muscular tissue. Underlined values are expressed as wet weigh.

	France a (5 regions) (*n* = 8 to 22). Mostly 21 or 22	**France b (Western Marshes, 3 regions) (*n* = 32)**	**Spain a (Doñana) (*n* = 5)**	**Spain b (whole) (*n* = 41: *n* = 19 for heavy metals)**	**Spain c (whole) (*n* = 10)**
PCBs	33.94[a] 13.99[g] (1.24 - 145.31)	26.19[a] (± 21.74)	2.44 [a] (2.40- 2.45)	78.30[a] 25.06 g (1.49 - 1005.59)	6.15 [a] (n.d. – 20.60)
DDTs	1.10[a] 0.38[g] (0.01 - 6.08)	(not detailed)	3.50 [a] (2.25 - 5.62)	14.79[a] 5.78 g (0.19 - 82.95)	1.28 [a] (0.38 – 2.97)
Oxychlordane	0.55[a] 0.28[g] (0.002 - 1.95)	-	-	-	-
BHC	0.17[a] 0.08[g] (0.005 - 0.67)	0.08[a] (± 0.07)	-	-	-
HEPO	0.124[a] 0.068[g] (0.009 - 0.546)	0.003[a] (± 0.010)	-	0.26[a] (n.d. - 1.53)	-
Dieldrin	0.70[a] 0.33[g] (0.12 - 2.91)	0.75[a] (± 1.45)	-	-	-
Aldrin	-	0.02[a] (± 0.07)	-	0.24[a] (n.d. - 5.84)	-
Lindane / HCH-γ	0.47[a] 0.10[g] (0.01 - 3.27)	0.18[a] (± 0.46)	0.02 [a] (0.01 -0.02)	1.95[a] 2.50 g (0.03 - 9.92)	-
Hg	9.46[a] 4.71[g] (0.50 - 41.00)	-	1.33 [a] (1.25 - 1.41)	0.99[a] (n.d. - 2.80)	-
Pb	0.55[a] 0.42[g] (0.12 - 1.58)	-	0.64 [a] (0.51 - 0.80)	0.09[a] (n.d. - 0.34)	-
Cd	0.35[a] 0.17[g] (0.01 - 2.03)	-	0.13 [a] (0-10 - 0.17)	0.04[a] (n.d. - 0.22)	-
Cu	28.78[a] 26.00[g] (11.00 - 53.00)	-	-	-	-
As	0.070[a] 0.055[g] (0.02 - 0.20)	-	-	-	-
Cr	1.51[a] 0.63[g] (0.07 - 4.90)	-	-	0.17[a] (n.d. - 0.49)	-
Ni	2.80[a] 1.51[g] (0.10 - 4.34)	-	-	-	-
Zn	77.33[a] 77.00[g] (70.50 - 90.70)	-	-	-	-
Reference	Lafontaine, 1995	Tans *et al*., 1995	Hernández *et al.*, 1985	Ruiz-Olmo *et al*., 1997	Ruiz-Olmo & López-Martín, unpublished
Period	1987-1995 except fpr one idividual in 1981	1987-1994	1982 -1983	1981-1993	1994-1999

On the other hand, the most recent studies on the effect of the pollutants on the American mink (KIHLSTRÖM *et al*., 1992; LEONARDS *et al*., 1994) have shown that the effect of PCBs on reproduction is progressive and asinthothic. Some American mink can breed with low success even with high pollutant levels in tissues. For this reason it seems appropriate to carefully consider the use of such static thresholds, especially when remembering that resistance to these levels could be variable in each species, according to the individuals and/or the populations (SMIT *et al.*, 1994). Organochlorine compounds can also be eliminated by female otters during gestation and weaning periods and by glandular secretions in both sexes. In addition, the body condition and the mobilisation of fat reserves and intoxication of the organism for these lipophilic pollutants must be considered in each case.

RUIZ-OLMO, LÓPEZ-MARTÍN and DELIBES (1998) found no correlation between the levels of any organochlorine compound or heavy metals, and the age of the otters (neither as a whole, nor by sex). This negative result could be affected by the great heterogeneity of the environments and to the great geographical dispersion of samples. LAFONTAINE (1995), found no significant correlation between the age and the levels of several organochlorine compounds (samples also originating from different zones). However, he found a positive significant correlation between the age of the otters and the levels of some heavy metals (mercury: $r^2 = 0.988$: $p < 0.09$ in liver; cadmium: $r^2 = 0.994$: $p < 0.05$ in kidney; lead: $r^2 = 0.976$: $p < 0.13$ in kidney).

6 CORRELATION BETWEEN POLLUTION LEVELS AND OTHER VARIABLES

In Table 2, the functions between the levels of main pollutants are shown. In the case of significant results, the high correlation found, allows us to use, for discussion, a single tissue of the most representative compounds (in this case, the PCBs).

Table 2. Correlation between the body condition index (KRUUK et al., 1987; LAFONTAINE, 1995; RUIZ-OLMO, 1995) and levels of several pollutants in otter tissues from France and Spain (after LAFONTAINE, 1995, and RUIZ-OLMO, LÓPEZ MARTÍN and DELIBES, 1998). (*) significant differences.

		France	**Spain**
PCBs	**Muscle**	$r = 0.580$ $p < 0.05$*	n.s.
	Liver	$r = -0.501$ $p < 0.05$*	n.s.
HCB	**Muscle**	$r = -0.498$ $p < 0.05$*	n.s.
	Liver	$r = -0.680$ $p < 0.01$*	n.s.
Cu	**Liver**	$r = -0.674$ $p < 0.01$*	n.s.
Hg	**Liver**	n.s.	$r = 0.561$ $p = 0.19$

LAFONTAINE (1995) found a significant correlation between the body condition index, *k* (KRUUK, CONROY and MOORHOUSE, 1987), for French otters (LAFONTAINE, 1995) and the levels of some pollutants (Table 3). However, RUIZ-OLMO, LÓPEZ-MARTÍN and DELIBES (1998) found no similar significant

correlation. Again, the geographical dispersion of the samples and their heterogeneity could explain the lack of correlation for this study.

Table 3. Functions, correlation coefficients and signification between the levels of some pollutants in the otter tissues from France and Spain (after LAFONTAINE, 1995 and RUIZ-OLMO, LÓPEZ MARTÍN and DELIBES, 1998). First pollutant is *y* and second *x*. (*) significant differences.

y / x	France	Spain
PCBs liver / PCBs muscle	log y = 0.967 log x + 0.012 $r = 0.57$: $p < 0.01$	log y = 0.758 log x + 0.441 $r = 0.81$: $p < 0.0001$
DDTs liver / DDTs muscle	log y = 0.426 log x + 1.729 $r = 0.50$: $p < 0.05$	log y = 0.667 log x + 0.585 $r = 0.65$: $p < 0.0001$
PCBs / DDTs (muscle)	$p > 0.25$	log y = 0.475 log x + 1.026 $r = 0.55$: $p < 0.0003$
PCBs / DDTs (liver)	$p > 0.25$	-
DDTs (muscle) / Hg (liver)	log y = 0.599 log x + 2.136 $r = 0.44$: $p < 0.05$	$p < 0.05$
DDTs (liver) / Hg (liver)	$p > 0.25$	$p < 0.05$

LAFONTAINE (1995) also found a positive correlation ($p < 0.02$) between the levels of lindane (γ-HCH) in the muscle of individual otters from Brittany, France, and the rate of maize culture (on a local scale), where lindane was widely used. A similar case could be found in South-west Spain, with a large agricultural area and higher use of some pesticides and where the levels of some pesticides (DDTs, lindane, etc.) were greater than those used in the north (RUIZ-OLMO, LÓPEZ-MARTÍN and DELIBES, 1998).

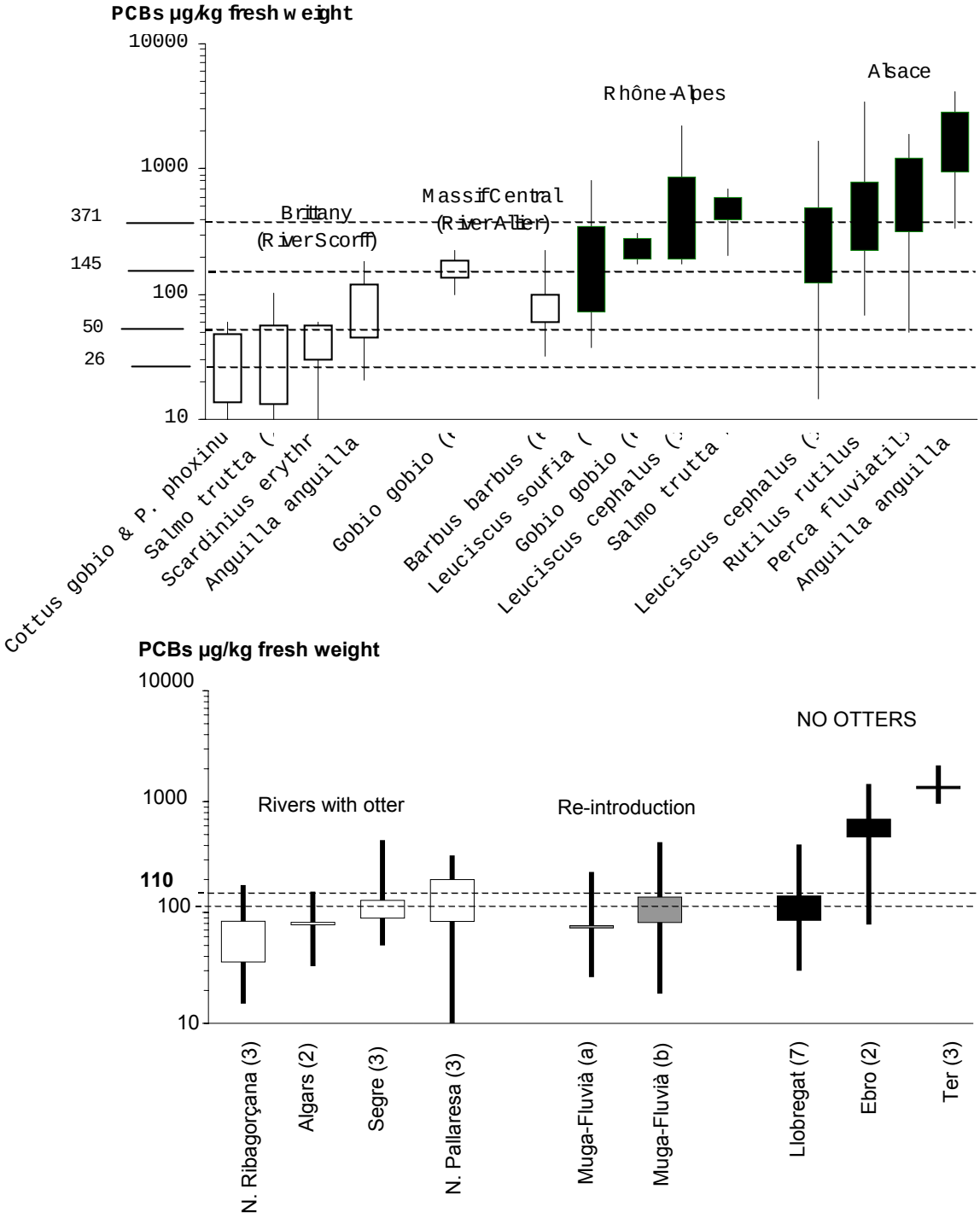
7 LEVELS OF ORGANOCHLORINES AND HEAVY METALS IN TISSUES OF FISH AND CRAYFISH AS OTTER PREY

The accumulation of organochlorines in otter tissues could only come from food, specifically fish, the main diet of the species. RUIZ-OLMO and LÓPEZ-MARTÍN (1994) found that the populations of otters from Catalonia (North-east Spain) were distributed in zones with less than the average level (arithmetic mean) of 0.1mg/kg: wet weight of PCBs in the muscle of fish (referring to the group of the fish consumed by the otter in each site).

LAFONTAINE and DE ALENCASTRO (2000) found quite a close relationship between the levels of PCBs in fish from different regions of France (Brittany, Massif Central, Rhône-Alpes and Alsace) and otter occurrence (Figure 2) (also see MICHELOT *et al*., 1998).

After these findings, the critical thresholds for contamination effects on fish could be between 0.15-0.20mg/kg: fresh weight of PCBs. In most areas, the average levels of PCBs in fish tissues from sites used by otters were under 0.10mg/kg, and are similar to those found in the previous work, and are comparable to the 0.145mg/kg proposed by LEONARDS *et al*. (1994); however, we need to keep in mind that there are differences between levels in muscle and the whole fish. They are higher than 0.026 - 0.05mg/kg levels presented by MACDONALD and MASON (1994), for the whole data, and for eels, respectively.

Figure 2. a) Synthesis of the average values, ranges ans interquartiles of PCBs in 11 fish species from four French regions, showing samples coming from the otter range (grey) and outside (white) (after LAFONTAINE & DE ALENCASTRO, 2000). b) Average values, ranges and interquartiles of PCBs levels in fish muscle from nine basins in Catalonia (N.E. Spain), showing samples coming from the otter range (grey), outside (white) and from the re-introduction area (recalculated data from LÓPEZ-MARTíN *et al*., 1995, and MATEO *et al*., 1999).



In North-east Spain, levels found by LÓPEZ-MARTÍN, RUIZ-OLMO and BORRELL, (1995), from the different basins (unpublished data), and plotted in Figure 2, coincide again with the results of LAFONTAINE and DE ALENCASTRO (2000), and with all otter populations in sites under 0.14 mg/kg: fresh weight of PCBs in fish muscle. In rivers not used by otters, average levels were often over 0.2mg/kg, reaching up to 1.34mg/kg. We must highlight the basins of the Muga and Fluvià Rivers, where, despite the fact that the otter became extinct toward the end of the 1970s (RUIZ-OLMO and GOSÁLBEZ, 1988), levels in fish tissues found at the beginning of the 1990s were compatible with the presence of the mustelid. This has allowed for the development of a reintroduction project (SAAVEDRA and SARGATAL, 1998) in which nearly 50 individuals have been relocated.

Results from France and Spain confirm those from Italy, since levels of PCBs within the otter range are much lower that those from zones without otters (VIVIANI *et al*., 1974; GALASSI and GANDOLFI, 1981; GALASSI, GANDOLFI and PACCHETTI, 1981; CANTONI, CATTANEO and FABRIS, 1985; COZZANI and PIETROGIACOMO, 1985; TURSI, CONSTANTINO and MATARRESE, 1989). In the Ticino River, where there is a reintroduction project, PCBs levels seem to be appropriate for their return (NARDI *et al*., 1993), while they could be less compatible with those of DDTs.

RUIZ and LLORENTE (1991), in the 1980s, found high levels of PCBs and DDTs in tissues of fish from Ebro delta (Northeast Spain), where otters had become extinct in the 1970s.

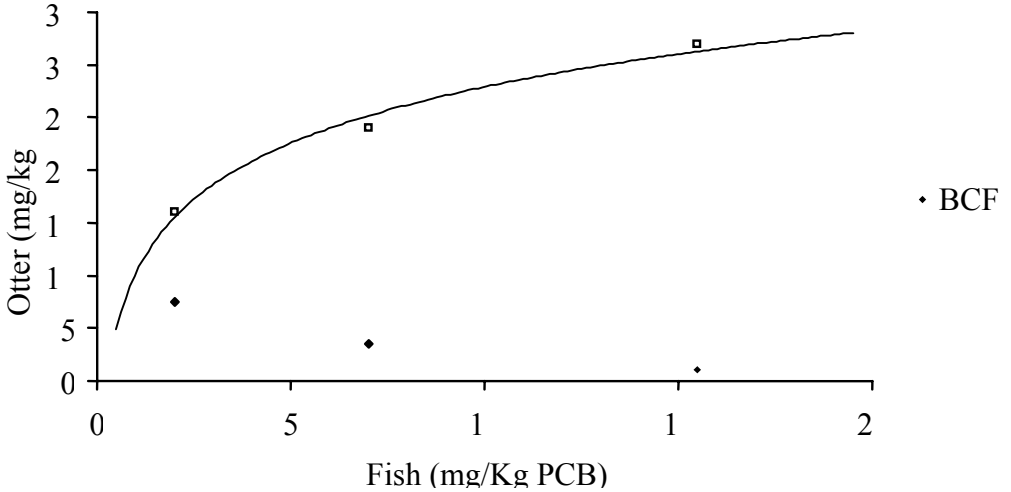
There are several studies on heavy metal levels in fish tissues from Italy; TURSI, CONSTANTINO and MATARESSE (1989) and FUMAGALLI and PRIGNIONI (1991) found very low levels in fish from Italian rivers with otters; the higher levels were mercury, being in almost all cases below the 0.2mg/kg: fresh weight. In other Italian rivers, with no otters, mercury levels were lower (BEGLIMONDI, FRAVOLINI and MOROZZI, 1975; LOCHT *et al.,* 1981). In Spain, RALDUA and PEDROCCHI (1996) found average mercury values between 0.74 and 2.80mg/kg: wet weight, in fish from Huesca (Aragon, Northeast Spain), outside the otter range or on the border of the otter distribution towards 1993. In Portugal, SANTOS-REIS, AFONSO and FREITAS, (1995), found high mercury levels in American crayfish, *Procambarus clarkii*, from the Tejo basin, this being the main otter prey in many areas; levels were highest in Almonda River (mean: 0.21mg/kg) and Server River (mean: 0.29mg/kg).

In some rivers, lead, chromium or nickel levels were very high (CAGGINO, 1982), and could have contributed in an important way to the decline of the otter.

8 BIOMAGNIFICATION FACTORS

In Spain, most of our studied otter populations feed mainly on fish (in the zones where we have studied bioconcentration factors: 90-95%; RUIZ-OLMO and PALAZÓN, 1997). This allowed us a better approach to the bioconcentration factors (considered as the ratio: levels in otter tissue/levels in fish tissue), than in other otter populations with a more complex diet. The only bioconcentration factors to PCBs and DDTs on the whole (LÓPEZ-MARTÍN and RUIZ-OLMO, 1996; RUIZ-OLMO, LÓPEZ-MARTÍN and DELIBES, 1998). These authors base their data on the analysis of the most-caught fish (the most abundant in the environment) and its importance in otter diet. Levels are drawn in Figure 3.

Figure 3. Biomagnification factors for otter and fish tissues in otters from NE Spain (RUIZ-OLMO *et al.,* 1995; LAFONTAINE & DE ALENCASTRO, 2000).



Biomagnification factors in PCBs were double those of DDTs (i.e., they accumulated double). For PCBs, a logarithmic function is followed (r = 0.99: p = 0.021), very similar to the results from the Canadian river otter *Lutra canadensis* (FOLEY *et al*., 1988). Bioconcentration values ranged between 2.2 and 8.2 in the case of PCBs (lipid weight), and between 0.9 and 4.8 in the case of DDTs.

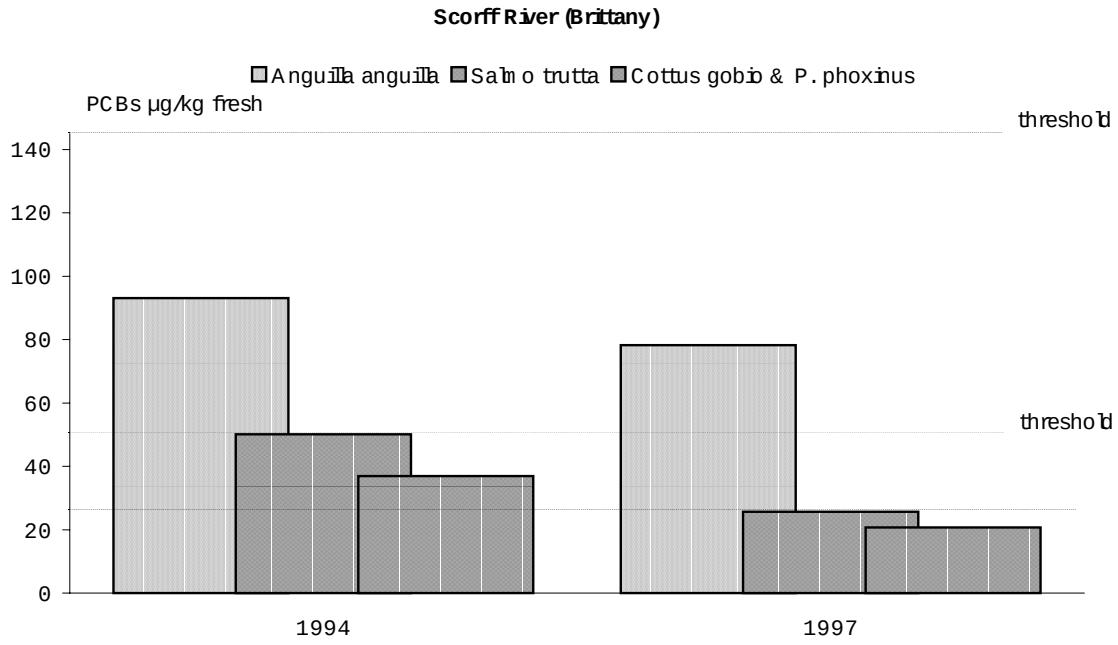
For the River Scorff, Brittany (North-west France), from 1994 to 1997, LAFONTAINE and DE ALENCASTRO (2000), found PCBs biomagnification factors varying from 22.2 to 44.9 (wet weight) for otter tissues *vs* all the fish species together, and from 584 to 3236 (wet weight) for otter tissues *vs* sediments.

RUIZ-OLMO, LÓPEZ-MARTÍN and DELIBES, (1998) found PCB congeners No.138, 153, 170, 180, 194, 195, 196/ 203 and 201, in greater proportion in otter tissues than in fish, there being a significant bioaccumulation among fish in congeners No. 101, 138, 141, 153, 170, 174 and 180. LAFONTAINE (1995) found greater prevalence of congeners 138, 153, 170 180, for the 12 analysed, the data agree with the Spanish results. MASON and RATFORD (1994) found that the congeners No 138, 153, 163, 180 and 187 are the most prevalent in British otters and Nos 118, 138, 153, 163 and 180 in Danish otters. For otters from Denmark, SMIT *et al*. (1996) found a greater bioconcentration of the congeners Nos. 138, 180, 156, 157, 189, 126 and 169. On consideration that they are the most bioacumulated congeners and could have greater weight in the toxicity, they utilise an index that includes these seven congeners only (∑ 7 PCBS), or with the Toxicity Equivalent Concentration (TEQ) (SAFE, 1994; LEONARDS, 1997). These methods allow the real power of toxicity for each concentration of PCB congeners, but differences between studies must be explained.

For the River Scorff, Brittany (North-west France), from 1994 to 1997, LAFONTAINE and DE ALENCASTRO (2000), analysed for 17 CB congeners, including three coplanars, and found the cumulative contribution (p <0.001) of the most toxic CBs (groups 1A + 1B + 2, according to the typology of MACFARLAND and CLARKE (1989) along the food chain, as follows: sediments > *Scardinius*

erythrophtalmus > *Salmo trutta* > *Cottus gobio* & *Phoximus phoxinus* > *Anguilla anguilla* > otter spraints > otter tissues (Figure 4).

Figure 4. Evolution of pollution levels in fish tissues from Scorff River (Brittany).



The proportion of congeners in each zone depends on the type of PCB (mixture) that the otters/fish have ingested (with the congeners 138, 153, and 180 found in great proportion in all the studies). The several industrial uses of the zones could characterise the proportion of each PCB in each zone.

9 EVOLUTION OF POLLUTION LEVELS

Unfortunately, otter carcasses were not analysed for contaminants between1950 and 1970 in the countries discussed here. This was the period when many of the organochlorine compounds and heavy metals were being used indiscriminately, widely and without control. The only data available refer to fish from the Po delta, Northern Italy. They show high levels (VIVIANI *et al*., 1974), with maxima up to 12mg/kg: wet weight for PCBs in the liver of *Gobius paganellus* and 6.13mg/kg in the liver of *Squalus acanthias*. If these species were consumed in significant numbers by otters, they could give rise to high concentrations in otter tissues (remember the thresholds for otter presence of 0.05-0.2mg/kg in fish). These authors found high levels of DDTs (up to 9mg/kg in some species) and lindane (up to 1.3mg/kg).

Lack of historical information prevents us from carrying out an analysis of the evolution and dynamics of several compounds in otters and their prey from those decades. Also, analytical methods and accuracies have changed during this time. However, we found a decrease in the levels of PCBs, DDTs and other pollutants in otter tissues (Table 1) and fish tissues (Figure 4: Table 4). These data agree with MASON (1998) in that they demonstrate a decrease in these pollutants.

Table 4.- Comparison between the average PCB levels in fish tissues from some rivers of NE Spain in two different periods (after LÓPEZ-MARTÍN & RUIZ-OLMO, unpublished).

		1990-92		1998-99		
Basin	**Site**	**PCBs**	**DDTs**	**PCBs**	**DDTs**	**Fish species**
N. Pallaresa	Gerri de la Sal	0.145 (0.06-0.30)	0.087 (0.02-0.2)	0.065 (0.04-0.09)	0.030 (0.01-0.05)	*S. trutta*
Segre	Seu d'Urgell (1)	0.207 (0.04-0.45)	0.235 (0.05-0.16)	0.212 (0.191-0.436)	0.128 (0.003-0.253)	*S. trutta* *C. gobbio* *C. toxostoma*
	Balira (1)			0.13 (0.07-0.19)	0.072 (0.037-0.107)	*S. trutta*
	Ponts	0.095 (0.05-0.24)	0.052 (0.02-0.07)	0.040 (0.005-0.08)	0.102 (0.01-0.02)	*S. trutta* *C. carpio* Others

(1) Sampling stations downstream Southern Andorra border

Heavy metal and other organochlorine compound levels found in Spain, France and Italy since the 1980s have been generally low, with the exception of mercury in some areas in France (LAFONTAINE, 1995) and Portugal (SANTOS-REIS, ALFONSO and FREITAS., 1995), in some small rivers of Tejo Basin and in Aveiro Ria, (Nuno Gomes, *pers. comm.*). This decrease in pollution levels (not only of PCBs but in general), would contribute to an explanation of otter recovery in several areas.

10 OTTER DISTRIBUTION 1995-2000: THE RESPONSE OF A BIOINDICATOR SPECIES

Otter distribution has changed since 1985, with a tendency towards increase and recovery in wide areas (ROSOUX, TOURNEBIZE and MAURIN, 1996; PRIGNIONI, 1997; RUIZ-OLMO and DELIBES, 1998; TRINDADE, FARINHA and FLORENCIO, 1998; CONROY and CHANIN, 2001). Figure 1 shows this increase in otter distribution, with an evident spread in several areas of central France and in zones of Spain (Pyrenees, Centre, Andalucia, etc.). Even in Portugal, where the otter was previously widespread, better results were recorded in 1995 (89% of positive sites).

However, the animal has not recovered in other areas of these countries, nor in Andorra or small states. In Andorra, the Balira Basin crosses the country and has high pollution levels which could explain the absence of otter in 1999, when we carried out an otter survey for the new Otter Action Plan (RUIZ-OLMO, unpublished). High contaminant values obtained in 1991 in fish tissues from the Segre River, just beyond the junction with Balirain Spanish stretch (LÓPEZ-MARTÍN, RUIZ-OLMO and BORRELL, 1995), are in accordance with the lack of otters. Even in 1998-99 we found 0.13-0.21 mg/kg: wet weight in muscle of brown trout from the Rivers Segre and Balira, just downstream of Andorra. The Balira River was determined to be the

most polluted in the Spanish Pyrenees by both the Water Suitability Index and water analysis (CONFEDERACIÓN HIDROGRÁFICA DEL EBRO, 2000). This could explain the absence of otters.

This otter recovery in wide areas is attributable to assistance in and an improvement of the general conditions for the species, especially pollution levels. On the other hand, the lack of otter recovery in other parts (with a good otter habitat index), could be a result of these conditions not improving enough or even worsening conditions, especially pollution.

11 DISASTERS AND OTHER CONTAMINANTS

Up to now, the effects of more widespread pollution types have been analysed. However, big disasters, producing negative effects on otters and their prey, should be emphasised. In analysing these types of occurrence, we can divide them into two types:

a) Oil spills. They have taken place mainly along the Atlantic coasts after ship accidents. In Spain, those from the *Urquiola* (May 1976) with 28.1 million gallons, and the *Amocco Cadiz* (1992) with 21.9 million gallons, can be highlighted. They affected the coast of Galicia. Recently (2000) an unidentified oil slick impacting several kilometres was found off the east coast of Cadiz (Southern Andalucia). Although in the first two instances, effects on some fish species, invertebrates and plants were important, no dead shore-living otters were found. These types of accidents have also occurred in rivers, like the Tajo River in Central Spain, in August 2000. There are no studies on the effects of these accidents on otter populations. However, years later, otters still inhabit some of these zones.

   A similar disaster occurred in December 1999, along the North-west coast of France (Brittany). The *Erika* oil spill polluted several hundreds of kilometres of sea-shore. Invertebrates, fish and especially several thousands of seabirds were affected. No dead shore-living otter was found however. This does not mean that this oil spill had nor will have no impact on otter survival, and, in terms of accumulation in the food chain, a study is now starting to analyse PAHs in both otter spraints (from coastal areas) and marine mammal tissues (LAFONTAINE and HASSANI, in progress).

b) Break-up of toxic reservoirs. This is especially highlighted by the case of the Aznalcollar mine (May 1998), on the Guadimar and Guadalquivir Rivers, Huelva (Southern Spain). A high quantity of heavy metals was released into these rivers as a result of mineral cleaning. Arsenic was the main metal, with more than 4000 mg/kg in sediments in three of nine sampling sites. Manganese, cadmium, chromium, copper, lead (more than 3000mg/kg at three of the sites), zinc and iron were also found at high levels. A monitoring program on physical and chemical parameter effects and on biotic elements has been carried out (Spanish Ministry of the Environment, in progress). In sediments, a decrease in levels was found in arsenic, iron, manganese and nickel, although no decrease was shown in the remainder. An increase in chromium, copper and lead was registered.

c) Mines. Heavy metals are found in high levels in waters used for mining activities, in Northern Portugal, for example, these affected pH levels and sediments (TRINDADE, FARINHA and FLORENCIO, 1998).

On the other hand, perhaps different pollutants are still active or could well begin to be so in the near future. A study of the effect of dioxines, furans, PVCs and organophosphorates, etc., should be made.

12 CONCLUSIONS

The results show the negative effect of contamination on otter distribution in France, Italy, Portugal, Spain and Andorra. This could explain the great decline during a relatively short period (three decades) and could have been coincidental with other factors such as habitat transformation, decrease of food availability and persecution, and other.

The study area is very extensive and is characterised by a high diversity of weather as well as hydrological, ecological and human characteristics. This is of great importance in understanding how pollution could affect otters. In fact, there are examples of pollution from several sources that have determined the distribution of the otter in some specific zones (organic pollution, acidification of water, detergents, etc.). However, only a limited series of compounds (micropollutants) producing lethal effects at a population level and sublethal (for example, affecting breeding) could have had a more global effect, in accordance with a disappearance on a regional scale (MASON, 1989; MACDONALD, 1991). These, especially, are the organochlorine compounds (from industrial sources and agriculture) and heavy metals. Of these, PCBs seem to be the most widespread in fish and otters, attaining higher levels of bioaccumulation in tissues. However, in specific regions, other biocides (mainly DDTs, oxychlordane, dieldrin and lindane) (CHANIN and JEFFERIES, 1978; MASON, 1989) and heavy metals (mainly mercury) (GUTLEB, 1995; KRUUK and CONROY, 1996; KRUUK, 1997) could have had a more important effect than PCBs.

The differences in toxicity need a more thorough analysis of pollutants, both with reference to the type of mercury (GUTLEB, 1995), and with reference to the congeners that make up PCBs (SMIT *et al*., 1994, 1996; LEONARDS, 1997).

There is increasing evidence of the effect on reproduction, and the pathological effects of these compounds on mammals (DELONG, GILMARTIN and SIMPSON, 1973; TANABE, 1988), and more specifically on otters and American mink (JENSEN *et al*., 1977; AULERICH and RINGER, 1977; KEYMER *et al*., 1988; MASON and O'SULLIVAN, 1992; MASON and MACDONALD, 1993). There is a correlation between PCB levels and the index of body condition, *k*, that has been demonstrated to determine the probability of mortality (KRUUK, CONROY and MOORHOUSE, 1987; LAFONTAINE, 1995). Lately, the effect on vitamin A (and subsequent effects) was shown (SMIT *et al*., 1996; SIMPSON *et al*., 2000).

This approach to PCBs congeners could bring with it some results applicable in the management and real understanding of the effects of these compounds. Our results also contribute to the idea of the existence of some thresholds of pollutant levels in prey, of some 0.1-0.2mg/kg: wet weight for PCBs, although important differences between the fish species exist (LAFONTAINE and DE ALENCASTRO, 2000).

Organochlorine levels have been diminishing world-wide, since preventive measures were undertaken (STOUT, 1986; BINGNERT *et al.,* 1993; NEWTON & WILLEY 1992). This decline has also occurred in the areas studied here, both in pollution levels of otter prey as well as in otter tissue levels. This decrease in levels coincides with a recovery in otter distribution. This fact tends to convince us of the effect of pollutants on otters, and also the bioindicator property of this mustelid. It

answers positively to the improvement in water quality, in pollution levels in prey tissues and in the environment in general. However, it is necessary to stay on guard since in many areas this recovery is not happening and, other new compounds or substances could have similar effects to those of organochlorine compounds and heavy metals.

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14 REFERENCES

Adrián, M. I., Wilden, W. & Delibes, M. (1985). Otter distribution and agriculture in South-Western Spain. In: *Proceedings of the XVIIth Congress of the International Union of Game Biologists*, Bruselas. Pp. 519-526.

Armitage, P. D. (1983). The perfomance of a new biological water quality score system based on macroinvertebrates over a wide range of unpolluted running-water. *Water Research*, **17**: 333-347.

Aulerich, R. J. & Ringer, R. K. (1977). Current status of PCB toxicity to mink and effect on their reproduction. *Archives of Environmental Contamination and Toxicology*, **6**: 279-292.

Beglimondi, A., Fravolini, A. & Morozzi, A. (1975). Rilevamenti quantitativi di alcuni metalli (mercurio, cromo, piombo e zinco) nei pesci delle principali acque di superficie dell'Umbria. *Archivi Veterinario da Italia*, 26: 55-65.

Bignert, A., Göthberg, A., Jensen, S., Litzen, K., Odsjo, T., Olson, M. & Reutegardh, L. (1993). The need for adequate biological sampling in ecotoxicological investigations: a retrospective study of twenty years pollution monitoring. *The Science of the Total Environment*, **128**: 121-139.

Bouchardy, C. (1986). *La loutre.* Ed. Sang de la Terre, París.

Caggino, G. F. (1982). Contenuti di alcuni metalli pesanti nella fauna ittica del fiume Po. *Inquinamento*, **6**: 25-28.

Cantoni, C., Cattaneo, P., Fabris, F. & Prina, G. (1985). Pesticidi organoclorti e PCB's nei pesci d'acqua dolce. *Industrie Alimentari*, ottobre, 808-810.

Cassola, F. (Editor) (1986). *La Lontra in Italia*. World Wildlife Fund. Serie Atti e Studi, **5**: 1-135. Roma.

Chanin, P. R. F. & Jefferies, D. J. (1978). The decline of the otter *Lutra lutra* L. in Britain: an analysis of hunting records and discussion of causes. *Biological Journal of the Linnaean Society*, **10**: 305-328.

Confederación Hidrográfica Del Ebro (2000). *Evaluación limnológica de la red fluvial del Ebro.* Confederación Hidrográfica del Ebro, Zaragoza.

Conroy, J. W. H. & Chanin. P. R. F. (2001). The status of the Eurasian otter (Lutra lutra) in Europe – a review. In: *Proceedings of the First Otter Toxicological Conference*. (Edited by Conroy, J. W. H., Yoxon, P. & Gutleb, A. C.). *Journal of the International Otter Survival Fund*, No 1, Xx-xx. Skye, September 2000. International Otter Survival Fund, Broadford, Isle of Skye.

Cozzani, R. & Pietrogiacomo, M. (1985). Determinazione di residui di pesticidi clorurati e policlorobifenili in anguille. *Industrie alimentari*, maggio, 454-456.

Delibes, M. (Editor) (1990). *La nutria (Lutra lutra) en España*. Serie Tècnica. ICONA, Madrid.

Delibes, M. & Callejo, A. (1983). On the status of the otter in Spain. *Paper presented at the IIIth International Otter Colloquium.* November 1983, Strasbourg.

Delong, R., Gilmartin, G. W. & Simpson, J. G. (1973). Premature births in California sea lions: Association with high organochlorine pollutant residue levels. *Science*, **181**: 1168-1169.

Elliot, K. M. (1983). The otter *Lutra lutra* in Spain. *Mammal Revue*, **13**: 25-34.

Foley, R. E., Jackling, S. J., Sloan, R. J. & Brown, M. K. (1988). Organochlorine and mercury residues in wild mink and otter: comparation with fish. *Environmental Toxicology and Chemistry*, **7**: 363-374.

Foster-Turley, P., MacDonald, S. M. & Mason, C. F. (Editors) (1990). *Otters. An Action Plan for their Conservation.* IUCN, Gland, Switzerland.

Fumagalli, R. & Prignioni, C. (1991). Livelli di accumulo di alguni netalli pesanti (Pb, Cd, Hg) in pesci predati dalla Lontra (*Lutra lutra*) in fiumi della Basilicata. *Atti II Convegno Nazionale Biologi della Selvaggina. Supplemento alle Ricerche di Biologia della Selvaggina*, **19**: 773-777.

Galassi, S. & Gandolfi, G. (1981). Variazioni del contenuto di pesticidi clourati e policlorodifenili nei cadevani (*Leuciscus cephalus*) e nelle alborelle (*Alburnus alburnus*) del Po. *Nouvi annali di Igieni e Microbilogia*, **32**: 393-408.

Galassi, S., Gandolfi, G. Pacchetti, G. (1981). Chlorinated hydrocarbons in fish from river Po (Italy). *The Science of the Total Environment*, **20**: 231-240.

Green, J. & Green, R. (1981). The otter (*Lutra lutra* L.) in Western France. *Mammal Revue*, **11**: 181-187.

Gutleb, A. C. (1995). *Umweltkontaminanten und Fischotter in Österreich eine Risikoabschätzung für Lutra lutra (L., 1758).* Ph. D. Thesis. University of Wien.

Hellawell, J. M. (1978). *Biological Surveillance of Rivers.* Water Research Centre, Stevenage.

Hernandez, L. M., Gonzalez, M. J., Rico, M. C., Fernandez, M. A. & Baluja, G. (1985). Presence and biomagnification of organochlorine pollutants and heavy metals in mammals of Doñana National Park (Spain), 1982-83. *Journal of Environmental Science and Health*, **b20**: 633-650.

Jensen, S., Kihlström, J. E., Olsson, M. & Orberg, J. (1977). Effects of PCB and DDT on mink (*Mustela vison*) during the reproductive season. *Ambio*, **6**: 239.

Keymer, I. F., Wells, G. A. H., Mason, C. F. & MacDonald, S. M. (1988). Pathological changes and organochlorine residues in tissues of wild otters (*Lutra lutra*). *The Veterinary Record*, **122**: 153-155.

Kihlström, J. E., Olsson, M., Jensen, S., Johanson, A., Ahibom, J. & Bergman, A (1992). Effects of PCB and different fractions of PCB on the reproduction of the mink (*Mustela vison*). *Ambio*, **21**: 563-601.

Kruuk, H. (1997). The significance of PCBs in otters: a reply. *I.U.C.N. Otter Specialist Group Bulletin*, **14**: 54-56.

Kruuk, H. & Conroy, J. W. H. (1996). Concentrations of some organochlorines in otters (*Lutra lutra* L.) in Scotland: implications for populations. *Environmental Pollution*, **92**: 165-171.

Kruuk, H., Conroy, J. W. H. & Moorhouse, A (1987). Seasonal reproduction, mortality and food of otters (*Lutra lutra* L.) in Shetland. *Sympium of the Zoological Society of London*, **58**: 263-278.

Lafontaine, L. (1995). *Contribution à la connaisance de la contamination des milieux aquatiques en France par les PCBs, les insecticides organoclorés et les métaux lourds, à partir d'un échantillon de 24 spécimens de loutre d'Europe Lutra lutra L. originaires des Bassins Loire-*

Bretagne, Adour-Garonne et Seine-Normandie. Ministère de l'Environement & Agence de l'Eau Loire-Bretagne.

Lafontaine, L. & de Alencastro, L. F. (2000). Statut de la loutre d'Eurasie (*Lutra lutra* L.) et contamination des poissons par les polychlorobiphényles (PCBs): éléments de synthèse et perspectives. *Actes XXIIIème Colloque Francophone de Mammalogie, Maisons-Alfort 1999*, SFEPM, Paris.

Lafontaine, L., Fortumeau, E. & Mainsant, S. (in press). Influence of habitat quality factors on otter *Lutra lutra* L. distribution in Brittany, NW France : a statistical approach for assessing recolonization probabilities. *Proceedings of the 7th International Otter Colloquium*. IUCN-SSC Otter Specialist Group, Trebon, Czech Rep, 14-20 march 1998.

Leonards, P. (1997). *PCBs in Mustelids. Analysis, Food Chain Transfer and Critical Lvels*. Ph. D. Thesis. Institute of Environmental Studies, University of Vrije (Amsterdam), 210 pp.

Leonards, P. E. G., Smit, M. D., de Jongh, A. W. J. J. & van Hattum, B. (1994). *Evaluation of dose-response relationships for the effects of PCBs on the reproduction of mink (Mustela vison).* Instituut voor Milieuvraagstukken. Vrije Universiteit Amsterdam, Amsterdam, 50 pp.

Locht, B., Muntau, H., Baudo, R., Galanti, G. & Varini, P. G. (1981). Detection of critical mercury concentrations in edibile fish from Pallanza basin, Lago Maggiore. *Memorie dell'Istituto Italiano di Idrobiologia "Dottor Marco De Marchi"*, **38**: 415-422.

Lodé, T. (1993). The decline of the otter *Lutra lutra* populations in the region of the Pays de Loire, Western France. *Biological Conservation*, **65**: 9-13.

López-Martín, J. M. & Ruiz-Olmo, J. (1996). Organochlorine residue levels and bioconcentration factors in otters (*Lutra lutra* L.) from North-East Spain. *Bulletin of Environmental Contamination and Toxicology*, **57**: 532-535.

López-Martín, J. M., Ruiz-Olmo, J. & Borrell, A. (1995). Levels of organochlorine compounds in freshwaters fish from Catalonia, N.E. Spain. *Chemosphere*, **31**: 3523-3535.

MacDonald, S. M. (1991). The status of the otter in Europe. In: *Proceedings of the Vth International Otter Collquium.* Hankensbüttel 1989 (Edited by Reuther, C & Röchert, R.). *Habitat*, **6**: 1-3.

Macdonald, S. M. & Mason, C. F. (1982). The otter *Lutra lutra* in central Portugal. *Biological Conservation,* **22**: 207-215.

MacDonald, S. M. & Mason, C. F. (1983). The otter *Lutra lutra* in Southern Italy. *Biological Conservation,* **25**: 95-101.

MacDonald, S. M. & Mason, C. F. (1994). *Status and conservation needs of the otter (Lutra lutra) in the Western Palearctic.* Council of Europe, Strasbourg.

MacFarland, V. A. & Clarke, J. U. (1989). Environmental occurence, abundance and potential toxicity of polychlorinated biphenyl congeners : considerations for a congener-specific analysis. *Environmental Health Perspecitives,* **81**: 225-239.

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

Mason, C. F. (1997). The significance of PCBs in otters at national and regional scales. *I.U.C.N. Otter Specialist Group Bulletin*, **14**: 3-12.

Mason, C. F. (1998). The decline in PCB levels in otters (*Lutra lutra*). *Chemosphere*, **36**: 1969-1971.

Mason, C. F. & MacDonald, S. M. (1986). *Otters. Ecology and Conservation*. Cambridge University Press, Cambridge.

Mason, C. F. & MacDonald, S. M. (1993). Pollution and otter conservation in a European context. In: *Proceedings of the National Otter Conference.* (Edited by Morris, P.A.). Pp. 17-20. Mammal Society, London.

Mason, C. F. & O'Sullivan, W. M. (1992). Organochlorine pesticide residues and PCBs in otters (*Lutra lutra*) from Ireland. *Bulletin of Environmental Contamination and Toxicology*, **48**: 387-393.

Mason, C. F. & Ratford, J. R. (1994). PCB congeners in tissues of European otter (*Lutra lutra*). *Bulletin of Environmental Contamination and Toxicology,* **53**: 548-554.

Mateo, R., Saavedra, D. & Guitart, R. (1999). Reintroduction of the otter (*Lutra lutra*) into Catalan Rivers, Spain: Assessing organochlorine residue exposure through diet. *Bulletin of Environmental Contamination and Toxicology*, **63**: 248-255.

Michelot, J.-L., de Alencastro, L. F., Laurent, L., Becker, K. & Randjean, D. (1998). Contamination par les PCBs et les metaux lourds de différents cours d'eau de la région Rhône-Alpes et potentialité de réintroduction de la Loutre (*Lutra lutra*). *Le Bièvre*, **16**: 3-27.

Nardi, P. A., Bragni, A., Tufaro, A. & Bernini, F. (1993). Dinamiche di accrescimento e fenomeni di accumulo di pesticidi in Icune specie ittiche del fiume Ticino. *Memoria della Società Ticcina da Sciencia Naturalle.*, **4**: 137-150.

Newton, I. (1979). *Population Ecology of Raptors*. T. & A.D. Poyser, Berkhamstead.

Newton, I. & Wyllie, I. (1992). Recovery of a sparrowhawk population in relation to declining pesticide contamination. *Journal of Applied Ecology,* **29**: 476 – 484.

Olsson, M. & Sandegren, F. (1983). The otter situation in Sweden and the Smaland-Sodermanland otter surveys of 1983. *Paper presented at the IIIrd International Otter Colloquium, November 1983, Strasbourg*

Prignioni, C. (1997). *La lontra, una vita silenziosa negli ambienti acquatici.* Edagricole, Bologna.

Raldua, D. & Pedrocchi, C. (1996). Mercury concentrations in three species of freshwater fishes from the Lowe Gállego and Cinca Rivers, Spain. *Bulletin of Environmental Contamination and Toxicology*, **57**: 597-602.

Reuther, C. (1980). Zur Situation des Fischotters in Europa. In: *Der Fischotter in Europa. Verbreintung, Bedrohung, Erhaltung*, (Edited by Reuther, C. & Festetics, A.). Pp. 71-92. Oderhaus und Göttingen.

Rosoux, R., Tournebize, T. & Maurin, H. (1996). Étude de la répartition de la loutre d'Europe (*Lutra lutra*) en France. Actualisation 1993. In: *La Loutre et le Vison d'Europe. Actes du XVII Colloque International de Mammalogie, Niort 23-25 octobre 1993.* (Edited by Gautier, J.Y., Libois, R. & Rosoux, R.). *Cahiers d'Ethologie*, **15**: 195-206.

Ruiz, X. & Llorente, G. (1991). Seasonal variation of DDT and PCB accumulation in muscle of carp (*Cyprinus carpio*) and eels (*Anguilla anguilla*) from Ebro Delta, Spain. *Vie Milieu*, **41**: 133-140.

Ruiz-Olmo, J. (1985). *Distribución, Requerimientos Ecológicos y Alimentación de la Nutria (Lutra lutra L., 1758) en el N.E. de la Península Ibérica*. Licenciature Thesis, Universitat de Barcelona, Barcelona.

Ruiz-Olmo, J. (in press). *Pla de conservació de la llúdriga a Catalunya: bases tècniques per a la seva gestió.* Departament de Medi Ambient, Barcelona.

Ruiz-Olmo, J. & Delibes, M. (Editors) (1998). *La Nutria en España ante el horizonte del año 2000.*SECEM, Málaga.

Ruiz-Olmo, J. & Gosálbez, J. (1988). Distribution of the otter, (*Lutra lutra* L. 1758) in the NE of the Iberian Peninsula. *Publicacions del Departament de Zoologia de Barcelona*, **14**: 121-132.

Ruiz-Olmo, J. & López-Martín, J. M. (1994). Otters and pollution in North-East Spain. In: *Seminar on the Conservation of the European Otter (Lutra lutra), Leeuwarden, the Netherlands, 7-11 June 1994*. Pp. 144-146.

Ruiz-Olmo, J. & Palazón, S. (1997). The diet of the European otter (*Lutra lutra* L.) in Mediterranean freshwater habitats. *Journal of Wildlife Research*, **2**: 171-181.

Ruiz-Olmo, J., López-Martín, J. M. & Delibes, M. (1998). Otters and pollution in Spain. In: *Behaviour and Ecology of Riparian Mammals.* (Edited by Dunstone, N. & Gorman, M). Pp.325-38. Cambridge Univiversity Press, Cambridge.

Ruiz-Olmo, J., Calvo, A., Palazón, S. & Arqued, V. (1998). Is the otter a bioindicator? *Galemys*, **10**: 227-237.

Saavedra, D., Sargatal, J. (1998). Reintroduction of the otter (*Lutra lutra*) in North-East Spain (Girona, province). *Galemys*, **10**: 191-200.

Safe, S. (1994). Polychlorinated biphenyls (PCBs): Environmental impact, biochemical and toxic responses, and implications for risk assessment. *Critical Reviews of Toxicology*, **24**: 87-149.

Santos-Reis, M. (1983). Present situation and conservancy of the river otter (*Lutra lutra* L., 1758) na Bacia Hidrografica do rio Tejo. *Paper presented at the IIIrd International Otter Colloquium.* November 1983, Strasbourg

Santos-Reis, M., Afonso, O. & Freitas, D. (1995). Estudo do impacte da Poluiçao por metais pesados na populaçao de lontra (*Lutra lutra* L., 1758) na bacia hidrográfica do Rio Tejo. *Protocolo Centro de Biologia Ambiental/Instituto da Caonservaçao da Natureza. Programa LIFE.*.

Simpson, V. R., Bain, M. S., Brown, R., Brown, B. F. & Lacey, R. F. (2000). A long-term study of vitamin A and polychlorinated hydrocarbon levels in otters *(Lutra lutra*) in South West England. *Environmental Pollution,* **110**: 267-275.

Smit, M. D., Leonards, P. E. G., van Hattum, B. & de Jongh, A. W. J. J. (1994). *PCBs in European otter (Lutra lutra) populations.* Instituut voor Milieuvraagstukken. Vrije Universiteit Amsterdam, Amsterdam.

Smit, M. D., Leonards, P. E. G., Murk, A. J., de Jongh, A. W. J. J. & van Hattum, B. (1996). *Development of otter-based quality objectives for PCBs.* Institute for Environment Studies, Vrije Universiteit, Amsterdam, Amsterdam.

Stout, V. (1986). What is happening to PCBs? Elements of the environmental monitoring as illustrated by an analysis of PCB trends in terrestrial and aquatic organisms. In: *PCBs and the Environment (Volume 2).* (Edited by Waid, J.S.). Pp. 164-205. CRC Press, Boca Raton, Fl.

Tanabe, S. (1988). PCB problems in the future: foresight from current knowledge. *Environmental Pollution*, **50**: 5-28.

Tans, M., Hugla, J.-L., Libois, R., Rosoux, R. & Thomé, J.-P. (1995). Étude du niveau de contamination par les PCBs et les pesticides orgabchlorés des loutres et d'anguilles issues des zones humides de l'ouest de la France. *Cahiers d'Ethologie*, **15**: 321-324.

Trindade, A., Farinha, N. & Florencio, E. (1998). *A Distribuiçao da Lontra, Lutra lutra, em Portugal. Situaçao em 1995.* Instituto da Conservaçao da Natureza. Ministério do Ambiente, Lisboa.

Tursi, A., Constantino, G. & Matarrese, A. (1989). *Relazione sulla qualità dell'ambiente fluviale relativa a studi condotti su paametri chimico-fisici delle acque, sul macrobentos e sulla fauna ittica di 5 fiumi dell'Italia centro meridionale nell'ambito del "Progetto Lontra Italia".* Studiottanta, Mestre.

Valentine, J. R. (1973). *Protection de la qualité des eaux et maitrise de la polution.* Eyroles, Paris.

Viviani, R., Crisetig, G., Cortesi, P. & Carpene (1974). Résidus de polychlorobyphenyls (PCBs) dans les poissons et les oisseaux du Delta du Po. *Revista Internationalle de Océanografia della Méditerranea*, **35-36**: 79-90.

Wells, D. E., Maier E. A. & Griepink, B. (1992). Developments in the analysis of chlorobiphenyls in environmental matrices for certification purposes. *International Journal of Environmental Analysis and Chemistry*, **46**: 265-275.

Westman, W. E. (1978). Measuring the inertia and resilence of ecosystems. *Bioscience*, **28**: 705-710.

DISTRIBUTION AND POPULATION DENSITY OF THE OTTER *LUTRA LUTRA* AND POLLUTION OF AQUATIC ECOSYSTEMS IN BELARUS

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1 INTRODUCTION

From the 1960s until the beginning of the 1990s, the otter *Lutra lutra* populations declined in Western Europe (MASON and MACDONALD, 1986; FOSTER-TURLEY, MACDONALD and MASON, 1990; REUTHER, 1993). According to MASON (1989), chemical pollution of aquatic ecosystems played a major role in the deterioration of the stock of water-living prey of the semi-aquatic predator and severely affected its distribution and reproduction. In Europe, monitoring of otter populations appeared to be quite often linked to contamination of aquatic ecosystems. In the last decade, however, otter numbers have been recovering in parts of Western Europe (MACDONALD, 1994; STUBBE and STUBBE, 1994; ROMANOWSKI, GRUBER and BRZEZINSKI, 1997; KRANZ and TOMAN, 2000; CONROY and CHANIN, 2001). The aim of this paper is to present current information on otter distribution in Belarus and discuss these results in connection with data on chemical pollution of aquatic ecosystems there.

2 MATERIALS AND METHODS

Information relating to otter presence at 243 sites along rivers in different parts of Belarus and several neighbouring areas (Eastern Poland, Northern Ukraine, and Pskov Region of Russia) was gathered in 1997-2000. In each place, a minimum 1km of river stretch was inspected. Because there was no financial support for this job, I carried out the otter surveys from time to time while doing another study on vertebrates, which determined the distribution of places inspected for otter presence (Figure 1).

Figure 1. Distribution of otter signs in catchments of rivers in Belarus, 1997-2000.

Multiannual (1984-2000) dynamics of otter density in the four rivers chosen as model aquatic ecosystems for long-term monitoring of the otter population were recorded. I did a census of otters on a small and medium-sized river in a protected area (respectively Volka and Zapadnaya Berezina Rivers, Naloboky Reserve, Grodno and Minsk Regions) and a hunting area (respectively Ahonka and Nischa Rivers in Vitebsk Region). A pronounced habitat-related difference in otter density was found (SIDOROVICH, 1992, 1997), and according to these results, both the small and medium-sized rivers chosen for the otter monitoring are characterised by a similar quality of habitat conditions. The rivers are moderately flowing and with moderately swamped floodplains, and the small river is a tributary of the medium-sized one. So, it is correct to compare otter distribution at similarly sized rivers in protected and hunting areas. In respect to several questions of otter distribution related pollution, other data on density of otters obtained in 1984-1996 in different regions of Belarus (SIDOROVICH and LAUZHEL, 1996; SIDOROVICH, 1997) were involved.

Each winter, on each river, more than 20km of the river bank were inspected. Otters were censused along the rivers during winter by searching the banks, ice covers and floodplains for their tracks. We tried to count the number of otters living on surveyed stretches of the rivers. To do this, we applied the following criteria. According to the methodological results related otter census (SIDOROVICH, 1992), the prints of hind feet were measured. In our field survey, differences in measurements of footprints of single otters consistently >1cm were accepted as criterion for differentiating individuals. Fresh marking places (with urine and faeces) were examined to determine sex. In relation to the prints of hind feet, males mainly leave urine marks on snow in front of a scat, whereas females defecate and urinate in the same place or urine marks are sprinkled behind the scat.

As is well known, the main contaminants are heavy metals, organochlorine pesticides, polychlorinated biphenyls (PCBs) and acid deposition. In addition to chemical pollution, many aquatic ecosystems in Belarus were strongly contaminated by the Chernobyl radionuclide fallout. All available data from both literature (KUZNETSOV and DOVNAR, 1984; JAKUSHKO *et al*., 1988; SAVCHENKO, 1992 *a* & *b*) as well as our own and joint studies (SAVCHENKO and SIDOROVICH, 1994; SIDOROVICH, SAVCHENKO and DENISOVA, 1996; SIDOROVICH, 1997) relating to the contamination of otters and aquatic ecosystems in Belarus were compared with information on otter distribution to determine the possible impacts of habitat pollution on otters. Data on the concentration of heavy metals (Pb, Cd, Zn, Cu, Mo, Cr, Ni, Ag, Sn, V, Co, Be, Ba, Mn, Ti, Zr, Y, Yb, Nb, Sc), acid deposition, organochlorine compounds (DDE, DDD, DDT, α-BHC, γ-BHC, PCBs), and radionuclides (Cs^{134} and Cs^{137}) were found in the literature or obtained in own and joint studies. Places sampled for any pollutants in either habitats (water and bottom sediment) or otters (mostly in muscle, liver and kidney) are shown in Figure 2.

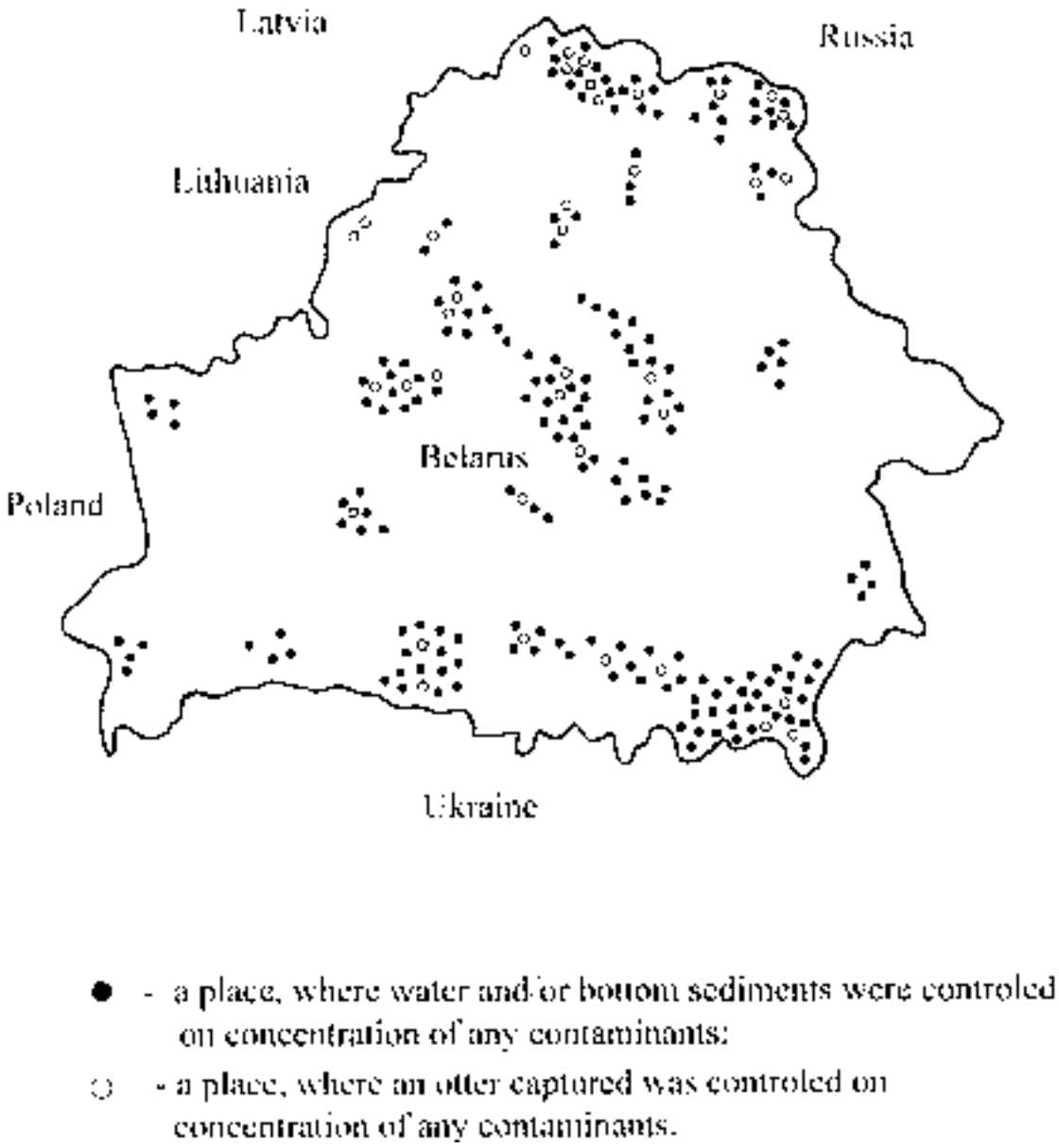

Figure 2. **Sites where otters, water and sediment samples were collected for pollutant analysis in Belarus between 1997 and 2000.**

Determination of organochlorine pesticides and PCBs were carried out by the method of gas-liquid chromatography (ROVINSKY *et al.,* 1990). The detection limit of DDE was 0.2μg/kg wet weight, DDD - 0.3, DDT - 0.4, α-BHC and γ-BHC - 0.1μg/kg: wet weight, PCBs in the water - 0.04 μg/l and bottom sediments - 0.01mg/kg: wet weight. The methods used to measure Cs134 and Cs137were described in detail by SUSCHENYA *et al.* (1995). To determine trace element contents, the samples were dried to constant weight at a temperature of 105°C. and then burnt in a muffle furnace at a temperature of 450°C, using the method described by NIKANOROV and ZHULIDOV (1991). The contents of Cu, Mn, Ti, Cr, Ni, Pb, Zn, Ag, Mo, Co, and V were measured in ash by means of atomic emission spectroscopy.

3 RESULTS

3.1 Otter distribution and population density

Data on otter presence gathered in 1997-2000 from 243 places located in river valleys of different part of Belarus and several neighbouring areas (Figure 1) suggest that otters seem to be largely distributed in the country and surrounding regions. During this otter survey, tracks and/or spraints were found at 229 places (94.2% of the total places investigated). Only in the Minsk urban area (mainly in its south-eastern part at the Svisloch River draining the city) was the otter considered a rare species, there no tracks were recorded in 14 out of the 17 sites inspected (73.7%).

The pronounced habitat-related difference in density of otters that was found, had been studied in detail before (SIDOROVICH, 1992, 1997), and, taking into account the aim of this paper, I will only pay some attention to that. According to the results, in Belarus, in both protected and hunting areas, otter density increased depending on the following favourable factors: natural riverbed, forested bank-side, bigger size of stream, faster flowing rate, intensive beaver construction activity, and abundance of meanders and old riverbeds.

Multi-annual (1984-2000) dynamics of otter population density in the four rivers chosen as model aquatic ecosystems for a long-term monitoring of the otter population in both protected and hunting areas are given in Figures 3 and 4. The density of otters was lower in the hunting areas: on average 7.2 versus 3.7 individuals per 10km of river stretch in medium-sized rivers ($t = 11.2$: $p < 0.001$) and 3.2 versus 1.8 individuals/10km of river stretch in small rivers ($t = 10.1$: $p < 0.001$). The density variation was substantially higher in the exploited otter population than in its protected population (22.2-32.4% versus 8.3-12.5%). These results and many other data obtained in 1984-1996 (SIDOROVICH, 1992, 1997) revealed a marked difference in the mean otter density and its year-to-year variation between protected and hunting areas. The main cause probably was an overexploitation i.e. quite often a year's hunting bag exceeds the yearly production of young. The otter is a protected species in Belarus, and individuals are usually killed by poachers and beaver trappers. Nevertheless, kills of otters in Belarus are still common. Moreover, in a part of the hunting areas, the abnormal population structure (i.e. low density and a predomination of females) is aggravated by the excessive selection for hunting males which directly, and possibly indirectly, negatively affected reproduction (SIDOROVICH, 1997). The direct impact appeared, because otter mothers were killed by hunters. The indirect influence may be a difficulty in mating in conditions of the altered population structure. The lowest otter density was registered in 1994-1996, when hunting of otters was particularly intensive. Then fur wearing declined in Belarus, and the otter population began to grow in number (Figures 3 and 4).

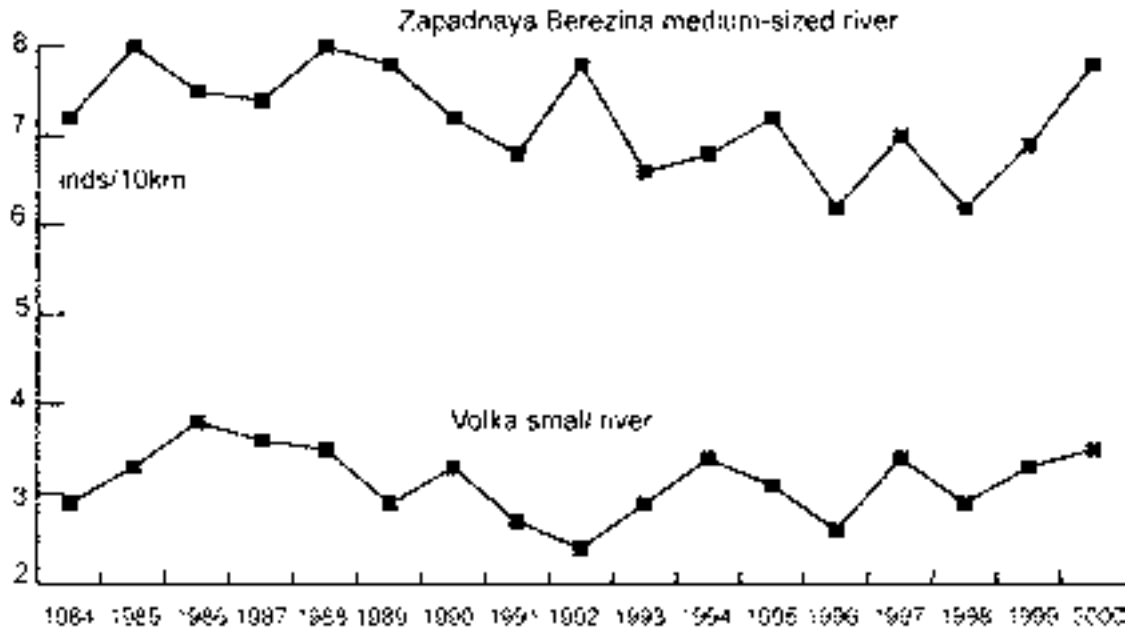

Figure 3. The estimated number of otters per 10 kilometres of river bank in protected areas of Belarus.

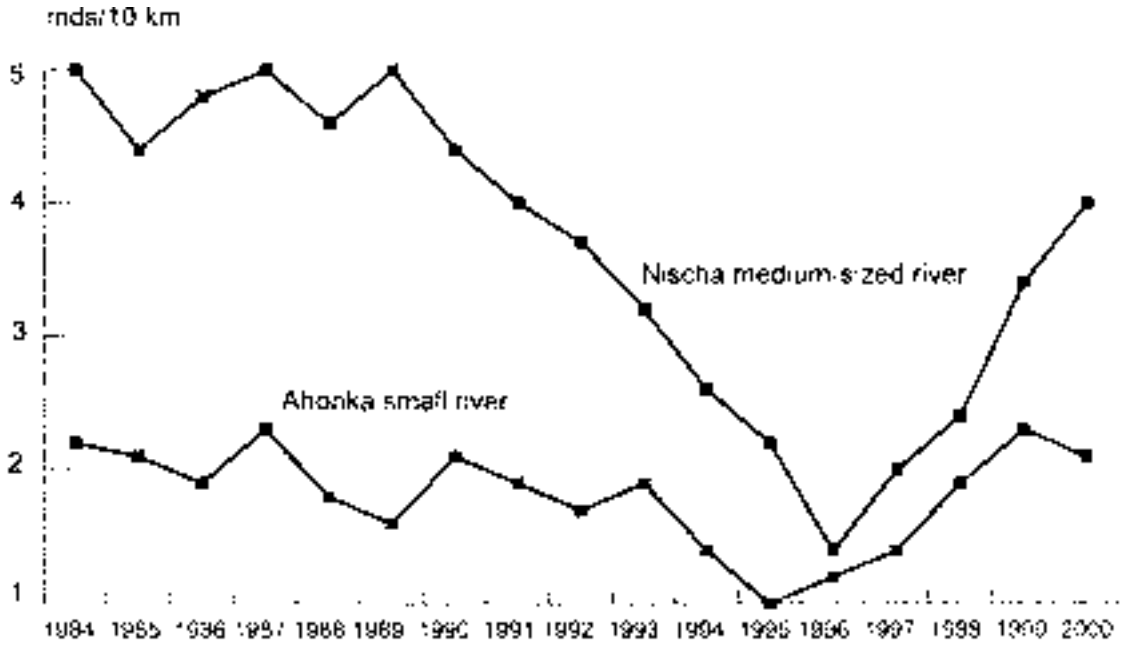

Figure 4. The estimated number of otters per 10 kilometres of river bank in areas of Belarus where hunting is permitted.

3.2 Pollution of aquatic ecosystems compared to the otter distribution

Acid deposition remains an unimportant pollution in Belarus. Only a small number of glacial lakes is characterised by naturally somewhat acidic water (JAKUSHKO *et al*., 1988).

According to the published data by SIDOROVICH, SAVCHENKOV and DENISOVA (1996), bottom sediments of the majority of the studied rivers (51 out of 58, 87.9%) located in different parts of Belarus contained natural values of trace element concentrations (5-28mg of Pb per kg dry weight, 5-45 of Cr, 2-33 of Cu, 3-53 of V, 4-27 of Ni, 120-4300 of Mn, 190-860 of Ba). The same levels of the trace element concentration occurred in the Berezina River and its tributaries draining the undisturbed forested area in the central part of Belarus (SAVCHENKO, 1992*b*) as well as in the Nischa and Drissa Rivers located in the large forest of central North Belarus (KUZNETSOV and DOVNAR, 1984; SAVCHENKO and SIDOROVICH, 1994) and the Lovat River draining its north-eastern part (SIDOROVICH, 1997). All these rivers were fairly densely populated by otters (SIDOROVICH, 1992, 1997). Some contamination by heavy metals was registered on seven rivers draining cities (SAVCHENKO, 1992*a*; SIDOROVICH, SAVCHENKOV and DENISOVA, 1996). In particular, the Svisloch River draining the City of Minsk was highly polluted by the town's sewage. The main heavy metal pollutants were Cr, Cu, Ni, Zn, Ag, Mo, found both in solid and liquid states in the river. The concentrations of heavy metal in water and bottom sediments were 2-160 times higher than the natural values. The mean concentration of zinc in shallow silts was 3500 mg/kg dry weight, Cr - 2400, Cu - 1400, Ni - 360, Sn - 170, Ag - 13, Cd - 24, Pb - 73, V - 100, Ba - 1200, Mn - 1300, Mo - 25, As - 6, W - 16, Sb - 6, Au - 2, Se - 0.034, La - 49, and Nb - 13. The Svisloch River ecosystem was also polluted by organochlorine pesticides. In the water of this river the concentration of DDE varied up to 0.02 μg/l, DDD - up to 0.89, DDT - 0.055-0.087, α-BHC - 0.004-0.023; γ-BHC - 0.007-0.033 μg/l, while in the water of the five other rivers already mentioned - Lovat, Drissa, Nischa, Volka and Zapadnaya Berezina Rivers, these contaminants were not detected. Nevertheless, the sample size (i.e. number of rivers controlled) is small, and therefore it is hard to say precisely, that aquatic ecosystems in Belarus are only slightly polluted with organochlorine pesticides.

Long-term studies on otter distribution in the Minsk urban area drained by the strongly polluted Svisloch River revealed that normally otters were rarely observed on the first 90km of the river downstream of the city. The otter density over the next 70km of this polluted river varied between 0.5 and 2.0, and on average was about one individual per 10km of river stretch. The stream and bank structure is similar in those parts of the Svisloch River, and this polluted river and many other medium-sized rivers densely populated by otters are also characterised by similar habitat structure. These allow the conclusion, that the rare presence of otters in the first 90km of the contaminated Svisloch River downstream of the City of Minsk appeared because possibly otters were severely affected by the pollution. The adult female otter caught in this polluted habitat was characterised by several morpho-physiological peculiarities. The uterus had a large cyst (4 x 1.5 x 0.8cm) with a long stalk (about 6cm). The liver weight was abnormally high. The ratio of liver weight to body weight (multiplied by 1000) of this female was 67.9, whereas normally in Belarus this index for an adult female otter (n = 33) ranged from 30.2 to 60.7, and on average was 46.5. The kidney weight was also fairly high. The ratio of kidney weight to body weight (multiplied by 1000) of this female was 15.9, whereas normally in Belarus this index for an adult female otter (n = 33) ranged from 6.9 to 17.2, and on average was 10.0.

PCBs were not found in both water and bottom sediments of seven rivers located in central and Northern Belarus including the most contaminated Svisloch River. The detection limit of the method used was 0.04μg/l for water and 0.01mg/kg of wet

weight for bottom sediments. Nevertheless, the seven rivers sampled is too small a sample size, and it is too early to summarise, that Belarus is an area relatively unpolluted by PCBs. However, the rather healthy population of otters and the data on PCB concentrations in the rivers investigated suggest this conclusion.

The radioactivity level of 80 out of 83 (96.4%) river ecosystems sampled was close to the natural value, 8-12μR/h for river watercourse, 9-34μR/h for banks and 9-68μ R/h for the floodplains. In 1990-1994 in the area of Chernobyl strongly contaminated by radionuclides, three rivers were investigated. There, levels ranged from 9 to 20μ R/h for the watercourses, from 33 to 42μR/h (average 11.98) on the banks (to 2m from a stream edge), from 26 to 486μR/h (average 51-89μR/h) on banks (from 2 to 10m); and from 29 to 67μR/h (average 44μR/h) in beaver burrows used by otters. In the floodplains, the radioactivity level was markedly higher and varied from 24 to 6231μR/h. Nevertheless, the otter density at the Rivers (Pripyat, Slovechna and Zhelon) in this area polluted by radionucludes reached 5.0, and on average was 2.5 individuals per 10km of river. These values were typical for the otter population in areas of Belarus, which were slightly contaminated by radionuclides (SIDOROVICH, 1997). Also before the Chernobyl radionuclide fallout, the density of otters on the similarly sized rivers in the Pripjat Catchment ranged between 0.7 and 5.4 individuals/10km (SIDOROVICH, 1988). Overall, no negative correlation between the radioactivity level and density of otters was found.

3.3 Contents of pollutants in otters

Out of the 11 heavy metals investigated, only levels of vanadium and cobalt in otters examined (n = 9) were below the limits of detection (respectively 1 and 3mg/kg of ash). Vanadium was measured in muscle and kidneys of only a single otter (0.16-0.35mg/kg: dry weight). Other heavy metal concentrations in muscle of otters (used as a basic tissue) may be arranged in the following decreasing order (SIDOROVICH, SAVCHENKO and DENISOVA, 1996).

Zn -- Cu -- Mn -- Ti -- Mo -- Cr -- Ni -- Pb -- Ag

10^{-2} ------- 10^{-3} ------- 10^{-4} ---------- 10^{-5} % dry weight.

A similar order might be formed in respect of otter liver and kidney. On average, the otter muscle contained 70 mg of Zn per kg of dry weight, 24 of Cu, 3.3 of Mn, 1.6 of Ti, 0.22 of Ag, 0.45 of Cr, 0.27 of Ni and 0.29 of Pb. The mean concentrations of the trace element in the otter livers and kidneys examined were as follows: 95 and 49 mg of Zn per kg dry weight, Cu - 32 and 25, Mo - 0.82 and 0.27, Mn - 13 and 4.5, Ti - 0.36 and 0.33, Ag - 0.84 and 0.022, respectively.

The levels of PCBs in all otters investigated (liver, fat and muscle from 16 individuals) was less than 0.01mg/kg wet weight (detection limit of the method used). The mean concentrations of organochlorine pesticides in the livers of the four otters studied was as follows: DDE - 22μg/kg: wet weight, DDD - 14, DDT - 14, α -BHC - 7.2, γ-BHC - 13.

In Belarus, the sampling of radioactivity in otter tissues had not been carried out before the Chernobyl accident in April 1986. Testes from five males and embryos from four pregnant females collected in 1983-1985 have only recently been measured for radioactive caesium. In the testes the concentrations of Cs^{137}ranged from 129 to 409Bq/kg (average 287) and in the embryos from 124 to 208Bq/kg (mean 178). After the Chernobyl radionuclide fallout, radioactive caesium in both otter testes (n = 14) and embryos (n = 4: pregnant females) was in markedly higher concentrations. In the areas where the radioactivity level was less than 20μR/h, the mean concentration of

^{137}Cs was 381Bq/kg (range 271-586) in the testes and 384Bq/kg (284-501) in the embryos; the mean concentrations of Cs^{134} was142Bq/kg (range 110-172) in the testes and 136Bq/kg (126-144) in the embryos examined. The increase of Cs^{137} concentrations in otter embryos is statistically significant ($t = 3.61$: $p = 0.02$). Substantially higher levels of radioactive caesium were found in the areas highly contaminated by radionuclides. In testes ($n = 4$) concentrations of Cs^{137} varied from 478 to 995Bq/kg (average 679Bq/kg) and in the single embryo examined it was 731Bq/kg. Concentrations of Cs^{134} were 130-276Bq/kg (average 194) in the testes and 232Bq/kg in the embryo. The differences between non-contaminated areas after the Chernobyl accident and before, compared with the areas highly polluted by radionuclide were statistically significant ($t = 2.25$-3.32: $p = 0.01$-0.05).

Also, various tissues and organs from a further six otters (three from the Chernobyl area and three otters from areas relatively non-contaminated by radionuclides) were measured for concentration of radioactive caesium. The content of Cs^{137} in otters that inhabited unpolluted rivers varied from 154 to 569Bq/kg. In contrast, the otters caught in the Chernobyl area contained 1.5-4.6 times higher concentrations of Cs^{137} (429-1122Bq/kg). The concentrations of Cs^{134} measured in otters caught in the Chernobyl area varied between 161 and 681Bq/kg. The concentration of Cs^{137} in otter faeces collected in the Chernobyl area varied from 324 to 6783 (average 2016Bq/kg) ($n = 19$). In rivers of Northern Belarus largely unpolluted by radioactivity, the concentration of Cs^{137} in the otter faeces examined ($n = 10$) varied from 84 to 201 (average 140)Bq/kg.

4 CONCLUSION

These data on concentrations of heavy metals and organochlorine compounds in aquatic ecosystems and otters, as well as information relating to acid deposition in otter habitats in different parts of Belarus are compared with levels in highly polluted regions of Western Europe (FORSTNER and WITTMANN, 1979; DISSANAYOKE, TOBSCHALL and PALME, 1983; MOORE and RAMAMOORTHY, 1984; WHITTON, 1984; HERNANDEZ, GONZALEZ and RICO, 1985; MASON and O'SULLIVAN, 1993; SMIT *et al*., 1994 and references therein). The comparison suggests that otter habitats in Belarus are relatively unpolluted by the above mentioned contaminants. Plausibly, this still allows for a healthy population of otters there. Only in the restricted urban area of the City of Minsk does the combined contamination by heavy metals and organochlorine pesticides seem to lead to the local otter decline recorded. Concerning radioactivity pollution, this seems not to have affected otters enough to be the cause of population density decrease, even in the Chernobyl area.

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6 REFERENCES

Conroy, J.W.H. & Chanin, P.R.F. (2001). The status of the Eurasian otter (*Lutra lutra*) – a review. In: *Proceedings of the First Otter Toxicology Conference.* (Edited by Conroy, J.W.H., Yoxon,

P. & Gutleb, A.C.). *Journal of the International Otter Survival Fund,* No 1, xx-xx. Skye, September, 2000. International Otter Survival Fund, Broadford, Skye.

Dissanayoke, C. B.; Tobschall, H. J. & Palme, H. B. (1983). The abundance of some major and trace elements in highly polluted sediments from the Rhine River near Mainz, West Germany. *The Science of the Total Environment*, **29**: 243-260.

Forstner, U. & Wittmann, G. (1979). *Metal Pollution in the Aquatic Environment*. New York: Springer-Verlag.

Foster-Turley, P., Macdonald, S. M. & Mason, C. F. **(Editors)**. (1990). *Otters - An Action Plan for their Conservation.* IUCN, Gland, Switzerland.

Hernandez, L. M., Gonzalez, M. J. & Rico, M. C. (1985). Presence and biomagnification of organochlorine pollutants and heavy metals in mammals of Donana National Park (Spain) 1982-1983. *Journal of Environmental Science and Health*, **20**: 633-650.

Jakushko, O. F., Myslivets, I. A., Rachevsky, A. N., Bogdel, I. I., Vlasov, I. P., Kalechits, V. A., Melnikov, O.K., Makritsky, A. M., and Mironov, V. G. (1988). [*Lakes of Belarus*.] Uradzhai, Minsk. [In Russian.]

Kranz, A. & Toman, A. (2000). Otters recovering in man-made habitats in central Europe. In: *Mustelids in a Modern World. Management and Conservation Aspects of Small Carnivore: Human Interactions*. (Edited by Griffiths, H.I.). Pp. 163-184. Backhuys Publishers, Leiden.

Kuznetsov, V. A. & Dovnar, V. V. (1984). [Trace elements in the contemporary sediment of the Drissa River.] In: *Geochemical Studies of Landscapes in Belarus*. Pp. 42-48. Nauka and Teknika, Minsk. [In Russian.]

Macdonald, S. (1994). Meeting of European Section OSG. *I.U.C.N. Otter Specialist Group Bulletin*, **10**:10-14.

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

Mason, C. F. & Macdonald, S. M. (1986). *Otters: Ecology and Conservation*. Cambridge University Press, Cambridge.

Mason, C. F. & O'Sullivan, W. M. (1993). Heavy metals in the livers of otters, *Lutra lutra*, from Ireland. *Journal of Zoology, London*, **231**: 675-678.

Moore, J. W. & Ramamoorthy, S. (1984). *Heavy Metals in Natural Waters*. Springer-Verlag, Berlin-Heeidelberg-New York-Tokyo.

Nikanorov, A. M., Zhulidov, A. V. (1991). [*Biomonitoring of Metals in Freshwater Ecosystems*.] Leningrad. [In Russian.]

Reuther, C. (1993). *Der Fischotter*. Naturbuch Verlag, Augsburg.

Romanowski, J., Gruber, B. & Brzezinski, M. (1997). The recovering otter population of central Poland. *I.U.C.N. Otter Specialist Group Bulletin*, **14**: 24-25.

Rovinsky, F. Y., Voronova, L. D., Afanasiev, M. I., Denisova, A.V., Pushkar, I.G. (1990). [*Background Monitoring of Terrestrial Ecosystem Contamination by Organochlorine Compounds*.] Nauka, Moskow. [In Russian.]

Savchenco, V. V. (1992*a*). [Technogeochemical sediment anomaly of the Svisloch river.] *Reports of the National Academy of Sciences of Belarus*, **36**: 67-71. [In Russian.]

Savchenco, V. V. (1992*b*). [Trace element distribution in alluvial sediments of the Berezina river.] *Journal of the National Academy of Sciences of Belarus*, biological seria, **5-6**: 92-98. [In Russian.].

Savchenco, V. V. & Sidorovich, V. E. (1994). [Heavy metal contents in the American mink (*Mustela vison*): penetrating, accumulating, and removal. Bioindication.] *Ekologia (Ekaterinburg):* 69-76. [In Russian.]

Sidorovich, V. E. (1988). [Otter population structure in the Pripyat river catchment.] *Proceeding of 5th conference: «Wild animals of the Polesse area in Belarus, their protection and sustainable use»*: 120-121. [In Russian.]

Sidorovich, V. E. (1992). [Otter *(Lutra lutra)* population structure in Belarus.] *Bulluten Moskovskogo Obschestva Ispytateley Prirody (Moskow),* **97**: 43-51. [In Russian.]

Sidorovich, V. E. (Editor). **(1997).** ***Mustelids in Belarus. Evolutionary Ecology, Demography and Interspecific Relationships*****. Zolotoy uley, Minsk.**

Sidorovich, V. E. & Lauzhel, G. O. (1996). Current status of habitat and abundance of the otter in Belarus: brief information. Report of the Seminar on the conservation of the European Otter (*Lutra lutra*). Leeuwarden, the Netherlands, 7-11 June 1994. Pp. 149-153. Council of Europe, Strasbourg.

Sidorovich, V. E., Savchenko, V. V. & Denisova, A. V. (1996). Heavy metals, organochlorine pesticides, PCBs and radionuclides in otters and their habitats in Belarus//Report of the

Seminar on the Conservation of the European Otter (*Lutra lutra*). Leeuwarden, the Netherlands, 7-11 June 1994. Pp. 126-133. Council of Europe, Strasbourg.

Smit, M. D., Leonards, P. E. G., van Hattum, B. & de Jongh, A. W. J. J. (1994). *PCBs in European Otter (Lutra lutra) Populations*. Amsterdam.

Stubbe, M. & Stubbe, A. (Editors). (1994). Saugetierarten und deren feldokologische Erforschung im ostlichen Deutschland *(Tiere im Konflikt* **3**). Halle (Saale), Martin-Luther-Universitat Halle-Wittenberg. [In German.]

Suschenya, L. M., Pikulik, M. M., and Plenin, A. E. (Editors). (1995). [Wild animals in the Chernobyl radionuclide contaminated area]. Nauka and Tehnika Publisher, Minsk.

Whitton, B. A. (Editor). (1984). *Ecology of European Rivers*. London: Blackwell Scientific Publications.

0THE ROLE OF DIELDRIN IN THE DECLINE OF THE OTTER *(LUTRA LUTRA)* IN BRITAIN: THE ANALYTICAL DATA

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1 INTRODUCTION

There were great changes in agriculture in Britain following the Second World War. One of these was the advent of chemical farming with widespread use of insecticides, fungicides and herbicides as well as fertilisers. The organochlorine insecticides, first DDT, then BHC and the 'second generation' and much more toxic cyclodienes, dieldrin and aldrin, were soon adopted in order to increase crop yields. These were very effective insecticides which were particularly valuable as they remained active for a very long time after application. For example, it takes 25 years for 95% of dieldrin in soil to disappear and 30 years for DDT (SHAROM and SOLOMON, 1981). However, this very persistence was the cause of their downfall as it allowed them to become global environmental contaminants. In addition, far from being only insecticides, they were found to be biocides, affecting all forms of animal life. The first wildlife casualties appeared in the late 1950s and soon the dead bodies of birds started to litter the fields and woods. This was worst in the Eastern counties of England. Further, persistence within tissues allowed secondary poisoning to occur and avian and mammalian predators began to be found dead as well.

I (DJJ) saw my first casualties in 1959 with the obliteration of my local rookery (*Corvus frugilegus*) in Oxfordshire, and soon became deeply involved. With official scepticism that all this could be due to poisoning rather than wildlife diseases, the Nature Conservancy set up the Toxic Chemicals and Wildlife Section in 1959 under Dr Norman Moore in order to provide the essential information required to obtain bans on their uses. I joined the Section as one of the first ecological toxicologists when it became based at the then new research station at Monks Wood. The full history of the 'battle' to prove the connection between insecticides and casualties and Monks Woods part in it has been written by SHEAIL (1985) and MOORE (1987).

Although employed to concentrate on the obvious avian casualties such as the peregrine falcon (*Falco peregrinus*), kestrel (*Falco tinnunculus*), sparrowhawk (*Accipiter nisus*) and heron (*Ardea cinerea*), I extended the research and analyses to British mammals. I had a longstanding interest in the otter (*Lutra lutra*) since the 1940s and could see that if the heron was seriously contaminated and foxes (*Vulpes vulpes*) were dying, then the otter population too could be affected. Indeed, the first people to notice that fewer otters were being caught were the otter hunters (LLOYD, 1962). However, otter bodies were very difficult to find in the early 1960s. The first one analysed by paper chromatography was a road casualty from Dorset in 1962, but this method did not allow accurate determination and all later organs were analysed by gas liquid chromatography. There followed over 200 post mortem examinations and 122 chemical analyses between 1965 and 1989. The overall results from the first 31 analyses up to 1973 were reported by JEFFERIES, FRENCH and STEBBINGS

(1974), but only to the effect that 81% contained measurable residues of dieldrin up to a wet weight concentration of 13.95µg/g. Apart from this note and the full analytical reports for seven individual otters published by JEFFERIES (1985, 1992), JEFFERIES and HANSON (1988, 1991) and SPALTON and CRIPPS (1989), the great majority of these analyses have remained unpublished. However, assertions made by much later analysts (MASON and MACDONALD, 1993) that it was the Polychlorinated biphenyls (PCBs) which affected otter numbers, probably caused its decline and slowed its recovery, have made it essential to place all the information on record. The analytical results, obtained first by myself working in the Nature Conservancy and Nature Conservancy Council and later by Hazel Hanson under contract from the Nature Conservancy Council and The Vincent Wildlife Trust, clearly supported the view that the organochlorine insecticides affected both individuals and the population of British otters just as much as they affected the peregrine falcon and sparrowhawk, and that the PCBs played little or no part in the otter decline. Further, the loss of the otter cannot be understood in isolation. It formed part of the great changes which were happening to other wildlife and the British countryside in the post-war decades. The analytical basis for this view is set out below.

2 METHODS

2.1 Obtaining bodies

British otters, particularly those from England, were very few for the first ten years following the population crash of 1957. Consequently, their bodies were difficult to find. One source I used during the 1960s was that of the packs of otter hounds which were still hunting until legal protection for the otter was obtained in England and Wales in 1978. As time progressed and numbers started to recover with the bans on the organochlorines, road traffic accident victims increased and provided the great majority of the specimens analysed. A few were found dead from various other causes, such as senility, fighting, starvation and disease, and at least two were killed in a dieldrin incident in Somerset. All were weighed, measured and given a post mortem examination (JEFFERIES and HANSON, 1987).

2.2 Chemical analysis

A sample of around two grams (mean: 1.774 ± 0.050g) was removed from the largest lobe of the liver and from beneath its surface (to eliminate possible contamination from gloves). The liver was chosen as the organ for monitoring analysis for all wildlife specimens at Monks Wood from 1963. This was because: (a) residues in this organ showed a close and significant relationship with dose rate in toxicological trials (JEFFERIES and WALKER, 1966), and (b) this organ is easy to find and remove and, unlike the brain, stays intact for a considerable period after death. It would have also been useful to have brain concentrations, because the relationship of brain to liver levels can provide a good indication that death was due to organochlorine poisoning [Dieldrin: JEFFERIES and DAVIS (1968); PCB: PRESTT, JEFFERIES and MOORE (1970)]. However, as said, there are problems with brain removal in all cases.

Analysis was by gas-liquid chromatography with an electron-capture detector. The method of sample clean-up, quantification and equipment used at Monks Wood has been published in detail elsewhere (JEFFERIES, STAINSBY and FRENCH, 1973; JEFFERIES and FRENCH, 1976; JEFFERIES and PARSLOW, 1976; JEFFERIES and FREESTONE, 1985). The residues were expressed in terms of µg organochlorine

per gram of wet weight tissue sample (parts per million or mg/kg) and quantifications against a standard were made down to 0.01μg/g. Sometimes only 'trace' amounts of dieldrin or DDE were found and remained unquantified (assigned a value of 0.005μg/g: wet weight).

With PCBs the standard used in early analyses was Aroclor 1254, as this was the commercial PCB mixture with the 'fingerprint' closest to that found in British wildlife specimens in the 1960s and 1970s (JEFFERIES and PARSLOW, 1976) and was used in toxicity trials (PRESTT, JEFFERIES and MOORE, 1970; JEFFERIES and PARSLOW, 1972, 1976). Some wildlife specimens (particularly from seabirds) showed an almost complete 'fingerprint' of Aroclor 1254 with all congeners, but in most specimens it was the constituent PCB congeners remaining after metabolism and passing through a food chain which were quantified. Early PCB analyses were quantified only as total PCB (PRESTT, JEFFERIES and MOORE, 1970) but quantification was later made to individual PCB congeners. Only total PCB residues are expressed here in order to allow analysis using early data and to show changes with time. Sometimes only 'trace' amounts of PCB were found and remained unquantified (assigned a value of 0.05μg/g: wet weight) (COOKE, BELL and HAAS, 1982).

The quantity of extractable lipid present was measured in all samples (in mg/g tissue) so that a lipid concentration could be expressed as well as that in wet weight of tissue.

2.3 Wet weight and Lipid weight concentrations

In the early days (i.e. 1960s) of wildlife analyses the concentrations of organochlorines were always expressed in terms of parts per million (ppm) or μg/g: wet weight of tissue [e.g. JEFFERIES and PRESTT (1966); JEFFERIES (1969)]. Subsequently, as these materials were concentrated in the lipid of the organ and this may vary in amount, then it became more usual in later years to express the concentrations in terms of μg/g of extractable lipid [e.g. MASON, FORD and LAST, (1986)] or indeed both (JEFFERIES, 1985, 1992). This use of lipid weight concentrations was particularly prevalent, and has more sense, when analysing eggs, when nearly all of the organochlorine content was in the yolk which was feeding the developing embryo [e.g. NEWTON, DALE and LITTLE, (1999)]. However, use of lipid concentrations really only inserts one more extraneous and unnecessary level of complexity and variation into the data for statistical analysis. As the amount of extractable lipid in an organ such as the liver is relatively small and measured in mg/g tissue, calculation of a lipid concentration obviously magnifies the organochlorine concentration considerably (e.g. with 50mg/g of lipid in a liver, a wet weight concentration of, say, 10μg/g PCB is multiplied 20 times (the wet to lipid conversion factor) to convert it to a lipid concentration of 200μg/g PCB). This may give concern that contamination is greater than it really is.

Other problems are that organ lipids may vary greatly with the nutritional state of the animal (i.e. with the amount of food available), with its age (i.e. lipid may be reduced with senility) and with the state of health of the animal (i.e. a sick or diseased animal may have very little organ lipid). Thus, JEFFERIES and HANSON (1987) concluded from a sample of 26 otters, that conversion factors for changing wet weight concentrations to lipid concentrations in otter liver varied from x 13.56 when with normally high levels of body fat to x 72.99 in an emaciated animal. This meant that an animal with 1μg/g PCB in fresh wet liver could have a lipid concentration of

13.56µg/g or 72.99µg/g without changing the amount of PCB present but purely on the degree of emaciation it had suffered. For example, one of the two otters found dead in North Norfolk (from Glandford and Blakeney) in 1984, showed very high concentrations of PCB in the liver [232µg/g: lipid weight MASON, FORD and LAST (1986)] but both were emaciated with little or no body fat (KEYMER *et al.*, 1988; SPALTON and CRIPPS, 1989). Unfortunately, the actual organ lipid density in mg/g was not given for this animal. It is essential that information on organ lipid density is given if only lipid pollutant concentrations are tabled.

From the point of view of the individual animal carrying the insecticide load it may matter considerably what the lipid organ concentrations are as there will be movement of the insecticide around the body and repartitioning between the organs and the more sensitive brain as the liver and other organ lipids are reduced (JEFFERIES and DAVIS, 1968; ECOBICHON and SASCHENBRECKER, 1969). However, from the point of view of monitoring the pollutant levels in otters and how these vary from place to place and year to year (which is what we are doing here), it is the amount of contaminant in the organ which is important and so wet weight concentrations provide a better comparative result than do lipid concentrations (i.e. one needs to know whether it is a heavy insecticide load or a small insecticide load redistributed).

In the present text both lipid and wet weight concentrations are given in the Tables 1, 2 and 3 of temporal changes. The former are given for purposes of comparison with other work and the latter because they provide better monitoring levels. Also, nearly all of the early toxicity trials related effect with organ residues expressed in terms of wet weight concentrations [e.g. BLACKMORE (1963); JEFFERIES (1972)].

The liver lipid levels in the 122 British otters analysed here ranged from 12.90 to 73.78mg/g of tissue with a mean value of 38.17 ± 1.11mg/g of tissue. These levels produce a mean wet to lipid concentration conversion factor of x 29.1348 with a range of x 13.5602 to x 77.5194. The mean conversion factor for the opposite direction, when calculating wet weight concentrations from lipid weight concentrations, was x 0.03817 with a range of x 0.01290 to x 0.07378. These are equivalent to dividing the lipid weight concentration by 26.20 (range ÷ 77.52 to ÷ 13.55) to get the wet weight concentration.

Table 1. The changing arithmetic mean wet weight concentrations (± Standard Error and ranges) of dieldrin, DDE and total PCB's found in 122 British otters in seven periods from 1965 to 1989. The percentage of each year group derived from Scotland is shown because this may apply a bias to the means (i.e. lower dieldrin and higher PCB's in Scotland). The otter A297 was killed in a lethal dieldrin incident in 1972. The very high residues present will bias the mean for 1972 to 1975 so is shown included and excluded from this mean. Dieldrin residues were highest at the start of the analysis (with 40% over µg/g) and then declined except for an increase with illicit use in the early 1980s. DDE increased from start of analysis to 1971 before declining, again with a short-term increase in the early 1980s. Total PCB's were absent to very low at the start with a dramatic increase in the late 1970s. They were still increasing at the end of the analysis.

Period	***n***	**Arithmetic mean wet weight residue ± standard error (µg/g)**	**% age over 1µg/g**	**Range of wet weight concentrations**	**% age of Scottish otters**
DIELDRIN					
1965-1969	5	1.146 ± 0.676	40.0	0.16 – 3.73	0
1970	10	0.525 ± 0.163	20.0	0 – 1.55	0
1971	7	0.457 ± 0.177	14.3	0.05 – 1.47	28.6
1972 – 1975 [A297]	8 +1 9	0.334 ± 0.071 13.95 1.847 ±1.514	11.1	0 - 0.62 0 -13.95	62.5
1977 – 1979	24	0.077 ± 0.015	0	0 – 0.22	87.5
1983 – 1986	43	0.436 ± 0.068	9.3	0 – 2.45	58.1
1987 – 1989	24	0.248 ± 0.044	0	0 – 0.96	37.5
DDE					
1965 – 1969	5	1.130 ± 0.561		0 – 3.16	0
1970	10	1.149 ± 0.444		0 – 4.50	0
1971	7	1.604 ± 0.660		0.20 – 5.39	28.6
1972 – 1975 [A297]	8 +1 9	1.192 ± 0.363 19.53 3.230 ± 2.062		0 – 2.49 0 – 19.53	62.5
1977 – 1979	24	0.492 ± 0.188		0 – 3.38	87.5
1983 – 1986	43	0.990 ± 0.234		0.05 – 8.52	58.1
1987 – 1989	24	0.640 ± 0.126		0.01 – 2.86	37.5
TOTAL PCBs					
1965 – 1969	5	0 ± 0		0 – 0	0
1970	10	0 ± 0		0 – 0	0
1971	7	0.014 ± 0.009		0 – 0.05	28.6
1972 – 1975 [A297]	8 +1 9	0.031 ± 0.009 0 0.028 ± 0.009		0 – 0.05 0 – 0.05	62.5
1977 – 1979	24	2.317 ± 0.384		0.18 – 8.13	87.5
1983 – 1986	43	2.200 ± 0.469		0 – 19.41	58.1
1987 – 1989	24	3.647 ± 0.694		0 – 14.92	37.5

Table 2. The changing geometric mean lipid weight concentrations (with range of one standard error and overall ranges) of dieldrin, DDE and total PCB's found in 122 British otters in seven periods from 1965 to 1989. The percentage of each year group derived from Scotland is shown because this may apply a bias to the means (i.e. lower dieldrin and higher PCB's in Scotland). The otter A297 was killed in a lethal dieldrin incident in 1972 and is shown separate so as not to bias the mean for 1972-1975. These concentrations show the same overall changes with time as shown by the arithmetic wet weight means given in Table 1, but these changes are less clear and smooth using this form of standard analysis (see text).

Period	*n*	Geometric mean lipid residue (µg/g)	Range of one standard error	Range of lipid weight concentrations	% age of Scottish otters
DIELDRIN					
1965-1969	5	14.34	7.55 - 26.49	3.93 – 103.60	0
1970	10	5.77	3.32 – 9.61	0 – 32.67	0
1971	7	7.26	4.76 – 10.83	0.82 – 36.41	28.6
1972 – 1975 [A297]	8 +1	7.09 236.96	4.88 – 10.14	0 – 15.50	62.5
1977 – 1979	24	1.67	1.30 – 2.10	0 – 11.74	87.5
1983 – 1986	43	8.37	7.38 – 9.47	0 – 50.56	58.1
1987 – 1989	24	5.68	4.63 – 6.94	0 – 21.07	37.5
DDE					
1965 – 1969	5	11.98	5.17 – 26.28	0 – 75.67	0
1970	10	9.35	4.83 – 17.39	0 – 139.23	0
1971	7	22.91	15.26 – 34.16	4.47 – 133.50	28.6
1972 – 1975 [A297]	8 +1	18.99 331.75	11.38 – 31.28	0 – 64.16	62.5
1977 – 1979	24	4.87	3.54 – 6.58	0 – 94.23	87.5
1983 – 1986	43	14.24	11.98 – 16.90	1.80 – 434.69	58.1
1987 – 1989	24	12.99	10.37 – 16.22	0.34 – 92.15	37.5
TOTAL PCBs					
1965 – 1969	5	0	0 – 0	0 – 0	0
1970	10	0	0 – 0	0 – 0	0
1971	7	0.22	0.07 – 0.40	0 – 1.24	28.6
1972 – 1975 [A297]	8 +1	0.75 0	0.48 – 1.06	0 – 1.90	62.5
1977 – 1979	24	50.48	41.24 – 61.73	6.37 – 266.10	87.5
1983 – 1986	43	32.10	26.45 – 38.91	0 – 984.56	58.1
1987 – 1989	24	70.95	54.72 – 91.91	0 – 507.41	37.5

Table 3. The arithmetic mean lipid and wet weight concentrations of total PCB's, dieldrin and DDE in otters found dead in England, Scotland and Wales for three separate periods in the 1970s and 1980s

Country	Period	*n*	*Lipid weight concentration*			**Wet weight concentration**		
			PCB	Dieldrin	DDE	PCB	Dieldrin	DDE
England	1977-79	3	117.18	4.96	29.41	3.577	0.153	0.913
	1983-86	11	51.11	15.02	19.46	1.906	0.612	0.777
	1987-89	11	128.27	8.64	15.86	3.958	0.247	0.479
Scotland	1977-79	21	69.03	2.17	11.07	2.137	0.066	0.432
	1983-86	25	81.80	10.08	38.96	2.352	0.376	1.194
	1987-89	9	137.27	6.01	27.56	3.872	0.181	0.856
Wales	1977-79	-	-	-	-	-	-	-
	1983-86	7	47.21	7.99	14.19	2.116	0.370	0.593
	1987-89	4	64.04	10.39	16.70	2.287	0.402	0.595

2.4 Use of arithmetic or geometric means for groups of residue values

It was noted early on that in any sample of eggs or livers, the distribution of the residue values was usually skewed to the right, so it became the convention to correct the residues to logarithmic values to give a distribution closer to the statistical normal curve. The mean residue was then quoted as a geometric rather than an arithmetic mean [e.g. COOKE, BELL and HAAS (1982); NEWTON, DALE and LITTLE (1999)]. However, as noted by FOWLER and COHEN (1992), if the geometric mean is used for population estimates it results in a corresponding underestimate and a much lower figure than the arithmetic mean. For instance, the geometric mean for lipid weight dieldrin concentrations in otter livers from England over 1965-89 is 9.71µg/g, whereas the arithmetic mean for the same set of values is much higher at 18.65µg/g (see Table 4a).

Table 4a. The mean levels of dieldrin in 62 Scottish, 17 Welsh and 43 English otters analysed from 1965 to 1989. Geometric and arithmetic means are shown with standard errors, as well as lipid and wet (arithmetic means only) weight concentrations and the range of residues found. As the geometric mean is based on logarithms, the upper and lower limits of one standard error are asymmetrical.

Country	***n***	**Geometric mean residue (µg/g)**	**Range of one St. Error**	**Range of residues**
Lipid weight concentrations of dieldrin				
Scotland	62	3.95	3.37 – 4.60	0 – 50.56
Wales	17	6.39	5.01 – 8.08	0 – 30.94
England	43	9.71	8.16 – 11.53	0 – 236.96

Country	**n**	**Arithmetic mean residue ± St. Error**	**Range of residues**
Lipid weight concentrations of dieldrin			
Scotland	62	6.94 ± 1.16	0 – 50.56
Wales	17	8.87 ± 1.86	0 – 30.94
England	43	18.65 ± 5.75	0 – 236.96
Wet weight concentrations of dieldrin			
Scotland	62	0.252 ± 0.048	0 – 2.45
Wales	17	0.359 ± 0.068	0 – 1.00
England	43	0.828 ± 0.327	0 – 13.95

Also, the use of geometric means for residue data provides certain problems in practice:

First, there are problems because not all the groups of residue values, i.e. those for a date or insecticide or country, have a skewed distribution or are skewed to the same extent. Some, indeed, have a normal distribution. Thus, the series of concentrations of total PCBs in liver lipid in 13 otters from South-west England in the period 1977 to 1989 was skewed (arithmetic mean ± s.e. = 126.92 ± 38.44µg/g: variance = 19,207.2: range = 2.41 - 507.41µg/g), whereas that for the seven otters from East Anglia in the same period was not (arithmetic mean ± s.e. = 43.43 ± 11.91µg/g: variance = 993.5: range = 0 - 81.04µg/g). It can be seen that in East Anglia the arithmetic mean is in the centre of the range and the variance is small in relation to this mean. Neither of these statements is true for the South-west of England. Another example is provided by the series of concentrations of dieldrin in liver lipid from 25 otters from Scotland in the period 1983-86. This had a skewed distribution (arithmetic mean = 10.079: range = 0 - 50.56µg/g), whereas that for the

11 otters from England in the same period (arithmetic mean = 15.019: range = 4.90 - 25.62μg/g) did not. There are many more examples. Comparative data analysis cannot be by geometric means in some cases and arithmetic means in others. Consequently, use of one form only produces some 'stresses' within the resulting analysis.

Second, but most important, although use of geometric means may provide a more accurate mean level for the contaminants of an individual within a population of otters where the data are skewed, it does not provide a satisfactory indication of the overall area contamination shown by the local otter population. This would perhaps be best provided by taking exactly two grams of tissue from the livers of all otters found dead, bulking it and then analysing the resulting sample in terms of micrograms of organochlorine per gram of bulk tissue; which is what we are doing by taking the arithmetic mean of individual wet weight samples. When geometric means are used in combination with lipid concentrations, the reduced value of the former, decreasing the contribution of all the higher contamination levels, together with the inherent variability of the latter, produces series which are difficult to analyse and compare.

The veracity of this statement can be seen in the results of the present work. Thus, (a) The series of changes in arithmetic means of wet weight concentrations of dieldrin and DDE with time show an expected stepwise progression (see Table 1) which correlates with that found in other species. The changes with time of the geometric means of the lipid concentrations of these two compounds, on the other hand, does not form such a smooth, stepwise progression (see Table 2). (b) The concentrations of DDE are seen to be approximately equal in England, Scotland and Wales when analysed in terms of wet weight and lipid weight arithmetic means (see Table 4b). This is to be expected with such a universal pollutant as DDE has become. However, when analysed as lipid concentrations with geometric means, this similarity disappears (see Table 4b). (c) The arithmetic means of lipid weight PCB concentrations in otters from Scotland show the expected stepwise increase with time (1977-79: 69.03; 1983-86: 81.80; 1987-89: 137.27μg/g), as do the arithmetic means of the wet weight PCB concentrations (1977-79: 2.137; 1983-86: 2.352; 1987-89: 3.872μg/g). However, if the analysis is completed using lipid weight concentrations and geometric means, then this stepwise increase with time is lost (1977-79: 44.89; 1983-86: 32.27; 1987-89: 98.1 μg/g).

In the present text, analytical results are given with both arithmetic and geometric means as well as both lipid and wet weight concentrations. This has been done for purposes of comparison with previous work and to illustrate the problems in their use.

3 RESULTS OF CHEMICAL ANALYSES

3.1 The organochlorine pollutants found in British otter tissue

Dieldrin/Aldrin (HEOD). The highly toxic cyclodiene group of organochlorine insecticides was introduced on a wide scale in Britain in 1956. The cyclodiene residue found most frequently in wildlife samples is 1,2,3,4,10,10-hexachloro-6, 7-epoxy-1,4,4a,5,6,7,8a-octahydro-1,4-endo, exo-5, 8-dimethanonaphthalene or HEOD, the active ingredient of the commercial insecticide dieldrin (contains 85% HEOD). HEOD is also produced as a metabolite of the cyclodiene, aldrin. Aldrin and dieldrin were used as cereal seed dressings and were applied as sprays and aldrinated fertilisers. Dieldrin was also available as a veterinary product for use in sheep dips to control fly strike. It is much more toxic than either DDT or gamma BHC with an

acute oral LD_{50} toxicity to rats of 46mg/kg body weight (MARTIN and WORTHING, 1977). It has caused much wildlife mortality (JEFFERIES, 1969).

Table 4b. The mean levels of DDE in 62 Scottish, 17 Welsh and 43 English otters analysed from 1965 to 1989. Geometric and arithmetic means are shown with standard errors, as well as lipid and wet (arithmetic means only) weight concentrations and the range of residues found. As the geometric mean is based on logarithms, the upper and lower limits of one standard error are asymmetrical.

Country	*n*	Geometric mean residue (µg/g)	Range of one St. Error	Range of residues
Lipid weight concentrations of DDE				
Scotland	62	10.40	8.61 – 12.53	0 – 434.69
Wales	17	15.23	11.18 – 20.62	0 – 139.23
England	43	12.79	10.44 – 15.63	0 – 331.75

Country	*n*	Arithmetic mean residue ± St. Error	Range of residues
Lipid weight concentrations of DDE			
Scotland	62	28.06 ± 7.60	0 – 434.69
Wales	17	27.33 ± 8.08	0 – 139.23
England	43	27.02 ± 7.81	0 – 331.75
Wet weight concentrations of DDE			
Scotland	62	0.946 ± 0.194	0 – 8.52
Wales	17	1.051 ± 0.264	0 – 4.50
England	43	1.177 ± 0.453	0 – 19.53

Heptachlor was another organochlorine insecticide of the same cyclodiene group as dieldrin/aldrin and which came into use at the same time. It was not so widely used as dieldrin (see Table 8). The residue usually found in wildlife samples is that of its metabolite, heptachlor epoxide. Heptachlor epoxide residues may also originate from the use of chlordane (JEFFERIES, 1985).

pp'-DDT was manufactured in large quantities in Britain during the Second World War for purposes of controlling the insect vectors of human diseases. From 1945 it was used increasingly for agricultural pest control. DDT itself is seldom found in wildlife tissues, partly because of its metabolism to DDE and partly because of post mortem breakdown (see TDE). When it is found it points to recent exposure to a treated crop. The acute oral LD_{50} toxicity of DDT to rats is 113 - 118mg/kg body weight (MARTIN and WORTHING, 1977).

pp'-DDE is the fat-soluble metabolite of DDT produced by the living animal. Most predators are exposed via th food to DDE ratheeirr than DDT. It has become a highly persistent universal global contaminant. It is still toxic (but less so than DDT) and can produce a range of sub-lethal effects in mammals and birds (JEFFERIES, 1975).

pp'-TDE is present in small amounts in technical DDT. However, it is produced by reductive dechlorination of DDT in the tissues of dead animals post mortem (WALKER and JEFFERIES, 1978). This anaerobic reaction proceeds even at -20°C. Consequently, the longer the body is stored before analysis the more TDE and the less DDT is found in the liver (JEFFERIES and WALKER, 1966; JEFFERIES, 1972). A

TDE concentration can be converted back to the original quantity of DDT mathematically (JEFFERIES and WALKER, 1966).

BHC (HCH). The gamma isomer of benzene hexachloride (lindane) (also known as gamma hexachlorocyclohexane) was first produced shortly after the end of the Second World War and was used increasingly for agricultural pest control. It has an acute oral LD_{50} toxicity to rats of 88 - 91mg/kg body weight (MARTIN and WORTHING, 1977). This is between that of DDT and dieldrin. The residues of BHC in watercourses are considered to have a domestic, rather than an agricultural or industrial origin, probably arising from the common use of wood preservatives in the home (HARPER, SMITH and GOTTO, 1977). It was thought not to be a serious environmental contaminant (because it is metabolised relatively rapidly) until it was found that it also degraded rapidly in animal tissues after death (even in deep freeze at -20°C) and so is lost to analysis (FRENCH and JEFFERIES, 1968). Thus, it can kill and then disappear from the body. Consequently, it is always under-represented in lists of pollutants. Rapid extraction is required.

HCB. The fungicide hexachlorobenzene has been found in some of the otter samples analysed here.

PCB. Unlike the agricultural insecticides, the polychlorinated biphenyls have an industrial origin and were not released to the environment intentionally. They have been manufactured since the 1930s by passing chlorine through biphenyl to produce mixtures of compounds chlorinated to varying degrees. These mixtures are marketed under a number of commercial names, e.g. Aroclor 1254 (biphenyl with 54% w/w of chlorine), and each mixture may have over a hundred different PCB congeners. They have many industrial uses, such as constituents of protective coatings, plasticisers, sealers, adhesives and printing inks. In liquid form they are used as hydraulic fluids, in cutting oils and grinding fluids and they are incorporated in electrical apparatus, such as transformers, as they are excellent dielectrics.

We had noted additional peaks in the chromatograms from wildlife specimens since the early 1960s. However, it was not until 1966 that Jensen identified a series of such peaks in Swedish wildlife as corresponding to polychlorinated biphenyls (JENSEN, 1966). HOLMES, SIMMONS and TATTON (1967) then showed that the compounds with long retention times found in British specimens were also PCBs and all wildlife samples (from 1966) were analysed for PCBs at Monks Wood as well as for organochlorine insecticides. Later work showed them to be widespread contaminants in British birds (PRESTT, JEFFERIES and MOORE, 1970) and a worldwide pollutant (RISEBROUGH *et al.,* 1968).

3.2 Temporal changes

Dieldrin. Examination of Table 1 shows quite clearly that dieldrin levels were still high in otters in the period 1965-69, eight years after the decline had started (in 1957) and after there had been a major ban on the use of aldrin/dieldrin in seed dressings in 1962 and a further major ban on their use in sheep dip in 1966. This suggests that dieldrin residues in otters would have been much greater in the period 1957 to 1962 and not much less in the period 1962 to 1966. Table 1 shows that there was a slow and progressive decline in wet weight residue levels of dieldrin up to the period 1977-79. There was then a sudden x 5.7 increase in the period 1983-86 before it started to decrease again in 1987-89. SIMPSON *et al*. (2000) showed that this decrease

continued in English otters from 1988 to 1996. It is likely that this decline continued in Wales and Scotland too after 1989. If a wet weight concentration of 1 μg/g dieldrin is taken to be a significant level (i.e. sufficient to kill a fox (BLACKMORE, 1963), though not an otter), then 40% of otters carried this concentration in 1965-69. This had decreased to 0% by 1987-89 (Table 1). However, the occasional otter exceeding this level still occurred in the period 1988-96 (one with 2.8 μg/g dieldrin out of 56 analysed, SIMPSON *et al.*, 2000).

The picture of decline, so clear when comparing arithmetic means of wet weight residues, is much less clear when comparing temporal changes in geometric means of lipid concentrations (Table 2) due to the insertion of further unnecessary extraneous factors (e.g. changes in lipid stores with disease and season) into the data set (see Sections 2.3 & 2.4).

The sudden increase in dieldrin levels in otters in the early 1980s is shown by birds too, so is likely to be real rather than an anomaly due to changes in sampling area. Thus, RATCLIFFE (1993) noted an unexplained upturn in the dieldrin concentrations in peregrine eggs in parts of Scotland between 1980 and 1986. NEWTON, DALE and LITTLE (1999) too showed a sudden increase in dieldrin levels in merlin (*Falco columbarius*) eggs from 1980 to 1985. The upturn in dieldrin levels in otters in 1983-86 was most marked in Scotland (x 5.7 over 1977-79) followed by those in England (x 4.0 over 1977-79) (Table 3). It was not obviously present in Wales (Table 3). RATCLIFFE (1993) considered that these dieldrin upsurges could reflect the using up of dieldrin stocks when the final restrictions on the use of these insecticides on cereals were announced. However, the size of the sudden increase in dieldrin found in Scottish otters and the fact that the Ministry of Agriculture, Fisheries & Food found dieldrin above the reporting limit (1μ/g) in 17 fleeces sampled during 1984 to 1986 as well as two sheep carcases with dieldrin above the maximum allowable residue levels (2.5mg/kg in mutton fat in 1985 and 0.5mg/kg in 1987) (CROSSETT, 1989) suggests a marked increase in illicit use of dieldrin sheep dips. This period of increased dieldrin use could have slowed the recovery of the otter.

DDE. This metabolite of DDT was found throughout the 25 years of the analytical study. Presumably it had been steadily increasing in wildlife samples, including otters, since production for agricultural uses started in 1946. Some evidence for this is seen in the progressively greater thinning of peregrine eggshells from 1946 to 1956 [Figure 9 in RATCLIFFE (1993)]. Thus, unlike dieldrin residues, which were already decreasing by the time the present study started in 1965, DDE residues were still increasing in 1965-69 (Table 1). DDE reached a peak in otter samples in 1971 (Table 1), before starting to decrease. Just as with dieldrin, there was an upturn in use and wildlife contamination (x 2.01: Table 1) in 1983-86, before DDE resumed its decline. A follow-on study (SIMPSON *et al.*, 2000) showed that this decline in otter DDE residues continued to 1996 and is presumably still declining. Again, this picture is much more clear, showing progressive steps, when analysed on the basis of arithmetic means of wet weight residues (Table 1), than it is with geometric means of lipid weight residues (Table 2).

As with dieldrin, the upsurge found in otter DDE residues in 1983-86 has been noted in both peregrine (RATCLIFFE, 1993) and merlin eggs (NEWTON, DALE and LITTLE, 1999). Examination of Table 3 shows that the upsurge only occurred in Scotland and not in England and Wales. Scottish wet weight residues increased by a factor of 2.76 between 1977-79 and 1983-86 (Table 3).

PCB. The picture of changes in PCB residues with time is different again from those of dieldrin and DDE. Thus, no PCBs were detected in the five otters from the period 1965-69 (only one otter from 1965 was not analysed for PCB) or the ten from 1970. The first residues found (in 1971) were only at trace level (trace = 0.05μg/g: wet weight). This was so in 1972-75 too, though more out of the nine analysed bore traces. The lethal incident otter, A 297, from Somerset did not contain any PCB. There was then a sudden, considerable and rapid rise in PCB residues by the end of the 1970s (period 1977-79) (see Tables 1 & 2). To some extent the size of the sudden increase is exaggerated by the fact that 87.5% of the otters of this time period were of Scottish origin whereas the two previous periods had many fewer Scottish animals. Scotland has the highest PCB levels (see Table 3 and Section 3.3). This leap in PCB residues in otters is not unique. There was a sudden increase (by a factor of 8.6 times) in the PCB residues in heron eggs from the Troy colony in Lincolnshire between 1970 and 1977 (COOKE, BELL and HAAS, 1982).

After the above leap, the levels of PCBs in otters continued to rise until the end of the study in 1989. This is shown most clearly by the lipid weight concentrations in Scottish otters (Table 3) which showed a progressive increase from 69.03 ± 15.07 (1977-79) to 81.80 ± 38.46 (1983-86) to 137.27 ± 36.93μg/g (1987-89). The mean for the period 1987-89 was significantly (t = 2.0600; d.f. 28: p <0.05) higher than that for the period 1977-79. Although it may appear in Table 3 that the PCB levels in England were higher in 1977-79 and then decreased to those in 1983-86, this is most likely due to the very small sample of three animals from which the former mean was calculated. A larger sample would, most likely, have shown a progression. The Welsh PCB residues increased from 1983-86 to 1987-89. The follow-on survey by SIMPSON *et al.* (2000) shows that from 1988 to 1996 the otter PCB residues were decreasing in South-west England. Thus, PCB residues were increasing in Britain at a time when the otter populations of England, Scotland and Wales were also showing marked increases [England: LENTON, CHANIN and JEFFERIES (1980); STRACHAN *et al.* (1990); STRACHAN and JEFFERIES (1996). Scotland: GREEN and GREEN (1980, 1987, 1997). Wales: CRAWFORD *et al.* (1979); ANDREWS and CRAWFORD (1986); ANDREWS, HOWELL and JOHNSON (1993)].

This finding that total PCB levels in otters were below detectable limits in the late 1960s and early 1970s was mirrored in all the analyses carried out on 14 species of other British mammals by DJJ at Monks Wood in that same period. These included: pipistrelle (*Pipistrellus pipistrellus*), brown long-eared bat (*Plecotus auritus*), Natterer's bat (*Myotis nattereri*), Daubenton's bat (*Myotis daubentonii*) (JEFFERIES, 1972); field mouse (*Apodemus sylvaticus*), bank vole (*Clethrionomys glareolus*), field vole (*Microtus agrestis*) (JEFFERIES, STAINSBY and FRENCH, 1973; JEFFERIES and FRENCH, 1976); water vole (*Arvicola terrestris*) (STRACHAN and JEFFERIES, 1993); polecat (*Mustela putorius*) (JEFFERIES, 1992); badger (*Meles meles*) (JEFFERIES, 1969); stoat (*Mustela erminea*), weasel (*Mustela nivalis*), fox (JEFFERIES, *unpublished*); wildcat (*Felis silvestris*) (JEFFERIES, 1991).

The above low or absent residues of PCBs in the livers of so many species of British mammals in the 1960s and early 1970s were not due to any practical difficulties in finding the residues at the time, as the same analysts were finding and quantifying PCBs in birds from 1966. Thus, PRESTT, JEFFERIES and MOORE (1970) reported the PCB contents of 196 avian livers from 33 species and 363 eggs from 28 species collected from April 1966 to August 1968, as well as the results of toxicity tests. Also, COOKE, BELL and HAAS (1982) reported the PCB contents of

727 herons, sparrowhawks, kestrels and barn owls analysed in the period 1967-1975, when 66.7% of them contained residues of more than 1μg/g: wet weight.

It was concluded at the time that the apparent lack of PCB in mammals was due to a more rapid breakdown in the tissues of this class compared to the situation in birds. There was some corroborating evidence supporting this point of view in the PCB levels in birds with different diets. Thus, the geometric mean liver (wet weight) concentrations of PCBs (with range of one standard error) were 4.41μg/g (3.39 - 5.75: n = 57) in herons, 2.29 (1.80 - 2.92: n = 83) in sparrowhawks, 0.62 (0.49 - 0.78: n = 125) in kestrels and 0.17 (0.13 - 0.22: n = 114) in barn owls (*Tyto alba*), during the years 1972-1975 (data from COOKE, BELL and HAAS, 1982). The diets of these four species are largely fish (herons), completely birds (sparrowhawks), largely mammals (kestrels) and completely mammals (barn owls). Consequently, it was thought that this ranking indicated a low PCB accumulation rate in mammals (probably due to a high rate of metabolism), a higher accumulation rate in birds and a very high accumulation rate in fish. A similar conclusion was reached by PRESTT, JEFFERIES and MOORE (1970) concerning the PCB residues in these four species for the years 1966 - 1968. Thus, otters could have a high intake rate but a high rate of metabolism. The latter may cope with the former until intake rises above a critical level exceeding the capacity of the species to metabolise and eliminate the toxins. The liver residue would then rise suddenly and rapidly.

Exactly the same ranking of heron (high), sparrowhawk, kestrel and barn owl (low) is found independently with DDE and dieldrin residues; again indicating differing accumulation rates between fish, birds and mammals (PRESTT, JEFFERIES and MOORE, 1970). It is known too that the organochlorine insecticides will induce the production of mixed function oxidase systems in the livers of both birds and mammals (JEFFERIES, 1975). These will then metabolise the introduced toxins but are also capable of bringing about hydroxylation and destruction of the steroid hormones. It is likely that similar hepatic enzyme systems are induced by PCBs and that their capacity for its metabolism could well differ between mammals and birds.

3.3 Country levels of organochlorines

Dieldrin. The mean levels of dieldrin in the 62 Scottish, 17 Welsh and 43 English otters analysed from 1965 to 1989 are shown in Table 4a. Geometric and arithmetic means as well as lipid and wet weight concentrations are shown. The lowest levels were found in Scotland, medium levels in Wales and the highest levels in England (x 2.45 to x 3.29 higher than the levels in Scotland). This ranking of dieldrin contamination correlates with the ranking of degree of reduction of the otter populations following the 1957 crash and the reverse ranking of the sizes of the remaining otter populations in terms of sites still occupied at the three national spraint surveys of 1977-79 [Scotland: 73%, GREEN and GREEN (1980); Wales: 20%, CRAWFORD *et al.* (1979); England: 5.78%, LENTON, CHANIN and JEFFERIES (1980)].

Table 4c. The mean levels of total PCB's in 62 Scottish, 17 Welsh and 43 English otters analysed from 1965 to 1989. Geometric and arithmetic means are shown with standard errors, as well as lipid and wet (arithmetic means only) weight concentrations and the range of residues found. As the geometric mean is based on logarithms, the upper and lower limits of one standard error are asymmetrical. The means for the periods 1987 – 1989 (lipid concentrations: geometric mean) and 1983

– 1986 (lipid and wet weight concentrations: arithmetic means) are shown separately to allow more accurate comparisons between countries.

Country	*n*	Geometric mean residue (µg/g)	Range of one St. Error	Range of residues	1987-1989 only	
					n	Geometric mean residue (µg/g)
Lipid weight concentrations of total PCB's						
Scotland	62	30.63	25.18 – 37.21	0 – 984.56	9	98.18
Wales	17	11.04	6.51 – 18.31	0 – 128.47	4	51.72
England	43	8.57	5.84 – 12.39	0 - 507.41	11	60.95

Country	*n*	Arithmetic mean residue ± St. Error	Range of residues	1983-1986 only	
				n	Arithmetic mean residue ± St. Error
Lipid weight concentrations of total PCB's					
Scotland	62	76.41 ± 17.48	0 – 984.56	25	81.80 ± 38.46
Wales	17	34.51 ± 8.90	0 – 128.47	7	47.21 ± 8.41
England	43	54.11 ± 14.33	0 – 507.41	11	51.11 ± 13.41
Wet weight concentrations of total PCB's					
Scotland	62	2.239 ± 0.380	0 – 19.41	25	2.352 ± 0.772
Wales	17	1.409 ± 0.334	0 – 4.07	7	2.116 ± 0.425
England	43	1.751 ± 0.438	0 – 14.92	11	1.906 ± 0.530

DDE. The mean levels of DDE in the otters of the three countries are presented in Table 4b in the same way as for dieldrin. The arithmetic means for lipid and wet weight concentrations are very similar in Scotland, Wales and England. This is particularly so for the arithmetic means of lipid weight concentrations in the three countries. The most variation is obtained by using the geometric means of lipid concentrations. The above similarity in residues could be expected with a long-term contaminant (some 43 years since the start of use in 1946) which is very persistent in the environment and with a very long retention time within the vertebrate body. The biological half-life of DDE in pigeon tissue has been estimated at 240 days compared to 47 days for dieldrin (WALKER, 1983).

PCB. The mean levels of total PCBs in the 122 otters of the three countries for the period 1965 to 1989 are shown in Table 4c presented as for dieldrin. All three forms of analysis show Scotland with the highest concentrations, with England having only 28% to 78% of that level. Wales has a level either slightly above or below that of England, depending on the analysis.

One problem in comparing countries in their PCB levels is that there were fewer Scottish animals in the early periods (see Table 1) when PCB was either absent or only present in trace amounts. So Scottish mean levels for otters could be falsely elevated over those of their southern counterparts. Thus, two short time periods, 1983-86 and 1987-89, have been analysed separately in Table 4c. All forms of analysis again show Scotland with the highest residues with England and Wales closer together. Arithmetic means of wet weight concentrations for 1983-86 show a progression with increasing PCB residues from England to Wales to Scotland. This may be the most accurate ranking in terms of relative contamination considering that 'England' includes Eastern England with its low PCB residues as well as the South-

west and Northern England (see Table 6). A list of all those otters found with very high total PCB residues (above 110µg/g: lipid concentration) is shown in Table 5. Most (64%) are Scottish with South-west England providing the majority of the remainder (27%).

Table 5. The geographical distribution of otters found dead between 1977 and 1989 with very high lipid concentrations of PCB in the liver (i.e. all those over 110µg/g lipid). The highest numbers and the highest residue levels are in Scotland and the South West of England, with only one in Wales. There was no indication that these levels were lethal. The distance (km) of each of these high PCB otters from the coast is also shown. The otters have been divided into two groups for analysis. The largest group of 15 otters (marked +) are from Western and Northern coasts of Britain, while those 7 marked 0 are from the East coast of Scotland with one from West Sussex (see text).

Country	County or Region	Group	Lipid concentration of PCB (µg/g) in liver	Distance from coast (km)
1977-1979				
Scotland	Grampian	0	116.47	40.14
	Shetland	+	121.26	0
	Shetland	+	128.21	0
	Borders	0	226.33	54.71
	Shetland	+	266.10	0
England	Somerset	+	132.46	16.28
	Somerset	+	138.00	16.28
1983-1986				
Scotland	Central	0	113.82	49.88
	Dumfries & Galloway	+	117.58	0.48
	Tayside	0	165.31	43.44
	Jura	+	984.56	0
England	West Sussex	0	120.07	29.12
1987-1989				
Scotland	Tayside	0	113.98	1.13
	Lewis (W. Isles)	+	180.13	2.57
	Lewis (W.Isles)	+	180.54	2.74
	Highland	0	256.61	9.98
	Lewis (W.Isles)	+	342.22	0.10
England	Cornwall	+	136.10	4.83
	Devon	+	233.44	13.68
	Devon	+	244.75	5.31
	Devon	+	507.41	6.44
Wales	Dyfed	+	128.47	9.65

Table 6. The arithmetic mean (± Standard Error) lipid and wet weight concentrations of total PCB's in otters found dead in four separate regions of England between 1977 and 1989.

Region of England	n	Arithmetic Means	
		Lipid PCB	Wet weight PCB
South West	13	126.92 ± 38.44	3.934 ± 1.129
South Coast	2	114.11	3.960
North West	3	47.48 ± 27.56	2.417 ± 1.283
Norfolk/Suffolk	7	43.43 ± 11.91	1.276 ± 0.447
All England	**25**	**92.99 ± 21.62**	**3.010 ± 0.648**

3.4 Within country variations in concentrations of total PCBs

The thesis presented by MASON and MACDONALD (1993) is that the area or country population declines of the otter are related to the lipid weight concentrations of total PCB found in the tissues of otters from those areas or countries. Thus, they show (in their Figure 1) a progression in PCB lipid residues decreasing from East Anglia to South-west England to Wales and to Scotland, with East Anglian otters having over five times the amount of PCB in their organ lipids as those from Scotland. This would, of course, correlate with the ranking of the highest otter population declines.

However, in the present analysis of a larger and different sample of otters, not only is the level of total PCB higher in Scotland than in England but the levels within England show the opposite trend to that given by MASON and MACDONALD (1993). Thus, the highest concentrations of total PCBs are to be found in South-west England, with the area of the extreme South Channel coast being close behind it. Otters from North-west England have much lower concentrations and those from East Anglia the lowest of all; only 34.2% of the south-west levels (Table 6). In addition, whereas many (46.2%) of the South-west (Cornwall, Devon, Somerset) otters have very high PCB residues (i.e. those above 110μg/g: lipid weight total PCB in their livers; see Tables 5 & 7), none of those from East Anglia (Norfolk, Suffolk) fall into this group (Table 7). Indeed, this region has PCB levels below average for England as a whole (Table 6). Thus, the rankings of PCB concentrations within England and between England and Scotland, which we present here, are the exact opposite of those shown by MASON and MACDONALD (1993).

What could be the reason for this anomaly? One problem is that MASON, FORD and LAST (1986) and MASON and MACDONALD (1993) provide analytical data in terms of amounts of PCB in liver lipid only, with no comparative data on wet weight concentrations or lipid content within that organ. There can be very large increases in the lipid concentrations of organochlorines with emaciation following disease or senility with no increases in their wet weight organ contents (see Section 2). At least one of the East Anglian otters analysed by MASON, FORD and LAST (1986) with a very high PCB lipid concentration was emaciated (KEYMER *et al.*, 1988; SPALTON and CRIPPS, 1989). In order to rule out such a problem in the present analysis we provide the wet weight PCB concentrations for the same otters from the same regions of England in Table 6. As can be seen, the rankings of the regional residues are almost the same as with the lipid concentrations, i.e. with East Anglian otters having 32.4% of the PCB concentrations of those inhabiting the south-west. Indeed, there are

meteorological reasons for PCBs to be distributed as we have shown in Tables 4c, 5 and 6 (see Section 7). Thus, as well as the timing of PCB increases coinciding with otter recovery, the distribution of high PCBs is negatively correlated with otter declines.

Table 7. The wet weight and lipid weight concentrations of total PCB's in the livers of all 7 otters found dead in the East Anglian counties of England (Norfolk and Suffolk) and all 13 of those from the counties of the South West (Somerset, Devon, Cornwall) in the years between 1977 and 1989. The East Anglian otters have much lower PCB residues than those from the South West of England and are below average for England as a whole.

Region of England	**County**	Wet weight **concentration of PCB (µg/g)**	**Lipid weightconcentration of PCB (µg/g)**
East Anglia	Suffolk	0	0
	Norfolk	0.29	5.67
	Norfolk	0.55	40.00
	Norfolk	0.93	42.29
	Norfolk	1.73	73.00
	Norfolk	2.09	81.08
	Suffolk	3.34	61.98
***n* = 7**	**Mean (Arith)**	**1.28**	**43.43**
South-west	Devon	0.09	2.41
	Devon	0.20	6.58
	Somerset	0.98	37.69
	Cornwall	1.22	39.30
	Somerset	1.22	52.72
	Cornwall	1.68	55.26
	Devon	2.94	63.82
	Somerset	4.30	138.00
	Somerset	4.34	132.46
	Cornwall	5.58	136.10
	Devon	6.78	233.44
	Devon	6.89	244.75
	Devon	14.92	507.41
***n* = 13**	**Mean(Arith)**	**3.93**	**126.92**

3.5 The minor organochlorine pollutants found in otters

Five other organochlorine pollutants occurred in the otters analysed but to a lesser extent than the main three pollutants described above. These were BHC, Heptachlor epoxide, HCB, TDE and DDT. They occurred in 25, 21, 20, 8 and 1 otters, respectively. These numbers amount to 20.5, 17.2, 16.4, 6.6 and 0.8% of the total 122 otters analysed and compare to 113 (92.6%), 113 (92.6%) and 96 (78.7%) containing DDE, dieldrin and PCBs.

BHC. was found in specimens dying from 1971 to 1987. The mean wet weight concentration was 0.085 ± 0.008µg/g and the mean lipid weight concentration was 2.13 ± 0.26µg/g in those 25 animals containing this pollutant. The overall means, if related to the total 122 otters analysed, were at 0.017 (wet weight) and 0.437µg/g (lipid weight).

Heptachlor epoxide was found in specimens dying from 1965 to 1986. The mean wet weight concentration was 0.133 ± 0.015µg/g and the mean lipid weight concentration was 3.94 ± 0.47µg/g in those 21 animals containing this pollutant. The overall means, if related to the total 122 otters analysed, were 0.023 (wet weight) and 0.679µg/g (lipid weight).

HCB was found in specimens dying from 1985 to 1989. The mean wet weight concentration was 0.050 ± 0.010µg/g and the mean lipid weight concentration was 1.64 ± 0.36µg/g in those 20 animals containing this pollutant. The overall means, if related to the total 122 otters analysed, were 0.008 (wet weight) and 0.269µg/g (lipid weight).

TDE was found in specimens dying from 1971 to 1985. The mean wet weight concentration was 0.959 ± 0.554µg/g and the mean lipid weight concentration was 19.84 ± 9.75µg/g in those eight animals containing this pollutant. The overall means, if related to the total 122 otters analysed, were 0.063 (wet weight) and 1.30µg/g (lipid weight). The TDE could have been derived from the post mortem breakdown of consumed DDT in the otter analysed (see above) or from feeding on carrion in which the breakdown had already occurred.

DDT Only one otter, which died in Wales in 1971, still contained a small amount of DDT (0.03µg/g: wet weight; 0.78µg/g: lipid weight). The same animal contained 0.05µg/g: wet weight (1.30µg/g: lipid weight) of TDE in the liver, indicating that post mortem breakdown was proceeding at the time of analysis (see Section 3.1).

3.6 The lethal concentration of dieldrin for otters: a recorded incident

The series of otter analyses reported in this paper contain one lethal incident involving dieldrin. In May 1972 a woollen mill at Tonedale on the River Tone, near Taunton, Somerset was reported to have had a spill of mothproofing fluids into the river. Dieldrin was used for mothproofing for longer than it was used in agriculture, i.e. until 1983. There was a fish kill and two otters were found dead about four miles (6.44km) apart and either side of the mill. The upstream otter was caught up in a riverside wire fence after a thunderstorm had raised the water level. This body was not retrieved and was swept away. The downstream otter (No. A297), found in a field below Clavenger Farm, was received from James Williams. It was a female of 14 lbs (6.35kg) weight in good body condition. It was not pregnant and there was no obvious cause of death at post mortem examination. There was some decomposition. The liver was removed and weighed 281.60g (4.43% of body weight). Samples of liver and brain were removed for analysis. They contained 58.87 and 89.26mg/g extractable lipid, respectively, i.e. this shows no sign of disease emaciation.

Analysis of the liver showed very high wet weight concentrations of 13.95 µg/g dieldrin and 19.53µg/g DDE (236.96µg/g and 331.75µg/g: lipid concentrations, respectively). No PCBs were found. The brain contained wet weight concentrations of 5.39µg/g dieldrin and 5.97µg/g DDE (60.39µg/g and 66.88µg/g: lipid concentrations, respectively). Again no PCBs were found. Heavy metal analyses of the liver showed dry weight concentrations of 28.34µg/g Hg, 212.53µg/g Zn, 72.36µg/g Cu and < 0.20µg/g Cd, with no lead.

The above dieldrin concentration of 13.95µg/g: wet weight is considered to be lethal. However, it could also be an ‘overkill’ and so falsely high, i.e. an animal can

consume more than the lethal dose of dieldrin before it dies. This level can be compared to those for other species. Thus, badgers have been found dead with 16.9 to 46.0µg/g dieldrin in the liver (JEFFERIES, 1969) and anything over 1µg/g is considered to be lethal to the very sensitive fox (BLACKMORE, 1963). Stoats may die at around 2 to 3µg/g (JEFFERIES, *unpublished*) and predatory birds at around 10µg/g (COOKE, BELL and HAAS, 1982). All are wet weight concentrations.

The total body load of dieldrin and the amount consumed by otter A297 can be estimated using data provided by acute toxicological trials with bank voles consuming dieldrin dressed wheat. Thus, the liver load formed 13% of the total body load of dieldrin in this species (JEFFERIES, 1969). As otter A297 had a concentration of 13.95µg/g in a liver weighing 281.60g, then the liver would have contained 3.9283mg dieldrin and the body load would have been 30.22mg. Further research (JEFFERIES, 1969) showed that in acute toxicological trials, mammals accumulated 55% of the dieldrin consumed in their body tissues. This would suggest an estimated dieldrin consumption of 54.94mg for otter A297. This compares to the female badger of 8.10kg found dead on 24 March 1967 containing 38.0µg/g dieldrin in a liver weighing 391g and which had a total body load of 114mg dieldrin after a consumption of 207mg of this compound (JEFFERIES, 1969).

3.7 Analyses of freshwater fish

Fish form 66.2 to 99.4% of the diet of the otter in Britain with eels (*Anguilla anguilla*) forming 16.2 to 54.4% of total dietary items (MASON and MACDONALD, 1986). There have been several published analytical studies of the concentrations of organochlorine insecticides and PCBs in British freshwater fish, some of which have included eels. The first study by PRESTT (1970) was made during the period when DDT and dieldrin were in considerable use. Prestt took 45 roach (*Rutilus rutilus*), seven bream (*Abramis brama*) and 18 eels from the rivers and drainage channels near to the Troy heron colony in Lincolnshire between 1964 and 1968. Analyses were of muscle and PCB levels were not measured (most of the study took place before analysis started). All the fish were contaminated with DDE and dieldrin. Roach muscle contained a mean of 0.048 (range: 0.003 - 0.213)µg/g DDE and 0.018 (0.003 - 0.139)µg/g dieldrin. Another cyprinid, the bream, contained 0.118 (0.043 - 0.358)µg/g DDE and 0.036 (0.012 - 0.055)µg/g dieldrin. The eels contained a mean of 0.398µg/g DDE + 0.049µg/g dieldrin in the muscle. All are wet weight concentrations.

Some calculations are necessary to convert these data to those for minced samples, as would be eaten by otters in the mid-1960s, and to determine the likely range maxima for eels. JEFFERIES and FREESTONE (1985) showed that in the cyprinid chub (*Leuciscus cephalus*), the muscle:mince ratio was 0.083 for total organochlorine concentration (i.e. a conversion factor of x 12.05 to estimate mince from muscle concentration). Also, an examination of the data from three species (PRESTT, 1970; JEFFERIES and FREESTONE, 1985) shows that, in the fish samples analysed, the range maxima were, on average, 3.4 times greater than the mean. Thus, the mean DDE levels in the minces of roach, bream and eels would have been 0.58, 1.42 and 4.80µg/g, respectively, (with range maxima of 2.6, 4.3 and 16.3µg/g: wet weight DDE) in 1964-68. In addition, the mean dieldrin levels in the minces of these three species in 1964-68 would have been 0.22, 0.43 and 0.59µg/g (with range maxima of 1.7, 0.7 and 2.0µg/g: wet weight dieldrin).

Further comparative analyses of the organochlorine residues in cyprinids and eels from Eastern England were made about twenty years after those of PRESTT (1970). JEFFERIES and FREESTONE (1985) analysed five chub taken from the River Black Bourn in Suffolk in 1982 as part of the preliminary work for an otter release project (JEFFERIES *et al.*, 1986). Also, SPALTON and CRIPPS (1989) examined 87 eels from the Rivers Glaven and Stiffkey as well as the Cley marshes of North Norfolk in 1986-87.

The study by JEFFERIES and FREESTONE (1985) sampled both muscle tissue and minces of whole fish. The mean muscle wet weight residues in chub were 0.008µg/g gamma BHC and 0.007µg/g total PCB, with no dieldrin or DDE at analysable quantities. The mean wet weight residues were much higher in mince at 0.036µg/g gamma BHC (range: 0.03 - 0.05), 0.065µg/g PCB (range: 0 - 0.22) and 0.080µg/g DDE (range: 0.02 - 0.17). Again no dieldrin was found.

SPALTON and CRIPPS (1989) showed mean wet weight dieldrin, DDE and PCB concentrations in eel mince to be 0.05 - 0.15, 0.06 - 0.15 and 0.07 - 0.21µg/g, respectively, depending on the river sampled. These levels are only 3.1% (DDE) and 25.4% (dieldrin) of the levels found in eels by PRESTT (1970) in Lincolnshire in the 1960s. SPALTON and CRIPPS (1989) noted that the concentrations found in North Norfolk eels were higher than those found in unpublished studies of eels sampled in mid and South Wales but lower than those of eels from the River Taw in North Devon and tributaries of the River Severn (*unpublished*).

4 THE BACKGROUND HISTORY TO THE OTTER DECLINE: THE KNOWN EFFECTS OF THE ORGANOCHLORINES ON OTHER BRITISH WILDLIFE AND THEIR AMELIORATION

4.1 Effects on birds

The initial domestic and agricultural uses of DDT following the end of the Second World War in 1945 soon contaminated pigeons and passerines and passed from these to predatory birds, such as the peregrine falcon, sparrowhawk and merlin. The first noticeable sub-lethal effect was that the eggshells of all three species started to thin and then break from 1946 (RATCLIFFE, 1970, 1993; NEWTON, 1973, 1986; NEWTON, ROBSON and YALDEN,1982) as physiological and endocrine changes occurred (JEFFERIES, 1975). These effects were also noticeable in seabirds, such as gannets (*Sula bassana*), from organochlorine pollution of sea fish (PARSLOW and JEFFERIES, 1977). When the much more toxic cyclodiene organochlorine insecticides, dieldrin and aldrin, first came into use as seed dressings in 1956 they were followed by very large kills of seed-eating birds, particularly in the Eastern counties. Dieldrin has six to 14 times the chronic lethal toxicity of DDT (DeWITT *et al.*, 1960). For example, John Ash (CRAMP, CONDER and ASH, 1962) recorded a kill of 5,668 woodpigeons (*Columba palumbus*), 118 stock doves (*Columba oenas*), 89 pheasants (*Phasianus colchicus*), 59 rooks and 104 birds of other species on a single Lincolnshire estate in spring 1961. Similar kills were so common that dead bodies littered the fields and woodland roosts in the late 1950s and early 1960s. These birds and their bodies contained very high concentrations of dieldrin (TURTLE *et al.*, 1963; JEFFERIES and PRESTT, 1966) as they could consume and carry much more than a lethal dose before death occurred. These very high dieldrin loads in prey species soon caused deaths and population crashes in the peregrine falcon (RATCLIFFE, 1993) and sparrowhawk (NEWTON, 1986). These crashes were most severe in the South and South-east of England. The British populations of the merlin

and kestrel too showed considerable declines (NEWTON, ROBSON and YALDEN, 1981; NEWTON and HAAS, 1988; VILLAGE, 1990). Dieldrin from sheep dipping accumulated in fleeces and mutton fat (CROSSETT, 1989) and passed into golden eagles (*Aquila chrysaetos*) from carrion feeding, so affecting their reproductive success (LOCKIE and RATCLIFFE, 1964; LOCKIE, RATCLIFFE and BALHARRY, 1969).

Dieldrin from sheep dips draining into ditches, streams and rivers and run-off rainwater from arable fields soon caused both coarse fish and eels to become heavily contaminated (see Section 3.7). Consequently, kingfishers (*Alcedo atthis*) and herons were also found dead with high levels of dieldrin in the body (PRESTT, 1970; COOKE, BELL and HAAS, 1982; HAAS and COOKE, 1983). Indeed, the heron carried higher organochlorine (and PCB) residues than any other species of wild bird in Britain (PRESTT, 1970; PRESTT, JEFFERIES and MOORE, 1970). Also, nearly every heron egg laid in the Eastern counties of England had an abnormally thin shell (PRESTT, 1970). One effect of this contamination was that the heron took many more years to recover from the severe winter of 1962-63 (when it showed a decrease of 41% below normal numbers) than was the case for other severe winters in the twentieth century (REYNOLDS, 1974). However, the heron population never crashed to the same extent as did those of the peregrine and sparrowhawk.

4.2 Effects on mammals

The most obvious casualty was the fox and some 1,300 were known to have died in the Eastern counties of England in just five months over the winter of 1959/60 (TAYLOR and BLACKMORE, 1961; THOMPSON and SOUTHERN, 1964). Many died in convulsions and disease (Fox encephalitis) was suspected until BLACKMORE (1963) showed experimentally that this mortality was linked to organochlorine seed dressings. The fox is very sensitive to dieldrin poisoning and any level above 1 μg/g: wet weight in the tissues could be considered a lethal concentration. Badgers were first reported among seed dressing casualties in 1961 (CRAMP, CONDER and ASH, 1962). JEFFERIES (1969) post-mortemed and analysed the bodies of 17 badgers found dead in South-east England in the 1960s and considered that six certainly and six others very probably died of dieldrin poisoning. The source of the dieldrin for both fox and badger was the large number of dead and dying woodpigeons lying in the woods at that time. Those badgers not succumbing to dieldrin poisoning may well have shown sub-lethal effects as there was a considerable drop reported in the numbers of badger cubs produced in some parts of Britain during the 1960s (NEAL, 1977). It is known that dieldrin-induced mortality was sufficient to exterminate the badger social groups in setts studied by JEFFERIES (1969) in Huntingdonshire and Norfolk in the 1960s. The sett in Monks Wood National Nature Reserve then remained empty for nearly 30 years and that in Woodwalton Fen for even longer (CRESSWELL, HARRIS and JEFFERIES, 1990).

The small mammals (field mice, bank voles and field voles) inhabiting the arable fields sown with winter wheat were shown to become rapidly contaminated with dieldrin after drilling, with up to lethal concentrations in the body (JEFFERIES, STAINSBY and FRENCH, 1973; JEFFERIES and FRENCH, 1976). Also, pipistrelle bats sampled in Cambridgeshire in 1968-69 were found to be carrying one third of the lethal level of organochlorine insecticides as their 'background' residue, with just under the lethal level after hibernation with loss of storage fat (JEFFERIES, 1972). However, as far as is known, none of these species showed population declines

similar to those shown by the peregrine falcon and sparrowhawk. The otter is the only known mammalian example (see Section 5).

4.3 Bans on the uses of organochlorines in Britain

Organochlorine insecticides. The very numerous and visible wildlife mortalities of the late 1950s and early 1960s caused great concern. Soon the new seed dressings were under suspicion. A joint committee of the Royal Society for the Protection of Birds, the British Trust for Ornithology and the Game Research Association (CRAMP and CONDER, 1960; CRAMP, CONDER and ASH, 1962) was set up to list the casualties and the Nature Conservancy formed a small research group (including both authors) at Monks Wood to study the effects of the new insecticides and to advise central government. The results of this research [reviewed by MOORE (1965, 1987); COOKE, BELL and HAAS (1982); SHEAIL (1985)] confirmed cause and effect, and, together with public concern, eventually brought about bans on all the uses of the organochlorine insecticides. Recommendations for such a series of progressive bans were made in three reports of the Advisory Committee on Poisonous Substances used in Agriculture but it took 24 years from 1962 to 1986 before all uses were removed. These progressive bans and their effective dates were as follows:

1962: Most of the deaths of seed eating birds were found to occur in cereal growing areas in spring, so a voluntary ban was imposed on the use of aldrin, dieldrin and heptachlor seed dressings on spring sown cereals and their autumn use was restricted to cereals in districts where there was a real danger from wheat bulb fly.
1966: The use of aldrin and dieldrin in sheep dip was banned with effect from 1 January 1966.
1975: A mandatory ban was imposed on the use of aldrin and dieldrin in all seed dressings in 1973. However, stock was allowed to be used up, so the ban was delayed until the end of 1974 with 1975 the first year with no cyclodiene seed dressings.
1981: All agricultural use of aldrin and dieldrin finally banned.
1982: All agricultural use of DDT was finally banned.
1983: Nearly all uses of the persistent organochlorine insecticides had ceased.
1985: Gamma BHC was allowed to be used longer than the other organochlorine insecticides because it was metabolised rapidly in the living vertebrate. However, it still has a higher vertebrate toxicity than DDT so a voluntary ban on its use in sheep dip formulations was brought in from 1 January 1985 (CROSSETT, 1989).
1986: The use of dieldrin for timber treatment ceased but gamma BHC use persisted for a few years longer.

Polychlorinated biphenyls. Around 20,000 guillemots (*Uria aalge*) died in a large wreck in the Irish Sea in the autumn of 1969. Analyses showed large amounts of PCBs (particularly Aroclor 1254) and post mortem examinations showed lesions of hydropericardia, as found in PCB toxicological tests (HOLDGATE, 1971; PARSLOW and JEFFERIES, 1973; PRESTT, JEFFERIES and MOORE, 1970). Monsanto (the manufacturers of the Aroclors) quickly responded by meeting Nature Conservancy staff (including the author, DJJ) and agreeing a ban on all further use of their PCBs in open systems in 1970 (SHEAIL, 1985). A ban on the further use of PCBs in closed systems (e.g. transformers) was brought in during 1986, with all PCBs in existing closed systems to be phased out by 1999 (Anonymous, 1990).

4.4 Recoveries and changes following the bans

There was a great and sudden reduction in the mortality among seed-eating birds in the spring following the introduction of the 1962 ban on the use of aldrin and dieldrin in spring sown seed dressings. However, seed dressing kills, particularly among woodpigeons in Eastern England, continued into the 1970s. Similarly, the deaths of foxes from secondary dieldrin poisoning, although much reduced after 1962, continued until the 1975 ban (D. J. JEFFERIES, *unpublished*). The eggshells of peregrine falcon and sparrowhawk started to increase in thickness again from 1966 and 1970, respectively, though thin eggshells were still found in both species in 1980 (RATCLIFFE, 1993; NEWTON, 1986). The recovery of the populations of the peregrine and sparrowhawk moved eastwards from the north and west and was virtually complete in the peregrine by 1985 (CRICK and RATCLIFFE, 1995) and in the sparrowhawk by 1990 (NEWTON and HAAS, 1984; STROUD and GLUE, 1991).

5 THE LETHAL AND SUB-LETHAL EFFECTS OF THE ORGANOCHLORINES ON OTTERS: THE MECHANISMS BY WHICH THE DECLINE WAS BROUGHT ABOUT

5.1 Lethal effects on otters

After analysing the bodies of several peregrine falcons and their prey in Britain, JEFFERIES and PRESTT (1966) came to the conclusion that the latter contained such high residues that the population crash of this species was most likely caused by the deaths of large numbers of breeding-aged adults after consumption of a few very highly contaminated avian prey. That is, contrary to one of the opinions of the time, that it was due to sub-lethal effects on breeding success. Some evidence for this can be seen from the fact that DDT and DDE were inducing sub-lethal effects in the peregrine and reducing breeding success by thinning their eggshells and causing egg breakage from as early as 1946 (RATCLIFFE, 1958, 1970, 1993). However, the population of the peregrine did not crash until after the introduction of the much more lethally toxic cyclodienes, such as dieldrin, in 1956 (RATCLIFFE, 1993). Sub-lethal effects, such as eggshell thinning, then continued to occur during the following period of lethal toxicity.

We suggest that the same could be said about the population crash of the otter; i.e. it was caused by insecticide-induced high mortality rather than by sub-lethal effects. The one lethal incident we know about in detail; that on the River Tone in Somerset in 1972 (see Section 3.6) showed how rapidly an otter can accumulate a lethal dose of dieldrin and succumb within days of the incident starting. This was an aquatic incident involving contaminated fish. There was, however, another route which would have involved even higher dose rates, i.e. the one which killed hundreds of foxes and badgers.

Thus, in the early 1960s, dead and dying birds, particularly woodpigeons, were lying, and sometimes flapping, in large numbers on the ground in woodland roosts after dieldrin dressed seed had been used locally. For example, MURTON and VIZOSO (1963) counted 50 dead woodpigeons in only 8.74hectares of a woodland roost in Eastern England in March and April 1961, after the spring drilling of dressed cereals. It should be remembered that animals have time to consume many times a lethal dose of dieldrin before it actually kills them ('overkill'). This applies to both prey and predators and analyses of these woodland roost woodpigeons showed residues up to 41μg/g: wet weight dieldrin in the muscle (TURTLE *et al.*, 1963).

Using the data from JEFFERIES and PRESTT (1966) and JEFFERIES and DAVIS (1968) it can be estimated that such a woodpigeon would have a total of 21 mg of dieldrin in the body, not including any gut contents (JEFFERIES, 1969). These are known to have been eaten by badgers in large numbers (woodpigeon feathers in the gut contents). Radio-tracked otters too spend up to 53% of the 24 hours in riparian woodlands (JEFFERIES *et al.*, 1986) and so would have come across many such dead and dying woodpigeons in the late 1950s and early 1960s. Otters are known to kill and eat birds of many species (HARRIS, 1968) and indeed birds may form up to 11% of the diet (MASON and MACDONALD, 1986). Also, they eat carrion (STEPHENS, 1957; CUTHBERT, 1973), so would be likely to consume many of the pigeons they found. As the one analysed poisoned otter (A 297 above) contained 30.22mg dieldrin as a total body load (and this could be an 'overkill') from a possible consumption of 54.94mg dieldrin (see Section 3.6), then it can be seen that an otter need only eat two heavily contaminated woodpigeons to kill it. Similar calculations have been made to show that only ten such woodpigeons would kill a badger (JEFFERIES, 1969) and three to six would kill a fox (BLACKMORE, 1963).

Freshwater fish have not been found to be so heavily contaminated as birds (see Section 3.7) so consumption of a lethal amount of dieldrin from this source would take longer, though it has been found to be possible in the field (see Section 3.6). The wet weight maxima in minced eels in the Lincolnshire dykes in 1964-68 was estimated to be 16.31μg/g DDE and 2.01μg/g dieldrin [see Section 3.7; PRESTT (1970)]. Two captive otters of mean weight 6.5kg consumed a total of 2.08kg of fish each day (STEPHENS, 1957). Thus, the female of 6.35kg which died in the 1972 Somerset dieldrin incident (see Section 3.6) may be expected to have consumed ca. 1kg of wet fish each day. Such a consumption of fish with the Lincolnshire 1964-68 maxima would provide a daily intake of 16.31mg DDE and 2.01mg of dieldrin (see Section 3.7). So, to achieve a 30.22mg dieldrin lethal body load would take 15 days and the 54.94mg dieldrin, considered to have been consumed to produce this body load, some 27 days, or longer with the lower dose rate. Other toxins (440mg DDE in the above case) would have been consumed at the same time. Obviously, the level of fish contamination in the River Tone incident in 1972 (see Section 3.6) was much higher even than this Lincolnshire sample as death occurred much more rapidly. However, this calculation provides an indication of the time it might take to achieve a lethal load when eating fish contaminated at the 'background' level of the time rather than in an 'incident'.

5.2 Vulnerability of the otter

Further to the calculations in Section 5.1 above, there are some potential problems for otters which suggest that, being aquatic, they may have been even more vulnerable to dieldrin poisoning than these figures would suggest and could have died at even lower levels (i.e. levels which would otherwise have been sub-lethal to the species). Thus, whereas badgers attempt to get to safety underground, even when intoxicated (JEFFERIES, 1969), otters naturally attempt to return to the safety of water. Consequently, it has been found essential that wild-caught otters which have been anaesthetised for the fitting of a radio-transmitter harness, should be restrained and kept under observation for five hours after injection. Otherwise, if released, they make straight for water where there would be a high risk of drowning while still inco-ordinated (MITCHELL-JONES *et al.*, 1984). Dieldrin poisoning too is known to produce inco-ordination and apparent blindness in mammals (BLACKMORE, 1963;

JEFFERIES, 1969) and the same risk of drowning would be present even in an aquatic animal.

In addition, laboratory mammals which have received sub-lethal doses of DDT, gamma BHC or dieldrin produce an increased volume of urine (NEGHERBON, 1959; HAYES, 1959) and show consequent thirst (JEFFERIES, 1975). Information from field situations too suggest that dieldrin produces increased thirst in such mammals as ground squirrels (*Citellus tridecemlineatus* and *C. franklinii*), foxes and badgers and there are records of many such inco-ordinated sub-lethally poisoned animals being found to have drowned whilst drinking (SCOTT, WILLIS and ELLIS, 1959; TAYLOR and BLACKMORE, 1961; JEFFERIES, 1969). Many sub-lethally poisoned barn owls were found to have drowned in cattle troughs in the early 1960s. Parallel symptoms to these are shown by animals made experimentally hyperthyroid (JEFFERIES, 1975) and organochlorines are known to produce hyperthyroidism at low dose rates (JEFFERIES, 1969; JEFFERIES and FRENCH, 1971, 1972). Consequently, sub-lethally poisoned otters may have had an additional drive to seek water even when none was nearby; so putting themselves at risk of drowning due to inco-ordination at the waters edge. This, and the low population size, may have been the main reasons why the otter was the only British wild mammal species known to have suffered a population crash through the environmental contamination with the organochlorine insecticides.

5.3 Low population size

Freshwater otters normally live at very low densities and in long linear rather than small 'two-dimensional' ranges as used by badgers and foxes. Thus, the alpha male otter studied by GREEN, GREEN and JEFFERIES, (1984) in Perthshire used a linear territory of good habitat measuring 39.1km (minimum polygon: 57.4km^2). Also, JEFFERIES *et al.* (1986) surveyed a similar sized territory (length: 39.2km; min. polygon: 74.7km^2) used by an adult male otter in Norfolk. The Perthshire territory was known to be used by a further one adult male, three breeding females, one sub-adult male/ adult female, one juvenile and approximately five cubs, besides the alpha male (GREEN, GREEN and JEFFERIES, 1984). Thus, each adult otter occupies 7.8km linear waterway and 11.5km^2 of ground. So, population size can never have been very large. I (DJJ) estimated it to be 7,350 breeding aged otters in the mid-1980s (for England, Scotland and Wales) (HARRIS *et al.,* 1995). Such a population structure, distribution and density is particularly vulnerable to removal of a large proportion of the breeding aged adults as by dieldrin poisoning (or indeed persecution). As adults are removed and territories become empty, the population fragments into smaller and smaller units separated by larger and larger distances (JEFFERIES, 1989*b*; STRACHAN and JEFFERIES, 1996). The remaining units do not join up to form smaller but viable populations but die out one by one as each becomes non-viable (e.g. JEFFERIES, 1988). The truth of this statement was successfully demonstrated by placing captive-bred breeding units of a male and two female otters in the spaces between the remaining fragments in East Anglia, when a rapidly declining regional population was reversed into a rapidly increasing one without any other outside influx (JEFFERIES, WAYRE and SHUTER, 2001). JEFFERIES (1989*a*) suggested that the English otter population was already in a 'stressed' state with a reduced proportion of mature adults due to long-term persecution before the start of dieldrin use added a further pressure which the population could not withstand.

Another problem related to a low population size and shown by both peregrine and otter, is that when a species is uncommon it is extremely difficult to find many or any of the lethally poisoned casualties. Only four peregrines were found dead with high residues (JEFFERIES and PRESTT, 1966) and two otters (this paper) from the large numbers which must have died during their population crashes. In contrast there were many hundreds of poisoned bodies found of some other similarly affected species, such as the fox (TAYLOR and BLACKMORE, 1961; BLACKMORE, 1963; THOMPSON and SOUTHERN, 1964; JEFFERIES, *unpublished*), badger (JEFFERIES, 1969 and *unpublished*) and sparrowhawk (COOKE, BELL and HAAS, 1982; NEWTON, 1986). However, the numbers of casualties found cannot be equated with casualty rate, but is largely a matter of numbers at risk. Thus, the British populations of fox, badger and otter were estimated to be 240,000, 250,000 and 7,350, respectively, in 1995 (HARRIS *et al.*, 1995). Those of the sparrowhawk (80,000 individuals in 1986; NEWTON, 1986) and peregrine falcon (2,340 individuals in 1991; RATCLIFFE, 1993) show a similar differential, explaining the relative lack of peregrine bodies. Another factor is the habitat used by that species. Thus, the otter living in and under water and the peregrine inhabiting mountains, moorlands and rocky coasts mediates against the ease of finding bodies.

5.4 Sub-lethal effects on otters

Research has shown that low doses of the organochlorine insecticides and PCBs produce a wide range of sub-lethal effects on birds and mammals (reviewed by JEFFERIES, 1975). The underlying effect, as shown by the unifying theory of JEFFERIES (1975), is on the transport of thyroid hormones and the production of hyper- (at low doses) and hypothyroidism (at high doses). One would expect that, as with the predatory birds, if many otters were dying from lethal levels of organochlorines, such as dieldrin, then many others must have been carrying sub-lethal residues. Thus, some sub-lethal effects should be observable if pollution was the problem.

This is indeed the case. There are several notable examples which suggest that sub-lethal effects were occurring throughout the period, though they were seldom measured. Thus, in parallel with the reported considerable decrease in the number of badger cubs produced in the early 1960s (NEAL, 1977; see Section 4.2), there was a shortage of otter cubs reported in the same period (CRANBROOK, 1977). Sub-lethal levels of DDT are known to reduce reproductive success (decreased fertility and absence of litters) in dosed mice (BERNARD and GAERTNER, 1964; WARE and GOOD, 1967) and dieldrin has a range of effects on the reproduction of mammals, including decreased litter size (GOOD and WARE, 1969; JEFFERIES, 1975). PCBs too have been found to reduce reproductive success in the mink (*Mustela vison*) in the laboratory, though there were no reports from the field of lower numbers of otter and badger cubs correlating with the period of high PCB levels in otters in the 1980s.

Another of the effects of the organochlorine insecticides and PCBs noted by JEFFERIES (1975) was that of changes in the level of vitamin A storage in the liver. This may be increased with hyperthyroidism in birds fed with low dose rates of DDT and decreased with hypothyroidism as dose rate increases (JEFFERIES and FRENCH, 1971). Low vitamin A stores are found too in DDT and dieldrin-dosed rats (*Rattus norvegicus*) (JEFFERIES, 1975) as well as rats and quail (*Coturnix coturnix*) receiving the PCB, Aroclor 1242 (CECIL *et al.*, 1973). SIMPSON *et al*. (2000) have analysed the vitamin A levels in the livers of 40 otters found dead in South-west England between 1988 and 1996. They found a marked increase in vitamin A levels

over this period which coincided with a significant decline in the levels of organochlorine pollutants in the bodies. Low vitamin A levels were prevalent in the early years of the study. This would, of course, correlate with the high PCBs found in the South-west of England in the 1980s (see above, Section 3.4), but there was a significant negative correlation with the remaining dieldrin in the organs too. Thus, one would expect very low vitamin A stores to have resulted from the high DDT, DDE and dieldrin levels found in the otters living in the late 1950s and 1960s before these residues decreased with time (see Section 3.2). These low vitamin A levels would have had a range of detrimental effects on individual otters (JEFFERIES, 1975).

One condition seen in otters in the years following the start of their population decline, and which may be linked with these sub-lethal effects on vitamin A storage levels, is that of blindness. Thus, WILLIAMS (1993) collected together all the records he could find on otters reported as 'blind', with any descriptions available. He collected 22 records from 11 different counties. The majority were blind in both eyes, so reducing the likelihood that the condition was due to injury. JEFFERIES (1996*b*) augmented these with a further four examples and reclassified them according to age, date and area. Cataracts can be regarded as a normal change associated with senility, as in a nine year old otter from Cley, Norfolk (WELLS, KEYMER and BARNETT, 1989). Eliminating aged otters produces 19 records from 1957 to 1980, which correlates with the period of use of aldrin/dieldrin in Britain, starting in 1956 (JEFFERIES, 1996*b*). There were no records outside these dates. Further, the association with the organochlorine insecticides is even more clear if the records are divided into three periods - in 1957-1959 there were four records (1.3 per year), in1960-1969 nine records (0.9 per year) and in 1970-1980 six records (0.5 per year). These follow the gradual reduction in organochlorine insecticide use in Britain (JEFFERIES, 1996*b*). Also, the distribution of the blind otters showed concentrations in the South of England (52.6%) with the lowest numbers in Scotland (15.8%).

To complete the picture, there are pathways, linked with the organochlorines and PCBs, by which obvious blindness could have been caused in otters. By obvious blindness we mean that which would be obvious to a casual observer. Typical descriptions, such as 'both eyes white' and 'both its eyes were completely white', are such conditions and do not suggest cataracts, but more a surface opacity due to serious corneal lesions. As noted above, hypothyroidism is the usual effect caused by the organochlorines and PCBs and as there is an inter-relationship between thyroid activity and vitamin A metabolism there is a secondary effect of Avitaminosis A (JEFFERIES, 1975). Xerosis of the epithelia and the more serious xeropthalmia and hyperkeratosis affecting the cornea are common in vitamin A deficient mammals. Hyperkeratosis in calves is often accompanied by a discharge from the eyes and followed by corneal opacity (DOXEY, 1971; WEST, 1992). There is a reduced immune system and an increased incidence of disease with hypothyroidism (JEFFERIES, 1975). These are the causal factors for the xerosis and hyperkeratosis of the epithelia of mammals and birds brought about by feeding DDT and dieldrin (NELSON *et al.*, 1944) and the adverse effects of DDT and PCB on the immune system, making them more susceptible to various diseases (NELSON *et al.*, 1944; FRIEND and TRAINER, 1970; JEFFERIES, 1975). Thus, the production of ocular discharge and conjunctivitis followed by keratitis and hyperkeratosis of the cornea caused by vitamin A deficiency, coupled with a reduction in the efficiency of the immune system with hypothyroidism, both induced by organochlorine insecticides, could explain the type of blindness observed in British otters for the two decades

following 1957 (JEFFERIES, 1996*b*). Also, it connects the disease with the organochlorine pollution thought to have caused the population decline.

The distribution and the timing of the blindness incidents suggests that those recorded were produced by dieldrin and DDT rather than the PCBs, though these are capable of causing a similar condition.

6 THE FACTORS LINKING THE DECLINE OF THE OTTER WITH THE USE OF THE ORGANOCHLORINE INSECTICIDES

6.1 The timing of the otter population decline and recovery

One of the major determinants for testing the likelihood that the start of use of the organochlorine insecticides was the trigger for bringing about the crash of the otter population is the timing of that decline in relation to the timing of insecticide use. The only data on the timing of the crash are provided by the records of the packs of otter hounds. CHANIN and JEFFERIES (1978) were able to analyse these records for the Joint Otter Group in terms of finds per 100 days hunting. Summing up the results over the 11 active hunts and plotting these from 1950 to 1976 (see Figure 1) shows quite conclusively that the otter decline started suddenly and was first measurable all over the country in late 1957.

The decline, as shown by the hunt statistics, was most severe in the south and east of England and least severe in the west and north (CHANIN & JEFFERIES, 1978). It was also synchronised all over England and Wales and Southern Scotland; which suggests a newly-introduced man-made factor rather than a disease epizootic. Habitat change and destruction and human disturbance do not act so suddenly nor in so synchronised a fashion all over the country. Dieldrin, aldrin and heptachlor, on the other hand, first came into use in 1955, with major usage by 1956. Highest usage was in the south and east (see Table 8). Thus, there is a correlation in time and area of greatest effect. It is remarkable that their effect on the otter should have been so sudden and so severe, with a noticeable decrease in population size within 18 months. This rapid effect occurred in the sparrowhawk too (NEWTON, 1986). It might be held that this correlation in time between cause and effect is circumstantial. If, on the other hand, there were changes in the rate of decline or in its cessation which correlated with the dates at which various uses of the organochlorine insecticides were banned, then one could say that the link was much firmer. Thus, STRACHAN and JEFFERIES (1996) have re-examined the long series of hunting data which were available to CHANIN and JEFFERIES (1978) (see Figure 1). They re-appraised the earlier section of the decline curve to see if there had been any reaction to the first voluntary ban on the use of aldrin/dieldrin on spring-sown cereals which took effect in 1962. There was indeed a marked change in the slope of decreasing hunting success in 1963, one year after the ban. The steep slope of rapidly decreasing hunting success suddenly slowed and although still decreasing, this continued at a reduced rate. Analysis showed that the slope of the regression line fitted to the total hunt data from 1956 to 1963 ($r = -0.9610$; d.f. 6: $p < 0.001$; equation: $y = 303.22 - 4.04x$, where y = finds/100 days hunting and x = date, i.e. 60 for 1960) was significantly ($t = 5.9328$; d.f. 18: $p < 0.001$) different to that for the period 1963 to 1976 ($r = -0.6722$; d.f. 12: $p < 0.01$; equation: $y = 101.91 - 0.83x$). During the years 1956 to 1963 hunting success was decreasing at the rate of 4.04 finds/100 days hunting per year, but this suddenly changed to a decrease of 0.83 finds/100 days hunting per year after 1963 (i.e. only 20.5% of the former rate of loss). The timing correlates so closely with that of the first ban taking effect that one can only conclude

that the two are connected.

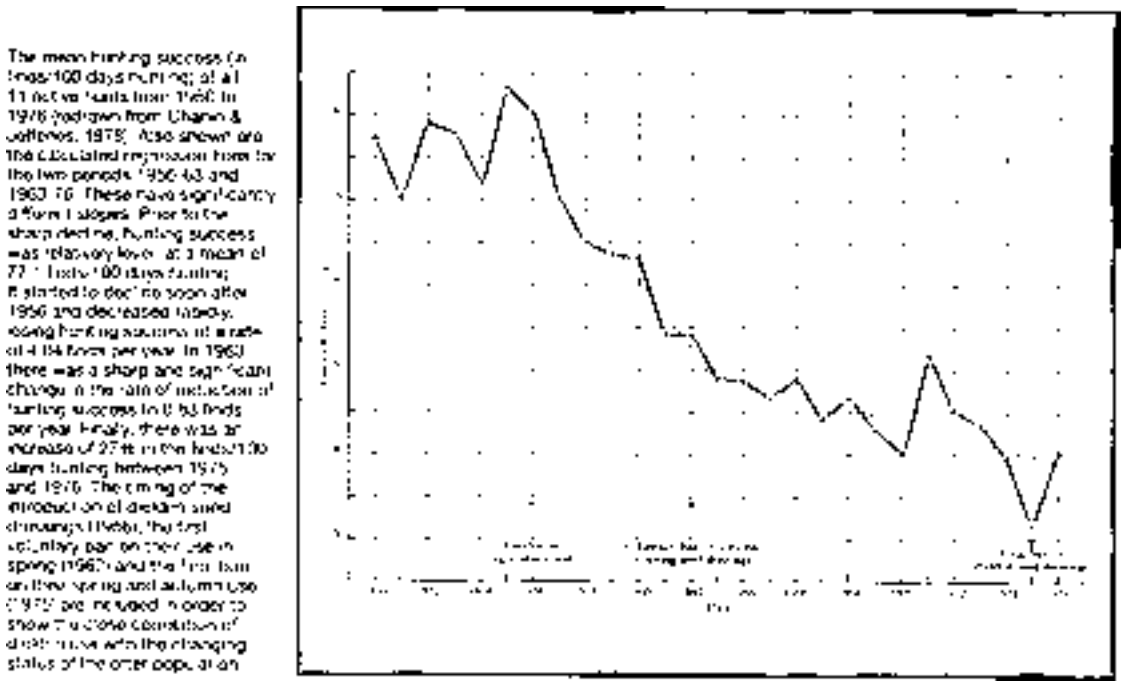


Figure 1.
The mean hunting success (in finds/100 days hunting) of all 11 active hunts from 1950 to 1976 (from CHANIN and JEFFERIES, 1978). Also shown are the calculated regression lines for the two periods 1956-1963 and 1963-1976 (STRACHAN and JEFFERIES, 1996). These have significantly different slopes. Prior to the sharp decline, hunting success was relatively level, at a mean of 77.1 finds/100 days hunting. It started to decline soon after 1956 and decreased rapidly, losing hunting success at a rate of 4.04 finds per year. In 1963 there was a sharp and significant change in the rate of reduction of hunting success to 0.83 finds per year. Finally, there was an increase of 27% in the finds/100 days hunting between 1975 and 1976. The timing of the introduction of dieldrin seed dressings (1956), the first voluntary ban on their use in spring (1962) and the final ban on their spring and autumn use (1975) are included in order to show the close correlation of dieldrin use with the changing status of the British otter population.

Further examination of the data on the hunting success of the western hunts suggested that the nadir of the western otter population decline occurred around 1968 (STRACHAN and JEFFERIES, 1996), after which it started to recover. The year 1968 is only two years after the 1966 ban on the use of dieldrin in sheep dips. The western and northern parts of Britain have the highest density of sheep and so sheep dip use (see Section 6.3).

Examination of the hunting records of the eastern hunts showed that the nadir of the eastern otter population decline did not occur until after hunting ceased in 1977 (STRACHAN and JEFFERIES, 1996). Indeed, examination of the results of the three national otter spraint surveys of England (1977-79: LENTON, CHANIN and JEFFERIES, 1980; 1984-86: STRACHAN *et al.*, 1990; 1991-94: STRACHAN and JEFFERIES, 1996) showed that the eastern nadir occurred between the surveys of 1977-79 and 1984-86, i.e. around 1980 (STRACHAN and JEFFERIES, 1996). This is five years after the final ban on dieldrin use in seed dressings (an eastern problem) in 1975. It was considered that the increasing delay between each dieldrin ban and an effect on the otter population was due to the accumulating dieldrin pollution of the

environment building up from past use (STRACHAN and JEFFERIES, 1996). This took time to wash out or become buried in the silts.

Table 8. The estimated annual area usage of the organochlorine insecticides, (a) aldrin/dieldrin, (b) DDT/BHC and (c) heptachlor in terms of acres treated. (One acre = 0.4047 hectares). The crops treated were largely cereals (wheat, barley, oats), sugar beet, potatoes and edible brassicas. The data are derived from Tables published in the Report by the Advisory Committee on Poisonous substances used in Agriculture and Food Storage (COOK, 1964) and were collected for the years 1962/1963. They cover England and Wales only and are divided into usage in four regions based on Ministry of Agriculture, Fisheries and Food Advisory Regions at that time. These were (1) East: the East Midlands, East Anglia and South East (Wye), (2) North: the Northern area, Yorkshire and Lancashire, (3) West: the West Midlands and the whole of Wales, (4) South: the South East (Reading) and the South West. They show that the Eastern Region had by far the heaviest usage of organochlorines. Note that the acreages of the three tables (a, b & c) are not strictly additive, as many crops may be treated with seed dressings against soil pests at drilling and also later sprayed or dusted with different insecticides to kill foliage pests.

Treatment	East	North	West	South
(a) Estimated acres treated annually with aldrin/dieldrin	438,650	151,371	65,522	57,680
(b) Estimated acres treated annually with DDT/BHC	1,465,100	365,330	284,400	587,200
(c) Estimated acres treated annually with heptachlor	199,000			

Thus, there is further correlation between the dates of the bans and the dates of corresponding positive effects on the otter population. What is more, the bans had their greatest effects on the otter population of certain areas (i.e. west or east) and these areas correlated with the areas of greatest reduction in local organochlorine insecticide usage provided by that particular ban.

It should be noted that the otter populations of Norfolk, Suffolk and Essex never decreased to a nadir and then recovered, but continued to decrease towards extinction. The populations of Suffolk and Norfolk were expected to reach extinction by 1984 and 1986, respectively (STRACHAN and JEFFERIES, 1996). This was not due to continued pollution. Dieldrin and DDE were very low and PCBs were lower than in the recovering west. The most likely cause was fragmentation of the distribution of a small and ageing population below the critical level for breeding and recovery (see Section 5.3) (JEFFERIES, 1989*b*). This was confirmed by placing the first release group of three young otters (male and two females) as a 'probe' into a Suffolk river in 1983 (JEFFERIES *et al.*, 1986). These bred and their progeny are still breeding in the same area (JEFFERIES, WAYRE and SHUTER, 2001). The very rapid recovery of the Norfolk, Suffolk and Essex otter populations since releases started (up to 34% site occupation in Norfolk by 1997; only 11 years after the date of predicted extinction; YAXLEY, 1997; JEFFERIES, WAYRE and SHUTER, 2001) provides further confirmation that moderate PCB levels of 50µg/g: lipid weight were not sufficient to prevent otters breeding and so causing population decreases, as has been suggested (MASON, 1989). Thus, 42.9% of otters analysed from Norfolk and Suffolk in recent years had residues above 50µg/g: lipid weight PCB (see Table 7).

6.2 The correlation between otter density as shown by survey and arable use of aldrin/dieldrin in the east, with particular reference to the wheat bulb fly areas

6.2.1 The two wheat bulb fly areas

Agriculture became sharply and increasingly polarised in Britain during the course of the 20th century, with arable concentrated in the fertile lowland east and stock grazing in the more upland west (TAPPER, 1992; STRACHAN and JEFFERIES, 1996). Wide fields were made by uprooting hedges and developed by the use of artificial fertilisers into large areas of monoculture. Consequently, most of the countries wheat, barley and oats are grown in the eastern half of the country with concentrations in East Anglia.

However, monocultures introduce new problems and research in the early 1950s showed that there were two areas of Eastern Britain which suffered most acutely from attack by the wheat bulb fly *Leptohylemyia coarctata* (GOUGH, 1957). The largest of these areas was in East Anglia, Lincolnshire and East Midlands (*ca.* 30,300km^2) with a smaller one (*ca.* 13,200km^2) in South-east Scotland. They were delineated by BELL (1975) and are shown in Figure 2. These areas were the ones treated most intensively with the new cyclodiene insecticides, dieldrin and aldrin, on their introduction as cereal seed dressings used with organo-mercury fungicides in 1956 (JEFFERIES, STAINSBY and FRENCH, 1973). This is demonstrated by the great differences in acreage treated with these chemicals in the East and West of Britain (see Table 8). In addition, when it was agreed in 1961 that a voluntary ban should be imposed on the use of aldrin, dieldrin and heptachlor seed dressings on spring-sown cereals from 1962, their autumn use was allowed to continue where there was a real danger of wheat bulb fly damage. Consequently, because of their vulnerable status, aldrin and dieldrin continued to be used in the wheat bulb fly areas until the complete and mandatory ban on all use of organochlorine seed dressings was applied from 1975.

6.2.2 Dieldrin residues in wildlife from the wheat bulb fly areas

The previously numerous otter populations had already largely disappeared from the two wheat bulb fly areas before chemical analysis of their bodies had got under way in 1965 (see Section below). Consequently, they are little represented in the series analysed here; completed at a time when most otter bodies were to be found in the west and north. However, the chemical analyses of birds, such as the heron and kestrel, started earlier at Monks Wood and these species were never completely eliminated from Eastern Britain, so we have considerable information on the residue levels carried in their bodies. Thus, the wet weight concentrations of dieldrin in the livers of 128 herons and 213 kestrels found dead from 1963 to 1975 are shown in Table 9 grouped into those found within the two wheat bulb fly areas and those found in the remainder of Britain. The numbers falling into the three residue groupings within and without the wheat bulb fly areas are significantly different in both species (Heron: $\chi^2 = 8.6705$; d.f. 2: $p < 0.02$; Kestrel: $\chi^2 = 58.8019$; d.f. 2: $p < 0.001$). It is clear too that not only were the mean dieldrin residues much higher in both species within the two wheat bulb fly areas, but the lethal casualties from dieldrin poisoning were greater in these areas also, both in actual numbers and on a percentage basis, although the areas occupy only 23.23% of England and 16.76% of Scotland.

Table 9. Variation in organochlorine residue levels between geographical areas: The wet weight concentrations of HEOD (dieldrin) in the livers of 128 herons and 213 kestrels divided into three ascending levels. The third level of over 10 µg/g is lethal. The numbers are further subdivided into

those bodies found within the two small areas with high incidence of wheat bulb fly attack (and so high usage of dieldrin) and those found in the remainder of Britain (see areas in Figure 2). The numbers falling into the three residue groups within and without the wheat bulb fly areas are significantly different in both species. Data are presented for the period 1963-1975 and are derived from COOKE *et al.*, 1982.

Heron (*Ardea cinerea*)				
HEOD residue concentrations µg/g	**0 – 0.9**	**1 – 9.9**	**10 or over**	**Totals**
Number outside WBF areas	37	27	5	69
Number inside WBF areas	29	15	15	59
				128

Kestrel (*Falco tinnunculus*)				
HEOD residue concentrations µg/g	**0 – 0.9**	**1 – 9.9**	**10 or over**	**Totals**
Number outside WBF areas	72	37	11	120
Number inside WBF areas	15	30	48	93
				213

For example, 48 kestrels were found dead containing lethal levels of dieldrin within the wheat bulb fly areas over the years 1963-1975, compared to only 11 in the remainder of Britain. There would, of course, have been many more casualties than 48 and 11 but their bodies remained unfound. The number 48 represents 52% of all those kestrels found dead in the wheat bulb fly areas, whereas only 9% of those found dead elsewhere died from this cause. This would have been so for otters too, with both high residues and numerous lethal casualties in these two small eastern areas.

6.2.3 Density of otters within and without the wheat bulb fly areas after the population crash

If we are correct and there were indeed numerous lethal casualties among otters in the arable areas of the east due to dieldrin poisoning, then, like the predatory birds, these would be particularly high in the wheat bulb fly areas and lowest away from cultivated Britain in the west. This can be checked because otters have been surveyed every seven years since 1977 by searching for their spraints (faeces) over 7,504 600-metre survey sites covering England, Scotland and Wales (JEFFERIES, 1997). Relative density can then be gauged by calculating the percentage of occupied sites in ordnance survey squares or regions (JEFFERIES in LENTON, CHANIN and JEFFERIES, 1980). Thus, this parameter can be used to compare the otter density remaining in each area or region after the population crash had occurred, i.e. in 1977-1979, when the first survey was made. This comparison is made in Table 10 between the wheat bulb fly areas, the remaining areas of the east and then the west separately for both Scotland and England with Wales (see Figure 2 for areas used in this analysis). This Table shows quite clearly a three-step gradient with highest otter density in the west, decreasing across the country to the wheat bulb fly areas of the east. This differential was present in Scotland, with a two-fold variation across the three-step gradient. Here, the difference in otter density between the west and the eastern remainder in 1977-1979 was significant ($\chi^2 = 179.51$; d.f. 1: $p < 0.001$) as was that between the eastern remainder and the wheat bulb fly area in the same period ($\chi^2 = 43.50$; d.f. 1: $p < 0.001$). However, it was most marked in England and Wales, where the variation across the gradient was 50-fold. Again, the difference in otter

density between the west and the eastern remainder in 1984-1986 was significant (χ^2 = 344.78; d.f. 1: $p < 0.001$) as was that between the eastern remainder and the wheat bulb fly area in 1984-1986 (χ^2 = 83.56; d.f. 1: $p < 0.001$; the gradient was present in 1977-1979 but the numbers then were too low for analysis by chi-squared test). Thus, the same answer is provided twice independently.

Figure 2 The agriculture of Britain polarised over the twentieth century into largely arable in the East and stock rearing in the West. The above figure shows the situation as it was around the late 1980s (data from TAPPER (1992) and STRACHAN and JEFFERIES (1996)) with areas (marked in black) to the West of the North-South line having less than 20% of farmland cultivated and areas to the East over 20% cultivation. Devon falls into the former and Cornwall into the latter category. The two Eastern areas within the broken lines (one, A, in Eastern Scotland and one, B, in Eastern England) are those known to suffer most acutely from attack by wheat bulb fly and would have been treated most intensively with organochlorine soil insecticides in the 1950s to 1970s. They are as delineated by BELL (1975) and based on the studies by GOUGH (1957). These wheat bulb fly areas have over 60% of the farmland cultivated (STRACHAN and , 1996). The Northern Isles (Orkney, Shetland), omitted from the above map, fall into the Western (black) category.

These analyses show a remarkable difference between otter occupation in the wheat bulb fly areas of both Scotland and England and that in their surrounding areas, still with high levels of farmland cultivation but with lower use of dieldrin. In England, this difference in dieldrin usage resulted in a large area, 23.2% of the total area of the country, having such a low otter population after the decline that only one in every 278 survey sites showed evidence of presence in the 1977-1979 survey. This very reduced population remained low, showing little recovery, at the second national survey of 1984-1986 and indeed would have remained low at the third survey of 1991-1994 if the numbers had not been augmented by release of captive-bred otters by the Otter Trust (JEFFERIES, WAYRE and SHUTER, 2001).

Thus, following the otter population crash which started in 1957, coincidentally with the start of dieldrin use in 1956 (see Figure 1), there is indeed a significant correlation between the degree of otter decline and the degree of dieldrin usage on each regions cultivated crops.

Table 10. Otter occupation related to the degree of cultivation and the wheat bulb fly areas: The percentage occupation by otters of sites surveyed in (a) Western Scotland, (b) Eastern Scotland and (c) the Scottish wheat bulb fly area in Eastern Scotland at the first survey in 1977-1979. Also, the percentage occupation by otters of sites surveyed in (d) Wales and Western England, (f) the English wheat bulb fly area in Eastern England and (e) the remainder of Eastern England in 1977-1979 and

1984-1986. The whole area of Scotland was not surveyed in 1984-1986. It can be seen that the lowest occupation by otters was in the two wheat bulb fly areas (c,f) (being very low in England with only 1 in 278 sites occupied) which, with the highest degree of cultivation and the greatest vulnerability to attack by wheat bulb fly, received the heaviest application of aldrin and dieldrin. The remainders of Eastern Scotland and England (b,e) were occupied by otters to a medium extent, while the Western areas of England, Scotland and Wales (a,d), with the lowest degree of arable cultivation and the lowest organochlorine applications on crops, had the highest density of otter occupation. See Figure 2 for areas used in this analysis.

Country/Region/ County	**Degree of cultivation**	**1977 - 1979**			**1984 - 1986**		
		No sites surveyed	**No occupied**	**% occupied**	**No sites surveyed**	**No occupied**	**% occupied**
SCOTLAND							
(a) Western Scotland							
Western Isles	< 20% of farmland cultivated	227	221	97.4			
Northern Isles		176	170	96.6			
Highland		1424	1313	92.2			
Dumfries & Galloway		414	341	82.4			
Strathclyde		875	532	60.8			
Overall		**3116**	**2577**	**82.70**			
(b) Eastern Scotland							
Grampian	20 – 60% of farmland cultivated	494	381	77.1			
Borders		279	87	31.2			
Overall		**773**	**468**	**60.54**			
(c) Scottish wheat bulb fly area							
Tayside	> 60% of farmland cultivated	410	251	61.2			
Central		146	71	48.6			
Fife		88	4	4.5			
Lothian		103	0	0.0			
Overall		**747**	**326**	**43.64**			
ENGLAND AND WALES							
(d) Western England and Wales							
Wales	< 20% of farmland cultivated	1030	210	20.3	985	388	39.4
Devon		228	43	18.9	228	89	39.0
North-west England		165	6	3.6	165	26	15.8
Overall		**1423**	**259**	**18.20**	**1378**	**503**	**36.50**
(e) Remainder of England	20-60% of farmland cultivated	1714	118	6.88	1778	165	9.28
(f) English wheat bulb fly area	> 60% of farmland cultivated	833	3	0.36	1017	6	0.59

6.2.4 Effects of the amount of arable on the hunting success of otterhounds

It is of value to examine and confirm the effects on the otter of the amount of arable, and so dieldrin use, in an area through a completely independent set of data than those provided by the national spraint surveys. The hunting success of the eleven packs of otterhounds in Britain south of lowland Scotland which were hunting up to legal protection in 1978, provide the means for such an examination.

The proportions of each hunt territory falling into each of three bands of cultivation intensity (less than 20%; 20 - 60%; over 60% of farmland cultivated; using data from Figure 38 of STRACHAN & JEFFERIES, 1996) were counted using a transparent square grid. Then scores of 10, 40 and 80 were attached to each of the above three levels of cultivation. Overall scores for each hunting territory could be

obtained by multiplying each of these cultivation intensity scores by the proportion of the hunt territory including them (e.g. Northern Counties Otterhounds: 0.0476 x 10 + 0.8730 x 40 + 0.0794 x 80 = overall score of 41.75). Using this method, the maximum score for an area with high intensity of cultivation would be 80 and the minimum score for an area with low intensity of cultivation would be 10. The higher the overall score of a hunt territory, the greater the amount of arable and the lower the score the greater the amount of pasture and livestock rearing.

Grouping of the six highest scoring hunting territories and the five lowest scoring territories provides significantly different (t = 3.5121; d.f. 9: $p < 0.01$) mean cultivation scores for the two groups (see Table 11).

If the mean hunting success of these two groups of hunts is calculated in terms of numbers of otters found per 100 days hunting for the period 1950-1955, i.e. before dieldrin was used on the arable areas of their territories, then although it is slightly lower in the high scoring group [probably due to high persecution of otters by gamekeepers in the south and east; STRACHAN and JEFFERIES (1996)], there is no significant difference (t = 1.2105; d.f. 9: not significant) between the two (see Table 11).

Table 11. The relationships between the degree of intensity of cultivation and hunting success in the 11 hunt territories before and after the population crash of 1957. Note that there is no significant difference in hunting success in areas with high and low amounts of arable in the period before dieldrin use and before the population crash. However, after the introduction of dieldrin in seed dressings in 1956 the difference in hunting success between areas with high and low amounts of arable, and so high and low amounts of dieldrin use, is statistically significant.

Grouping	**Otter Hound hunt territories**	**Score for intensity of cultivation**	**Hunting success 1950-1955**	**Hunting success 1966-1971**	**Percentage decrease in hunting success**
Group with high intensity of cultivation	Eastern Counties Buckingham Courtenay Tracy Northern Counties Culmstock Dartmoor	49.70 ± 8.93	70.50 ± 4.66	33.17 ± 3.10	53.03 ± 2.87
Group with low intensity of cultivation	Hawkstone Border Counties Kendal and District Dumfriesshire Pembroke and Carmarthen	14.34 ± 2.21	83.60 ± 10.54	58.40 ± 8.36	28.51 ± 9.88
	Significance of difference				

If, on the other hand, this calculation is made for the period 1966-1971, i.e. after the high use of dieldrin in the arable areas of their territories, there is a significant difference between the hunting success of the two groups of hunts (t = 3.0447; d.f. 9: $p < 0.02$). Those hunts with the lowest amounts of arable land have the highest success (see Table 11). The hunting success of both groups of hunts decreased but the percentage decrease of the low intensity cultivation group was significantly *(*t = 2.5905; d.f. 9: $p < 0.05$) lower than that of the high intensity cultivation group (see Table 11). This link between fewer otters to hunt and degree of arable cultivation after, but not before, dieldrin use provides independent confirmation of the

importance of organochlorine insecticides in the otters decline. The use of the industrial PCBs does not correlate with agricultural practices in any way.

Further use of these cultivation scores and the hunting success of the above 11 packs of hounds indicates that there is a significant linear relationship between the cultivation score and the estimated nadir for the otter decline (from hunting records; STRACHAN and JEFFERIES, 1996) in each hunt territory. The higher the cultivation score (i.e. the more towards the east and south), then the higher and longer the use of dieldrin and the later the nadir in the otter decline before recovery started (r = + 0.8655; d.f. 10: $p < 0.001$; equation: $y = 64.37 + 0.2615x$, where y = date of nadir (1978 = 78) and x = score for intensity of cultivation). The final mandatory ban on dieldrin use in seed dressings was not imposed until 1975, whereas the final ban on dieldrin use in sheep dips was imposed much earlier in 1966. Thus, use of a different approach and the form of the curve of changing hunting success with time in each hunt territory, provides additional strong evidence that use of dieldrin in agriculture and the banning of it were the major causes of the decline and recovery of the British otter population.

6.3 The effect on otters of the veterinary use of dieldrin in sheep dips in the north and west

Although the major effects of the cyclodiene organochlorine insecticides were to be seen in the arable east and south of Britain, particularly in the two wheat bulb fly areas (see Section 6.2), smaller but by no means inconsequential amounts were used for veterinary purposes in the livestock rearing areas of the north and west. Thus, dieldrin was used extensively in sheep dips to control fly strike because its persistence provided a long period of larvicidal activity (12 - 20 weeks). This long persistence meant that dipping was only necessary once a year rather than the usual twice and COOK (1964) noted that "the majority of farmers are using those single dips which contain dieldrin". Their recommended use was intended to provide bath concentrations of 0.02 to 0.05% of active ingredient. After dipping, these baths were often allowed to run into ditches from which they polluted local waterways. They also resulted in a mean level of 2.4μg/g: wet weight of dieldrin being found in British-produced mutton kidney fat (COOK, 1964). Most of this veterinary use was in the west because of the polarisation of British agriculture (TAPPER, 1992; STRACHAN and JEFFERIES, 1996). The resulting pollution did not have such a marked effect on the otter as did that of seed dressings in the east, but a considerable effect can be demonstrated nevertheless.

The presence of an effect and its degree can be gauged by correlating the amount of decline of the otter population in the western and northern areas of Britain with the use of dieldrin in sheep dips in those areas. THOMPSON and BADDELEY (1991) mapped area sheep densities from the 1960s over the whole of Britain in terms of four grades, i.e. less than 1, 1 to 2, 2 to 3 and greater than 3 sheep per hectare. It follows that these sheep densities can be used as indicators of the relative amounts of insecticide used in sheep dipping in different regions, counties and countries. The post-decline otter densities to compare with these regional sheep densities are provided by the results of the first national otter survey carried out over 1977-1979 (LENTON, CHANIN and JEFFERIES, 1980; CRAWFORD *et al.*, 1979; GREEN and GREEN, 1980). Only the information from Wales, Scotland outside the wheat bulb fly area and the English counties of Cornwall, Devon, Cumbria and Northumbria can be used in order to avoid confusion with the seed dressing effect on the eastern and

southern areas of Britain. Thus, the percentage occupation of survey sites by otters in these regions, counties and countries is shown in Table 12 divided into four groups according to the sheep density of those areas.

Table 12. The percentage occupation of survey sites by otters in areas and regions of Northern and Western Britain in 1977-1979, i.e. the sheep rearing areas. These areas are further divided into four groups with regard to the density of sheep maintained in that area in the 1960's. The density of sheep reared in an area is an indicator of the relative amount of insecticide, e.g. dieldrin, used in sheep dipping in that area. This is highest in Borders, Wales and Cumbria and lowest in Northern Scotland, the Western and Northern Isles.

Country	**County/Region**	**Sheep per hectare**			
		< 1	1-2	2-3	> 3
Scotland	Shetland	97.52			
Scotland	Western Isles	97.36			
Scotland	Orkney	94.55			
Scotland	Highland	92.21			
Scotland	Dumfries and Galloway			82.37	
Scotland	Grampian		77.13		
Scotland	Strathclyde		60.80		
England	Cornwall		31.54		
Scotland	Borders				31.18
England	Devon			23.63	
Wales					20.39
England	Northumbria		17.11		
England	Cornwall		11.86		
England	Cumbria			5.26	
England	Cumbria				2.77
England	Cumbria			2.70	
England	Northumbria			2.38	

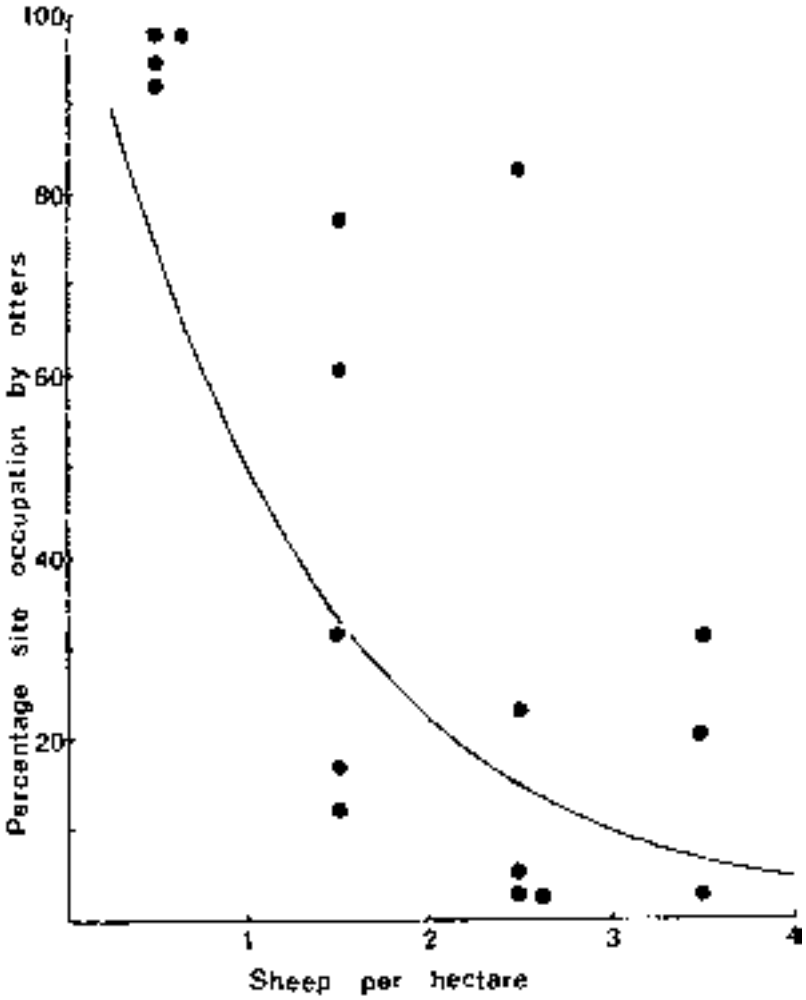
It can be seen from Table 12 that sheep dip and so dieldrin use was highest in Borders, Wales and Cumbria and lowest in Northern Scotland, the Western and Northern Isles, and that in general these usages relate to low and high otter densities and so survival after the 1957 crash.

Further, the form of the Table suggests a relationship between sheep dip use and otter occupation of an area; i.e. as the former increases then the latter decreases. This suggestion can be tested by assigning four values to the four grades of sheep densities (i.e. <1, 1 - 2, 2 - 3 and >3 are given values of 0.5, 1.5, 2.5 and 3.5 sheep per hectare) and calculating the regression correlation coefficient. This is shown to be significant (r = -0.6272; d.f. 15: $p < 0.01$) with an equation: $y = 2.0414 - 0.3460x$, where x = sheep/hectare and y = log percentage site occupation by otters in 1977-1979 (see Figure 3).

The above proven relationship between amount of sheep dip and so dieldrin used in the west and north and the degree of otter decline explains several features of otter distribution following the 1957 crash. Thus, the different otter densities of the adjacent Borders (31.18%) and Dumfries and Galloway (82.37%) regions in the lowlands can be attributed to their high and low sheep densities (see Table 12). Similarly, in the North of England, the different otter densities of the adjacent counties of Cumbria (2.70 - 5.26%) and Northumbria (2.77 - 17.11%) are a result of their relatively high and low sheep densities. It is the reason too, why, although all are sheep areas, Devon and Cornwall had higher otter densities than Wales after the crash and all three had lower otter densities than Highland Region of Scotland with its

extremely low sheep density. There are other features of detail which fit the hypothesis. Pembrokeshire, at the tip of the Dyfed peninsula in Wales, had the lowest sheep density in that country (<1 sheep/hectare; THOMPSON and BADDELEY, 1991) and also the highest level of otter survival (CRAWFORD *et al.*, 1979). It was also the area where the feral American mink first became established in Wales (CRAWFORD *et al.*, 1979). Similarly, the north-east tip of Caithness had a higher sheep density than neighbouring Sutherland (THOMPSON and BADDELEY, 1991), with a consequent comparatively reduced otter survival (GREEN and GREEN, 1980, 1997).

Figure 3



The relationship between percentage survey site occupation by otters in areas of Western and Northern Britain in 1977-1979 and the density of sheep (and so dieldrin sheep dip use) in those areas in the 1960s. The calculated regression line is shown (see text). There is a significant ($p<0.01$) correlation.

One other feature of this negative correlation between sheep and otter densities is the apparent anomaly still remaining between England and Wales on the one hand and Scotland on the other, when all the other comparisons between area sheep and otter differences hold good. Thus, why are all the otter densities for the same grade of sheep density higher in Scotland than in England and Wales (see Table 12)? This cannot be solely because the western and northern areas of England and Wales also had more mixed farms, with consequently more arable and seed dressing use, than in

Scotland as the otter survival in the wheat bulb fly area of Scotland was much greater than that in the wheat bulb fly area of England (see Section 6.2). This is so although dieldrin use was similar in the two areas. The most likely explanation is that put forward by JEFFERIES (1989*a*, 1996*a*). The population crash of the otter caused by dieldrin use would not have been so severe if the population had not been stressed by elimination of many mature individuals due to years of persecution by gamekeeping and hunting interests. This was much greater in England and Wales than in Scotland (STRACHAN & JEFFERIES, 1996).

7 DISCUSSION AND CONCLUSIONS

7.1 A summary of the reasons why the otter decline is considered to have been caused by the organochlorine insecticides dieldrin/aldrin

(1) The otter population crash started in 1957, within 18 months of the introduction of the highly toxic cyclodiene organochlorine insecticides, aldrin, dieldrin and heptachlor. It started simultaneously over England, Wales and Southern Scotland, indicating the sudden countrywide introduction of a new, probably man-made, environmental factor.

(2) Otter decline and recovery showed many correlations with agricultural practices.

(3) The decline started at a time when there were very high casualties among seed eating birds in the South and East of England. Also, the populations of two predatory birds, the peregrine falcon and the sparrowhawk, crashed at exactly the same time and with the same area of greatest effect. These two avian declines are independently considered to have been caused by dieldrin use. The otter is particularly vulnerable to lethal dieldrin poisoning, which is known to have occurred.

(4) In England, the greatest effect on the otter population was seen in the arable south and east, which coincided with the region having the greatest acreage treated with organochlorine insecticides used as seed dressings. The two small wheat bulb fly areas of South-east Scotland and South-eastern England received the highest and longest dieldrin use for this purpose and showed the highest wildlife residues and fatalities and the greatest otter declines.

(5) Relating hunting success to intensity of arable cultivation showed that, whereas before dieldrin use started in 1956, there was no significant correlation between otter hunting success and the amount of arable in an area, after 1956 there was a significant inverse relationship between the two. As arable cultivation (and dieldrin use) increased, then hunting success decreased. (See Section 6.2).

(6) There was a sudden change in the rate of otter decline following the first, 1962, ban on some dieldrin uses in seed dressings. The start of recovery shown by the western and eastern otter population curves (i.e. the nadirs of decline), occurred at dates which were correlated with the introduction of each of the main bans on organochlorine usage.

(7) There is a significant linear relationship between the intensity of arable cultivation of an area (and so dieldrin usage) and the date of the nadir of the decline curve for that area. This is earliest where the amount of cultivation is low, so decline changed to recovery earlier in the west than in the east. Thus, otter recovery is now progressing from the west to the east, as was the case with the two predatory birds.

(8) In the north and west of Britain the degree of decline shown by the otter population is directly related to sheep density as this reflects the amount of dieldrin used for sheep dipping in each region. (See Section 6.3).

(9) Unlike other pollutants analysed (i.e. DDT, DDE, PCB), dieldrin residues in otter tissues were highest close to the date when the population crash started (i.e. 1957). Their decline in otter tissues (following bans on usage) coincided with the recovery of the population.
(10) The highest dieldrin residues were found in English otters where the decline was most severe and the lowest were found in Scottish otters where the decline was not so great.
(11) The only major actions taken to stop and reverse the otter decline and so bring about recovery were, first, bans on the uses of the organochlorine insecticides and, second, providing legal protection to reduce persecution and allow the maximum rate of repopulation.

7.2 The reasons why the PCBs are considered not to have been major causal agents in the 1957 otter decline

(1) PCBs were first produced and used in 1930 and there are no data suggesting that use suddenly increased in 1956-1957.
(2) This paper shows that PCB concentrations in otters did not start to increase above trace levels until the late 1970s (1977-1979), i.e. long after recovery had already started in the west of Britain (1966-1970). PCB levels increasing over the 1980s then coincided with otter recovery in most of the remaining areas.
(3) The highest PCB levels occur in the areas inhabited by the strongest otter populations, both in England and Scotland.
(4) The timing of otter recovery cannot be correlated with any of the bans on PCB use whereas there are correlations with organochlorine insecticide bans.
(5) PCBs, as found in Britain, do not have the lethal toxicity of dieldrin (PRESTT *et al.*, 1970) and it is the killing of breeding-aged adults which causes sudden population crashes rather than small reductions in breeding success.
(6) PCBs are industrially-used chemicals whereas all the correlations with effects on the otter show links with agriculture.
(7) Ornithologists using much larger data bases on predatory birds, with many hundreds of analyses [e.g. NEWTON, DALE and LITTLE (1999) analysed 630 merlin eggs alone), could find no correlations between PCB concentrations and population declines (NEWTON and HAAS, 1984; NEWTON, 1986; RATCLIFFE, 1993). Indeed, NEWTON, DALE and LITTLE (1999) remark that 'to our knowledge, PCBs have not been implicated in the population declines of raptors'. It is inconceivable that sudden pollution-driven declines in otters and predatory birds, occurring at exactly the same dates and concentrated in the same areas of Britain, had different causes. Use of Occam's Razor would suggest that the main causal agents were likely to be similar in the two cases.
(8) Laboratory tests have shown that the American mink is very sensitive to some PCB mixtures and that these reduce reproductive success (JENSEN *et al.*, 1977; AULERICH and RINGER, 1977; BLEAVINS, AULERICH and RINGER, 1980; KIHLSTROM *et al.*, 1992). This sensitivity has been extrapolated to the otter by some authors (MASON and MACDONALD, 1993), but as LEONARDS *et al.* (1994) have pointed out, this extapolation is highly speculative. The otter may be more like the ferret (*Mustela furo*) which is known to be much less sensitive to PCBs than the mink (LEONARDS *et al.*, 1994). Because of this doubtful extrapolation MASON and MACDONALD (1993) use a level of 50μg/g of PCB in tissue lipid as that likely to be associated with reproductive failure in the otter. However, a liver lipid concentration of 56.7μg/g PCB was found in a trapped otter from Islay in 1985 which was pregnant

with three healthy embryos and even higher residues have been found in lactating animals which have recently given birth (D. J. JEFFERIES and H. M. HANSON, *unpublished*). KRUUK, CONROY and CARSS (1993) have other similar examples. It should be remembered too that the feral mink population became established at the time of rapid otter decline and was then expanding eastwards over England at the same time that very high PCB levels were found in otters (STRACHAN and JEFFERIES, 1993).

(9) Although analysis of a male cub road casualty derived from the Minsmere (Suffolk) otter release of 1985 showed a total PCB level of 61.98μg/g in the liver lipid (JEFFERIES and HANSON, 1988), the captive-bred otters released there continued to breed and the release area is still populated 16 years afterwards by their descendants. The rebuilding of the otter population of East Anglia with only 36 otters in ten years (JEFFERIES, WAYRE and SHUTER, 2001) shows that this level of PCB was unimportant to the population.

(10) PCB toxicology is complex. One problem is that whereas many laboratory toxicity trials have been carried out with 'complete' commercial mixtures with all congeners and dioxin and dibenzofuran impurities present, the end analyses have been used to determine the likely effects of residues found in wild animals which have very different relative concentrations of a reduced range of congeners after passing through the environment and metabolism by several layers of the food chain.

(11) All the above remarks and the lack of any definite effects of the PCBs on the British otter and predatory bird populations relate to the particular commercial PCB mixtures (namely the Aroclors, particularly 1254; PRESTT, JEFFERIES and MOORE, 1970) used in this country. They cannot necessarily be extrapolated to the situation in other countries.

7.3 Was there a delay in the recovery of the British otter population ?

There is a widely held view that, because the other main organochlorine casualties, the peregrine and the sparrowhawk, recovered by 1985 and 1990, respectively, whereas the otter is still (in 2001) slowly expanding its area of occupation, there must have been another anthropogenic factor holding the species back, particularly in the east (discussed by JEFFERIES, 1997). This was one reason for all the research on the PCBs, i.e. these might have been such a factor as they were increasing during the recovery period. However, PCB levels were highest in otters in the west and north where recovery was most rapid (STRACHAN and JEFFERIES, 1996).

The sudden increase in environmental dieldrin and DDE in the period 1983-1986, on the other hand, may well have slowed the recovery of the otter. There are strong indications that they had such an effect on the recovering peregrine falcon. Thus, the numbers of occupied territories, pairs producing eggs and pairs rearing young all showed a marked 'plateau' from 1983 to 1989 in the otherwise continuously increasing peregrine population curves from 1966 to 1991 (Figure 20 of RATCLIFFE, 1993). This incidentally provides yet one more indicator that dieldrin, and to a lesser extent DDT, were the causal agents in the decline of this species. However, again, this late increase in dieldrin in the environment, probably from its illicit use, affected the peregrine as well as the otter so would not have selectively slowed the latter species.

What did slow the recovery of the otter relative to the two predatory birds was that whereas the two birds were largely affected by the use of dieldrin in seed dressings in the east, the otter was affected by dieldrin in seed dressings in the east and sheep dips in the west and north, so had no unaffected populations from which it could recover

and expand. Obviously, too, the fact that otters are not so mobile as birds and use linear ranges would slow their rate of re-colonisation over large distances.

Beyond these factors, STRACHAN and JEFFERIES (1996) have remarked that the otter population was recovering and expanding as rapidly as could be expected. The declines halted and recovery started in each affected area soon after the ban which stopped dieldrin use in that area. The movement eastward of the 'rolling front' of the established breeding otter population occurs at 3.6km/year (STRACHAN and JEFFERIES, 1996), which is very close to that of the 'rapidly recovering' polecat population [3.5km/year between 1975 and 1985; BIRKS (1999)]. Indeed, further analysis (JEFFERIES, 1997) has shown that the otter population of England was expanding at the rate of 39.1 newly occupied 10km squares every year between 1984 and 1994. Comparison with the calculated expansion rates of other mammals which were released by man at a restricted number of sites shows that this rate is very close to that of the American mink (41.4/year; over 1984-1994; in England) and not far short of that of the grey squirrel (*Sciurus carolinensis)* (52.4/year; over 1937-1945; in England) (JEFFERIES, 1997). It would appear then that populations of many mammals, particularly mustelids, do not colonise rapidly [compare the very slow re-occupation of areas by the badger after their clearance for TB control; CRESSWELL, HARRIS and JEFFERIES (1990)]. Thus, there is no need to invoke another anthropogenic factor, such as PCBs, to explain the comparatively (to that of birds) slow recovery of the otter.

7.4 The reason why very high PCB levels show a westerly and northerly distribution

We have shown in Sections 3.3 and 3.4 above, that the high PCB levels, i.e. those above 110μg/g: lipid weight, tend to have a westerly and northerly distribution. Thus, in England they are all but one (i.e. 6 of them) from the South-west (Cornwall, Devon, Somerset). There is one example in Dyfed in Wales and 14 in Scotland, the Western and Northern Isles. The only southern animal in the high PCB group was one from West Sussex in England. There were none from Central and Eastern England (see Table 5).

Further examination of these high PCB otters shows that they have a coastal distribution [see Table 5 and Table 13(1)]. 17 out of the 22 (77.3%) were within 17km (10.56 miles) of the coast while five out of 22 (22.7%) were actually on the coast itself. Indeed the geometric mean distance from the sea was only 5.58km [Table 13(1)], which, if compared to the width across Britain of 240km (Scotland) and 395km (England & Wales) shows how close to the coast these otters were living.

However, if the high PCB data are subdivided into those otters from western and northern coasts of Britain, including the Western and Northern Isles (i.e. all those marked + in Table 5), and those down the east coast of Scotland plus the individual from West Sussex (marked o in Table 5), it can be seen that the western group were living nearer to the coast (2.66km) than were the eastern group (22.23km) [see Table 13 (2,3)]. Indeed, one of the latter group was living 54.7km from the coast, whereas all the immediately coastal otters were in the first group. This difference in coastal distance is significant (t = 4.9012; d.f. 20: p <0.001). There is also a difference in PCB levels in the western and eastern groups with the former having the greater residues [see Table 13 (4,5)], though this difference, although large, is not statistically significant.

Table 13. The coastal distribution and contamination levels of otters with very high PCB concentrations (above 110 μg/g lipid weight) in the liver. The geometric mean distance from the coast (km) of (1) all 22 otters, (2) those 15 otters from the Western and Northern coasts, and (3) those 7

otters from the Eastern and Southern coasts. The geometric mean PCB levels (µg/g lipid) of (4) those 15 otters from the Western and Northern coasts and (5) those 7 otters from the Eastern and Southern coasts.

	n	**Geometric Mean**	**Range of one St. Error**	**Range of values**
1	Distance from the coast (km) of all 22 otters with very high lipid concentrations of PCB			
	22	5.58	3.86 – 7.92	0 – 54.71
2	Distance from the coast (km) of western and northern otters with very high PCB concentrations			
	15	2.66	1.73 – 3.89	0 – 16.28
3	Distance from the coast (km) of eastern and southern otters with very high PCB concentrations			
	7	22.23	13.83 – 35.39	1.13 – 54.71
4	Mean PCB levels (µg/g lipid) in the above 15 western and northern otters			
	15	205.69	175.61 – 240.89	117.58–984.56
5	Mean PCB levels (µg/g lipid) in the above 7 eastern and southern otters			
	7	150.46	131.84 – 171.70	113.82-256.61

What could be the reason for this distribution of high PCB otters? Examination shows that high PCBs in the South-west of England, Wales, Scotland and the Western and Northern Isles correlates exactly with the area of high rainfall in Britain. This is westerly and northerly [see Figure 41 of MANLEY (1952)]. Indeed, this map showing the distribution of high rainfall in Britain in December shows a narrow band of high rainfall along the south (Channel) coast of England, which would incorporate the West Sussex otter too. It is known that the organochlorine insecticides and PCBs have been found to contaminate rain (WHEATLEY and HARDMAN, 1965; TARRANT and TATTON, 1968; RISEBROUGH *et al*., 1968) and that the biota of continents such as the Antarctic and Arctic, many thousands of miles from application, have been found to be contaminated due to aerial and sea current distribution (MELLANBY, 1967). The prevailing winds in Britain are from the south south west in winter and from the west south west in summer (MANLEY, 1952). It is known too, from analysis of guillemot eggs, that the Irish Sea has been three times more contaminated with PCBs than has the North Sea along the Eastern coast of Britain (PARSLOW and JEFFERIES, 1975). This contamination is thought to be due to the dumping of industrial sewage sludge in the 1960s [around 1000kg of PCB isomers were being discharged into the Clyde Estuary and Liverpool Bay every year; HOLDEN (1970)]. Thus, the air over the Irish Sea and its approaches would become saturated with contaminated water which would then be deposited over Western and Northern Britain as rain and snow.

If the distribution of the highest PCB contaminated group of 15 western otters is examined (i.e. those with the most coastal distribution and marked + in Table 5), it can be seen that these are all from peninsulas (South-west, Dyfed, Dumfries and Galloway) or archipelagos (Western Isles, Shetland) with a south-west orientation pointing into the Irish Sea, the Minch and their southern and northern approaches. Thus, not only do these areas have the highest PCB contaminated rainfall, they will also receive sea spray in the wind from the most highly contaminated Irish Sea water. There are fewer highly contaminated otters on the eastern side of Britain. These have a lower PCB contamination than those of the west and are further inland (up to 54.7km). These Scottish animals would receive much of their PCBs in rainfall from the west. This is not to say, of course, that all of the PCB has a rainfall or spray-borne origin.

There are some indications that the above situation has been developing and changing since the mid-1960s. PRESTT, JEFFERIES and MOORE (1970), analysing sedentary British birds dying in the period 1966-1968, could find no obvious area biases in the distribution of their PCB contamination. Also, the PCB contamination of guillemot eggs collected from Shetland and St Kilda was still low (14 & 17µg/g; lipid weight, respectively) at a time (1972-1973) when it was very high in the Irish Sea area itself (216µg/g; St Bees Head, Cumbria) (PARSLOW & JEFFERIES, 1975). Yet by 1977-1979 the above (this paper) distribution pattern of high PCBs had developed, with Shetland otters having as high residues as those from Wales and the South-west. This suggests a north-easterly drift of PCB contaminated sea water in the intervening period. From this time (1977-1979) the PCBs were increasing in Scottish otters over the whole remaining period of this analytical survey (i.e. to 1989).

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9 REFERENCES

Anonymous (1990). *This Common Inheritance: Britain's Environmental Strategy*. Her Majesty's Stationery Office, London.

Andrews, E. M. & Crawford, A. K. (1986). *Otter Survey of Wales 1984-85*. The Vincent Wildlife Trust, London.

Andrews, E., Howell, P. & Johnson, K. (1993). *Otter Survey of Wales 1991*. The Vincent Wildlife Trust, London.

Aulerich, R. & Ringer, R. (1977). Current status of PCB toxicity to mink, and effects on their reproduction. *Archives of Environmental Contamination and Toxicology,* **6**: 279-292.

Bell, A. A. (1975). Dieldrin residues in the livers of kestrels and barn owls found dead in 1970-1973. A*nnual Report of the Institute of Terrestrial Ecology,* **1974**: 27-28.

Bernard, R. F. & Gaertner, R. A. (1964). Some effects of DDT on reproduction in mice. *Journal of Mammalogy,* **45**: 272-276.

Birks, J. D. S. (1999). A survey of polecat distribution in Britain, 1993-1997. In: *The Distribution and Status of the Polecat* Mustela putorius *in Britain in the 1990s.* (Edited by Birks, J.D.S. & Kitchener, A.C.). Pp. 28-54. The Vincent Wildlife Trust, London.

Blackmore, D. K. (1963). The toxicity of some chlorinated hydrocarbon insecticides to British wild foxes (*Vulpes vulpes*). *Journal of Comparative Pathology & Therapeutics,* **73**: 391-409.

Bleavins, M. R., Aulerich, R. J. & Ringer, R. K. (1980). Polychlorinated biphenyls (Aroclors 1016 and 1242): Effects on survival and reproduction in mink and ferrets. *Archives of Environmental Contamination and Toxicology,* **9**: 627-635.

Cecil, H. C., Harris, S. J., Bitman, J. & Fries, G. F. (1973). Polychlorinated biphenyl-induced decrease in liver vitamin A in Japanese quail and rats. *Bulletin of Environmental Contamination & Toxicology,* **9**: 179-185.

Chanin, P. R. F. & Jefferies, D. J. (1978). The decline of the otter *Lutra lutra* L. in Britain: an analysis of hunting records and discussion of causes. *Biological Journal of the Linnean Society,* **10**: 305-328.

Cook, J. W. (Chairman) (1964). *Review of the Persistent Organochlorine Pesticides: A Report of the Advisory Committee on Poisonous Substances used in Agriculture and Food Storage.* Her Majesty's Stationery Office, London.

Cooke, A. S., Bell, A. A. & Haas, M. B. (1982). *Predatory Birds, Pesticides and Pollution.* Institute of Terrestrial Ecology, Huntingdon.

Cramp, S. & Conder, P. J. (1960). *The Deaths of Birds and Mammals connected with Toxic Chemicals in the First Half of 1960: Report No.1 of the BTO - RSPB Committee on Toxic Chemicals.* Royal Society for the Protection of Birds, London.

Cramp, S., Conder, P. J. & Ash, J. S. (1962). *The Second Report of the Joint Committee of the British Trust for Ornithology and the Royal Society for the Protection of Birds on Toxic Chemicals, in collaboration with the Game Research Association.* Royal Society for the Protection of Birds, Sandy.

Cranbrook, The Earl of (1977). The status of the otter (*L.lutra* L.) in Britain in 1977. *The Biological Journal of the Linnean Society,* **9**: 305-322.

Crawford, A., Evans, D., Jones, A. & McNulty, J. (1979). *Otter Survey of Wales 1977-78*. Society for the Promotion of Nature Conservation, Nettleham, Lincoln.

Cresswell, P., Harris, S. & Jefferies, D. J. (1990). *The History, Distribution, Status and Habitat Requirements of the Badger in Britain.* Nature Conservancy Council, Peterborough.

Crick, H. Q. P. & Ratcliffe, D. A. (1995). The peregrine *Falco peregrinus* breeding population of the United Kingdom in 1991. *Bird Study,* **42**: 1-19.

Crossett, R. N. (Chairman) (1989). *Report of the Working Party on Pesticide Residues: 1985-88*. Food Surveillance Paper No. 25. Her Majesty's Stationery Office, London.

Cuthbert, J. H. (1973). Some observations on scavenging of salmon *Salmo salar* carrion. *Western Naturalist,* **2**: 72-74.

DeWitt, J. B., Menzie, C. M., Adomaitis, V. A. & Reichel, W. L. (1960). Pesticidal residues in animal tissues. *Transactions of the North American Wildlife Conference,* **25**: 277-285.

Doxey, D. L. (1971). *Veterinary Clinical Pathology.* Bailliere Tindall, London.

Ecobichon, D. J. & Saschenbrecker, P. W. (1969). The redistribution of stored DDT in cockerels under the influence of food deprivation. *Toxicology & Applied Pharmacology,* **15**: 420-432.

Fowler, J. & Cohen, L. (1992). *Practical Statistics for Field Biology*. John Wiley, Chichester.

French, M. C. & Jefferies, D. J. (1968). Disappearance of gamma BHC from avian liver after death. *Nature, London,* **219**: 164-166.

Friend, M. & Trainer, D. O. (1970). Polychlorinated biphenyl: interaction with duck hepatitis virus. *Science, New York,* **170**: 1314-1316.

Good, E. E. & Ware, G. W. (1969). Effects of insecticides on reproduction in the laboratory mouse. IV Endrin and dieldrin. *Toxicology &Applied Pharmacology* **14**: 201-203.

Gough, H. C. (1957). Studies on wheat bulb fly (*Leptohylemyia coarctata* (Fall.). IV. The distribution of damage in England and Wales in 1953. *Bulletin of Entomological Research,* **48**: 447-457.

Green, J. & Green, R. (1980). *Otter Survey of Scotland 1977-79.* The Vincent Wildlife Trust, London.

Green, J. & Green, R. (1987). *Otter Survey of Scotland 1984-85.* The Vincent Wildlife Trust, London.

Green, J., Green, R. & Jefferies, D. J. (1984). A radio-tracking survey of otters *Lutra lutra* on a Perthshire river system. *Lutra* **27**: 85-145.

Green, R. & Green, J. (1997). *Otter Survey of Scotland 1991-1994.* The Vincent Wildlife Trust, London.

Haas, M. B. & Cooke, A. S. (1983). Pollutants in kingfishers. *Annual Report of the Institute of Terrestrial Ecology,* **1982**: 64-65.

Harper, D. B., Smith, R. V. & Gotto, D. M. (1977). BHC residues of domestic origin: a significant factor in pollution of freshwater in Northern Ireland. *Environmental Pollution,* **12**: 223-233.

Harris, C. J. (1968). *Otters: a Study of the Recent Lutrinae.* Weidenfeld & Nicolson, London.

Harris, S., Morris, P., Wray, S. & Yalden, D. (1995). *A Review of British Mammals: Population Estimates and Conservation Status of British Mammals other than Cetaceans.* Joint Nature Conservation Committee, Peterborough.

Hayes, W. J. (1959). Pharmacology and Toxicology of DDT. In: *DDT: the Insecticide Dichlorodiphenyltrichloro-ethane and its Significance.* Volume 2. (Edited byMuller, P. & Simmons, S.W.). Pp 11-247. Birkhauser Verlag, Basel.

Holden, A. V. (1970). Source of polychlorinated biphenyl contamination in the marine environment. *Nature, London,* **228**: 1220-1221.

Holdgate, M. W. (Editor) (1971). *The Seabird Wreck of 1969 in the Irish Sea.* Natural Environment Research Council, London.

Holmes, D. C., Simmons, J. H. & Tatton, J. O'G. (1967). Chlorinated hydrocarbons in British wildlife. *Nature, London,* **216**: 227.

Jefferies, D. J. (1969). Causes of badger mortality in Eastern counties of England. *Journal of Zoology, London,* **157**: 429-436.

Jefferies, D. J. (1972). Organochlorine insecticide residues in British bats and their significance. *Journal of Zoology, London,* **166**: 245-263.

Jefferies, D. J. (1975). The role of the thyroid in the production of sublethal effects by organochlorine insecticides and polychlorinated biphenyls. In: *Organochlorine Insecticides: Persistent Organic Pollutants*. (Edited by Moriarty, F.), Pp. 131-230. Academic Press, London.

Jefferies, D. J. (1985). An otter casualty from Breydon Water, Norfolk. *Journal of the Otter Trust,* **1** (8) **1984**: 23-24.

Jefferies, D. J. (1988). Postscript: The demise of the otter population of the Rivers Glaven and Stiffkey and adjacent coastal marshes. In: *The Otter*, 2nd edition. (Weir, V.). The Vincent Wildlife Trust, London.

Jefferies, D. J. (1989*a*). The changing otter population of Britain 1700 - 1989. *Biological Journal of the Linnean Society,* **38**: 61-69.

Jefferies, D. J. (1989*b*). Fragmentation of the English otter population and its effect on viability. *Journal of the Otter Trust,* **2** (2) **1988**: 20-22.

Jefferies, D. J. (1991). Some observations on Scottish wildcats *Felis silvestris* based on the results of autopsies. *Glasgow Naturalist,* **22**: 11-20.

Jefferies, D. J. (1992). Polecats *Mustela putorius* and pollutants in Wales. *Lutra,* **35**: 28-39.

Jefferies, D. J. (1996*a*). Decline and recovery of the otter - a personal account. *British Wildlife,* **7**: 353-364.

Jefferies, D. J. (1996*b*). Blindness in British otters: further incidence, possible causes and correlation with the period of organochlorine pollution. *Journal of the Otter Trust* **2** (9) **1995**: 14-22.

Jefferies, D. J. (1997). The changing status of the otter in the British Isles as revealed by spraint surveys. In: *The Vincent Wildlife Trust Review of 1996.* (Edited by Weir, V.). Pp. 19-23. The Vincent Wildlife Trust, London.

Jefferies, D. J. & Davis, B. N. K. (1968). Dynamics of dieldrin in soil, earthworms and song thrushes. *Journal of Wildlife Management,* **32**: 441-456.

Jefferies, D. J. & Freestone, P. (1985). Chemical analysis of some coarse fish from a Suffolk river carried out as part of the preparation for the first release of captive-bred otters. *Journal of the Otter Trust* **1** (8) **1984**: 17 - 22.

Jefferies, D. J. & French, M. C. (1971). Hyper- and hypothyroidism in pigeons fed DDT: an explanation for the 'thin eggshell phenomenon'. *Environmental Pollution,* **1**: 235-242.

Jefferies, D. J. & French, M. C. (1972). Changes induced in the pigeon thyroid by pp-DDE and dieldrin. *Journal of Wildlife Management,* **36**: 24-30.

Jefferies, D. J. & French, M. C. (1976). Mercury, cadmium, zinc, copper and organochlorine insecticide levels in small mammals trapped in a wheat field. *Environmental Pollution,* **10**: 175-182.

Jefferies, D. J. & Hanson, H. M. (1987). Autopsy and chemical analysis of otter bodies. *The Vincent Wildlife Trust Report,* **1986**: 42-44.

Jefferies, D. J. & Hanson, H. M. (1988). The Minsmere otter release and information gained from a detailed examination and analysis of the two casualties. *Journal of the Otter Trust,* **2** (1) **1987**: 19-29.

Jefferies, D. J. & Hanson, H. M. (1991). Evidence of fighting in a juvenile male otter road casualty bred from the otters released at Minsmere in 1985 and 1987. *Journal of the Otter Trust,* **2** (4) **1990**: 13-24.

Jefferies, D. J. & Parslow, J. L. F. (1972). Effect of one polychlorinated biphenyl on size and activity of the gull thyroid. *Bulletin of Environmental Contamination and Toxicology,* **8**: 306-310.

Jefferies, D. J. & Parslow, J. L. F. (1976). Thyroid changes in PCB-dosed guillemots and their indication of one of the mechanisms of action of these materials. *Environmental Pollution,* **10**: 293-311.

Jefferies, D. J. & Prestt, I. (1966). Post-mortems of peregrines and lanners with particular reference to organochlorine residues. *British Birds,* **59**: 49-64.

Jefferies, D. J. & Walker, C. H. (1966). Uptake of pp-DDT and its postmortem breakdown in avian liver. *Nature, London,* **212**: 533-534.

Jefferies, D. J., Stainsby, B. & French, M. C. (1973). The ecology of small mammals in arable fields drilled with winter wheat and the increase in their dieldrin and mercury residues. *Journal of Zoology, London,* **171**: 513-539.

Jefferies, D. J., French, M. C. & Stebbings, R. E. (1974). Pollution and Mammals. In: *Monks Wood Experimental Station Report for 1972-73.* Pp. 13-15. Natural Environment Research Council, Huntingdon.

Jefferies, D. J., Wayre, P. & Shuter, R. (2001). A brief history of the Otter Trusts successful programme of repopulating lowland England with otters bred in captivity with a special emphasis on East Anglia. *Journal of the Otter Trust,* **3** (4) **2000:** 105-117**.**

Jefferies, D. J., Wayre, P., Jessop, R. M. & Mitchell-Jones, A. J. (1986). Reinforcing the native otter *Lutra lutra* population in East Anglia : an analysis of the behaviour and range development of the first release group. *Mammal Review,* **16**: 65-79.

Jensen, S. (1966). Report of a new chemical hazard. *New Scientist,* **32**: 612.

Jensen, S., Kihlstrom, J. E., Olsson, M., Lundberg, C. & Orberg, J. (1977). Effects of PCB and DDT on mink (*Mustela vison*) during the reproductive season. *Ambio,* **6**: 239.

Keymer, I. F., Wells, G. A. H., Mason, C. F. & Macdonald, S. M. (1988). Pathological changes in wild otters (*Lutra lutra)* and organochlorine tissue residues. *Veterinary Record,* **122**: 153-155.

Kihlstrom, J. E., Olsson, M., Jensen, S., Johansson, A., Ahlbom, J. & Bergman, A. (1992). Effects of PCB and different fractions of PCB on the reproduction of the mink (*Mustela vison*). *Ambio,* **21**: 563-569.

Kruuk, H., Conroy, J. W. H. & Carss, D. N. (1993). *Otters, eels and contaminants*. (Report to Scottish Natural Heritage), Institute of Terrestrial Ecology, Banchory.

Lenton, E. J., Chanin, P. R. F. & Jefferies, D. J. (1980). *Otter Survey of England 1977-79.* Nature Conservancy Council, London.

Leonards, P. E. G., Smit, M. D., De Jongh, A. W. J. J. & Van Hattum, B. (1994). *Evaluation of Dose-response Relationships for the effects of PCBs on the Reproduction of Mink (*Mustela vison*)*. Institute for Environmental Studies, Vrije Universiteit, Amsterdam.

Lloyd, J. L. (1962). Where are the otters ? *Gamekeeper and Countryside,* **65**: 299-300.

Lockie, J. D. & Ratcliffe, D. A. (1964). Insecticides and Scottish golden eagles. *British Birds,* **57**: 89-102.

Lockie, J. D., Ratcliffe, D. A. & Balharry, R. (1969). Breeding success and organochlorine residues in golden eagles in West Scotland. *Journal of Applied Ecology,* **6**: 381-389.

Manley, G. (1952). *Climate and the British Scene.* Collins, London.

Martin, H. & Worthing, C. R. (1977). *Pesticide Manual*, 5th edition. British Crop Protection Council, London.

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

Mason, C. F. & Macdonald, S. M. (1986). *Otters: ecology and conservation*. Cambridge University Press, Cambridge.

Mason, C. F. & Macdonald, S. M. (1993). Pollution and otter conservation in a European context. In: *Proceedings of the National Otter Conference, Cambridge, September 1992*. Edited by Morris, P.A,). Pp. 17-20. The Mammal Society, Bristol.

Mason, C. F., Ford, T. C. & Last, N. I. (1986). Organochlorine residues in British otters. *Bulletin of Environmental Contamination and Toxicology,* **36**: 656-661.

Mellanby, K. (1967). *Pesticides and Pollution*. Collins, London.

Mitchell-Jones, A. J., Jefferies, D. J., Twelves, J., Green, J. & Green, R. (1984). A practical system of tracking otters *Lutra lutra* using radiotelemetry and 65-Zn. *Lutra,* **27**: 71-84.

Moore, N. W. (1965). Pesticides and birds: A review of the situation in Great Britain in 1965. *Bird Study,* **12**: 222-252.

Moore, N. W. (1987). *The Bird of Time: The science and politics of nature conservation, a personal account*. Cambridge University Press, Cambridge.

Murton, R. K. & Vizoso, M. (1963). Dressed cereal seed as a hazard to woodpigeons. *Annals of Applied Biology,* **52**: 503-517.

Neal, E. G. (1977). *Badgers.* Blandford, Poole.

Negherbon, W. O. (1959). *Handbook of Toxicology,* Vol. III *Insecticides.* W.B. Saunders, Philadelphia.

Nelson, A. A., Draize, J. H., Woodard, G., Fitzhugh, O. G., Smith, R. B. & Calvery, H. O. (1944). Histopathological changes following administration of DDT to several species of animals. *Public Health Reports, Washington,* **59**: 1009-1020.

Newton, I. (1973). Egg breakage and breeding failure in British merlins. *Bird Study,* **20**: 241-244.

Newton, I. (1986). *The Sparrowhawk.* T. & A.D. Poyser, Calton.

Newton, I. & Haas, M. B. (1984). The return of the sparrowhawk. *British Birds,* **77**: 47-70.
Newton, I. & Haas, M. B. (1988). Pollutants in merlin eggs and their effects on breeding. *British Birds,* **81**: 258-269.
Newton, I., Dale, L. & Little, B. (1999). Trends in organochlorine and mercurial compounds in the eggs of British merlins *Falco columbarius*. *Bird Study,* **46**: 356-362.
Newton, I., Robson, J. E. & Yalden, D. W. (1981). Decline of the merlin in the Peak District. *Bird Study,* **28**: 225-234.
Newton, I., Bogan, J., Meek, E. & Little, B. (1982). Organochlorine compounds and shell-thinning in British merlins *Falco columbarius. Ibis,* **124**: 328-335.
Parslow, J. L. F. & Jefferies, D. J. (1973). Relationships between organochlorine residues in livers and whole bodies of guillemots. *Environmental Pollution,* **5**: 87-101.
Parslow, J. L. F. & Jefferies, D. J. (1975). Geographical variation in pollutants in guillemot eggs. *Annual Report of the Institute of Terrestrial Ecology* **1974**: 28-31.
Parslow, J. L. F. & Jefferies, D. J. (1977). Gannets and toxic chemicals. *British Birds,* **70**: 366-372.
Prestt, I. (1970). Organochlorine pollution of rivers and the heron (*Ardea cinerea* L.). *11th Technical Meeting, International Union for the Conservation of Nature & Natural Resources, New Delhi,* **1**: 95-102.
Prestt, I., Jefferies, D. J. & Moore, N. W. (1970). Polychlorinated Biphenyls in wild birds in Britain and their avian toxicity. *Environmental Pollution,* **1**: 3-26.
Ratcliffe, D. A. (1958). Broken eggs in peregrine eyries. *British Birds,* **51**: 23-26.
Ratcliffe, D. A. (1970). Changes attributable to pesticides in egg breakage frequency and eggshell thickness in some British birds. *Journal of Applied Ecology,* **17**: 67-107.
Ratcliffe, D. **A.** (1993). *The Peregrine Falcon.* 2nd edition. T. & A.D. Poyser, London.
Reynolds, C. M. (1974). The census of heronries, 1969-73. *Bird Study,* **21**: 129-134.
Risebrough, R. W., Rieche, P., Peakall, D. B., Herman, S. G. & Kirven, M. N. (1968). Polychlorinated biphenyls in the global ecosystem. *Nature, London,* **220**: 1098-1102.
Scott, T. G., Willis, Y. L. & Ellis, J. A. (1959). Some effects of a field application of dieldrin on wildlife. *Journal of Wildlife Management,* **23**: 409-427.
Sharom, M. S. & Solomon, K. R. (1981). Absorption, desorption, degradation and distribution of permethrin in aqueous systems. *Journal of Agriculture and Food Chemistry,* **29**: 1122-1125.
Sheail, J. (1985). *Pesticides and Nature Conservation: The British experience 1950-1975.* Clarendon Press, Oxford.
Simpson, V. R., Bain, M. S., Brown, R., Brown, B. F. & Lacey, R. F. (2000). A long-term study of vitamin A and polychlorinated hydrocarbon levels in otters (*Lutra lutra*) in South West England. *Environmental Pollution,* **110**: 267-275.
Spalton, J. A. & Cripps, S. J. (1989). *The Report of the North Norfolk Otter Project 1986-1987*. The Vincent Wildlife Trust, London.
Stephens, M. N. (1957). *The Natural History of the Otter*. Universities Federation for Animal Welfare, London.
Strachan, R. & Jefferies, D. J. (1993). *The Water Vole* Arvicola terrestris *in Britain 1989 - 1990: its Distribution and Changing Status.* The Vincent Wildlife Trust, London.
Strachan, R. & Jefferies, D. J. (1996). *Otter Survey of England 1991-1994.* The Vincent Wildlife Trust, London.
Strachan, R., Birks, J. D. S., Chanin, P. R. F. & Jefferies, D. J. (1990). *Otter Survey of England 1984-1986*. Nature Conservancy Council, Peterborough.
Stroud, D. A. & Glue, D. (1991). *Britain's Birds in 1989/90: the Conservation and Monitoring Review*. British Trust for Ornithology and Nature Conservancy Council, Thetford.
Tapper, S. (1992). *Game Heritage: An Ecological Review from Shooting and Gamekeeping Records.* Game Conservancy, Fordingbridge.
Tarrant, K. R. & Tatton, J. O'G. (1968). Organochlorine pesticides in rainwater in the British Isles. *Nature, London,* **219**: 725-727.
Taylor, J. C. & Blackmore, D. K. (1961). A short note on the heavy mortality in foxes during the winter 1959 - 60. *Veterinary Record,* **73**: 232-233.
Thompson, D. B. A. & Baddeley, J. (1991). Some effects of acidic deposition on montane *Racomitrium lanuginosum* heaths. In: *The Effects of Acid Deposition on Nature Conservation in Great Britain.* Focus No. 26. (Edited by Woodin, S.J. & Farmer, A.M.). Pp. 17-28. Nature Conservancy Council, Peterborough.
Thompson, H. V. & Southern, H. N. (1964). Red fox *Vulpes vulpes* (L.). In: *The Handbook of British Mammals*, 1st edition. (Edited by Southern, H.N.). Pp. 352-357. Blackwell, Oxford.

Turtle, E. E., Taylor, A., Wright, E. N., Thearle, R. J. P., Egan, H., Evans, W. H. & Soutar, N. M. (1963). The effects on birds of certain chlorinated insecticides used as seed dressings. *Journal of the Science of Food & Agriculture,* **14**: 567-577.

Village, A. (1990). *The Kestrel*. T. & A.D. Poyser, London.

Walker, C. H. (1983). Pesticides and birds - mechanisms of selective toxicity. *Agriculture Ecosystems and Environment,* **9**: 211-226.

Walker, C. H. & Jefferies, D. J. (1978). The postmortem reductive dechlorination of pp-DDT in avian tissues. *Pesticide Biochemistry and Physiology* **9**: 203 - 210.

Ware, G. W. & Good, E. E. (1967). Effect of insecticides on reproduction in the laboratory mouse. II. Mirex, Telodrin and DDT. *Toxicology & Applied Pharmacology,* **10**: 54-61.

Wells, G. A. H., Keymer, I. F. & Barnett, K. C. (1989). Suspected Aleutian Disease in a wild otter (*Lutra lutra*). *Veterinary Record,* **125**: 232-235.

West, G. P. (1992). *Black's Veterinary Dictionary*. 17th edition. A. & C. Black, London.

Wheatley, G. A. & Hardman, J. A. (1965). Indications of the presence of organochlorine pesticides in rainwater in Central England. *Nature, London,* **207**: 486-487.

Williams, J. (1993). Blindness in otters. *Mammal News,* **92**: 8.

Yaxley, R. (1997). *Report of the Norfolk Otter, Mink and Water Vole Survey.* Norfolk Wildlife Trust, The Environment Agency, Essex & Suffolk Water, Norwich.

REVIEW OF CURRENT KNOWLEDGE OF PHAH TOXICITY AND VITAMIN HOMEOSTASIS IN THE EURASIAN OTTER (*LUTRA LUTRA*)

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1 INTRODUCTION

Over the last decades otter (*Lutra lutra*) populations have declined markedly in Europe. In addition to physical threats, such as habitat destruction, traffic accidents and drowning in fish nets and fykes also polyhalogenated aromatic hydrocarbon- (PHAH), and more specifically polychlorinated biphenyl- (PCB) pollution is considered to be one of the major factors in this decline (MASON, 1989). This assumption was based on toxicological studies with the mink (*Mustela vison*) that is often used as a model for the otter (JENSEN *et al*., 1977; review in LEONARDS *et al*., 1994), and on associations between high PCB levels in otters and declining or endangered populations (OLSSON and SANDEGREN, 1983; BROEKHUIZEN, 1989; MASON, 1989). For practical and ethical reasons no toxicological experiments have been conducted with the Eurasian otter itself. Because of the lack of necessary data, a field study was performed to derive a no observed effect level for otters in the otter itself, its food and sediment (MURK *et al*., 1998). In addition, the possible use of the applied parameters as potential non-destructive biomarkers was studied; as such biomarkers will be needed to monitor exposure and health status of otters after re-introduction in their natural environment.

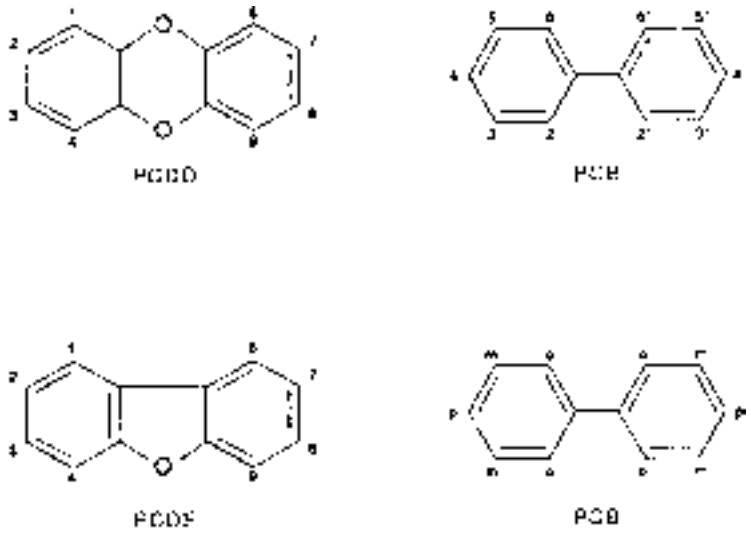

Figure 1. Chemical structure of polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and polychlorinated biphenyls (PCBs)

Polyhalogenated aromatic hydrocarbons (PHAHs) are known for their persistence, their plethora of toxic effects and enrichment in food chains in numerous areas and species (IPCS, 1993; TANABE, IWATA and TATSUKAWA, 1994; WANIA and MACKAY, 1996). All PCDD, PCDF and PCB structures consist of two halogenated phenylrings, with 75, resp. 135 and 209 possible congeners dependent on the degree and place of chlorine substitution (Figure 1).

The mechanisms of toxicity of PHAHs such as polychlorinated dibenzo-p-dioxins (PCDDs), dibenzofurans (PCDFs) and polychlorinated biphenyls (PCBs) are well investigated. In addition to the arylhydrocarbon receptor (AhR)-mediated induction of liver cytochrome P450 enzymes, PHAHs are able to disrupt metabolism and storage of retinoids (ZILE, 1992) and thyroid hormone homeostasis (BROUWER *et al*., 1998). The parent compounds alter metabolism (MURK *et al*., 1994*b*), whereas the hydroxylated metabolites disrupt the plasma transport of T_4 and all-*trans*-retinol (BROUWER and VAN DEN BERG, 1986).

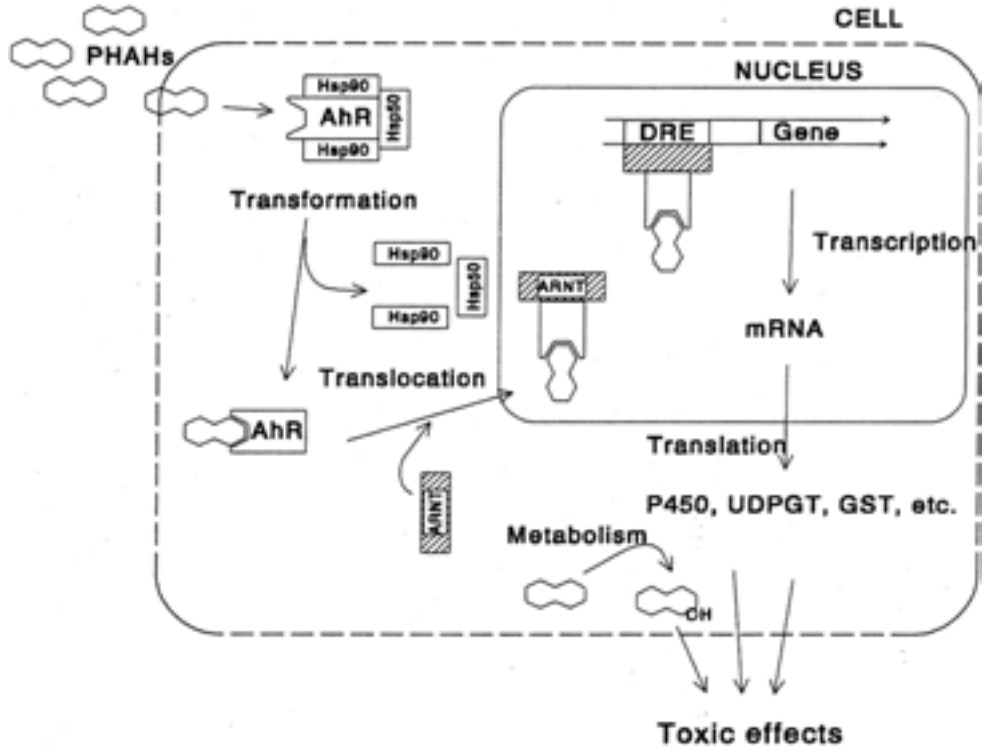

Figure 2. Schematic representation of the Ah-receptor mediated mechanism of action for 2,3,7,8-tetrachlorodibenzo-p-dioxin and related toxic PHAHs. Abbreviations: PHAHs: polyhalogenated aromatic hydrocarbons; AhR: arylhydrocarbon receptor; Hsp: heat shock protein; ARNT: Ah-receptor nuclear translocator; DRE: dioxin responsive enhancer; P450: cytochrome P450 1A; UDPGT: uridine-5-diphosphate-glucuronyltransferase; GST: glutathione S-transferase

The Ah-receptor is a cytosolic protein (POLAND and KNUTSON, 1982), which forms a complex with the chaperone protein hsp90. This complex dissociates when a ligand binds to the Ah-receptor. After binding of the compound the receptor-ligand complex is translocated to the nucleus by the Ah-receptor nuclear translocator (Arnt) protein. In the nucleus the ligand-Ah-Arnt complex binds selectively to

dioxin responsive elements (DREs) on the DNA. To date, 26 genes have been identified as having a DRE upstream, although the molecular mechanism involved in the toxicity following their transcription remains elusive (SUTTER and GREENLEE, 1992). This interaction induces expression of DRE-regulated genes, such as the marker gene cytochrome p4501A1 (CYP1A1), which causes an increased expression of CYP1A1 mRNA and protein, a phase 1 biotransformation enzyme. Dependent on the chlorine substitution other genes under control of the Ah-receptor pathway can be induced such as CYP1A2 and phase 2 biotransformation enzymes such as UDP-glucuronyltransferase 1 (UGTs) and glutathion-S-transferase π (SUTTER and GREENLEE, 1992). A scheme of the Ah-receptor-mediated mechanism is given in Figure 2.

In general, biotransformation of xenobiotics leads to detoxification and their enhanced excretion. PHAH metabolites, however, are described to own specific biological activities. Although the Ah-receptor mediated induction of CYP1A1/2 is necessary for the formation of hydroxylated and methylsulphonated metabolites, these metabolites may cause non-Ah-receptor mediated responses. A hydroxylated 3,3′,4,4′-tetrachlorinated biphenyl(CB) metabolite, 4-OH-3,3′,4′,5-tetraCB, was described to interact with transthyretin (TTR), the major plasma thyroid hormone transport protein, in rats (BROUWER, 1989; BIRGELEN, 1994), marine mammals (REIJNDERS, 1986; BROUWER *et al*., 1989*b*) and birds (MURK *et al*., 1994*a*). The effect on circulating thyroid hormones is not dependent on the parent PCB-congeners but on the presence of *para*-hydroxylated metabolites. BROUWER and VAN DEN BERG (1986) suggested a mechanism based on disturbed plasma transport for the decrease in plasma T_4 levels in rats exposed to TCB, a coplanar PCB-congener. These metabolites bear a strong structural resemblance to T_4 and exhibit competitive binding for transthyretin (LANS, 1995). A hydroxylated TCB metabolite (4-OH-3,3′,4′,5-tetraCB) present in plasma interacted with TTR *in vivo*, resulting in the competitive displacement of T_4 from TTR. Metabolites are known to selectively accumulate in the mammalian foetus (MORSE *et al*., 1996).

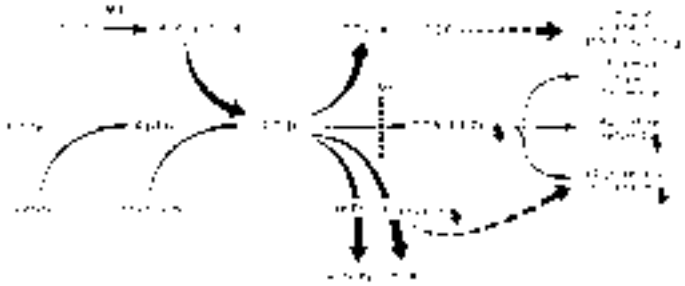

Figure 3. Model of interference of thyroxin (T_4) and retinol transport in serum after binding of a PCB metabolite to transthyretin (TTR), and its effect on T_4, retinol and retinol binding protein (RBP) levels (BROUWER, 1987). The thin lines represent the normal situation with TTR transport T_4 and retinol (bound to RBP), the solid and interrupted bold lines represent the interaction of the 4-OH-3,3′,4′,5-tetraCB (4-OH-TCB) metabolite with TTR after exposure of rats to 3,3′,4,4′,-tetraCB (TCB) and subsequent predicted effects. MFO: mixed function oxidases; b: blockage.

Via a second pathway PCBs are able to increase the glucuronidation and deiodination of T_4, which will also result in lower T_4 levels (MURK *et al*., 1994*b*). In addition to the interference of hydroxylated metabolites with plasma T_4 levels,

induction of T_4- or T_3 (triiodothyronine)-glucuronidation may enhance hepatic elimination of thyroid hormones. Hydroxylated metabolites have also been shown to competitively inhibit hepatic T_4-5´deiodinase activity, involved in the conversion of T_4 to T_3, and to uncouple mitochondrial oxidative phosphorylation (BROUWER *et al*., 1994).

In addition the interaction of 4-OH-3,3´,4´,5-tetraCB with TTR, diminished RBP-TTR binding (BROUWER, 1987), which successively led to decreases in plasma RBP and retinol (BROUWER and VAN DEN BERG, 1986). The binding of metabolites results in a decrease of T_4 and retinoid levels in blood and tissues (BROUWER and VAN DEN BERG, 1986; BROUWER *et al*., 1986; BROUWER, REIJNDERS and KOEMAN, 1989*a*). The proposed mechanism is given in detail in Figure 3.

Marine mammals, birds and rats, have all been shown to have the capacity to metabolise the model PCB-77 *in vitro* into hyroxylated metabolites, which are able to compete with T_4 for the TTR binding site (MURK *et al*., 1994*b*).

The reduction of T_4 levels may also be a result of metabolic activity and elevated levels of T_4-uridine-diphosphoglucoronyl-transferases activity due to pollutants such as Aroclor 1254, a commercial mixture of PCBs (BARTER and KLAASSEN, 1992). T_4-UGTs catalyses the forming of T_4-glucuronides, which are excreted via the gallbladder (BIRGELEN, 1994).

Many toxic symptoms of PCB-exposure resemble those of vitamin A deficiency (BROUWER, 1991; NORD, 1992), a vitamin which plays an important role in tissue development in foetuses, reproduction, and resistance against infectious diseases. As a consequence of both mechanisms of toxic action of PCBs mentioned above, the vitamin A homeostasis will be disturbed and the vitamin A storage in liver reduced, as has been demonstrated in several experimental and field studies (JENSEN *et al*., 1987; SPEAR, GARCIN and NARBONNE, 1988; SPEAR *et al*., 1989; BROUWER *et al*., 1989*a* & *b*; ZILE, 1992; BRUNSTRÖM, HAKANSSON and LUNDBERG, 1991; CHEN *et al*., 1992; MURK *et al*., 1994*a* & *b*). Therefore, reduction in hepatic vitamin A levels is expected to be a sensitive, and physiologically relevant marker for the toxic action of PCBs.

Based on the so-called 'dioxin-like' effects the TCDD- (2,3,7,8-tetrachlorodibenzo-*p*-dioxin) or toxic equivalency factor- (TEF) concept was introduced (SAFE, 1987) to be able to estimate the AhR-related toxic potency of a mixture of PCBs based on chemical data. For this purpose the concentrations of individual PHAHs are multiplied by their respective TEF value, and added up to give the total TCDD toxic equivalency of the mixture (AHLBORG *et al*., 1994).

This review summarises the results of a recently published joint study that has been performed with environmentally exposed feral and captive otters (MURK *et al*., 1998). In this paper the comparison of hepatic retinoid levels, as a potential biomarker for effect, with the AhR-related toxic potency of PCBs in the same otter livers has been described. In addition, these results were compared with information on the health status of the dead otters, which has been presented in more detail by LEONARDS *et al*. (1996).

For the animals from which livers as well as whole blood samples have been collected, TEQ levels were determined in both matrices to be able to determine whether TEQ levels in blood predict TEQ levels in liver.

2 The CALUX (chemical-activated luciferase expression) Assay

The CALUX-Assay has recently been developed, based on AhR-mediated firefly (*Photinus pyralis*) luciferase gene expression in genetically modified cell lines (AARTS *et al*., 1995). To produce the CALUX cells, a vector containing the luciferase gene under transcriptional control of DREs was stably transfected into rat (H4IIE) hepatoma cell lines (Fig. 4). Luciferase induction by TCDD appeared to be dose-dependent, and saturates at ligand concentrations greater than 100-1000nM. For the PCDD, PCDF and PCB-congeners tested so far, the relative potency to induce *CALUX* activity correlated well with proposed TEF values (AARTS *et al*., 1995; GARRISON *et al*., 1996; SANDERSON *et al*., 1996). These TEF values are based on a number of endpoints, but the cytochrome IA-inducing potency in H4IIE hepatoma cells is most important (AHLBORG *et al*., 1994). The luciferase induction of an unknown sample is expressed as so called *CALUX*-TEQ, using a TCDD standard curve (MURK *et al*., 1996, 1997).

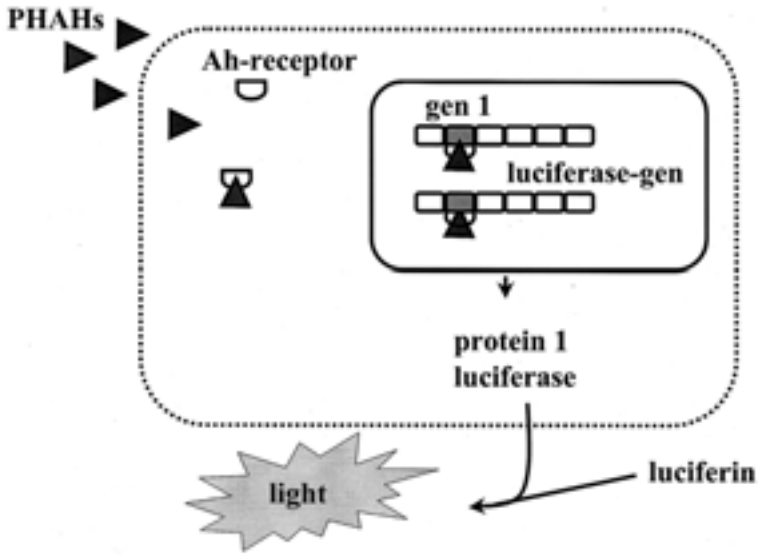

Figure 4. Schematic representation of Ah-receptor mediated luciferase production in stably transfected H4IIE cells (CALUX-Assay).

The CALUX-Assay proved to be a sensitive, fast and relatively easy method to determine the total AhR-related toxic potency of mixtures of PHAHs in different environmental matrices, expressed in TCDD equivalents (TEQs). The usefulness of the CALUX-Assay is especially evident when, due to small sample size or small concentrations of individual congeners, samples would have to be pooled, thus losing information, or animals have to be killed to get enough material. The CALUX-Assay can also be used for rapid screening of large quantities of samples.

3.1 Retinoid and TEQ levels in otter tissues

In the environmentally-exposed dead otters the hepatic TEQ levels based on non- and mono-ortho PCBs, ranged from 0.1-56 ng/g lipid. Hepatic retinylpalmitate levels, ranging from 20100-1 µg/g lipid (600-0.03 µg/g wet weight) were negatively correlated with these TEQ levels (Fig. 5a). Hepatic retinol levels ranged from 2611-4.1 µg/g lipid (94-0.1 µg/g wet weight). The negative correlations of hepatic retinol (Fig. 5b) and retinyl palmitate levels with TEQ levels were all comparable and statistically significant, either expressed on a lipid or on a fresh weight basis. Based on the fitted curve the no observed effect concentration (NOEC) and 90% effect concentration (EC90) for retinol were resp. 1 and 5 ng TEQ/g lipid and for retinylpalmitate resp. 2 and 5 ng TEQ/g lipid.

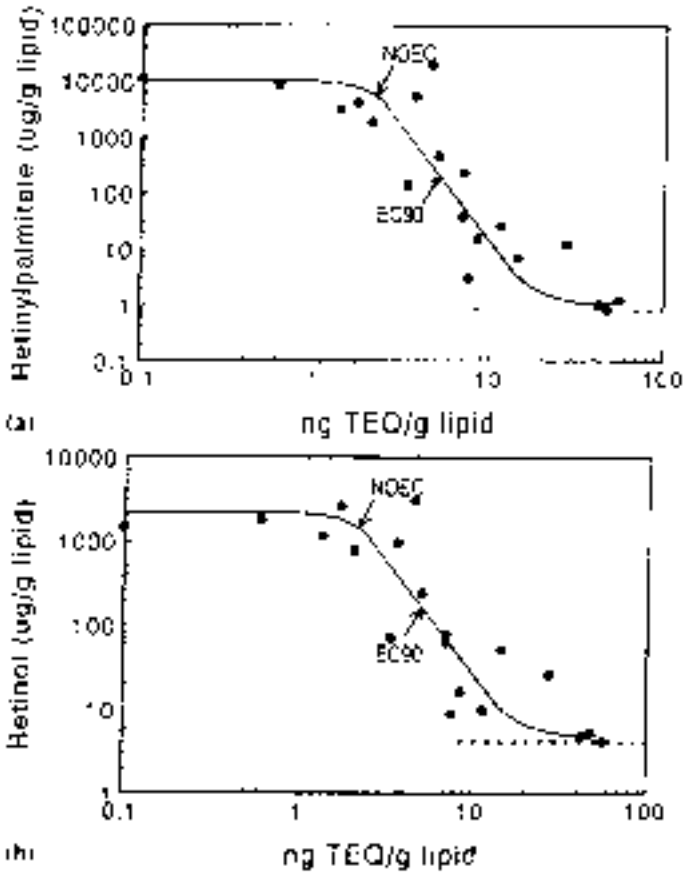

Figure 5 (a) Hepatic retinylpalmitate levels (µg/g lipid) and (b) hepatic retinol levels (µg/g lipid) in environmentally exposed otters plotted against hepatic TEQ levels (ng/g lipid; based on non- and mono-ortho PCBs). The calculated no observed effect concentration (NOEC) and 90% effect concentration (EC90) for retinylpalmitate are 2 and 5 ng TEQ/g lipid (see arrows).

The correlations with PCBs expressed as ng Σ7-PCB showed comparable patterns but were less clear (data not shown). Although both hepatic retinol and hepatic retinylpalmitate levels strongly decrease with increasing hepatic TEQ level, the ratio of hepatic retinol over retinylpalmitate increases from 13% in relatively clean, to 730% in highly exposed otters (Figure 6). The strongest increase in ratio was found in otters with hepatic PCB concentrations of about 5 ng TEQ/g lipid and higher.

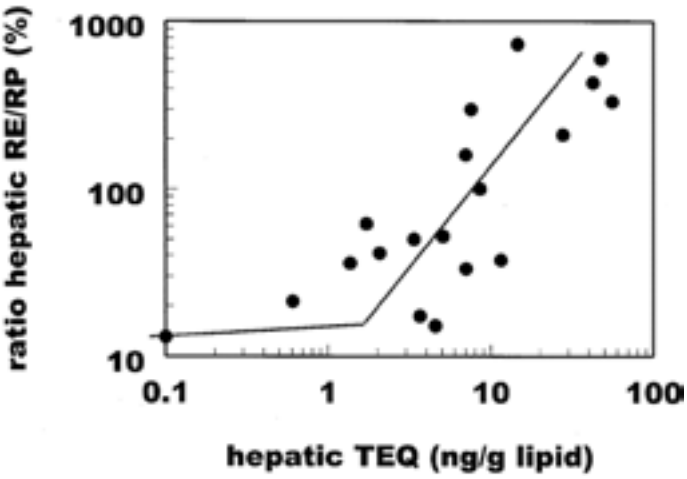

Figure 6. The hepatic retinol/retinylpalmitate (RE/RP) ratio expressed as % (w/w) in environmentally exposed otters plotted against hepatic GC-TEQ levels (ng/g lipid; based on non- and mono-ortho PCBs).

A statistically significant, and very strong correlation was observed between hepatic *CALUX*-TEQ and hepatic GC-TEQ levels (Figure 7). Hepatic GC-TEQ levels correlated with the GC-TEQ levels in blood from the same animals (Figure 8).

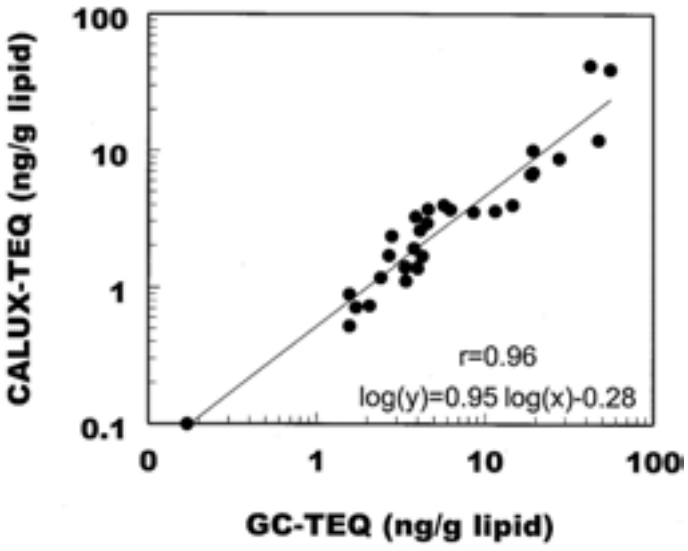

Figure 7. Correlation between TEQ levels measured with the Ah-receptor mediated expression of the luciferase reporter gene (CALUX-TEQ) and calculated based on non- and mono- ortho PCB levels (GC-TEQs) in liver of environmentally exposed, dead otters (P<0.0001).

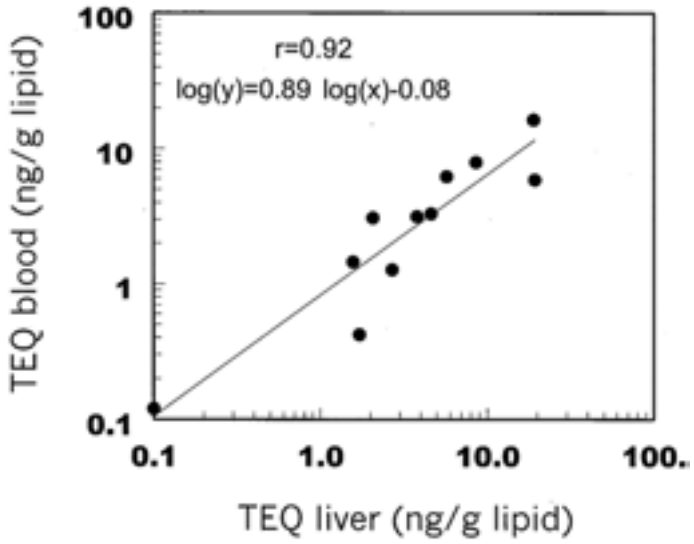

Figure 8. Correlation between TEQ levels (ng/g lipid; based on non- and mono-ortho PCBs) in liver and blood plasma of environmentally exposed otters (P<0.001).

4 Discussion

4.1 AhR-related decrease in retinoid levels

The results of MURK *et al*. (1998) suggest that otters are sensitive to compounds acting via the AhR. In combination with the selective accumulation by otters of the most toxic, planar PHAHs (LEONARDS *et al.*, 1997), current environmental concentrations appear to be high enough to almost cause a retinoid depletion. Although dietary intake of vitamin A can also influence hepatic vitamin A levels, disturbances in vitamin A homeostasis have also been associated with PHAH-exposure in experimental setups where the food quality was kept constant for all experimental groups, for example doves (SPEAR *et al*., 1989), harbour seals (BROUWER *et al*., 1989*a*), eider ducks (MURK *et al*., 1994*b*) and mink (BRUNSTRÖM *et al*., 1991). The increased ratio of retinol over retinylpalmitate with internal exposure observed in the feral otters, suggests that either the storage capacity or the mobilisation of retinoids is disturbed in a dose-related manner. In the study described by BRUNSTRÖM *et al* (1991) the hepatic vitamin A content was reduced by 48 % in adult female mink fed 2 mg Clophen A50 during 12-14 weeks. The vitamin A level was determined after complete hydrolyses, so it is not possible to distinguish between retinyl esters and retinol. An important difference with feral otters is that the mink were fed vitamin supplements, which may at least partially compensate adverse effects of the PCBs on vitamin A homeostasis. Nevertheless, in the lungs of the same animals vitamin A levels were reduced by 67%. This shows that in a target tissue vitamin A levels can already be reduced before the liver, the main vitamin A storage organ, is depleted. In Sprague-Dawley rats dosed once with 10 ng 2,3,7,8-TCDD/g body weight, retinoids were mobilized from hepatic and extrahepatic storage sites already within 14 days after exposure. Retinylpalmitate levels were decreased in liver (to 2.4%) and lung (to 20%), whereas retinyl plamitate and retinol levels were increased in kidney (to 850% and 245% respectively) and retinol levels in serum (to 145%) (BROUWER *et al*., 1989*b*).

4.2 Possible consequences of disturbance of vitamin A homeostasis

LEONARDS *et al*. (1996) studied the frequency and severity of diseases in feral Danish otters, and found that these increased with increasing hepatic TEQ-levels (see also GUTLEB, 2001). Animals were divided in three exposure groups of comparable size: low (0-5 ng TEQ/g lipid), middle (5-10 ng TEQ/g lipid) and high (>10 ng TEQ/g lipid). Table 1 presents the average concentrations of hepatic TEQ and retinoid levels for all animals tested in this study, divided in the same exposure groups as in LEONARDS *et al*. (1996). The concentration ranges at which increased disease rates occurred, correlated with ranges at which the hepatic retinol and retinylpalmitate levels decreased. This is to be expected, as vitamin A is not only essential for normal growth and development, but also for the resistance against infections. There is a bidirectional relationship between vitamin A deficiency and increased infection, which may result in a vicious cycle: vitamin A deficiency increases the risk for infection, which in turn decreases vitamin A levels (SOMMER, KATZ and TARWIOTO, 1984). Vitamin A functions in maintaining anatomical barriers of the body against microbial colonisation and infection, especially the epithelial lining of the respiratory, genitourinary and gastrointestinal tracts. Vitamin A also influences the systemic immune response, including antibody production and T lymphocyte proliferation and activity (DAVIS and SELL, 1989; FRIEDMAN and SKLAN, 1989; SIJTSMA *et al*., 1989). Immune responses were often affected before other manifestations of vitamin A deficiency were observed.

Table 1. Average GC-TEQ, hepatic retinol and retinylpalmitate levels, the average ratio hepatic retinol (RE) over retinylpalmitate (RP) (all ± standard deviation), and infection incidence in environmentally exposed dead otters grouped after their exposure levels. N is the number of otters in each group

Ranges	GC-TEQ (ng/g lipid)	Retinol (ug/g liver)	Ret.palm. (ug/g liver)	Ratio RE/RP (%)	Infection incidence[1]	N
0-5	2.2 ± 1.6^{A}	58 ± 34^{A}	273 ± 238^{A}	32 ± 18^{A}	low	8
5-10	7.0 ± 1.3^{A}	2.4 ± 2.9^{B}	4.4 ± 5.9^{B}	129 ± 107^{B}	middle	5
>10	33.2 ± 18.1^{B}	0.4 ± 0.4^{C}	0.2 ± 0.3^{C}	392 ± 254^{B}	high	6

[1] More information on the infection incidence is described in LEONARDS *et al*. (1996)

[A,B,C] Statistically significant ($P<0.05$) different group averages are indicated with different letters

In otters from England a correlation of decreasing PCB concentrations and increasing retinoid concentrations in liver tissues was found for an observation period of about a decade (SIMPSON *et al*., 2000). In a Danish survey for the health status of feral otters, especially pneumonia was a frequently occurring infectious disease, while this was never registered for otters living in captivity (MADSEN *et al*., 1999). This difference could be caused by a combination of a much lower PCB-exposure, less challenging conditions of living, and addition of vitamin A supplements to the food. As a consequence, animals living in captivity will not so easily be a victim of the vicious cycle mentioned above, and therefore experimental studies with wildlife species may result in an underestimation of the risks involved.

4.3 CALUX-TEQ-levels as a measure of internal dose

The almost linear, strong correlation between hepatic *CALUX*-TEQ and hepatic GC-TEQ levels indicates the TEF-values chosen for calculation of the GC-TEQ levels are a good measure of the toxic potency of these PCBs, which is measured directly in the *CALUX* assay. Former experiments, with single compounds, already indicated a good correlation between the TEF values of several PCDDs, PCDFs and PCBs as proposed by AHLBORG *et al*. (1994) and the toxic potency relative to TCDD as measured in the *CALUX* assay (AARTS *et al*., 1995; GARRISON *et al*., 1996; SANDERSON *et al*., 1996).

The good correlation between blood and hepatic TEQ levels indicates that TEQ levels in blood samples can be used as a measure for internal dose, in this case TEQ levels in livers. This is in accordance with earlier results with experimentally exposed eider ducks, describing a good correlation between *CALUX*-TEQ levels in blood plasma and PCB levels in abdominal lipids (MURK *et al*., 1997). The possibility to determine the internal dose based on a blood sample offers the possibility of non-destructive monitoring of exposure.

5 Conclusions

- A strong negative correlation was observed between hepatic vitamin A levels and TEQ levels in environmentally-exposed Eurasian otters. These results indicate that otters are sensitive for AhR-related toxic effects of PCBs, and that current environmental PCB levels are high enough to cause adverse effects.

- Otters exposed to more than 2 ng TEQ/g lipid had strongly reduced hepatic retinoid levels, which coincided with a higher incidence of infectious diseases.

- The toxicological potency of PCBs acting via the AhR, expressed as *CALUX*-TEQs, correlated well with the TEQ-levels estimated based on chemical PCB measurements in otter liver.

- The TEQ levels in the otter blood can be used to predict the TEQ levels in otter liver.

- The internal dose expressed as TEQs can be quantified with the *CALUX* assay using 0.5 ml of blood plasma. For determination whether an otter has an internal dose of less than 2 ng TEQ/g lipid (5 pg TEQ/ml plasma), and for quantification of higher TEQ levels, an aliquot of 50 µl blood plasma is enough.

6 REFERENCES

Aarts, J. M. M. J. G., Denison, M. S., Cox, M. A., Schalk, M. A. C., Garrison, P. A., Tullis, K., de Haan, L. H. J. & Brouwer, A. (1995). Species-specific antagonism of Ah receptor action by 2,3',5,5'-tetrachloro- and 2,2',3,3',4,4'-hexachlorobiphemyl. *European Journal of Pharmacology and Environmental Toxicology,* Section 293: 463-474.

Ahlborg, U. G., Becking, G. C., Birnbaum, L. S., Brouwer, A., Derks, H. J. G. M., Feeley, M., Golor, G., Hanberg, A., Larsen, J. C., Liem, A. K. D., Safe, S. H., Schlatter, C., Waern, F. & Younes, Yränheikki, E. (1994). Toxic equivalency factors for dioxin-like PCBs. *Chemosphere* **28**: 1049-1067.

Barter, R. A. & Klaassen, C. D. (1992). UDP-glucuronosyl-transferase inducers reduce thyroid hormone levels in rats by an extrathyroidal mechanism. *Toxicology and Applied Pharmacology*, **113**: 36-42.

Birgelen, A. P. J. M. (1994). *Toxic and biochemical effects of polychlorinated biphenyls (PCBs) relative to and in combination with 2,3,7,8-tetrachlorodibenzo-p-dioxin in the rat. Possible implications for the risk assessment*. Thesis, University of Agriculture, Wageningen, The Netherlands.

Broekhuizen, S. (1989). Belasting van otters met zware metalen en PCBs. *De Levende Natuur,* **90 (2)**: 43-47 [in Dutch].

Brouwer, A. (1987). *Interference of 3,4,3′,4′-tetrachlorobiphenyl in vitamin A (retinoids) metabolism: possible implications for toxicity and carcinogenicity of polyhalogenated aromatic hydrocarbons*. Thesis, Leiden University.

Brouwer, A. (1989). Inhibition of thyroid hormone transport in plasma of rats by polychlorinated biphenyls. *Archives of Toxicology*, **13**: 440-445.

Brouwer A. (1991). The role of enzymes in regulating the toxicity of xenobiotics. Role of biotransformation in PCB-induced alterations in vitamin A and thyroid hormone metabolism in laboratory and wildlife species. *Biochemical Society Transactions* **19**: 731-738.

Brouwer, A. & Reijnders, P. J. H., Koeman, J. H. (1989*a*). Polychlorinated biphenyl (PCB) - contaminated fish induces vitamin A and thyroid hormone deficiency in the common seal (*Phoca vitulina*). *Aquatic Toxicology*, **15**: 99-106.

Brouwer, A. & van den Berg, M. (1986). Binding of a metabolite of 3,4,3′,4′-tetrachlorobiphenyl to transthyretin reduces serum vitamin A transport by inhibiting the formation of the protein complex, carrying both retinol and thyroxin. *Toxicology and Applied Pharmacology*, **85**: 301-312.

Brouwer, A. & van den Berg, M., Blaner, W. S., Goodman, D. W. S. (1986). Transthyretin (prealbumin) binding of PCBs, a model for the mechanism of interference with vitamin A and thyroid hormone metabolism. *Chemosphere,* **15**: 1699-1706.

Brouwer, A., Hakansson, H., Kukler, A., van den Berg, K. J. & Ahlborg, U. G. (1989*b*). Marked alterations in retinoid homeostasis of Sprague-Dawley rats induced by a single i.p. dose of 10 ug/kg of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Toxicology*, **58**: 267-283.

Brouwer, A., Lans, M. C., de Haan, L. H. J., Murk, A. J. & Morse, D. C. (1994). Formation and toxicological aspects of phenolic metabolites of polychlorobiphenyls (PCBs) and related compounds. *Organohalogen Compounds,* **20**: 465-469.

Brouwer, A., Morse, D. C., Lans, M. C., Schuur, A. G., Murk, A. J., Klasson-Wehler, E., Bergman, Å. & Visser, T. J. (1998). Interactions of persistent environmental organohalogens with the thyroid hormone system: mechanisms and possible consequences for animal and human health. *Toxicology and Industrial Health,* **14**: 59-84.

Brunström, B., Hakansson, H. & Lundberg, K. (1991). Effects of a technical PCB preparation and fractions thereof on ethoxyresorufin O-deethylase activity, vitamin A levels and thymic development in the Mink (*Mustela vison*). *Pharmacology and Toxicology*, **69**: 421-426.

Chen, L-C., Berberian, I., Koch, B., Mercier, M., Azais-Braesco, V., Glauert, H. P., Chow, C. K. & Robertson, L. W. (1992). Polychlorinated and polybrominated biphenyl congeners and retinoid levels in rat tissues: structure-activity relationships. *Toxicology and Applied Pharmacology*, **114**: 47-55.

Davis, C. Y. & Sell, J. L. (1989). Immunoglobulin concentrations in serum and tissues of vitamin A-deficient broiler chicks after Newcastle disease virus vaccination. *Poultry Science*, **68**: 136-144.

Friedman, A. & Sklan, D. (1989). Antigen-specific immune response impairment in chicks as influenced by dietary vitamin A. *Journal of Nutrition*, **119**: 790-795.

Garrison, P. A., Tullis, K., Aarts, J. M. M. J. G., Brouwer, A., Giesy, J. P. & Denison, M. S. (1996). Species-specific recombinant cell lines as bioassay systems for the detection of 2,3,7,8-tetrachlorodibenzo-*p*-dioxine-like chemicals. Fund. Applied Toxicology, **30**: 194-203.

Gutleb, A. C. (2001). The role of pollutants in the decline of the otter**.** In: *Proceedings of the First Otter Toxicological Conference.* (Edited by Conroy, J. W. H., Yoxon, P. & Gutleb, A. C.). Pp. Xx-xx. Skye, September 2000. International Otter Survival Fund, Broadford Isle of Skye.

IPCS, (1993). Polychlorinated Biphenyls and Terphenyls (second edition). *Environmental Health Criteria,* **140**. WHO, Geneva, pp. 682.

Jensen, R. S., Cullum, M. E., Deyo, J. & Zile, M. H. (1987). Vitamin A metabolism in rats chronically treated with 3,3',4,4',5,5'-hexabromobiphenyl. *Biochim. Biophys. Acta,* **926**: 310-320.

Jensen, S., Kihlström, J. E., Olsson, M., Lundberg, C. & Örberg, J. (1977). Effects of PCB and DDT on mink (*Mustela vison*) during the reproductive season. *Ambio* **6**: 239.

Lans, M. C. (1995). *Thyroid hormone binding proteins as novel targets for hydroxylated polyhalogenated aromatic hydrocarbons (PHAHs): possible implications for toxicit*y. Thesis, University of Agriculture, Wageningen.

Leonards, P. E. G., Smit, M. D., de Jongh, A. W. J. J. & van Hattum, B. (1994). *Evaluation of Dose-response Relationships for the Effects of PCBs on the Reproduction of Mink (Mustela vison)*. VU Boekhandel/Uitgeverij, Amsterdam, 47 pp.

Leonards, P. E. G., Elmeros, M., Cofino, W. P., Conroy, J., Gutleb, A. C., Mason, C. F, Murk, A. J., Smit, M., van Hattum, B. & Madsen, A. B. (1996). Toxic PCBs in European otter populations in relation to biological factors and health status. In: *Development of Otter-based Quality Objectives for PCBs* (Edited by Leonards, P. E. G., Murk, A. J., de Jongh, A. W. J. J. and van Hattum, B.). IVM 96/11, 47-62.

Leonards, P. E. G., Zierikzee, Y., Brinkman, U. A. Th., Cofino, W. P., van Straalen, N. M. & van Hattum, B. (1997). The selective dietary accumulation of planar polychlorinated biphenyls in the otter (*Lutra lutra*). *Environmental Toxicology and Chemistry*, **16**: 1807-1815.

Madsen, A. B., Dietz, H. H., Henriksen, P. & Clausen, B. (1999). Survey of Danish free living otters (*Lutra lutra*) - a consecutive collection and necropsy of dead bodies. *I.U.C.N. Otter Specialist Group. Bulletin,* **16**: 65-75.

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

Morse, D. C., Klasson-Wehler, E., Wesseling, W., Koeman, J. H. & Brouwer, A. (1996). Alterations in rat brain thyroid hormone status following pre- and postnatal exposure to polychlorinated biphenyls (Aroclor 1254). *Toxicology and Appllied Pharmcology*, **136**: 269-279.

Murk, A. J., Bosveld, A. T. C., van den Berg, M. & Brouwer, A. (1994*a*). Effects of polyhalogenated aromatic hydrocarbons (PHAHs) on biochemical parameters in chicks of the common tern (*Sterna hirundo*). *Aquatic Toxicology*, **30**: 91-115.

Murk, A. J., van den Berg, M., Fellinger, M., Rozemeijer, M. J. C., Swennen, C., Duiven, P., Boon, J. P., Brouwer, A. & Koeman, J. H. J. (1994b). Toxic and biochemical effects of 3,3',4,4'-tetrachlorobiphenyl (CB 77) and Clophen A50 on eider ducklings (*Sommateria mollissima*) in a semi-field experiment. *Environmental Pollution*, **86**: 21-30.

Murk, A. J., Legler, L., Denison, M. S., Giesy J. P., van der Guchte, C. & Brouwer, A. (1996). Chemical Activated Luciferase Expression *(CALUX):* a novel *in vitro* bioassay for Ah receptor active compounds in sediments and pore water. *Fundamentals of. Applied Toxicology*, **33**: 149-160.

Murk, A. J., Leonards, P. E. G., Bulder, A. S., Jonas, A., Rozemeijer, M. J. C., Denison, M. S., Koeman, J. H. & Brouwer, A. (1997). The *CALUX* (chemical-activated luciferase expression) assay adapted and validated for measuring TCDD equivalents in blood plasma. *Environmental Toxicology and Chemistry*, **16**: 1583-1589.

Murk, A. J., Leonards, P. E. G., van Hattum, B., Luit, R., van der Weiden, M. & Smit, M. (1998). Application of biomarkers for exposure and effect of polyhalogenated aromatic hydrocarbons in naturally exposed European otters (*Lutra lutra*). *Environmental Toxicology and Pharmacology*, **6** : 91-102.

NORD (1992). Risk assessment of polychlorinated biphenyls (PCBs). *Nord,* **26**: 1-99.

Olsson, M. L. & Sandegren, F. (1983). Is PCB partly responsible for the decline of the otter in Europe? In: *Proceedings of the 5th International Otter Symposium*. (Edited by Reuther, C., Röchert R). Strasbourg, November 24-27, 1983. *Habitat* **6**: 223-227.

Poland, A. & Knutson, J. C. (1982). 2,3,7,8-tetrachlorodibenzo-p-dioxin and related halogenated aromatic hydrocarbons: examination of the mechanism of toxicity. *Annual Review of Pharmacology and Toxicology*, **22**: 517-554.

Reijnders, P. J. H. (1986). Reproductive failure in common seals feeding on fish from polluted coastal waters. *Nature,* **324**: 456-457.

Safe, S. (1987). Determination of 2,3,7,8-TCDD toxic equivalent factors (TEFs), support for the use of the in vitro AHH induction assay. *Chemosphere,* **16**: 791-802.

Sanderson, J. T., Aarts, J. M. M. J. G., Brouwer, A., Froese, K. L., Denison, M. S. & Giesy, J. P. (1996). Comparison of Ah receptor-mediated luciferase and ethoxyresorufin O-deethylase induction in H4IIE cells: implications for their use as bioanalytical tools for the detection of polyhalogenated aromatic hydrocarbons. *Toxicology and Applied Pharmacology*, **137**: 316-325.

Sijtsma, S. R., West, C. E., Rombout, J. H. W. M. & van der Zijpp, A. J. (1989). Vitamine A en Newcastle diseas virusinfectie bij kippen. *Voeding,* **50**: 182-187. [in Dutch].

Simpson, V. R., Bain, M. S., Brown, R., Brown, B. F. & Lacey, R. F. (2000). A long-term study of vitamin A and polychlorinated hydrocarbon levels in otters (*Lutra lutra*) in South West England. *Environmental Pollution*, **110**: 267-275.

Sommer, A., Katz, J. & Tarwioto, I. (1984). Increased risk of respiratory disease and diarrhoea in children with pre-existing mild vitamin A deficiency. *American Journal of Clinical Nutrition*, **40**: 1090-1095.

Spear, P. A., Garcin, H. & Narbonne, J. F. (1988). Increased retinoic acid metabolism following 3,3',4,4',5,5'-hexabromobiphenyl injection. *Canadian Journal of Physiology and Pharmacology*, **66**: 1181-1186.

Spear, P. A., Bourbonnais, D. H., Peakall, D. B. & Moon, T. W. (1989). Dove reproduction and retinoid (vitamin A) dynamics in adult females and their eggs following exposure to 3,3',4,4'-tetrachlorobiphenyl. *Canadian Journal of Zoology,* **67**: 908-913.

Spitsbergen, J. M., Walker, M. K., Olson, J. R. & Peterson, R. E. (1991). Pathological alterations in early life stages of lake trout, *Salvelinus namaycush*, exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin as fertilized eggs. *Aquatic Toxicology*, **19**: 41-72.

Sutter, T. R. & Greenle, W. F. (1992). Classification of members of the Ah gene battery. *Chemosphere,* **25**: 223-226.

Tanabe, S., Iwata, H. & Tatsukawa, R. (1994). Global contamination by persistent organochlorines and their ecotoxicological impact on marine mammals. *The Science of the Total Environment***, 154**: 163-171.

Wania, F. & Mackay, D. (1996). Tracking the distribution of persistent organic pollutants. *Environmental Science and Technology*, **30**: 390-396.

Zile, M. H. (1992). Vitamin A homeostasis endangered by environmental pollutants. *Proceedings of the Society of Experimental Biology and Medicine*, **201**: 141-153.

POST MORTEM PROTOCOL FOR OTTERS

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1 INTRODUCTION

Otters found dead in the field, or which die in captivity, are of great research value. However, in order to obtain maximum information it is important that carcases are handled correctly, and that post mortem examinations are carried out in a consistent, detailed and methodical way. This sub-chapter describes a post mortem protocol designed to give optimal results, and also to ensure that meaningful comparisons can be made when carcases are examined in different laboratories.

Ideally, carcases should be examined immediately after being found. This is often not possible in which case they should be placed in a refrigerator (4-6°C), but for no more than three days. Where it is apparent that a carcases cannot be examined within this timescale they should immediately be placed in a deep freeze (-18° to -20°C). Freezing however, produces gross and histopathological alterations, thus reducing the value of the post mortem examination.

All post mortem observations, laboratory results and details of tissues stored in deep freeze or in preservative should be recorded in a consistent way on standard forms. Appendix I is an example of the form used by the author to record organ weights, etc. Provided the data is recorded the precise layout is not important and individual investigators will have their own preferences.

Post Mortem Protocol

1. Give the carcase a unique laboratory reference number. This number must be used to identify all samples taken from the carcase.

2. Record the date when the otter was found, location (with National Grid Reference if possible), name and address of finder, and apparent cause of death.

3. Record the date of the post mortem examination and the names of participating veterinarians.

4. Weigh the carcase (dry) to the nearest 100g for adults and to the nearest 10g for cubs.

5. With the animal on its back, and using a rigid rule, measure the length from nose to anus and from anus to tail tip. In the case of road traffic accident specimens check that the spine is not fractured, as this can give erroneous results.
6. If radiographic facilities are available consider making x-ray plates, especially if shooting or unexplained trauma is suspected. X-rays are also very helpful in diagnosing cases of hydrocephalus. Scan the carcase for the presence of a microchip.

7. Examine all external features, checking for scars and bite wounds, especially to the head, feet and perineum. Check teeth for wear, calculus formation, abscesses and fractures. Note skin condition and condition of foot pads and claws. Examine eyes and ears. Record sex, noting position/development of testicles and appearance of *os penis*. Place any external parasites in 70% ethanol.

8. Make a mid-line incision from the point of jaw to the anus. Reflect skin and observe fat deposits over flanks. Record extent of fat on a scale of 1 to 3. Remove fat samples (10-20g), wrap in aluminium foil and place in deep freeze. In the case of females observe mammary gland development and check for presence of milk/colostrum.

9. Remove lower rib cage and abdominal wall. Observe organs *in situ*. If any appear abnormal consider taking samples for bacteriological examination. Photograph any abnormalities, remembering to include the case reference number in the photograph.

10. Remove and weigh all organs, including thyroid glands, thymus, adrenal glands and pancreas. Weigh small organs to two decimal places.

11. Where resources permit, place a section of all tissues in buffered formal saline (BFS)* for histological examination. Samples should not exceed 5mm in thickness, except lung which may be up to 10mm in thickness. Small organs, such as thyroid glands, may be fixed whole. Any tissues showing pathological lesions should always be placed in fixative. If there is a history of the animal having shown neurological signs place at least one side of the brain in BFS.

12. If organs show lesions suggestive of a viral infection, e.g., canine distemper or Aleutian disease place small, uncontaminated, samples in sterile containers and hold at 4°C prior to contacting a virology reference laboratory for their advice on storage and handling. If this is not possible place samples directly in deep freeze.

13. In order to minimise contamination, delay opening the alimentary tract until all other organs have been examined. Examine, and if possible identify, any stomach contents before placing them in deep freeze. Always freeze stomach contents in cases of suspected poisoning.

14. Cut both kidneys longitudinally, using at least two parallel cuts, and check for renal calculi. If present, place calculi in a clean bijou bottle (do not place in 10% BFS).

15. Place duplicate samples (approximately 20g) of liver, kidney, skeletal muscle and brain in aluminium foil and transfer to deep freeze for future toxicological examination.

16. If the otter is freshly dead, place approximately 20g of liver in deep freeze for vitamin A analysis. This should be carried out within one month.

17. For genetic analysis collect a piece of kidney or tongue (approximately 5g). Store either in 95% ethanol or, without preservative, in deep freeze. Take care to use clean instruments on uncontaminated surfaces.

18. Remove at least one upper incisor tooth for age determination. This may be stored frozen or in BFS.

19. If possible collect a serum sample for future antibody studies. Pericardial fluid, if available, is a good alternative. Hold in deep freeze.

20. If the animal is pregnant, or if neonates/foetuses are submitted, the weight, sex and crown – rump length should be recorded. It is particularly important to look for evidence of developmental defects, e.g. anophthalmia, hydrocephalus, cleft palate. Any abnormal looking organs, including placenta, should be cultured for evidence of bacterial infection. Samples should also be placed in BFS. If possible examine liver for vitamin A status.

*BFS : This is usually 10% but concentrations between 5 and 10% are satisfactory.

2 DISEASES

2.1 Non-infectious Diseases

Traumatic injuries, principally caused by road traffic accidents and intra -specific aggression are common (SIMPSON, 1997). Bite wounds are mostly to the face, feet and anus/genitals (SIMPSON and COXON, 2000). In some cases bites may result in fracture of the baculum (os penis) (STEPHENS, 1957). In *L. canadensis*, defective development of the baculum, as well as small or missing testes, has been linked to polychlorinated hydrocarbon pollutants (HENNY, GROVE and HEDSTROM, 1996). A cryptorchid otter in Cornwall had a nil detectable hepatic Vitamin A level (SIMPSON *et al.,* 2000). Hydrocephalus has been recorded in cubs, but the cause is obscure (GREEN, 1998). Five out of thirteen otters that died following an oil spill in Shetland were shown to be suffering from haemorrhagic gastroenteritis, believed to be due to ingestion of oil (BAKER *et al.*, 1981).

Urolithiasis is common, especially in captive otters (STEPHENS, 1957; KEYMER, LEWIS and DON, 1981). Salivary calculi, or sialolyths, have been reported in a number of otters in South West England (SIMPSON, 1998) and in a single case from Shetland (BAKER *et al.*, 1981). As with urolithiasis, the aetiology is unknown. Gall stones, or choleliths, have been noted by a number of investigators but their significance is obscure (MADSEN *et al.*, 1999; WELLS, KEYMER and BARNETT, 1989). Cases of cystic or/and convoluted uteri have been recorded in Norway, England and Denmark and although they appear pathological this is not proven (HEGGBERGET, 1988; SIMPSON, 1997; ELMEROS and MADSEN, 1999).

Blindness was reported to be common in otters in England between 1957 and 1980. One or both eyes were affected, appearing white, but they were not examined by a pathologist (WILLIAMS, 1989). A similar case has been reported recently in Denmark (MADSEN *et al.*, 1999). The precise nature of the lesion in both countries remains uncertain. However, lenticular cataracts were seen in a single case in Norfolk, England (WELLS *et al.*, 1989). Recent investigations in South West England showed clear evidence of retinal dysplasia in approximately 12% of cases and suspected lesions in a further 25% (WILLIAMS, FLINDALL and SIMPSON, 1998).

Otters have been observed showing signs of inco-ordination/disorientation in Ireland and England (MASON and O'SULLIVAN, 1992; WELLS *et al.*, 1989) but neuro-histological examinations were either not carried out or no lesions were seen.

Adrenal hyperplasia was reported in a single case in Norfolk (KEYMER *et al.*, 1988) and in a number of otters in South West England. In the latter cases it appeared that males dying of bite wounds and females in late pregnancy/lactating were most likely to be affected (SIMPSON, 1997). However, although stress may be implicated there was a positive correlation between adrenal size and hepatic concentration of some PCB congeners (SIMPSON, 1998). Adrenal aplasia, together with renal aplasia, has been reported in *L. canadensis* in the USA and appears to be linked to levels of polyhalogenated hydrocarbons in the environment (HENNY *et al.*, 1996).

Chronic mercury poisoning has been suspected in otters in Shetland (KRUUK and CONROY, 1991) and high tissue levels have also been recorded in England (MASON, LAST and MACDONALD, 1986). The highest levels in these cases were similar to those seen in experimental poisoning in *L. canadensis* (O'CONNOR and NIELSEN, 1981). Unfortunately, brains were not examined histologically.

2.2 Infectious Disease

There is little evidence of significant infectious disease in wild otters. STEPHENS (1957) referred to a case of tuberculosis in Cornwall, England but the organism was not typed. More recently *Mycobacterium avium* ssp. *avium* was shown to be the cause of massive lesions involving the mesenteric lymph nodes in an otter in Scotland (A. PATTERSON, *pers. comm.*). Small greyish granulomata, which may resemble those of tuberculosis, are sometimes seen in the lungs. These are due to inhaled spores of the fungus *Emmonsia* sp. The condition is referred to adiaspiromycosis and is common in otters in England (SIMPSON and GAVIER-WIDEN, 2000) and in Finland (RUDBACK and STJERNBERG, 1998). Other bacterial infections occasionally recorded are pseudotuberculosis, caused by *Yersinia pseudotuberculosis* (KEYMER, 1992) and salmonellosis. *Salmonella binza* was isolated from the gut of an otter in Norfolk and could possibly have been derived from poultry. *S. enteritidis*, phage type 6, caused fatal gastroenteritis in a captive Asian small clawed otter (*Aonyx cinerea*) which had been fed on day old chicks (V. R. SIMPSON, *unpublished data*) and *S. enteritidis* was also isolated from a wild otter in Russia (BENKOVSKII, GOLOVINA and SCHERBINA, 1973).

An otter which had apparently died after eating toads had multiple haemorrhages in the lungs and *Aeromonas hydrophila* was isolated on culture (SIMPSON and RULE, *unpublished data*). The same organism was isolated from the heart and lungs of an otter which died from severe adiaspiromycosis (SIMPSON and GAVIER-WIDEN, 2000).

Leptospirosis has been suggested as a possible cause of jaundice in otters (KEYMER, 1992). However, there is, as yet, no supporting evidence for this condition in otters, and histological examination of a large numbers of livers and kidneys from South West England showed no lesions suggestive of leptospirosis (SIMPSON, 1998).

As yet unnamed *Brucella* sp. has been isolated from otters, as well as various pinnipeds and cetaceans, in Scotland (FOSTER *et al.*, 1996). The significance of this isolate is as yet uncertain. *Plesiomonas shigelloides* was implicated as a probable cause of abortion in an otter foetus in Scotland (WEBER and ROBERTS, 1989)

Viral infections of otters appeared to be very uncommon. Although there are records of canine distemper affecting captive otters in Germany (GEISEL, 1979), and distemper virus inclusion bodies have been seen in otherwise healthy wild otters in Denmark, there do not appear to be reports of it causing clinical disease in wild otters. There is a single record of rabies in a wild otter, also in Germany (WILHELM and VOGT, 1981). A tentative diagnosis of Aleutian disease was made histologically on an otter from Norfolk, England (WELLS *et al.*, 1989). Feline infectious peritonitis has been suspected in a captive *A. cinerea* (VAN de GRIFT, 1976).

Although various parasites have been recorded in otters there is little evidence that they cause disease. Infection of *L .canadensis* with the kidney worm *Dioctophyme renale* is not uncommon in North America and the parasite has been recorded in *L. lutra* in the UK (CORBET and HARRIS, 1991). An unidentified strongyle larva was seen histologically in the renal pelvis of an otter from South West England (SIMPSON, 1998). Another animal in the same study had *Sarcocystis* sp. in the external eye muscles. In a study in Denmark *Angiostrongylus vasorum* larvae were identified in the lungs of a single otter (MADSEN *et al.,* 1999).

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4 REFERENCES

Baker, J. R., Jones, A. M., Jones, T. P. & Watson, H. C. 1981. Otter *Lutra lutra* mortality and marine oil pollution. *Biological Conservation* **20**: 311-321.

Benkovskii, L. M., Golovina, T. I. & Scherbina, R. D. 1973. Studying the diseases and parasites of the otter from Sakhalin Island. *Vestnik Zoologie*. **7**: 21-24.

Corbet, G. B. & Harris, S. 1991. *The Handbook of British Mammals*. Blackwell, Oxford.

Elmeros, M. & Madsen, A. B. 1999. On the reproductive biology of otters (*Lutra lutra*) from Denmark. *Zeitschrift fur Saugetierkunde* **64**: 193-200.

Foster, G., Jahans, K. L., Reid, R. J. & Ross, H. M. 1996. Isolation of Brucella species from Cetaceans, Seals and an Otter. *Veterinary Record*, **138**: 158-586.

Geisel, O. 1979. Staupe bei Fischottern (*Lutra lutra*). *Berliner und Munchener Tierarztliche Wochenschrift*. **92**: 304.

Green, R. 1998. Proceedings of the 1998 IUCN Otter Colloquium, Trebon.

Grift, van de, E. R. 1976. Possible feline infectious peritonitis in short clawed otters, A. cinerea. *Journal of Zoo Animal Medicine,* **7**: 18.

Heggberget, T. M. 1988. Reproduction in the female European otter in central and northern Norway. *Journal of Mammalogy,* **69**; 164-167.

Henny, C. J., Grove, R. A. & Hedstrom, O. R. 1996. *A Field Evaluation of Mink and River Otter on the Lower Columbia River and the Influence of Environmental Contaminants*. Final report to The Lower Columbia River Bi-State Water Quality Program, February 12, 1996.

Keymer, I. F., Lewis, G., & Don, P. 1981. *Sonderdruck aus Verhandlungsbericht des XXII Internationalen Symposiums uber die Erkrankungen der Zootier, Halle/Saale*, 391-401.

Keymer, I., Wells, G., Mason, C. & Macdonald, S. 1988. Pathological changes and organochlorine residues in tissues of wild otters (*Lutra lutra*). *Veterinary Record,* **112**: 153-155.

Keymer, I. 1992. Diseases of the otter (*Lutra lutra*). *Proceedings of the National Otter Conference, Cambridge, September 1992* Pp. 30-33.

Kruuk, H. & Conroy, J. 1991. Mortality of otters (*Lutra lutra*) in Shetland. *Journal of Applied Ecology*, **28**: 83-94.

Madsen, A. B., Dietz, H. H, Henriksen, P. & Clausen, B. 1999. Survey of free-living otters (Lutra lutra) – a consecutive collection and necropsy of dead bodies. *I.U.C.N. Otter Specialist Group Bulletin,* **16**: 65-75.

Mason, C., Last, N. & Macdonald, S 1986. *Bulletin of Environmental Contamination and Toxicology,* **37**: 844-849.

Mason, C. F. & O'Sullivan, W. M. 1992. Organochlorine pesticide residues and PCBs in otters (*Lutra lutra*) from Ireland. *Bulletin of Environmental Contamination and Toxicology,* **48**: 387-393.

O'Connor, D. & Nielsen, S 1981. *Proceedings of the First Worldwide Furbearers Conference, American Forestry Association*. Pp 1728-1745.

Rudback, E. & Stjernberg, T. 1998. Trauma is the major cause of mortality in Finnish otters. *Proceedings of EWDA 3rd International Conference, Edinburgh, 16-20 September 1998*

Simpson, V. R. 1997. Health status of otters (*Lutra lutra*) in South West England based on post mortem findings. *Veterinary Record,* **141**: 191-197.

Simpson, V. R. 1998. *A Post Mortem Study of Otters (Lutra lutra) found dead in South West England*. R&D Technical Report W148, Environment Agency, Rio House, Waterside Drive, Aztec West, Bristol BS32 4UD

Simpson, V. R. & Coxon, K. E. 2000. Intraspecific aggression, cannibalism and suspected infanticide in otters. *British Wildlife,* **11**; 423-426.

Simpson, V. R. & Gavier-Widen, **D.** 2000. Fatal adiaspiromycosis in a wild European otter (*Lutra lutra*). *Veterinary Record*, **147**: 239-241.

Simpson, V., Bain, M., Brown, R., Brown, B. & Lacey, R. 2000. A long-term study of vitamin A and polychlorinated hydrocarbon levels in otters (*Lutra lutra*) in South West England. *Environmental Pollution,* **110**: 267-275.

Stephens, M. M. 1957. *The Otter Report*. University Fund for Animal Welfare, Potters Bar, Herts.

Weber, J. M. & Roberts, L. 1989. A bacterial infection as a cause of abortion in the European otter, Lutra lutra. *Journal of Zoology,* **219**: 688-690.

Wells, G., Keymer, I. & Barnett, K. 1989. Suspected Aleutian disease in a wild otter (*Lutra lutra*). *Veterinary Record,* **125**: 232-235.

Wilhelm, A., & Vogt, D. 1981. Rabies in an otter, *Lutra lutra*. *Monatshefte fur veterinarmedizin*, **36**: 361.

Williams, J (1989). Blindness in otters. *I.U.C.N. Otter Specialist Group Bulletin*, **4**: 29-30.

Williams, D. W., Flindall, A. & Simpson, V. R. 1998. Retinal dysplasia in wild otters: a pathological survey. In: *Abstracts of the 3rd European Wildlife Disease Association Conference, Edinburgh, 1998*

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Otter *Post Mortem* Data – sample record sheet

Name of laboratory:

Case Reference No: **Pathologist**

Total Length:	cm		Date Submitted:
Nose to anus:	cm		Date of PM:
Anus to Tail:	cm		Sex:
Body Weight:	kg		Fresh or Frozen:

Organ	Weight (gm)	Histol.	Freeze	Notes/special instructions
Heart				
Liver				
Spleen				
Right Kidney				
Left Kidney				
Right Thyroid				
Left Thyroid				
Right Adrenal				
Left Adrenal				
Lung				
Cardiac Thymus				
Pancreas				
Right testis				
Left testis				
Foot Pad	---		---	
Eye/s	---		---	
Fat	---	---		
Muscle	---			
Uterus/Gonads	---		---	
Brain/spinal cord	---			
Salivary Gland	---		---	
Bladder	---		---	
Stomach Contents		---		
Rib/bone	---	---		
Incisor Tooth	---		---	
Blood/serum	---	---		
Liver: Vitamin A	---	---		
Urine	---	---		

DNA FINGERPRINTING OF OTTER SPRAINT

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1 INTRODUCTION

Over the past 25 years a great deal of time and effort has been put into studying the Eurasian otter (*Lutra lutra*). However a high proportion of this work is concerned with the otter's diet, with its distribution and status or with toxic chemicals and otters. Research on 'behavioural' aspects of the otter's life, such as foraging, population structure, territories, movements etc are relatively few in number and a high proportion of these come from studies of coastal otters on Scottish Islands where otters are easier to study because they are active during the day.

This situation is not unique amongst European carnivores, which are generally more difficult to study than smaller mammalian species such as rodents, but both red foxes and badgers, which are similar in size to otters, have been well studied over many years. Otters are much more scarce than these species and possibly more difficult to capture. In addition, their endangered status in many countries creates legal and ethical constraints to capturing animals for marking or radio- tagging. All of these factors conspire to make the study of otter behaviour difficult to carry out, particularly in areas where the need for conservation is greatest as a result of past declines in population.

The discovery that the DNA of otters can be recovered from their spraint opens up a wide range of opportunities for ecologists by enabling them to record locations visited by individual otters over a period of time without any direct interference to the animals themselves and also to monitor the activities of a considerable number of individuals. The purpose of this paper is to summarise the results of a pilot study of the technique carried out in the UK during 1997 and 1998 (COXON *et al*., 1998).

2 TECHNICAL BACKGROUND

DNA fingerprinting of otter spraint is based on the fact that chromosomes carry sections of DNA which do not form part of the genes but consist of short sequences of DNA repeated few to many times. These are known as satellites (micro-satellites if less than ten base pairs per repeat, mini-satellites if more). The most significant features of these are that the number of repeats is variable – giving rise to the equivalent of gene alleles - and that each is flanked by a characteristic sequence of DNA known as a primer (see box).

Micro-satellite with 5 repeats (primer underlined)

tgtctacgt*cacacacaca*ctcgtacgt

Same micro-satellite with 4 repeats:

tgtctacgt*cacacaca*ctcgtacgt

The discovery that DNA from cells lining the gut of an animal could be recovered from faecal material opened up the possibility of using the technique of DNA fingerprinting as a means to study animal ecology. Research carried out by John Dallas and colleagues at Aberdeen University led to the identification of 13 micro-satellites in otters (DALLAS and PIERTNEY, 1998) and in preliminary laboratory trials they were able to successfully extract intact otter DNA from the spraints of captive animals. However they also discovered that the DNA was rapidly broken down once the spraint had been deposited indicating that only spraint collected within hours of deposition could be used.

Analysis of collected spraint consists of two stages:

1 Extraction and amplification of the DNA fragments using the primers to locate the micro-satellites and the Polymerase Chain Reaction (PCR) to increase the quantities of each to measurable levels;

2 'Typing' – typically using gel electrophoresis to separate the alleles at each locus and determine their size.

3 POTENTIAL FOR ECOLOGICAL RESEARCH

The ability to identify the location of individual animals (albeit only at sites where they defecate) at intervals over a period of time offers considerable scope for adding to our knowledge of otter ecology and behaviour. The following list indicates a number of obvious possibilities:

- Minimum numbers of otters present;
- Proportion of these that are resident;
- Turnover of residents;
- Home range size and distribution;
- Territoriality;
- Movements of non-residents;
- Dispersal of young;
- Sex differences in the above;
- Sex ratios;
- Differences between areas where otters are established and re-colonising.

In addition to these there are opportunities for genetic studies, for example comparing the genetic variability in different areas. The number of loci available for DNA fingerprinting to date does not permit the determination of relatedness between individuals although it is possible to exclude relationships in some instances.

The use of DNA fingerprinting in toxicological studies may be limited, at least until the relationships (if any) between levels of pollutants in spraints, levels in the diet and the pollutant burden within the animal is better understood. Clearly it would

permit the study of changes of contamination of spraints over time, which might, in itself, give some indication of the nature of these relationships.

4 THE PILOT PROJECT

In 1997 a project was devised to field test this technique. Funded by the Environment Agency, the consortium of collaborators involved staff of the Agency and the Universities of Aberdeen and Exeter, together with staff and volunteers from local Wildlife Trusts and Otter Groups in Hampshire, Somerset and Devon.

The project had four principal objectives:

1 To fingerprint tissue samples from at least 100 otters from southwest England to obtain data on levels of genetic diversity in the area being studied.

2 To collect and analyze approximately 500 spraints from four study areas across southern England.

3 To produce a report assessing the feasibility of the technique.

4 To identify resource needs and a protocol for collection and analysis which could be used by researchers in the future.

5 FIELDWORK

Four rivers were selected, three in the southwest; the Torridge, the Brue and the Tone and one in central southern England; the Itchen. Groups of volunteers were assigned sites to be searched once a month on a predetermined day. The sites were spaced at an average of one per 3km of river on all but the Itchen where the density was 1.6 sites per km.

Only spraint known to have been deposited the previous night was collected and placed in chilled absolute alcohol until it could be taken to a freezer for long term storage. Collecting normally ceased at 10.00am to minimize the amount of degradation of DNA.

Volunteers for the Brue, Tone and Itchen were members of existing otter groups who had been working in these areas previously. Most were amateur naturalists but with experience of finding and identifying otter spraints. No such group existed on the river Torridge and rather than train new field workers a small number of people living nearby who already had some experience with otter surveying were recruited.

6 PRELIMINARY RESULTS

6.1 Summary of Achievements

Fifty volunteers visited 150-200 sites per month on the four catchments for 12 to 15 months, achieving 2667 site-visits. A total of 622 spraints were collected and 119 of these were typed (*ca.* 20%) yielding 57 different genotypes and, therefore, a minimum of 57 otters on these four waterways.

6.2 Ecological Data

Table 1 and Figures 1 and 2 illustrate the nature of data recorded and show that although a large number of positive identifications could be made, the amount of information recorded for each otter was relatively small. Forty-three otters were only identified once and only three otters were identified more than five times, one of these being recorded three times at the same site.

Table 1 Summary of collections for each river

	Itchen	**Brue**	**Torridge**	**Tone**
Samples	261	97	89	175
Fingerprints	53	16	15	35
Spraints/visit	0.13	0.28	0.55	0.46
Genotypes	13	12	10	22

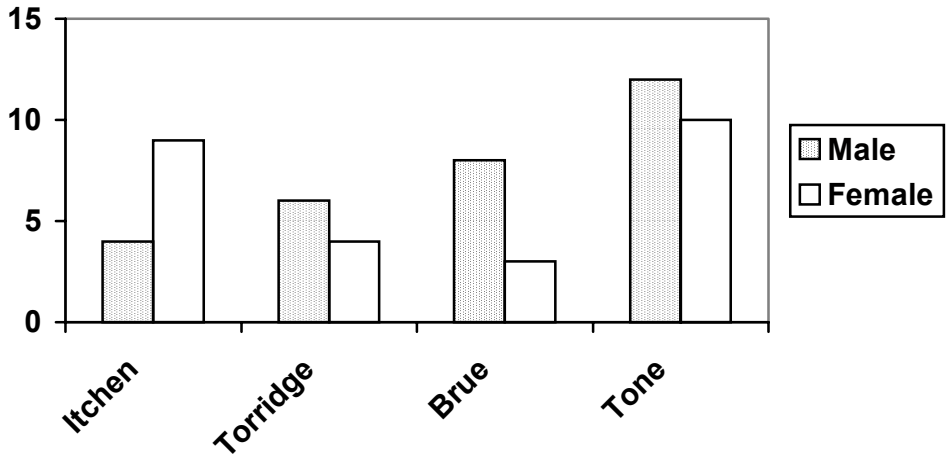

Figure 1. Number of otters of each sex

ID	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul
S22F		X		X										X	
S25M		X													
S26F		X											X		
S27M		X													
S23F				X											
S07F				X				X							
S21M				X											
S28F					X										
S01M						X					X				X
S09M								X							
S10M									X						
S12F									X						
S13F										X	X				
S14F										X					
S15M										X	X				
S20F										X					
S03M											X				
S18F											X				
S19M											X				
S34M													X		
S39M													X		
S38M															X

Figure 2. Dates on which each otter was recorded for the Tone. Female otters are identified by the ID suffix F and males by the ID suffix M.

6.3 Genetic Information

Analysis of the tissue samples from otters in the southwest showed that the genetic variability in the area was considerably lower than in Scotland. The number of alleles at most loci fell consistently within the range three to five, indicating low to intermediate levels of variability. However one locus had a very high frequency of one allele and of the nine loci tested only six were used for the analysis of DNA from otter spraint.

Variability on the Itchen was particularly low - three was the highest number of alleles recorded and one locus was monomorphic.

Table 2. Number of alleles at each locus on the Tone and Itchen

	701	**715**	**717**	**832**	**833**	**902**
Tone	4	3	4	4	4	3
Itchen	2	1	2	3	3	3

7 PROBLEMS AND OBSTACLES TO PROGRESS

Three main ‘problems’ were identified by the pilot project.

1 Only 20% of spraints could be typed

2 Low levels of genetic variability
- •SW otters show about half the variation of Scottish otters
- •Itchen otters are much less variable than those from the Southwest

3 Retention of volunteers

Given the effort put in by everyone contributing to the project it was disappointing that only 20% of the spraints collected could be successfully typed. The data presented shows tantalizing indications of what might be available but the fact that some otters were caught two or three times over a period of up to a year indicates that more is needed to fully interpret this. Were the animals present in the study area but not recorded or did they leave the area and then return?

A young otter that was found dead on the Itchen proved to have a genotype identical to those from spraints collected both before it could have been born and after it had died. Clearly in this case the genetic variability was not sufficient to reliably discriminate between some individuals on the basis of the six loci used. Subsequent reanalysis of DNA from spraints collected on this river using further loci revealed the fact that one other otter, thought to have been recorded 19 times over *ca.* 40km of river, was in fact two individuals, although one of these was only recorded twice.

Although the groups of volunteers already in existence continued with some changes in personnel, it proved more difficult to maintain the small group working on the Torridge, not through lack of commitment but because several of the members had to give up due to changes in personal circumstances (obtaining jobs and moving houses). This was related to the younger average age of members of this group.

8 SUBSEQUENT DEVELOPMENTS AND THE FUTURE.

Since the project was completed techniques for extracting and analysing DNA have continued to develop. In a recent small-scale trial a commercial company was able to extract DNA from 75% of a batch of otter spraints and also to obtain higher quantities. Rather than the limited selection of six loci that were used in the pilot project, nine loci will be examined in an effort to reduce the risk of misidentifying otters with similar profiles. A batch of 300 spraints from the Tone, Brue and Itchen has now been sent to this company for analysis and the results are expected to be available during 2001.

Should it prove possible to extract and type a higher proportion of spraints with increased confidence due to more loci being used, there are plans for further studies using the technique in Scotland and Cornwall and interest has been expressed by a number of people studying otters elsewhere in Europe.

9 CONCLUSION

This paper is intended to provide a simple background to the process and pitfalls of DNA fingerprinting. For a more detailed explanation the paper by COXON *et al*., (1998) should be consulted. The Executive Summary of this together with names of people interested in the technique, the address of the commercial company and some other information may be found on the world wide web at http://www.ex.ac.uk/mammals/dna/.

10 REFERENCES

Coxon, K., Chanin, P., Dallas, J. F. & Sykes, T. (1998). *The Use of DNA Fingerprinting to Study the Population Dynamics of Otters (*Lutra lutra*) in Southern Britain: a Feasibility Study.* R&D Technical Report W202. Environment Agency, Swindon. 123 pages.

Dallas, J.F. & Piertney, S.B. (1998). Microsatellite primers for the Eurasian otter. *Molecular Ecology* **7**: 1248-1251.

INTEGRATED APPROACHES
TO THE ANALYSIS OF CONTAMINANTS IN OTTERS

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ABSTRACT
Spatial and temporal analyses of contaminant levels in otters are often essential for determining whether pollutants exert an effect at the population level. Such analyses usually require comparison and integration of data from different studies and laboratories but this can be problematic if the ways in which contaminants have been quantified has varied markedly. In this paper, we reviewed 33 studies on contaminant levels in otters, quantified how the approach, quality and reporting of the chemical analysis varied, and highlighted the critical factors that need to be incorporated into the analysis so that data from different studies can be integrated. The types of contaminants, the samples analysed, and the analytical methods used differed substantially between otter studies. However, none of these factors were likely to prevent integration of contaminant data for otters provided that good quality assurance and control procedures were carried out and reported. Provision of quality assurance data was inconsistent in the otter studies that were reviewed and it is recommended that reporting of sample weights, moisture and lipid content of samples, limits of detection and recovery data should be considered an essential requirement for contaminant studies.

1 INTRODUCTION

The effects of pollutants on otters have been a particular cause for concern. In Europe, this is because of the role that organochlorine (OC) pesticides and polychlorinated biphenyls (PCBs) are thought to have played in contributing to the decline of Eurasian otter (*Lutra lutra*) populations (MACDONALD and MASON, 1983; JEFFERIES, 1989). There has also been much attention paid to the possible effects of contaminants on otter and other mustelid species in the USA and elsewhere (MASON and WREN, 2001). As a consequence, there have been a considerable number of studies carried out to determine the exposure of otters to various contaminants and assess the associated impacts on populations [see review by MASON and WREN (2001)].

Most contaminant studies on otters involve measurement of pollutant levels in the diet or body tissues. Dietary pollutant concentrations can be compared to doses known to cause effects in captive individuals or in test species, such as American mink (*Mustela vison)*. Similarly, tissue burdens in free-living animals can be compared with levels associated with organ-specific or more general toxic insult in experimentally dosed laboratory species. Such extrapolations between species and between captive and free-living animals can be problematic (FORSYTH, 2001) but are usually the only means of assessing whether contaminants adversely affect free-living animals. These studies are totally dependent upon high quality chemical analysis to correctly identify the pollutants involved and accurately quantify the magnitude of exposure and/or subsequent accumulation by otters. Furthermore, as the

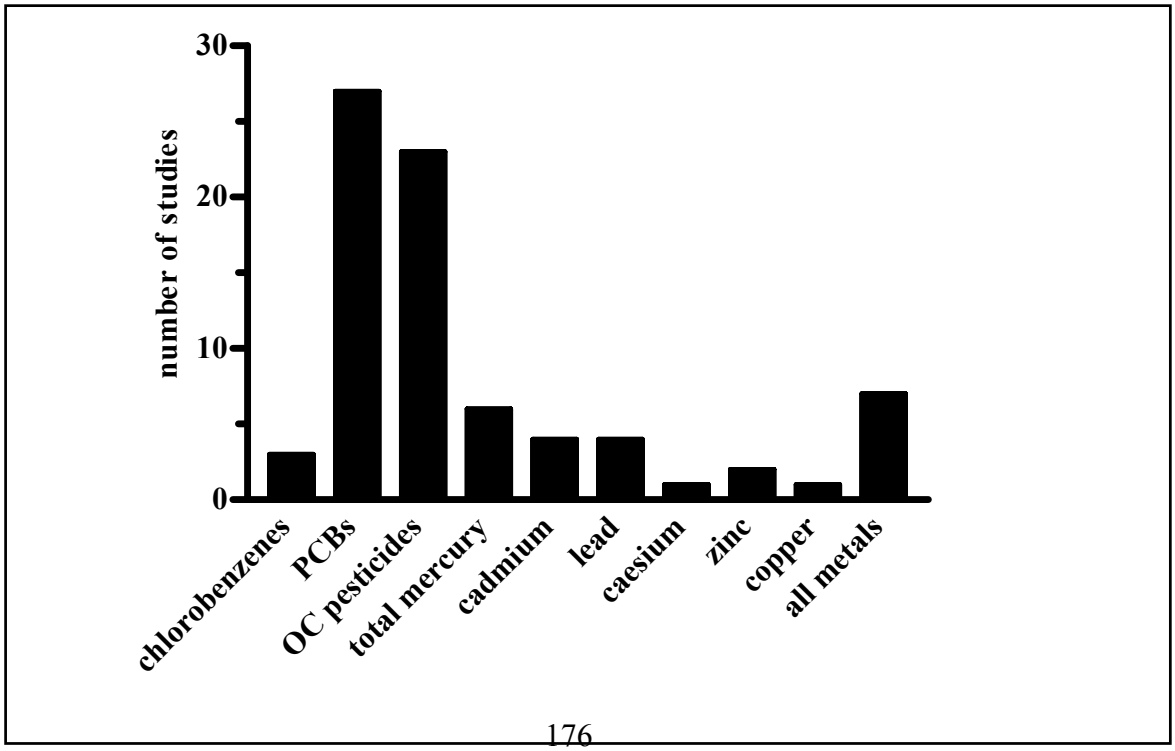
cumulative number of studies has grown, it has become increasingly possible to assess how the levels of contaminants in otters and their diet differ between regions and countries, and has changed over time. These spatial and temporal analyses are often key in determining whether pollutants exert an effect at the population scale and are again reliant upon high quality chemical analyses that are consistent between studies and laboratories.

In this paper, we review a number of major studies on contaminant levels in otters and describe how the approach, quality and reporting of the chemical analysis have varied between studies. The aim is to highlight the main factors that should be incorporated into studies and routinely reported when analysing contaminant data, so that valid comparisons can be made between different studies.

2 METHODS

Thirty-three studies (Figure 1) that reported contaminant levels in Eurasian otters or their diets and spraints were reviewed. These studies were published in peer-reviewed journals between 1981 and 2000. Details of contaminant and tissue type analysed, sample preparation, methods of analysis and the ways in which data and quality control measures were reported, were all recorded.

Figure 1. Types of contaminants determined in thirty-three peer-reviewed studies on otters published between 1981 and 2000. The papers reviewed were: MASON and REYNOLDS, 1988; SKARÉN, 1988; DELIBES, MACDONALD and MASON, 1991; KRUUK and CONROY, 1991; MASON *et al*., 1992; MASON and MADSEN, 1992; MASON and O'SULLIVAN, 1992; MASON, 1993*a* & *b*; MASON and MACDONALD, 1993*a*, *b* & *c*; MASON and MADSEN, 1993; MASON and O'SULLIVAN, 1993*a & b*; O'SULLIVAN, MACDONALD and MASON, 1993; BERGMAN *et al*., 1994; MASON and MACDONALD, 1994; MASON and RATFORD, 1994; LÓPEZ-MARTIN, RUIZ-OLMO and BORRELL, 1995; KRUUK and CONROY, 1996; LÓPEZ-MARTIN and RUIZ-OLMO, 1996; TANS *et al.,* 1996; BOON *et al*., 1997; KRUUK, CONROY and WEBB, 1997; LEONARDS *et al*., 1997; SJOASEN *et al*., 1997; GUTLEB and KRANZ, 1998; GUTLEB *et al*., 1998; MASON, 1998; MURK *et al*., 1998; MATEO, SAVEDRA and GUITART,1999; SIMPSON *et al*., 2000.



3 RESULTS AND DISCUSSION

3.1Contaminants and tissue types analysed

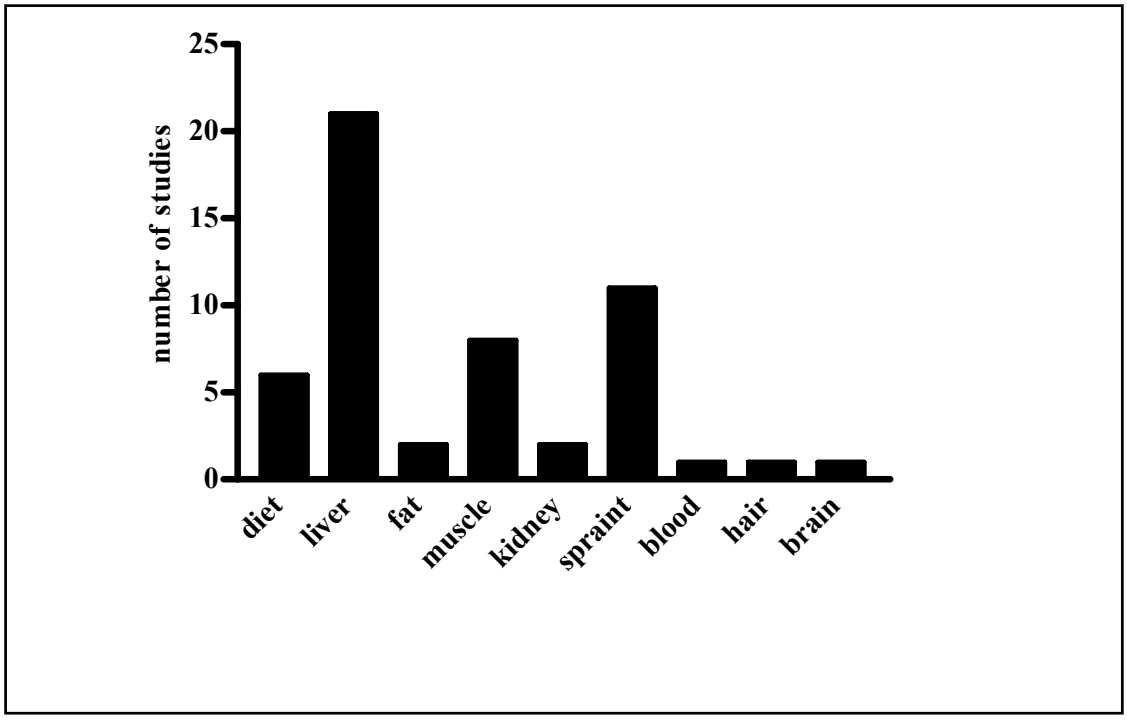
In the 33 studies overall, organic pollutants and pesticides were the contaminants of major interest (Figure 1). PCBs were the most frequently determined contaminants, although the concentrations of various OC pesticides were usually reported simultaneously. This reflected the fact that the two sets of compounds can be determined in the same analytical run and both pose a toxic hazard to otters.

PCBs are theoretically comprised of 209 different congeners that differ in the number and position of chlorine atoms on their biphenyl rings and, hence, have different physico-chemical and toxicological properties. PCB concentrations can be reported as concentrations of individual congeners, as the sum of congeners that were determined, on a matched or total PCBs basis, or as toxic equivalents (TEQs). A matched concentration is the sum concentration for those congeners detected that also occur in a technical-grade PCB mixture, such as Aroclor 1260. Total PCBs are usually calculated as the sum concentration of all detected compounds other than those known to be OC insecticides or other compounds. TEQs are a means of expressing the relative toxicity of compounds that act via the *Ah* receptor. Toxic Equivalency Factors (TEFs) are an order of magnitude estimate of the toxicity of a compound relative to that of 2, 3, 7, 8 -tetrachlorodibenzo-*p*-dioxin (TCDD), the most potent dioxin congener (AHLBORG *et al*., 1992; VAN DEN BERG *et al*., 1998). TEQs are calculated by multiplying the TEF by the concentration for each individual congener and summing the resultant values. In the otter studies that were reviewed, eight reported total PCB concentrations only and eleven solely gave matched PCB concentrations. Nine other studies reported individual congener concentrations. Two of these gave no other PCB data, five reported both congener and total PCB concentrations and two gave TEQ, congener and total PCB values. Overall, therefore, there was relatively little consistency in the way PCB concentrations were quantified and this did not facilitate cross-study comparisons.

Metal concentrations in otters were described in seven studies. Mercury concentrations were reported in all but one and different forms of mercury were not distinguished, despite methyl mercury being far more toxic to otters than inorganic mercury (MASON and WREN, 2001). Cadmium, lead and caesium (all toxic elements) and copper and zinc (essential trace elements) were quantified less frequently (Figure 1). Four of the papers reported more than one metal contaminant, the remaining three gave only mercury or caesium concentrations.

The type of sample that was analysed for contaminants varied widely between studies. Pollutant concentrations were determined non-destructively in diet, spraint, hair and blood (although blood may also sometimes be obtained from fresh carcasses) and destructively in several body tissues (Figure 2). The variation in the sample type analysed occurred because studies differed in their scientific aims. Usually, the most appropriate sample type was used to meet the specific study objectives.

Figure 2. Types of samples analysed for contaminants in thirty-three peer-reviewed



studies on otters published between 1981 and 2000 (see Figure 1 for the list of studies reviewed).

Of the non-destructive measures, spraint, and to a lesser extent diet, were most frequently analysed (Figure 2), reflecting an interest in quantifying the oral exposure of otters to contaminants. The difficulties with such analyses are in ensuring that they are truly representative as there may be considerable uncertainty that separate spraint samples are from different individual otters and that diet samples are truly what otters are eating. Pollutant concentrations in otter hair have occasionally been used as a non-destructive measure of contaminant assimilation (Figure 2) but such information is largely qualitative and only generally applicable for inorganic contaminants. Analysis of blood also provides a non-destructive measure of assimilation and is better than hair because it gives an instantaneous measure of circulating levels of organic and inorganic compounds. Relatively few studies have analysed blood contaminant levels however (Figure 2), perhaps because obtaining blood from otters can be difficult, and is both time consuming and labour-intensive.

Body tissues are usually destructive samples obtained from otter carcasses. These are relatively easy to obtain because it is not unusual for otters to be killed on roads and in other accidents. Of all the body organs, the liver accumulates some of the highest levels of both inorganic and organic contaminants, is actively involved in toxicant metabolism and detoxification, and is often itself a site of toxicity. For these reasons, it is the most frequently analysed body tissue (Figure 2), although large-scale intra- and inter-individual variation in relative weight (liver weight expressed as a % of body weight) can occur which complicates interpretation of residue data. Quantification of residues in other body tissues may be justified on the basis of less marked variation in relative weight, concerns over toxicity in specific organs, or better measures of long-term exposure (e.g., PCB residues in fat). However, all such

measures have associated problems in interpreting their significance. Overall, the lack of consistency in choice of body tissue for analysis means that it can be difficult or impossible to compare data between studies.

3.2 Sample preparation and analysis method

The way in which samples or sub-samples are initially taken, the actual weight of material analysed and the way in which samples are subsequently prepared can all affect the quality and sensitivity of the analysis. The distribution of contaminants within large organs, such as the liver, may be heterogeneous, although such intra-tissue variation does not appear to have been investigated. Homogenising samples overcomes potential problems with residue heterogeneity and is probably the best means of sub-sampling organs although care is needed; practices such as freeze-drying that are sometimes carried out to aid sample homogenisation can alter the tissue concentrations of PCBs and possibly other chlorinated compounds (WALKER *et al*., 1999).

Sample weight can affect the sensitivity of the analysis because generally, there is an inverse relationship between sample weight and the limits of detection (LoDs) for the sample, sample LoDs increasing as sample weights become smaller. The upper limit for sample weights may be dictated by a variety of analytical and operational factors and there is no single optimal weight. This was reflected to some extent in the otter studies in which sample weights ranged 40 fold (0.5g to 20g) in the 21 studies in which contaminant levels in the liver were quantified. This does not pose a significant barrier to integrating data from different studies provided that the limits of detection are given. However, it is good practice to report summary data on sample weights although this was only done in just over a third (38%) of the otter studies in which livers were analysed for contaminants.

Extraction and solubilisation techniques, clean-up methods and chromatography are all likely to vary between laboratories and to improve in precision and sensitivity over time. All potentially can affect the sensitivity and accuracy of chemical analysis. However, it is impracticable and undesirable to try and achieve an integrated approach to analysis by recommending particular types of techniques. The influence of different techniques on the analytical data can be determined to a large extent by implementation of good quality assurance and control and thorough reporting.

The analytical techniques used to analyse compounds also vary between laboratories and change with time as technology advances. Analysis of metal contaminants in otters to date has been generally consistent and largely involved atomic absorption spectrophotometry based methods. These may be replaced by newer technologies, such as inductively-coupled plasma mass spectrometry in which several elements can be determined simultaneously, but this is unlikely to substantially alter the precision or sensitivity of the analysis. Analysis of organic contaminants in the otter studies that were reviewed all involved gas chromatography with electron capture detection (GC-ECD), although three studies also used gas chromatography-mass spectrometry (GC-MS). GC-MS has the advantage that compound identification is based on mass and has greater certainty than GC-ECD techniques that rely largely on matching the retention times of chromatographic peaks in samples with those in standards. GC-MS also makes the identification of unknown compounds easier. The preference for GC-ECD over GC-MS in otter contaminant studies is likely to be cost-related and because ECD techniques are more sensitive for halogenated organic compounds. With the recent improvement of clean-up and large-

volume injection techniques, GC-MS methods can achieve the levels of sensitivity that are fit for purpose for environmental studies but are likely to remain more expensive. The consequence may be that while improvement in the certainty of compound identification may be desirable, its benefit may be outweighed by that conferred from maximising the number of samples analysed using cheaper GC-ECD methods. Overall, as with sample preparation methods, any variation between studies in the analytical method is unlikely to hamper integration of otter contaminant data provided that adequate quality assurance and control data are reported.

3.3 Quality assurance and control

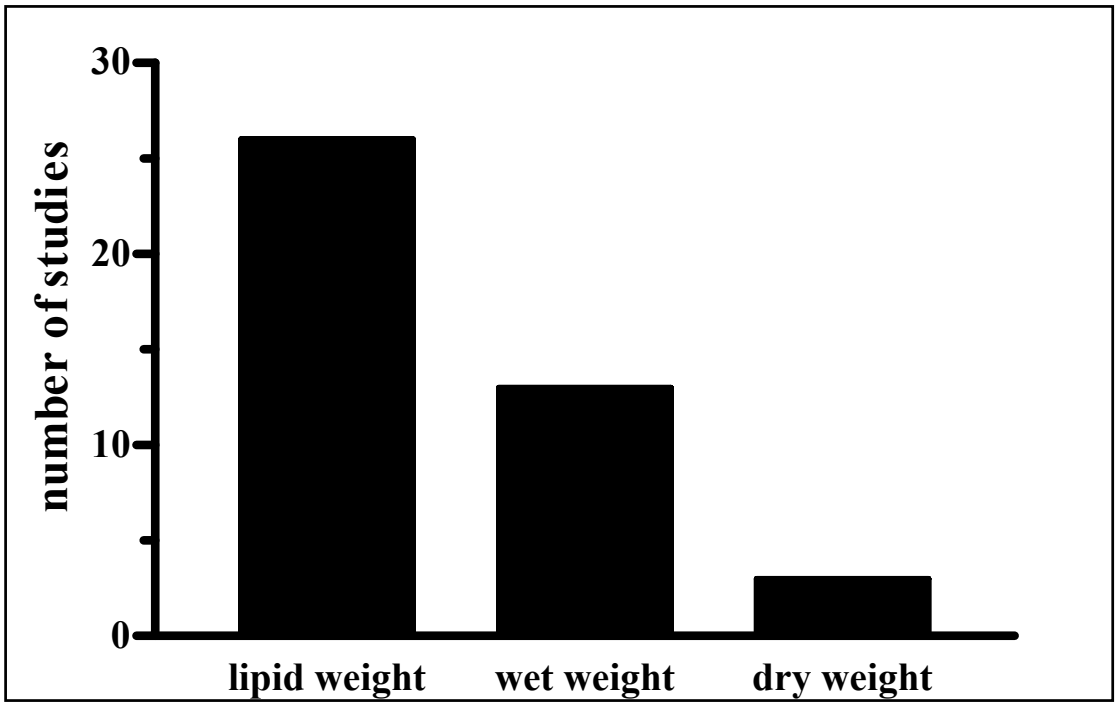
Consistent implementation and reporting of quality assurance and control data is the key to an integrated approach to quantifying contaminants in otters. Analysis of blanks alongside unknowns to determine the occurrence and scale of background contamination should routinely be carried out. Similarly, repeat analysis of some samples is also necessary to quantify variation caused by heterogeneity of residues within tissues and/or variability in the efficiency of sample preparation and extraction. Although analysis of blanks and repeat samples form part of good analytical practice, it is the clear reporting of LoDs, recovery data and the basis on which concentration data are expressed that are the main requirements when comparing data from different studies.

Differences in LoD values between studies become important when a high proportion of the samples in one or more studies have non-detected contaminant concentrations, as comparisons may be flawed if a broadly similar LoD is not applied to all data sets. LoDs can be calculated in a variety of ways and there is no standard method (MILLER and MILLER, 1993). Similarly, there is no standard way of expressing LoDs. They can be given as the instrumental LoD, as a concentration in the sample extract, or take into account the average sample weight and be expressed as a concentration in the sample. Variation in the way LoDs are calculated is unlikely to affect comparisons of data between studies provided that the LoD for each compound is stated in each study; routinely expressing the LoD on a sample concentration basis facilitates such comparisons. In the otter studies, 12 of the 21 studies on liver contaminants quoted LoDs and all did so on a sample concentration basis. Of the nine studies that did not give LoDs, three measured only total or matched PCB concentrations, for which LoDs either cannot be calculated (total PCBs) or are relatively meaningless because of the way concentrations are calculated (matched PCBs). However, individual congeners were quantified in the other six studies and it would be expected that LoDs would have been given.

Recovery data are usually generated using spiked samples or a certified reference material (CRM). The amount of analyte detected in the spiked sample or the CRM is determined and can be expressed as a percentage of that added as the spike or certified to be present in the reference material. CRMs are probably the best way of generating recovery data but are often not available for the sample matrix of interest. Spiking samples has the benefit that it involves the actual sample matrix and so any matrix-specific effects should be revealed, but has the disadvantage that the spike is applied topically and recoveries may be artificially high. Recoveries cannot be calculated for total PCB concentrations because of the way these are calculated but they can be generated for congeners, matched PCB concentrations, individual compounds and elements. Such data are usually produced to demonstrate the effectiveness of the analytical method, but also provide a means of normalising data from different studies, thereby eliminating biases that arise solely from variation in

the efficacy of the analytical method. However, approximately half (10 out of 19) of the studies on liver contaminants in otters that quantified compounds for which recoveries could be generated did not provide any recovery data. Failure to report such information limits the effectiveness with which comparisons can be made between contaminant concentrations in otter samples collected at different times or locations.

Figure 3. Number of studies on liver contaminants in Eurasian otters that expressed pollutant data on a wet weight, dry weight and lipid weight basis. Some studies expressed data in more than one way. See Figure 1 for the list of all studies that were reviewed.



Tissue concentrations of pollutants in otters (and other species) are variously reported on a wet weight, dry weight or lipid weight basis (Figure 3). Organic contaminant concentrations are usually reported on a wet weight and/or lipid weight basis whereas levels of inorganic contaminants are usually expressed on a wet weight and/or dry weight basis. Variability in reporting methods can again hamper comparisons between studies when concentrations are not reported on the same basis and data on the water and lipid content of the samples are not given. Routine reporting of such data, either on an individual sample basis or as average values, should be the norm. Where it is necessary to transform data from different studies to a standard format before comparisons can be made, it can be argued that data on organic and inorganic contaminants should be recalculated on a lipid weight and dry weight basis respectively. This would eliminate biases caused by inter-study variability in lipid extraction efficiency (organic contaminants only) and liver water content.

4 CONCLUSIONS

Overall, there are multiple sources of methodological variability that influence the way in which contaminant data in otters (and animals generally) are reported. The specific scientific aims of a project and the practicality of obtaining samples largely dictate which compounds and sample matrices are analysed, as is evident from this review of contaminant studies on the Eurasian otter over the last 20 years. However, the use of spraints and liver samples, where possible, is likely to enhance the value of the data in terms of comparability to previous studies.

The methods of sample preparation and analysis that can be used are often dependent on the availability of analytical facilities and resources. Attempts to standardise analytical methods are therefore unlikely to succeed, although the use of mass spectrometry is likely to become more common (and is desirable) because of the improved certainty in compound identification. Variation between studies in analytical methods is not a bar to integrating contaminant data for otters, provided that there is a harmonised and effective general approach to quality assurance and control. Routine generation and reporting of quality assurance data allows the quantification of variability introduced by the analytical process and facilitates the normalisation of data to eliminate spurious bias. To date, the provision of quality assurance data has been relatively inconsistent in studies on contaminants in the Eurasian otter. Reporting of the sample weights, moisture and lipid content of samples, LoDs (on a sample concentration basis) and recovery data should be considered an essential requirement for all such studies in the future.

5 REFERENCES

Ahlborg, U. G., Brouwer, A., Fingerhut, M. A., Jacobson, J. L., Jacobson, S. W., Kennedy, S. W., Kettrup, A. A. F., Koeman, J. H., Poiger, H., Rappe, C., Safe, C. H., Seegal, R. F., Tuomisto, J. & van den Berg, M. (1992). Impact of polychlorinated dibenzo-p-dioxins, dibenzofurans and biphenyls on human and environmental health, with special emphasis on the toxic equivalency factor concept. *European Journal of Pharmacology,* **228**: 179-199.

Bergman, A., Norstrom, R. J., Haraguchi, K., Kuroki, H. & Beland, P. (1994). PCB and DDE methyl sulfones in mammals from Canada and Sweden. *Environmental Toxicology and Chemistry,* **13**: 121-128.

Boon, J. P., van der Meer, J., Allchin, C. R., Law, R. J., Klunsoyr, J., Leonards, P. E. G., Spliid, H., Storr Hansen, E., McKenzie, C. & Wells, D. E. (1997). Concentration-dependent changes of PCB patterns in fish-eating mammals: structural evidence for induction of cytochrome P450. *Archives of Environmental Contamination and Toxicology* **33**: 298-311.

Delibes, M., Macdonald, S. M. & Mason, C. F. (1991). Seasonal marking, habitat and organochlorine contamination in otters *(Lutra lutra*) - a comparison between catchments in Andalusia and Wales. *Mammalia*, **55**: 567-578.

Forsyth, D. J. (2001). Extrapolation of laboratory tests to field populations. In: *Ecotoxicology of Wild Mammals***.** (Edited by Shore, R.F. & Rattner, B.A.). Pp. 577-634. John Wiley & Sons. Chichester, UK.

Gutleb, A. C. & Kranz, A. (1998). Estimation of polychlorinated biphenyl (PCB) levels in livers of the otter (*Lutra lutra*) from concentrations in scats and fish. *Water Air and Soil Pollution,* **106**: 481-491.

Gutleb, A. C., Kranz, A., Nechay, G. & Toman, A. (1998). Heavy metal concentrations in livers and kidneys of the otter (*Lutra lutra*) from central Europe. *Bulletin of Environmental Contamination and Toxicology,* **60**: 273-279.

Jefferies, D. J. (1989). The changing otter population of Britain 1700-1989. *Biological Journal of the Linnean Society,* **38**: 61-69.

Kruuk, H. & Conroy, J. W. H. (1991). Mortality of otters (*Lutra lutra*) in Shetland. *Journal of Applied Ecology,* **28**: 83-94.

Kruuk, H. & Conroy, J. W. H. (1996). Concentrations of some organochlorines in otters (*Lutra lutra* L) in Scotland: implications for populations. *Environmental Pollution,* **92**: 165-171.

Kruuk, H., Conroy, J. W. H. & Webb, A. (1997). Concentrations of mercury in otters (*Lutra lutra* L) in Scotland in relation to rainfall. *Environmental Pollution,* **96**: 13-18.

Leonards, P. E. G., Zierikzee, Y., Brinkman, U. A. T., Cofino, W. P., van Straalen, N. M. & van Hattum, B. (1997)**.** The selective dietary accumulation of planar polychlorinated biphenyls in the otter (*Lutra lutra*). *Environmental Toxicology and Chemistry,* **16**: 1807-1815.

López-Martin, J. M. & Ruiz-Olmo, J. (1996). Organochlorine residue levels and bio-concentration factors in otters (*Lutra lutra* L) from northeast Spain. *Bulletin of Environmental Contamination and Toxicology,* **57**: 532-535.

López-Martin, J. M., Ruiz-Olmo, J. & Borrell, A. (1995). Levels of organochlorine compounds in fresh-water fish from Catalonia, NE Spain. *Chemosphere,* **31**: 3523-3535.

Macdonald, S. A. & Mason, C. F. (1983). Some factors influencing the distribution of otters (*Lutra lutra*). *Mammal Review***, 13**: 1-10.

Mason, C. F. (1993*a*). PCB and organochlorine pesticide residues in a sample of otter (*Lutra lutra*) spraints from Northern Ireland. *Biology and Environment-Proceedings of the Royal Irish Academy,* **93B**: 111-112.

Mason, C. F. (1993*b*). Regional trends in PCB and pesticide contamination in northern Britain as determined in otter (*Lutra Lutra*) scats. *Chemosphere,* **26**: 941-944.

Mason, C. F. (1998). Decline in PCB levels in otters (*Lutra lutra*). C*hemosphere,* **36**: 1969-1971.

Mason, C. F. & Reynolds, P. (1988). Organochlorine residues and metals in otters from the Orkney Islands. *Marine Pollution Bulletin***, 19**: 80-81.

Mason, C. F. & Madsen, A. B. (1992). Mercury in Danish otters (*Lutra lutra*). *Chemosphere,* **25**: 865-867.

Mason, C. F. & Macdonald, S. M. (1993*a*). Impact of organochlorine pesticide-residues and PCBs on otters (*Lutra lutra*) - a study from western Britain. *The Science of the Total Environment,* **138**: 127-145.

Mason, C. F. & Macdonald, S. M. (1993*b*). Impact of organochlorine pesticide-residues and PCBs on otters (*Lutra lutra*) in eastern England. *The Science of the Total Environment,* **138**: 147-160.

Mason, C. F. & Macdonald, S. M. (1993*c*). PCBs and organochlorine pesticide-residues in otter (*Lutra lutra*) spraints from welsh catchments and their significance to otter conservation strategies. *Aquatic Conservation-Marine and Freshwater Ecosystems,* **3**: 43-51.

Mason, C. F. & Macdonald, S. M. (1994). PCBs and organochlorine pesticide-residues in otters (*Lutra lutra*) and in otter-spraints from southwest England and their likely impact on populations. *The Science of the Total Environment,* **144**: 305-312.

Mason, C. F. & Madsen, A. B. (1993). Organochlorine pesticide-residues and PCBs in Danish otters (*Lutra lutra*). *The Science of the Total Environment,* **133**: 73-81.

Mason, C. F. & O'Sullivan, W. M. (1992). Organochlorine pesticide-residues and PCBs in otters (*Lutra lutra*) from Ireland. *Bulletin of Environmental Contamination and Toxicology,* **48**: 387-393.

Mason, C. F. & O'Sullivan, W. M. (1993*a*). Further observations on PCB and organochlorine pesticide residues in Irish otters *Lutra lutra*. *Biology and Environment-Proceedings of the Royal Irish Academy*, **93B**:187-8.

Mason, C. F. & O'Sullivan, W. M. (1993*b*). Heavy-metals in the livers of otters, *Lutra lutra*, from Ireland. *Journal of Zoology, London*, **231**: 675-678.

Mason, C. F. & Ratford, J. R. (1994). PCB congeners in tissues of European otter (*Lutra lutra*). *Bulletin of Environmental Contamination and Toxicology***, 53**: 548-554.

Mason, C. F. & Wren, C. D. (2001). Carnivora. In: *Ecotoxicology of Wild Mammals***.** (Edited by Shore, R.F. & Rattner, B.A.). Pp. 315-370. John Wiley & Sons. Chichester, UK.

Mason, C. F., Macdonald, S. M., Bland, H. C. & Ratford, J. (1992). Organochlorine pesticide and PCB contents in otter (*Lutra lutra*) scats from western Scotland. *Water Air and Soil Pollution,* **64**: 617-626.

Mateo, R., Saavedra, D. & Guitart, R. (1999). Reintroduction of the otter (*Lutra lutra*) into Catalan rivers, Spain: assessing organochlorine residue exposure through diet. *Bulletin of Environmental Contamination and Toxicology,* **63**: 248-255.

Miller, J. C. & Miller, J. N. (1993). *Statistics for Analytical Chemistry*: Third edition. Ellis Horwood Limited, London.

Murk, A. J., Leonards, P. E. G., van Hattum, B., Luit, R., van der Weiden, M. E. J. & Smit, M. (1998). Application of biomarkers for exposure and effect of polyhalogenated aromatic hydrocarbons in naturally exposed European otters (*Lutra lutra*). *Environmental Toxicology and Pharmacology,* **6**: 91-102.

O'Sullivan, W. M., Macdonald, S. M. & Mason, C. F. (1993). Organochlorine pesticide residues and PCBs in otter spraints from southern Ireland. *Biology and Environment-Proceedings of the Royal Irish Academy,* **93B**: 55-7.

Simpson, V. R., Bain, M. S., Brown, R., Brown, B. F. & Lacey, R. F. (2000). A long-term study of vitamin A and polychlorinated hydrocarbon levels in otters (*Lutra lutra*) in south west England. *Environmental Pollution*, **110**: 267-275.

Sjoasen, T., Ozolins, J., Greyerz, E. & Olsson, M. (1997). The otter (*Lutra lutra*) situation in Latvia and Sweden related to PCB and DDT levels. *Ambio*, 26: 196-201.

Skarén, U. (1988**).** Chlorinated hydrocarbons, PCBs and cesium isotopes in otters (*Lutra lutra* L) from central Finland. *Annales Zoologici Fennici*, **25**: 271-276.

Tans, M., Hugla, J. L., Libois, R. M., Rosoux, R. & Thome, J. P. (1996). Contamination of European otters (*Lutra lutra*) by PCB congeners and organochlorinated pesticides in the wetlands of western France. *Netherlands Journal of Zoology,* **46**: 326-336.

Van den Berg, M., Birnbaum, L., Bosveld, A. T. C., Brunstrom, B., Cook, P., Feeley, M., Giesy, J. P., Hanberg, A., Hasegawa, R., Kennedy, S. W., Kubiak, T., Larsen, J. C., van Leeuwen, F. X. R., Liem, A. K. D., Nolt, C., Peterson, R. E., Poellinger, L., Safe, S., Schrenk, D., Tillitt, D., Tysklind, M., Younes, M., Waern, F. & Zacharewski, T. (1998). Toxic Equivalency Factors (TEFs) for PCBs, PCDDs, PCDFs for humans and wildlife. *Environmental Health Perspectives,* **106**: 775-792.

Walker, L. A., Shore, R. F., Wright, J. & Wyatt, C. L (1999). Changes in PCB congener concentrations and profile in avian liver due to freeze-drying. *Chemosphere,* **38**: 1947-1956.

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