

POLYCHLORINATED BIPHENYLS AND ORGANOCHLORINES IN
BY-CAUGHT HARBOUR PORPOISES *PHOCOENA PHOCOENA*
AND COMMON DOLPHINS *DELPHINUS DELPHIS* FROM IRISH
COASTAL WATERS

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ABSTRACT

Concentrations of 11 organochlorine (OC) pesticides and 10 individual polychlorinated biphenyls (PCB) in blubber and liver from 12 harbour porpoise *Phocoena phocoena* and eight common dolphins *Delphinus delphis* incidentally caught in fishing nets in Irish waters are presented. Female harbour porpoises had highest concentrations of OC in blubber and male common dolphins in liver. Harbour porpoises had higher mean concentrations of lindane (121-154 ng/g extractable lipid), dieldrin (116-121 ng/g) and γ -BHC (54-128 ng/g) but common dolphins had greater overall concentrations of DDT (9444-3998 ng/g). The predominant DDT metabolite was pp-DDE and for the chlordanes was t-nonachlor. Concentrations of ICES 7 PCB (liver-blubber) were similar in both species (4075-7999 ng/g in harbour porpoise and 4076-8945 in common dolphins). The sum of ICES 7 PCB in porpoises ranged from 3041-12270 ng/g extractable lipid in the blubber of females and from 2911-10429 ng/g in males and 798-11074 ng/g in the blubber of female common dolphins and 1555-15883 ng/g in males.

Contaminant levels were generally similar to those reported from Scotland but lower than reported from Scandinavia. Ratios of DDT to DDE suggests that there are limited new sources of DDT into the Irish marine environment. These results provide a baseline for monitoring of persistent pollutants in the Irish marine environment.

INTRODUCTION

The role of persistent organochlorines in the health and disease prevalence of marine mammals is still uncertain. Since Reijnders (1986) showed a link between polychlorinated biphenyls (PCB) and reproductive failure in common seals *Phoca vitulina* there have been increased concerns over the impact of these and other persistent pollutants on marine mammal populations. The epizootic in the Mediterranean during 1990-1992 when hundreds, perhaps thousands, of striped dolphins *Stenella coeruleoalba* died of a morbilli virus was thought to have been precipitated by elevated PCB concentrations in the dolphins blubber (Aguilar and Borrell 1994). Numerous studies have shown elevated levels of organochlorines (OC) and PCB in a wide range of cetacean species (see reviews by O'Shea and Brownell 1994; Aguilar and Borrell 1995) but the pathological implications of contaminant burdens is still equivocal (Kuiken *et al.* 1994; de Guise *et al.* 1995).

Concentrations of OC residues in marine mammals tissues may vary with a range of physiological factors including age, sex, maturity and body condition (Addison and Smith 1974; Borrell *et al.* 1995; Cockcroft *et al.* 1989; 1990; Muir *et al.* 1992) which complicates inter-species and inter-site comparisons. Males tend to accumulate lipophilic substances throughout their life but females offload them to their young during gestation and lactation (Addison and Brodie 1977; 1987). Organochlorine burdens also vary considerably between species and location. Coastal species such as harbour porpoises *Phocoena phocoena*, may accumulate higher concentrations than more pelagic or oceanic species such as striped or common dolphins *Delphinus delphis* (e.g. Cockcroft *et al.* 1990; Kuiken *et al.* 1994; Marsili and Forcadi 1997) and Odontocetes accumulate higher levels than Mysticetes (Borrell and Aguilar 1987; Kleivane and Skaare 1998).

Due to the wide variations in contaminant burdens in cetaceans more species from a wider geographical spread as possible need to be analysed. Most early studies of pollutants in cetaceans were based on the analyses of tissues from animals washed up on the coast (e.g. Wells *et al.* 1994; Marsili and Forcadi 1997). The cause, location and time of death of stranded animals is largely unknown which limits interpretation of the data and, in addition, there can also be significant post-mortem changes in OC levels (Borrell and Aguiar 1990). In recent years animals incidentally caught in fishing nets have become available for sampling (e.g. Kleivane *et al.* 1995; Tanabe *et al.* 1997) which has greatly enhanced studies on the effect of pollutants on marine mammals as the time and cause of death is known.

The prevailing weather and currents off the Irish coast are westerly and pollutant burdens in cetaceans may be expected to be some of the lowest in Europe, however studies of persistent pollutants in any mammal species from Ireland are scarce. Mason and O'Sullivan (1992; 1993) recorded levels of PCB in the liver and kidney of otters *Lutra lutra* and mink *Mustela vison* high enough to physiologically compromise some individuals but pesticide levels were not thought to be of concern. Nixon (1991) analysed samples from common seals *Phoca vitulina* from the northwest coast of Ireland for a variety of OC and PCB contaminants. Total DDT and PCB were some 2.3 times lower than levels from the east and northeast coast of England, dieldrin 3.7 times and HCB around 5 times lower. Other OC and most CB congeners analysed were similar to levels from England (Nixon 1991). Concentrations of OC and PCB in a group of white-sided dolphins *Lagenorhynchus acutus* mass stranded in Killala Bay on the northwest coast of Ireland were similar to those recorded from other studies on this species in the Atlantic (McKenzie *et al.* 1998). They suggested OC contamination was ubiquitous in this species, with these pelagic dolphins integrating OC contaminants from different areas making patterns indistinguishable from each other.

The aim of this study was to establish baseline levels of persistent OC and PCB in harbour porpoises and common dolphins from Irish coastal waters. Both species are widespread and abundant off the Irish coast (Evans 1980; Berrow and Rogan 1997). Harbour porpoises tend to be more coastal, occurring exclusively over the continental shelf, feeding on a variety of mainly demersal Gadoids (*Trisopterus* spp., *Merlangius merlangus*) and Clupeids (*sprattus*, *Clupea harengus*) (Rogan and Berrow 1995). Common dolphins occur more offshore in deeper water off the shelf edge as well as over the continental shelf and exhibit seasonal inshore movements from May to September (Evans 1980). Dietary studies (Rogan and Berrow 1995) have shown common dolphins in Irish waters feed on a variety of Gadoids (*Trisopterus* spp., *Merlangius merlangus*), Clupeids (*Clupea harengus*) and cephalopods (*Gonatus*, *Histioteuthis* and *Toderopsis* spp.). Both harbour porpoise and common dolphins are frequently caught in fishing nets (Berrow and Rogan 1999) which were landed for this study, providing valuable samples for analyses.

METHODOLOGY

SAMPLE COLLECTION

Samples of blubber and liver were collected from 12 harbour porpoise and eight common dolphins incidental caught in fishing nets off the south and southwest coast of Ireland (Fig. 1) between 1990 and 1994. Post-mortem examination of all dolphins and seven porpoises were carried out within 48 hours of being landed and between 2-8 days after being caught. The remaining five harbour porpoises were frozen at -20°C, prior to post-mortem examination, then defrosted. A strip of blubber (c10-20g), removed immediately posterior to the dorsal fin, and a sample of liver were collected with a stainless steel knife, placed in aluminum foil and stored at -20°C until analysed. Sex, length (in a straight line from tip of beak to the centre of the tail fluke) and blubber thickness (immediately posterior to the dorsal fin) were recorded during post-mortem examination. Age was calculated from examination of GLG layers in teeth (after Lockyer 1995a). Sexual maturity of males was determined by a combination of testes weight and body length and for females from evidence of ovulation (ovarian corpora), lactation and pregnancy (after Lockyer 1995b).

SAMPLE PREPARATION

All glassware used during chemical analysis was washed with detergents and high purity pestiscan grade organic solvents (a mixture of one part dichloromethane and one part methanol and then rinsed with hexane). All apparatus used in the soxhlet extraction was pre-run with a 50:50 hexane/dichloromethane mixture before extractions (the sodium sulphate used was also hexane washed).

The OC analysed followed the methods described by Allchin *et al.* (1989). Eleven OC (HCB, a-HCH, g-HCH, pp-DDE, pp-DDT, pp-DDD, op-DDE, dieldrin, a-chlordane, g-chlordane, t-nonachlor) and 10 individual chlorobiphenyls (IUPAC nos: 28, 31, 52, 101, 118, 153, 105, 138, 156, 180 numbered according to Ballshmiter and Zell (1980)) were examined. The sum of the seven congeners (CB 28, 52, 101, 118, 138, 153, 180) recommended by ICES (International Council for Exploration of the Sea) for monitoring purposes (ICES 1986) was calculated (ICES 7 PCB). PCB analysis was adapted from Allchin *et al.* (1989) and de Boer (1989). For soxhlet extraction, 3g of blubber or liver tissue was ground with 15-20g sodium sulphate and stored in the desiccator overnight and then ground to a free-flowing sample. The sulphated and dehydrated samples were exhaustively extracted with 70mls of dichloromethane/hexane (ratio 1:1) for a minimum of 6 hours at c20 cycles per hour. A combination of polar and apolar solvents was used, as apolar solvents alone may not extract CBs and OCPs exhaustively from mammalian tissue. A higher content of the polar solvent increases the extraction potency.

For lipid determination, extracts were evaporated on a rotary evaporator. 1ml aliquots of the reduced extracts were dried to a constant weight in an oven at 105°C and reweighed and the lipid concentration determined as a percentage of the original wet sample weight. These values were then used to express the final results on a lipid

weight basis. The activated alumina columns were prepared by dry packing with 3g of 5% deactivated alumina and topped with a 1cm layer of sodium sulphate. A suitable aliquot, based on the lipid content and expected PCB and OC content, of the sample extract was evaporated to 1ml and quantitatively transferred to the top of the column. The sample was eluted with 20ml of n-hexane and collected in 20ml test-tubes. This was then reduced to 1ml under a nitrogen stream and further fractionated on a silica column. Columns for silica fractionation were dry packed with 2g of silica topped with a 1cm layer of sodium sulphate and the 1ml of sample transferred to the top. The sample was eluted with 20ml of n-hexane containing 2% tetrahydrofuran, two fractions were collected: a 6ml sample containing HCB, all the PCBs, pp DDE, and op DDE and a 10ml sample containing a-BHC, lindane, a-chlordane, g-chlordane, t-nonachlor, ppDDT, ppDDD. After fractionation, 50ng/ml of the internal standard TCN (tetrachloronaphthalene) was added and the cleaned up extract was reduced, under nitrogen, to 1ml approximately. Every tenth sample was a total method blank of sodium sulphate and treated the same as the samples throughout the cleanup and fractionation.

CHEMICAL ANALYSES

Two high resolution capillary columns were used to give confirmative identification where peaks co-elute on one or other of the columns. The overall optimization of the GC parameters were carried out prior to running samples according to guidelines given in Quasimeme Intercomparison exercises (Megginson *et al.* 1994). Multi-level calibration curves were created using 10 standards for each of the 26 components in the standards. A complete set of calibration standards were run after every 20 samples with a single calibration level being run after every 10 vials to adjust retention time drift in samples.

Most of the congeners reported here had well resolved chromatograph peaks obtained from the CP-Sil 8 column. Data obtained on the CP-Sil 19 column of CBs -52, -101, -118, -153, -180 were within 10% of the data from the CP-Sil 8 column. Data on two mono-ortho substituted congeners with a "dioxin-type toxicity", the CBs -105 and -156, were well separated on the CP-Sil 19 column. Some co-eluting pairs on the CP-Sil 8 column that were also co-eluting on the CP-Sil 19 column are indicated by a double identity. The overlapping peaks were quantified on the basis of the first mentioned congener in each pair. Of the pair CB101/90 the presence of the second congener was neglected as Wells *et al.* (1992) reported that the contribution of the second congener was very small in marine mammals.

A certified reference material, CRM 349 Cod liver oil, containing seven certified CB congeners was run every 10 samples to assess the accuracy and precision of the determinations being carried out. To minimise errors due to the non-linearity of the ECD detector a multi-level calibration curve was used for each component with standards spanning a range 0.5-500 ng/ml (Wells *et al.* 1992). The calibration curve linearity was determined by correlating the amount injected with the detector response in peak height after correction using the internal standard.

RESULTS

BIOLOGICAL CHARACTERISTICS OF SAMPLED ANIMALS

Six male and six female harbour porpoises, measuring 94 - 155cm and 115 - 151cm in length respectively, were sampled (Table 1). Most of these animals (91%) were juveniles between 1 - 4 years of age and all of the female porpoises and three of the males were sexually immature. Three female common dolphins measuring between 186 and 202cm, and five male dolphins, between 129 - 214cm in length, were also sampled and ranged from 2 - 11 years of age (Table 1). Three of the female dolphins were sexually immature, one was pregnant and lactating and two had reproduced previously. Three of the five male dolphins were juveniles, one was approaching sexual maturity at 7 years old and one sexually mature. Blubber thickness ranged from 9-22mm in harbour porpoises and 10-15mm in common dolphins (Table 1).

EXTRACTABLE LIPID CONTENT

The lipid content in blubber ranged from 57.1 to 97.2% in harbour porpoises (Table 2a) and 47.9 to 94.8% in common dolphins (Table 3a). Only two immature female porpoises had a lipid content in the blubber less than 85%. As expected, lipid content in liver was an order of magnitude lower and ranged from 3.8 to 8.0% in harbour porpoises (Table 2a) and 2.1% to 7.9% in dolphins (Table 3a). There was no significant correlation between blubber thickness and % lipid in either harbour porpoises (blubber: $r^2 = 0.9\%$, $P = 0.79$, liver: $r^2 = 0.5\%$, $P = 0.88$) or common dolphins (blubber: $r^2 = 2.3\%$, $P = 0.56$, liver: $r^2 = 84.2\%$, $P = 0.26$). Due to the small age range sampled and low sample sizes it was not possible carry out much statistical analyses on the data, however concentrations of CB153, CB138, CB180 did increase ($P < 0.25$, $r^2 > 60\%$) and DDT and dieldrin declined ($P < 0.40$, $r^2 > 0.35\%$) with age in male common dolphins but these trends were not significant.

MEAN CONCENTRATIONS OF PESTICIDES, DDT AND METABOLITES

The results of the OC analyses expressed as ng per gram of extractable lipid are shown in Table 2a and 3a. In general all contaminants were at greater concentrations in the blubber in both species sampled. Female harbour porpoises had the highest concentrations of all OC in blubber analysed while male common dolphins had the highest concentrations of OC in the liver. In comparison with common dolphins, harbour porpoises had higher mean concentrations (males-females) of the pesticides lindane (121-154 ng/g compared to 42-45 ng/g), dieldrin (116-121 ng/g compared to 8-20 ng/g) and Σ -BHC (54-128 ng/g compared to 37-41 ng/g) but common dolphins had greater overall concentrations of DDT (9444-3998 ng/g compared to 346-4664 ng/g).

The predominant DDT metabolite was pp-DDE which is probably due to its resistance to further metabolism.

Concentrations of pp-DDE in the blubber of female porpoises ranged from 926-3658 ng/g extractable lipid and in males from 1178-3124 ng/g extractable lipid (Table 2a). The sum of DDT metabolites measured in harbour porpoises ranged from 1640-5989 ng/g in blubber and 43-8910 ng/g in liver (Table 2a). Concentrations of pp-DDE in the blubber of female common dolphins ranged from 2633-3658 ng/g extractable lipid and in males from 926-2938 ng/g extractable lipid and concentrations in liver ranged from 113-2922 ng/g extractable lipid in females and from 654-7046 ng/g extractable lipid in males. The sum of DDT metabolites gave a range of concentrations from 244-12743 ng/g extractable lipid in blubber tissue and from 131-8306 ng/g extractable lipid in liver tissue of common dolphins. The predominant chlordanes metabolite in both tissues of both species sampled was t-nonachlor. The sum of chlordanes metabolites in harbour porpoises ranged in concentration from ng/g wet wt in blubber and 0.9-95 ng/g wet wt in liver and in dolphins was 77-3153 ng/g wet wt in blubber and 1-84 ng/g wet wt in liver. Higher concentrations of cyclodienes occurred in female harbour porpoises compared to males and both sexes of common dolphins but concentrations were greater in male common dolphins compared to females.

CONCENTRATIONS OF PCB

The mean concentrations of ICES 7 PCBs were similar in both species (4075-7999 ng/g in harbour porpoise and 4076-8945 in common dolphins) but there were large differences in individual congeners (Tables 3a and 3b). ICES 7 PCB levels in blubber were 10-20 fold greater than liver when expressed on a wet weight basis. When normalised on a lipid basis, the differences between tissues were much less pronounced. Concentrations of PCB and OC between liver and blubber differed by as much as a factor of five for some individuals of the same species but often less than twofold between tissues. Although 10 PCB congeners were identified only three predominated in both liver and blubber tissues. These were chlorobiphenyls CB138, CB153 and CB180, which are considered to be resistant to metabolism (Boon *et al.* 1992) and are generally the most abundant chlorobiphenyl congeners in marine mammal blubber (Law *et al.* 1995). The sum of ICES 7 PCB in individual porpoises ranged from 3041-12270 ng/g extractable lipid in the blubber of females and from 2911-10429 ng/g in males. In the liver of individual dolphins the concentrations in females ranged from 187-9237 ng/g and in males 1112-11135 ng/g. The sum of the ICES 7 PCB in the blubber of female common dolphins ranged from 798-11074 ng/g and in males from 1555-15883 ng/g. In the liver of female dolphins concentrations ranged from 2148-13867 ng/g extractable lipid and in males from 1798-7032.

OC AND PCB CONCENTRATIONS IN INDIVIDUAL HARBOUR PORPOISES

There were a wide range of OC and PCB concentrations in individual harbour porpoises (Table 2a and 2b). The highest OC concentrations were recorded in two immature females (CBC1 and CBC5, Table 1). CBC1 had elevated levels of Σ -BHC (693.3 ng/g extractable lipid), lindane (281.8) and pp-DDT (1590.5) in blubber and Σ -BHC (1267.6) in liver compared to the other dolphins sampled while CBC5 had elevated levels of Σ -BHC (736.5) and t-nonachlor (1622.1) in blubber. All other individual harbour porpoises had elevated levels of single OC but there were no obvious trends with sex or age. In CBC13, the oldest porpoise sampled (a 6 year old male) only t-nonachlor (1557.5) and pp-DDE (3124.4) were elevated relative to other individuals sampled. ICES 7 PCB levels were highest in the blubber of CBC3, CBC11 and HP2/94 and these individuals together with CBC1 in the liver. HP2/94 had the thinnest layer of blubber (9mm) recorded and had elevated levels of t-nonachlor (1718.7) in the blubber and Σ -BHC (2664.4), op-DDE (536.4), pp-DDE (7692.3) in the liver but especially high levels of ICES 7 PCB in the blubber (ICES 7 PCB 12269.6 ng/g extractable lipid). This may suggest that these high levels are related to the poor body condition of this individual, however CBC2 which also had only 9mm blubber thickness (Table 1) only showed elevated levels of Σ -BHC (1593.3) in the liver and nothing in blubber tissue.

OC AND PCB CONCENTRATIONS IN INDIVIDUAL COMMON DOLPHINS

Similar to harbour porpoises there was a large range in the concentrations of individual OC and PCB in dolphins but no consistent patterns. Highest levels of t-nonachlor (1622.1 ng/g extractable lipid) and PCB (ICES 7 11074.2 ng/g extractable lipid) were recorded in the blubber of CD5, t-nonachlor (1498.5) and pp-DDT (1689.7) in the blubber of CBC8 and t-nonachlor (1718.7) and Σ -BHC (315.4) in the blubber of CD9. Dieldrin (454.1) and ICES 7 PCB (10706.8 ng/g extractable lipid) were highest in the blubber of CBC7. CBC9 and CBC10 also had elevated levels of ICES 7 PCB (9237.1 and 11135.4 ng/g extractable lipid) in the blubber and pp-DDE (7046.4) in the liver respectively. Two dolphins (CBC7 and CBC8) were thought to be adult and calf. They had similar concentrations of Σ -BHC in blubber but the mother had higher levels of pp-DDT and t-nonachlor than the calf and the calf higher concentrations of dieldrin (both elevated) relative to other individuals and all CB congeners compared to the adult dolphin. In liver, the calf had higher levels of five OC (Σ -BHC, lindane, dieldrin, pp-DDT and t-nonachlor) and all CB congeners, despite extractable lipid content in both tissues sampled from both animals being the similar (83.9% and 90.5% in blubber and 2.5% and 2.1% in liver).

A foetus weighing just 3.0g was recovered from a lactating common dolphin (CBC6). This dolphin had elevated levels of DDT (1590.5 ng/g extractable lipid) in blubber and Σ -BHC (2223.5) in the liver. Dieldrin in the adult occurred at 6.3 ng/g extractable lipid in the blubber and 11.1 ng/g in the foetus, DDT at 2.6 (adult) 37.2 (foetus) ng/g and DDE at 174.6 (adult) 25.3 ng/g (foetus). Sum of ICES 7 PCB amounted to 796.3 ng/g extractable lipid in the

blubber of the adult and 135.2 ng/g in the foetus with CB 138, 153 and 180 accounting for 174.7, 220.5 and 304.8 in the adult blubber and 23.1, 36.1 and 45.5 ng/g respectively in the foetus. Concentrations of DDT (3192.0 ng/g) and ICES 7 PCB (9236.9 ng/g extractable lipid) were much higher in the liver of the adult dolphin suggesting there was considerable mobilisation of lipid reserves. A sample of milk from CBC6 was analysed and dieldrin was recorded at 1.5 ng/g extractable lipid, Σ -BHC at 11.1, t-nonachlor at 22.0 and DDT and DDE at 13.8 and 68.7 ng/g respectively (sum 95.5). The sum of ICES 7 PCB was 222.0 ng/g extractable lipid with the congeners CB 138, 153 and 180 accounting for 55.1, 72.3 and 27.6 ng/g respectively. Extractable lipid was only 11.4% in the dolphins milk and 0.8% in the foetus which would account for the relatively low OC and PCB concentrations compared to the adult dolphin.

DISCUSSION

In order to accumulate persistent pollutants through ingestion, intake rate must exceed metabolism and elimination. As both porpoises and dolphins are relatively long lived marine species (mean longevity for harbour porpoises is around 10-12 years and around 20-25 years for common dolphins) they can accumulate high concentrations of organochlorines relative to levels in the environment (Law *et al.* 1995). It has been suggested that marine mammals are good 'averagers' of water quality, especially for persistent pollutants that may occur below detectable levels in seawater (Calmet *et al.* 1992) and have been used as bio-markers in Ireland (Berrow *et al.* 1998). Fish absorb the majority of organic contaminants via an equilibrium partitioning between gill membranes and the surrounding water (Duinker *et al.* 1989). This results in limited variability in the concentrations of OC and PCB accumulation unlike marine mammals which can show large variation within species and between geographical locations. However concentrations of OC residues in marine mammals tissues may vary with a range of physiological factors which must be taken into account when comparing burdens between species and between sites.

CONCENTRATIONS OF ORGANOCHLORINES, PCB AND METABOLITES

Kleivane *et al.* (1995) found PCB levels increased with age in male porpoises but concentrations of PCB for three immature animals and three mature males sampled in the present study did not differ substantially. Concentrations of organochlorine pesticides in common dolphins generally followed the expected patterns for age and sex (Borrell *et al.* 1995; Cockcroft *et al.* 1989) but PCB congeners and OC did not differ greatly between male and female immature dolphins. Dieldrin increased with age in the females until after breeding suggesting that it was not as easily metabolised as the HCB and Σ -BHC isomers. Concentrations of pesticides were high in the immature female sampled (CD5) but declined in the two females that had reproduced, presumably due to transfer of their pollutant burden to their young (Addison and Brodie 1977; 1987). Lower concentrations of HCB, Σ -BHC, lindane and pp-DDT in the same individual suggests that these compounds may have been metabolised and eliminated or that

uptake was insufficient to maintain the concentration and offset the effect of dilution as blubber was laid down. The chlordanes were also quite high in CD5 but were similar to those of mature females CBC6 and CBC8 suggesting little had been transferred to their calves during lactation. Tanabe (1988) showed as much as 90% of the females OC burden could be transferred across the placenta and through lactation and the first descendent often receives the highest amounts of pollutants (Fukushima and Kawai 1981; Cockcroft *et al.* 1989). Cockcroft *et al.* (1990) estimated that 4% transfer of the mother's total contaminant burden was transferred per day, in her milk, to dependant young resulting in 80-85% PCB and 69% DDT of residues transferred within seven weeks post-partem. With such high transfers following first or second ovulation, the first descendent is subjected to a rapid high dosage of PCB and OC soon after birth. The effects of such high doses of contaminants is unknown, however work done on rhesus monkeys ingesting mother's milk with PCB concentrations (0.154-0.397 µg/g) showed signs of intoxication within two months of birth. On this basis Carstens *et al.* (1979) suggested that foetus and neonates were more susceptible to PCB intoxication. Marine mammals have a very small capacity to metabolise organochlorines resulting in potentially deleterious effects at low concentrations (Aguilar and Borrell 1995). Two dolphins (CBC8 and CBC7) were caught together which suggests they may be related. The age of the calf was not determined, however from its length it can be estimated to be 1-2 years. PCB and organochlorine burdens were similar to two other males in this age-class. If this was a first calf this would support that as much as 80% of the adults load is transferred to their calf as PCB and OC in the blubber of CBC8 were between 5-20% of CBC7.

Residue concentrations in blubber are often inversely related to body condition and growth as the potential to store lipophilic pollutants increases as the animal lays down layers of blubber. As fat is metabolised for energy production, the remaining OC become more concentrated and conversely, as fat is laid down any existing OC become diluted (Addison and Smith 1974; Drescher *et al.* 1977; Donkin *et al.* 1981; Van de Zande and Ruiter 1983). Of the two harbour porpoise with the thinnest blubber layer one had elevated levels of some OC and all PCBs but this pattern was not consistent in the other individual. Abnormally high lipid content in the liver is considered to be characteristic of animals exposed to high levels of PCB and OC (Yoshioka 1976) but other factors such as a high fat diet and a variety of diseases may also effect hepatic lipidosiis but there is no evidence of this in the present study. The immature dolphin CD5 had very low extractable lipid in its blubber (47.9%) which may indicate poor condition which may have led to fat mobilisation, however pollutant levels were similar to one sexually mature male (CBC9). One mature male (CD10) had highest concentrations of all PCBs and pesticides although it was younger (by 4 years) than CBC9. This may have been due to the older dolphin having laid down more blubber, diluting the contaminant burden or that CD10 had been exposed to greater concentrations of contaminants. Lipid concentrations were greater in CD10 (90%) than CBC9 (84%) suggesting the latter.

RATIO OF DDT TO DDE

The ratio of DDE to DDT acts as a chronology of contaminant input into the ecosystem. The relative amount of DDE with respect to DDT increases with time following its release into the environment and as DDT moves through trophic webs (Aguilar 1984). DDE is the chief metabolic product of DDT in biological samples and is difficult to degrade, or excrete due to its low water solubility (Aguilar 1984). A high ratio > 0.6 implies that the organism has not been subjected to recent inputs of DDT. The ratio of DDE to DDT increases with age in the females and decreases with maturity, which is thought to be the result of the detoxification activity of the liver and differential transfer across the placenta, but not during lactation (Borrell *et al.* 1995).

The ratio of DDT to DDE in male dolphins, in the present study, ranged from 0.63-0.91 and in harbour porpoises the ratio in males and females ranged from 0.53-0.68. Borrell and Aguilar (1987) speculated that as the total DDT burden increased it was likely that the male enzymatic system was more highly activated, and the rate of conversion of DDT to DDE was enhanced. In all cases in this study the ratio exceeded 0.5 indicating that the DDT inputs were aging in the environment of these dolphins and porpoises and that limited new sources of DDT existed. This is consistent with previous studies in Ireland (Mason and O'Sullivan 1992; 1993) and the fact that DDT is no longer used in the Northern hemisphere.

RATIO OF DDT/PCB

In dolphins, the ratio of DDT to ICES 7 PCB did not present any clear trend in immature animals and mature males. In mature females the ratio drops after reproductive transfer, as seen in CBC6 (0.31) and CBC8 (0.52), compared to the ratio in the immature female CD5 (0.90). Borrell and Aguilar (1987) found that the ratio decreased with age in females but this trend was probably the result of different transfer rates of PCB and DDT during gestation and lactation. The highly lipophilic chemicals such as PCB were found to be less transferable from the pregnant female to the foetus than DDT (Borrell and Aguilar 1987). This would favour a decrease in the ratio of DDT to PCB as a female reproduces successively and grows older. This differential transfer has been observed in other species of marine mammals (Addison and Brodie, 1987; Holden 1978; Kawai *et al.* 1981; Tanabe *et al.* 1982). No trend for DDT to PCB ratios were seen in porpoises. In females ratios ranged from 0.41 to 0.83 in the immature sample and ranges in the males were from 0.41 to 0.95 not necessarily increasing relative to age.

CONCENTRATIONS OF CHLORDANE

Technical chlordane contains a number of distinct compounds, the most notable of which are two stereoisomers, alpha-chlordane (cis-isomer) and gamma-chlordane (trans-isomer). The remainder of the technical material comprises

of isomers of chlordane; chlordene; heptachlor; nonachlor; and multiple other OC compounds. The chlordane compounds looked at in this study were alpha-chlordane and gamma-chlordane and trans-nonachlor. Trans-nonachlor was the major metabolite detected in all the dolphins, contributing to 70-80% of the total chlordane concentrations. Trans-nonachlor is metabolized to oxy-chlordane, however the low percentage of oxy-chlordane found in cetaceans from other studies suggested that cetaceans have a relatively low capacity to metabolize trans-nonachlor compared to marine birds (Muir *et al.* 1992).

Alpha-chlordane and gamma-chlordane were at much lower concentrations in all the animals, they form a major proportion of technical chlordane. Both chlordane isomers have been shown to be transformed to oxychlordane via the trans-nonachlor route in mammals, with gamma-chlordane being oxidised more rapidly than alpha-chlordane (Tashiro and Matsumura 1977). This is consistent with the findings of this study in that alpha-chlordane was at a higher concentration in every animal than the gamma-isomer. Accumulation of chlordanes occurred with age, increasing in immature animals and adult males and decreasing in mature females as evidenced by the both species in this study.

COMPARISON WITH OTHER SITES IN EUROPE *Harbour porpoise* " \ 3 Generally contaminant levels in harbour porpoises caught off the south-west coast of Ireland were similar to that reported from Shetland Islands (Law and Allchin 1994) and the Moray Firth (MAFF 1994) suggesting these are relatively clean marine environments. Male porpoises from the Shetland Islands have slightly higher overall concentrations of PCB, DDT and DDE metabolites (Table 4) which may be due older age profile of the animals analysed. Levels of DDE and PCB in females were relatively high in Irish porpoises but this may also be age related as the Irish porpoises analysed were generally sexually immature individuals. A recent study by Kleivane *et al.* (1995) determined OC residues and PCB in harbour porpoises from 3 locations along the Norwegian and Danish coasts. Although our sample is relatively small a similar distribution of age-classes were analysed. Scandinavian porpoises accumulated much higher concentrations of pesticides (γ -BHC: 250 ng/g extractable lipid, lindane: 190 ng/g), cyclodienes (dieldrin: 3050 ng/g, t-nonachlor: 1720 ng/g) as well as DDT (sum 16390 ng/g) and PCB (sum 23270 ng/g). Ranges from Ireland were close to the minimum values recorded by Kleivane *et al.* (1995). Even allowing for the fact that the PCB concentration in the Norwegian sample was based on the sum of 47 detected PCB, the major PCB congeners 147/123, 153, 138/163/164, 182/187, 180 represented 44-53% of the total sum, so that even if we halve their values, PCB was still far in excess of those levels recorded this study. The inputs of dieldrin into the Irish environment would seem to be quite small relative to Scandinavia, as were γ -BHC and lindane. Lindane is predominant in both studies suggesting there has been recent marine inputs, since it is rapidly transformed into γ -HCH in nature. The only source of chlordane in Ireland is from atmospheric transport most likely from America and Canada but the level of t-nonachlor in our study, at its maximum, is only a fifth of that from porpoises caught off the Norwegian coast (Kleivane *et al.* 1995).

A comparison of mono-ortho CB (CB105, CB118 and CB156) values to those presented by de Boer *et al.* (1993) and Wells *et al.* (1994) showed that overall levels (680, 391 and 11 ng/g wet weight) were similar to animals

from the North Sea (210, 690 and 18 ng/g wet weight) and Scotland (129, 408 and 5 ng/g wet weight) though differences in individual congeners (e.g. CB 105 and CB CB118) were apparent.

Common dolphin

Organochlorine levels in common dolphins stranded along the UK coast between 1988 and 1992 (MAFF 1994) were considerably higher (DDE: 7109 - 9132 ng/g extractable lipid, n = 34) than levels reported from Irish waters (Tables 3a and 3b) however the cause of death of these animals was not known. Values for ICES 7 PCB were also elevated (22366 - 27126 ng/g extractable lipid) in the UK study which would indicate that there is much less exposure to PCB around Ireland. Concentrations were close to those recorded (DDE: 2664 - 6556 ng/g extractable lipid, sum PCB: 4936 - 7800 ng/g extractable lipid) in a population of bottlenose dolphins *Tursiops truncatus* in the Moray Firth, Scotland (Law and Allchin 1994) but well below values (DDE: 61111 - 41000 ng/g extractable lipid, sum PCB: 110000 - 90000 ng/g extractable lipid) in the same species in Cardigan Bay, Wales (Law and Allchin 1994). The Welsh study recorded concentrations in bottlenose dolphins amongst the highest recorded anywhere in the world.

Mono-ortho PCB congeners (CB105, CB118, CB156) in common dolphins from Irish waters (116, 387 and 23 ng/g extractable lipid) were of the same order of magnitude (305, 359 and 21 ng/g extractable lipid) as those obtained by de Boer *et al.* (1993) from the west coast of Ireland in 1992. Mono-ortho PCB are toxicologically important because of their similarity in structure to TCDD (2,3,7,8-tetrachloro dibenzo-p-dioxin). These dioxin like CB congeners are capable of inducing the production of aryl-hydrocarbon hydroxylase and ethoxy resorufin 0-diethylase or thymic atrophy in an analogous fashion. Levels in both bottlenose dolphins from Scotland (290, 324 and 24 ng/g extractable lipid) (Wells and Echarri 1992) were of similar magnitude to common dolphins in the present study but levels in white-beaked dolphins *Lagenorhynchus albirostris* stranded on the Dutch coast (410, 1800 and 350 ng/g extractable lipid) (de Boer 1993) were up to two orders of magnitude higher. Cockcroft *et al.* (1990) reported DDT concentrations of 4000 ng/g wet weight in immature bottlenose dolphins from southeast Africa and 6400 and 900 ng/g in mature males and females and PCB concentrations of 900 - 6400 ng/g wet weight which are around 25-50% lower than that reported from Ireland. Concentrations of total ICES 7 PCB ranged from 773 to 63424 ng/g wet weight and DDT 160 to 54550 wet weight in white-sided dolphins live stranded in County Mayo, Ireland and washed up on the Scottish coast but concentrations were highly dependant on age, sex, reproductive and nutritional condition (McKenzie *et al.* 1998) but are still considerably higher than reported here.

COMPARISON WITH OTHER MAMMALS IN IRELAND

The only survey in Ireland of PCB concentrations in marine mammals was carried out by Nixon, (1991) on common seals after an out break of phocine distemper virus. Overall levels of all organochlorines were higher in dolphins and

porpoises sampled compared to seals but concentrations of the ICES 7 PCB ranged from 994-10664 ng/g extractable lipid basis for females which are similar to those recorded in common dolphins (798-11074 ng/g extractable lipid) and harbour porpoises (3041-12270 ng/g extractable lipid for female animals) in this study. These values are similar despite ecological differences including diet, habitat and metabolism. Levels of persistent pollutants in cetaceans off the east coast of Ireland are likely to be much higher (e.g. Berrow *et al.* 1998; Troisi *et al.* 1998) than off the west coast. Concentrations of dieldrin, DDE and PCB in marine mammals were much higher than that reported in terrestrial mammals (dieldrin: 0.37 - 14.70 ng/g extractable lipid, DDE: 0.60 - 42.60 and total PCB: 2.00 - 215.0) in Ireland (Mason and O'Sullivan 1992; 1993) but further studies are required before the extent and impact of these pollutant burdens can be assessed.

The persistent OC and PCB quantified in this study are known to have weak estrogenic capabilities and concern exists over the capability of these synthetic compounds to induce reproductive effects. It is likely that the fish diet of dolphins and porpoises plays a major role in the uptake and maintenance of contaminant burdens in marine mammals in Ireland. Although there is still insufficient knowledge about the feeding behaviour and migratory habits of cetaceans in Irish waters and their prey species, OC concentrations reported here are among the lowest values reported from Europe. These values provide a baseline against which temporal trends in water quality may be determined.

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Figure legends

Fig. 1 - Location of by-caught harbour porpoise and common dolphins sampled.

Table 1. Location, date of incidental capture and biological parameters of harbour porpoises *Phocoena phocoena* and common dolphins *Delphinus delphis* sampled for organochlorine and PCB contamination.

<i>Species</i>	<i>Code</i>	<i>Location of capture</i>	<i>Date of capture</i>	<i>Sex</i>	<i>Length (cm)</i>	<i>Blubber thickness (mm)</i>	<i>Age (years)</i>	
Harbour porpoise	CBC 1	NW Blaskets	32939	F	125	11	2	
	CBC 3	51°08'N, 11°06'W	14/04/94	F	151	17	4	
	CBC 4	Seven Heads	21/04/93	F	124	21	2	
	CBC 15	Loop Head	14/04/94	F	133	17	2+	
	CBC 5 ¹	Sligo Bay	27/04/93	F	117	12	2	
	HP 2/94	V4699	34517	F	115	9	2	
	CBC 2	Loop Head	33972	M	119	9	2	
	CBC 11	51°06' N, 6°43'W	34130	M	121	20	2	
	CBC 12	G5637	18/08/93	M	94	18	1	
	CBC 13	52°09' N, 10°47'W	16/01/94	M	155	22	6	
	CBC 14	51°36'N, 8°20'W	23/01/94	M	130	21	4	
	HP 16	W 5543	33520	M	145	18	ND	
	Common dolphin	CD 5	Blasket Islands	33000	F	186	ND	4
		CBC 6 ²	Loop Head	24/11/93	F	202	15	10
CBC 8 ³		49°04'N, 13°16'W	34067	F	201	10	10	
CBC 9		51°03'N 15°39'W	34280	M	197	14	11	
CD 9		45°50'N, 15°55'W	33487	M	145	ND	2	
CD 10		52°45'N, 10°09'W	33396	M	214	ND	7	
CBC 7 ³		49°04'N, 13°16'W	34067	M	129	10	ND	
CBC 10		48°52'N, 13°49'W	17/09/93	M	149	ND	36161	

¹ lactating ² pregnant, ³ possible mother and calf, ND - Not determined

Table 2a. Mean (\pm SD) organochlorine burden of harbour porpoise *Phocoena phocoena* from Irish coastal waters. Concentrations expressed as ng per g extractable lipid, ranges in parentheses.

		<i>Blubber</i>		<i>Liver</i>	
		<i>Male (n = 6)</i>	<i>Female (n = 6)</i>	<i>Male (n = 6)</i>	<i>Female (n = 6)</i>
Lipid	(%)	96 \pm 4 (88 - 97)	81 \pm 16 (57 - 93)	5 \pm 1 (4 - 8)	5 \pm 1 (4 - 7)
HCH - group	∞ - BHC	54 \pm 25 (28 - 99)	128 \pm 101 (32 - 315)	29 \pm 499 (20 - 56)	32 \pm 25 (8 - 79)
	lindane	121 \pm 100 (16 - 256)	154 \pm 84 (73 - 282)	32 \pm 17 (12 - 56)	24 \pm 25 (2 - 57)
Cyclodienes	∞ - Chlordane	205 \pm 42 (137 - 235)	419 \pm 303 (160 - 978)	124 \pm 77 (61 - 168)	158 \pm 168 (7 - 417)
	∞ - Chlordane	27 \pm 11 (8 - 42)	59 \pm 51 (19 - 158)	13 \pm 5 (6 - 30)	16 \pm 10 (7 - 29)
	☺ - Nonachlor	897 \pm 462 (323 - 1558)	1270 \pm 487 (429 - 1719)	351 \pm 211 (151 - 755)	428 \pm 389 (10 - 1025)
	Dieldrin	121 \pm 174 (5 - 401)	116 \pm 175 (6 - 175)	6 \pm 454 (13 - 10)	3 \pm 1 (3 - 4)
DDT - group	p,p' - DDT	527 \pm 246 (249 - 927)	1003 \pm 537 (360 - 1690)	48 \pm 24 (18 - 76)	58 \pm 19 (30 - 75)
	p,p' - DDE	1943 \pm 721 (1178 - 3124)	2593 \pm 913 (926 - 3658)	897 \pm 456 (583 - 1750)	2253 \pm 2831 (32 - 7692)
	o,p' - DDE	12 \pm 6 (8 - 24)	18 \pm 14 (9 - 45)	9 \pm 3 (4 - 13)	99 \pm 214 (7 - 536)
	p,p' - DDD	665 \pm 211 (325 - 880)	1050 \pm 375 (342 - 1348)	300 \pm 148 (114 - 552)	487 \pm 519 (11 - 1414)
	sum - DDT	346 \pm 1132 (1838 - 4941)	4664 \pm 1581 (1640 - 5989)	1246 \pm 614 (770 - 2392)	2878 \pm 3310 (43 - 8910)

Table 2b. Mean (\pm SD) PCB burden of harbour porpoise *Phocoena phocoena* from Irish coastal waters. Concentrations expressed as ng per g lipid, ranges in parentheses.

<i>Congener</i>	<i>Blubber</i>		<i>Liver</i>	
	<i>Male (n = 6)</i>	<i>Female (n = 6)</i>	<i>Male (n = 6)</i>	<i>Female (n = 6)</i>
CB 28	22 \pm 11 (10 - 34)	22 \pm 6 (15 - 31)	13 \pm 2 (9 - 15)	27 \pm 40 (2 - 106)
CB 31	1.0 \pm 1.0 (0.5 - 3.0)	-	2.0 \pm 1.0 (1.0 - 3.0)	-
CB 52	351 \pm 90 (236 - 498)	452 \pm 147 (239 - 695)	193 \pm 43 (130 - 247)	242 \pm 174 (5 - 514)
CB 101	300 \pm 204 (189 - 715)	348 \pm 79 (280 - 500)	179 \pm 129 (68 - 428)	250 \pm 213 (13 - 612)
CB 105	68 \pm 38 (41 - 141)	84 \pm 16 (55 - 101)	43 \pm 25 (21 - 86)	50 \pm 33 (3 - 104)
CB 118	391 \pm 244 (255 - 880)	425 \pm 90 (333 - 351)	268 \pm 168 (99 - 168)	304 \pm 205 (11 - 601)
CB 138	1957 \pm 933 (860 - 3136)	2534 \pm 1146 (876 - 4087)	1108 \pm 484 (519 - 1863)	1708 \pm 1495 (29 - 3935)
CB 153	2583 \pm 1280 (1076 - 4320)	3342 \pm 1593 (983 - 5449)	1719 \pm 781 (760 - 2946)	3161 \pm 2029 (751 - 6211)
CB 156	11 \pm 16 (4 - 33)	8 \pm 1 (8 - 10)	6 \pm 2 (5 - 9)	5 \pm 2 (1 - 8)
CB 180	545 \pm 267 (248 - 988)	875 \pm 417 (253 - 1371)	595 \pm 294 (208 - 1001)	967 \pm 966 (23 - 2624)
Sum ICES 7	6148 \pm 2802 (2911 - 10429)	7999 \pm 3282 (3041 - 12270)	4075 \pm 1875 (1798 - 7032)	6659 \pm 4582 (2148 - 13867)

Table 3a. Mean (\pm SD) organochlorine burden of common dolphin *Delphinus delphis* from Irish coastal waters. Concentrations expressed as ng per g extractable lipid, ranges in parentheses.

		<i>Blubber</i>		<i>Liver</i>	
		<i>Male (n = 5)</i>	<i>Female (n = 3)</i>	<i>Male (n = 4)</i>	<i>Female (n = 3)</i>
Lipid	(%)	90 \pm 5 (84 - 95)	76 \pm 25 (48 - 95)	3 \pm 1 (3 - 4)	4 \pm 3 (2 - 8)
HCH - group	⌘ - BHC	41 \pm 24 (32 - 315)	37 \pm 50 (58 - 145)	54 \pm 17 (35 - 76)	33 \pm 20 (11 - 49)
	lindane	45 \pm 47 (69 - 256)	42 \pm 65 (130 - 282)	32 \pm 36 (1 - 82)	16 \pm 19 (4 - 38)
Cyclodienes	⌘ - Chlordane	302 \pm 221 (160 - 978)	209 \pm 329 (308 - 480)	306 \pm 213 (116 - 539)	240 \pm 202 (13 - 400)
	⌘ - Chlordane	66 \pm 21 (19 - 158)	30 \pm 45 (40 - 65)	49 \pm 15 (34 - 67)	23 \pm 22 (7 - 45)
	⊙ - Nonachlor	1543 \pm 1237 (429 - 1719)	685 \pm 1062 (1378 - 1622)	900 \pm 728 (223 - 1681)	592 \pm 508 (31 - 1020)
	Dieldrin	20 \pm 21 (5 - 454)	8 \pm 2 (6 - 158)	23 \pm 3 (21 - 26)	-
DDT - group	p,p' - DDT	932 \pm 871 (334 - 585)	598 \pm 942 (974 - 1690)	91 \pm 45 (25 - 118)	60 \pm 10 (53 - 67)
	p,p' - DDE	7674 \pm 5273 (926 - 2938)	2381 \pm 3734 (2633 - 3658)	3713 \pm 3160 (654 - 7046)	1721 \pm 1448 (113 - 2922)
	o,p' - DDE	89 \pm 41 (11 - 24)	37 \pm 48 (9 - 45)	46 \pm 21 (23 - 74)	11 \pm 10 (4 - 18)
	p,p' - DDD	749 \pm 627 (342 - 1287)	534 \pm 841 (1011 - 1348)	678 \pm 518 (205 - 1181)	625 \pm 534 (14 - 1009)
	sum - DDT	9444 \pm 6812 (2385 - 15115)	3998 \pm 9951 (244 - 12743)	4528 \pm 3700 (999 - 8306)	2293 \pm 1987 (131 - 3857)

Table 3b. Mean (\pm SD) PCB burden of common dolphin *Delphinus delphis* from Irish coastal waters. Concentrations expressed as ng per g lipid, ranges in parentheses.

<i>Congener</i>	<i>Blubber</i>		<i>Liver</i>	
	<i>Male (n = 5)</i>	<i>Female (n = 3)</i>	<i>Male (n = 4)</i>	<i>Female (n = 3)</i>
CB 28	21 \pm 1 (15 - 31)	18 \pm 24 (3 - 45)	16 \pm 7 (7 - 22)	6 \pm 7 (2 - 13)
CB 31	2 \pm 1 (1 - 4)	3 \pm 3 (1 - 6)	3 \pm 3 (1 - 6)	2 \pm 1 (1 - 3)
CB 52	306 \pm 299 (77 - 817)	211 \pm 353 (5 - 619)	164 \pm 3 (40 - 499)	218 \pm 199 (4 - 390)
CB 101	411 \pm 315 (221 - 970)	399 \pm 633 (22 - 1129)	321 \pm 296 (122 - 759)	250 \pm 213 (13 - 612)
CB 105	111 \pm 80 (57 - 251)	100 \pm 161 (5 - 286)	67 \pm 64 (25 - 161)	49 \pm 59 (4 - 148)
CB 118	155 \pm 245 (178 - 822)	333 \pm 529 (18 - 944)	245 \pm 228 (90 - 582)	305 \pm 272 (10 - 566)
CB 138	2547 \pm 1891 (399 - 4874)	1144 \pm 1696 (155 - 3102)	1104 \pm 1390 (246 - 3182)	1410 \pm 1303 (40 - 2621)
CB 153	3030 \pm 2758 (478 - 2257)	1530 \pm 2257 (221 - 8943)	1548 \pm 1938 (348 - 4445)	2156 \pm 2049 (58 - 4148)
CB 156	22 \pm 19 (9 - 55)	20 \pm 29 (43 - 54)	19 \pm 16 (9 - 43)	19 \pm 15 (1 - 36)
CB 180	1300 \pm 906 (182 - 2105)	590 \pm 443 (305 - 1100)	678 \pm 651 (253 - 1646)	763 \pm 670 (60 - 1395)
Sum ICES 7	8945 \pm 5945 (1555 - 15883)	4225 \pm 5932 (798 - 11074)	4076 \pm 4726 (1112 - 11135)	5051 \pm 4589 (187 - 9237)

Table 4. Comparisons in concentrations of OC and PCB (expressed as ng per g wet weight) in the blubber of harbour porpoises *Phocoena phocoena* from the UK and Ireland.

<i>Organochlorine</i>	<i>Ireland</i>	<i>Moray Firth</i> ¹	<i>Shetland Islands</i> ²
	<i>Female - Male</i>	<i>Female - Male</i>	<i>Female - Male</i>
Dieldrin	31 - 51	419 - 1559	-
HCB	400 - 357	166 - 444	340 - 410
DDE	2207 - 1771	688 - 1960	1502 - 3002
sum DDT	3823 - 3011	1377 - 4180	-
ICES 7 PCB	6574 - 5697	1240 - 5500	3320 - 7887

¹ Law and Allchin (1994), ² MAFF (1994)