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.

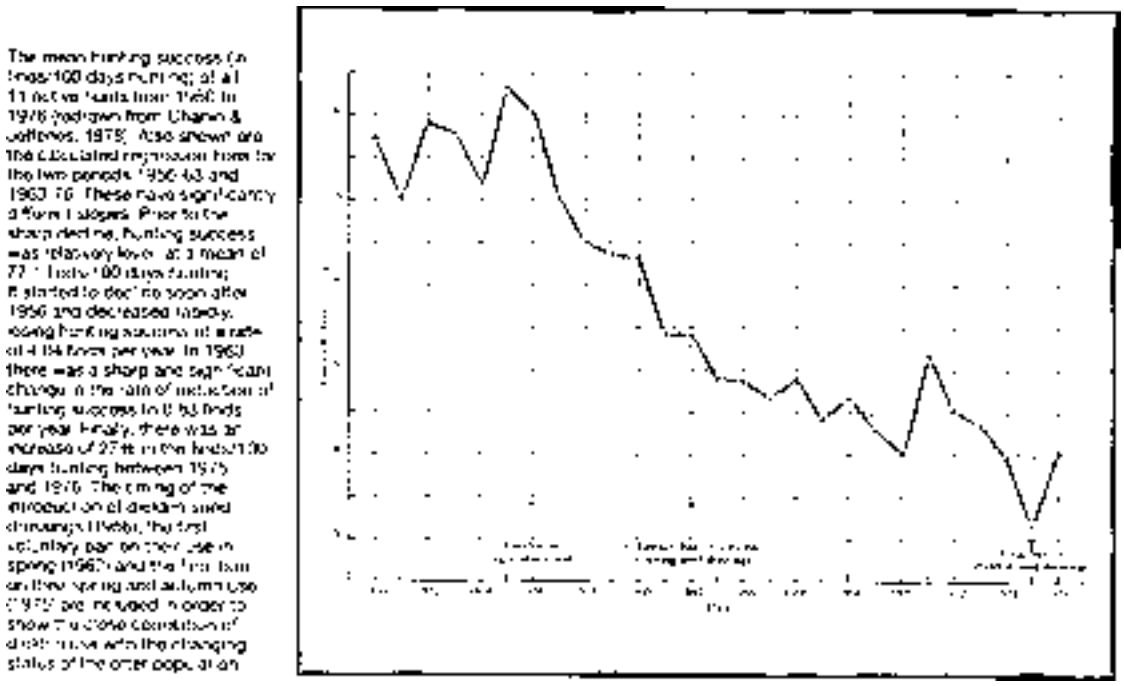


Figure 1.
The mean hunting success (in finds/100 days hunting) of all 11 active hunts from 1950 to 1976 (from CHANIN and JEFFERIES, 1978). Also shown are the calculated regression lines for the two periods 1956-1963 and 1963-1976 (STRACHAN and JEFFERIES, 1996). These have significantly different slopes. 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	

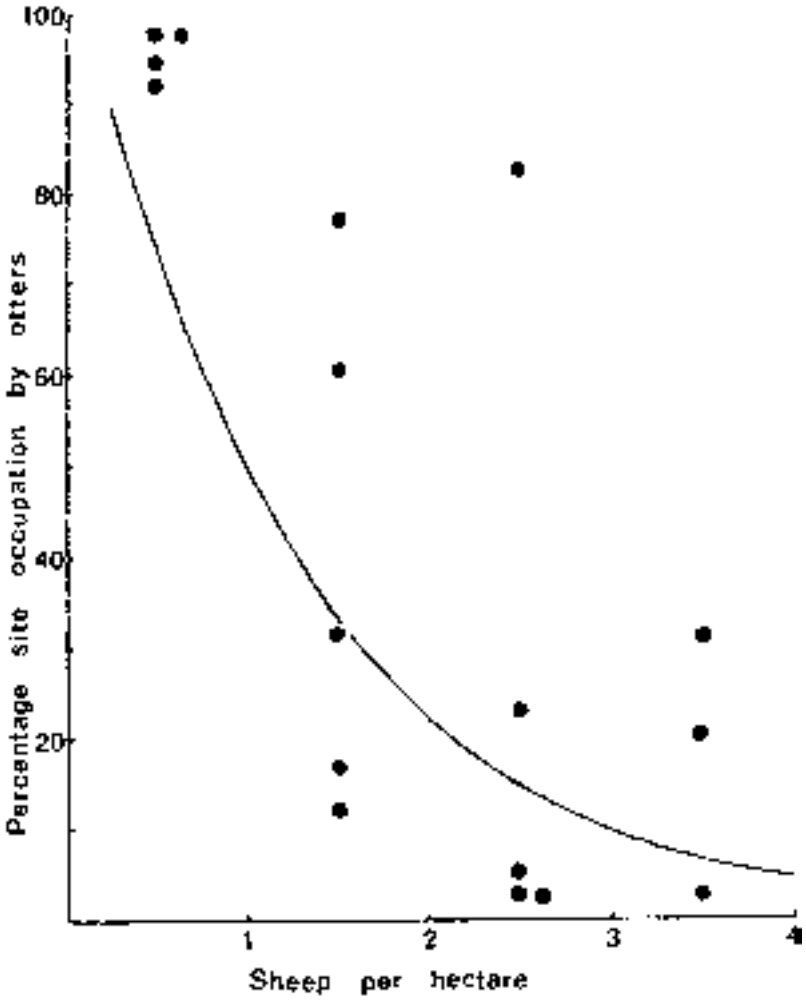
It can be seen from Table 12 that sheep dip and so dieldrin use was highest in Borders, Wales and Cumbria and lowest in Northern Scotland, the Western and Northern Isles, and that in general these usages relate to low and high otter densities and so survival after the 1957 crash.

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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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.

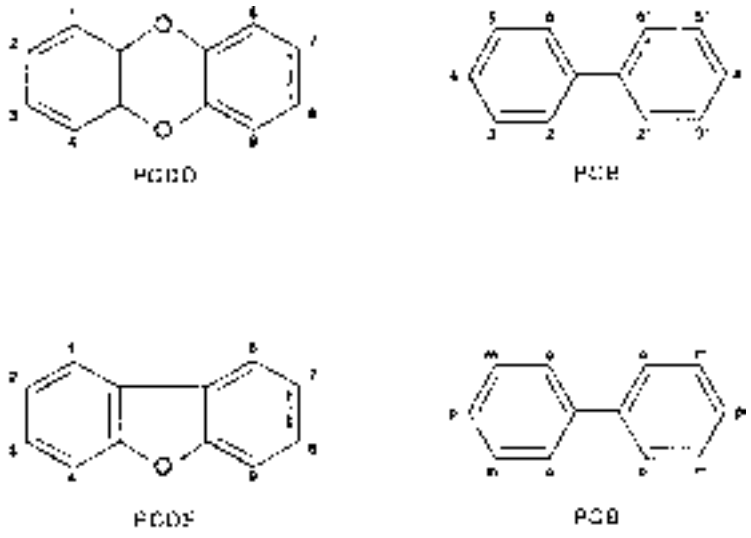

Figure 1. Chemical structure of polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and polychlorinated biphenyls (PCBs)

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).

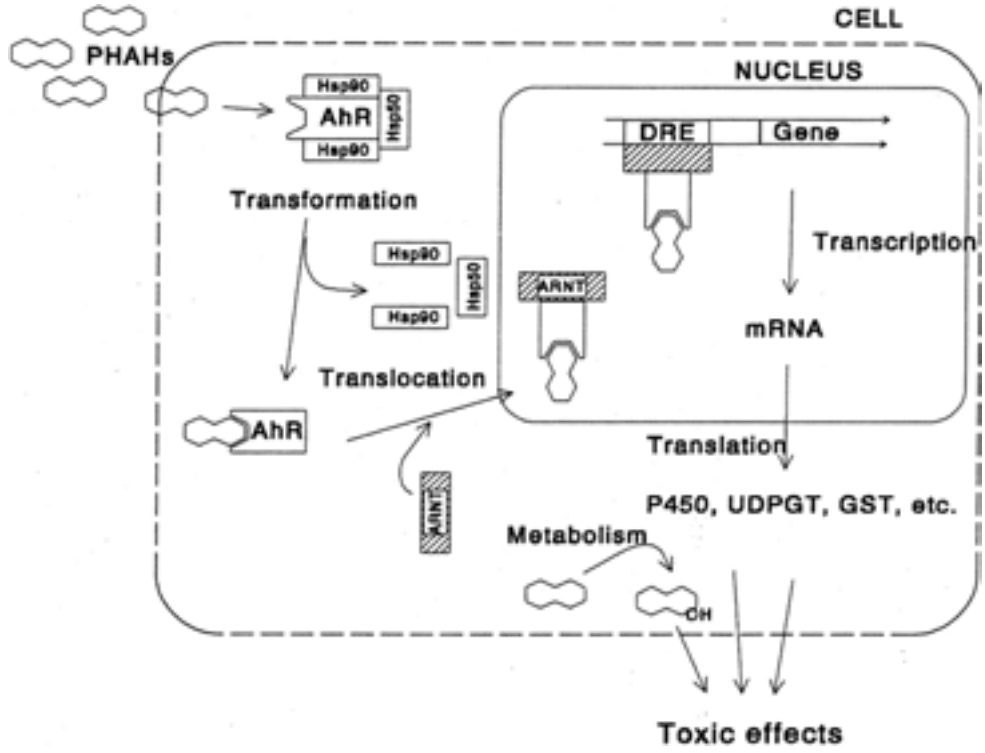

Figure 2. Schematic representation of the Ah-receptor mediated mechanism of action for 2,3,7,8-tetrachlorodibenzo-p-dioxin and related toxic PHAHs. Abbreviations: PHAHs: polyhalogenated aromatic hydrocarbons; AhR: arylhydrocarbon receptor; Hsp: heat shock protein; ARNT: Ah-receptor nuclear translocator; 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).

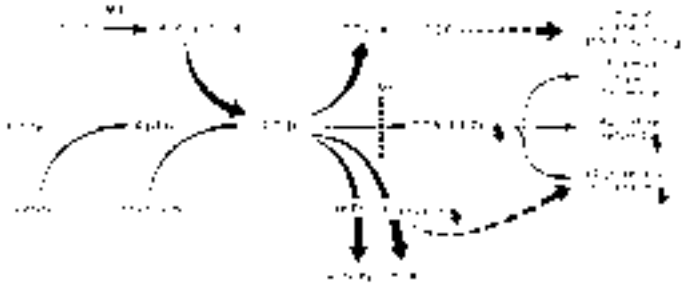

Figure 3. Model of interference of thyroxin (T_4) and retinol transport in serum after binding of a PCB metabolite to transthyretin (TTR), and its effect on T_4, retinol and retinol binding protein (RBP) levels (BROUWER, 1987). The thin lines represent the normal situation with TTR transport T_4 and 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).

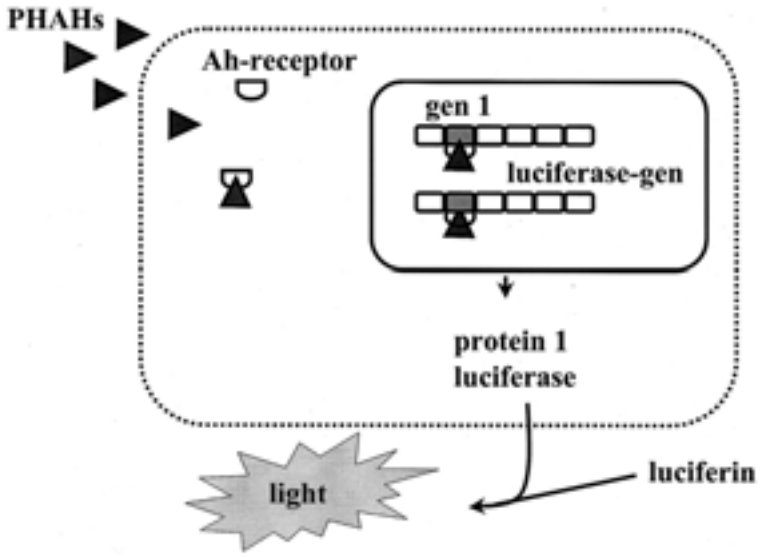

Figure 4. Schematic representation of Ah-receptor mediated luciferase production in stably transfected H4IIE cells (CALUX-Assay).

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.

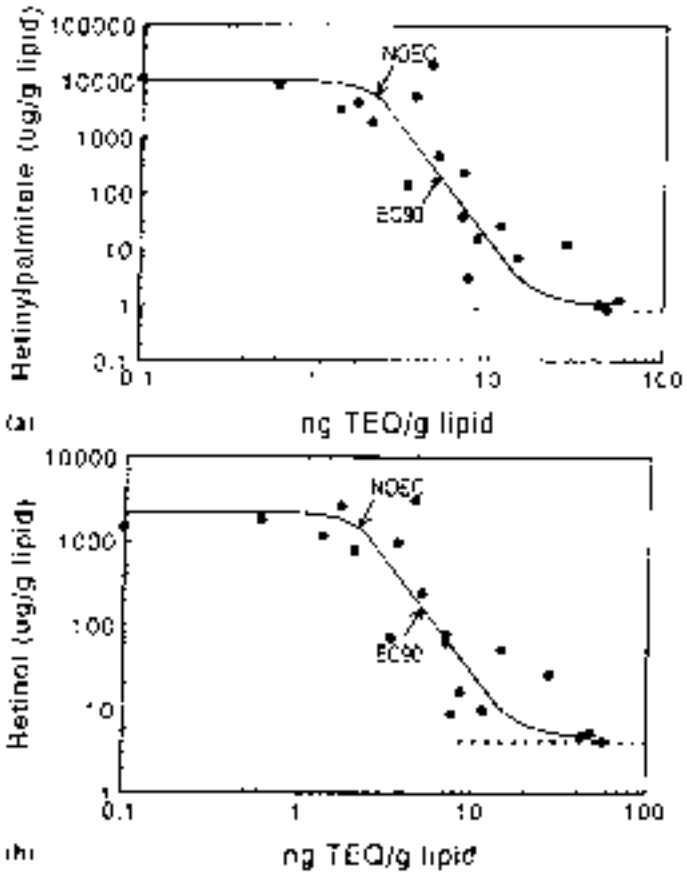

Figure 5 (a) Hepatic retinylpalmitate levels (µg/g lipid) and (b) hepatic retinol levels (µg/g lipid) in environmentally exposed otters plotted against hepatic TEQ levels (ng/g lipid; based on non- and mono-ortho PCBs). The calculated no observed effect concentration (NOEC) and 90% effect concentration (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.

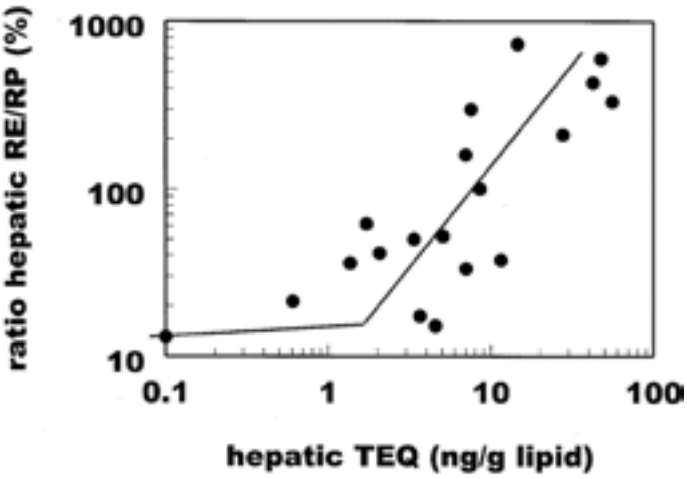

Figure 6. The hepatic retinol/retinylpalmitate (RE/RP) ratio expressed as % (w/w) in environmentally exposed otters plotted against hepatic GC-TEQ levels (ng/g lipid; based on non- and mono-ortho PCBs).

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).

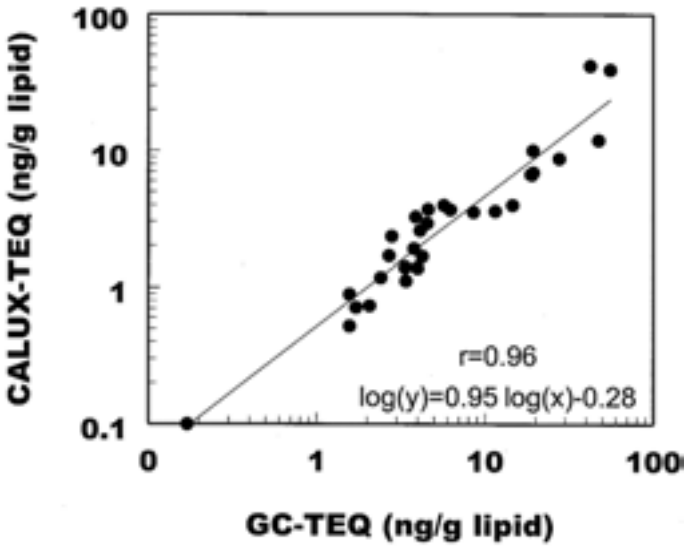

Figure 7. Correlation between TEQ levels measured with the Ah-receptor mediated expression of the luciferase reporter gene (CALUX-TEQ) and calculated based on non- and mono- ortho PCB levels (GC-TEQs) in liver of environmentally exposed, dead otters (P<0.0001).

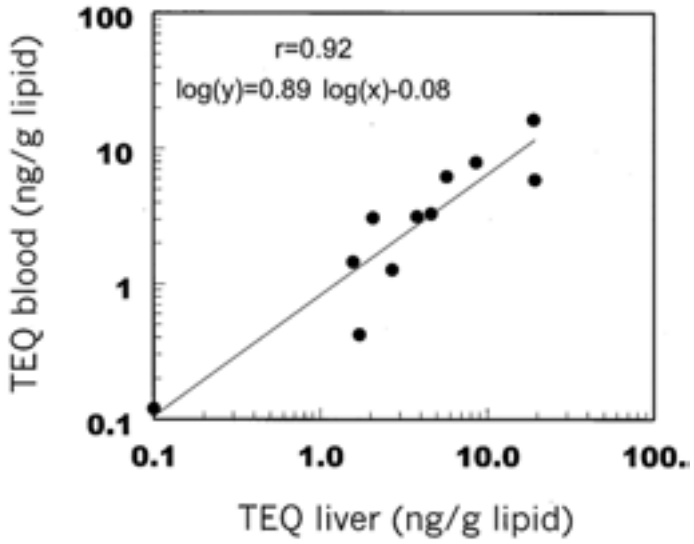

Figure 8. Correlation between TEQ levels (ng/g lipid; based on non- and mono-ortho PCBs) in liver and blood plasma of environmentally exposed otters (P<0.001).

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.

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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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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

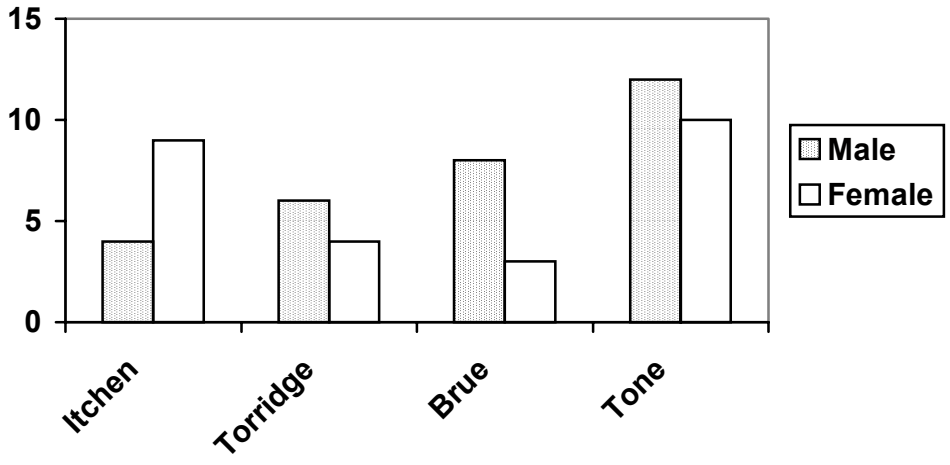

Figure 1. Number of otters of each sex

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/.

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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

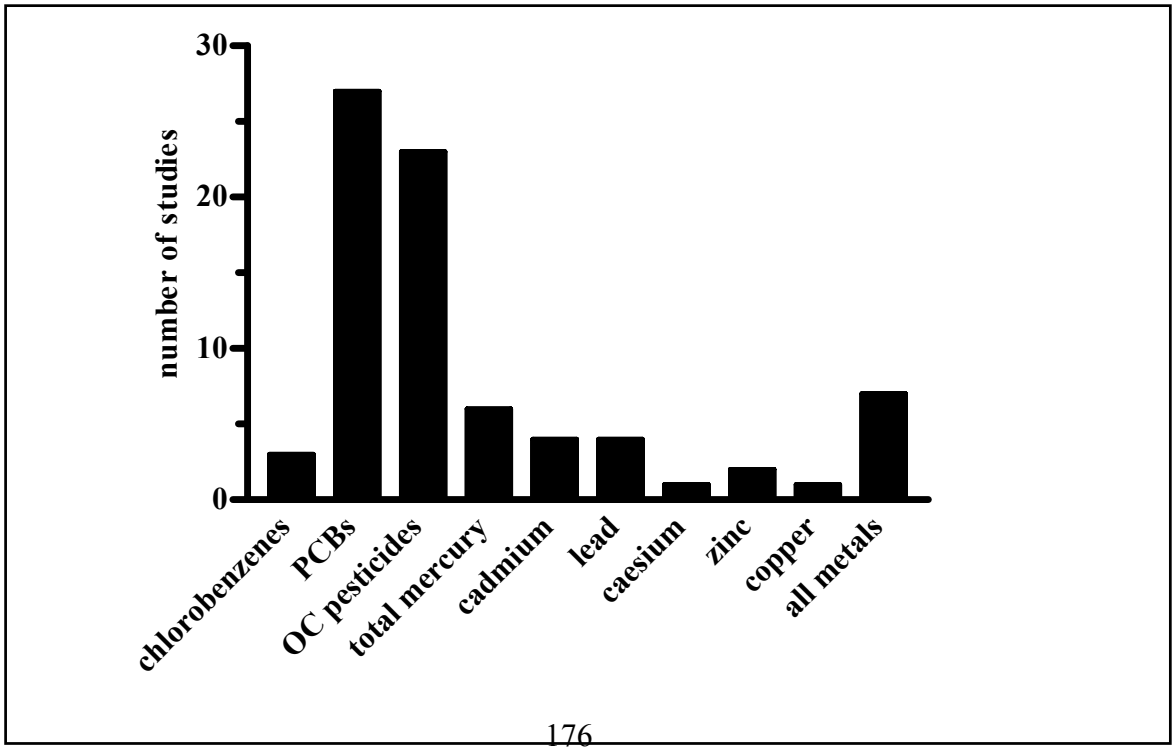
cumulative number of studies has grown, it has become increasingly possible to assess how the levels of contaminants in otters and their diet differ between regions and countries, and has changed over time. These spatial and temporal analyses are often key in determining whether pollutants exert an effect 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

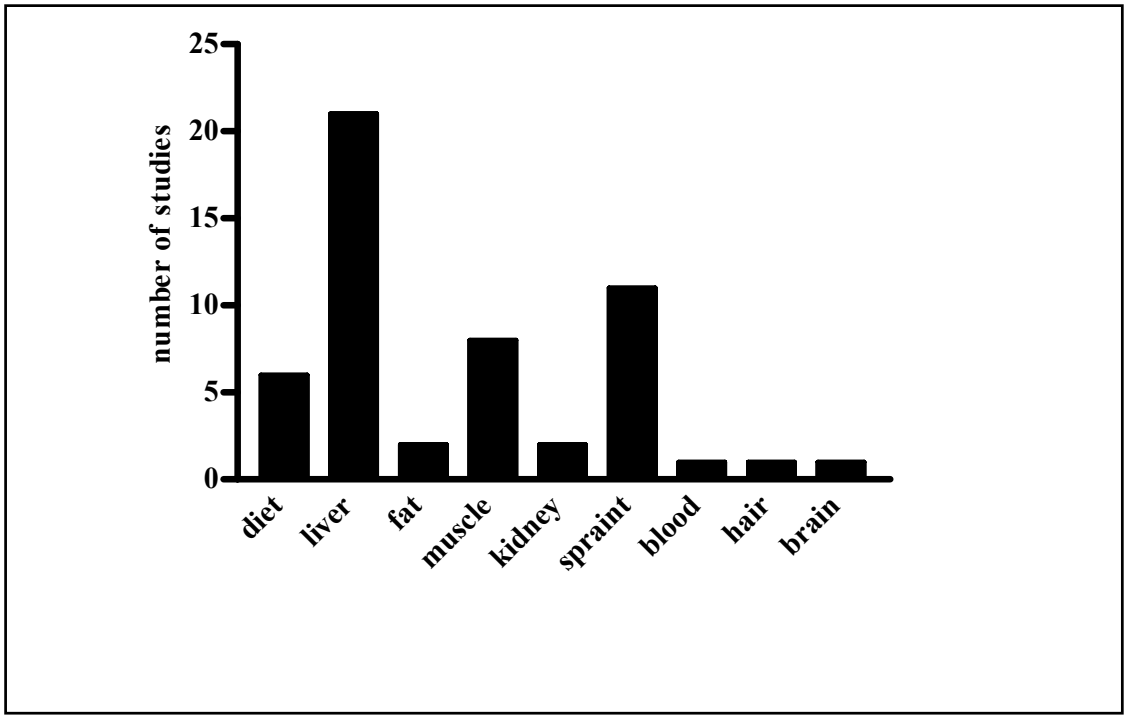
In the 33 studies overall, organic pollutants and pesticides were the contaminants of major interest (Figure 1). PCBs were the most frequently determined contaminants, although the concentrations of various OC pesticides were usually reported simultaneously. This reflected the fact that the two sets 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

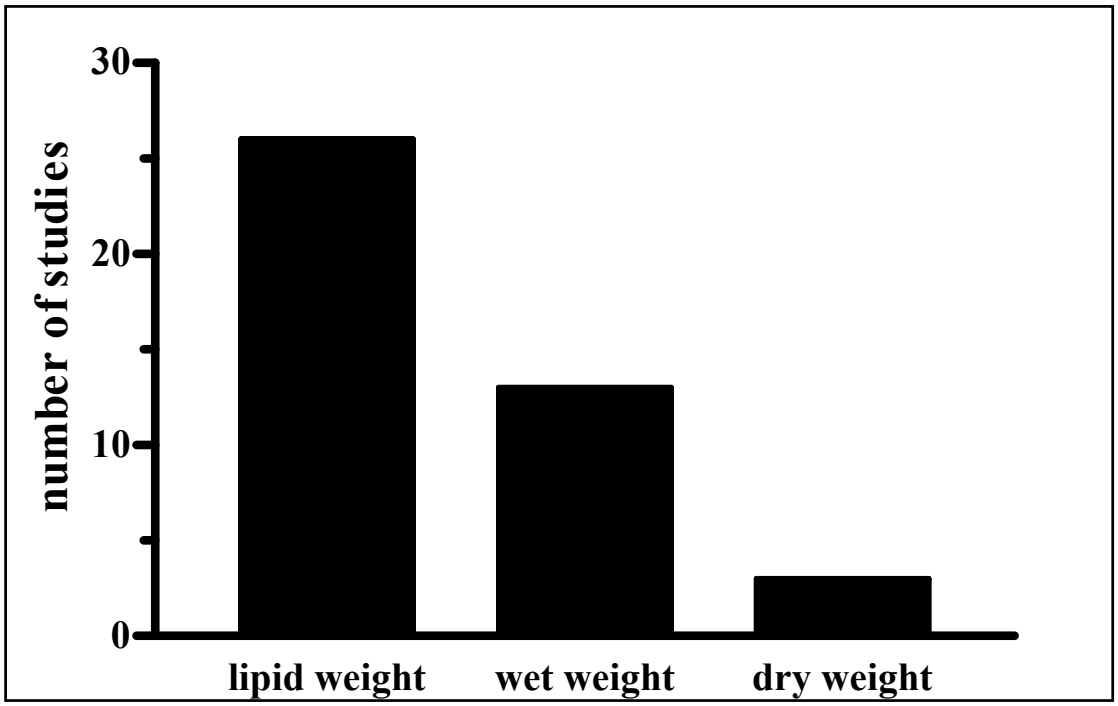
Consistent implementation and reporting of quality assurance and control data is the key to an integrated approach to quantifying contaminants in otters. Analysis of blanks alongside unknowns to determine the occurrence and scale of background contamination should routinely be carried out. Similarly, 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.

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