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Potential modifications to the Predatory Bird Monitoring Scheme (PBMS): second report

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Predatory Bird Monitoring Scheme

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Contents

1. Executive summary

The Predatory Bird Monitoring Scheme (PBMS) covers a long-term monitoring programme that examines the levels of pollutants in avian wildlife species in Britain. It was instrumental in securing the phased withdrawals of the permitted uses of organochlorine insecticides and has since provided a measure of the effectiveness of regulatory bans in reducing the exposure of wildlife. The PBMS has expanded over the years and currently monitors carcasses and/or eggs of various (terrestrial, freshwater and marine) predatory birds for organochlorine (OC) pesticides and their metabolites, polychlorinated biphenyls (PCBs), mercury and second-generation rodenticides (carcasses only). Since 1974, the PBMS has been the subject of a series of contracts (known as the Wildlife & Pollution contracts) from the Nature Conservancy Council, as was, and subsequently from the Joint Nature Conservation Committee (JNCC). This enables the JNCC and country agencies to monitor trends and advise on the effectiveness of measures to restrict the use and entry into the environment of some of these compounds.

The concentrations of organochlorine pesticides in many predatory bird species have declined to levels below those likely to have toxic effects. As a result, concerns about the environmental hazards posed by these pesticides have, at least in part, been superseded by those posed by new groups of chemicals. In the last review of the work of the PBMS (Shore *et al.* 2002a), a number of possible new activities that could be incorporated into the PBMS were specified. Any such refocusing of the PBMS within existing resources would require revision of the current sampling intensity of organochlorine pesticides in order to free resources for new monitoring. The first objective of the current report was to assess the implications of reducing sampling intensity on the ability to detect long-term trends in the extent of wildlife contamination. This is described in Section 3.

The other objectives of the current report were to consider in greater detail some of the potential activities that had been outlined in the original review. These were specifically to: explore whether monitoring for polybrominated diphenyl ethers (PBDEs) and polycyclic aromatic hydrocarbons (PAHs) could be incorporated into the PBMS (Section 4); describe how the link between environmental residue data and toxicity could be improved for PCBs (Section 5); demonstrate the ways in which the PBMS data can be used to identify hotspots of contamination and so provide information on potential sources (Section 6); examine the frequency with which unknown compounds occur in predatory birds and describe a strategy by which they might be identified (Section 7).

Statistical analyses of the trends in contaminants in Eurasian sparrowhawks *Accipiter nisus*, merlins *Falco columbarius* and barn owls *Tyto alba* suggest that such studies require annual sampling for probably a minimum of ten years to detect long-term changes. The sensitivity with which trends are likely to be detected is reduced if sampling is on a biennial or triennial basis or carried out for a shorter period. Implementation of new monitoring programmes designed to determine whether contaminant levels in birds are changing should therefore envisage a commitment of at least ten years. The data collected over this initial period can be used to determine whether there is evidence of any change, the power of the study to detect such change, and the likely impacts of subsequent changes in sampling frequency on ability to detect trends. Large-scale contaminant studies that are carried out over shorter periods of time or with lower sampling frequency are likely to have relatively low power and only reveal large changes.

Analysis of PBDEs and PAHs by the PBMS is feasible. PBDEs could be monitored in both livers and eggs of samples currently collected under the PBMS. PAH analysis would be better restricted to eggs. The costs of incorporating these analyses within the PBMS could be met within current resourcing if the sampling strategy of the scheme is revised.

Preliminary analysis of congener concentrations and associated Toxicity Equivalents Quotient (TEQ) values in sparrowhawk livers indicated that although there was some correspondence between total PCB concentrations and TEQ values, total PCB concentration was not necessarily a good predictor of TEQ. This is because the TEQ value is particularly influenced by the concentrations of the dioxin-like congeners (such as congeners 77 and 126) that have relatively high toxicity equivalency factor (TEF) values. Monitoring TEQ values as well as total PCB values in future would be worthwhile so that changes over time in PCB accumulation can be more clearly linked to likely toxicity. This could be done equally well for PCB monitoring in eggs as well as livers and may be more important when examining the eggs for some species, such as merlin and particularly white-tailed eagle *Haliaeetus albicilla* eggs, where PCB concentrations remain relatively high. The costs of doing this within the scheme would be relatively minor as all the congeners are determined simultaneously within the same analytical run and the only additional costs are relatively minor ones in terms of using extra standards and in data-handling and analysis.

Preliminary analysis of unknown peaks on chromatograms of sparrowhawk livers has demonstrated that up to 40 unknown compounds occur in individual samples. Individuals that have high concentration of total PCBs also have the highest number of unknown compounds. The frequency with which each unknown compound, as identified by its retention time, occurred in birds was highly variable and ranged from 3.7% to 85.7%. On average, each compound occurred in 18-28% of birds. A strategy as to how to identify unknown compounds is outlined.

If PBDEs and PAHs are to be incorporated into the monitoring of the PBMS, it is recommended that this is done in the first instance using the species and samples that are currently collected. Sampling would be maintained on an annual basis but analysis would be carried out on a triennial (or equivalent) basis. This would mean that the sensitivity with which long-term trends could be detected would lower than if analysis was carried out on all samples but this strategy would allow PBDEs and PAHs to be monitored and PCBs to be reported on a congener and TEQ basis. Samples not analysed immediately would be archived for future use in other studies or future monitoring should there be a requirement to increase the number of samples analysed. It is also recommended that mercury, lead and cadmium residues are monitored by the PBMS as the additional costs of doing so are minimal. Sampling and analysis for rodenticides should continue unchanged.

Further investigation of unknown compounds in birds and identification of contaminant hotspots is also recommended. These studies would require additional, one-off, funding.

2. Background and aims

The Bird of Prey Monitoring Scheme (PBMS) covers a long-term monitoring programme that examines the levels of pollutants in selected avian wildlife species in Britain. The programme was started in the early 1960s when there were serious concerns about the effects of organochlorine (OC) insecticides and organomercury fungicides on various bird and mammal species. This early work demonstrated the effects of the OCs, particularly on raptors, and was instrumental in securing the phased withdrawals of the permitted uses of these insecticides in the UK and abroad. The programme has subsequently assessed the effectiveness of these bans in the UK by measuring whether there has been a decline in the concentrations of organochlorine pesticides in the livers and eggs of predatory and freshwater fish-eating birds.

Since 1974, the PBMS has been the subject of a series of contracts (known as the Wildlife & Pollution Contracts) from the Nature Conservancy Council (NCC), as was, and subsequently from the Joint Nature Conservation Committee (JNCC). These contracts have been carried out by the Institute of Terrestrial Ecology (now the Centre for Ecology & Hydrology (CEH)). Over the years, the PBMS expanded to encompass a range of other contaminants and pesticides, thereby reflecting contemporary conservation and regulatory concerns. Investigations have been made into the levels of industrial polychlorinated biphenyls (PCBs), following their identification as pollutants in 1966. These have been measured in both birds of prey and in the eggs of marine predators; northern gannet *Morus bassanus* eggs are collected approximately biennially from two colonies and, when available, from other sites. The levels of mercury, which may derive from past agricultural and both past and current industrial sources, have also been tracked in various bird species. Since 1982, investigations have been made into the effects of the newest generation of rodenticides on barn owls *Tyto alba*. As a result of recent pilot studies that found a high proportion of red kites *Milvus milvus* and common kestrels *Falco tinnunculus* contained residues of second-generation rodenticides (Shore *et al.* 2000; Shore *et al.* 2001b), the monitoring of rodenticides was widened in 2001 to include both these species as well as barn owls, although kites thought to have actually died as a result of poisoning are usually examined by the Department of Environment and Rural Affairs (Defra) Wildlife Incident Investigation Scheme (WIIS).

The current long-term monitoring activities undertaken under the PBMS are summarised in Table 2.1. Carcasses or eggs of various other predatory species not listed have also in the past been the subject of long-term monitoring for organochlorines and mercury, although sampling intensity has not always been so great. Most notably, these species include kestrel, peregrine falcon *Falco peregrinus*, great crested grebe *Podiceps cristatus* and kingfisher *Alcedo atthis*. These species no longer form part of core monitoring for organochlorines or mercury although, in some cases, they are now monitored for other contaminants (e.g. kestrels for rodenticides), and body tissues and egg contents of all species received at Monks Wood are archived in a deep-freeze tissue bank. This tissue archive represents a unique means of examining historical changes in environmental levels of new contaminants that have become cause for concern and it has been used for dedicated studies on certain species (Shore *et al.* 2002b) or on contaminants not normally covered under the PBMS (Erry *et al.* 1999).

Table 2.1: Current monitoring carried out under the PBMS

Compound	Species[1]		Tissue type	Start of monitoring
DDE	Eurasian sparrowhawk	*Accipter nisus*	Liver	1963
HEOD	Grey heron	*Ardea cinerea*	Liver	1963
Total PCBs	Merlin	*Falco columbarius*	Egg	1963
Total mercury[2]	Golden eagle	*Aquila chrysaetos*	Egg	1963
	White-tailed eagle	*Haliaeetus albicilla*	Egg	1986
	Northern gannet	*Morus bassanus*	Egg	1971
Second-generation rodenticides	Barn owl	*Tyto alba*	Liver	1982
	Common kestrel	*Falco tinnunculus*	Liver	2001
	Red kite	*Milvus milvus*	Liver	2001

[1] other species have been subject to long-term monitoring for organochlorine pesticides, PCBs and mercury in the past (see text for details).

[2] not monitored in birds collected in 2001

Some of the core activities undertaken by the PBMS address issues that have declined in conservation and policy significance. For instance, dichlorodiphenyldichloroethylene (DDE) and 1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4á,5,6,7,8,8á-octahydro-1,4-endo,exo-5,8-dimethanonaphthalene (HEOD) are the major metabolites that are detected in the tissues of animals exposed to dichlorodiphenyltrichloroethane (DDT) and to one or more of dieldrin, aldrin or endrin, respectively. The PBMS has demonstrated that, following the implementation of voluntary and then mandatory bans on the use of the parent chemicals, average annual concentrations of DDE and HEOD have largely declined to non-toxic levels in the tissues and eggs of most of the predatory birds that are monitored (Shore *et al.* 2005a). Concerns about the environmental hazards posed by the organochlorine pesticides have, at least in part, been superseded by those posed by newer groups of compounds; for example, there is mounting evidence of widespread exposure of vertebrate wildlife to second-generation rodenticides (McDonald *et al.* 1998; Newton *et al.* 1999b; Shore *et al.* 1999a; Shore *et al.* 2000; Shore *et al.* 2001b; Shore *et al.* 2003a; Shore *et al.* 2003b). The possible impacts of endocrine disrupting chemicals on wildlife are also a current concern. Therefore, as part of the last review of the work undertaken by the PBMS, crucial gaps in the understanding of contemporary wildlife and pollution issues were highlighted and ways in which the PBMS could be used to address these gaps were identified (Shore *et al.* 2002a). A number of activities that could be usefully incorporated into the PBMS were specified. These included widening the numbers and types of compounds monitored; improving understanding of the link between environmental residue data and toxicity for compounds that are already monitored and are still of concern; using data from the scheme to identify contamination hotspots on a national scale; and identifying unknown compounds that occur in birds. These issues are considered in more detail in Sections 4-7 of the current report.

If the flexibility of the PBMS was to be enhanced and resources liberated for the kinds of new activities outlined above, it was recognised that some of the current monitoring would have to

be modified or stopped (Shore *et al.* 2002a). However, terminating the monitoring of organochlorine pesticides would do little to increase the flexibility of the PBMS if PCBs were still monitored because PCBs and organochlorine pesticides are determined simultaneously. PCBs are still of current interest because residues have not declined significantly in many of the birds that are monitored and remain of toxicological significance in some species (Shore *et al.* 2005a). Termination of the monitoring of both organochlorine pesticides and PCBs was considered undesirable, not only because of the remaining environmental concerns over PCBs but also because it would result in the loss of a unique long-term database (Shore *et al*. 2002a). Such large-scale, long-term data sets are invaluable for understanding the processes by which chemicals move through the environment and exert effects on individuals and populations. One possible solution that was identified was that the flexibility and scope of the PBMS could be enhanced by carrying out analysis of organochlorine pesticides, PCBs and mercury on a multi-year (for instance every two or three years) rather than an annual basis, thereby freeing resources in the intervening years for other monitoring studies or specific, one-off investigations. It was recommended that a statistical evaluation of the existing long-term PBMS datasets should be carried out to determine the impact of sampling frequency on the ability to detect long-term trends in contamination. This evaluation forms Section 3 of the current report.

The specific aims of the present report were to:

i. quantify the effects of sampling frequency on the speed and precision with which long-term changes in patterns of contamination can be detected with statistical rigour,

ii. investigate how the monitoring of new compounds, particularly polybrominated diphenyl ethers (PBDEs) and polycyclic aromatic hydrocarbons (PAHs), can be incorporated into the PBMS,

iii. demonstrate how the link between environmental residue data and toxicity can be improved for PCBs, which are currently reported as total PCBs,

iv. demonstrate the ways in which the PBMS data can be used to identify hotspots of contamination, and so information on potential sources,

v. examine the frequency with which unknown compounds occur in predatory birds and outline the ways in which they can be identified.

These five objectives form the basis of Sections 3-7 of the present report. A summary of the overall findings of the PBMS to date, and recommendations as to the way the PBMS can be adapted to widen its scope, and hence maximise its usefulness to regulators and policy-makers, is given in Section 8.

3. Statistical evaluation of existing long-term datasets

3.1. Introduction

Currently, the Predatory Bird Monitoring Scheme (PBMS) monitors contaminant residues on a yearly basis. All carcasses and eggs of the core monitoring species that are sent to Monks Wood each year are analysed. To enhance the flexibility of the PBMS so that specific one-off studies can be accommodated and the range of contaminants monitored widened, it may be necessary to reduce the frequency with which some compounds are currently monitored (see Section 2 of the present report for details). The aim of the current section was to examine the consequences of changing sampling frequency on the sensitivity with which changes in long-term contaminant trends can be detected. This involved re-examining a number of the long-term datasets of the PBMS to address the following questions:

i. how long did it take to detect statistically significant, progressive changes in the exposure of birds monitored under the PBMS to DDE, HEOD, PCB, mercury and anticoagulant rodenticides, as determined either by residue magnitude or proportion of individuals that were contaminated?

ii. how is ability to detect annual rate of change in contaminant burden affected by sampling frequency?

3.2. Methods

Three discrete datasets were used in the analysis:

a) liver concentrations of DDE, HEOD, PCB and mercury in Eurasian sparrowhawks *Accipiter nisus* over a 31-year period (1970–2000). There was an average of 54-60 samples per year for each of the four compounds. Data were reduced to annual geometric means prior to analysis (Figure 3.1) because within-year observations were less likely to be independent than annual mean values.

b) DDE, HEOD, PCB and mercury concentrations and shell indices in merlin *Falco columbarius* eggs covering a 30-year period (1970–2000 but no data for 1972) for all determinations except mercury (which was monitored from 1979). There was an average of 21-30 determinations per year for each of the five variables. Data were reduced to annual means prior to analysis and all were geometric means except those for shell index (Figure 3.2). Means were used in preference to data for all individuals in each year because within-year observations were less likely to be independent than annual mean values.

c) second-generation anticoagulant rodenticides in the livers of barn owls *Tyto alba* covering a 19-year period (1982–2000). An average of 50 birds per year were analysed. Data that was used was the proportion of birds in each year containing detectable amounts of one of more rodenticide (Figure 3.3).

Changes over time in annual mean contaminant concentration or proportion of birds containing rodenticide were analysed by linear regression models. Recent assessment of these

trends has shown that they are suitably described by linear regression analyses using log-transformed data (Shore *et al*. 2005a) and geometric means were used in the current analyses. It was assumed that there were no significant changes over time in sampling or detection efficiency over the time period analysed.

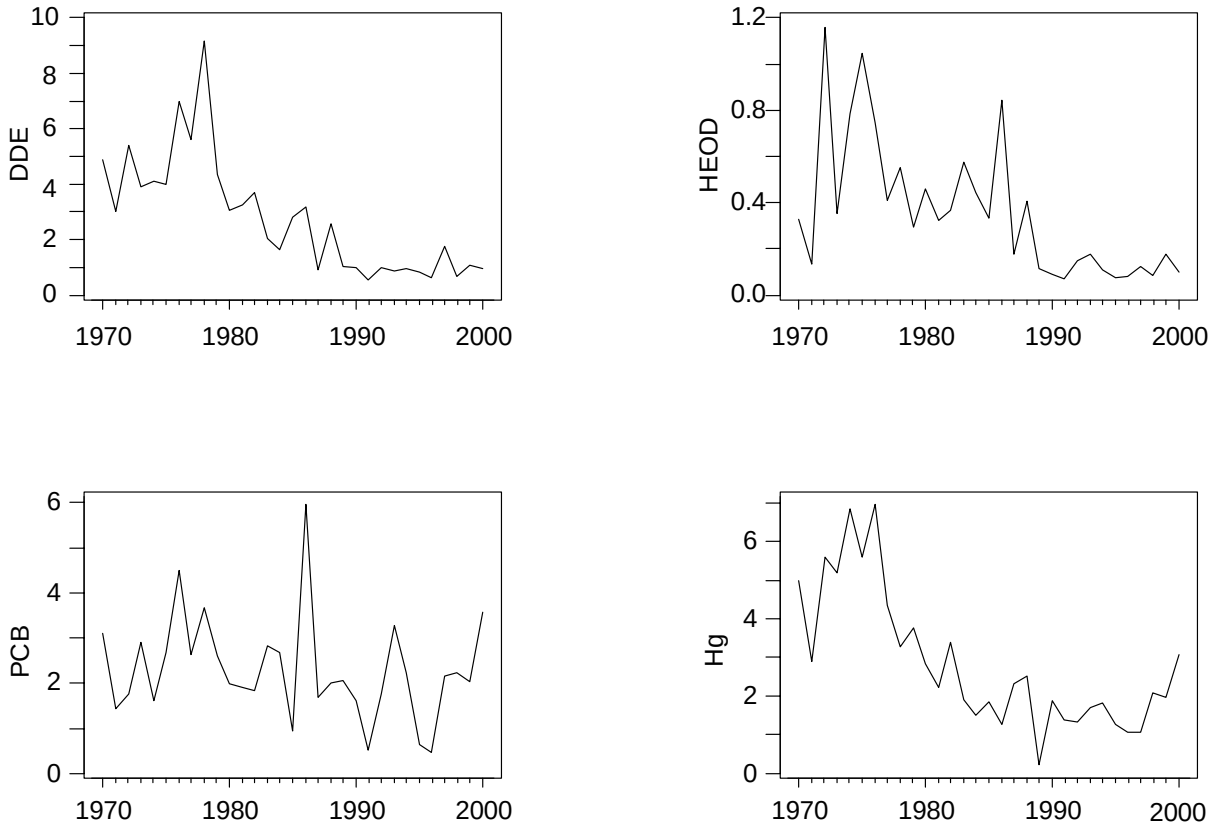

Figure 3.1: Annual geometric mean concentrations of DDE, HEOD, PCB (all µg/g wet weight) and mercury (µg/g dry weight) in the livers of Eurasian sparrowhawks analysed between 1970 and 2000.

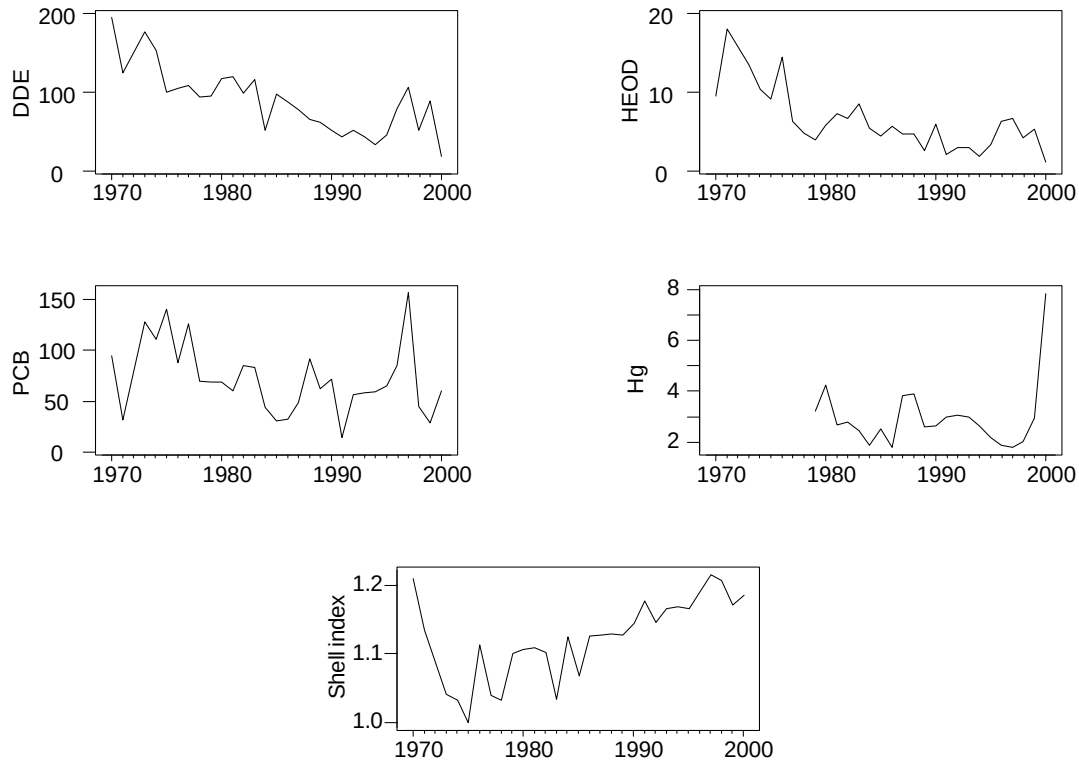

Figure 3.2: Annual geometric mean concentrations of DDE, HEOD, PCB (all µg/g lipid) and mercury (µg/g dry weight) and arithmetic mean shell indices for merlin eggs analysed between 1970 and 2000.

Figure 3.3: Percentage of barn owls containing one or more second-generation anticoagulant rodenticide.

3.3. Results

3.3.1. How long did it take to detect statistically significant, progressive changes?

Using data for all the years that were available, it was calculated when the downward trends in organochlorine pesticides, PCBs, mercury and shell index and the upward trend in the proportion of barn owls containing rodenticide residues could be first detected. This was when the gradient of the regression line in the linear regression model was first significantly different ($P<0.05$) from zero. The years when this first occurred are shown in Table 3.1.

The time taken to detect significant trends in observations varied markedly between species and contaminants, ranging between 6 (rodenticides in barn owls) and 24 years (shell index in merlin eggs). The average period of time taken to demonstrate significant reductions in contaminant levels in sparrowhawks and merlins was 17 years and 10 years, respectively. The figures in Table 3.1 can be considered conservative as, in some cases, the statistical significance of a trend may have been lost in the year following first detection (because of inherent variation in the data) and only become re-established, and remained so, in subsequent years. Contemporary analysis of such data would normally be cautious and general acceptance that apparent trends are real may only be achieved once trends prove statistically significant over several years.

In general, identification of trends could be achieved two years earlier if weighted linear regression rather than simple linear regression analysis was used. This process uses the number of samples in each year to modify the regression analysis; greater importance is given to years with larger sample sizes. Years with small samples, and potentially unreliable values, are down-weighted. Thus, even with annual sampling and weighting of data for sampling intensity, detection of significant trends requires monitoring for considerable periods of time.

Table 3.1: Year when statistically significant annual changes in residue magnitude or proportion of birds contaminated could first be detected.

	Sparrowhawk			**Merlin**			**Barn owl**		
	start of data run	yr trend detected	Time (years)	start of data run	yr trend detected	Time (years)	start of data run	yr trend detected	Time (years)
DDE	1970	1987	17	1970	1978	8			
HEOD	1970	1991	21	1970	1979	9			
PCB	1970	no trend	no trend	1970	1986	16			
mercury	1970	1983	13	1979	1986	7			
shell index				1970	1994	24			
% rodenticide							1982	1988	6

3.3.2. How is ability to detect annual rate of change in contaminant burden affected by sampling frequency?

The time taken to detect significant trends is largely a function of the frequency of sampling, the magnitude of the trend and the intra and inter-year variability in the data, although, in some cases, it may also be dependent on the timing of specific large-scale events that trigger changes in trend direction. Overall, there is no simple relationship between frequency of sampling and time taken to detect trends.

To illustrate how sampling frequency can affect the sensitivity with which annual changes in residue magnitude or proportion of birds contaminated can be detected, trend detection values were calculated for each contaminant using data for every year (annual sampling), every other year (biennial sampling) and every third year (triennial sampling) over the first 10, first 20 and all 30 years of sampling; data for barn owls only covered a 20-year span. Thus, there were nine trend values for each contaminant in sparrowhawks and merlin eggs (Table 3.2) and six for rodenticides in barn owls (Table 3.3). These detection figures are the annual rate of change that would have been detectable with 85% power (equivalent to three times the expected standard error of the calculated regression slope).

Table 3.2: Annual rate of change in DDE, HEOD, total PCBs and mercury concentrations (μg/g for contaminants) and shell index in sparrowhawk livers and merlin eggs that would have been detectable with 85% power under different sampling strategies (annual, biennial, triennial) over three different time periods.

	interval	years	DDE	HEOD	PCB	mercury	Shell index
Sparrowhawk	annual	30	0.088	0.015	0.072	0.077	
		20	0.162	0.027	0.133	0.142	
		10	0.459	0.076	0.376	0.403	
	biennial	30	0.125	0.021	0.102	0.109	
		20	0.229	0.038	0.188	0.201	
		10	0.659	0.110	0.541	0.579	
	triennial	30	0.153	0.025	0.125	0.134	
		20	0.263	0.044	0.215	0.231	
		10	0.621	0.103	0.510	0.546	
Merlin	annual	30	1.661	0.173	2.088	0.083	0.0029
		20	3.054	0.318	3.839	0.152	0.0053
		10	8.670	0.903	10.900	0.432	0.0151
	biennial	30	2.353	0.245	2.958	0.117	0.0041
		20	4.335	0.452	5.450	0.216	0.0076
		10	12.451	1.297	15.653	0.620	0.0217
	triennial	30	2.890	0.301	3.633	0.144	0.0050
		20	4.961	0.517	6.236	0.247	0.0086
		10	11.739	1.223	14.758	0.585	0.0205

The analysis of annual trends in sparrowhawks and merlins demonstrated three major points.

First, the sensitivity with which annual rates of change were detected increased with the duration of monitoring but the relationship was not linear. For example, the annual rate of change in liver mercury concentrations in sparrowhawks that could be detected after annual sampling for 10, 20 and 30 years were 0.403, 0.142 and 0.077 μg/g respectively. Thus the overall sensitivity of detection was improved almost 3-fold when monitoring was carried out for 20 rather than 10 years but only 5-fold when monitoring data were available for 30 rather than 10 years.

The second point was that reducing sampling frequency over any one specific timeframe decreased the sensitivity with which trends could be detected but again in a non-linear way. Thus, for example, over the 30-year period in which mercury was measured in sparrowhawks, sampling on an annual basis gave a detectable rate of change of 0.077 μg/g. Sampling on a biennial or triennial basis would have given comparative figures of 0.109 μg/g (1.4-fold less sensitive) and 0.134 μg/g (1.7-fold less sensitive), respectively. Interestingly, the effect of reducing sampling intensity from biennial to triennial did not always reduce sensitivity over

the shortest (10-year) period but this is likely to have been because the within year variability in some of the years sampled under the triennial scheme was by chance smaller than in other intervening years that were sampled under the biennial scheme.

The third major point revealed by the analysis was that although the annual rate of change in contaminant concentration varied both with species and contaminants, the effect of reducing the duration or frequency of sampling always largely had the same impact on sensitivity of detection. However, changing duration had far greater effect than changing frequency. For example, for a fixed amount of sampling points (say 10), trend detection was always better if sampling was carried out biennially over 20 years compared with annual sampling over 10 years.

Table 3.3: Annual rate of change in the % of barn owls contaminated with second-generation rodenticides that would have been detectable under different sampling strategies (annual, biennial, triennial) over two different time periods.

Interval	Period of years	change in the % of birds containing rodenticide
Annual	20	1.087
	10	3.086
Biennial	20	1.543
	10	4.432
Triennial	20	1.766
	10	4.179

The data for barn owls differ in nature from that for sparrowhawks and merlins because they are simply the proportion of birds contaminated rather than an annual rate of change in residue magnitude. However, the effect of reducing the duration or intensity of sampling was the same as that for other species in that both reduced the sensitivity of analysis. For example, an increase of $\geq$ 3.1%/year in the proportion of birds with residues could be detected by annual monitoring over 10 years; in contrast, this figure would only have been $\geq$ 4.4% if monitoring was biennial (Table 3.3). Over the whole 10 year period, this means that an increase of 31% or more in the proportion of birds with residues would have been detected by annual sampling, but only an increase of 44% or more would have detected by biennial sampling.

3.4. Conclusions and recommendations for monitoring frequency

It is clear from the analysis outlined above that it is necessary to collect relatively long series of monitoring data if the aim is to determine whether progressive changes in residue magnitude or the proportion of birds contaminated are real. The analysis of the trends in contaminants in sparrowhawks, barn owls and in merlin eggs suggest that, when annual sampling is carried out, monitoring needs to be continued for probably a minimum of ten years in order to detect statistically significant trends. The regression analysis used to determine the significance of such trends should be weighted to account for inter-year variation in sampling intensity. Even with this intensity of monitoring, the sensitivity with

which trends are likely to be detected remains relatively coarse but is markedly better than if sampling is on a biennial or triennial basis over the same time period or if annual monitoring is carried out for a shorter period.

It is also apparent from the analysis that for a fixed number of sampling periods, the sensitivity with which trends can be detected is better when sampling is carried out biennially or triennially over 20 or 30 years respectively, compared with when it is carried out annually over 10 years. While this suggests that reducing the frequency of monitoring but increasing its duration may be a sensible option, a major disadvantage is that the time taken to first detect significant trends will be longer than if annual sampling is carried out. Given that annual sampling for at least 10 years may be necessary to first detect trends with statistical significance, further delays in such detection may be problematic in that large-scale changes in exposure levels will have occurred before they are first detectable. The key issue here is the trade-off between speed to first detect trends, and sensitivity of trend detection over the longer-term.

Given this analysis above, it would seem clear that implementation of new monitoring programmes, where the aim is to determine as rapidly as possible whether contaminant levels in birds are changing, should carry out annual monitoring and probably envisage a commitment of at least ten years. Data collected in this way over this initial period can be used to determine if there is evidence of any change, the power of the study to detect change, and the likely impact of changing sampling frequency on subsequent trend detection. Large-scale contaminant studies that are carried out over shorter periods of time or with lower sampling frequency are likely to have low power and only reveal changes that are large.

Incorporation into the PBMS of monitoring for new chemicals should, therefore, probably envisage an initial 10-year commitment to annual monitoring, once pilot studies have established the practicalities of determining residues in PBMS samples. Such monitoring could most readily be accommodated within the current PBMS resources by continuing existing annual sample collection (and carcass post-mortems) but reducing the analytical effort devoted to currently monitored compounds (such as PCBs) that remain of interest but for which rapid trend detection is not a priority. This could be achieved by determining the residues of such compounds either in only a proportion of birds received each year (for instance every second or third bird) or in all birds received in every second or third year (biennial or triennial sampling). Either mechanism would involve the same number of analyses over a two- or three-year period but a reduction in analytical effort overall compared with the current practice of analysing all birds each year. The resultant extra analytical resources that would be available could be used to analyse new chemicals of interest in samples collected each year. Reduced sampling intensity for PCBs and other currently monitored compounds would, however, reduce the ability of the PBMS to carry out some specific investigations, such as determining whether there are spatial patterns in residues in birds.

It has not been possible within the scope of the current report to carry out an analysis of within-year and between-year variation in residues. This analysis is needed to compare the possible impacts on trend detection of either analysing a proportion of birds received each year or analysing all birds received every second or third year. However, it is evident that where there may be an interest in detection of possible multi-annual cycles in contaminant residues, such as for PCBs (Shore *et al.* 2005a), preservation of annual sampling, even with reduced sample size, is likely to be important. Thus, with the current state of knowledge available, reduced annual sampling is considered preferable to biennial or triennial sampling

as a means of liberating analytical resources in the PBMS. Suggestions and rationale for which areas of current monitoring should be reduced are discussed further in Section 8 of the present report.

4. The potential for analysing new compounds using the Predatory Bird Monitoring Scheme, with particular reference to polybrominated diphenyl ethers (flame-retardants) and polycylic aromatic hydrocarbons (PAHs)

4.1. Introduction

There are a number of policy-drivers that could suggest a need for more extensive monitoring of a wider range of compounds than those currently covered by the PBMS. Also, there are various problems and challenges in the management of risks associated with newly-introduced and existing chemicals that need to be examined if national and international chemical risk assessment itself is not to lose credibility. In addition, there is a range of scientific uncertainties and unknowns related to chemical manufacture, use and disposal that need to be addressed if policies and regulations relating to chemicals are to operate in an atmosphere of increasing scientific certainty. Monitoring a wider range of chemicals in top predators could help reduce these uncertainties and fill in important gaps in knowledge. Major areas in need of consideration on a policy and strategic science front include the need:

- to avoid or minimise threats from chemicals whose presence in animals could indicate that the terms of the Birds Directive, Habitats Directive, Integrated Pollution Prevention and Control Directive, Water Framework Directive and Biodiversity Action Plans were not being met. This might be especially important where the Directives indicated a need to minimise the level of toxic chemicals in protected species or where contamination or biological effects might impinge adversely on protected species or habitats.
- to understand more about the true extent to which animals are exposed to mixtures of chemicals, especially because many groups of chemicals affect common biochemical receptors and functional physiological systems, with considerable potential for synergism.
- to enable risk assessment process for chemicals to focus more attention on the chemicals that are actually found in wildlife, rather than attempting to rely solely on limited data from tests of uncertain environmental and ecological relevance.
- under cost-benefit considerations to demonstrate the effectiveness of environmental regulations by showing the benefits they bring (e.g. monitoring can demonstrate falling residue levels after control measures have been taken; monitoring could help demonstrate that pesticide use was being minimised).

Bird tissues and eggs provide a number of opportunities for analysing new groups of compounds, either for the purposes of chemical monitoring, for monitoring potential toxicity, or monitoring actual biological effects. Eggs can be used to assess embryotoxic impacts, carcasses to assess impacts on adults and juveniles; either could be used for chemical monitoring.

The range of compounds that could be studied is considerable. Traditionally, attention within the PBMS has focused on 'lipid-soluble' persistent chemicals, but there is plenty of scope for analysing more water-soluble (e.g. rodenticide) and even less environmentally-persistent materials (e.g. organophosphorous pesticides), **provided that** the chemical of interest is the **parent** compound. There is still a substantial challenge to be faced if the compounds of interest are toxic metabolites. Analytical methods for such compounds could be developed, and in some instances may need to be.

In the first report that considered the ways in which the PBMS could be modified (Shore *et al.* 2002a), a number of compounds of current environmental concern that could be monitored using the PBMS were specifically identified. These included two classes of compound, brominated flame-retardants, especially polybrominated diphenyl ethers (PBDEs), and polycyclic aromatic hydrocarbons (PAHs), which were of particular concern because of their toxic hazard and known presence in some environmental compartments. Here, we focus on these two series of compounds to address the following questions:

(i) what would the value of monitoring be in policy terms?

(ii) if these compounds were monitored using the PBMS, what species would be appropriate, and what pathways of chemical movement would we be gaining knowledge of?

(iii) what toxicity data is available for these compounds?

(iv) what analytical developments would be required to launch a monitoring programme?

(v) how compatible would this be with current analytical capabilities?

(vi) what costs would be incurred by monitoring for these compounds?

4.2. Polybrominated diphenyl ethers (PBDEs)

4.2.1. Chemistry and toxicology

PBDEs are low vapour pressure compounds with log octanol-water partition coefficients of between *c*. 6-10. Broadly speaking this means they may persist in soils and sediments and accumulate in wildlife, unless they are metabolised by organisms or are unstable in light. PBDEs are not light-stable, and debromination of the higher halogenated forms seems to occur reasonably readily under experimental conditions.

Evidence for biological breakdown of these compounds suggests metabolism is complex, but it certainly occurs. Some of the metabolites are toxic, and the metabolic pathway can involve cyclical processes between the gut and hepatic system of higher organisms. Turnover of these compounds is rapid. This means that exposure must clearly exceed excretion if residues are to accumulate in tissues. The dynamics of this will be complex, involving at least five body compartments.

The toxicology of PBDEs is uncertain in many respects (WHO 1997). In toxicological terms PBDEs and the other brominated flame-retardants exhibit activity at the aryl hydrocarbon (Ah) receptor and affect the functioning of the complex suite of genes controlled via this receptor. PBDEs and other polyhalogenated aromatic hydrocarbons can also inhibit the binding of thyroxine (T4) to transthyretin (the T4-plasma transport protein) and so affect thyroid-mediated physiological function. Thus, like their metabolism, their toxicology is likely to be subtle and complex, and, due to their high lipid solubility, is likely to involve

central nervous system (CNS) level impacts. In respect of toxicity they represent a potential threat akin to that posed by several groups of halogenated diphenyl ring compounds.

4.2.2. Monitoring information

Indicative levels in wild species are shown in Table 4.1. From this it can be seen that almost all the major sentinel groups of organisms (e.g. birds, mussels, sea mammals – data not given) can be used for monitoring. The main pathways being examined are:

i. releases from point sources (such as industrial producers and major users),
ii. diffuse pollution from commercial and domestic use and disposal.

Both temporal and spatial information is available for parts of North America and Europe (de Wit 2002). Levels are highest near sources and a sharp upward trend with time is discernable in many but not all environmental compartments. In many cases PBDE-47 is the compound found at highest levels. In other cases PBDE-99 or even PBDE-153 is dominant. There is great debate as to whether 'deca' PBDE (PBDE-209) is found in wildlife. If PBDE-209 occurs in wildlife, risk management action might be needed under EU regulatory procedures. The concerns are based not only on the possibility that PBDE-209 bioaccumulates, but that it can breakdown to other less brominated but biologically active materials. There are differing views about the environmental and analytical stability of PBDE-209. This compound may have been voluntarily withdrawn from manufacture in any case (de Wit 2002).

Table 4.1: PBDE concentrations in wildlife (largely drawn from de Wit 2002)

Species	Tissue	Level found	Reference
Starling (juvenile)	Muscle	6-13 ng/g lipid	(Sellström *et al.* 1993; Sellström 1996)
Rabbit	Muscle	Not detected	(Jansson *et al.* 1993; Sellström *et al.* 1993; Sellström 1996)
Deer	Muscle	0.5-1.7 ng/g lipid	(Jansson *et al.* 1993; Sellström *et al.* 1993; Sellström 1996)
Mussels	Whole soft tissue	Range from not detected up to 17 ng/g dry wt	(de Boer *et al.* 2000)
Fish (background)	Muscle	26-1200 ng/g lipid	(de Wit, 2002)
Fish (industrial)	Muscle	950-27,000 ng/g lipid	(Andersson & Blomkvist 1981)
Osprey	Pooled muscle	2100 ng/g lipid	(Jansson *et al.* 1993; Sellström *et al.* 1993; Sellström 1996)
Seabirds (UK)	Liver	300-6400 ng/g lipid	(Allchin *et al.* 2000)
Predatory bird eggs	Eggs	6-1300 ng/g lipid	Sellström *et al*., unpub. data

4.2.3. Analytical considerations

Analysis for many brominated flame-retardants can be done by gas chromatography–mass spectrometry techniques. For some compounds derivitisation techniques are required during sample preparation. This added step will increase analytical uncertainties. Techniques not involving such steps are possible but less sensitive. GC-MS procedures are quite consistent with current approaches. Although GC-ECD techniques could be used these are probably not as acceptable in modern quality assurance procedures. Analytical costs would be similar to those associated with PCBs.

4.2.4. Recommended way forward

Precautionary monitoring for PBDEs would seem justified given the large-scale production of these compounds, the fact that environmental concentrations appear to be rising, and that may have immune, hormonal and neurotoxic effects in vertebrates. Data for the UK environment is currently limited (Table 4.1) and there is little information on the extent of exposure and any trends over time. Monitoring for PBDEs could be carried out using the types of biological samples already gathered by the PBMS. The value of such monitoring in policy terms would be that it would establish whether there was evidence of significant and widespread transfer of PBDEs through terrestrial food chains (and marine food chains if gannet eggs were also used) to top predators and whether the degree of exposure is changing over time. Such information is obviously crucial for assessing the potential threat of these compounds for wildlife. Monitoring may need to be extended beyond the PBDEs as several other brominated compounds are used as flame-retardants and there is only limited information on these.

An initial pilot study, which forms part of CEH's core science programme, is currently underway and involves the scanning of wildlife samples for PBDEs. This work is being carried out in collaboration with Lancaster University and involves small numbers of eggs and tissues. The results of this study will indicate the ease with which the PBMS could accommodate monitoring for PBDEs. The development of an integrated sampling strategy in co-operation with other research organisations (e.g., CEFAS, Lancaster University) would be worthwhile to maximise the information gathered on the environmental behaviour of these compounds.

4.3. Polycyclic aromatic hydrocarbons (PAHs)

4.3.1. Chemistry and toxicology

The properties, environmental fate and toxicology of polycyclic aromatic hydrocarbons have been documented and summarised in a substantial World Health Organisation Environmental Health Criteria document (WHO 1998). PAHs are a large group of multi-carbon ring compounds produced by natural combustion processes and human activities involving combustion. They are lipophilic and semi-volatile to some extent (log octanol-water-c.3.5-7.5 with variable volatility across the structural range). Despite microbial degradation, some PAHs persist in the environment and thus have the potential to bioaccumulate. They are not readily broken down by visible light, and are metabolised in multi-celled organisms. There are a large number of compounds in this series. About 20 attract attention, in part due to ease of analysis. They are mostly transported around the environment in particulate-bound form.

Of all the PAHs, the known human carcinogen benzo [a] pyrene and similar compounds attract most attention because of their toxicity. Some metabolites are also toxic and bind to DNA causing mutations. PAHs are highly embryotoxic to birds. Recent findings indicate that UV-B radiation can enhance PAH toxicity in amphibians and in marine organisms by perhaps as much as three orders of magnitude (Malcolm & Shore 2003; WHO 1998). This suggests a need to quantify the risks to freshwater and marine organisms.

4.3.2. Monitoring information

PAHs have been detected in wildlife and in the eggs of birds (Table 4.2). However, in general, levels of parent PAHs in vertebrates are limited by the fact that extensive metabolism occurs. Analytical methods for metabolites are complex. Measuring adducts, where PAH metabolites become bound to macromolecules, is often the method of choice because of its relevance to effects, but the analytical methods are complex and expensive.

PAH concentrations tend to be lower in the livers of birds than in eggs, by up to some two or three orders of magnitude (Hallett & Brecher 1984). Bird eggs would therefore be the choice tissue to study in wildlife although the pathway here would probably mostly reflect oiling. However, it has been shown that even tiny quantities of oil (of which PAHs are a major toxic component) and trace amounts of PAHs themselves can cause embryotoxic effects (WHO 1998). Thus, there is every justification for monitoring eggs as the **potential** for biological impacts is very high, although what data there is for the UK (the eggs of coastal-nesting birds from largely uncontaminated sites) suggests residues are currently low (Shore *et al.* 1999b). Data on other wildlife species is very scarce, despite evidence that relatively low-level exposure rates can have a range of important sub-lethal effects (Malcolm & Shore 2003).

4.3.3. Analytical considerations

No analytical development would be required for bird eggs beyond some minor adjustment and quality assurance of clean-up. This is because techniques for PAH parent compounds are already in place and adequately sensitive. The analytical technique of choice is GC-MS as other methods are too prone to interference. Analytical costs would be similar to those for PCBs.

Table 4.2. Concentrations of selected PAHs in eggs of various bird species (µg/kg)[1]

PAH	Concentration range (µg/kg)	Notes
Naphthalene	12-400	The most volatile PAH. Surprising to find any in eggs. Presence suggests recent exposure.
Benzo [a] pyrene	Not detected – *c*. 50	Known human carcinogen. Importance for wildlife unknown. Found in 17 of 19 species examined in 2 studies.
Indeno [1,2,3-cd] pyrene	Not detected – 2 0	The least volatile of the standard PAHs. Present in 16 of 19 examined in two studies.

[1]Data summarised from Malcolm & Shore (2003) reviewing available information on PAHs in wildlife. These data are mostly on a dry weight basis.

4.3.4. Recommended way forward

Despite the known potential for embryotoxic effects, there is little useful data on the levels of PAHs in vertebrates in Britain and almost nothing is known about how concentrations vary in either a temporal or spatial sense. Monitoring of PAH concentrations in bird eggs collected by the PBMS could begin immediately. Merlin and gannet eggs are those that are regularly collected in the largest numbers by the PBMS and would provide information on levels in the eggs moving though terrestrial and aquatic food chains to top predators. The resultant data could be linked to that on spatial variation in soil PAHs that has been collected recently (Osborn, unpublished data) to explore whether there is any evidence of a correlation between environmental concentrations (as determined from soil measurements) and levels in top predators.

The value of monitoring for PAHs in policy terms would be that it would establish whether there was evidence of significant accumulation of embryotoxic compounds in bird eggs in both the terrestrial and marine environments and whether the extent of exposure is changing over time. The toxicological significance of individual PAH concentrations in eggs could be inferred by comparisons to egg-dosing studies that have been carried out on model species in the laboratory. The significance of PAHs mixtures in eggs is uncertain and so would be more difficult to ascertain – a problem not peculiar to PAHs.

Monitoring using merlin and/or gannet eggs would provide little information on spatial variation in PAH levels, at least in the short-term, and samples may not be collected from areas where contamination may be highest, such as in industrialised regions and around the estuaries of major rivers that may be contaminated by industrial discharges. If the aim was to determine the extent of spatial variation in PAH residues in eggs and to examine levels in species likely to suffer the highest extent of exposure, sampling of eggs of appropriate sentinel species found in contaminated regions may be necessary. Species may include feral pigeon *Columba livia* in urban areas and common eider *Somateria mollissima* and great cormorant *Phalacrocorax carbo* in and around estuaries; eider may be a particularly good

sentinel species as it feeds extensively on bivalve molluscs that are themselves good accumulators of PAHs (Bender *et al.* 1988; Naes *et al.* 1995). Sampling networks would have to be developed by the PBMS although it is probable that some of the existing network could be used to collect the eggs of the coastal birds. In the first instance, pilot studies to measure the PAH concentrations in the eggs of these species from areas that are likely to be relatively highly contaminated would be necessary. Such studies could be carried out as one-off exercises to determine the suitability of widening the PBMS to include these species.

In conclusion, a sensible first step in monitoring PAHs using the PBMS would be to analyse merlin and gannet eggs for these compounds. This would determine whether detectable concentrations of PAHs are found in the eggs of these species and so whether they can be used to measure temporal trends in exposure. Subsequent studies might involve the analysis of eggs of other species to determine whether they might be more suitable as sentinel species of exposure and effects.

5. Modifications to how polychlorinated biphenyl (PCB) concentrations are reported

5.1. Introduction

PCBs are highly persistent compounds with known toxicological properties (WHO 1993). Because of their persistence and biological activity, their use in open systems was prohibited in many countries in the early 1970s and their production in most industrial countries was terminated later in the same decade (Hoffman *et al.* 2001); their use in new equipment was banned from 1986 in the UK. The target for disposal of all remaining PCBs was mid-2000 in the UK and is 2010 for the EU as a whole. If the recent targets for disposal of all remaining PCBs are successful, this should aid the elimination of PCBs from the environment in forthcoming years, although the actual and future rate of release of PCBs from landfill and sealed systems is uncertain.

PCBs are comprised of 209 different congeners that vary in the number and position of chlorine atoms attached to the biphenyl rings. Congeners differ in their physico-chemical and toxicological properties. The toxicity of compounds such as PCBs that act through the *Ah* receptor is often expressed as a Toxic Equivalency Factor (TEF). This is an order of magnitude estimate of the toxicity of that compound relative to that of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), the most potent dioxin congener (Ahlborg *et al.* 1994; Ahlborg *et al.* 1992; van den Berg *et al.* 1998). TEF values are used in conjunction with concentration data to calculate Toxic Equivalents (TEQ) in environmental samples. The TEQ for each compound is calculated as the product of the TEF and the concentration of the compound. Amongst the different PCB congeners, the TEQ values are highest for the co-planar, non-mono and di-*ortho* substituted coplanar congeners (also sometimes called dioxin-like polychlorinated biphenyls).

It would be expected that the exposure of wildlife to PCBs and the subsequent concentrations accumulated in biota would have decreased since manufacture and use was phased out. PCB concentrations are currently monitored as part of the Predatory Bird Monitoring Scheme (PBMS), thereby providing a quantitative assessment of whether regulatory efforts to remove these compounds from the environment have resulted in reductions in wildlife exposure. Since the late 1970s, PCB concentrations have declined in some avian species but not in others (Shore *et al.* 2005a). This suggests that the fate, behaviour and persistence of PCBs in the environment is complex, may vary between different trophic pathways, and that the success to date in reducing wildlife exposure has been patchy.

PCBs in birds are currently quantified by the PBMS as total PCB concentrations and are measured by gas chromatography with electron-capture detection. Total PCB concentrations are calculated as the sum area under all quantifiable peaks on the gas chromatogram other than those peaks known to be organochlorine pesticides. Thus, total PCB concentrations provide a measure of the total levels of all PCB congeners in the sample (although peaks for unidentified compounds that may not be PCBs are also included – see Section 7 of the present report), and hence a measure of exposure. However, such measurements do not provide particularly good information as to whether there have been concomitant changes in the toxicity associated with that exposure. This is because changes in total PCB concentrations

may not necessarily reflect decreases in those congeners with significant toxic potency. Indeed, it is possible that total PCB concentrations may fall but PCB TEQ values rise if fate, behaviour and persistence vary markedly between congeners with different toxic potencies.

In the previous report on ways that the PBMS might be modified (Shore *et al.* 2002a), the potential for both reporting TEQ values as part of the PBMS and for examining whether there are likely to have been have been long-term changes in TEQ values over the last 30 years, was highlighted. In this section of the current report, the aim was carry out a retrospective analysis of individual PCB congeners and TEQs to determine whether:

(i) total PCB concentrations give an accurate indication of TEQs,

(ii) trends in TEQ values were similar to those for total PCBs in birds monitored by the PBMS.

5.2. Methods

The livers from 321 sparrowhawks that died between 1991-98 were analysed for total PCB concentrations as part of the PBMS. Congener concentrations were not analysed in birds prior to 1991. They had also been analysed for concentrations of congeners 77, 118, 126 and 169, four congeners with assigned (and, apart from 118, relatively high) TEF values and which regularly occur in sparrowhawk livers; the TEFs are 0.05, 0.00001, 0.1 and 0.001, respectively. The methods used to quantify congener and total PCB concentrations in wildlife samples are described in detail elsewhere (Pain *et al.* 1999; Shore *et al.* 2001a). The relationships between concentrations of individual congeners, summed congeners, total PCBs and TEQ values were explored and variations over time in total PCB concentrations and TEQ values were compared.

As the analytical methods have been developed over the last few years, the capacity to analyse more of the congeners that now have TEF values ascribed to them has increased. For sparrowhawk livers that were analysed most recently (birds that died in 2000), the concentrations of eleven congeners that contribute to the overall TEQ value were determined. These included the four congeners analysed in birds that had died in earlier years and also congeners (associated TEF value in parenthesis) 105 (0.0001), 114 (0.0001), 123 (0.00001), 156 (0.0001), 157 (0.0001), 167 (0.00001), and 189 (0.00001). The relationship between TEQ value and total PCB concentration was again quantified in these birds.

5.3. Results

The relationship between the summed concentration of the four PCB congeners and the associated TEQ value was plotted for each of the 321 sparrowhawks analysed between 1991 and 1998 (Figure 5.1). The birds fell into four distinct groups that were categorised by the PCB congeners detected in the liver (Figure 5.1). None of the four congeners were detected in 134 birds (just over 40% of the sample). Congener 118 only was detected in various concentrations in 140 birds, congeners 118 and 169 in 38 birds and either all three or four congeners were present in the remaining nine birds; congeners 77, 126 and 169 never occurred in livers when congener 118 was absent.

When TEQ values were plotted against total PCB concentrations, these four clusters of birds remained reasonable distinct (Figure 5.2). It was evident that birds with the same TEQ values

could differ in their liver total PCB concentrations by some 2-3 orders of magnitude, and birds with the same total PCB concentrations could vary in their TEQ value by up to four orders of magnitude. When annual mean liver PCB concentrations and TEQ values were plotted against each other for the years 1991-98 (Figure 5.3), there was some measure of correspondence but the correlation was not statistically significant and was highly dependent on the apparently unusually high values for 1993. When data for 1993 were excluded from the analysis, there was in fact no correspondence between annual mean total PCB concentration and annual mean TEQ ($r_{(7)}$= 0.059, P = 0.90).

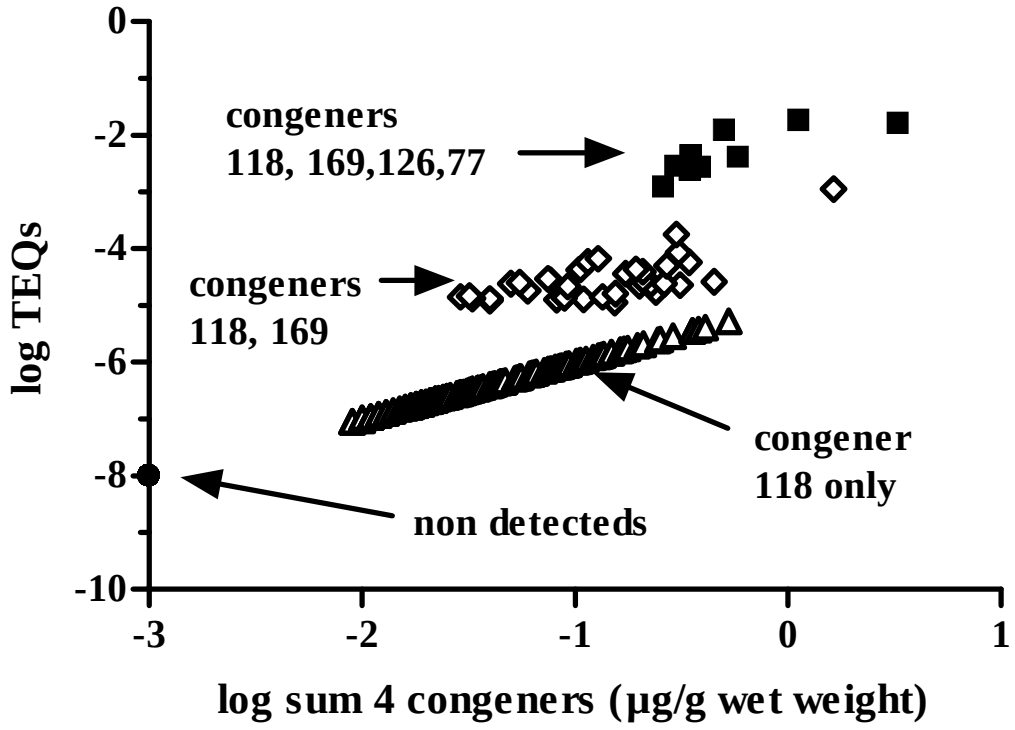

Figure 5.1: Relationship between the summed concentrations of congeners 77, 118, 126 and 169 used to calculate toxic equivalents and total toxic equivalent (TEQ) value in the livers of Eurasian sparrowhawks analysed between 1991 and 1998.

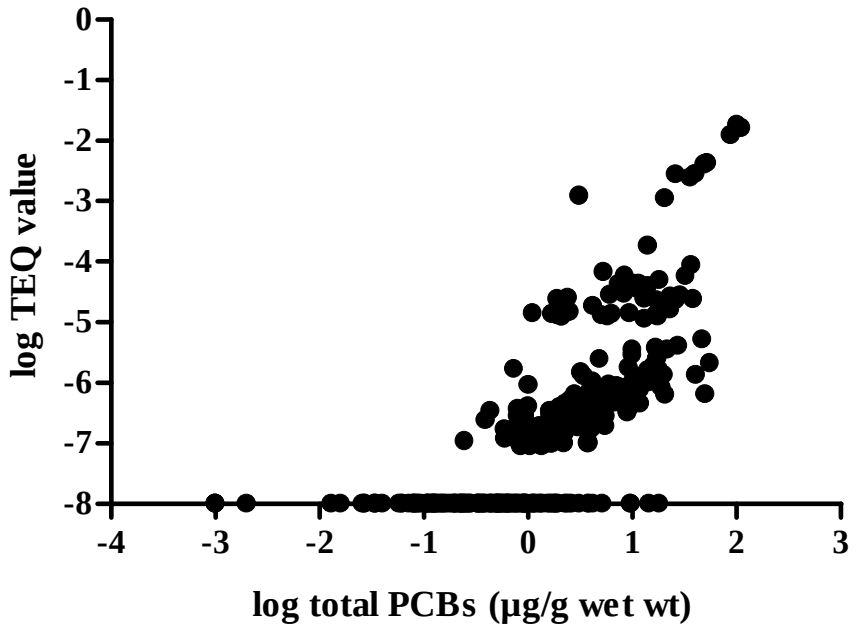

Figure 5.2: Relationship between log10 liver concentration of total PCBs and log10 TEQ (calculated as the summed toxic equivalents of PCB congeners 77, 118, 126 and 169) for 321 Eurasian sparrowhawks analysed between 1991 and 1998.

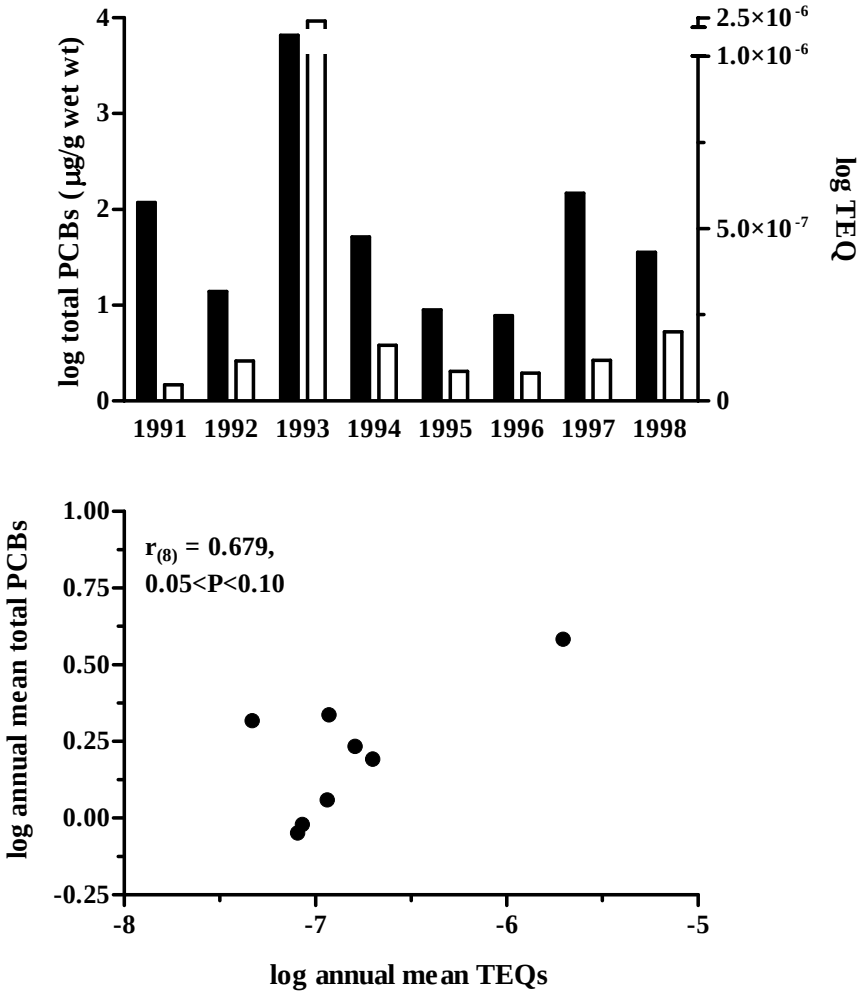

Figure 5.3: Upper graph: annual mean liver log total PCB concentrations (filled bars) and annual mean log TEQ values (open bars) for Eurasian sparrowhawks that died between 1991 and 1998; lower graph: the relationship between the two variables.

When the relationship between total PCB concentration and TEQ value, as determined using 11 rather than 4 congeners, was examined for birds that died in 2000, total PCB concentration proved to be a good predictor of the TEQ value (Figure 5.4). However, this did not appear to be the result of including more congeners in the TEQ calculation but due to the absence in all birds of congeners 77 and 126. These two congeners have TEF values (0.05 and 0.1, respectively) that are 50–100-fold greater than congener 169 (which was present) and at least 500–1000-fold greater than the TEFs of the other eight congeners. Relative to their concentrations, they therefore have a disproportionately large influence on the TEQ value compared with the other congeners.

5.4. Discussion

From this preliminary analysis, it is apparent that although there was some correspondence between total PCB concentrations and TEQ values, total PCB concentration was not

necessarily a good predictor of TEQ, at least in sparrowhawks. This is because the TEQ value is particularly influenced by the concentrations of the dioxin-like congeners (such as congeners 77 and 126) that have relatively high TEF values. During the 1990s, these congeners occurred in a small percentage of all birds that were analysed; when these congeners were absent, there was a strong correlation between total PCBs and TEQs. Thus, it is likely that it will be changes over time in the accumulation of dioxin-like congeners, rather than total PCB accumulation that will most affect any temporal patterns in TEQ.

It would clearly be worthwhile to monitor TEQ values as well as total PCB values in future monitoring so that changes over time in PCB accumulation can be more clearly linked to the likely toxicity. This could be done equally well for PCB monitoring in eggs as well as livers and may be more important when examining the eggs for some species, such as merlin and particularly white-tailed eagle *Haliaeetus albicilla* eggs, where PCB concentrations remain relatively high. The costs of doing this within the scheme would be relatively small as all the congeners are determined simultaneously within the same analytical run. Additional costs are only those associated with the use of extra standards and with data-handling and analysis. Determination of historic changes in TEQ values in the livers or eggs of species that have been monitored would require separate investigation. As part of its core programme, CEH has gathered concentration data for congeners 77, 118, 126 and 169 for all livers and eggs analysed since 1992. Analysis of trends in TEQ values in different species over the last decade could be carried out as a one-off exercise using the archived data at relatively little cost. Determination of longer-term patterns of change would require archived samples for key species to be reanalysed in order to quantify congener patterns. This would involve more substantial costs that would be the same (unit cost/per sample) as those incurred for PCB analyses currently.

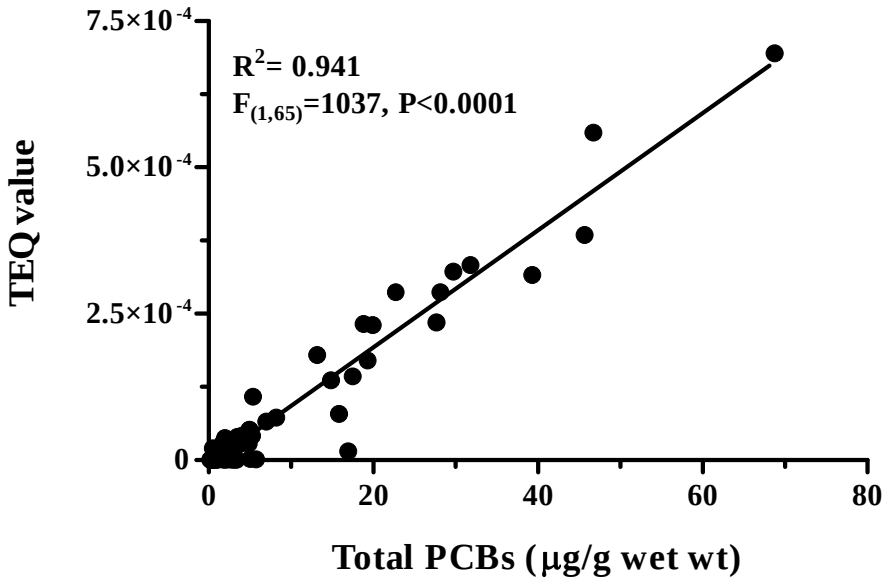

Figure 5.4: Relationship between TEQ values and total PCB concentration in the livers of Eurasian sparrowhawks that died in 2000.

6. Spatial variation in PCB concentrations in common kestrels *Falco tinnunculus* and Eurasian sparrowhawks *Accipiter nisus*

6.1. Introduction

Although temporal trends in contaminant levels in predatory birds in Britain have been well documented (Newton *et al.* 1999a; Newton & Galbraith 1991; Newton *et al.* 1999b; Newton *et al.* 1993), spatial patterns have been less thoroughly investigated. Such studies have often been restricted to studies of differences in contaminant levels between certain regions that may vary in environmental concentrations of contaminants (Erry *et al.* 1999; Newton *et al.* 1999a), between inland and coastal birds, and between colonies of seabirds (Newton *et al.* 1999a; Newton & Galbraith 1991; Newton *et al.* 1990; Sparks *et al.* 1999). There has been little attempt to examine spatial variation in contaminant burdens at a countrywide scale to determine whether contamination occurs in specific areas (that may be related to key sources) or is distributed diffusely through the environment. Knowledge of the spatial extent and intensity of environmental contamination is crucial to understanding the processes by which contaminants are distributed in and move through the environment. Such knowledge is also a key requirement if regulation or remediation measures to curb environmental pollution are to be effective.

In this section, we describe a preliminary study that has been carried out to examine whether there is any evidence of contaminant clusters in birds of prey in Britain. We report on the development of the techniques used to identify contaminant clusters in wildlife and describe the spatial distribution of liver PCB concentrations in common kestrels *Falco tinnunculus* and Eurasian sparrowhawks *Accipiter nisus* found dead from throughout Britain between 1993 and 1997.

6.2. Data sets

Spatially-resolved contaminant data were available for 309 sparrowhawks and 203 kestrels that had died in the five-year period 1993 to 1997. There was no significant difference between the species in either the sex ratio or the proportion of first year to adult birds (Fisher's Exact tests, $P>0.05$ in both cases). Liver PCB concentrations (expressed as mg/kg wet weight) were calculated as the sum of the five predominant PCB congeners (congeners 128, 138, 153, 170, 180) that occurred in the two species and were plotted as a dot map that used scaled symbols to represent the liver PCB concentration in each bird (Figure 6.1). The data were apparently clustered, indicating that the distribution of carcasses that were collected was not uniform. The mapping also revealed apparent clusters of high PCB concentrations in both species. These clusters were most notable in north-west England but were also scattered throughout Great Britain, particularly for sparrowhawks (Figure 6.1).

6.3. Statistical evaluation of apparent clusters

Identification and verification of contamination clusters or hotspots is complex. Pollution data with a spatial or geographical aspect is most commonly encountered in 'point data' form, as this usually reflects the method of data collection. The simplest and most obvious technique employed to visualise such information is a 'dot map' that may have scaling or other differentiations between dots that indicate specific attributes. Determining whether spatial patterns exist in the data, or are the result of random variation, is problematic. Intuitive ideas about what constitutes a pattern or random variation can be misleading, and it is notoriously difficult to reach a conclusion purely on the basis of a visual analysis (Bailey & Gatrell 1995).

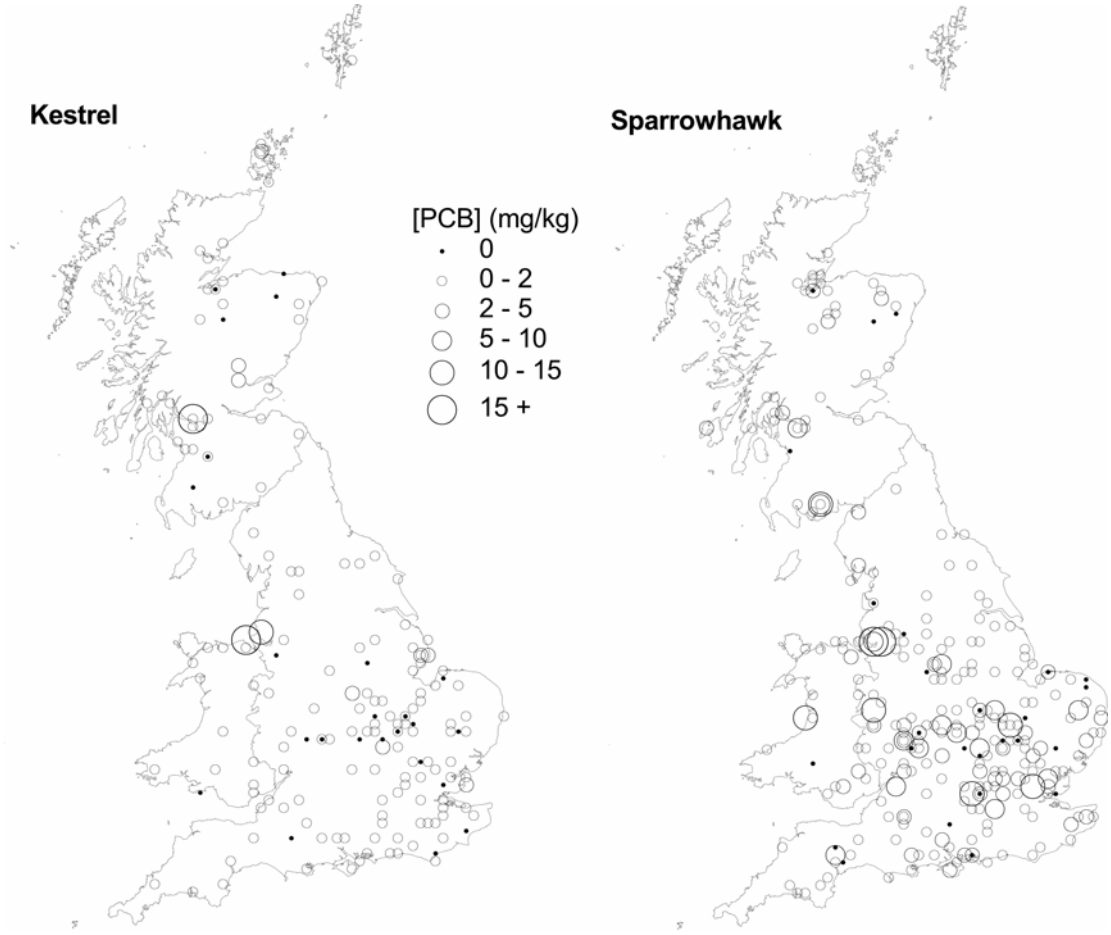

Figure 6.1: Distribution of common kestrel and Eurasian sparrowhawk carcasses recovered between 1993 and 1997. The magnitude of the liver PCB concentrations (sum of five congeners) that were detected within individual birds is indicated by the scaling.

Techniques for identifying clusters in spatially-resolved, point-based data sets have largely emerged from the fields of human epidemiology and medical geography. The absolute nature of human disease, in that a disease event either occurs or it does not, lends itself well to analysis of the binomial and Poisson distribution or incidence rates, and these techniques are particularly common (Marshall 1991). Other frequently used methods include various empirical measures of the relative locations of disease events, such as the K-function, which can be compared with theoretical distributions derived from the Poisson model (Gatrell & Rowlingson 1994). A particularly substantial body of work incorporating these methods has grown around the investigation of the incidence of rare diseases (such as leukaemia) near

industrial installations (Hills & Alexander 1989; Thomas 1991). Geographical Analysis Machines (GAMs), models that systematically search a study area for patterns in point data sets, have been utilised in the search for cancer clusters, and these are also reliant on the use of Poisson distributions for significance testing (Openshaw *et al.* 1987).

Studies to identify disease clusters in humans use spatially-resolved census data to determine the 'population at risk', the census data (e.g. for age or sex) being used to take into account the risk factors for population sub-groups. This is the basis for the binomial and Poisson distribution analysis that is carried out. However, this kind of detailed population data is all but impossible to collect for wild species and was unavailable for the PCB data for kestrels and sparrowhawks. Furthermore, the use of Poisson distribution is only justified if the spatially-resolved data are randomly distributed and this was not the case for the PBMS data sets. An additional complication was that the PCB concentration data are a continuous variable rather than 'presence or absence' data (as is collected for disease events) and virtually all established statistical techniques fail or struggle to identify the presence of a cluster against the background of a population with varying attributes, such as a range of PCB concentrations. Thus, a bespoke tool was generated to determine the statistical significance of the PCB clusters.

The initial analysis treated kestrels and sparrowhawks separately, although there was concern that several very large PCB concentration values in each data set (>10 mg/kg in three kestrels and >15 mg/kg in two sparrowhawks) may dominate the analysis and mask clusters of a lesser magnitude. Each species data set was therefore analysed twice, first using the full complement of records, then again with the large values removed.

A Monte Carlo (MC) simulation and significance test procedure was adopted and combined with a so-called 'brute force' methodology (Openshaw *et al.* 1987) to develop an analysis tool that would 'search' each data set for clusters of high PCB concentrations at a variety of spatial scales. This approach was similar to the original GAMs approaches that have been used in human epidemiology but, fundamentally, was not reliant on census or other population data. In all analyses, the geographical location of the data points (the location in which the bird carcasses were found) was fixed during the MC simulations. The attribute values of the data points (the PCB concentrations) were then randomly re-allocated to the fixed-point locations, effectively 'shuffling' the attribute values amongst the spatial location data set. This process was repeated 250 times, resulting in a series of realistic, yet synthetic, PCB concentration distribution maps. The synthetic spatial patterns in these data sets could then be compared with the original data set to determine the probability of the apparent clusters of high PCB concentrations occurring by chance.

The 'brute force' technique was employed to systematically search through the sparrowhawk and kestrel datasets (those with and without their largest values removed) and compare them with the synthetic data sets. Each search looked for an arbitrary number of five clusters at spatial scales ranging from a radius of 5.5 km to 25.5 km. The bottom of this range was dictated by the nature of the bird of prey data sets, with the geographical information for each bird being only at 10 km resolution. A radius of 5.5 km therefore ensured that each search would visit at least two potential data points. The upper limit was reached via trial and error. During preliminary trials, it was found that any clusters over a spatial scale of 45 km or more were never statistically significant, so it was decided to limit searches to a maximum radius of 25.5 km to comfortably encompass this range. This was adjudged to be a feature of the scale of the study area (Great Britain) and the distribution of the data points.

The 'brute force' method worked by systematically visiting the centre of each 1 km square in the study area (in this case 230,006 locations). As the location of the bird data was known only to the nearest 10 km, centres at 1 km intervals avoided the possibility of rounding errors. At each centre, the numbers of birds within the minimum radius were counted. The sum of the PCB concentrations of any birds within that radius was calculated and the weighted centroid (centre of gravity) of the cluster identified. The sum concentration was then written to file and compared to that of any existing clusters already detected in that search, in descending order. If the sum concentration was greater than the current maximum it became the new maximum sum value. In the simplest case, the previous maximum value then became the second-largest; the second became the third and so on. In the case of the previous maximum sum value being located in the same area and holding many of the same birds as the new current maximum, an arbitrary rule was applied that stated that two clusters would be considered independent only if their centroids were at least one radius apart. Applying the separation rule then meant that the previous maximum sum value only became the second largest if it was spatially distinct from the new, current maximum. This was repeated for all ranks of cluster (largest to fifth-largest). Once the minimum radius had been examined, the search radius was increased by a small amount (1 km) and the process repeated. This continued until the maximum radius was reached. The search then moved to the next 1 km centre and the process was repeated. In total, over 10^9 comparisons were performed for the two species.

The outcome of the 'brute force' process was the identification of the maximum, second, third, fourth and fifth largest clusters as determined using the different search radii. Information on the coordinates for the centroid of each cluster and the identity of the birds within each cluster were also available. To determine whether a cluster in the observed data set was real, the observed values were compared with the synthetic values. Starting with the largest cluster at the smallest radius, the observed sum PCB concentration for birds in the cluster was compared to the 250 values for the highest concentration at the smallest radius in the synthetic data sets. Clusters were considered real if less than 5% of the values from the synthetic data sets were larger than the observed value; this approximates to a 5% probability level. This process was then repeated for all the other radii and then for the second, third, fourth and fifth-largest clusters.

6.4. Results

The median (and inter-quartile range) wet weight liver PCB concentration was 0.404 µg/g (0.107-1.50) in sparrowhawks and 0.213 µg/g (0.05-0.65) in kestrels. The difference between the two species was statistically significant (Mann-Whitney U test, U= 24520, P<0.0001). Output from the spatial analyses is summarised in Table 6.1 and displayed graphically in Figures 6.2 and 6.3.

The only statistically significant cluster detected during the analysis of the complete sparrowhawk data set was the largest cluster, at radii of 5.5 km to 17.5 km inclusive. This was located in Merseyside, north-west England. When two very large PCB concentration values were removed from the sparrowhawk data set (birds from Merseyside) and the data reanalysed, no statistically significant clusters were detected (Figure 6.2).

Table 6.1: Statistically-significant clusters detected at various radii during analysis of liver PCB concentrations (sum of five predominant congeners) in Eurasian sparrowhawks and common kestrels in Britain. (1) denotes complete data set, (2) denotes data set with maximum PCB values removed (no significant clusters when the sparrowhawk data was reanalysed with the maximum values removed). A filled square denotes statistically significant at 95th percentile, a dash denotes not statistically significant.

		search radius (km)																							
dataset	cluster rank	5.5					10.5					15.5					20.5								
sparrowhawk 1	1	■	■	■	■	■	■	■	■	■	■	■	■	■	-	-	-	-	-	-	-				
kestrel 1	1	-	-	-	-	-	-	■	■	■	■	■	■	■	-	-	-	-	-	-	-				
	2	-	-	■	■	■	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-				
	3	■	■	■	■	■	■	-	-	-	-	-	-	-	-	-	-	-	-	-	-				
	4	■	■	■	■	■	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-				
kestrel 2	1	■	■	■	■	■	■	-	-	-	-	-	-	-	-	-	-	-	-	-	-				
	2	■	■	■	■	■	■	-	-	-	-	-	-	-	-	-	-	-	-	-	-				
	3	-	-	-	-	-	■	-	-	-	-	-	-	-	-	-	-	-	-	-	-				

Figure 6.2: Distribution of Eurasian sparrowhawk carcasses recovered between 1993 and 1997 with significant clusters (large black circles) of high liver PCB concentrations (sum of five congeners) indicated. Left-hand map shows that a single significant cluster was revealed when the complete dataset was analysed, the right-hand map shows that there were no significant clusters when the data were reanalysed with the largest concentrations (>15 mg/kg wet weight) removed (see text for details).

In contrast to the data for sparrowhawks, analysis of the complete kestrel data set indicated that there were four clusters (Figure 6.3, left-hand map) that were statistically significant at a range of search radii (Table 6.1). The largest, in terms of sum PCB concentration, was again located in Merseyside in north-west England. The second-largest cluster was located in south-west Scotland (near Glasgow), the third on the coast of eastern England and the fourth on the Orkneys.

When the three largest PCB values (>10 mg/kg wet weight in birds from the Merseyside region) were removed from the dataset and the data were reanalysed, three statistically significant clusters were detected (Figure 6.3, right-hand map). The eastern England cluster (originally ranked third, now the largest) and the Orkney cluster (originally ranked fifth, now second) were both still apparent at a range of radii (Table 6.1). A new cluster, in eastern Scotland (near Perth), was also detected but was significant only at a 10.5 km radius. The south-west Scotland cluster near Glasgow that was detected in the original analysis was also detected but was not quite statistically significant ($0.05<P<0.1$) and so is not shown on the revised map (Figure 6.3, right-hand map).

6.5. Discussion

The overall higher liver concentrations of the predominant PCB congeners in sparrowhawks than kestrels was consistent with the long-term national picture for total PCBs and other contaminants in that sparrowhawks are generally more contaminated than kestrels (Shore *et al.* 2005a). This has been attributed to food chain differences in exposure (Newton *et al.* 1993), and the inherently greater ability of kestrels to metabolise xenobiotics (Walker *et al.* 1987).

The regional picture in contamination was more complex and ran counter to the broad national picture in important respects. Hotspots, or clusters, of PCB residues occurred, but largely only in kestrels. The diet is thought to be the main route of exposure of raptors to PCBs (and other environmental contaminants) and the more frequent occurrence of PCB clusters in kestrels than sparrowhawks is likely to be diet-related. Kestrels mainly take small mammals whereas sparrowhawks feed almost exclusively on small birds, some of which may be migratory (Newton 1986; Village 1990). The home ranges of the small mammals are likely to be smaller than those of avian prey; furthermore, kestrels hunt over smaller (mostly <2 km^2) areas than sparrowhawks (>10 km^2), although this can vary with prey availability (Newton 1986; Village 1990). Because of these factors, it might be expected that localised hotspots of contamination would be more clearly reflected in kestrel than sparrowhawk diet. Hence, spatially resolved data for contamination in kestrels would also be the more likely to reveal clusters than similar data for sparrowhawks. The finding that spatial variation in environmental arsenic concentrations was reflected by regional differences in arsenic residues in kestrels but not sparrowhawks (Erry *et al.* 1999) is consistent with this concept.

The most marked PCB cluster revealed by the spatial analysis was for Merseyside. This was, in fact, the only cluster that was statistically significant in both kestrels and sparrowhawks. The fact that the highest PCB concentrations occurred in individuals from the same location in both species, despite the differences in their diet, strongly suggests that there is an environmental source of PCBs in the Merseyside region that is sufficiently large and/or widespread to have affected multiple food chains. The presence of a cluster in kestrels from south-west Scotland, close to Glasgow, may have been indicative of another localised source associated with regional industrial development. However, the significance of this cluster was equivocal as it was not quite statistically significant in the second analysis of the data.

Figure 6.3: Distribution of common kestrel carcasses recovered between 1993 and 1997 with significant clusters (large black circles) of high liver PCB concentrations (sum of five congeners) indicated. Left-hand graph shows the clusters revealed when the whole dataset was analysed, the right-hand graph the clusters detected when the highest residue values that may have masked other clusters were removed from the dataset (see text for details).

Although there were no other statistically significant clusters in sparrowhawks, two other clusters were apparent for kestrels but were not associated with industrialised regions. The first was on the coast of eastern England and the second on Orkney. Both clusters were robust in that they were detected in both sets of analysis, and may reflect localised sources of contamination. The Orkney cluster was consistent with previous finding that total PCB concentrations in merlin eggs were on average higher on Orkney than from elsewhere in Britain (Newton *et al.* 1999a). The occurrence of a cluster on the coast of eastern England might also be due to the presence in the sample of migrating birds; kestrels migrate to Britain from Sweden (Village 1990). Migrants may have elevated liver PCB concentrations because they have accumulated high PCB burdens prior to migration and/or mobilised PCB-rich fat depots during the migration passage. However, sparrowhawks also partially migrate and would similarly deplete PCB-rich fat depots. Thus, if migration were a major cause of east-coast clusters, they might be expected to be apparent for sparrowhawks as well; no such clusters were apparent in this species.

The specific sources of PCBs to kestrels and sparrowhawks are not known. However, Merseyside, the area with the most marked cluster, contains heavy industries that made substantial use of PCBs (including PCB manufacture and disposal operations and the

electrical sector of the economy). Interestingly, small mammals around a Merseyside landfill have been shown to be contaminated with PCBs (Johnson *et al.* 1996). The source of PCBs in kestrels in other clusters and in individuals of both species (from both urban and rural areas) that have relatively high residues (Figure 6.1) is uncertain. The toxicological significance of the PCB residues in cluster birds is also uncertain and cannot be readily inferred from the current data. This is because the five congeners used to calculate the sum PCB concentrations do not necessarily have the greatest toxic potency compared with other congeners that may occur in smaller amounts. Studies using TEQ data are needed to determine whether toxicity clusters occur and, if so, whether their location mirrors those detected for the five congeners used in the current study.

In conclusion, these preliminary results suggest that (a) significant regional sources of PCBs still exist for UK wildlife some 30 years after use began to be restricted, and (b) are consistent with a previously unidentified role for home range size as a determinant of exposure to pollutants. Spatial analysis of PCB residues top avian predators may be a useful means of identifying hotspots of PCB contamination. However, the identification of clusters in the five-year sample of birds used in this study, although statistically valid, is based on data for a small numbers of individuals. Further studies are needed to determine whether the geographical location of clusters detected in kestrels that died between 1993 and 1997 also occur in birds that died in other years. Such studies on birds that died in earlier years would require analysis of data for total PCBs rather than the sum of the five predominant congeners, as this is the only consistent dataset available over a longer time period.

7. Unknown compounds in predatory birds

7.1. Background and aims

In the previous report on ways that the PBMS might be modified (Shore *et al.* 2002a), total PCBs in the livers of sparrowhawks that died during the 1990s were compared with Aroclor 1254-matched PCB concentrations[2]. This analysis indicated that Aroclor 1254-matched PCB concentrations accounted on average for only approximately 75% of the total PCB concentration. The difference between Aroclor 1254-matched and total PCB concentrations may in part result from congeners in the total PCB values that are not present in Aroclor 1254 but may also be due to other, unknown compounds. The actual identity of these compounds and the potential toxic risk they may pose to birds are uncertain

The aim of the present study was to re-examine gas-chromatograph traces that contained unknown peaks to determine whether there is merit in closer examination and identification of the unknown compounds they represent. The specific objectives were to:

(i) determine which birds were likely to contain large numbers of unknown compounds

(ii) carry out a preliminary assessment of the frequency with which different unknown compounds occurred.

7.2. Methods

The difference between Aroclor 1254-matched and total PCB liver concentrations were quantified (as a % of the total PCB concentration) for sparrowhawks that had been analysed at Monks Wood between 1992 and 1998. Frequency distributions of the data were calculated for birds (Figure 7.1) and individuals within the top 25% of the distributions in each year were selected for further investigation. The use of these selection criteria helped ensure that the birds were likely to contain significant numbers or concentrations of unknown compounds and that selection of birds was independent of overall level of contamination. Archived gas chromatograph data were not available in electronic form for all these individuals, particularly in some of the earlier years, and, in total, data were available for 49 birds.

The electronically-archived gas chromatogram data were re-examined. The number of unknown peaks present, and their associated retention times relative to the internal dichlobenil standard, was recorded for each bird. Unknown peaks were defined as peaks that represented compounds that were not present in Aroclor 1254 nor were one of a suite of organochlorine pesticides (DDT and its metabolites, HEOD, HCB, gamma-hexachlorocyclohexane (g-HCH)) that are routinely determined in these birds.

Subsequent data analysis involved batching the data into three groups defined by the year(s) of analysis: 1992-95, 1996 and 1997-98. This was because there were two changes to analytical instrumentation during the time period and these potentially could have influenced

[2]Aroclor-1254 is a commercial mixture of individual PCB congeners used in some manufactured goods and processes. Matching congeners in tissue samples with those found in the Aroclor mixture is one recognised means of providing a quantitative measure of total PCBs in a sample

the quantification of unknowns. Prior to 1996 samples, ageing electron capture detectors were replaced as part of an equipment maintenance programme. This may have enhanced the sensitivity with which some compounds were detected and potentially resulted in a net increase in the number of unknowns observed on the chromatographic traces. The second change, made just before the 1997 samples were analysed, was of the GC column. This may have altered the absolute retention times of unknown compounds and so peaks with the same retention time in pre- and post-1997 samples may not necessarily represent the same compound.

7.3. Results

The difference between Aroclor 1254-matched and total PCB concentration for all sparrowhawks ranged from 0 to 100%. There was some variation between years but the difference was between 0 and 40% in approximately two-thirds of the birds and higher in the remainder (Figure 7.1).

Analysis of the relationship between the extent of contamination and total PCB concentration in the 49 birds selected for more detailed analysis revealed that there was a significant positive relationship between the total PCB concentration and the number of unknown peaks in the liver (Figure 7.2). This pattern occurred in each of the three time periods that were examined. Overall, it was the most-contaminated birds, as defined by high liver total PCB concentrations, which had the highest number (up to approximately 40) of unknown compounds in the liver.

Birds with high total PCB concentrations, and hence with large numbers of unknown compounds, did not necessarily have the highest % difference between total and Aroclor 1254-matched PCBs, however. High % differences were also recorded in birds with relatively low levels of contamination (Figure 7.3). This was because the PCBs that were present in some relatively uncontaminated birds were not necessarily congeners found in Aroclor 1254. Thus, the % difference between Aroclor 1254-matched and total PCB concentrations could be as high as 100% in these birds but the difference was small in absolute terms and the result of a small number of unknown compounds. Therefore, the % difference between Aroclor 1254-matched and total PCB concentrations was not necessarily a good indicator of the presence of unknown compounds. In contrast, the size of the absolute difference between these two measures was significantly correlated with the number of unknown compounds (Figure 7.4) but the number of unknowns in birds with small differences was highly variable, ranging from 0 to 25 peaks.

The frequency with which each unknown compound, as identified by its retention time, occurred in birds was highly variable (Figure 7.5) and ranged from 3.7% to 85.7%. On average, each compound occurred in 18-28% of birds (range of the median values for the three time periods analysed). The retention times of unknown compounds ranged between approximately 5 and 65 minutes and there was no obvious clustering in the data in terms of the retention times of most peaks or the retention times of the most frequently-occurring peaks (Figure 7.5).

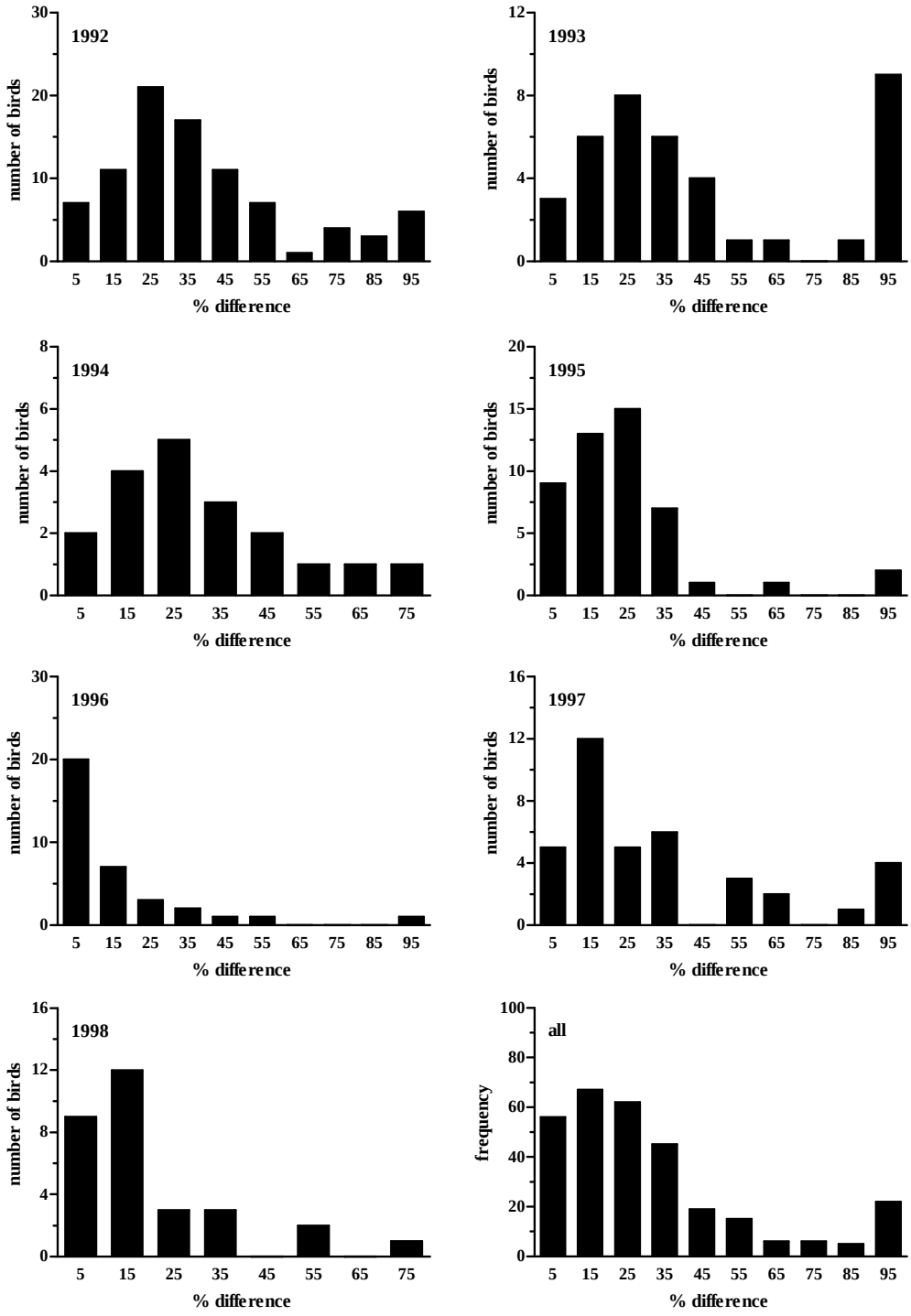

Figure 7.1: Frequency distribution of the difference between the total PCB concentration and the Aroclor 1254-matched PCB concentration (expressed as a % of total PCB concentration) in the livers of Eurasian sparrowhawks for years 1992 to 1998 inclusive.

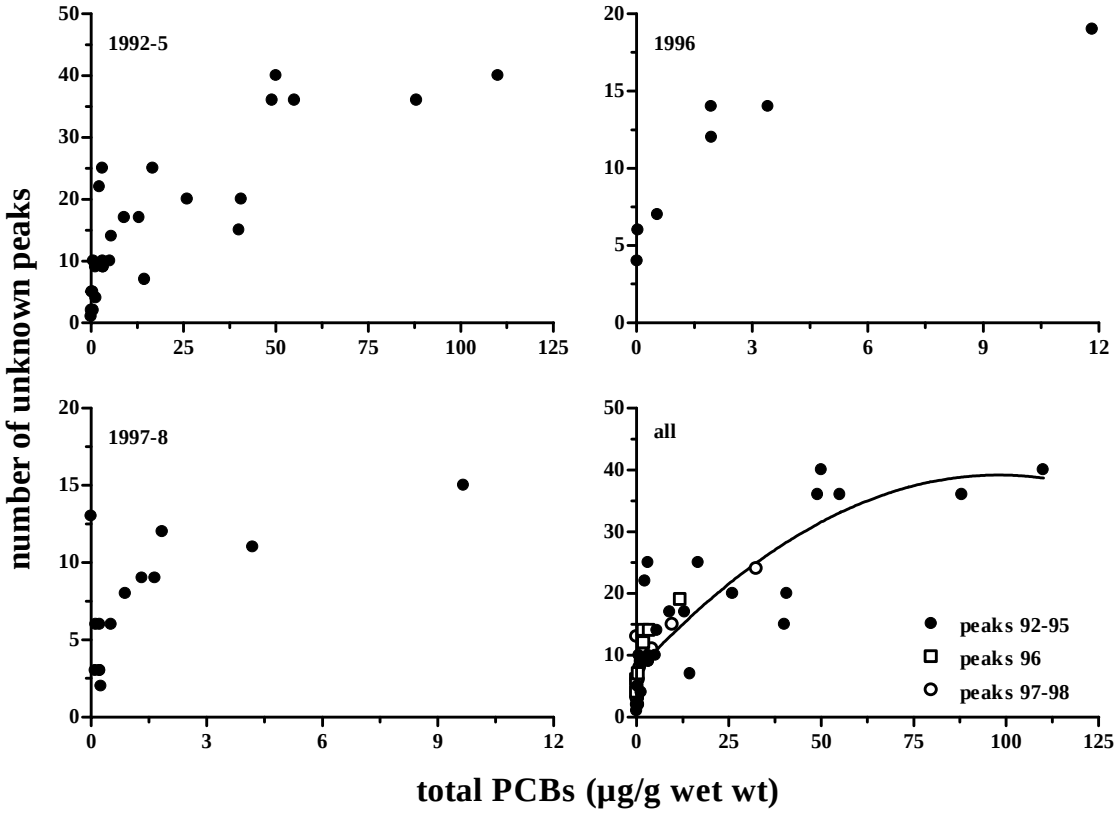

Figure 7.2: Relationship between total PCB concentration and the number of unknown peaks in Eurasian sparrowhawks analysed in different groups of years and over the whole period 1992-98. The regression line for number of unknowns against total PCB concentration (all years combined) is given by the equation $y = 7.46 + 0.648\ x - 0.003\ x^2$ (R^2= 0.745, $F_{(1,46)}$ = 134.7, P<0.001) where x is the total PCB concentration and y is the number of unknowns.

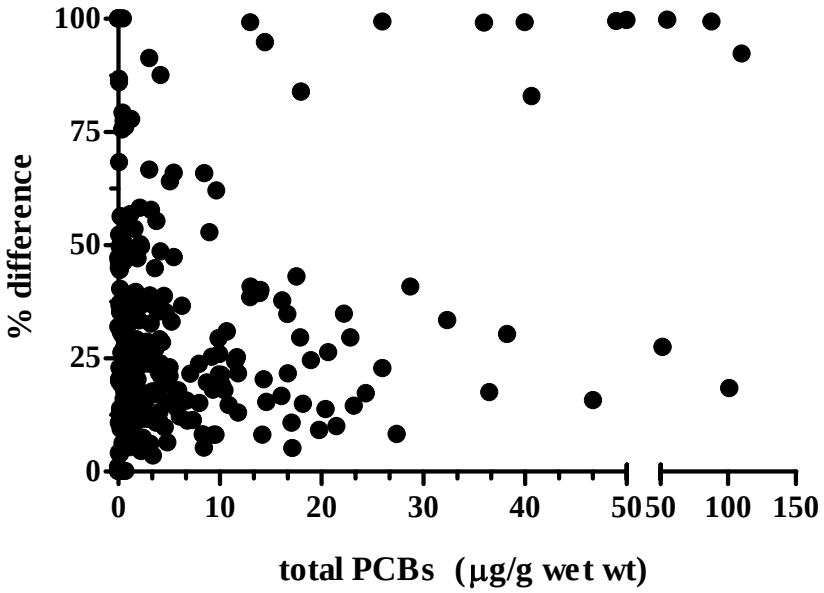

Figure 7.3: Difference between Aroclor 1254-matched and total PCB concentration (expressed as % of total PCB concentration) plotted against total PCB concentration in the livers of Eurasian sparrowhawks that died between 1992 and 1998.

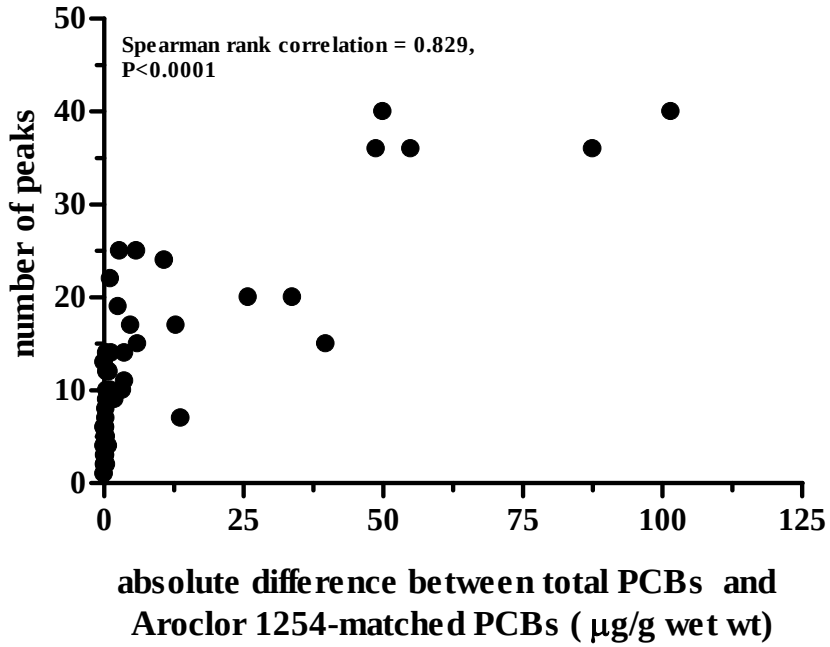

Figure 7.4: The number of unknown compounds (expressed as number of peaks on the gas chromatogram) plotted against the absolute difference between Aroclor 1254-matched and total PCB concentration in the livers of Eurasian sparrowhawks that died between 1992 and 1998.

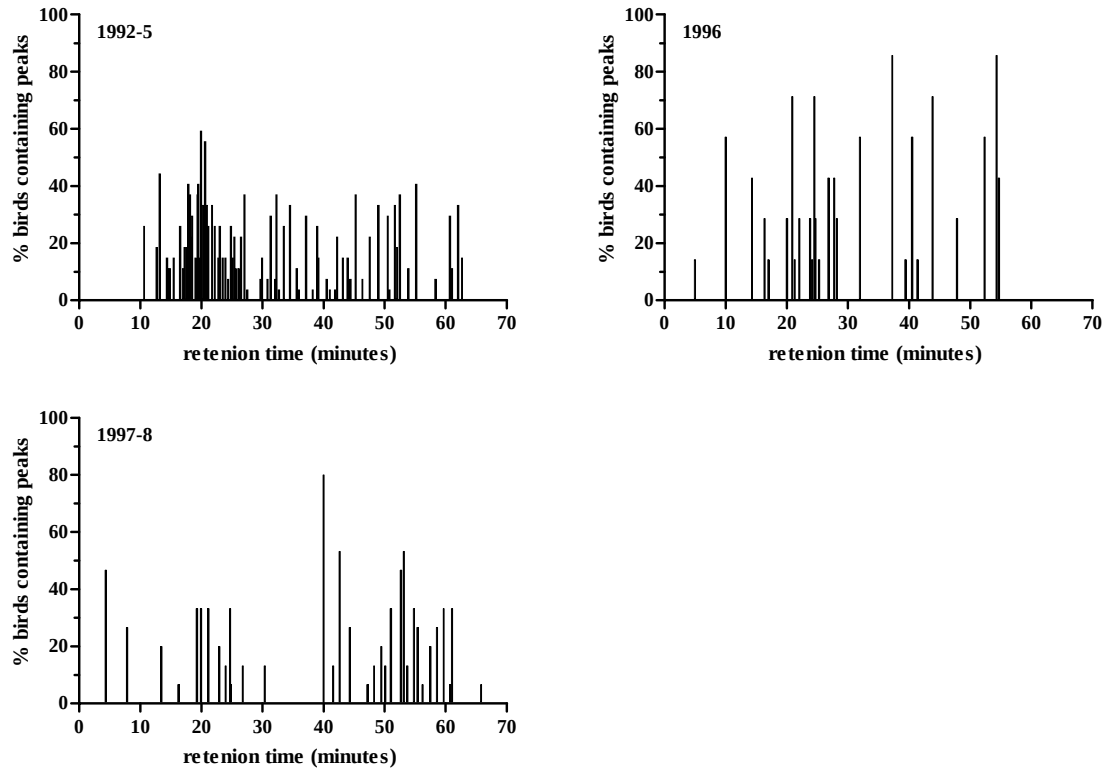

Figure 7.5: Retention times of unknown peaks in a selected sample of Eurasian sparrowhawks, and the % of birds in that sample that contain the peaks. Data are broken down for three year-groups (see text for details) and the sample size for each group are 27 (1992-95), 7 (1996) and 15 (1997-98).

7.4. Discussion

Birds of prey may be exposed to a wide range of organic contaminants. Only some of these contaminants are likely to be detectable by the analytical extraction, clean up and quantification methods currently used for the PBMS. Thus, although it is evident from the present study that large numbers of unknown organic compounds do occur in sparrowhawks (and presumably other predatory birds), the extent to which this occurs has almost certainly been underestimated.

Individual birds that had high concentration of total PCBs also had the highest number of unknown compounds. This may in part occur because a proportion of the unknown compounds are PCB congeners that have not been specifically quantified and may not be present in Aroclor 1254. However, it is also possible that exposure to PCBs and non-PCB compounds occurs simultaneously because there is a common source of exposure, or that individuals with high PCB concentrations also accumulate other compounds because they have poor general capacity to metabolise and eliminate xenobiotics.

This study has identified which individual birds are likely to have the highest number of unknown compounds (that are detectable using current methods) and has quantified the relative frequency with which compounds with different retention times occur. Both pieces of

information are essential for planning how to carry out a study to investigate the identity and toxicological significance of these compounds in birds of prey. In any such study, birds that would be studied would be individuals known to have high liver concentrations of PCBs and most effort would be given to identifying compounds that occur with the greatest frequency generally. The liver tissues from these birds would be analysed by GC-MS and the peaks of unknown compounds compared to library spectra. This would provide a provisional identification of the unknown compounds with an associated estimate of the certainty of that identification. For the compounds that occur most frequently in the birds, and others that may have high toxic potency, the appropriate standards would be purchased and run alongside the tissue extracts so that the ionisation patterns could be compared and the unknown compounds identified with greater certainty.

The likely outcome of any such studies would be to identify compounds with known toxicological properties that occur with the greatest frequency in birds; of the compounds detectable using current methods, it is these that may pose the greatest risk. If appropriate, 'potentially high-risk' compounds might then be incorporated into the PBMS so that temporal trends, species differences and regional variation in exposure can be quantified. Thus, the scope of the PBMS could be increased in a focussed way that sets the priority on toxic organic compounds that are known to occur frequently in birds. However, development of the scheme in this manner would not prevent the incorporation of other compounds that may *currently* occur rarely in birds but about which there are concerns over future exposure scenarios and patterns.

One difficulty with the approach outlined above is that the compounds that are currently seen as unknowns on gas-chromatographic traces are only those that have been successfully captured with the extraction techniques used to extract organochlorine pesticides and PCBs. Thus, discovery of new compounds relies to some extent on serendipity. Clearly, a more rigorous means of finding out which chemicals are present in wildlife is needed, especially as some whole groups of compounds, such as veterinary medicines and pharmaceuticals generally, have hardly been studied from an environmental perspective.

A generalised approach to analysis could be developed to deal with this issue. This could involve the capture of many chemicals and allow broad spectrum 'hunting' for new chemicals. The approach could be an interesting and exciting one combining many new aspects of sample preparation, clean-up and analysis not readily available elsewhere in the UK (see Figure 7.6).

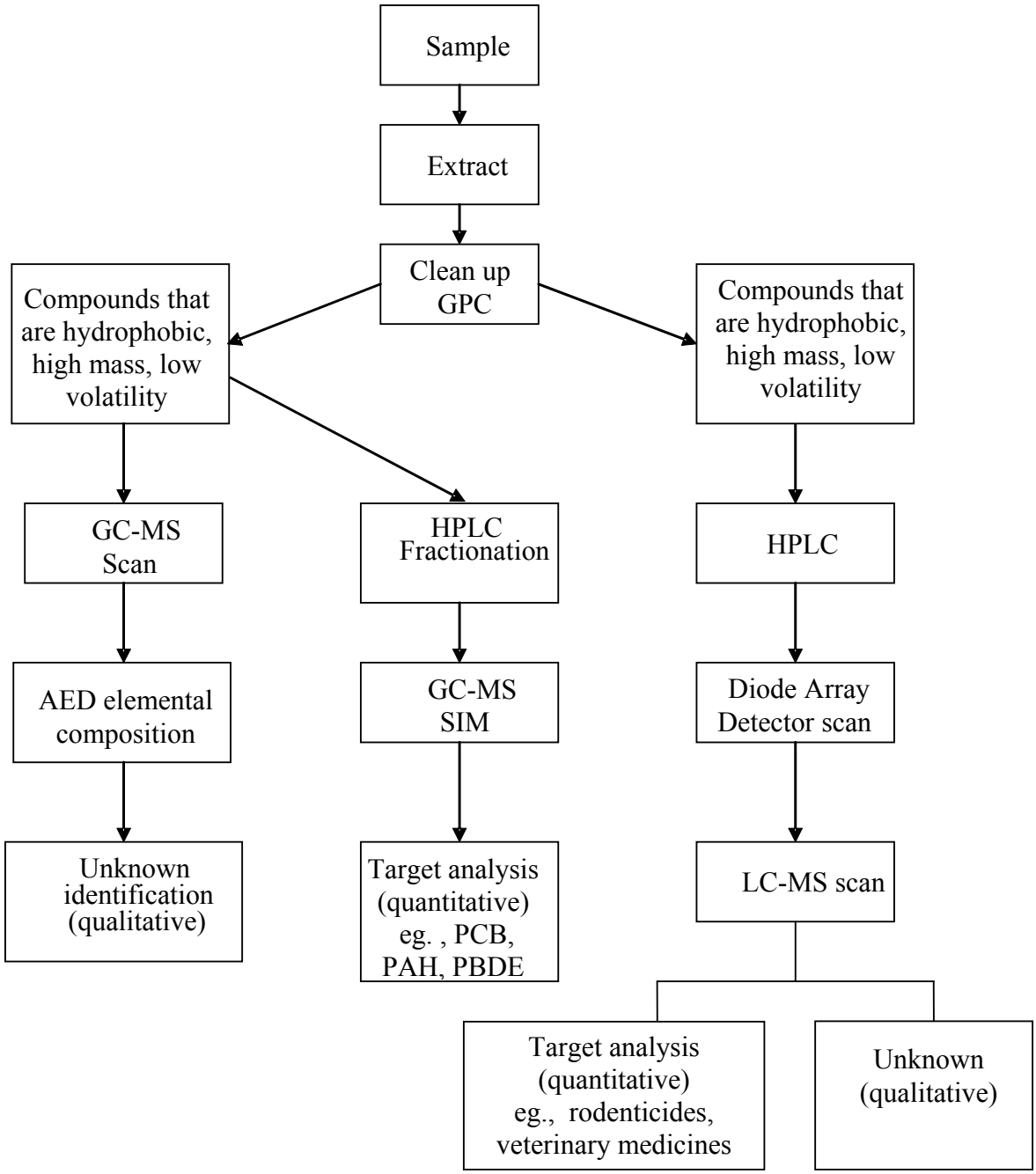

Figure 7.6: Schematic diagram outlining potential generalised analytical procedure for monitoring bird tissues and eggs.
AED: atomic emission detector; GC-MS: gas chromatography-mass spectrometry; GPC: gel permeation chromatography; HPLC: high-pressure liquid chromatography; LC-MS: liquid chromatography-mass spectrometry; SIM: select ion monitoring.

8. Discussion

8.1. Summary of long-term trends in contaminant levels in predatory birds

The most recent review of the long-term data of the PBMS (Shore *et al.* 2005a) confirmed the long-term decline in the concentrations of organochlorine pesticides in the livers and eggs of the predatory birds that are monitored. Liver and egg concentrations of DDE and HEOD have largely levelled off and are not thought to be at levels that are likely to have significant biological effects. High (and possibly toxic) concentrations are still found in occasional samples, however. For species such as the peregrine falcon *Falco peregrinus* and merlin *Falco columbarius*, eggshell indices have now recovered to (peregrine falcon) or are just below (merlin) pre-DDT values. The white-tailed eagle *Haliaeetus albicilla* is the exception to this general pattern; two of the eight eggs analysed to date from birds reintroduced to western Scotland have contained high DDE concentrations.

In contrast to the organochlorine pesticides, there has been no uniform decline in liver and egg PCB concentrations across species. Declines have occurred in some species (such as the heron) but not in many others. The environmental persistence, and possible associated toxic effects in birds, of PCBs is uncertain.

Liver mercury concentrations have undergone a long-term decline in some species, such as the Eurasian sparrowhawk *Accipiter nisus*, common kestrel *Falco tinnunculus* and grey heron *Ardea cinerea*, but there has not been a general pattern of decline in concentrations in the eggs of other species. Mercury concentrations in some eggs have been of similar magnitude to those associated with embryotoxic effects in test species (Shore *et al.* 2005a). Mercury contamination in predatory birds remains a current international concern (for example see Palma *et al*. 2005; Tavares *et al*. 2005) but the toxicological implications are uncertain, partly because residues are often measured as total mercury (as in the PBMS) and the more toxic organomercury fraction is not distinguished from the far less toxic inorganic fraction.

The latest review of the PBMS (Shore *et al*. 2005a) also confirmed that the proportion of barn owls containing second-generation anticoagulant rodenticides has risen to approximately 40%. Other studies have demonstrated widespread exposure in a variety of predatory birds and mammals, and non-target exposure to anticoagulant rodenticides is a growing area of conservation concern.

8.2. Recommendations for future monitoring – compounds to be monitored

8.2.1. Anticoagulant rodenticides

Given the major wildlife concerns over anticoagulant rodenticides, monitoring for these compounds is currently of high conservation value and remains a priority. We recommend that monitoring of barn owls should remain at the current level. In fact, recent analytical developments that involve transferring quantification techniques to a mass spectrometry basis means that residues of some first-generation anticoagulant rodenticides, such as warfarin and

coumatetralyl, are also likely to be reported for barn owls in future years. This will help provide a more complete picture of possible risk posed by anticoagulant rodenticides generally.

The recent widening of the PBMS monitoring to incorporate the analysis of up to 30 kestrels per year for second-generation rodenticides further reflects the importance of this issue, and concerns over recent findings that an unexpectedly high proportion of kestrels may be exposed to rodenticides (Shore *et al.* 2001b). The first set of birds to be analysed are those collected in 2001 (Shore *et al*. 2005b). The costs of the additional rodenticide monitoring has been partly met by not analysing any samples collected in 2001 for mercury, although this is equivalent to only approximately one-third of the analytical cost associated with the rodenticide analysis. The remaining resource has been provided as part of CEH's collaborative input to the programme and has been met from its core science funding.

8.2.2. PCBs (and organochlorine pesticides)

Because there are major uncertainties about long-term trends, toxicity and spatial variation in PCBs in wildlife, we believe there is a strong argument for retaining monitoring of these compounds. Our ability to now identify hotspots of contamination (Section 6) will allow us to focus attention in future years on potential problem areas and regions. The lack of evidence for rapid changes in concentrations per year at a national scale suggests, however, that the intensity of monitoring can probably be reduced. The case for retaining the monitoring of organochlorine pesticides is much weaker, apart from in species such as white-tailed eagle. However, because chemical analysis of organochlorine pesticides and PCBs are carried out simultaneously in the same sample, organochlorine pesticide residues will be quantified and reported along with PCB residues, although this is not the main purpose of this monitoring. We recommend that monitoring of PCB residues in the livers and eggs of predatory birds is maintained, but at a reduced level so that resources are freed for other monitoring purposes (see Section 8.3 below), and that PCB concentrations are reported as congener concentrations and TEQs as well as total PCBs.

Given continuing concerns over the breeding success of white-tailed eagles, we would recommend that the current monitoring of organochlorine pesticide and PCBs in eggs should continue.

8.2.3. PBDEs and PAHs

The potential for analysing these compounds has been discussed in Section 4 of the present report. Both sets of compounds can feasibly be incorporated into the monitoring of the PBMS and would provide the first information on long-term trends in resides and potential toxic effects in top predators in Britain. If such monitoring were to be carried out, we would recommend that sparrowhawk and heron livers (representative of terrestrial and freshwater environments) and merlin, golden eagle and gannet eggs (representative of terrestrial and marine environments) be monitored for these compounds in the first instance. Data should be reviewed after the first three years to determine whether the selected species are suitable biomonitors for these compounds. This monitoring can be achieved within current resources of the PBMS if the sampling strategy is revised (see Section 8.3 below). Initial investigations in collaboration with the University of Lancaster are underway to determine whether current analytical procedures used in the PBMS are broadly suitable for PBDE analysis.

8.2.4. Mercury and other metals

Monitoring of mercury concentrations in tissues and eggs was dropped for samples collected in 2001 but concerns over long-term trends and possible effects remain. Arguably, reinstatement of mercury monitoring in tissues and eggs on a reduced sampling basis (see Section 8.3 below) would be beneficial, particularly if the organomercury fraction was quantified; this would be of particular relevance to aquatic species. The costs of quantifying total mercury in the numbers of samples collected under a reduced sampling strategy would be minor (approximately £700 per year). The resources required for quantification of organomercury concentrations are uncertain at present as an analytical procedure has yet to be established in the Monks Wood laboratory.

Cadmium and lead are other toxic metals that occur widely in wildlife and it was recommended previously that analysis of these metals as part of the PBMS was feasible (Shore *et al.* 2002a). Because of the inputs of lead into the environment as lead shot and fishing weights, there have been particular concerns over lead levels in birds, including predatory birds (Pain *et al.* 1995). Analysis of these elements requires the same sample preparation as for mercury. Therefore, it would be possible and relatively inexpensive (*c.* £300 per year) to monitor for these two additional elements in samples that are analysed for mercury. Thus, all three elements could be incorporated into a PBMS for approximately an additional £1K per year and would provide information on current levels that can be assessed against a large body of information available on the toxic effects associated with such residues.

We would recommend that total mercury, lead and cadmium levels in tissues and eggs should be monitored by the PBMS if the small additional resources can be found. The possibilities for incorporating analysis of organomercury, particularly in aquatic birds, merit further exploration.

8.3. Recommendations for future monitoring – sampling and analysis strategy

Given the importance of anticoagulant rodenticide monitoring, it is recommended that the sampling and analysis strategy for rodenticides in barn owls and kestrels are not changed. The remaining discussion in this Section (8.3) refers only to liver and egg samples collected for the analysis of PCBs and associated compounds.

The analysis of sampling frequency carried out in Section 3 of the present report pointed the way in which the scope of the PBMS can most readily be widened to include other organic contaminants. The strategy would be to maintain annual sampling of tissues and eggs but to reduce the number of samples that are chemically analysed each year for PCBs and organochlorine pesticides. The remaining samples can be archived or used for other analyses. By maintaining a programme of annual sample collection, the continuity of sampling, which relies upon a volunteer network, will be maintained and all volunteers will continue to receive information on the findings of the PBMS. This feedback of information is considered vital for the maintenance of the collection network (Shore *et al.* 2002a) and its importance cannot be over-emphasised.

8.3.1. Sparrowhawk and heron livers

The largest single saving that can readily be achieved is to reduce the analysis of sparrowhawk and heron livers by up to two-thirds from the currently-commissioned 60 down to 20 per year. Every third sample to arrive at Monks Wood would be analysed, the others archived. Overall, it would be expected that some 10-15 sparrowhawks per year and 5-10 herons per year would be analysed. This sampling strategy has essentially the same impact on resources as reducing monitoring of sparrowhawks and herons from an annual to a triennial basis but has better statistical power (see Section 3).

The resultant savings in analytical costs could be used to monitor the livers for PBDEs (see Section 4) and to report PCB concentrations on a congener and TEQ as well as a total PCB basis (see Section 5). If PBDEs were not to be monitored, the resource savings might be used to provide funding flexibility within the PBMS so as to enhance the ability to initiate new monitoring or specific one-off investigative studies.

8.3.2. Eggs

In contrast to the strategy for livers, there is little scope for reducing the intra-year replication for analysis of merlin, golden eagle and gannet eggs. This is because few eggs of each species are analysed each year and there is relatively large variation in residue magnitude between eggs because of regional differences in exposure (Newton *et al.* 1999a; Newton & Galbraith 1991; Newton *et al.* 1990). There are three alternatives strategies:

i. monitoring could continue as present with no saving on resources.

ii. all eggs received in any one year would be analysed but this would only be done every third year; eggs collected in intervening years would be archived (in deep freeze) for possible future analysis. It is anticipated that, given recent developmental work in the Monks Wood analytical laboratory, it would be possible to report organochlorine pesticide, PCB, PAH and PBDE concentrations in eggs collected every third year for the same cost that is currently incurred by annual analysis of eggs for organochlorine pesticides and PCBs.

iii. This strategy of increasing the number of compounds determined but at reduced sampling intensity would dramatically widen the breadth of the PBMS. The concomitant disadvantage would be that the speed and sensitivity with which long-term environmental trends are detected would be poorer than if monitoring was annual (see Section 3). If the aim were to monitor trends in some compounds with as much precision as possible, annual monitoring would be more appropriate. This could be achieved *post-hoc* if the need for more detailed monitoring became apparent after triennial monitoring was begun as samples archived in intervening years could be analysed.

iv. monitoring organochlorine and PCB concentrations in eggs could be terminated, and the savings switched to other analyses. However, long-term trends in PCB concentrations remain uncertain, and levels in eggs from some areas are a cause for concern (Shore *et al.* 2005a).

8.3.3. Recommendation on sampling and analysis strategies for sparrowhawk and heron livers and for eggs

If it is assumed that inclusion of PAHs and PBDEs in the PBMS is desirable, we would recommend that **sampling** of livers and eggs is maintained on an annual basis but that **analysis** is carried out on a triennial basis (eggs) or equivalent (every third liver sample analysed). Analysis would include PCBs (congener, TEQ, total concentration), PBDEs, PAHs and organochlorine pesticides. These changes would be cost-neutral within the current resources of the PBMS

We would also recommend that these samples are analysed for mercury, lead and cadmium if additional small-scale resources are available.

8.4. Additional studies

Section 6 has highlighted the approaches that can be used to examine spatial variation in residues and identify hotspots of contamination. This approach needs to be extended for PCBs to look at total PCB concentrations over a longer time period (to examine the permanence of hotspots) and to TEQ data to see if TEQ clusters occur and, if so, whether they coincide with those for total PCBs. This approach to spatial analysis can also be extended to other contaminants, such as rodenticides.

Section 7 has described the extent to which unknown compounds occur in predatory birds and has outlined the procedure that could be followed to identify the most frequently-occurring unknown compounds.

We recommend that both sets of studies are pursued as they will provide much better quantification of hazard and risk and insights into ways risk may be mitigated. The revised sampling structure outlined in Section 8.3 above does not have sufficient funding to support such studies. Additional funding would be needed to progress these projects.

8.5. Summary of recommendations

An overall revised strategy for continued monitoring of anticoagulant rodenticides and for monitoring PCBs, PBDEs, PAHs, organochlorine pesticides and heavy metals on the basis of triennial sampling or its equivalent is summarised in Table 8.1. We recommend this as a feasible means of extending the scope of the PBMS, and concomitantly increasing its relevance and flexibility. The proposed changes to the core monitoring would be cost-neutral in terms of the current levels of resourcing, apart from the minor additional costs associated with the monitoring for heavy metals. Additional specific investigative studies, such as detailed analysis of spatial variation in residues and investigation of unknown compounds (see Sections 6 and 7), would require dedicated resources.

Table 8.1: Current and suggested future monitoring for the PBMS

Current monitoring	Species	Tissue type	Current monitoring	Suggested future monitoring	Funding required
Second-generation rodenticides	Barn owl	liver	30	30	Within current resources
	Common kestrel	liver	30	30	
	Red kite	liver	up to 5/yr	up to 5/yr	
g-HCH, DDE, HEOD total PCBs	Eurasian sparrowhawk/ Grey heron	liver	60/yr	20/yr	Within current resources
PBDEs, PCB congeners and TEQs,	Eurasian sparrowhawk/ Grey heron	liver	–	20/yr	Within current resources
DDE, HEOD, total PCBs	Merlin Golden eagle Northern gannet	egg	20-40/yr	20-40/3-yr	Within current resources
PBDEs, PAHs, PCB congeners and TEQs	Merlin Golden eagle Northern gannet	egg	–	20-40/3-yr	Within current resources
DDE, HEOD, total PCBs	White-tailed eagle	egg	1-5/yr	1-5/yr	Within current resources
PBDEs, PAHs, PCB congeners and TEQs	White-tailed eagle	egg	–	1-5/yr	Within current resources
mercury lead cadmium	Eurasian sparrowhawk/ Grey heron	liver	–	20/yr	Additional £1K / yr
	Merlin Golden eagle Northern gannet	egg	–	20-40/3-yr	
Spatial monitoring Identification of unknowns	Various	various	–	–	Additional funding required

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Appendix 1: Abbreviations used in the text

AED	atomic emission detector
Ah	arylhydrocarbon
CEFAS	Centre for Environment, Fisheries and Aquaculture Science
CEH	Centre for Ecology and Hydrology
CNS	Central Nervous System
Defra	Department for Environment, Food and Rural Affairs
DDE	dichlorodiphenyldichloroethylene
DDT	dichlorodiphenyltrichloroethane
EU	European Union
GAM	Geographical Analysis Machine
GB	Great Britain (England, Scotland and Wales, excluding Northern Ireland)
GC	gas chromatography
GC-ECD	gas chromatography with electron-capture-detection
GC-MS	gas chromatography with mass spectrometry
GIS	geographic information system
GPC	gel permeation chromatography
HCB	hexachlorobenzene
g-HCH	gamma-hexachlorocyclohexane
HEOD	1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4á,5,6,7,8,8á-octahydro-1,4-endo,exo-5,8-dimethanonaphthalene [dieldrin]
HPLC	high-pressure liquid chromatography
LC-MS	liquid chromatography–mass spectrometry
JNCC	Joint Nature Conservation Committee
MC	Monte Carlo
NCC	Nature Conservancy Council
OC	organochlorine
PAH	polycyclic aromatic hydrocarbons
PBDE	polybrominated diphenyl ethers
PBMS	Predatory Bird Monitoring Scheme
PCB	polychlorinated biphenyls
PCDD	polychlorinated dibenzodioxins
PCDF	polychlorinated dibenzofurans
SIM	select ion monitoring
TCDD	2,3,7,8-tetrachlorodibenzo-p-dioxin
TEF	Toxic Equivalency Factor
TEQ	Toxic Equivalent
T4	thyroxine
UK	United Kingdom (England, Scotland, Wales and Northern Ireland)
UV	ultra violet
WHO	World Health Organization
WIIS	Wildlife Incident Investigation Scheme