Slipping Away

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# Swimming in Chemicals

## Widespread presence of brominated flame retardants and PCBs in eels (Anguilla anguilla) from rivers and lakes in 10 European countries

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Technical Note 12/2005/ October 2005

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# Preface

European eels from 20 locations in 10 countries across Europe donated by members of the fishing and science community or purchased in local markets were found to contain varying levels of brominated flame retardants (BFRs) and/or polychlorinated biphenyls (PCBs). Some of the chemicals analysed are in current use while others have been prohibited either in recent years or, as in the case of PCBs, more than 20 years ago. The results provide a snapshot of hazardous chemicals in eels from a random selection of urban and rural fresh and brackish ecosystems and indicate the degree to which their habitat has been contaminated with the target substances.

This study shows that contamination of freshwater ecosystems with persistent and bioaccumulative man-made chemicals remains a problem in Europe and indicates the failure of regulations, past and current, to control chemical pollution of our environment. The results further give insight into the chemical contamination of a European species whose population is now in rapid decline. Findings highlight the need for precautionary measures, the early identification of chemical contaminants and the substitution of the most hazardous substances with safer alternatives.

The European eel (Anguilla anguilla) is an important part of fresh and brackish water ecosystems. Capable of living up to 20 years, it spends a great deal of this long lifespan in localised habitats. As a relatively fatty species, the European eel absorbs and concentrates the bioaccumulative organic pollutants that may be present in lower concentrations in its diverse diet of crustaceans, worms, snails, larvae and even small fish. For these reasons eels have long been recognized as a "bioindicator" species able to reveal the contaminants present in local habitats.

As well as providing insight into the environmental quality of their freshwater ecosystems, the presence of hazardous chemicals in the European eel is significant because of the potential adverse impact on the species itself. European eel populations are in severe decline across the continent and a precautionary approach towards protecting this species is critical for its survival. In some European waters, numbers of young eels are estimated to be as low as 1 % of historic levels.

The mysterious lifecycle of the European eel raises questions as to what impacts hazardous chemicals have on its reproductive cycle. Eels reach sexual maturity at the end of their life, when they return to sea to breed and die. Some scientists have theorized that the hazardous chemicals stored in the sexually maturing eels' body fat may be mobilized during this final stage of migration and breeding, releasing large quantities of chemicals that could undermine successful reproduction and healthy offspring.


This study does not attempt to provide definitive answers to these questions. Rather our hope is that it will provide a substantial piece of the puzzle regarding a little understood species and provide important information about the legacies of poor environmental regulation, past and present. To our knowledge, this represents geographically the most extensive body of data to date on the distribution of the commonly used brominated flame retardant HBCD in an aquatic species.

Other analyses commissioned by Greenpeace over recent years have confirmed the presence of substances with hazardous properties in consumer products, such as electronics, toys, cosmetics, and textiles. Chemical additives may leak out of products over time and Greenpeace has found such chemicals are typically present in house dust in European homes, in rainwater and in umbilical cord blood. This current study adds to this work by documenting the environmental distribution of a subset of tracked chemicals, some of which are prohibited and others still in routine use, notably HBCD, and showing their presence in European freshwater ecosystems.

Recognizing the need for better chemicals control, the European Union (EU) is now debating a new legislative proposal known as REACH (Registration, Evaluation, Authorisation of Chemicals). In the autumn of 2005, politicians and governments will take important decisions to either strengthen or weaken this proposal for greater protection from hazardous chemicals. Hopes are high among green, health, consumer, women's and labour organizations for this long awaited reform. Yet a number of politicians and governments have proposed to exempt requirements for industry to provide basic toxicity information for 2/3 or more of the 30,000 chemicals that would fall under the scope of REACH.

To be effective REACH must require industry to identify and provide basic health, safety and environmental data currently lacking for the majority of marketed chemicals. Only then can we identify the chemicals that are persistent, bioaccumulative, that may cause cancer, birth defects, reproductive illnesses or harm future development and fertility by disrupting hormonal functions. For REACH to drive solutions, it must then require the phase-out and substitution of these most hazardous substances wherever possible, authorizing their use only on a time-limited basis and when absolutely necessary.

Greenpeace urges European leaders to heed the lessons learned from poor regulations of the past. Chemicals such as PCBs still contaminate European wildlife and ecosystems despite discontinued use as early as the 1970s. The uncertain future of the European eel throws the fragility of biodiversity into contrast against long lasting chemical persistence.

A strong REACH offers a mechanism to prevent chemical contamination before it occurs. Greenpeace calls on the European Union to show leadership and support legislature that will keep our freshwater ecosystems and wildlife healthy and protect us all for future generations.

Helen Perivier
Greenpeace International

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# Executive Summary

A study of 20 pooled samples of muscle tissue from European eels (Anguilla anguilla) which had recently been caught in rivers or lakes in 10 countries across Europe[1] during the summer of 2005 demonstrated the widespread presence of some brominated flame retardants (including tetra- and pentabrominated diphenyl ethers, or PBDEs, and hexabromocyclododecane, or HBCD) in this species. Results also indicated that PCBs remain a significant contamination issue in many water bodies.

To our knowledge, this is the most geographically extensive survey to date of the presence of brominated flame retardants in the European eel and should add significantly to the body of data both on the distributions of these persistent environmental contaminants and on the chemical body burden of this species. Since the study was based on the analysis of only 20 pooled samples, it clearly does not represent an exhaustive survey, nor give levels representative of all catchments in those countries as a whole. Rather the data provide a snapshot of contaminant levels across Europe in what is a keystone species in many aquatic ecosystems, and a species under severe threat from a number of other pressures including overfishing, habitat loss, parasite outbreaks and climate change.

Levels of tetra-BDE (BDE-47) varied from <0.1 ppb (ng/g fresh weight), in one of two samples from France and one of two from Ireland, to 46 ppb (256 ppb on a fat weight basis) in a single sample from the River Thames in the UK. Concentrations of penta-brominated congeners (BDE-99 and 100) were generally slightly lower. Higher brominated congeners (hexa- to octa-) were detected less frequently at concentrations above 0.1 ppb, probably as these partition more readily to liver tissue than to muscle. Overall, these levels and patterns of contamination are similar to those reported for eels and other species in the limited number of other (more regionally focused) studies available, other than where major industrial point sources of these chemicals are present.

Tissues concentrations of HBCD were of a similar order (<1 - >50 ppb fresh weight, nd - >278 ppb lipid weight), with the UK sample again showing the highest levels (quantitation subject to confirmation), and two samples showing no detectable residues (one of two samples from the Czech Republic and again one of two from Ireland). Intermediate concentrations were found in samples from other regions, with some evidence for higher levels in samples from predominantly industrial or urban locations than in those from more rural catchments. Taken together, these data indicate that the ranges of levels previously recorded over more limited geographical areas (other than those studies conducted downstream from plants manufacturing or using brominated flame retardants) may be typical for eel populations across Europe.

Although action has recently been taken across Europe to stop the use of "penta" and "octa" BDE formulations, the legacy of their previous use (in existing products and already in the environment) can be expected to continue for some time. Some evidence suggests that "deca-BDE", still widely used within Europe, can degrade in the environment to form some of the less-brominated (and more bioaccumulative) forms. HBCD also remains in continued use despite its recognised aquatic toxicity and potential hormone disrupting properties.

[1] Belgium, Czech Republic, France, Germany, Ireland, Italy, Netherlands, Poland, Spain, and UK


Tetrabromobisphenol-A (TBBP-A) was not detected in any of the samples, though this may result from the higher detection limits (3-5 ppb) achievable in this study. Other studies have previously reported the presence of TBBP-A residues in fish muscle, though at levels below 3 ppb. Residues of deca-BDE (BDE-209) were not analysed for in the current study.

Although concentrations varied widely, data from the current study also reaffirmed the ongoing legacy of contamination with PCBs, commonly present at concentrations between 10 and 50 times higher than for the tetra- and penta-BDEs and HBCD. Highest concentrations (expressed as the sum of the ICES 7 congeners) were recorded for one of three samples from the Netherlands (Hollandsdiep), at over 1500 ppb fresh weight and almost 10 000 ppb (10 parts per million) fat weight.

This study demonstrates once again that the risks presented by persistent and bioaccumulative chemicals cannot be deemed to be adequately controlled. At the same time, the continued presence, and in some cases high levels, of PCBs in the eel tissues, despite the fact that their use was prohibited more than twenty years ago, illustrate the very long-term consequences of recognising a problem too late, or at least of acting too late.

There appears to be no clear relationship between contaminant levels (of PBDEs, HBCD or PCBS) and the average lengths or weights of the pooled eel samples, despite the fact that concentrations might have been expected to have varied with age. This awaits confirmation when age data are available. At this stage, however, the lack of any consistent pattern suggests that local levels of contamination in the aquatic environment may be a more dominant factor than body size in determining relative tissue levels in the eels

It is not possible to determine from these data what the consequences of such contamination might be for the eels themselves. Although there are indications that, during the sexually immature ("yellow eel") life stage, eels are commonly able to tolerate high levels of chemical pollution, what impact this might have when fat reserves are mobilised as the eels reach maturity and migrate to the open ocean to spawn is simply not known. The possibility remains that pollutants such as PCBs and brominated flame retardants may be contributing to the observed declines in European eel populations by reducing adult survival or spawning success.

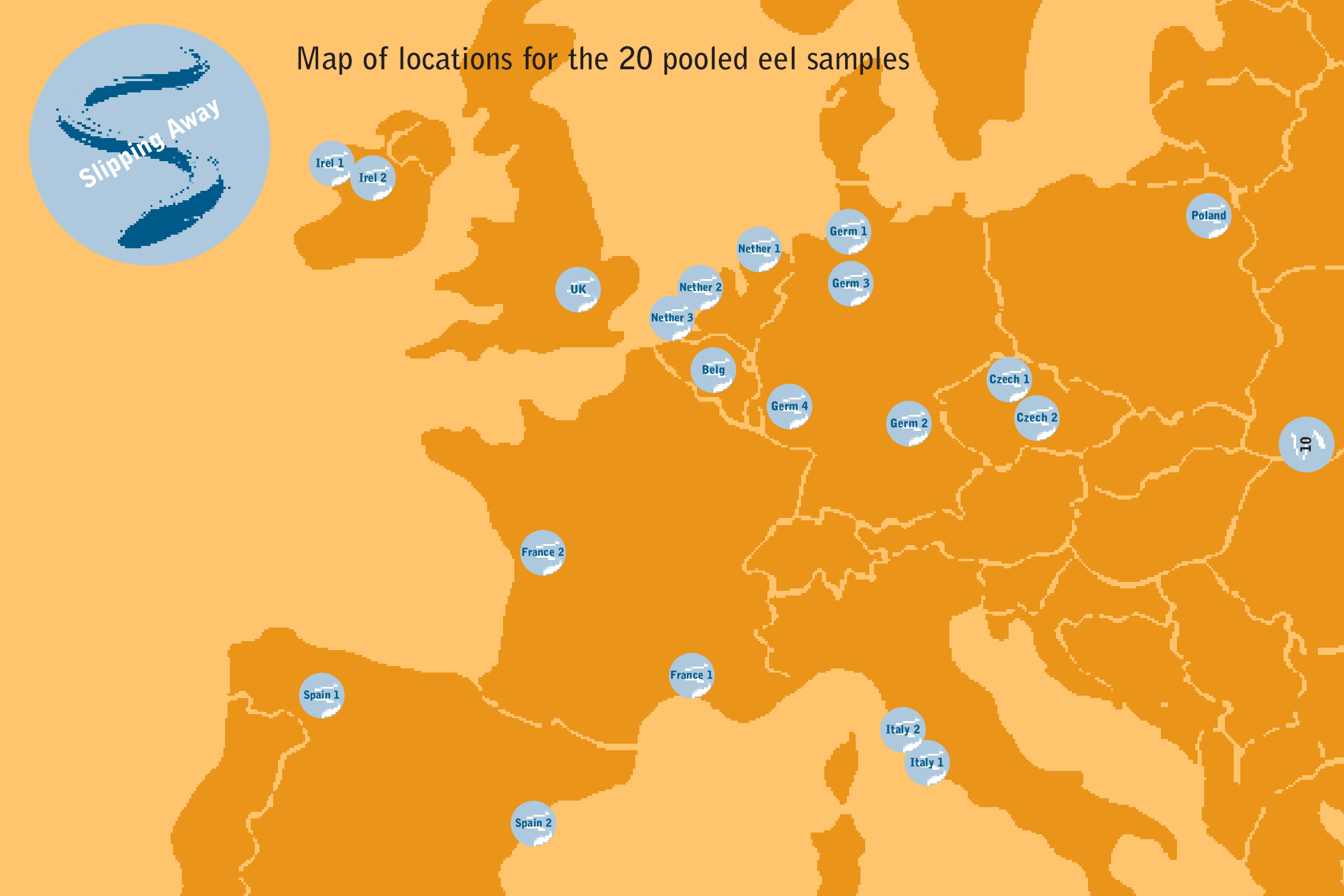
Similarly, the scale of the threat to consumers of eels, whether their natural predators or human consumers, is also unknown. Although some studies have attempted to calculate risks, often in terms of margins of safety, these assessments are inevitably limited by the lack of data on the effects of long-term, low level exposure to chemicals such as HBCD.

History tells us that the consequences of large-scale use of chemicals which are persistent and bioaccumulative, though difficult to predict, are all too often severe. Once in the environment, the fate and effects of these chemicals cannot be controlled. With the new chemicals legislation under development in Europe (REACH), there is an opportunity for all European countries to begin to address the problem of persistent and bioaccumulative chemicals effectively by requiring that such chemicals be replaced with less hazardous alternatives wherever and whenever those alternatives exist (the principle of substitution) and by ensuring that no chemicals are marketed in the future unless information on these and other basic properties is available (the principle of "no data, no market").

All components of the environment stand to benefit from such controls. While it is unlikely that actions on chemicals alone will be sufficient to reverse the demise of the European eel, such action will remain one essential component of a precautionary approach to their protection.



# Map of locations for the 20 pooled eel samples



# Introduction

Alongside the ongoing development of analytical methods has come increasing recognition of the significance of brominated flame retardants as widespread environmental contaminants (see de Wit 2002 and Law et al. 2003 for recent reviews). Brominated flame retardants are a diverse group of man-made bromine-containing chemicals which are either incorporated into materials such as polymers or used to treat textiles and other products in order to make them resistant to the break-out and spread of fire. As such they currently serve an important role in product fire safety, albeit at the expense of substantial environmental releases and health concerns and notwithstanding the growing availability of non-brominated (often halogen-free) alternatives which serve the same function.


# What are brominated flame retardants?

The term brominated flame retardants encompasses more than 70 individual chemicals or chemical groups, although the bulk of global production (estimated in 1999 to be in excess of 200 thousand tonnes per year) currently focuses on just three main groups, namely the polybrominated diphenyl ethers (PBDEs), tetrabrominated bisphenol-A (TBBP-A) and hexabromocyclododecane (HBCD) (Alaee et al. 2003).

Within the PBDE group, many individual chemical forms are possible, ranging from those with a single bromine atom (monobromodiphenyl ethers) to a form with the maximum of ten bromine atoms (decabromodiphenyl ether). Three commercial PBDE formulations have dominated the market, namely "penta", rich in tetra and pentabrominated forms, "octa", rich in octabrominated forms, and "deca", comprising almost exclusively the decabrominated form (often termed BDE-209). While concerns over the toxicity of the "penta" and "octa" formulations, coupled with the documented exponential accumulation of "penta"-related forms in human breast milk, have resulted in bans on the marketing and use of these formulations in Europe (EU 2003), their manufacture and use continues in North America, Asia and other parts of the world (Alaee et al. 2003). Within Europe, "deca" is now the only PBDE produced commercially. Nevertheless, because of their resistance to degradation, tetra and penta forms may be expected to persist in the environment for some time to come, especially as these forms (in common with HBCD) have a strong tendency to build up in fatty tissues of animals. Furthermore, it is possible that, once released to the environment, "deca" may become progressively debrominated, contributing further to environmental levels of the more bioaccumulative lower-brominated forms (Sellström et al. 1998).

Another group of brominated flame retardants, the polybrominated biphenyls (PBBs) had widespread use in the past, and are undoubtedly still present in some older products, but have been prohibited from manufacture and use in most countries for some time.

The PBDEs and HBCD are commonly used in additive mode, i.e. simply blended with polymers, such that their propensity to leach out of products during use and after disposal is particularly high. Although TBBP-A can also be used as an additive flame retardant, it is more often used in reactive mode, such that it becomes much more tightly bound to the polymer matrix and consequently less mobile. Nevertheless, research has confirmed the presence of residues of all of these three main groups of brominated flame retardants in the environment, including in animals.

## Toxicity concerns

Along with the recognition of their widespread presence in environmental samples and, in the case of HBCD and some PBDEs, an ability to bioaccumulate in animal tissues to much higher levels than in the surrounding water, air, soil or sediment, have come increasing concerns regarding the potential toxicity of these chemicals. Overall, toxicity data for the brominated flame retardants remain very limited (Vos et al. 2003, Birnbaum and Staskal 2004), not least because the problems they present have been recognised only relatively recently, while analytical methods remain very much under development (Covaci et al. 2003). Nevertheless, those data which are available suggest a range of potential adverse effects in humans and wildlife. Along with the acute toxicity of some forms to aquatic life, which for HBCD seems relevant even at low concentrations, there is growing evidence to suggest impacts of several PBDEs and HBCD on neurobehavioural development in mammals. There are also indications that elements of the thyroid hormone system may be susceptible to exposure to PBDEs, HBCD and TBBP-A (Darnerud 2003, Legler and Brouwer 2003, Birnbaum and Staskal 2004; see also Boxes on pages 38 and 39).


# Environmental trends in flame retardant contamination

Within freshwater and marine systems, residues of PBDEs and, more rarely, HBCD and TBBP-A have previously been reported in sediments and in biota (both invertebrate and vertebrate animals, including fish). Concentrations are still generally significantly below those of some of the better known "legacy" persistent organic pollutants, notably the polychlorinated biphenyls (PCBs) historically used in transformer fluids and a variety of other industrial and commercial applications until their phase-out in the late 1970s. However, increasing evidence suggests that, whereas levels of PCBs in most environmental compartments have declined substantially in the decades since their phase-out, levels of brominated flame retardants may be showing the opposite trend, at least in some species and regions, especially in top predators (Hites 2004, Lebeuf et al. 2004, Elliott et al. 2005, Klamer et al. 2005).

A small number of intensive local studies on freshwater and/or marine systems are available, most notably those on the Western Scheldt estuary in Belgium (Voorspoels et al. 2003, Janak et al. 2005), the Tees estuary in the UK (Allchin et al. 1999, Food Standards Agency 2004) and the River Viskan in Sweden (Sellström et al. 1998). In these cases, however, the focus has largely been on determining impacts from specific point sources (e.g. brominated flame retardant manufacturing sites in the case of the Scheldt and Tees and several textile plants using these compounds in the Scheldt and Viskan). Boon et al. (2002) provide a more general overview of levels of PBDEs in animals representing different levels in the food web in the North Sea, confirming the biomagnification of six of these chemicals from fish to marine mammals, while Law et al. (2002) report PBDE levels in 47 cormorants and 60 harbour porpoises from various locations around England and Wales. More recently, Lacorte et al. (2003) reported PBDE levels in 40 samples representing the eight major river basins of Portugal, though data are for sediments only. Studies which include analysis for HBCD and TBBP-A are becoming more frequent, though data sets for these chemicals are still inevitably far smaller than for the PBDEs.

## The bigger picture

Moreover, for all brominated flame retardants, determination of more widespread geographical distributions and trends remains limited. Given the likely long-term persistence of these chemicals in the aquatic environment, their potential toxicity and capacity to bioaccumulate and their ongoing high volume production and use, there is an urgent need for more information concerning their levels and trends in environmental samples over wider areas. The value of broader geographical surveys was exemplified recently by Ueno et al. (2004), for example, using muscle of skipjack tuna (a species with a global distribution) to demonstrate the ubiquitous presence of PBDEs even in species frequenting offshore waters and apparent higher levels in specimens from the northern hemisphere (possibly reflecting differences in scale of uses and releases).

# Eels as biomonitors of flame retardant contamination


Since brominated flame retardants are only sparingly soluble in water, preferentially dissolving into fats and oils, measurements of concentrations in biological tissues such as fish muscle or liver can provide an invaluable monitoring tool. For species which are important food sources, whether for wildlife, humans or both, such analyses can also give an indication of likely intakes and threats to such consumers. Ideal species for biomonitoring are those which are relatively high in fat with a sufficiently long lifecycle to ensure that fluctuating contaminant levels in water, sediments and/or prey species can be integrated or averaged out over time. In this regard, the European eel (Anguilla anguilla), in addition to its key ecological role in freshwater and brackish ecosystems throughout Europe, also represents a valuable indicator species for water quality.

Although they have a complex lifecycle, for which the very early (larval) and late (sexually mature adult) stages remain remarkably poorly understood, European eels spend a large proportion of their lives localised in brackish or freshwater systems. During this phase, the eels develop from "glass eels" through elvers to "yellow eels", with which we are probably most familiar. In the "yellow eel" stage, so-called because of their yellow-brown colouration, the eels grow substantially, gaining weight and accumulating large fat reserves, but remaining sexually immature. This phase can last between 3-8 years for males and far longer (8-15 years) in females (Feunteun 2002). It is this long lifecycle stage, coupled with local habit and diverse feeding at various levels in the food web (crustaceans, worms, snails, insect larvae and even small fish) (Versonnen et al. 2004), which can result in the eel accumulating substantial body burdens of a variety of heavy metals and persistent organic pollutants symptomatic of local water quality conditions and pollution sources.

The use of the European eel as a species for pollution biomonitoring was initially proposed more than 20 years ago. In that time, a number of studies have included locally caught eels in surveys to determine levels of heavy metal compounds, especially the toxic and bioaccumulative methlymercury (Collings et al. 1996, Edwards et al. 1999, Yamaguchi et al. 2003), and organochlorine contaminants such as PCBs and pesticide residues (de Boer & Hagel 1994, Weatherly et al. 1997, Bordajandi et al. 2003, Versonnen et al. 2004). To date, however, relatively few studies have used eels to monitor for organobromine compounds, including the brominated flame retardants.

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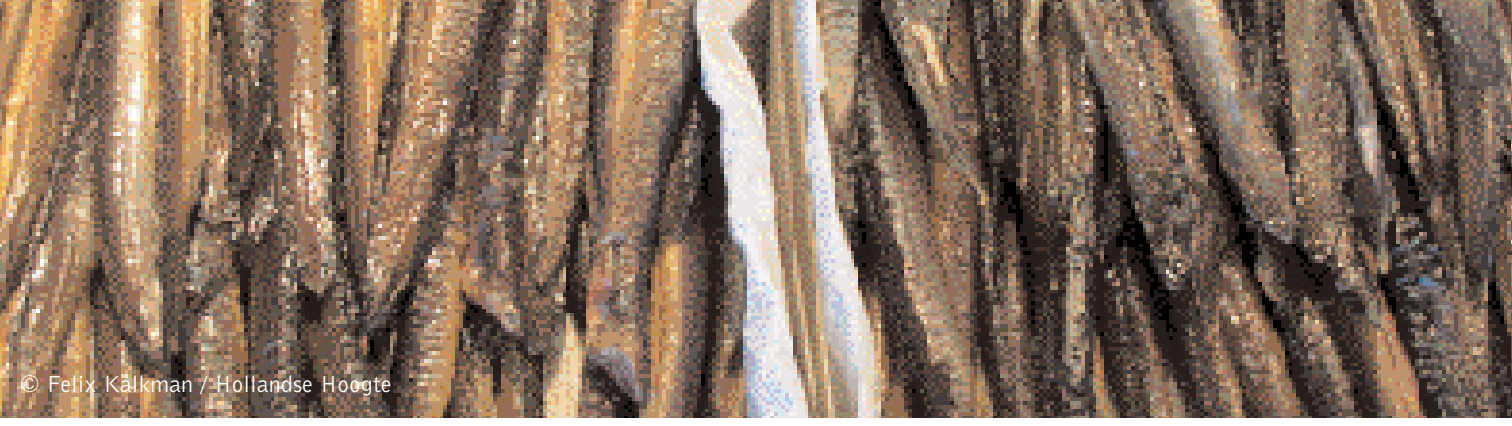
Of those studies which have, that of de Boer (1990) is probably still one of the most extensive to date, providing early indications of the widespread presence of PBDEs in eels from a total of 10 Dutch rivers and lakes and suggesting the significance of local point sources as well as more regional contamination. Much more recently, Morris et al. (2004) reported HBCD and TBBP-A levels for a number of pooled eel samples collected from several locations in the Belgian and Dutch Scheldt Basin, while a study for the UK Food Standards Agency (FSA 2004) used eels as well as trout as biomonitors in a study of PBDE and HBCD contamination associated with point source releases from a manufacturing plant located on the River Skerne in the Tees basin (NE England).

Our current study was designed to extend the geographical spread of data on levels of brominated flame retardants in European eels considerably, by analysing pooled samples of eel muscle from one or more catchments in a total of 10 countries around Europe. Levels of PCBs were determined in the same pooled samples for comparative purposes and to provide PCB data for some European countries for which contemporary data are lacking. Despite the long-standing phase-out of all new uses, PCBs remain significant environmental pollutants, probably maintained in part by ongoing leakage from obsolete equipment and waste dumps, and can still present substantial threats to aquatic ecosystems (see box, page 40).




# European eels under threat

Underlying this study, however, is another worrying trend. Since the European eel was first proposed as a biomonitor of pollution more than two decades ago, severe population declines have been recorded right across its natural European range (Feunteun 2002). In some areas, populations have declined as much as ten-fold in the last twenty years. Declines in Northern European populations were evident even several decades earlier, as documented by local catch statistics since the 1940s. In some European waters, numbers of young eels joining existing depleted stocks are estimated to be as low as 1% of historic levels.

Although not commonly recognised, it has therefore been clear for some considerable time that the European eel is under severe threat throughout its entire range, leading to increasing calls for urgent management action to reverse declines (ICES 2002, Wirth and Bernatchez 2003, Laffaille et al. 2005). In October this year, The European Commission proposed seasonal closure of all eel fisheries until such time as Member States have in place national plans to ensure recovery of stocks (mandatory from July 2007) (EC 2005).

There may be many factors contributing to the observed population declines, including climate change (impacting primarily on the oceanic stages of the lifecycle), overfishing, loss of habitat, construction of physical barriers to upstream or downstream migration, explosions in parasite populations and poor water quality, including chemical pollution (Feunteun 2002). Identifying the principal causal factors in any one region is hindered in part by the large gaps in knowledge concerning the lifecycle of the European eel. For example, from what little is known of the oceanic stages of the lifecycle, it is certainly possible that changes in climatic conditions, particularly shifts in currents, could have profound impacts on breeding success and recruitment of young eels to existing populations in inland waters of Europe (Wirth and Bernatchez 2003). Following many years in a river or lake system in the immature "yellow eel" stage, and triggered by as yet unidentified factors, the European eel metamorphoses to a markedly different "silver eel" stage, mobilising fat reserves and migrating downstream and out into the Atlantic ocean. Spawning is thought to take place in warmer waters at lower latitudes and at depths of around 400m (Feunteun 2002). However, despite the hypothesised location for this mass spawning being the Sargasso Sea, based on observations and predictions made in the 1920s, this has never been reliably confirmed. What is known, however, is that after spawning, the eel larvae (leptocephali) which hatch depend on the prevailing currents of the North Atlantic to return to European waters. The time taken for this journey remains disputed (somewhere from less than a year to three years), as does the degree of control the larvae are able to exert on their trajectory, but what is very clear is that any substantial change in the strength or direction of currents could result in a large proportion of larvae failing to reach inland waters within their natural range and therefore to replenish declining populations.

On a regional level, overfishing has undoubtedly contributed to the observed declines or, at the very least, hindered the recovery of populations depleted for other reasons. Part of the problem in fishery management terms has been the long periods between recorded failures in recruitment by young eels and the decline in catches in fisheries targeting older eels, a factor resulting from the long life history of the species.

Many current management programmes focus on restocking rivers and lakes with farm-raised eels, though this seems to have been insufficient in itself to reverse the downward trend. It has to be hoped that greater controls on fishing indicated by the Commission's recent decision will have considerably greater success.

## Sleeping poisons?

Despite the current focus on the direct population impacts of fishing, on the one hand, and restocking on the other, the possibility remains that chemical contamination of Europe's waterways, and consequent accumulation of persistent contaminants in eels, may also share the blame. Some previous studies have suggested that the European eel, being able to occupy waters with a wide variation in overall water quality, have a low sensitivity to chemical pollution. Knights (1997) concluded that organochlorine contaminants, including quite high recorded levels of PCBs, were not a major cause behind the decline in recruitment. Nevertheless, the same author stressed that any determinations of critical environmental levels or body burdens designed to ensure protection of eels and their predators would need to be developed with considerable caution given the very substantial data gaps. For further information on the uses, hazards and controls of PCBs.

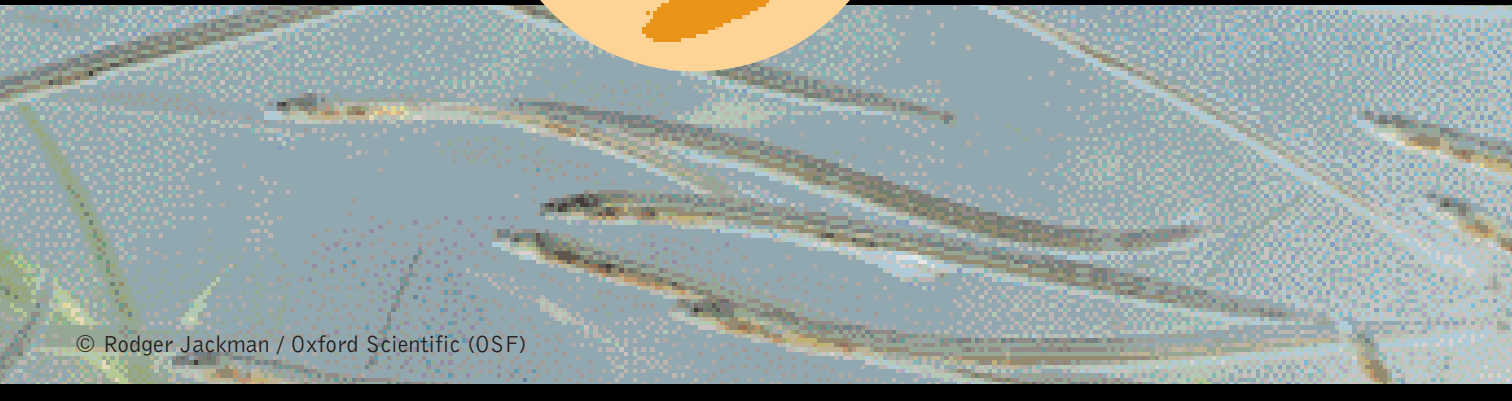
Indeed, while lethal effects in adults may generally only occur at very high exposure levels, sublethal effects of common current body burdens on physiology and on spawning success once sexual maturity is reached certainly cannot be ruled out (Feunteun 2002, Versonnen et al. 2004). The mobilisation of fat reserves which occurs during the transition from "yellow" to "silver" stages and the subsequent migration could well release substantial quantities of persistent organic pollutants and heavy metals into the circulatory system, potentially impacting on the gonads during the most critical period in their development (Robinet and Feunteun 2002). In this way, the body burdens of various contaminants in maturing eels at the time at which they are leaving inland waters for the spawning grounds may be critical in determining the success of the spawn and subsequent returns of healthy larvae and young eels.

## Scope of the current study

With a single study, however wide the geographical spread and range of contaminants considered, it is clearly not possible to determine the relative significance of chemical contamination compared to the numerous other pressures on populations of European eels. Nevertheless, in such a data poor environment, it is hoped that the results of our current study will provide another substantial piece of the puzzle by giving a contemporary snapshot of body burdens of brominated flame retardants and PCBs in samples of eels from various parts of Europe. For example, to our knowledge, this represents the most extensive body of data to date on the distribution of HBCD in European eels.

The mere presence of persistent organic contaminants in itself, whatever the concentrations, clearly does not represent proof of a cause-effect relationship of this nature. Nevertheless, in line with a precautionary approach to management of European eel stocks, as advocated by Russell and Potter (2003), every effort must be made to address all possible threats to populations in the wild, including chemical pollution, if the future of the European eel is to be secured.

# Slipping Away





## Materials and Methods

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# Materials and Methods

## Study design

Samples of freshly caught European eels (A. anguilla), in the "yellow eel" life stage, were obtained from a total of 20 locations across 10 countries in Europe (Belgium, Czech Republic, France, Germany, Ireland, Italy, Netherlands, Poland, Spain and UK) during late July and early August 2005.

Initially it was intended that each sample should consist of five eels, from which a single pooled muscle sample would be prepared in each case. In practice it was not possible to obtain five eels from every location. A summary of the numbers of samples collected from each country, along with the numbers of individual eels in each sample, their average lengths and weights and average lipid (fat) contents is given in Table 1. All lipid (fat) contents in Table 1 and figure 1(b) and used throughout this report to calculate lipid normalized concentrations of contaminants are extractable lipid levels, i.e. lipids which could be extracted using standard methods. In total, including all samples from all countries, a total of 80 individual eels were analysed in the current study.

The catchments and types of water body from which the samples were collected ranged from rural and relatively remote from development (such as the single site selected in Poland) to sites within urban and/or industrial zones (such as the sample collected from the Tevere River in the centre of Rome and some of the samples collected in Germany and the Netherlands). A list of the sample sites and their descriptions is given in Table 2.

Following pooling, all samples were analysed for a total of:

- 11 PBDE congeners (BDE#17, 28, 47, 66, 85, 99, 100, 138, 153, 154 and 183)
- the 3 isomers of HBCD ( $\alpha$, $\beta$ and $\gamma$)
- tetrabromobisphenol-A (TBBP-A)
- 25 PCB congeners (CB#28, 52, 101, 118, 153, 138, 180, 31, 105, 128, 149, 170, 183, 187, 18, 44, 47, 49, 66, 110, 158, 141, 151, 156 and 194)

## sample codes and sample sizes

| Sample code | Number of eels in sample | Average length (cm) | Average weight (g) | Average lipid content (%) |
|---|---|---|---|---|
| Belgium | 4 | 58.3 | 415.2 | 19.1 |
| Czech 1 | 2 | 47.0 | 165.9 | 4.7 |
| Czech 2 | 2 | 52.0 | 249.8 | 14.2 |
| France 1 | 5 | 47.4 | 178.7 | 2.8 |
| France 2 | 5 | 36.7 | 88.0 | 11.6 |
| Germany 1 | 5 | 59.0 | 378.7 | 19 |
| Germany 2 | 5 | 67.8 | 579.6 | 22 |
| Germany 3 | 5 | 56.5 | 308.7 | 17 |
| Germany 4 | 5 | 59.9 | 326.1 | 15.3 |
| Ireland 1 | 6 | 46.0 | 177.0 | 3.5 |
| Ireland 2 | 6 | 26.7 | 30.5 | 15.4 |
| Italy 1 | 5 | 36.3 | 96.3 | 22.2 |
| Italy 2 | 2 | 57.1 | 374.4 | 25.8 |
| Netherlands 1 | 2 | 37.6 | 107.6 | 9.9 |
| Netherlands 2 | 2 | 39.1 | 113.7 | 15.8 |
| Netherlands 3 | 2 | 40.3 | 125.8 | 15.2 |
| Poland | 5 | 50.5 | 217.7 | 6 |
| Spain 1 | 4 | 44.0 | 152.2 | 4.6 |
| Spain 2 | 5 | 35.5 | 84.0 | 19.7 |
| UK | 5 | 58.8 | 474.5 | 18 |

*Table 1: Sample codes and sample sizes (number of individual eels in pooled sample) for each of the 20 samples collected, including average lengths and weights determined from individuals in each pooled sample and the percentage lipid (fat) content of muscle tissue determined after pooling of tissue samples as described below.*

## Sample collection

Other than the two samples from Ireland and the single sample from the UK, all sample collections were arranged by staff from the respective national offices of Greenpeace. The two pooled samples from the Republic of Ireland were kindly provided by the Marine Institute, Ireland. The single pooled sample from the River Thames in the United Kingdom was kindly supplied by staff at the Centre for Environment, Fisheries and Aquaculture Science (CEFAS), Burnham-on-Crouch.

# Collection dates and locations for the 20 pooled eel samples

| Sample code | Date of collection | Location |
|---|---|---|
| Belgium | 01-03/08/05 | Canal Charleroi-Bruxelles, near Arquennes (S of Brussels), Central Belgium |
| Czech 1 | 31/07/05 | River Elbe, at Hřensko (N of Děčín, near border with Germany), N Czech Republic |
| Czech 2 | 03/08/05 | River Otava, at junction of Otava and Vltava Rivers (S of Prague), Central Czech Republic |
| France 1 | 26/07/05 | Etang de Thau, between cities of Meze and Sete (SW of Montpellier), S France |
| France 2[1] | 30/07/05 | Nantes, W France |
| Germany 1 | 27/07/05 | River Elbe, near Hoopte (S of Hamburg), N Germany |
| Germany 2 | 01/08/05 | River Main, near Bamburg (N of Nürnberg), S Germany |
| Germany 3 | 25-27/07/06 | River Weser, at Nienberg (between Bremen and Hannover), N Germany |
| Germany 4 | 02/08/05 | River Rhein, Riedstadt (near Darmstadt), S Germany |
| Ireland 1 | 11/08/05 | Lake Furnace (partially tidal brackish lough), Newport (County Mayo), W Ireland |
| Ireland 2 | 11/08/05 | Owengarve River, Glenthomas, near Newport (County Mayo), W Ireland |
| Italy 1 | 01/08/05 | Tevere River, central Rome, Central Italy |
| Italy 2 | 03/08/05 | Bracciano Lake (Anguillara), Central Italy |
| Netherlands 1 | 27/07/05 | Harinxmakanaal, Leeuwarden, N Netherlands |
| Netherlands 2 | 19/07/05 | Noordzee Kanaal, Ijmuiden, W Netherlands |
| Netherlands 3 | 03/07/05 | Hollandsdiep, Hoge Zwaluwe, S. Netherlands |
| Poland | 26/07/05 | Lake Druglin Duży, close to small village of Rożyñsk (Great Mazurian Lakes region), NE Poland |
| Spain 1 | 25/07/05 | River Miño, at Forchadela Tomiño, near A Guarda (Pontevedra region, Galicia), NW Spain |
| Spain 2[2] | 21/07/05 | River Ebro, 800 meters from the mounth of the river. (SW of Tarragona, Cataluña), E Spain |
| UK | 16/08/05 | River Thames, off Canvey Island (E of London), SE England |

*Table 2: Collection dates and locations for the 20 pooled eel samples analysed in the current study*

[1] was labeled France 3 on arrival – renumbered as France 2 as only two samples were provided
[2] was labeled Spain 3 on arrival – renumbered as Spain 2 as only two samples were provided

In all cases, samples of freshly caught eels were provided by local anglers or retailers, taking all due care to verify the precise catch location. In order to avoid contamination or cross-contamination of the samples, eels were wrapped either individually or as a pooled sample in sheets of new, clean aluminium foil and placed inside transparent polyethylene bags. All samples were frozen as soon as possible after collection (and in all cases within 24 hours of the eels having been caught) and stored frozen and in the dark.

Samples were transported by courier to the Greenpeace Research Laboratories, University of Exeter (UK) in insulated boxes packed with artificial ice packs or dry ice. All samples were verified as being still frozen on arrival at our laboratory, from which they were dispatched (again by courier) to the CEFAS Burnham Laboratory for analysis.


# Analytical methods

## Sample preparation

For each sample, all individual eels were thawed, separated, weighed and their lengths recorded before preparing a single pooled (composite) sample using equal quantities of muscle tissue from each specimen in the sample. All remaining tissues from each specimen were retained to enable further analyses and age determination (not yet undertaken).

## Sample analysis

Pooled eel muscle samples were extracted utilising an automated Soxhlet procedure. Extracts were cleaned and fractionated using alumina and silica columns (for PCBs and PBDEs) or gel permeation chromatography followed by sulphuric acid clean-up (for HBCD). Analyses were performed using either a negative ion chemical ionisation mass spectrometry (NICI-GC/MS, for PBDEs), gas chromatography with electron capture detection (GC-ECD, for PCBs) or liquid chromatography – mass spectrometry (LC-MS, for HBCD).

Full details of the methods employed in the preparation, extraction, clean-up and analysis of the samples are provided in Annex A

# Results and Discussion

As may be expected, a fairly strong (though non-linear) relationship exists between average eel length and average weight within the group of 20 pooled samples (Figure 1a). Length initially increases steadily with weight, approaching a maximum length (in all but one pooled average) of the order of 60 cm. Above 300g in weight, however, length increases only marginally. Lipid content shows no consistent trend with average weight (Figure 1b). The significance, if any, of an apparent minimum in lipid content in eels with an average weight in the region of 150-200g is not known. Once eels have been aged it may be possible to determine if this reflects a real minimum in lipid content associated with a particular stage in the lifecycle or if it is merely an artifact.

Concentrations of PBDEs, HBCD isomers, TBBP-A and PCBs were initially determined on a wet weight (fresh weight) basis. A summary of the results is provided in Table 3. This table shows concentrations for the three most abundant PBDE congeners identified in the sample, namely BDE#47 (tetra-BDE), BDE#99 and BDE#100 (both penta-BDEs), as well as sum values for all three isomers of HBCD and a sum of concentrations of the so-called ICES 7 PCBs (CB#28, 52, 101, 118, 153, 138, 180), indicative of broader PCB contamination and useful for purposes of comparison with previously published studies. TBBP-A was not detected in any of the samples in this study (see below).

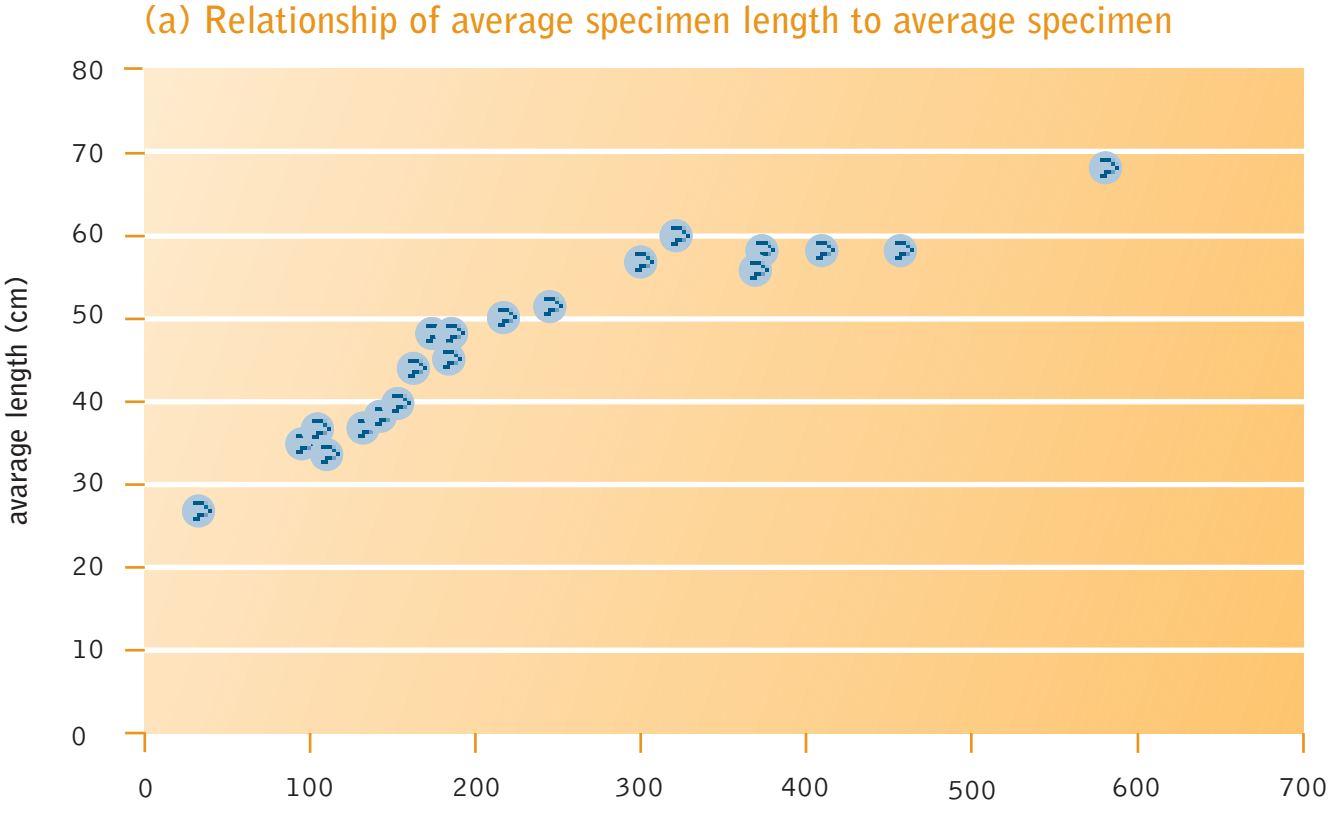


Figure 1a: relationship of average specimen length to average specimen weight for all 20 pooled samples.


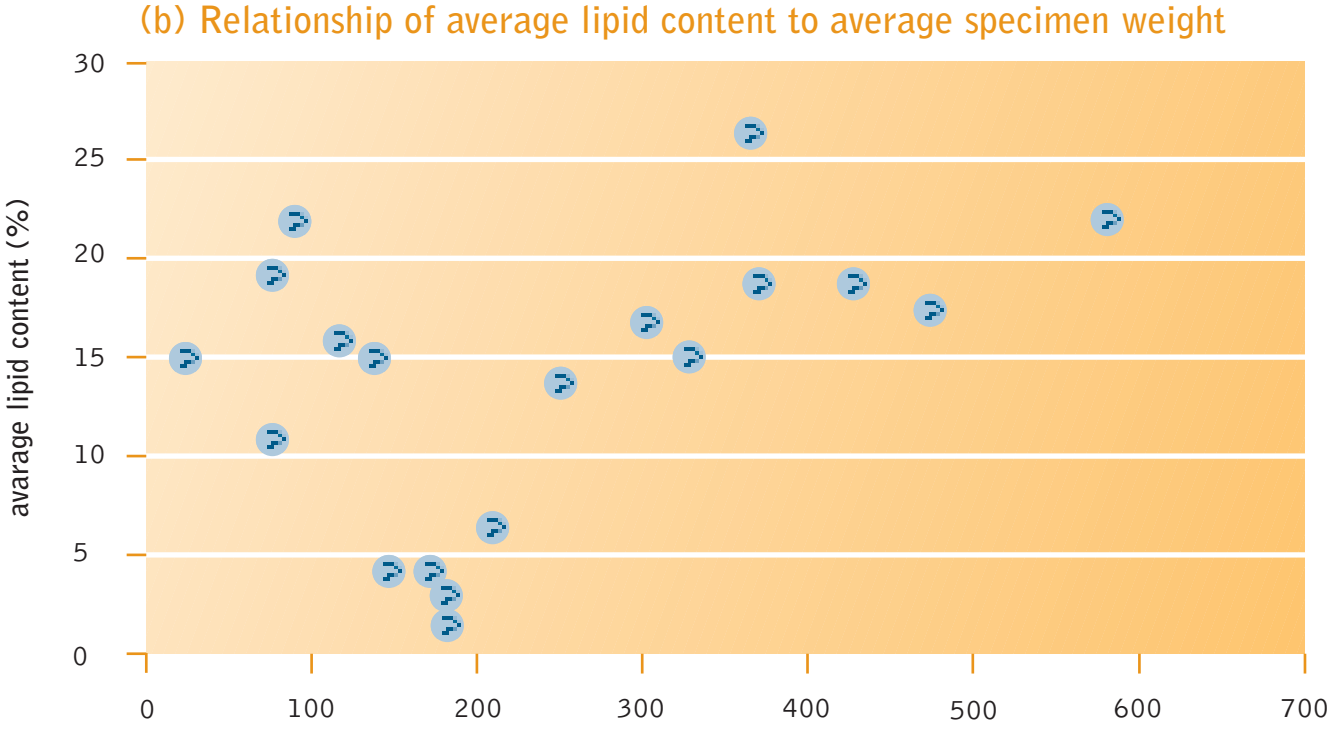


Figure 1b: relationship of average lipid content to average specimen weight for all 20 pooled samples

# Levels of some key brominated diphenyl ethers (fresh weight)

| Sample code | Number in pooled sample | BDE-47 (tetra) | BDE-99 (penta) | BDE-100 (penta) | Σ HBCD | Σ PCBs (ICES 7) |
|---|---|---|---|---|---|---|
| Belgium | 4 | 4.7 | nd | 2.0 | 5 | 97 |
| Czech 1 | 2 | 4.3 | 0.2 | 1.4 | 4 | 184 |
| Czech 2 | 2 | 1.0 | nd | nd | nd | 66 |
| France 1 | 5 | nd | nd | nd | 3 | 29 |
| France 2 | 5 | 0.5 | nd | nd | 2 | 5 |
| Germany 1 | 5 | 7.9 | 0.7 | 0.9 | 2 | 327 |
| Germany 2 | 5 | 17.0 | nd | 3.9 | 15 | 566 |
| Germany 3 | 5 | 9.5 | 0.6 | 2.0 | 9 | 196 |
| Germany 4 | 5 | 9.3 | 0.6 | 3.1 | 37 | 381 |
| Ireland 1 | 5 | 0.2 | nd | nd | nd | 4 |
| Ireland 2 | 5 | nd | nd | nd | 3 | 5 |
| Italy 1 | 5 | 24.0 | 2.1 | 6.8 | 26 | 483 |
| Italy 2 | 2 | 1.8 | nd | nd | 4 | 120 |
| Netherlands 1 | 2 | 0.4 | nd | nd | 9 | 16 |
| Netherlands 2 | 2 | 3.2 | nd | 1.0 | 2 | 165 |
| Netherlands 3 | 2 | 17.0 | 0.6 | 7.7 | 9 | 1512 |
| Poland | 5 | 0.2 | nd | nd | 1 | 2 |
| Spain 1 | 4 | 1.2 | nd | 0.5 | 7 | 54 |
| Spain 2 | 5 | 2.7 | nd | 0.9 | 4 | 123 |
| UK | 5 | 46.0 | 3.2 | 12.0 | >50 | 136 |

*Table 3: levels of some key brominated diphenyl ethers (PBDEs), hexabromocyclododecane (HBCD) and polychlorinated biphenyls (sum of ICES 7 PCBs) per unit fresh weight of eel muscle (all values expressed as ng/g fresh weight, parts per billion or ppb); nd – not detected (below limit of detection); detection limits for PBDEs – 0.125 ppb, HBCD – 1 ppb, PCBs – 1 ppb.*

Table 4 shows the same summary data recalculated (normalised) on a lipid (fat) weight basis, again to aid cross-comparison with some previously published studies and in recognition that the contaminants of interest are expected to accumulate primarily in the fatty component of the muscle tissues.

Expressing results on a fresh weight (wet weight) basis gives an indication of the total body burden of the fish and the levels to which consumers of the muscle (flesh) may be exposed (be they natural predators or human consumers). However, in any particular water body, fatty or oily fish will accumulate more of these persistent organic contaminants than non-fatty fish such that any indications of relative water quality status based on fresh weight concentrations will inevitably be highly species-specific. Moreover, as is apparent from the pooled eel samples analysed in the current investigation (see Table 1, Figure 1b), fat (lipid) content can vary substantially between individuals of the same species. Expressing results on a fat (lipid) weight basis can therefore enable more valid intercomparison of levels of exposures to persistent organic chemicals between species and/or individuals with markedly differing tissue fat contents (from different locations, for example).

# PBDEs

Of the 11 PBDE congeners quantified, 3 (BDE#17, 85 and 138) were not found in any of the twenty pooled samples at levels above the limit of detection (0.125 ng/g fresh weight, ppb). A further 3 congeners (BDE#28, 66 and 183) were found in only one or two of the samples. The predominant congeners, as may be expected from their historical use, bioaccumulative potential and resistance to degradation were the tetra and pentabrominated congeners shown in Tables 3 and 4 (BDE#47 and BDE#99/100).

For BDE#47, concentrations ranged from below limits of detection in two samples (France 1, from the Etang de Thau, and Ireland 2, from the Owengarve stream) to 46 ppb in the single pooled sample from the UK (River Thames). Levels of BDE#99 and 100 were generally lower (<0.125–3.2 ppb and <0.125-12 ppb respectively), consistent with commonly reported patterns. Aside from the UK sample, the next highest concentrations were recorded in samples from the Tevere River in the centre of Rome (Italy 1), from the Hollandsdiep (Netherlands 3) and from the Main River near Bamburg (Germany 2). Lowest levels, aside from samples from France and Ireland, were recorded for sample Netherlands 1 (collected from the Haringsmakanaal) and the single pooled sample from Poland (collected from the relatively rural Great Mazurian Lakes region). Results for BDE#47 and for BDE#99/100 are shown in Figure 2a below.

## Levels of some key brominated diphenyl ethers (lipid weight)

| Sample code | Number in pooled sample | BDE-47 (tetra) | BDE-99 (penta) | BDE-100 (penta) | Σ HBCD | Σ PCBs (ICES7) |
|---|---|---|---|---|---|---|
| Belgium | 4 | 24.6 | nd | 10.5 | 24 | 508 |
| Czech 1 | 2 | 91.5 | 4.0 | 29.8 | 79 | 3915 |
| Czech 2 | 2 | 6.8 | nd | nd | nd | 465 |
| France 1 | 5 | nd | nd | nd | 111 | 1036 |
| France 2 | 5 | 4.1 | nd | nd | 13 | 43 |
| Germany 1 | 5 | 41.6 | 3.8 | 4.6 | 9 | 1721 |
| Germany 2 | 5 | 77.3 | nd | 17.7 | 69 | 2573 |
| Germany 3 | 5 | 55.9 | 3.6 | 11.8 | 56 | 1153 |
| Germany 4 | 5 | 60.8 | 3.7 | 20.3 | 239 | 2490 |
| Ireland 1 | 5 | 4.9 | nd | nd | nd | 114 |
| Ireland 2 | 5 | nd | nd | nd | 20 | 32 |
| Italy 1 | 5 | 108.1 | 9.5 | 30.6 | 117 | 2176 |
| Italy 2 | 2 | 7.0 | nd | nd | 15 | 465 |
| Netherlands 1 | 2 | 3.8 | nd | nd | 90 | 162 |
| Netherlands 2 | 2 | 20.3 | nd | 6.1 | 11 | 1044 |
| Netherlands 3 | 2 | 111.8 | 4.1 | 50.7 | 61 | 9947 |
| Poland | 5 | 3.8 | nd | nd | 25 | 33 |
| Spain 1 | 4 | 26.1 | nd | 10.7 | 161 | 1174 |
| Spain 2 | 5 | 13.7 | nd | 4.7 | 22 | 624 |
| UK | 5 | 255.6 | 17.8 | 66.7 | >278 | 756 |

*Table 4: levels of some key brominated diphenyl ethers (PBDEs), hexabromocyclododecane (HBCD) and polychlorinated biphenyls (sum of ICES 7 PCBs) per unit lipid (fat) weight of eel muscle (all values expressed as ng/g lipid weight, parts per billion or ppb); nd – not detected (below limit of detection); calculated LODs vary according to lipid content.*

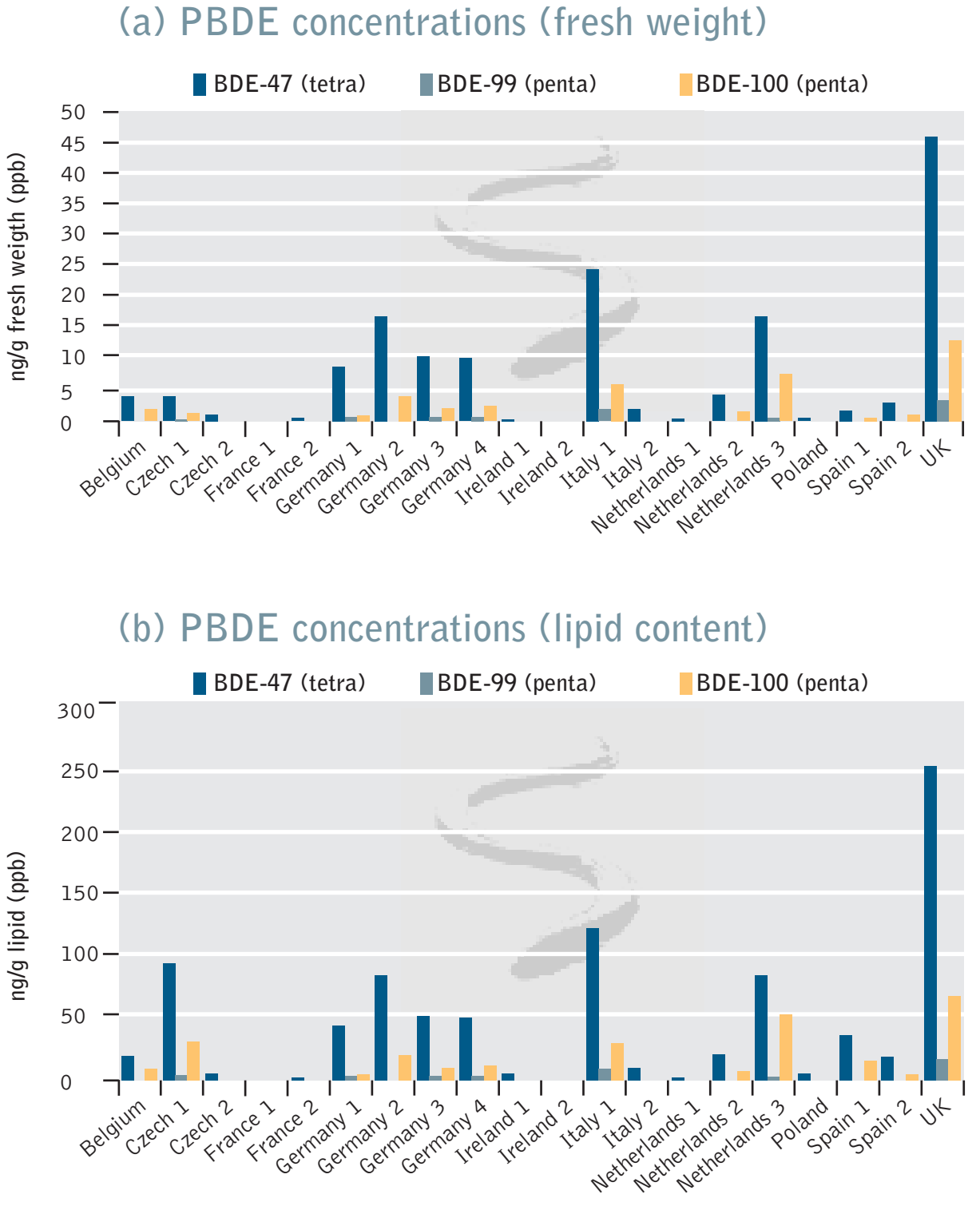


*Figure 2: concentrations of the three most abundant PBDE congeners identified in the pooled eel muscle samples normalized to (a) fresh weight (wet weight) and (b) lipid (fat) content.*

As noted above, relatively few data have been published to date for PBDEs in European eels and most are expressed only on a lipid weight basis. The UK Food Standards Agency (FSA 2004) report wet-weight levels for BDE#47, 99 and 100 of 34, 2 and 12 ppb respectively for a single control sample of eels from the River Skerne (Tees Basin) in the NE of England. Levels for this control sample, collected upstream from a major brominated


flame retardant manufacturing facility which was known to be releasing brominated compound wastes at the time of sampling, are towards the upper end of the range of values recorded for eels across Europe in the current study. As expected, levels recorded in close proximity to and downstream from this manufacturing facility were far higher (e.g. BDE#47 264-579 ppb, FSA 2004).

Expressed on a lipid weight basis (Table 4 and Figure 2b above), the concentrations are obviously higher but exhibit a similar pattern (though concentrations for the sample Czech 1 increase disproportionately as a result of the relatively low lipid of this pooled sample). Levels for BDE#47 (<0.8-256 ppb), BDE#99 (<0.6-17.8 ppb) and BDE#100 (<0.5-66.7 ppb) are once again within a similar range to those recorded for eels from the control site on the River Skerne in the UK (152, 8.9 and 17.8 ppb respectively, FSA 2004).

Although few other eel data are available for direct comparison, lipid normalized data do compare well with levels reported for other species. For example, Sellström et al. (1993) reported sums of BDE#47, 99 and 100 of 17-62 ppb (lipid basis) for Baltic herring muscle, similar to the 3.2-32 ppb reported by Haglund et al. (1997) and the 8.4-100 ppb reported by de Boer (1990) for the same species. Baltic roach muscle revealed similar levels (Burreau et al. 2004), as did samples of cod and whiting muscle sampled from the North Sea by Boon et al. (2002).

## HBCD

Of the three isomers, $\alpha$–HBCD was commonly the most abundant, with $\gamma$ and $\beta$ present in the majority of samples but at levels only marginally above the limit of detection. This pattern of contamination is as expected given the specific properties of the three isomers (de Wit 2002). $\alpha$-HBCD was found in 14 of the 20 samples at concentrations from 1 to more than 50 ppb (fresh weight). Levels in the single pooled sample from the UK (River Thames) significantly exceeded the maximum reporting limit for $\alpha$-HBCD (50 ppb); final quantitative data for this sample are still pending.

Aside from this UK sample, values were notably higher than the majority of other samples in Germany 4 (from the Rhein near Darmstadt) and Italy 1 (from the Tevere River as it passes through the centre of Rome), with summed concentrations of HBCD isomers ($\Sigma$-HBCD) of 37 and 26 ppb (fresh weight basis) respectively (Table 3, Figure 3a). In contrast, HBCD was below detection limits in two samples, namely Czech 2 (from Hrensko in the far north of the country) and Ireland 1 (from Lough Furnace on the west coast). Concentrations in the majority of the other samples ranged from 1-10 ppb. Within Germany, site 1 revealed the lowest HBCD levels (River Elbe, near Hoopte), while in the Netherlands, site 2 showed the lowest levels (the Noordzee Kanaal at Ijmuiden).

Total HBCD concentrations reported for the single control sample of eels from the River Skerne in the UK (FSA 2004) were significantly higher (at 159 ppb fresh weight) than the highest quantitative value currently available in our study (though the value for the Thames sample is currently only a lower bound figure at >50 ppb).

Concentrations in tissues of eels sampled below the manufacturing site on the Skerne were, as expected, extremely high, ranging from 570-9432 ppb in samples collected in closest proximity to the plant and remaining clearly elevated above control values at all sites downstream as far as the Tees Barrage (40-951 ppb). It is not clear whether this primarily results from transfer of HBCD downstream by water or suspended particulates or reflects the migration of eels along the length of the river.

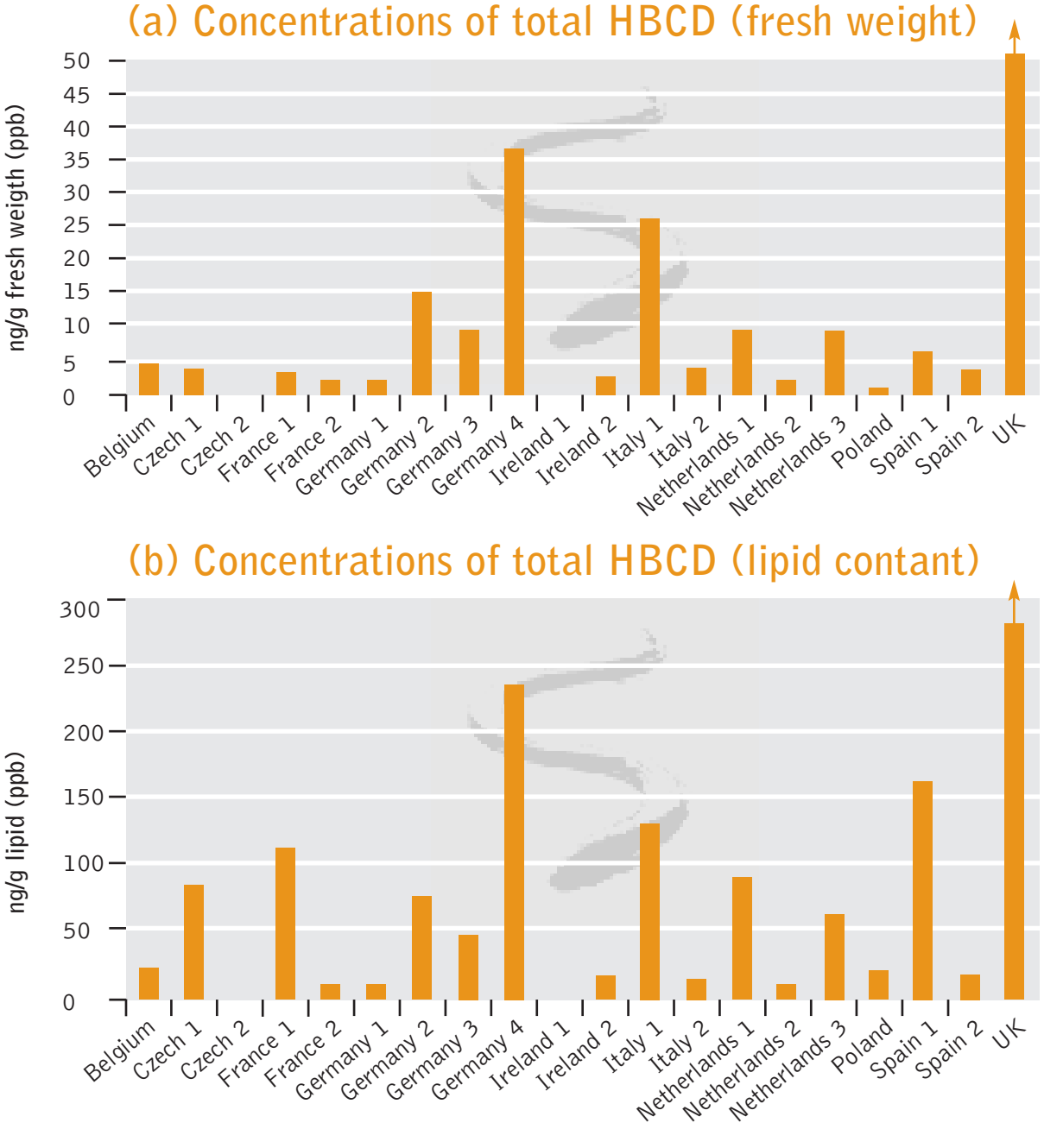


*Figure 3: concentrations of total HBCD (sum of all isomers) in the pooled eel muscle samples normalized to (a) fresh weight (wet weight) and (b) lipid (fat) content. Arrows denote that values for UK sample are currently lower bound estimates only.*

Moreover, given the likely scale of atmospheric emissions of HBCD from the plant during its operation, it may well be that the control site upstream from the plant may not represent background levels of contamination expected for more remote sites. Expressing HBCD concentrations on a lipid normalized basis (Table 4 and Figure 3b above) results in marked increases in relative concentrations for some samples, especially France 1 (Etang de Thau) and Spain 1 (River Miño), as a consequence of the very low lipid content of the eels sampled from these locations (2.8% and 4.6% respectively). On a lipid basis, 5 of the 20 samples analysed contained total HBCD concentrations of above 100 ppb (total range nd->278 ppb).

Morris et al. (2004) reported concentrations for HBCD isomers in similarly pooled samples of "yellow eels" from Dutch rivers (collected in 1999) and from the Scheldt basin in Belgium (collected in 2000). Levels in the Dutch samples ranged from 25-359 ppb lipid weight (and for the Scheldt 29-266 ppb), comparable with the range for all samples in our study, though substantially higher than the range for the three samples from the Netherlands alone (11-90 ppb). Indeed the concentration in the pooled sample from Hollandsdiep in our study (61 ppb) is approximately four times lower than the pooled sample collected from the same water body in 1999 and analysed by Morris et al. (2004), though clearly it is not possible to draw any firm conclusions from these limited comparisons.

## TBBP-A

Tetrabromobisphenol-A was not detectable in any of the 20 pooled samples at a limit of detection of 3-5 ppb fresh weight (equivalent to between 10 and 180 ppb on a lipid normalized basis). While some previous studies have reported the presence of traces of TBBP-A in eel muscle, e.g. from Berlin in 1998/99 (Kemmlein 2000, in OSPAR 2001), and in the sample set from the Scheldt Basin and various Dutch rivers discussed above (Morris et al. 2004), it has generally been found in only a subset of samples and at maximum concentrations below the detection limits of the method employed in our current study. Concentrations appear to be similar or even lower in other freshwater and marine species (de Boer et al. 2002, 2003).


# PCBs

For ease of comparison, a subset of seven key PCB congeners, referred to as the ICES 7, have been selected from the total of 25 congeners quantified in the current study. These congeners (CB#28, 52, 101, 118, 153, 138 and 180) are recognized as among the most commonly found in environmental samples and are used as convenient markers of relative degree of contamination. The sums of the concentrations of these individual ICES 7 PCBs are reported in Table 3 (fresh weight) and Table 4 (lipid weight) and illustrated in Figure 4a and b respectively.

Total ICES 7 PCB concentrations on a fresh weight basis ranged from low ppb levels (in France 1, Ireland 1 and 2, and Poland) to low ppm (mg/kg) levels (Netherlands 3, Hollandsdiep). The concentration in eel tissue from the pooled Hollandsdiep sample was almost three times higher than the next highest level recorded in our study (Germany 2, from the Main River), suggestive of historical or ongoing localized point sources of PCBs. A single sample from Italy (Italy 1) and two additional samples from Germany (Germany 1 and 4) also contained total ICES 7 PCB concentrations of greater than 200 ppb fresh weight.

That the European eel in its "yellow" stage can accumulate (and seemingly tolerate) quite high tissue levels of PCBs has been known for some time. Concentrations from the ppb to low ppm range have also been reported for this species from the River Po basin in Italy (Bressa et al. 1997), waterways in Berlin (Fromme et al. 1999), several rivers in Wales (Weatherly et al. 1997) and the Vanajevesi River in Finland (Tulonen and Vuorinen 1996).

Although the PCB concentrations recorded for the two Spanish pooled samples in the current study were at the lower end of the reported range (54 and 123 ppb wet weight respectively), muscle samples from 14 eels collected in the Turia estuary (approximately 200km SW from the Ebro delta) in 2000 yielded still lower levels, at 29.3 ppb fresh weight (Bordajandi et al. 2003).

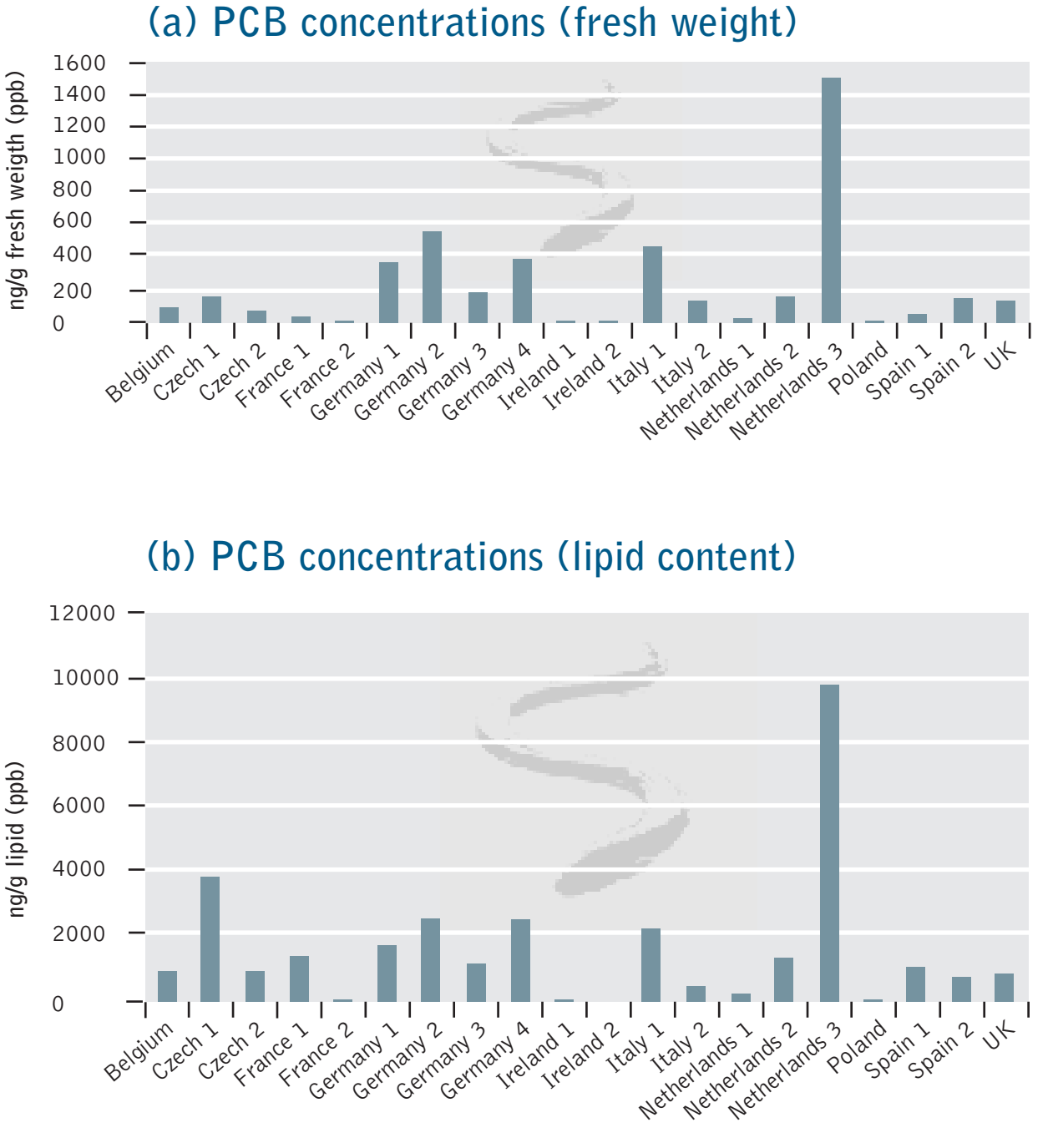


*Figure 4: PCB concentrations (sum of ICES 7 PCBs) in the pooled eel muscle samples normalized to (a) fresh weight (wet weight) and (b) lipid (fat) content.*

In contrast to its relatively high concentrations of both PBDEs and HBCD, the single pooled sample from the Thames (UK) did not contain particularly high levels of PCBs. Furthermore, within the sample sets for Germany and the Netherlands, those samples with the highest HBCD concentrations were not those showing the highest concentrations of PCBs. Excluding the single high value for sample Netherlands 3, PCB concentrations show some relationship with those of BDE#47 (tetra-BDE). However, there is no evidence of any such association between concentrations of HBCD and those of PCBs.

It may be expected that the age of the eels, governing the length of time to which each specimen had been exposed to water quality conditions in any one area, would have a substantial impact on body burden of persistent organic pollutants such as PBDEs, HBCD and PCBs. Until such time that the eels have been accurately aged, it is not possible to determine the significance of this factor in controlling the tissue levels found in the current study. Nevertheless, if a strong age-dependency of body burden did exist then some correlation of concentration with either fish length or fish weight might be expected. No such correlation was evident for any of the contaminants determined in the pooled samples. This seems to suggest, therefore, that local conditions, including the proximity of habitat to urban and/or industrial sources of pollution, may have a large role to play in determining tissue levels of persistent organic pollutants in the European eel.

Concentrations of lipid normalized PCBs (total ICES 7) for the 20 pooled samples ranged from 32 ppb in sample Ireland 2 (Owengarve stream) to 9947 ppb (almost 10 parts per million) in sample Netherlands 3 (from Hollandsdiep). In one of the most extensive previous studies to date concerning PCBs in eels (142 samples from 20 locations around the Netherlands), de Boer et al .(1994) reported lipid normalized concentrations ranging from 274±176 ppb (in the Canal Leopold) to 14400±9700 ppb (for eels taken from the Zuid Willemsvaart Canal), a similar order of magnitude to those recorded in our current study. Versonnen et al. (2004) note that, whereas even at these very high concentrations, the European eel appears to remain insensitive to the potential effects on hormone systems, nothing is known about how that sensitivity might change as the eels mature and mobilize fat reserves. Furthermore, even if "yellow eels" are themselves able to tolerate high levels of persistent organic pollutants such as PCBs, such levels could have more severe consequences for their predators, including aquatic mammals (otters, mink) and birds (Yamaguchi et al. 2003).


# Conclusions

Overall, these data illustrate the widespread nature of contamination of aquatic systems with brominated flame retardants, especially the more bioaccumulative forms tetra-BDE and HBCD, and their ability to accumulate in the muscle tissues of the European eel (Anguilla anguilla). All 20 pooled samples analysed contained detectable residues of at least one brominated flame retardant compound.

The predominance of tetra- and penta-brominated congeners of the diphenyl ethers is consistent with their known properties and with the results of previous studies in fish. More heavily brominated congeners such as hexa- and octa-BDE have been reported to accumulate more readily in fish livers than in muscle tissue. Levels of the PBDE most widely used across Europe, namely deca-BDE (BDE-209), were not determined in this study but might also be expected to be relatively low in eel muscle tissue, in common with other fish species, despite its proven bioavailabilty and accumulation in other organisms.

HBCD was detectable in eels from all but two locations while PCBs, as may be expected from their ubiquitous presence in the environment, were detectable in all samples.

By far the highest levels of both PBDEs and HBCD were recorded in the single pooled sample collected from the River Thames in the UK, for which the HBCD levels exceeded the maximum which could be determined using the standard method employed by the analytical laboratory (and which is currently undergoing confirmatory analysis). However, this same sample did not contain the highest level of PCBs, recorded instead for one of the three samples from the Netherlands (Hollandsdiep), with concentrations of over 1 part per million on a fresh weight basis and almost 10 parts per million on a lipid (fat) weight basis.

A few samples contained low levels of all the contaminants measured, most notably the two samples collected in the west of Ireland, one of two samples collected in France (close to Nantes) and the single sample collected in a rural area of Poland (the Great Mazurian Lakes). Samples from all other locations contained intermediate concentrations, within the ranges previously reported for these contaminants in the few other studies available.

With exception of the UK sample, there appeared to be some positive correlation between concentrations of tetra-BDE and those of PCBs (though current PBDE concentrations are roughly 10-50 times lower than those of the PCBs). No evidence was found for a similar association between levels of HBCD and those of either the PBDEs or PCBs, suggesting different environmental distributions, driven perhaps by differences in primary sources or pathways of movement through ecosystems.

Furthermore, there appears to be no clear relationship between contaminant levels (of PBDEs, HBCD or PCBS) and the average lengths or weights of the pooled eel samples, despite the fact that concentrations might have been expected to have varied with age. This awaits confirmation when age data are available. At this stage, however, the lack of any consistent pattern suggests that local levels of contamination in the aquatic environment may be a more dominant factor in determining relative tissue levels in the eels than age.

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To our knowledge, this is the most geographically extensive survey to date of the presence of brominated flame retardants in the European eel (Anguilla anguilla) and should add significantly to the body of data both on the distributions of these persistent environmental contaminants and on the chemical body burden of the eel. Levels of PBDEs and HBCD were generally lower than those previously reported in eels sampled close to known point sources of discharges of emissions of these compounds (particularly manufacturing facilities in the UK and Netherlands) but nevertheless demonstrate that their presence in European river systems is ubiquitous. This is likely to have resulted from a combination of direct releases from large industrial facilities manufacturing or using brominated flame retardants and more diffuse releases from products during manufacture, use and/or disposal.

Since the study was based on the analysis of only 20 pooled samples, it clearly cannot provide an exhaustive survey. Neither can the results for the small number of samples from each country be taken as average or indicative levels representative of all catchments in those countries as a whole. It is probable that, in any one of the countries included in the survey, it would be possible to find both more and less contaminated populations of eels in other waterways. Rather the data provide a snapshot of contaminant levels across Europe in what is a keystone species in many aquatic ecosystems, and a species under severe threat from a number of pressures.

It is not possible to determine from these data what the consequences of such contamination might be for the eels themselves. Although there are indications that, during the "yellow" life stage, eels are commonly able to tolerate high levels of chemical pollution, what impact this might have when the fish enter their "silver" stage, and contaminant-loaded fat reserves are mobilised during the most sensitive period of sexual development, is simply not known. Nevertheless, recognising their abilities to interfere with developmental processes in other organisms, the possibility remains that pollutants such as PCBs and brominated flame retardants may be contributing to the observed declines in populations of the European eel by reducing mature adult survival or spawning success.

The scale of the threat to consumers of eels, whether their natural predators (including birds, such as herons, and mammals, such as otters) or human consumers, is also unknown. Although some studies have attempted to calculate risks, often in terms of margins of safety, these assessments are inevitably limited by the lack of data on the effects of long-term, low level exposure to chemicals such as HBCD.

This study demonstrates once again that the risks presented by persistent and bioaccumulative chemicals, including HBCD which remains in commercial use across Europe, cannot be deemed to be adequately controlled. At the same time, the continued presence, and in some cases high levels, of PCBs in the eel tissues, despite the fact that their use was prohibited more than twenty years ago, illustrate the very long-term consequences of recognising a problem too late, or at least of acting too late.

Action has recently been taken across Europe to stop the continued use of "penta" and "octa" BDE formulations, and this has already led in some cases to declining levels. Nevertheless, the legacy of their previous use, both from the quantities already released to the environment and the additional quantities which will ultimately be released from obsolete products, can be expected to continue for some time. Futhermore, since there is evidence to suggest that residues of the PBDE formulation which is still commercially used in Europe, namely "deca" BDE, can degrade in the environment to form some of the less-brominated (and more bioaccumulative) forms, it is unlikely that actions taken to date will resolve the totality of the problem. HBCD remains under assessment within Europe. In the mean time, despite the great uncertainties surrounding its toxicity and environmental fate and despite observations of its ability to interfere with hormone systems in vertebrates, its widespread use and release to our environment continues.

History tells us that the consequences of large-scale use of chemicals which are persistent and bioaccumulative, though difficult to predict, are all too often severe. Once in the environment, the fate and effects of these chemicals cannot be controlled. It is becoming increasingly widely recognised, including within some governments and even some sectors of the chemical industry, that action must be taken to phase-out the use of all chemicals which confound the ability of natural systems to degrade them and which consequently build up in the environment, in wildlife and in our food.

With the new chemicals legislation under development in Europe (REACH), there is an opportunity for all European countries to begin to address the problem effectively and consistently by requiring that all chemicals exhibiting these properties (e.g. PBT and vPvB chemicals) are replaced with less hazardous alternatives wherever and whenever those alternatives exist (the principle of substitution). It is also essential that this legislation will be capable of preventing the manufacture and use in the future of chemicals for which basic data on hazardous properties, including their environmental persistence and ability to bioaccumulate, are not available (the principle of "no data, no market").

All components of the environment stand to benefit from such controls and from more sustainable production and use of chemicals in the future. While it is unlikely that actions on chemicals alone will be sufficient to reverse the demise of the European eel, such action will remain one essential component of a precautionary approach to their protection.

# Polybrominated diphenyl ethers (PBDEs)

Polybrominated diphenyl ethers are one of several classes of brominated compound in widespread use as flame retardant additives in plastics and foams, including plastic casings of electronic equipment (OECD 2003). PBDEs are environmentally persistent chemicals. Some, especially the lower brominated congeners (e.g. "penta-BDE"), are also highly bioaccumulative. Their manufacture and use as additives in plastics and other polymers, in which they are not tightly bound to the polymer matrix, has led to their widespread presence in the environment. PBDEs can be detected in indoor air and dusts in the workplace and in the home. They also occur in almost every part of the environment, including sediments (Allchin et al. 1999), freshwater and marine fish (Asplund et al. 1999a, b), birds eggs (Hites 2004) and even whales from the deep oceans and the Arctic (de Boer et al. 1998, Ikonomou et al. 2002).

PBDEs have also been reported as common contaminants in humans, including reports from Sweden, Spain, Finland and North America (Lindstrom et al. 1997, Meneses et al. 1999, Strandman et al. 1999, She et al. 2000). Concentrations of lower brominated PBDEs have shown increasing levels in both blood and breast milk in recent decades, particularly in regions in which "penta" remains in commercial use (Alaee et al. 2003, Meironyte et al. 1999, Thomsen et al. 2002). Workers in electronics recycling facilities in Europe have been found to have higher blood levels of PBDEs than other workers, probably as a result of inhalation of contaminated dust (Sjödin et al. 2001, Sjödin et al. 2003). For the general population, exposure to PBDEs probably occurs through a combination of food contamination and direct exposure the chemicals from consumer products and/or contaminated dusts (Harrad et al. 2004).

While their acute toxicity is considered low, chronic exposure to certain PBDEs (especially in the womb) has been associated with abnormal brain and skeletal development in animals with possible long-term impacts on memory, learning and behaviour (Darnerud 2003, Eriksson et al. 2001). There are concerns that similar effects may be of relevance in humans (Branchi et al. 2003). PBDEs also exhibit endocrine disrupting properties, interacting with both oestrogen and thyroid hormone systems either as the parent compound or as metabolites (Meerts et al. 1998, 2001, Legler & Brouwer 2003). Effects on the immune system have also been reported (Birnbaum & Staskal 2004, Darnerud 2003). Furthermore, when plastics containing PBDEs are burned, either in an incinerator or by open burning, the potential exists for formation of brominated dioxins (IPCS 1998), which may be of equivalent toxicity to chlorinated dioxins.

Because of these environmental and human health concerns, controls are increasingly being placed on the use of PBDEs (along with some other brominated fame retardants) in some regions. Penta-BDE is included as a "priority hazardous substance" under the EU Water Framework Directive (EC 2001) and has been proposed for inclusion as a POP (persistent organic pollutant) under the 2001 global Stockholm Convention (Peltola & Yla-Mononen 2001). Both "penta" and "octa" are now banned from use in Europe (EC 2003). Within the electronics sector, use will be prohibited from July 2006 under the Directive on Restrictions on Hazardous Substances (RoHS), associated with the WEEE Directive (EC 2002a, b). Nevertheless, even when such controls take full effect, a substantial legacy of PBDEs will remain in obsolete electrical and electronic equipment. Moreover, the widely used decabromodiphenyl ether ("deca") will not be included in this prohibition, despite concerns regarding its widespread environmental distribution, accumulation in raptorial birds and tendency to undergo partial degradation to less-brominated, more bioacumulative, forms.

# Hexabromocyclododecane (HBCD)

HBCD remains in widespread use in all regions, primarily as an additive flame retardant in thermoplastic polymers though also in certain other plastics, textile coatings, cables etc.. Historically its production volumes have been somewhat lower than those for most PBDEs, though insufficient information exists to determine any recent trends, particularly in response to the introduction of prohibitions within Europe on the marketing and use of penta- and octa-BDE.

HBCD is environmentally persistent and highly bioaccumulative, showing a bioconcentration factor in fathead minnows (a freshwater fish species) of more than 18 000 (Sellstrom et al. 1998). Although its high fat solubility makes it relatively insoluble in water, some studies indicate that the aquatic toxicity of HBCD is high, with LC50 concentrations (concentrations lethal to 50% of test organisms) in the low parts per billion range (ug/l) for some invertebrate and fish species (OECD 2003).

Despite its widespread use, little information exists on potential exposures to, and toxicity of, HBCD in other animals. Whereas chronic toxicity to the liver, considered for some time to be the primary target organ, seems to occur only following relatively high doses, impacts on levels of thyroid hormone in the blood have been reported at somewhat lower doses, raising the possibility that HBCD has endocrine (hormone) disrupting properties (Birnbaum & Staskal 2004). In addition, other studies have reported fundamental changes in neurological and behavioural characteristics (spontaneous behaviour, memory and learning), associated with impacts on certain nervous system receptors, in mice exposed to HBCD during the first few days of their lives (Eriksson et al. 2002). Exposure at the same time to elevated PCB concentrations increased the severity of these impacts further. Studies using one particular in vitro test system of neurological damage (employing cultured cells from a certain portion of the brain) concluded that HBCD yielded the most potent response of all brominated flame retardants tested by this method to date (Reistad et al. 2002). Information regarding possible carcinogenicity remains very scarce.

HBCD is not currently subject to restrictions on marketing and use within the EU. Assessments of the environmental risks posed by this chemical are ongoing and will undoubtedly benefit from any further information concerning its environmental distribution.

# Polychlorinated Biphenyls (PCBS)

Polychlorinated biphenyls (PCBs) are a group of synthetic organic chemicals that contain 209 individual compounds (known as congeners) with varying patterns of chlorine substitution. PCBs have been used in a wide variety of applications, including transformer oils, hydraulic fluids, plasticisers and carbonless copy papers. They were also used in capacitor dielectrics, heat transfer fluids, lubricating and cutting oils, and in paints and printing inks (ATSDR 2000). Use in transformer oils (frequently with tri- and tetrachlorobenzenes as solvents, Swami et al. 1992) and capacitors accounted for the greatest tonnages (de Voogt & Brinkman 1989). Production of PCBs was banned in 1977 when their ability to accumulate in the environment and to cause harmful effects became apparent (ATSDR 2000).

PCBs enter the environment as mixtures containing a variety of individual components and impurities. At least one third of the PCBs that have been produced are now estimated to have entered the environment (Swedish EPA 1998). The other two thirds remain in old electrical equipment and in waste dumps, from which they continue to leach into the environment (for example, when obsolete electrical and electronic equipment is dismantled, recycled and/or disposed of).

Once released to the environment from whatever source, PCBs are highly persistent. Furthermore, PCBs that are taken up by aquatic organisms and fish accumulate in them, reaching levels that may be thousands of times higher than in water (ATSDR 2000). PCBs are bioconcentrated to a factor of 6000 for fish and 47 000 for invertebrates (Jones et al. 1988). Train (1979) reports bioconcentration factors of between 2500 and 100 000.

PCBs can be absorbed through the skin as well as through ingestion and inhalation. For the general population today, food is undoubtedly the primary route of exposure to PCBs (see e.g. review by Allsopp et al. 2000), although dermal exposure may be dominant amongst those directly handling PCBs or PCB-contaminated materials (Lees et al. 1987).

PCBs exhibit wide range of toxic effects in animals, including immunosuppression, liver damage, tumour promotion, neurotoxicity, behavioural changes and damage to both male and female reproductive systems (Seegal and Shain 1992, Safe 1993, Rice 1999). PCBs may affect not only the oestrogen system, but also the androgen system, the thyroid hormone system, the retinoid system, the corticosteroid system and several other endocrine pathways (Brouwer et al. 1999). Although it is difficult to assess the impact of contaminants on populations in the wild, not least because they are exposed to complex mixtures of chemical contaminants, some immunological and reproductive disorders in marine mammals have nevertheless been linked to elevated levels of persistent organochlorines, in particular the PCBs (see reviews by Allsopp et al. 1999, 2001, Haave et al. 2003).

In humans, the greatest body of research on the toxic effects of PCBs has come from two incidents in Japan and Taiwan where people consumed cooking oil that was contaminated with PCBs and other organochlorines. A recent review of data for children born to mothers exposed to PCBs and PCDFs in the Taiwan incident notes higher incidences of retarded growth, delayed cognitive development and behavioural problems than in children of unexposed mothers (Guo et al. 2004). In young men with prenatal exposure there was also significantly increased abnormal morphology of sperm.

Studies on the general population of the Netherlands and the Arctic and families of Swedish fishermen (reviewed by Allsopp et al. 1999, 2001) suggested that even relatively low levels of exposure to PCBs can result in impacts on the immune system (see also Weisglas-Kuperus et al. 2004) growth retardation and neurological effects.

The control of PCBs is addressed under many international legal instruments relating to environmental pollution (inter alia, the Barcelona, Helsinki, Basel, Bamako, Rotterdam, OSPAR and LRTAP Conventions and the International Joint Commission on the Great Lakes). In addition, PCBs are targeted for global production ban under the 2001 Stockholm Convention on persistent organic pollutants (POPs), an instrument which also requires proper controls on destruction of stockpiles and the handling of wastes.


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# Annex A: details of sample preparation and analytical methods

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# Annex A: details of sample preparation and analytical methods

Pooled eel muscle samples were extracted utilising an automated Soxhlet procedure using a 1:1 (v/v) acetone:n-hexane mix (de Boer et al. 2001). Thoroughly homogenised samples of tissue were mixed with sodium sulphate, transferred to the Soxhlet thimble and topped with 1 cm of sodium sulphate. Extraction took place over a 4 h period with an average of 9 - 10 cycles h-1.

### Sample extract cleanup for PBDEs and PCBs

Aliquots of each of the Soxhlet extracts were cleaned up and fractionated using alumina and silica columns, and the PBDEs and PCBs determined using gas chromatography with detection by negative ion chemical ionization mass spectrometry (GC-MS-NICI) and Electron Capture Detector (GC-ECD) respectively.

### Analysis of PBDEs using GC-MS-NICI

Residues of selected PBDEs were determined by GC-MS-NICI after the method of De Boer et al. (2001). In brief, sample extracts in iso-octane were analysed by gas chromatography mass spectrometry in the negative ion chemical ionisation mode. A seven point calibration curve was constructed using BDEs17, 28, 47, 66,100, 99, 85, 154, 153 & 138, representing the dominant congeners in "penta" mix formulations, plus BDE183, representative of "octa" mix formulations. Samples were injected in the pulsed splitless mode onto a 50m x 0.25mm x 0.25µm DB-5 column and bromine ions at 79/81 amu were monitored in selected ion monitoring (SIM) mode. Quantitation was performed by internal standard procedures using CB#200 as a reference.

### Analysis of PCBs using GC-ECD

An Agilent 6890 GC with microcell ECD was used to determine PCBs. The separation was performed on a 50.0 m _ 200 µm, 0.33-µm-film-thickness DB-5 capillary column supplied by Agilent Technologies (Waldbronn, Germany). The carrier and ECD make-up gas were hydrogen (32.2 psi constant pressure, initial velocity 50 cm/s) and argon/methane (95:5), respectively. The initial oven temperature was 90°C, held for 2.00min, then increased to 165°C at 15°C/min, to 285°C at 2°C/min, and finally held for 23 min. The injector temperature and detector temperature were 270°C and 300°C, respectively. A 1-µl extract was injected in splitless mode with a purge time of 2 min. The identification of CBs was based on the retention time of individual standards in the calibration mixtures.

### Sample extract cleanup for HBCD

Sample extract clean-up was performed by gel permeation chromatography (GPC) followed by sulphuric acid cleanup.

GPC cleanup was performed on a Agilent Series 1100 HPLC system (Agilent Technologies, Waldbronn, Germany) consisting of an autosampler, a quaternary pump, a vacuum degasser, a variable wavelength UV detector set at 254 nm, a thermostated column department set at 25°C and a fraction collector with 20 ml capacity vessels (30 mm _ 48 mm), all controlled by ChemStation software (A.09.03). The GPC columns used were two Envirogel™ columns (Waters Corporation, Milford, MA, USA) (19_150 mm and 19_300 mm) in series.

The mobile phase was composed of ethyl acetate:cyclohexane [1:1] heated to 25°C before being run through the columns at a flow rate of 5ml/min for 40 min.

A volume of Soxhlet extract, determined as a function of lipid content, and 20µl of surrogate internal standards (containing 2500 ng/ml $d_{18}$-α-HBCD, $d_{18}$-β-HBCD, $d_{18}$-γ-HBCD and $^{13}C_{12}$ TBBP-A), were concentrated to 1.5 ml. 900 µl of the 1.5 mL concentrated sample extract were injected onto the calibrated GPC system. Fractions were collected from ca 15 to 22 minutes, evaporated to dryness and reconstituted to 1 ml using HPLC grade η-hexane before sulphuric acid cleanup.

Sulfuric acid cleanup of the GPC fraction was performed following EPA Method 3665A. For this, the GPC fractions were concentrated to dryness using a Turbo-Vap concentrator at 37°C (5-10 psi) and the residues were transferred quantitatively into a stoppered tube using a maximum of 1 ml η-hexane. 5 ml aqueous sulphuric acid solution (1:1) were added to the 1 ml η-hexane extract and vortexed for 1 minutes. The upper solvent layer was transferred to a clean tube and the sulphuric acid treatment was repeated until the solvent phase was colourless. After the last clean-up step, the sulphuric acid was extracted twice with η-hexane and combined with the cleaned up η-hexane extract. The final hexane extract was reduced to near dryness and reconstituted to 120 µl using methanol.

### HBCD analysis by LC-MS

The LC-MS system used was a Surveyor® HPLC system (ThermoFinnigan, San Jose, CA, USA) consisting of a quaternary pump equipped with a vacuum-membrane degasser and an autosampler equipped with a column heater. Detection was performed using an LCQ Advantage ion trap mass spectrometer (ThermoFinnigan, San Jose, CA, USA), equipped with an electrospray (ESI) interface operated in the negative ionisation mode. The Xcalibur software package version 1.2 (ThermoFinnigan, San Jose, CA, USA) was used for instrument control, data acquisition and processing. The HPLC column used was a 100 mm _ 2.0 mm i.d. (3µm particle size) Luna C18(2) column protected by a SecuriGuard™ cartridge and the injection volume was 15µl.

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# Annex B: detailed data for PBDEs, HBCD, TBBP-A and PCBs

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# Annex B: detailed data for PBDEs, HBCD, TBBP-A and PCBs

## Concentrations of 11 PBDEs (ng/g fresh weight,ppb)

| Sample code | % Lipids (wt/wt) | Number in pooled sample | BDE#17 | BDE#28 | BDE#47 | BDE#66 | BDE#85 | BDE#99 | BDE#100 | BDE#138 | BDE#153 | BDE#154 | BDE#183 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Belgium | 19.1 | 4 | nd | nd | 4.7 | nd | nd | nd | 2.0 | nd | nd | nd | nd |
| Czech 1 | 4.7 | 2 | nd | nd | 4.3 | nd | nd | 0.2 | 1.4 | nd | 0.2 | 0.2 | nd |
| Czech 2 | 14.2 | 2 | nd | nd | 1.0 | nd | nd | nd | nd | nd | nd | nd | nd |
| France 1 | 2.8 | 5 | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd |
| France 2 | 11.6 | 5 | nd | nd | 0.5 | nd | nd | nd | nd | nd | nd | nd | nd |
| Germany 1 | 19.0 | 5 | nd | nd | 7.9 | nd | nd | 0.7 | 0.9 | nd | nd | nd | nd |
| Germany 2 | 22.0 | 5 | nd | nd | 17.0 | nd | nd | nd | 3.9 | nd | 0.5 | 0.7 | nd |
| Germany 3 | 17.0 | 5 | nd | nd | 9.5 | nd | nd | 0.6 | 2.0 | nd | nd | nd | nd |
| Germany 4 | 15.3 | 5 | nd | nd | 9.3 | nd | nd | 0.6 | 3.1 | nd | 0.4 | 0.4 | nd |
| Ireland 1 | 3.5 | 5 | nd | nd | 0.2 | nd | nd | nd | nd | nd | nd | nd | nd |
| Ireland 2 | 15.4 | 5 | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd |
| Italy 1 | 22.2 | 5 | nd | nd | 24.0 | nd | nd | 2.1 | 6.8 | nd | 1.4 | 0.9 | nd |
| Italy 2 | 25.8 | 2 | nd | nd | 1.8 | nd | nd | nd | nd | nd | nd | nd | 2.1 |
| Netherlands 1 | 9.9 | 2 | nd | nd | 0.4 | nd | nd | nd | nd | nd | nd | nd | nd |
| Netherlands 2 | 15.8 | 2 | nd | nd | 3.2 | nd | nd | nd | 1.0 | nd | nd | nd | nd |
| Netherlands 3 | 15.2 | 2 | nd | 0.4 | 17.0 | nd | nd | 0.6 | 7.7 | nd | 1.2 | 1.0 | nd |
| Poland | 6.0 | 5 | nd | nd | 0.2 | nd | nd | nd | nd | nd | nd | nd | nd |
| Spain 1 | 4.6 | 4 | nd | nd | 1.2 | nd | nd | nd | 0.5 | nd | nd | nd | nd |
| Spain 2 | 19.7 | 5 | nd | nd | 2.7 | nd | nd | nd | 0.9 | nd | nd | nd | nd |
| UK | 18.0 | 5 | nd | 0.5 | 46.0 | 0.8 | nd | 3.2 | 12.0 | nd | 1.1 | 2.1 | nd |

*Table B1: concentrations of 11 PBDE congeners in eel muscle tissue (ng/g fresh weight, ppb)*
*nd – below detection limit (0.125 ppb)*

## Concentrations of 11 PBDEs (ng/g lipid weight,ppb)

| Sample code | % Lipids (wt/wt) | Number in pooled sample | BDE#17 | BDE#28 | BDE#47 | BDE#66 | BDE#85 | BDE#99 | BDE#100 | BDE#138 | BDE#153 | BDE#154 | BDE#183 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Belgium | 19.1 | 4 | nd | nd | 24.6 | nd | nd | nd | 10.5 | nd | nd | nd | nd |
| Czech 1 | 4.7 | 2 | nd | nd | 91.5 | nd | nd | 4.0 | 29.8 | nd | 3.2 | 5.1 | nd |
| Czech 2 | 14.2 | 2 | nd | nd | 6.8 | nd | nd | nd | nd | nd | nd | nd | nd |
| France 1 | 2.8 | 5 | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd |
| France 2 | 11.6 | 5 | nd | nd | 4.1 | nd | nd | nd | nd | nd | nd | nd | nd |
| Germany 1 | 19.0 | 5 | nd | nd | 41.6 | nd | nd | 3.8 | 4.6 | nd | nd | nd | nd |
| Germany 2 | 22.0 | 5 | nd | nd | 77.3 | nd | nd | nd | 17.7 | nd | 2.3 | 3.1 | nd |
| Germany 3 | 17.0 | 5 | nd | nd | 55.9 | nd | nd | 3.6 | 11.8 | nd | nd | nd | nd |
| Germany 4 | 15.3 | 5 | nd | nd | 60.8 | nd | nd | 3.7 | 20.3 | nd | 2.6 | 2.7 | nd |
| Ireland 1 | 3.5 | 5 | nd | nd | 4.9 | nd | nd | nd | nd | nd | nd | nd | nd |
| Ireland 2 | 15.4 | 5 | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd |
| Italy 1 | 22.2 | 5 | nd | nd | 108.1 | nd | nd | 9.5 | 30.6 | nd | 6.3 | 4.1 | nd |
| Italy 2 | 25.8 | 2 | nd | nd | 7.0 | nd | nd | nd | nd | nd | nd | nd | 8.1 |
| Netherlands 1 | 9.9 | 2 | nd | nd | 3.8 | nd | nd | nd | nd | nd | nd | nd | nd |
| Netherlands 2 | 15.8 | 2 | nd | nd | 20.3 | nd | nd | nd | 6.1 | nd | nd | nd | nd |
| Netherlands 3 | 15.2 | 2 | nd | 2.5 | 111.8 | nd | nd | 4.1 | 50.7 | nd | 7.9 | 6.6 | nd |
| Poland | 6.0 | 5 | nd | nd | 3.8 | nd | nd | nd | nd | nd | nd | nd | nd |
| Spain 1 | 4.6 | 4 | nd | nd | 26.1 | nd | nd | nd | 10.7 | nd | nd | nd | nd |
| Spain 2 | 19.7 | 5 | nd | nd | 13.7 | nd | nd | nd | 4.7 | nd | nd | nd | nd |
| UK | 18.0 | 5 | nd | 2.8 | 255.6 | 4.2 | nd | 17.8 | 66.7 | nd | 6.1 | 11.7 | nd |

*Table B2: concentrations of 11 PBDE congeners in eel muscle tissue (ng/g lipid weight, ppb)*

## Concentrations HBCD, total HBCD and TBBP-A (fresh weight)

| Sample code | % Lipids (wt/wt) | Number in pooled samples | α HBCD | β HBCD | γ HBCD | Σ HBCD | TBBP-A |
|---|---|---|---|---|---|---|---|
| Belgium | 19.1 | 4 | 2 | 1 | 1 | 5 | nd |
| Czech 1 | 4.7 | 2 | 4 | nd | nd | 4 | nd |
| Czech 2 | 14.2 | 2 | nd | nd | nd | nd | nd |
| France 1 | 2.8 | 5 | nd | 3 | nd | 3 | nd |
| France 2 | 11.6 | 5 | nd | 2 | nd | 2 | nd |
| Germany 1 | 19.0 | 5 | 2 | nd | nd | 2 | nd |
| Germany 2 | 22.0 | 5 | 12 | 2 | 1 | 15 | nd |
| Germany 3 | 17.0 | 5 | 7 | 2 | 1 | 9 | nd |
| Germany 4 | 15.3 | 5 | 26 | 3 | 8 | 37 | nd |
| Ireland 1 | 3.5 | 5 | nd | nd | nd | nd | nd |
| Ireland 2 | 15.4 | 5 | nd | 2 | 1 | 3 | nd |
| Italy 1 | 22.2 | 5 | 23 | 2 | 1 | 26 | nd |
| Italy 2 | 25.8 | 2 | 2 | 2 | nd | 4 | nd |
| Netherlands 1 | 9.9 | 2 | 6 | 2 | 1 | 9 | nd |
| Netherlands 2 | 15.8 | 2 | 2 | nd | nd | 2 | nd |
| Netherlands 3 | 15.2 | 2 | 6 | 2 | 1 | 9 | nd |
| Poland | 6.0 | 5 | nd | 1 | nd | 1 | nd |
| Spain 1 | 4.6 | 4 | 2 | 3 | 2 | 7 | nd |
| Spain 2 | 19.7 | 5 | 1 | 2 | 1 | 4 | nd |
| UK | 18.0 | 5 | >50 | 2 | 2 | >50 | nd |

*Table B3: concentrations of HBCD isomers, total HBCD and TBBP-A in eel muscle tissue (ng/g fresh weight, ppb).*

## Concentrations HBCD, total HBCD and TBBP-A (lipid weight)

| Sample code | % Lipids (wt/wt) | Number in pooled samples | α HBCD | β HBCD | γ HBCD | Σ HBCD | TBBP-A |
|---|---|---|---|---|---|---|---|
| Belgium | 19.1 | 4 | 11 | 8 | 6 | 24 | nd |
| Czech 1 | 4.7 | 2 | 79 | nd | nd | 79 | nd |
| Czech 2 | 14.2 | 2 | nd | nd | nd | nd | nd |
| France 1 | 2.8 | 5 | nd | 111 | nd | 111 | nd |
| France 2 | 11.6 | 5 | nd | 13 | nd | 13 | nd |
| Germany 1 | 19.0 | 5 | 9 | nd | nd | 9 | nd |
| Germany 2 | 22.0 | 5 | 57 | 7 | 5 | 69 | nd |
| Germany 3 | 17.0 | 5 | 40 | 9 | 7 | 56 | nd |
| Germany 4 | 15.3 | 5 | 167 | 18 | 54 | 239 | nd |
| Ireland 1 | 3.5 | 5 | nd | nd | nd | nd | nd |
| Ireland 2 | 15.4 | 5 | nd | 11 | 9 | 20 | nd |
| Italy 1 | 22.2 | 5 | 103 | 7 | 6 | 117 | nd |
| Italy 2 | 25.8 | 2 | 7 | 9 | nd | 15 | nd |
| Netherlands 1 | 9.9 | 2 | 61 | 16 | 13 | 90 | nd |
| Netherlands 2 | 15.8 | 2 | 11 | nd | nd | 11 | nd |
| Netherlands 3 | 15.2 | 2 | 40 | 11 | 9 | 61 | nd |
| Poland | 6.0 | 5 | nd | 25 | nd | 25 | nd |
| Spain 1 | 4.6 | 4 | 46 | 66 | 48 | 161 | nd |
| Spain 2 | 19.7 | 5 | 6 | 8 | 7 | 22 | nd |
| UK | 18.0 | 5 | >278 | 13 | 12 | >278 | nd |

*Table B4: concentrations of HBCD isomers, total HBCD and TBBP-A in eel muscle tissue (ng/g lipid weight, ppb)*

## Concentrations of ICES 7 PCBs (ng/g fresh weight, ppb)

| Sample code | % Lipids (wt/wt) | Number in pooled sample | CB#28 | CB#52 | CB#101 | CB#118 | CB#138 | CB#153 | CB#180 | SUM ICES7 |
|---|---|---|---|---|---|---|---|---|---|---|
| Belgium | 19.1 | 4 | nd | nd | 6 | 10 | 28 | 38 | 15 | 97 |
| Czech 1 | 4.7 | 2 | 2 | 7 | 11 | 9 | 51 | 69 | 35 | 184 |
| Czech 2 | 14.2 | 2 | 8 | 4 | 4 | 4 | 14 | 20 | 12 | 66 |
| France 1 | 2.8 | 5 | nd | nd | nd | 3 | 6 | 17 | 3 | 29 |
| France 2 | 11.6 | 5 | nd | nd | nd | nd | 2 | 2 | nd | 5 |
| Germany 1 | 19.0 | 5 | 2 | 8 | 33 | 26 | 89 | 120 | 49 | 327 |
| Germany 2 | 22.0 | 5 | 5 | 16 | 49 | 59 | 160 | 200 | 77 | 566 |
| Germany 3 | 17.0 | 5 | nd | 2 | 14 | 17 | 57 | 77 | 28 | 196 |
| Germany 4 | 15.3 | 5 | 5 | 15 | 34 | 44 | 95 | 150 | 38 | 381 |
| Ireland 1 | 3.5 | 5 | nd | nd | nd | nd | 2 | 2 | nd | 4 |
| Ireland 2 | 15.4 | 5 | nd | nd | nd | nd | 2 | nd | 2 | 5 |
| Italy 1 | 22.2 | 5 | 7 | 16 | 39 | 41 | 120 | 160 | 100 | 483 |
| Italy 2 | 25.8 | 2 | nd | 4 | 12 | 21 | 34 | 37 | 12 | 120 |
| Netherlands 1 | 9.9 | 2 | 2 | nd | 2 | nd | 4 | 4 | 3 | 16 |
| Netherlands 2 | 15.8 | 2 | 8 | 14 | 12 | 19 | 38 | 55 | 19 | 165 |
| Netherlands 3 | 15.2 | 2 | 7 | 45 | 140 | 120 | 360 | 670 | 170 | 1512 |
| Poland | 6.0 | 5 | nd | nd | nd | nd | 1 | 1 | nd | 2 |
| Spain 1 | 4.6 | 4 | nd | nd | nd | 3 | 15 | 27 | 8 | 54 |
| Spain 2 | 19.7 | 5 | 8 | 4 | 6 | 8 | 26 | 43 | 28 | 123 |
| UK | 18.0 | 5 | nd | 6 | 19 | 17 | 37 | 43 | 14 | 136 |

*Table B5: concentrations of ICES 7 PCB congeners in eel muscle tissue (ng/g fresh weight, ppb)*

*nd – below detection limit (1 ppb)*

## Concentrations of ICES 7 PCBs (ng/g lipid weight, ppb)

| Sample code | % Lipids (wt/wt) | Number in pooled sample | CB#28 | CB#52 | CB#101 | CB#118 | CB#138 | CB#153 | CB#180 | SUM ICES7 |
|---|---|---|---|---|---|---|---|---|---|---|
| Belgium | 19.1 | 4 | nd | nd | 31.4 | 52.4 | 146.6 | 199.0 | 78.5 | 507.9 |
| Czech 1 | 4.7 | 2 | 42.6 | 148.9 | 234.0 | 191.5 | 1085.1 | 1468.1 | 744.7 | 3914.9 |
| Czech 2 | 14.2 | 2 | 56.3 | 28.2 | 28.2 | 28.2 | 98.6 | 140.8 | 84.5 | 464.8 |
| France 1 | 2.8 | 5 | nd | nd | nd | 107.1 | 214.3 | 607.1 | 107.1 | 1035.7 |
| France 2 | 11.6 | 5 | nd | nd | nd | nd | 17.2 | 17.2 | nd | 43.1 |
| Germany 1 | 19.0 | 5 | 10.5 | 42.1 | 173.7 | 136.8 | 468.4 | 631.6 | 257.9 | 1721.1 |
| Germany 2 | 22.0 | 5 | 22.7 | 72.7 | 222.7 | 268.2 | 727.3 | 909.1 | 350.0 | 2572.7 |
| Germany 3 | 17.0 | 5 | nd | 11.8 | 82.4 | 100.0 | 335.3 | 452.9 | 164.7 | 1152.9 |
| Germany 4 | 15.3 | 5 | 32.7 | 98.0 | 222.2 | 287.6 | 620.9 | 980.4 | 248.4 | 2490.2 |
| Ireland 1 | 3.5 | 5 | nd | nd | nd | nd | 57.1 | 57.1 | nd | 114.3 |
| Ireland 2 | 15.4 | 5 | nd | nd | nd | nd | 13.0 | nd | 13.0 | 32.5 |
| Italy 1 | 22.2 | 5 | 31.5 | 72.1 | 175.7 | 184.7 | 540.5 | 720.7 | 450.5 | 2175.7 |
| Italy 2 | 25.8 | 2 | nd | 15.5 | 46.5 | 81.4 | 131.8 | 143.4 | 46.5 | 465.1 |
| Netherlands 1 | 9.9 | 2 | 20.2 | nd | 20.2 | nd | 40.4 | 40.4 | 30.3 | 161.6 |
| Netherlands 2 | 15.8 | 2 | 50.6 | 88.6 | 75.9 | 120.3 | 240.5 | 348.1 | 120.3 | 1044.3 |
| Netherlands 3 | 15.2 | 2 | 46.1 | 296.1 | 921.1 | 789.5 | 2368.4 | 4407.9 | 1118.4 | 9947.4 |
| Poland | 6.0 | 5 | nd | nd | nd | nd | 16 | 16 | nd | 33 |
| Spain 1 | 4.6 | 4 | nd | nd | nd | 65.2 | 326.1 | 587.0 | 173.9 | 1173.9 |
| Spain 2 | 19.7 | 5 | 40.6 | 20.3 | 30.5 | 40.6 | 132.0 | 218.3 | 142.1 | 624.4 |
| UK | 18.0 | 5 | nd | 33.3 | 105.6 | 94.4 | 205.6 | 238.9 | 77.8 | 755.6 |

*Table B6: concentrations of ICES 7 PCB congeners in eel muscle tissue (ng/g lipid weight, ppb)*

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