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A long-term study of vitamin A and polychlorinated hydrocarbon levels in otters (*Lutra lutra*) in south west England

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"Capsule": *Vitamin A deficiency may have been common in otter populations in Britain and may have influenced declines.*

Abstract

Seventy-seven wild otters (*Lutra lutra*) found dead in south west England between 1988 and 1996 were examined post mortem. Liver samples were analysed from 56 otters for polychlorinated hydrocarbons and from 40 for vitamin A (retinol). There was a significant decline in the levels of pollutants over the study period and this coincided with a marked increase in vitamin A levels. However, a causal relationship was not established. Low vitamin A levels were prevalent in the early years of the study but no conclusive pathological lesions due to deficiency were seen. It is suggested that vitamin A deficiency may have been widespread in Britain's otter population until recently and may have been implicated in the earlier decline.

Keywords: Otters; *Lutra*; Vitamin A; PCBs; Organochlorines

1. Introduction

It is widely accepted that the massive decline in the population of otters (*Lutra lutra*) in Europe in the 1950s and 1960s was caused by polychlorinated hydrocarbons, such as organochlorine pesticides (OCs) and polychlorinated biphenyls (PCBs) (Jefferies et al., 1974; Chanin and Jefferies, 1978; Mason, 1989; Anon, 1996). However, the evidence against them is largely circumstantial, with high tissue levels recorded in otters in areas where populations have declined and low levels observed in areas where populations have remained stable (Olsson et al., 1981; Mason, 1989). There does not appear to be any documented proof that these compounds cause pathological lesions in otters. However, there are many reports, mostly on experimental animals, which describe how they interfere with vitamin A metabolism (Kimbrough, 1974; Jefferies, 1975; Bröuwer et al., 1986; Bank et al., 1989; Brunström et al., 1991; Håkansson et al., 1992).

Isolated populations of otters survived in the more rural areas of Britain, including south west England. In a study which started in 1988, otters found dead in this area have been submitted to Polwhele Veterinary Investigation Centre (Truro, UK) where they have been subjected to detailed post mortem examination (Simpson, 1997). This paper describes the results of analyses of liver samples for polychlorinated hydrocarbons and vitamin A (retinol) and the relationship between these compounds over an 8-year period.

2. Materials and methods

Seventy-seven otters were examined between December 1988 and March 1996. With the exception of two cases from Hampshire they all came from Cornwall, Devon and Somerset. The majority (83%) had been killed in road traffic accidents and most were apparently healthy animals. In some cases the carcases were too badly damaged or too autolysed for detailed examination but liver samples were collected from 56 otters for pollutant analysis. These were placed in aluminium foil and stored at −20°C. Liver samples from 40 animals which were

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judged to be in reasonably fresh condition and not autolysed were either dispatched immediately to Shrewsbury Veterinary Investigation Centre for vitamin A analysis or held overnight at −20°C and posted the following day.

2.1. Pollutant analysis

2.1.1. Method

Samples were analysed in three batches for OCs, hexachlorobenzene and the standard set of 10 PCBs as defined in the 1974 Paris Commission. Each sample was thawed, mechanically homogenised and a 5 g aliquot dried using anhydrous sodium sulphate before being left to stand overnight in 25 ml iso-hexane. The mixture was then mechanically shaken for 10 min and the solvent decanted off and retained. The process was repeated on the same aliquot with a further 25 ml iso-hexane. The two portions of extract were then combined and concentrated to 5 ml under nitrogen. A 1 ml aliquot of the extract was cleaned up on two columns (100 mm×10 mm internal diameter). The first was filled with 5 g of 10% water-activated florisil (chromatographic analysis grade) and eluted with a 20 ml solution of 5% diethyl ether in iso-hexane. The eluent was collected, concentrated to 2 ml, internal standard (pentachloronitrobenzene) added and a portion used for the analysis of OCs by gas chromatography–electron capture detection (GC–ECD/ECD). One millilitre of this eluent was quantitatively transferred on to a second column containing 2 g of 3.5% water-activated silica gel (high purity) capped with 5 mm sodium sulphate. The extract was eluted with 9 ml iso-hexane and evaporated to 1 ml. Hexachlorocyclohexane (HCH)-delta was added as the internal standard and the extract analysed for PCBs using GC–ECD/ECD. Any positive results were confirmed using gas chromatography–mass selective detector (GC–MSD). The laboratory holds UKAS accreditation for this method.

2.1.2. GC conditions

The GC conditions for GC–ECD\ECD were: Varian 3500 using 50 m×0.25 mm CP SIL 8CB and 60×0.25 mm DB17 columns and fitted with an 8100 autosampler; injection: 1 μl splitless; injection temperature: 270°C; detector temperature: 300°C; carrier gas: helium at 1.2 ml/min; makeup gas: nitrogen at 30 ml/min; temperature programme: OCs, 4 min at 45°C, 15°C/min to 150°C, 5°C/min to 175°C, 3 min at 175°C, 5°C/min to 210°C, 210°C for 3 min, 5°C/min to 270°C, 270°C for 10 min; PCBs, 1 min at 45°C, 50°C/min to 230°C, 230°C for 5 min, 3°C/min to 250°C, 250°C for 3 min, 3°C/min to 270°C, 270°C for 35 min.

A PE Nelson 900 Series was used as an interface for a PC with Perkin Elmer Turbochrom 3 software for data calculation. The system was re-calibrated in batches of 10–20 samples and each calibration curve contained four data points. Results were corrected for recovery.

The GC conditions for GC–MSD were: Hewlett Packard 5972 fitted with a 5890 GC with autosampler and using a 50 m×0.25 mm CP SIL 8CB column; injection: 2 μl splitless; injection temperature: 270°C; temperature programme: 2 min at 65°C, 10°C/min to 270°C, 270°C for 5 min; Carrier gas: helium at 1 ml/min; detector temperature: 280°C, selected ion mode; tuning conditions: heptacosa used to tune at 69, 219 and 502 m/z; scan parameters: 30–425 amu with scan time 0.55 s and interscan time of 0.05 s.

Confirmation is a qualitative process, using retention times and the following ions: dieldrin — 79, 81, 263; hexachlorobenzene (HCB) — 284, 282; HCH-gamma — 181, 219; DDT and TDE — 237, 235; DDE — 246; PCB congeners — 118–326, 254; 138–360, 290; 153–360, 260, 290; 156–360, 290; 180–394, 324. Data calculation is on-line with the HP proprietary data system.

2.1.3. Quality assurance

GC–ECD/ECD — procedural quality control standards were run at least every 10 samples and each batch contained one procedural blank. Certified reference materials were not used as none could be obtained in a suitable matrix. Recovery correction and limits of detection were calculated by performance testing the method to NS30 standards. The method is controlled to a variability of less than 50% (Table 1).

2.2. Vitamin A analysis

2.2.1. Method

The vitamin A status was determined by measurement of retinol following complete hydrolysis. Procedures were carried out under subdued lighting and samples held in amber glassware. The liver sample was thawed, chopped, and a 2 g aliquot homogenised in a tube containing 8 ml deionised water. One millilitre of homogenate was added to a freshly prepared mixture of 4 ml ethanol, 1 ml 25% ascorbic acid and 2 ml 12% potassium hydroxide. After incubation at 70°C for 30 min the sample was cooled for 10 min at 4°C, 4 ml hexane added and then shaken for 3 min at the 1400 setting on a Vibrax shaker. It was left to stand for 10 min before being centrifuged at 2500 rpm for 5 min at 4°C. Hexane supernatant (0.8 ml) was placed in a high performance liquid chromatography (HPLC) vial, capped and sealed. One-hundred microlitres of sample was automatically injected into an HPLC column with a 97% methanol/water mobile phase at 1.3 ml/min. Retinol was detected by fluorescence using 325-nm excitation and 480-nm emission wavelengths. The laboratory holds UKAS accreditation for this method.

2.2.2. Equipment

The equipment used was as follows: HPLC: Kontron Autosampler 360, Kontron pump 320; column:

Table 1
Performance characteristics of analytical method

	p,p′-DDE	*p,p′*-DDT	*p,p′*-TDE	γ-HCH	Dieldrin	HCB	PCB105	PCB118	PCB138	PCB153	PCB156	PCB180
LOD (μg/kg)	1.75	1.50	1.50	1.50	1.75	1.75	1.0	1.16	0.96	1.24	1.12	0.97
% recovery	67.0	82.4	68.6	82.6	72.6	82.0	71.8	83.3	81.1	86.4	77.2	75.9

Phenomenex Bondclone 10, 150×3.9 mm; spectrofluormetric detector: Shimadzu RF 551.

2.2.3. Quality assurance

Equipment was calibrated using retinol (Sigma Chemicals) standards solutions of 1.0, 2.5 and 5.0 μg/ml, coefficient of variation 2.1%. Frozen aliquots of a bovine liver were used as internal controls at the beginning of each batch and between every 10 samples, coefficient of variation 18.7%; initial value of control liver was determined using 10 replicates.

2.3. Statistics

Statistical analyses of the data were carried out using GENSTAT 5 Release 3.1 (DEC Alpha AXP/Open VMS).

3. Results

The hepatic concentrations of the various pollutants and vitamin A are summarised in Table 2. PCB congeners are identified by their IUPAC numbers (Ballschmiter and Zell, 1980). Values for congeners 28, 52 and 101 are not shown as 28 was not detected in any sample and 52 and 101 were each present in three samples only. Congener 31 is also excluded as levels were not measured in batch I and it was not detected in any samples in batches II and III. Congener 105 values were not measured in batches I and II but it was present in all but three samples in batch III. Similarly, 156 levels were not measured in batch I but it was present in all but four samples in batches II and III.

The OC pesticide *p,p′*-DDT and its derivatives *p,p′*-DDE and *p,p′*-TDE were recorded in almost all samples

Table 2
Concentrations of vitamin A and polychlorinated hydrocarbons in otter's livers[a]

Determinand	Range	Mean	SD	NA/*n*	*n* < dl
Vitamin A	Nd–1836.0	282.0	430.1	0/40	1
p,p′-DDE	13.8–2397.0	355.0	468.7	0/56	0
p,p′-DDT	Nd–270.0	13.54	38.2	0/56	9
p,p′-TDE	Nd–960.0	85.17	157.8	0/56	5
γ-HCH	Nd–17.7	1.125	2.9	0/56	42
Dieldrin	13.4–2801.1	301.7	528.5	0/56	0
PCB105	Nd–35.2	6.08	8.3	39/56	3
PCB118	Nd–455.0	38.84	70.6	0/56	4
PCB138	Nd–553.0	109.6	123.7	3/56	1
PCB153	Nd–625.0	116.2	149.1	3/56	3
PCB156	Nd–47.4	14.8	13.4	23/56	4
PCB180	Nd–514.0	81.75	107.2	3/56	1
HCB	2.7–148.0	20.74	24.1	0/56	0

[a] PCBs, OCs and HCB measured in μg/kg wet matter; vitamin A in μmol/kg. Nd, nil detected; NA/*n*, number not analysed/number of samples; *n* < dl, number below detection limit.

but their *ortho para* isomers were either not present or were at low levels and are therefore not shown. Lindane (γ-HCH) values in many cases were below the limit of detection. The α and β isomers are excluded as α-HCH was not detected in any sample and β-HCH was present in only two. No samples contained endrin or endosulphan. Aldrin was only detected twice and the dieldrin levels in these two cases were not noticeably higher than in the rest.

The concentrations of the individual pollutants in each otter were initially plotted in chronological sequence and most compounds showed a similar pattern of apparent decline over the study period. This was most noticeable in the case of *p,p′*-DDE and dieldrin. The data were therefore analysed by simple linear regression of the logarithms of the concentrations of the determinands against the date of death. The decision to use the logarithms of the pollutant concentrations was taken because values appeared to be skewed. One consequence of this decision was the exclusion of a small number of otters from the calculations where the values could not be ascribed because the concentration of the determinand was below the limit of detection. This involved one animal each for congeners 138 and 180, three for 153 and four for 118. It was considered that these omissions would not materially affect the results for these compounds. However, in the case of γ-HCH, 42 out of the 56 samples were below the limit of detection and the γ-HCH statistical analysis is therefore not reported.

The regression coefficients are given in Table 3. They show highly significant declines over the study period in the concentrations of *p,p′*-DDE, dieldrin and HCB.

Table 3
Regression coefficient of log concentration of polychlorinated hydrocarbons on time (in years)

Determinand	Regression coefficient	Standard error	Significance[a]	Annual rate of change (%)[b]
p,p′-DDE	−0.150	0.039	***	−29
p,p′-DDT	−0.083	0.049	#	−17
p,p′-TDE	−0.131	0.042	**	−26
Dieldrin	−0.170	0.036	***	−32
PCB118	−0.068	0.031	*	−14
PCB138	−0.079	0.031	*	−17
PCB153	−0.083	0.031	*	−17
PCB180	−0.070	0.031	*	−15
HCB	−0.112	0.024	***	−23

[a] $***p \leqslant 0.001$; $**0.001 < p \leqslant 0.01$; $*0.01 < p \leqslant 0.05$; $\#0.05 < p \leqslant 0.1$.
[b] Compound rate applicable to concentrations on a non-logarithmic scale.

There were less pronounced, but nevertheless significant, downward trends in the concentrations of individual PCBs. The declines through time for *p,p′*-DDE, dieldrin and PCB congener 153 are illustrated in Figs. 1–3.

The liver vitamin A levels showed considerable variation, with values below 1 μmol/kg in some cases and over 1000 μmol/kg in others. However, when the values were ranked it was apparent that there was a markedly skewed distribution, with most otters having vitamin A levels of less than 100 μmol/kg (Fig. 4). Seven animals had values below 7 μmol/kg and the majority of these low values occurred in the early years of the study. Four had values of approximately 1000 μmol/kg or more. Regression analysis of the logarithm of the vitamin A

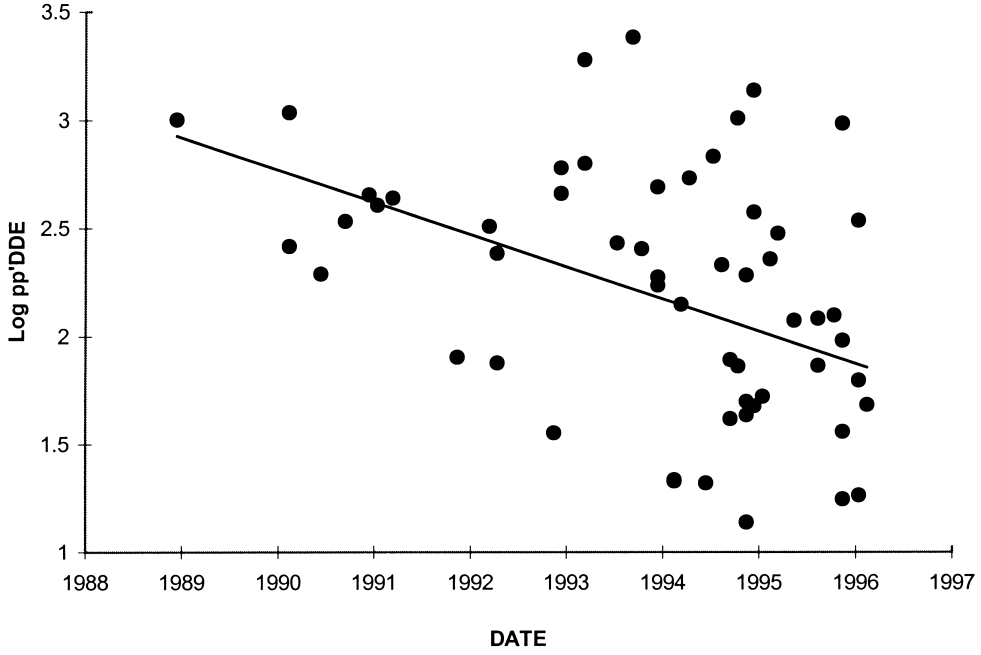

Fig. 1. Log hepatic concentration of *p,p′*-DDE (μg/kg) through time.

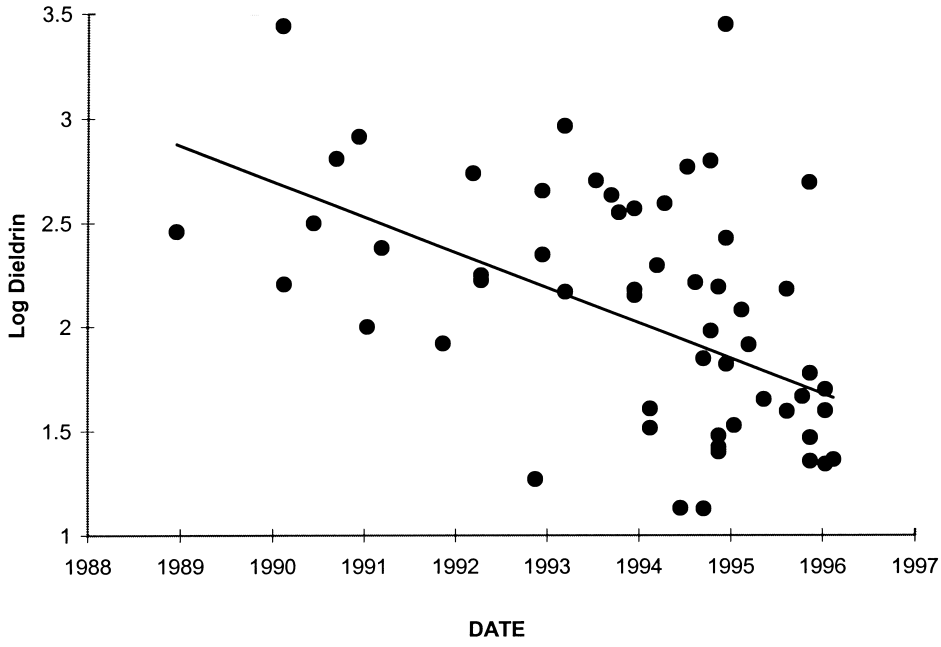

Fig. 2. Log hepatic concentration of dieldrin (μg/kg) through time.

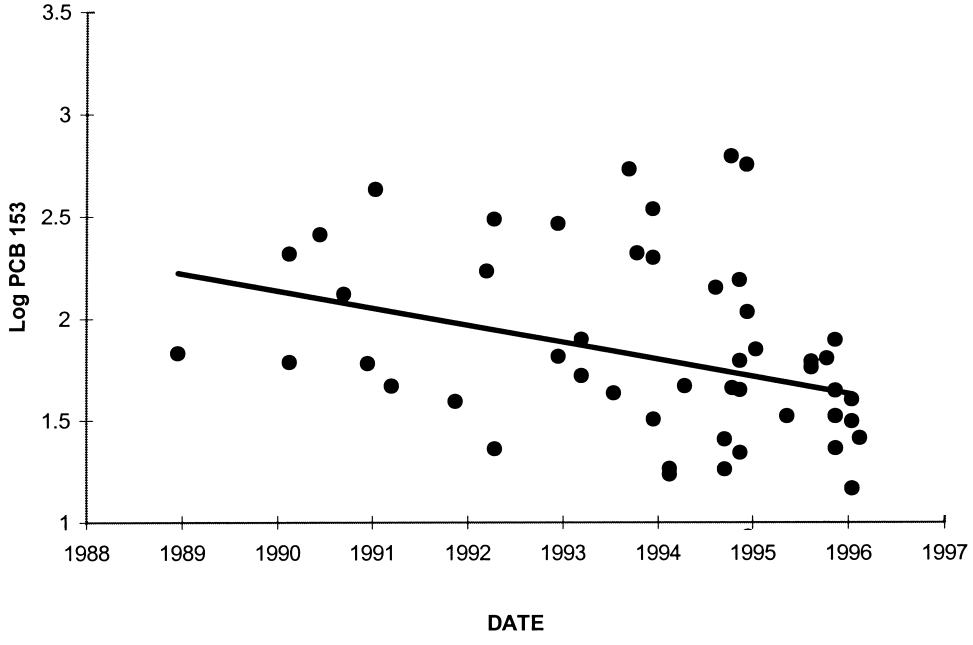

Fig. 3. Log hepatic concentration of PCB153 (μg/kg) through time.

concentration against time showed a highly significant upward trend (regression coefficient 0.28, standard error 0.064, $p < 0.001$). This would correspond to a rate of increase of +94% per year on the unlogged scale. The increase in vitamin A levels through time is illustrated in Fig. 5.

In view of the evidence for declining PCB and OC levels, increasing vitamin A levels, and the fact that many chlorinated hydrocarbons have been shown to interfere with vitamin A metabolism, the data were re-examined for evidence of a possible relationship between these pollutants and vitamin A. Calculation of correlation coefficients confirmed a significant negative association between vitamin A and dieldrin but not *p,p′*-DDE (Table 4). There was also a significant negative association between vitamin A and PCB congener 138 but not congeners 118, 153 and 180 (Table 5). When time was included in a multiple regression model neither dieldrin nor congener 138 continued to show a significant relationship to vitamin A. The inclusion of

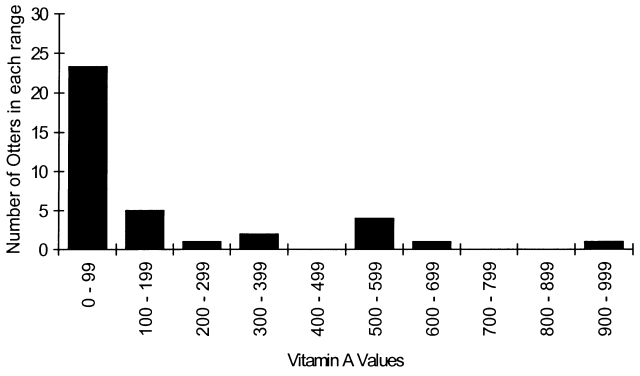

Fig. 4. Hepatic concentrations of vitamin A showing distribution in the population (μmol/kg). Note: Four otters had values in excess of 1000 μmol/kg.

dieldrin (or PCB138) did not, however, diminish the significance of the relationship between vitamin A and time. Therefore, the upward trend in vitamin A over time could not be adequately explained by the changes in these pollutants alone.

4. Discussion

This study shows that the levels of polychlorinated hydrocarbons in otters in south west England declined markedly between 1988 and 1996. Although most research on otters in recent years has focused on PCBs, particularly on the role of specific congeners, the two chemicals in the present study which showed the most significant decline were dieldrin and DDT. The decline in the populations of peregrine falcons (*Falco peregrinus*) and sparrowhawks (*Accipiter nisus*) in Britain in the 1950s and 1960s has been attributed to the effects of the OC pesticides. When the OCs were progressively withdrawn from agricultural use the populations of these raptors recovered. Although DDT was involved it is considered that dieldrin and aldrin (which is metabolised to dieldrin) played the major role (Ratcliffe, 1980; Newton, 1986). There is also well-documented evidence that the sharp decline in the English otter population started immediately after dieldrin was introduced in 1956 (Chanin and Jefferies, 1978). There is now evidence of a population recovery following the almost complete ban on the use of dieldrin/aldrin in 1981 and the total ban in 1989 (Strachan and Jefferies, 1996; Simpson, 1997). Whilst the toxic nature of some PCB congeners is not in doubt, healthy otter populations exist in places like Shetland where PCB burdens are high but dieldrin levels are low (Kruuk and Conroy, 1996). The importance of the OCs should not be ignored and, in particular, it is suggested that dieldrin may exert a synergistic effect. In experiments on mink (*Mustela vison*) mortality increased from 71 to 100% when a small amount of dieldrin was added to a diet containing Aroclor 1254 (Aulerich and Ringer, 1977).

The second observation in this study is the prevalence of low vitamin A levels in the early years followed by a steady, marked rise. Examination of stomach contents showed that the otters' diet throughout was mostly fish, which should be a good source of vitamin A, and it is unlikely that a dietary deficiency occurred. However,

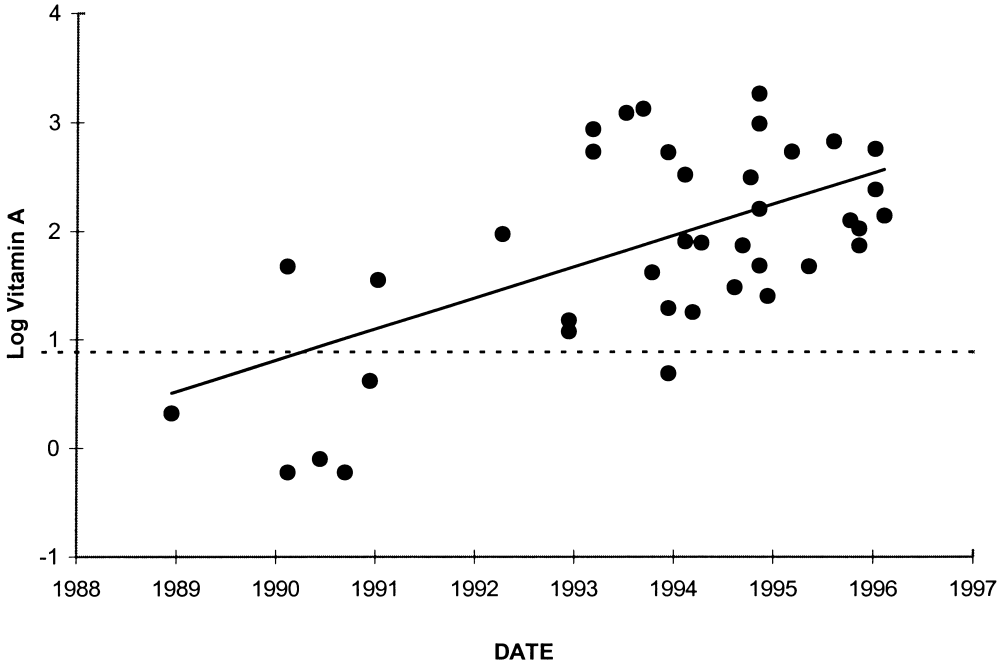

Fig. 5. Log hepatic concentration of vitamin A through time. The dotted line represents the critical value of 7 μmol/kg.

Table 4
Coefficients of correlation between log concentrations of vitamin A, *p,p′*-DDE, dieldrin and date[a]

	Vitamin A	*p,p′*-DDE	Dieldrin	Date
Vitamin A	1.00			
p,p′-DDE	−0.17	1.00		
Dieldrin	−0.44	0.81	1.00	
Date	0.67	−0.46	−0.61	1.00

[a] Degrees of freedom = 33.

Table 5
Coefficients of correlation between log concentrations of vitamin A, PCB congeners and date[a]

	Vitamin A	PCB118	PCB138	PCB153	PCB180	Date
Vitamin A	1.00					
PCB118	−0.20	1.00				
PCB138	−0.34	0.91	1.00			
PCB153	−0.30	0.90	0.99	1.00		
PCB180	−0.30	0.86	0.93	0.95	1.00	
Date	0.65	−0.34	−0.36	−0.34	−0.33	1.00

[a] Degrees of freedom = 33.

the OCs and the PCBs have both been shown experimentally to affect vitamin A status. Early studies on pigeons (*Columba livia*) dosed with either DDT or dieldrin showed an initial increase in hepatic vitamin A levels followed by decreased levels on prolonged exposure (Jefferies, 1975). Similarly, PCBs also cause reduced hepatic storage and increased renal excretion (Bank et al., 1989; Brunström et al., 1991). In addition, PCB metabolites block serum transportation of retinol by competing for binding sites on a transport protein complex (Bröuwer et al., 1986). Most of this work has been carried out on experimental animals but recent studies on road casualty wild otters from Denmark have shown low vitamin A levels associated with high PCB levels (Murk et al., 1998).

Liver vitamin A (retinol) values in domestic animals are normally in excess of 200 μmol/kg (Blood et al., 1983). Values below 7 μmol/kg are considered to be critical (Blood et al., 1983) and below 2 μmol/kg are evidence of severe deficiency (Doxey, 1983). Sixty-eight per cent of the otters in this study were below 200 μmol/kg and seven were below 7 μmol/kg, including four below 2 μmol/kg. The above-quoted normal values should only be applied to otters with caution but there is a report of vitamin A values in apparently normal wild otters (Stephens, 1957). Although the sample was small, the results are particularly valuable as the analyses were carried out in 1952–54, i.e. immediately before the decline of the otter population in Britain. Six samples were analysed, including two from Cornwall, and the values ranged from 28.8 to 540 μmol/kg and a mean of 176.6 μmol/kg. The values in the present study were therefore more extreme, ranging from less than 1 to 1836 μmol/kg, with a mean of 282 μmol/kg. In view of the ability of the halogenated hydrocarbons to increase as well as to decrease vitamin A levels this extreme range of values is of interest, particularly as very high vitamin A levels may also cause physiological effects.

The otters in this report formed part of a wider study which included detailed pathological examination (Simpson, 1997). The pathology of vitamin A deficiency varies to some degree according to species but typically includes foetal resorption, abortion and stillbirth. However, events such as these are most unlikely to be observed in wild otters and no cases were seen. Mammalian foetuses and neonates normally have low reserves but high demands for vitamin A (Håkansson et al., 1987) and deficiency at this age may cause a variety of developmental defects, such as abnormal bone modelling, hydrocephalus, gonadal hypoplasia and cryptorchidism. Cubs with severe defects are unlikely to survive for long and most will therefore go undetected. Only one otter had developmental defects consistent with vitamin A deficiency. This was a young male (< 1 year) which appeared stunted and was a cryptorchid. The hepatic vitamin A level was less than 1 μmol/kg but the pollutant levels were not notably high.

Adequate vitamin A is also required to maintain the integrity of epithelia, particularly of mucous membranes, and deficiency may lead to increased predisposition to infections, formation of renal calculi and eye conditions such as xerophthalmia, keratitis and corneal ulceration. Retinal degeneration may also occur (Jubb and Kennedy, 1963; Jefferies, 1975). Several of the otters had occasional, small, white, focal lesions in the lungs. On histopathological examination they were found to be cases of adiaspiromycosis caused by inhalation of fungal spores. The lesions were considered to be of little pathologial significance and apart from this there was little evidence of infectious disease. Renal calculi are commonly seen in captive otters and were systematically looked for in this study. None were seen on gross examination although a small calculus was observed histologically in a renal calyx of one otter. Unfortunately, the liver of this animal was too autolysed for vitamin A analysis. No otters had gross eye lesions suggestive of vitamin A deficiency but histopathological results will be reported elsewhere.

At the present time there would not appear to be any conclusive evidence of gross pathology in otters due to vitamin A deficiency, either in this country or elsewhere. However, in the past very few have been examined by pathologists. If the annual trend in vitamin A levels in this study is projected back in time it suggests that prior to the mid-1980s hypovitaminosis A may have been prevalent in otters in south west England. In the period

1957–80, i.e. immediately after the crash in Britain's otter population and before any evidence of a recovery, an experienced field observer recorded whitish opaque eyes in 22 otters, some of which were blind (Williams, 1989). Unfortunately, none were examined post mortem.

There are few data on specific PCB congener levels in Britain but the mean levels of 118, 138, 153 and 180 detected in the otters in this study are all lower than seen in Scotland in the period 1987–92 (Kruuk and Conroy, 1996). The mean OC levels are similar to those reported previously in other parts of Britain (Mason, 1989; Kruuk and Conroy, 1991), although lower than in *L. lutra* in Spain (Hernandez et al., 1985). They are also similar to mean concentrations seen in *Lutra canadensis* in North America (Henny et al., 1981; Foley et al., 1988). However, the maximum values in individual otters are lower than in most of these reports. Nevertheless, nine had total DDT derivatives in excess of 1000 μg/kg and two had dieldrin levels in excess of 2000 μg/kg wet matter. These levels could possibly be expected to have had an effect on vitamin A metabolism. However, although there were significant negative associations between dieldrin and vitamin A, and between PCB138 and vitamin A, the results from multiple regression suggest that the observed correlations between these pollutants and vitamin A may not be causal but due to their separate relationships with time. The possibility of other, confounding, factors cannot be ruled out. In the present study the toxic coplanar congeners 77, 126 and 169 were not measured but in studies on mink they were shown to cause the most pronounced depression of vitamin A levels (Håkansson et al., 1992). Furthermore, recent research in the Netherlands has demonstrated selective retention of congeners 126 and 169 by otters (Smit et al., 1998). The Dutch research group has also reported that the correlation between high PCB and low vitamin A levels was much clearer when PCBs were expressed as the Toxic Equivalent of seven of the most toxic congeners (Safe, 1990; Ahlborg et al., 1994), and not as the sum of the concentrations of the individual congeners (Murk et al., 1998).

This study has shown clear evidence of decreasing concentrations of polychlorinated hydrocarbons in otters between 1988 and 1996 in south west England. At the same time there have been very significant increases in their vitamin A levels. These changes have coincided with an increase in the number of otters submitted for examination each year and it would appear that the population may be increasing, not only in south west England (Simpson, 1997) but also nationally (Strachan and Jefferies, 1996). Hypovitaminosis A may have been a significant factor in the decline of the European otter population, but whether this was induced by high concentrations of OC and PCBs — acting separately or in concert—or by some other factors in the environment, remains uncertain.

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