Organohalogen chemicals in human blood from the United Kingdom

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Abstract

Blood serum from 154 volunteers at 13 UK locations in 2003 were analysed for a range of PCBs, organochlorine pesticides and PBDEs. HCB, *p,p'*-DDE and *p,p'*-DDT and β-HCH were the dominant organochlorine pesticides in most samples. BDEs 47, 99, 100, 153, 154 and 183 were the most regularly detected PBDEs. This study is the first report of BDE209 in UK human blood (found in 11 samples, range <15-240 ng/g lipid). Concentration and age correlated for the less easily metabolised PCBs, *p,p'*-DDT and *p,p'*-DDE, HCB and HCHs. With increasing age females tended to have lower concentrations of the more chlorinated PCBs than males. Similar PBDE concentrations, and distributions, to those reported in the general population in Sweden in 2002 were found, despite differences in historical PBDE production and usage. There is increasing regulation to control persistent and bioaccumulative chemicals, and establishing human exposure will help to identify substances which should be urgently phased out.

Capsule: A survey of PBDE, PCB and OC pesticide concentrations in human blood helps identify baseline concentrations in the UK population and found decabromodiphenyl ether in non-occupationally exposed individuals.

KEYWORDS: Polychlorinated biphenyl, chlorinated pesticides, polybrominated diphenyl ether, brominated flame retardants, serum

Introduction

Humans are exposed to man-made chemicals from a variety of environmental sources. Non-occupational exposure of the general human population to a range of man-made lipophilic organic chemicals, such as those classed as 'persistent organic pollutants' (POPs), and similar chemicals, has been of increasing concern over the last several decades. This concern is based on the realisation that chemicals with these properties can have serious effects in wildlife and humans, and moreover, that once such effects become apparent, ongoing exposure cannot be prevented in the short term. Concern is also linked to the realisation that many chemicals have been inadequately tested for their safety and the increasing sophistication of toxicology. In particular, there is increasing evidence that environmentally relevant concentrations of some chemicals may have measurable toxic (e.g. endocrine effects) and genotoxic effects in humans and other animals (Golden et al., 1998; Oberdörster and Cheek, 2000; Legler and Brouwer, 2003; Welshons et al., 2003; Kalantzi et al., 2004a; Kalantzi et al., 2004b). This concern is also fed by the ability to demonstrate widespread contamination because of the increasing sophistication and sensitivity of analytical techniques and instruments.

Some organochlorine pesticides (OCPs) were banned or restricted in North America and Europe in the 1970s, but some, such as DDT and HCHs continue to be used in some countries. After widespread commercial and industrial use in a great variety of products, the production of polychlorinated biphenyls (PCBs) was banned in many countries in the 1970s.

However, PCBs are still in service in capacitors and transformers in many countries, although the Stockholm Convention contains provisions to help ensure the safe disposal of existing stocks, and requires that countries aim to eliminate discharges of PCBs formed as by-products. The persistence of PCBs and other POPs means that they are still present, and mobile, in the environment, despite the international restrictions agreed under the Stockholm Convention.

Two of the three main polybrominated diphenyl ether (PBDE) commercial mixtures (known as pentaBDE and octaBDE) were banned from the European market in 2004. These products are now also facing bans in several states in the US, and will be removed from the North American market by 2008. However, many products containing these chemicals are still in use, and many end their useful lives in land-fill sites, from where PBDEs may be able to enter the wider environment. Strict furniture fire resistance laws in the UK and some states of the USA (e.g. California) have meant that pentaBDE was probably used more extensively in polyurethane foam products in these countries. A third commercial BDE mixture, decabromodiphenyl ether (decaBDE), is used in high-impact polystyrene for electronic enclosures and as a flame retardant in upholstery textiles (Hardy, 2002) at an estimated rate of 56,100 tonnes, in 2001, worldwide (Voorspoels et al., 2003).

The general human population is predominantly exposed to PCBs and OCPs through foodstuffs (Duarte-Davidson and Jones, 1994) but a significant proportion of certain PBDE mixtures may enter humans through the inhalation of dust or vapour-phase PBDEs in the indoor environment (Bergman et al., 1997; Sjödin et al., 2001; Wilford et al., 2004). Human exposure to these chemicals has generally been investigated by analysing the chemicals of interest in a variety of tissue samples. This gives what could be viewed, simplistically, as an ‘integrated’ view of the exposure of the subject, since lipophilic chemicals are predominantly stored in the lipid deposits of the body, found throughout most tissue types. However, the

mechanisms of uptake and storage of these chemicals are not well understood, and indeed neither the mechanism, nor the efficiency, of absorption of these chemicals across the human gastro-intestinal tract has been characterised to a satisfactory degree (Kelly et al., 2004). There are many complicating factors which impact on the chemical concentrations measured in the tissue of a subject, including: individual biological variation; temporal exposure pattern (e.g. sporadic, evenly distributed, or following a smoothly changing curve over the subject's life); lifestyle and personal factors (e.g. age, children carried and breastfed, habits and diet, current body-mass index and changes in this over time). The differences in chemical behaviour in people with only slight differences in the characteristics outlined above can be appreciated from reports of both net absorption and net excretion of individual PCB congeners in adult male humans under similar conditions (e.g. Juan et al., 2002).

Man-made lipophilic chemicals are present in all tissue types that have been investigated, and it has been shown that, with the general exception of the brain, liver and kidneys, different tissues contain similar concentration of these chemicals, when expressed on a fat weight basis (Mussalo-Rauhamaa, 1991; Schecter et al., 1991; Schecter et al., 1998; Wingfors et al., 1998; Aylward et al., 2003).

A range of study designs has been used to investigate chemical concentrations in human populations in the past, and these have used a variety of sample media, including: tissues from cadavers (Duarte-Davidson et al., 1994b; Quintana et al., 2004); milk from nursing mothers (Schoula et al., 1996; Harris et al., 1999a); blood (Mussalo-Rauhamaa, 1991; Wingfors et al., 1998); hair (Tsatsakis and Tutudaki, 2004); and adipose and other tissues by biopsy or from resectional surgery (Covaci et al., 2002a; Quintana et al., 2004). Sampling human tissues gives rise to issues related to patient care and ethical considerations, and each tissue which can be sampled has advantages and disadvantages. Blood has distinct advantages over other tissues for survey studies because it is relatively easy to sample. It is also relatively easy to get

ethical approval and to recruit volunteers. Blood can be taken from people of all backgrounds and ages and, importantly, from both sexes, and does not necessarily preclude or augment the inclusion of volunteers with illnesses. In taking blood samples, however, care should be taken to exclude the effects of recent meals on chemical concentrations in the blood. In addition, the low lipid concentration, and complex matrix, can lead to difficulty in achieving sufficient analytical sensitivity.

It is important to have knowledge of the distribution of pollutant concentrations within a population, so that changes can be monitored, and comparisons drawn. In Europe and North America concentrations of PCBs and OCPs in human tissues have generally been declining since the 1970s (Norén and Meironyté, 2000; Sjödin et al., 2004). Concentrations of PBDEs in human tissues are currently changing in different ways in different regions - for example, it appears that PBDE concentrations are reaching a plateau in Sweden, but may still be rising in North America (Ryan et al., 2002; Sjödin et al., 2004). Concentrations of PBDEs found in human milk from the UK have recently been reported to be substantially lower than concentrations found in the USA, but somewhat higher than Finland, Japan and Sweden (Kalantzi et al., 2004c). We have also found statistically different concentrations of PCBs, OCPs and BDE47 in human milk samples taken between 2001 and 2003 in two regions of the UK (Kalantzi et al., 2004c).

In this study we determined PCBs, OC pesticides and PBDEs in blood from 154 people, in 13 cities and towns, in the UK. The volunteers represented a wide range of ages, socio-economic backgrounds, occupations, and lifestyle patterns. A range of lifestyle, occupation and personal factors may influence chemical exposure and concentrations in an individual, so the responses to questions on these parameters were investigated.

Materials and Methods

Methodological tests

Before samples were taken for the survey a range of methodological tests were performed to optimise the study design. In order to determine the optimum amount of blood required for analysis of the target compounds a large (approx. 80 g) blood serum sample was prepared for analysis using the analytical methods detailed below. To determine the analytical variability, and whether the preferred sampling medium should be plasma or serum, one volunteer gave three samples of serum and three of plasma. Procedural blanks, consisting of blood collection tubes (Vacutainers®) filled with purified water, were treated and analysed as samples, to investigate whether the collection tubes contaminated the samples with the chemicals of interest. Analytical blanks consisted of purified water.

There was no statistical difference, for any chemical group or individual chemical, between serum and plasma, on either a whole weight or an extracted lipid weight basis, so either could be chosen as the sampling medium. However, the plasma BD Vacutainers®, treated with lithium heparin, showed detectable concentrations of PCBs, whereas the serum BD Vacutainers® showed no detectable concentrations of the chemicals of interest. Therefore, serum was chosen as the sampling medium. From the results of the large blood sample, 10 g was chosen as the practical amount of serum to extract and prepare for analysis, which allowed the dominant constituents of each chemical class (CBs 28, 74, 118, 153, 180, 194; p,p'-DDT; p,p'-DDE; HCB; β-HCH; BDEs 47, 99, 153 - each PCB or PBDE congener representing a halogenation level) to be reliably detected (at least 10 times the standard deviation of the blank, or 3 times the detection limit, in the absence of blank concentrations). The analytical variability, based on concentrations expressed per g lipid in serum, was close to 30% (RSD - relative standard deviation) for all analytes.

Choice of volunteers and ethical approval

154 volunteers provided samples, between 9 and 19 (median 11) volunteers from each of 13 locations in the UK. The volunteers were sampled in Belfast, Birmingham, Cardiff, Edinburgh, Exeter, Godalming, Huntingdon, Leeds, London, Manchester, Newcastle-upon-Tyne, Nottingham and Winchester (see Figure 1 for locations), however, they did not necessarily live in these towns. Of the volunteers, 50 were male and 104 female and the median age was 40.5 (range 22 to 80). The body mass index (BMI) ranged between 17.7 and 42.8 (median 23.4). 1 volunteer was vegan, 19 vegetarian, 23 predominantly vegetarians who also ate fish, and 110 were omnivores. 54 of the female volunteers had not had a child, 11 had one, 18 had 2 children, 10 had 3, and 6 had 4 or more children. The majority of women breastfed all of their children.

The only selection criteria for volunteers were that they were over 18 years old, weren't knowingly pregnant or had any illness or heart condition that might increase the risks associated with having a sample of blood taken. The volunteers were sampled during the day, mostly on week days, which might have selected against people at work. No claim is made about the representativeness of the sample to the general adult population, but the higher proportion of women and vegetarians sampled might suggest that the self-selecting group is not truly representative.

Sample collection

Blood samples were taken by medical professionals in March, June and July 2003. 40 ml whole blood was taken from each volunteer. Volunteers were asked not to take a meal, or drink any milk, for at least several hours before the samples were taken so that their blood-lipid chemical concentrations should represent their 'stable' levels (unaffected by recent ingestion). Blood samples were taken into BD Vacutainer® serum tubes (with clotting agent and polymer separator). Samples were mixed by inverting the tubes several times immediately after blood collection, and, after 10-15 minutes, centrifuged at 3000 rpm for 10

min, to separate the serum from the blood cells. Samples were immediately frozen and transported to the laboratory. All samples were still frozen upon receipt at the laboratory, where they were defrosted and the serum transferred to clean glass containers before being frozen at –20 °C until analysis.

Analysis

Sample analysis was performed at Lancaster University using methods based on those developed by Hovander *et al.* (2000), briefly entailing denaturation of the sample with hydrochloric acid and propan-2-ol, followed by extraction with a hexane:MTBE mixture. Samples were then cleaned using concentrated sulphuric acid, followed by gel permeation chromatography (Biobeads S-X3), before the addition of internal standards (CB30, $^{13}C_{12}$-labelled CB141 and $^{13}C_{12}$-labelled CB208), final volume reduction, and analysis by GC-MS. Samples were analysed for 43 PCBs (CBs 18, 22, 28, 31, 41/64, 44, 49, 52, 54, 70, 74, 87, 90/101, 95, 99, 104, 105, 110, 114, 118, 123, 138, 141, 149, 151, 153, 155, 156, 157, 158, 167, 170, 174, 180, 183, 187, 188, 189, 194, 199 and 203) and 12 organochlorine pesticides (α- and γ-chlordane, HCB, *o,p'*- and *p,p'*-DDD, *o,p'*- and *p,p'*-DDE, *o,p'*- and *p,p'*-DDT, and α-, β- and γ-HCH) using a Finnigan TRACE GC- MS. The gas chromatograph used splitless injection and was fitted with a 50 m CPSil8 capillary column. The mass spectrometer used an electron impact (EI+) source in selected ion monitoring (SIM) mode, monitoring the relevant molecular ion m/z ratios for PCBs and dominant fragments for OCPs.

21 PBDEs (BDEs 17, 28, 32, 35, 37, 47, 49, 71, 75, 77, 85, 99, 100, 119, 138, 153, 154, 166, 181, 183 and 190) were analysed using a Fisons MD800 GC-MS. The GC used splitless injection and was fitted with a 30 m DB5 capillary column. The MS used a negative ion chemical ionisation (NICI) source in SIM mode, and used ammonia as the reagent gas, monitoring m/z ratios 79 and 81 for the PBDEs.

BDE209 was analysed using a Micromass Autospec Ultima GC-HRMS in EI mode, using a resolution of at least 10,000, tuned using perfluorokerosene (PFK). The GC used cool on-column injection and was fitted with a 15 m DB5 capillary column. The MS used an EI+ source in selected ion monitoring (SIM) mode, monitoring m/z ratios 797.3355 and 799.3334 for BDE209 and m/z ratios 809.3757 and 811.3737 for ^{13}C-labelled BDE209.

Quality control

PCBs and OCPs were analysed using CB30 and ^{13}C-CB141 as internal standards, while PBDEs were analysed using ^{13}C-CB141 and ^{13}C-CB208 as internal standards. All samples were spiked with ^{13}C-labelled recovery standards (6 ng total), in 25 µl acetone, before extraction. The standards included $^{13}C_{12}$-labelled CBs 28, 52, 101, 138, 153, 180 and 209 and $^{13}C_{12}$-labelled BDE209. Concentrations were not corrected for the recoveries of these standards, but the recoveries were monitored to assess the effectiveness of the analytical methods. ^{13}C-labelled PCB recoveries averaged 79-84 % and ^{13}C-labelled BDE209 recovery averaged 107%.

One laboratory blank, consisting of purified water, was analysed for every 10 samples analysed, and average blank concentrations were subtracted from the concentrations found in each sample before application of the detection limit.

Control samples, consisting of the BD Vacutainer® tubes used for sample collection were prepared using purified water. After drawing the water into the tube the water was decanted and treated as a normal sample for analysis. One control sample was prepared for each 25 blood samples analysed.

One in-house reference material, consisting of an equivalent amount of homogenised seal blubber lipid to that which was normally extracted from a blood sample, was analysed for each 25 blood samples. Concentrations of selected analytes were compared to a control chart based on previous analyses of the reference material. They did not exceed the specified upper

or lower action limit concentrations, which were defined as the mean ± 3 times the standard deviation.

Detection limits

For chemicals detected in the blank samples the method detection limit was defined as three times the standard deviation of the blank value. In the absence of detectable concentrations in the blank samples the method detection limit was defined as the instrument detection limit. Method detection limits were typically (in ng/g lipid) 0.6 for PCBs, 0.9 for OCPs, 0.2 for PBDEs (excluding BDE209) and 36 for BDE209. The relatively high detection limit obtained for BDE209 is due to a combination of the instrument detection limit and the concentrations which were found in the analytical blanks.

Questionnaire

A questionnaire, completed by each volunteer, included a range of questions relating to personal characteristics, habits, lifestyle and their home. The questionnaire results were transferred into a spreadsheet, and, where appropriate, processed and categorised to a small number of discrete bands. Questionnaire data used in the statistical analysis included: age; gender; body mass index (BMI); current weight trend (gaining/losing/stable); sampling location (i.e. place of main residence); age of main residence; type of area resided in (urban/rural); areas previously resided in; visits to malarial countries; new furniture/carpet purchases (in the last decade); general diet (omnivore, vegetarian, vegan, fish consumption); consumption of organic foodstuffs; number of children carried; number of children breastfed; main occupation type; computer usage (hours per day).

The entire data-set, formed by the chemical concentration data and the questionnaire data, was analysed using the ‘SPSS’ statistical software package.

Results and discussion

The chemical concentrations found in the serum samples are summarised in Table 1. There is evidence, as expected in biological samples, that the concentrations do not conform to a Normal distribution (see later discussion), so the data has been summarised using the median, ranges and quartile values. It should be noted that the LOD is calculated by dividing the method detection limit (ng or pg per sample) by the weight of sample analysed, and is therefore different for each sample. Values below the LOD are treated as being zero for calculating total chemical group concentrations (i.e. 'Total PCB' etc.).

We believe this study represents the most comprehensive survey of organohalogen chemicals in humans in the UK in at least the last 10 years, and includes the first report of PBDEs in human blood serum samples from the general population in the UK.

PCB, organochlorine pesticides and PCDD/F concentrations have been shown to be similar, when expressed as concentration per g lipid, in human adipose and blood serum or plasma (Mussalo-Rauhamaa, 1991; Schecter et al., 1991; Wingfors et al., 1998) and also, for PCBs, between human milk and blood (Schecter et al., 1998). It is likely that most PBDEs also follow this pattern because they have similar physical-chemical properties to the other chemicals studied. We have, therefore, included published concentrations in human adipose and milk in further discussion, since there is a lack of human serum concentration data in the literature for some of the chemicals tested.

PCBs

The total PCB concentrations (defined as the sum of all congeners detected) ranged from 14 to 670 ng/g lipid, with a median of 170 ng/g lipid. As expected, CBs 153, 180 and 138 (in that order) were the dominant congeners, each contributing more than 10% to the median total PCB concentration.

Previously published concentrations of PCBs in the western European population are summarised in Table 2. Only data with acceptable data presentation (i.e. range and median or geometric mean - the arithmetic mean concentration alone is not useful for samples with non-Normal distributions) have been used for this comparison. All published studies of PCBs in the UK population are included. It can be seen that the median total PCB serum concentration in 2003 is approximately five times lower than the concentration in adipose taken from (predominantly male) corpses in Wales in 1990/91 (Duarte-Davidson et al., 1994b) and approximately 2.5 times lower than in milk-fat samples from Wales in the same period (Duarte-Davidson et al., 1994a). The range of total PCB concentrations is somewhat broader in relation to the median in this study than was found in either of the Welsh studies. Since it has been shown that human exposure to PCBs is predominantly through the diet (Duarte-Davidson and Jones, 1994), the decrease in PCB concentration since 1990/1 is likely to be due to a reduction in environmental (and particularly human foodstuffs) concentrations over the past decade.

The median and range of values found in this study are, however, very close to those found in human breast milk-fat collected in London and Lancaster between December 2001 and January 2003 (Kalantzi et al., 2004c). The median total PCB concentration found in this study is 2 to 3 times lower than found in studies of human serum (from women aged 50-65 (Koppen et al., 2002) and from pregnant women (Covaci et al., 2002b) in Belgium) and adipose from a varied population (Chu et al., 2003) in other European studies (between 1991 and 2002). The range of concentrations found in this study has the same upper limit, but a substantially (by a factor of 8 or more) smaller lower limit than found in the other European studies (see Table 2). The cause of the difference in PCB concentrations between this study and those in these mainland European countries cannot be determined, but may be related to different dietary or agricultural practices, or different characteristics of the sample populations.

Organochlorine pesticides

Of the organochlorine pesticides analysed HCB, *p,p'*-DDE, *p,p'*-DDT and β-HCH were most commonly detected and were dominant in almost all samples. α-Chlordane and γ-chlordane were not detected in any of the samples analysed.

The range of concentrations found for HCB was relatively small, whilst the range of concentrations found for *p,p'*-DDE was the largest of any chemical analysed. The median concentrations of HCB, *p,p'*-DDE, *p,p'*-DDT and β-HCH were very close to median concentrations found in milk samples taken from London and Lancaster between 2001 and 2003 (Kalantzi et al., 2004c) although the ranges of values found for HCB and β-HCH in this study are slightly narrower. Organochlorine pesticide concentrations found in other studies of western Europeans are summarised in Table 3. It can be seen that *p,p'*-DDE concentrations found in this study are between a factor of 1.1 and 9.9 lower than found in other studies conducted since 1990, and in particular that concentrations are somewhat lower than recently found in Belgium. Again, the cause of the difference in concentrations between this study and the other studies cannot be determined. HCB concentrations in this study were rather lower than found in the UK in 1997 or in Belgium in 1999, and β-HCH concentrations in this study were rather lower than found either in the UK in 1997.

o,p'-DDD, *o,p'*-DDT, *o,p'*-DDE, *p,p'*-DDD, α–HCH and γ–HCH were detected in few samples. In all cases the *p,p'*-DDE concentration greatly exceeded (almost always by more than an order of magnitide) the *p,p'*DDT concentration, indicating that exposure to the DDT pesticide was either indirect (e.g. through the diet) or historical. In seven cases, however, the concentration of *p,p'*-DDE was less than ten times the concentration of *p,p'*-DDT, which may indicate more recent exposure. Six out of these samples came from volunteers who had spent time in areas where malaria is prevalent (and where DDT may still be in current use),

although other volunteers who had also spent time in malarial areas did not show increased DDT/DDE ratios.

PBDEs

BDEs 47, 99, 100, 153, 154 and 183 were regularly detected. All of these, except BDE183, are constituents of pentaBDE commercial products, whereas BDEs 153 and 183 are constituents of the octaBDE commercial product. Neither product is now used in the European Union (Renner, 2004). The median concentration of BDE153 was higher than the median concentration of BDE47, which is unusual compared to other published data on humans, but was also found in Belgian adipose samples (Covaci et al., 2002b) and in serum from some occupational groups in Sweden between 1997 and 2000 (Jakobsson et al., 2003). A similar pattern was found in milk-fat samples from Lancaster in the UK taken between 2001 and 2003 (Kalantzi, 2004). The values and the distribution of the concentrations of the sum of BDEs 47, 99, 100, 153 and 154 were very similar to that found in Swedish blood samples taken from the general population (van Bavel et al., 2002), with approximately 5% of samples from this UK study and the Swedish study having concentrations higher than 30 ng/g lipid (more than five times the median value). High concentration ‘outlier’ samples have also been noted in a number of separate studies of PBDEs in humans (Hites, 2004). This may indicate that there is a ‘normal PBDE exposure’ population and a ‘higher PBDE exposure’ population within each country, but the source of the higher exposure is not evident from the personal information provided. Another possibility is that some individuals have markedly different capacities to metabolise and/or excrete PBDEs (for example, human polymorphism in cytochrome P-450 isoenzymes has been documented (e.g. Bolt et al., 2003)).

BDE209, the predominant congener in the deca-BDE commercial product, was found in 11 samples, ranging in concentration from close to the detection limit (3 samples) to a maximum

of 240 ng/g lipid (230 pmol/g lipid). It is likely that improvements in the detection limit will result in BDE209 being found in a higher proportion of samples.

PBDE concentrations in humans in western Europe and North America have been summarised in Table 4. It can be seen that the total PBDE concentrations found in this study are very similar to concentrations found in other European countries in recent years, and rather higher than were found in the late 1970s and early 1980s in Scandinavia. Median total BDE concentrations in the UK are approximately ten times lower than recently found in North America (She et al., 2002; Mazdai et al., 2003; She et al., 2004), although the maximum BDE concentration is similar in the UK, California and Indiana, and the minimum value is similar in the Pacific North-West of America and the UK. Both the UK and California have very strict fire resistance laws for furniture which is likely to have led to greater exposure of the general population to the pentaBDE product (previously used to treat polyurethane foam for furniture) than people living in other countries or states. This appears not to have led to higher median serum concentrations in the UK, although the range of detected values is broader than in other countries – perhaps because a small number of people may have particularly high exposure to these chemicals, either at home or through their occupation. BDE183 (indicative of octaBDE) was found in this study at a similar median concentration, and with a similar range, to recent studies in Sweden (Jakobsson et al., 2003) and North America (Mazdai et al., 2003; She et al., 2004), whereas we found BDE209 (indicative of decaBDE) at a higher median concentration than was found in Sweden and North America, but with a similar overall range of concentrations to Sweden. The highest BDE209 concentrations reported in the Swedish study were from occupationally exposed people (in the flame retarded rubber industry), and are similar to those found in the UK in this study, although the people with the highest concentrations in this study did not work in industries noted for BDE209 use (such as plastic, rubber and curtain fabric processing).

Statistical analysis

As previously noted, there was evidence that the chemical concentrations did not conform to the Normal distribution. Q-Q plots confirmed that the data was well described by the log-Normal distribution for all chemicals except several PBDEs (BDEs 47, 99, 100 and 153) for which there were a small number of 'outlying' points at the upper extreme of the distribution. Chemical concentration data were therefore transformed to their natural logarithms (ln) for further statistical analysis, and the tests were performed both including and excluding the 'outlying' points for the relevant PBDEs. To reduce the complexity of the analysis one PCB was chosen to represent each chlorination level represented in the PCBs analysed (CBs 28, 52, 118, 153, 180 and 194), six PBDEs were chosen (BDEs 47, 99, 100, 153, 154 and 183 – BDE209 was not detected in enough samples for statistical tests to be performed), and the OC pesticides which were most commonly detected (total HCH, *p,p'*-DDE, *p,p'*-DDT and HCB) were included.

Highly significant correlations (Pearson correlation coefficients 0.34-0.52, significant at the 99 % level) were found between all 'old use' chemicals, i.e. PCBs and OCPs, (indicating that they are likely to have reached the volunteers through similar exposure patterns, histories and routes) and between individual PBDEs. However, no significant correlations were found between PBDEs and either PCBs or OCPs. For further statistical analysis the chemicals were, therefore, represented by *p,p'*-DDE, total HCHs, total PCBs, HCB, BDE47, BDE183 and total PBDEs (BDEs 47 and 183 are retained separately because BDE183 did not exhibit 'outliers' from the log-Normal distribution as BDE47 did, and because of their different historical usage patterns).

Chemical concentration data were compared to categorised 'lifestyle' factors (age was grouped by decade from ages 20 to 60, using age >60 as a single group, BMI was grouped as <20, 20-24.9, 25-29.9 and >30) using a univariate ANOVA, removing factors stepwise until

all remaining factors were significant. Using this method it was found that volunteer age-group (to a highly significant degree – 99%) and location (95%) had the greatest impact on total PCB concentrations, although no regional differences could be identified (i.e. significant differences between sampling sites did not follow any geographical, or other, pattern). Significantly different (99%) HCB concentrations were found in volunteers from different locations, with gender also having a significant impact on the differences seen. The ANOVA model for *p,p'*-DDE concentrations showed variability may be related to age (99% significance), and for total HCH may be related to both location and age (99% significance). The ANOVA model could not identify any significant differences between concentrations of any PBDEs which could be related to the questionnaire results.

It is likely that the sample population in each location (median 11 individuals) is too small to reliably identify concentration differences between regions of the UK, although the sample population in London (19 males and females) was little lower than in a previous study of breast milk (27 females) which did show regional differences (Kalantzi et al., 2004c). However, principal components analysis of the HCB and HCH concentrations, based on location, showed that concentrations found in serum from volunteers sampled in Belfast and Newcastle could be substantially separated from samples from the rest of the UK (a small number of 'the rest of the UK' samples overlapped with some Belfast and Newcastle samples). No particular factors associated with samples taken in these cities could be found which might explain this observation.

Linear regression analysis showed that many PCBs (generally excluding $Cl_{3\text{-}4}$CBs), *p,p'*-DDE, *p,p'*-DDT and HCH concentrations correlated highly significantly (at the 99% level) with age, and that HCB correlated significantly (at the 95% level) with age (see Table 5 and Figure 2 for examples). The particular PCBs which correlated well with age were CBs 74, 99, 105, 118, 138, 153, 156, 157, 167, 170, 180, 187 and 194, and those not correlating well were

CBs 28 and 183, the remaining PCBs also did not correlate well with age but were detected in less than half of the samples. PCB metabolism in mammals is related to the chlorination pattern (see Strandman et al., 2000), and the easily metabolised congeners generally accumulate less than more persistent congeners. This could explain the lack of correlation between the less chlorinated PCBs and age, and similarly for some of the more chlorinated PCBs which are more easily metabolised due to their chlorination pattern. For persistent $Cl_{6\text{-}8}$CBs, being female had a significant (at the 95% level) negative impact on the slope of this regression (see Figure 2 – CB180) i.e. with increasing age females tended to have lower concentrations than males. Since we do not expect a difference in exposure, or metabolism, between males and females in the general population, we presume that this is related to the transfer of chemical to children during gestation and lactation, which is indirectly related to age, as that is the only major excretion route different between females and males. However, significant correlations between chemical concentrations in females and the number of children carried (or breast fed) were not found.

Concluding remarks

This study was, we believe, the first survey of blood serum concentrations of PCBs, organochlorine pesticides and PBDEs in the UK. The data-set produced may be seen as an indicator of human concentrations of this range of chemicals in the UK, although it cannot be considered representative of the UK population in general. In particular, we have shown a correlation between age and concentrations of the following chemicals: persistent PCBs; *p,p'*-DDT; *p,p'*-DDE; HCB; HCHs. For the more chlorinated persistent PCBs we have also shown a significant difference in the age related concentration difference between males and females. We have also found that the UK population in 2003 had similar PBDE concentrations, and a similar concentration distribution, to that found in the general population in Sweden in 2002, with approximately 5% of the population having more than five times the median PBDE

concentration, which may indicate a sub-population, in both countries, which is more exposed to these chemicals than the general population. This is somewhat surprising because of the history of PBDE production and use in the UK compared to Sweden. We report BDE209 concentrations in humans in the UK for the first time, with a similar overall range of concentrations to that found in Sweden recently, although, in contrast to the Swedish study, the people with the highest concentrations in this study did not work in industries noted for BDE209 use.

There is increasing regulatory pressure to control persistent and bioaccumulative chemicals more effectively - for instance in the forthcoming European Union 'REACH' chemicals legislation, and under the Stockholm Convention on persistent organic pollutants. Establishing levels of contamination in humans and wildlife is an important way of verifying the environmental behaviour of chemicals approved for use. Such monitoring helped to identify penta- and octa-BDE as priorities for banning in the EU, and their proposed addition to the list of POPs. Further monitoring will be useful to determine the effect of the EU ban on human exposure to these chemicals compared to, for instance, the US where restrictions on PBDE use have been introduced more recently.

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Table 1 – Summary of PCB concentrations found in blood serum

	Median	**Range**	**1st/3rd Quartiles**	**N[b]**
	ng/g lipid	ng/g lipid	ng/g lipid	
CB 18	<0.38	<0.38-2.9	<0.38/<0.38	15
CB 22	<0.33	<0.33-4.8	<0.33/<0.33	19
CB 28	2.1	<1.0-14	1.2/3.0	128
CB 31	<0.36	<0.36-10	<0.36/0.95	55
CB 41/64	<0.24	<0.24-21	<0.24/<0.24	20
CB 44	<0.24	<0.24-1.6	<0.24/<0.24	28
CB 49	<0.38	<0.38-3.9	<0.38/<0.38	30
CB 52	<0.25	<0.25-4.7	<0.25/0.5475	67
CB 54	<0.24	<0.24-0.5	<0.24/<0.24	1
CB 70	<0.24	<0.24-19	<0.24/0.58	54
CB 74	4.0	<0.3-27	2.2/8	144
CB 87	<0.24	<0.24-57	<0.24/<0.24	12
CB 90 + 101	<1.3	<1.3-9	<1.3/<1.3	13
CB 95	<0.98	<0.98-9	<0.98/<0.98	17
CB 99	3.1	<3.3-17	2.1/5.1	153
CB 104	<0.24	NA	NA	0
CB 105	1.0	<0.29-6.5	<0.29/2.1	114
CB 110	<0.32	<0.32-2.1	<0.32/<0.32	6
CB 114	0.16	<0.25-9.2	<0.25/0.81	77
CB 118	6.1	<0.52-29	3.5/10	153
CB 123	<0.24	<0.24-0.66	<0.24/<0.24	4
CB 138	27	<7.1-110	18/47	149
CB 141	<0.67	<0.67-3.6	<0.67/<0.67	5
CB 149	<4.1	<4.1-12	<4.1/<4.1	8
CB 151	<1.6	<1.6-6.7	<1.6/<1.6	10
CB 153	41	<9.3-200	28/70	152
CB 155	<0.24	NA	NA	0
CB 156	4.6	<0.41-18	3/7.7	151
CB 157	1.1	<0.25-3.8	0.48/1.7	121
CB 158	<0.28	<0.28-5	<0.28/0.62	68
CB 167	1.65	<0.3-6.1	0.9/3	134
CB 170	13	<2.6-61	8/21	150
CB 174	<0.65	<0.65-6.8	<0.65/<0.65	9
CB 180	33	<4.7-200	20/53	151
CB 183	3.0	<0.91-16	<0.91/5	104
CB 187	6.6	<2.1-57	3.3/11	129
CB 188	<0.24	NA	NA	0
CB 189	<0.24	<0.24-1.2	<0.24/<0.24	8
CB 194	5.1	<0.61-39	2.5/8	147
CB 199	<0.24	NA	NA	0
CB 203	<2.8	<2.8-37	<2.8/5.8	71
Total PCB	**170**	**14-670**	**100/270**	**154**

Table 1 cont'd

	Median	**Range**	**1st/3rd Quartiles**	**N[b]**
	ng/g lipid	ng/g lipid	ng/g lipid	
α- chlordane	<0.48	NA	NA	0
γ- chlordane	<0.48	NA	NA	0
HCB	11	<4.8-72	<4.8/20	108
o,p'- ddd	<0.48	<0.48-49	<0.48/<0.48	13
o,p'- dde	<0.48	<0.48-3.9	<0.48/<0.48	7
o,p'- ddt	<0.51	<0.51-8.4	<0.51/<0.51	19
p,p'- ddd	<0.51	<0.51-20	<0.51/<0.51	8
p,p'- dde	100	<11-2600	59/200	153
p,p'- ddt	2.9	<0.64-73	1.6/5.1	140
Total DDX*	**100**	**1.3-2600**	**61/210**	**154**
α-HCH	<0.48	<0.48-23	<0.48/<0.48	23
β-HCH	12	<0.68-80	7/22	151
γ-HCH	<1.7	<1.7-110	<1.7/<1.7	17
Total HCH	**15**	**<8.9-120**	**7.8/29**	**154**
BDE 17	<0.13	<0.13-0.3	<0.13/<0.13	2
BDE 28	<0.14	<0.14-10	<0.14/0.2	42
BDE 32	<0.16	<0.16-11	<0.16/<0.16	16
BDE 35	<0.12	<0.12-1.1	<0.12/<0.12	3
BDE 37	<0.12	<0.12-0.4	<0.12/<0.12	4
BDE 47	0.82	<0.30-180	<0.3/1.9	105
BDE 49	<0.13	<0.13-1.7	<0.13/<0.13	3
BDE 71	<0.12	<0.12-0.3	<0.12/<0.12	1
BDE 75	<0.12	<0.12-0.3	<0.12/<0.12	1
BDE 77	<0.12	<0.12-17	<0.12/<0.12	6
BDE 85	<0.13	<0.13-5.3	<0.13/<0.13	4
BDE 99	<0.16	<0.16-150	<0.16/0.93	63
BDE 100	0.76	<0.17-390	0.53/1.1	142
BDE 119	<0.12	<0.12-1.4	<0.12/<0.12	5
BDE 138	<0.13	<0.13-1.5	<0.13/<0.13	3
BDE 153	1.7	<0.26-87	1.2/2.4	152
BDE 154	0.60	<0.15-4.4	0.37/0.84	132
BDE 166	<0.12	<0.12-2.9	<0.12/<0.12	2
BDE 181	<0.12	<0.12-0.9	<0.12/<0.12	1
BDE 183	0.30	<0.14-1.8	<0.14/0.62	84
BDE 190	<0.12	NA	NA	0
BDE 209	<15	<15-240	<15/<15	11
Total PBDE	**5.6**	**0.63-420**	**3.6/9.6**	**154**

Total DDX = sum of *o,p'*-DDD, *o,p'*-DDE, *o,p'*-DDT, *p,p'*-DDD, *p,p'*-DDE and *p,p'*-DDT; NA = not applicable (concentrations all below LOD); [b] Number of samples with concentrations above LOD (out of 154)

Table 2 – PCB concentrations previously found in tissue samples from the European population

Region	**Year**	**Sample Type**	**Total PCB concentration ng/g lipid** *median (range)*	**Reference**
Wales, UK	1990-1	Adipose	850 (260-2600)	(Duarte-Davidson et al., 1994b)
Wales, UK	1990-1	Milk	440 (140-1700)	(Duarte-Davidson et al., 1994a)
London & Lancaster, UK	2001-3	Milk	180 (26-530)	(Kalantzi et al., 2004c)
UK	2003	Serum	170 (14-670)	This study
Belgium	1999	Serum	350 (110-1000)[a]	(Covaci et al., 2002b)
Belgium	1999	Serum	530 (450-680[b])	(Koppen et al., 2002)
Belgium	2002	Adipose	480[c] (190-1100)	(Chu et al., 2003)
Netherlands	1991	Serum	550[a,c]	from (Covaci et al., 2002b)
Netherlands	1995	Serum	450 [a,c]	from (Covaci et al., 2002b)
Sweden	1996	Plasma	350-640	(Norén et al., 1999)

[a] = Calculated assuming 0.5% lipid in blood; [b] = 95th Percentiles; [c] = Arithmetic mean

Table 3 – Organochlorine pesticide concentrations previously found in tissue samples from the European population

Region	Year	Sample Type	Concentration (ng/g lipid) Median (Range) *p,p'*DDE	HCB	β-HCH	Reference
Wales, UK	1990-1	Adipose	990 (110-6400)			(Duarte-Davidson et al., 1994b)
Wales, UK	1990-1	Milk	310 (35-6000)			(Duarte-Davidson et al., 1994a)
UK	1997-8	Milk	280 (<60-4000)	250 (<12-330)	50 (<8-750)	(Harris et al., 1999b)
London & Lancaster, UK	2001-3	Milk	150 (22-1600)	18 (ND-180)	17 (1.2-1500)	(Kalantzi et al., 2004c)
UK	2003	Serum	100 (15-2600)	14 (5.4-72)	12 (1.9-80)	This study
Belgium	1999	Serum	310 (110-4100)[a]	36 (12-100)[a]		(Covaci et al., 2002b)
Belgium	1999	Serum	870 (730-1200[b])	110 (90-132[b])		(Koppen et al., 2002)
Belgium	2002	Adipose	480[c] (84-1800)			(Chu et al., 2003)
Sweden	1996	Plasma	110-880	16-56		(Norén et al., 1999)

[a] = Calculated assuming 0.5% lipid in blood; [b] = 95th Percentiles; [c] = Arithmetic mean

Table 4 – PBDE concentrations previously found in human tissue samples

Region	Year	Sample Type	Concentration (ng/g lipid) Median (Range) ΣBDE[a]	BDE183	BDE209	Reference
London & Lancaster, UK	2001-3	Milk	5.7[b] (0.7-28)			(Kalantzi et al., 2004c)
UK	2003	Serum	4.7 (0.52-420)	0.59 (0.19-1.8)[f]	83 (35-240)[g]	This Study
Sweden	2001-2	Blood	4.9[c,h]			(van Bavel et al., 2002)
Belgium	2000	Adipose	4.8[c]			(Covaci et al., 2002a)
Finland	1994-98	Milk	2.1[c]			(Strandman et al., 2000)
Germany	1985	Blood	3.1[c,i]			(Thomsen et al., 2002)
	1990		3.6[c,i]			
	1995		3.7[c,i]			
	1999		3.9[c,i]			
Norway	1977	Serum	0.44[c]			(Thomsen et al., 2002)
	1986		1.1[c]			
	1995		3.1[c]			
	1999		3.1[c]			
Sweden	1972	Milk	0.07[c]			Summarised in (Sjödin et al., 2003)
	1980		0.45[c]			
	1990		1.2[c]			
	2000		2.6[c]			
Sweden	1997	Serum		11 (3.0-25) ED	4.8 (<0.29-9.5) ED	(Jakobsson et al., 2003)
	1998			<0.38 (<0.38-1.6) CB	2.3 (<0.96-5.6) CB	
	2000			<0.48 (<0.48-1.1) RM	28 (1.2-140) RM	
	2000			<1.9 RW	34 (6.7-280) RW	
	1999			1.2 (0.23-6.1) CT	1.5 (<0.96-6.8) CT	
	1997			0.23 (<0.02-1.3) C	<0.67 (<0.67-7.7) C	
	1997			0.15 (0.029-0.37) CL	<0.67 (<0.67-3.7) CL	
	2000			<0.38 AB	2.4 (0.92-9.3) AB	
USA	1985-89	Serum	9.6 (4.6-74)[j]			(Sjödin et al., 2004)
	1990-94		48 (7.5-86)[j]			
	1995-99		71)42-120)[j]			
	2000-2		61 (47-160)[j]			
USA (CA)	1995-99	Breast tissue	41 (17-462)			(She et al., 2002)
USA (IN)	2001	Serum	37 (15-580)[k]	<LOD (<LOD-2.7)		(Mazdai et al., 2003)
North American Pacific NW	2003	Milk	49 (6.3-309)[l]	0.2 (0.006-1.6)	0.25 (0.05-1.5)	(She et al., 2004)

[a] = sum of BDEs 47, 99, 100, 153 and 154 unless otherwise indicated; [b] = Geometric mean; [c] = Arithmetic mean; [d] = Calculated assuming 0.5% lipid in blood; [e] = 95th Percentiles; [f] = only detected values used (N = 85); [g] = only detected values used (N = 11); [h] = sum of BDE47, 99, 153; [i] = BDE47 only; [j] = sum of BDE47, 85, 99, 100, 153, 154; [k] = sum of BDE47, 99, 100, 153, 154, 183; [l] = sum of BDE28, 33, 71, 47, 66, 85, 99, 100, 153, 154, 183, 209; ED = Electronics dismantlers; CB = Circuit board recycling workers; RM = Rubber mixers; RW = Rubber wire production; CT = Computer technicians; C = Clerks; CL = Cleaners; AB = Abattoir workers

Table 5 – Regression slopes and coefficients for the relationships between chemical concentrations and the age of the volunteers (figures in brackets are calculated with outliers removed)

Chemical	All volunteers (N=154) Slope ng/(g lipid . year)	r^2	Males only (N=50) Slope ng/(g lipid . year)	r^2	Females only (N=104) Slope ng/(g lipid . year)	r^2	Significantly (95% level) lower slope for females
CB28	0.015 (0.015)	0.009 (0.009)	-0.032 (-0.032)	0.025 (0.025)	0.025 (0.025)	0.026 (0.026)	
CB74	**0.19 (0.20)**	**0.31 (0.40)**	0.07 (0.13)	0.029 (0.25)	0.21 (0.21)	0.41 (0.41)	
CB118	**0.24 (0.25)**	**0.33 (0.38)**	0.26 (0.26)	0.32 (0.32)	0.23 (0.24)	0.32 (0.38)	Y (Y)
CB153	**1.3 (1.4)**	**0.40 (0.43)**	2.4 (2.4)	0.52 (0.52)	1.11 (1.18)	0.41 (0.48)	Y (Y)
CB180	**1.1 (0.96)**	**0.39 (0.48)**	2.1 (1.5)	0.50 (0.55)	0.89 (0.89)	0.51 (0.51)	Y (Y)
CB194	**0.17 (0.17)**	**0.19 (0.51)**	0.39 (0.30)	0.26 (0.70)	0.14 (0.15)	0.39 (0.56)	Y (Y)
HCB	*0.21 (0.21)*	*0.043 (0.043)*	-0.017 (-0.017)	0.0 (0.0)	0.25 (0.25)	0.062 (0.062)	
p,p'-DDE	**9.7 (7.2)**	**0.21 (0.34)**	9.1 (8.5)	0.30 (0.37)	9.7 (7.0)	0.19 (0.32)	
*p,p'*DDT	**0.099 (0.067)**	**0.032 (0.075)**	0.16 (0.081)	0.066 (0.076)	0.082 (0.061)	0.023 (0.068)	
b-HCH	**0.55 (0.55)**	**0.33 (0.33)**	0.35 (0.35)	0.24 (0.24)	0.57 (0.57)	0.33 (0.33)	
BDE47	-0.16 (0.009)	0.011 (0.002)	-0.29 (0.067)	0.013 (0.065)	-0.11 (-0.008)	0.016 (0.002)	
BDE100	-0.19 (-0.001)	0.006 (0)	-0.49 (0.016)	0.008 (0.038)	-0.065 (-0.003)	0.010 (0.005)	
BDE153	-0.059 (-0.002)	0.014 (0)	0.026 (0.046)	0.013 (0.097)	-0.078 (-0.006)	0.020 (0.007)	

Bold figures are Significant at the 99% level; *Italic figures are significant at the 95% level*

Figure legends

Figure 1 - Map of the UK, showing sampling locations (shaded areas indicate major urban centres)

Footnote: Numbered crosses indicate sampling sites - (1) Edinburgh, (2) Newcastle-upon-Tyne, (3) Leeds, (4) Belfast, (5) Manchester, (6) Nottingham, (7) Birmingham, (8) Huntingdon, (9) Cardiff, (10) London, (11) Godalming, (12) Winchester and (13) Exeter

Figure 2 – Concentration of selected chemicals plotted against age, with the genders differentiated

Footnote: Results of the linear model: **CB28** (Cl_3CB) – no correlation with age, representative of metabolised and less chlorinated PCBs; **CB118** (Cl_5CB) – positive correlation, no difference between the genders; **CB180** (Cl_7CB) – positive correlation, significantly (at the 99% level) lower slope for females, representative of non-metabolised and more chlorinated PCBs; **HCB** – no correlation; ***p,p'*-DDE** - positive correlation, no difference between the genders; **Total HCH** - positive correlation, no difference between the genders.

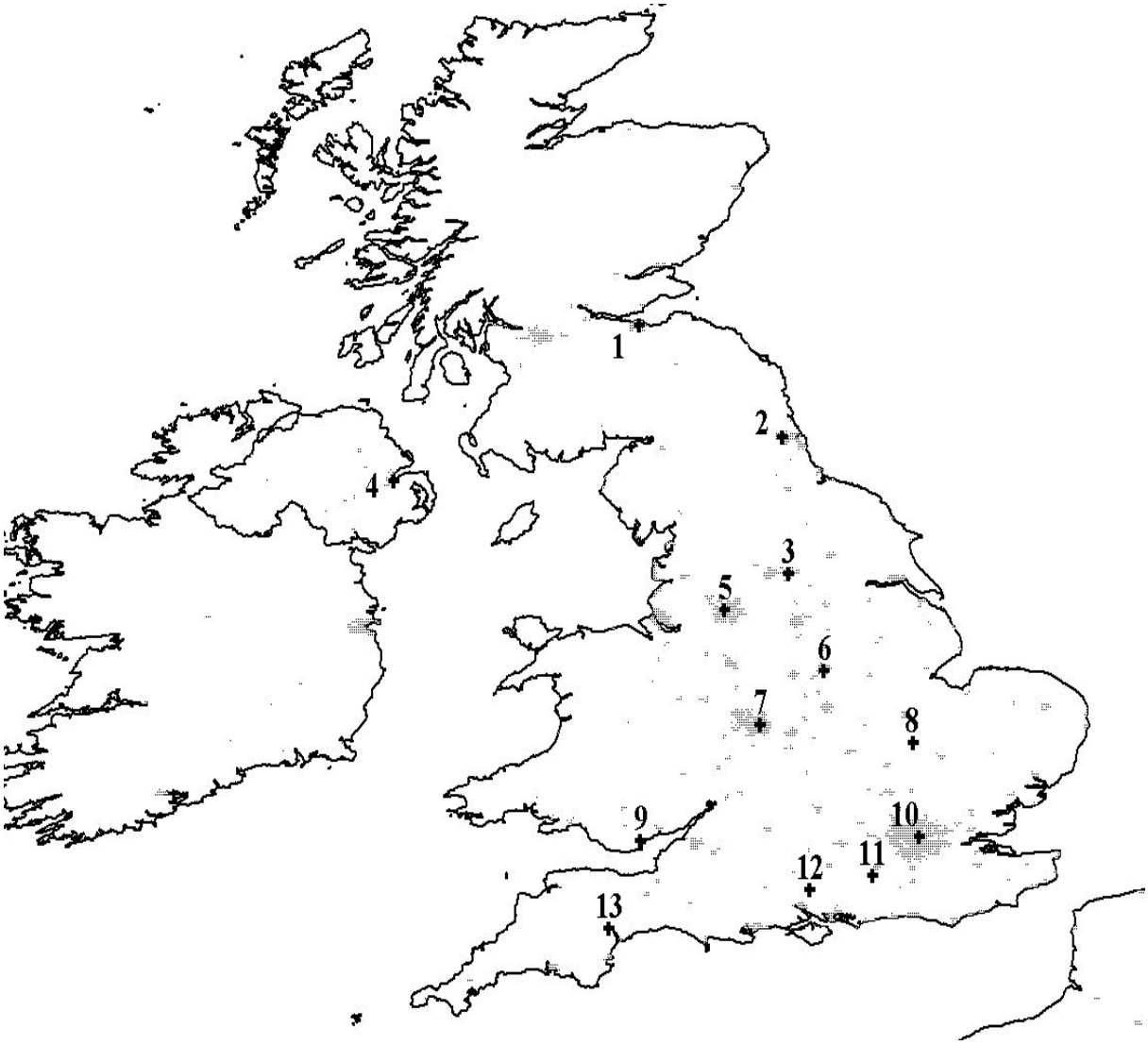

Figure 1

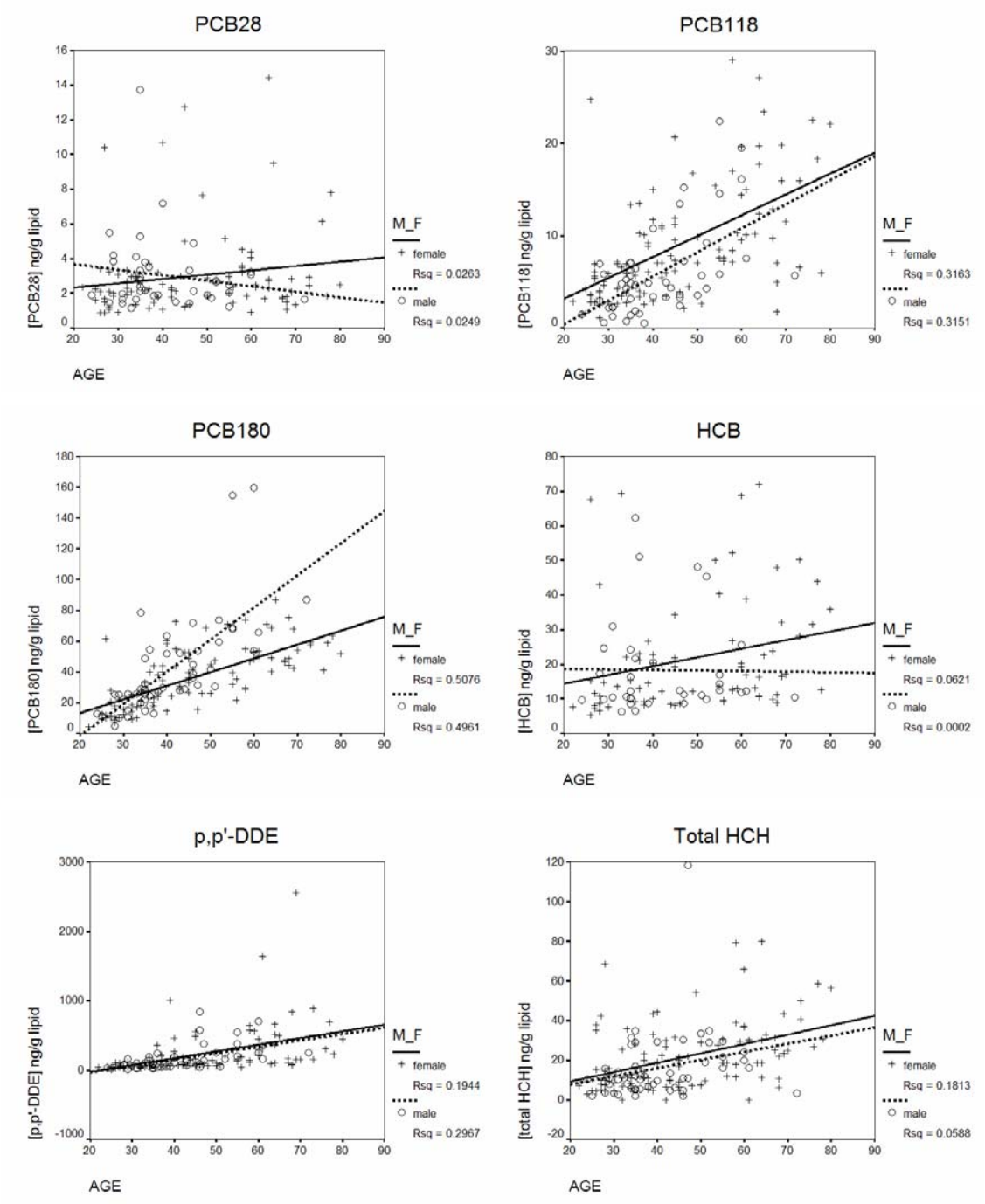
PCB28
[PCB28] ng/g lipid
AGE
M_F
female
Rsq = 0.0263
male
Rsq = 0.0249
PCB118
[PCB118] ng/g lipid
AGE
M_F
female
Rsq = 0.3163
male
Rsq = 0.3151
PCB180
[PCB180] ng/g lipid
AGE
M_F
female
Rsq = 0.5076
male
Rsq = 0.4961
HCB
[HCB] ng/g lipid
AGE
M_F
female
Rsq = 0.0621
male
Rsq = 0.0002
p,p'-DDE
[p,p'-DDE] ng/g lipid
AGE
M_F
female
Rsq = 0.1944
male
Rsq = 0.2967
Total HCH
[total HCH] ng/g lipid
AGE
M_F
female
Rsq = 0.1813
male
Rsq = 0.0588

Figure 2