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The Bioaccumulation of Persistent Organic Pollutants in Marine Species from Irish and Surrounding Waters

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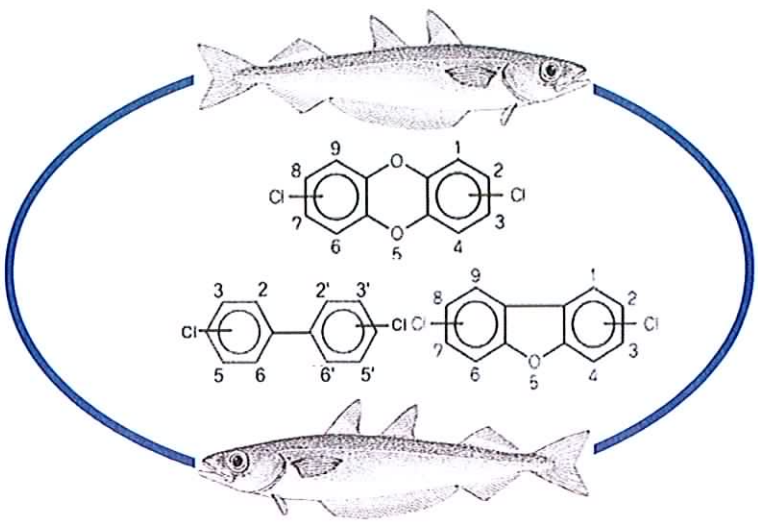
The bioaccumulation of persistent organic pollutants in marine species from Irish and surrounding waters.



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For the Award of PhD.

Dublin Institute of Technology.

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Abstract

The presence of anthropogenic pollutants throughout all compartments of the marine environment have been of international concern for a number of decades. While a great number of datasets documenting "absolute" concentrations of persistent organic pollutants in a variety of marine biota are available, bioaccumulation, biomagnification and the fate of these compounds in the marine food web or marine ecosystem is often not possible. This thesis reports various analytical methodologies employed to determine levels of a wide scope of persistent organic pollutants in marine biota and traces the flow of carbon and nitrogen isotopes through the marine food web, ultimately applying bioconcentration and biomagnification modelling techniques to these data.

Standardisation of sampling procedures and baseline levels of a wide range of lipophilic contaminants in a variety of marine species are reported. This thesis (and associated publications) report a number of new contaminant datasets in Irish marine species and reports that no adverse effects to the consumer of Irish fishery produce are currently expected from a number of contaminant groups including toxaphene, PBDEs and HBCD. Enantiospecific enrichment and trophic level status related accumulation of OCP compounds in Killer whales is reported, with the first known use of stable isotope techniques to identify marine mammal dietary influences in a killer whale from British and Irish waters. Factors influencing pollutant bioaccumulation in commercially important and/or biologically sensitive species including, blue whiting, eels and killer whales are discussed. The role of stable isotopes in modelling contaminant bioaccumulation, trophic transfer within the marine web, as a means to establish dietary and/or habitat preference and the implications of findings on environmental and food safety monitoring programmes are discussed.

Datasets and techniques reported demonstrate that stable isotope based techniques are powerful tools in environmental and food safety monitoring programmes thereby continuing to further protect both the consumer of marine produce, marine species and marine biodiversity.

I certify that this thesis which I now submit for examination for the award of PhD, is entirely my own work and has not been taken from the work of others save and to the extent that such work has been cited and acknowledged within the text of my work.

This thesis was prepared according to the regulations for postgraduate study by research of the Dublin Institute of Technology and has not been submitted in whole or in part for an award in any other Institute or University.

The work reported on in this thesis conforms to the principles and requirements of the Institute's guidelines for ethics in research.

The Institute has permission to keep, to lend or to copy this thesis in whole or in part, on condition that any such use of the material of the thesis be duly acknowledged.

Signature _____

Date 27/05/09

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Scope of this thesis

The presence of industrial and other anthropogenic pollutants throughout all compartments of the marine environment has been of national and international concern for a number of decades. A great number of regional and international contaminant monitoring programs currently exist with primarily goals to; identify pollutants of immediate concern and to assess both temporal and spatial aspects of contamination, usually with the ultimate aim to reduce contaminant inputs and to minimize impacts of pollution on the marine environment, on biodiversity and to the consumer of marine produce.

The majority of these programmes are well established and are of sufficient resolution power to allow temporal and spatial trend assessments completed for a number of so-called "priority" or "pollutants of concern" in the water column, sediment and within marine biota. While national and international programmes provide vast datasets documenting "absolute" concentrations of persistent organic pollutants in a variety of marine biota, relatively few request the collection and reporting of appropriate supporting parameters to enable scientists model pollutant biomagnification and fate through each of the trophic levels of the marine food web.

It is crucial that such bodies strive to maintain/improve the current state of the marine environment, to further develop tools and mechanisms to assist in maintaining biodiversity and continue to protect both the health status of marine inhabitants and the consumer of marine produce. Therefore it is fundamental that chemists, biologists, environmental managers and consumer risk assessors develop an understanding of the

underlying principles and mechanisms resulting in the biomagnification of pollutants through marine ecosystems and food-webs.

This thesis reports various analytical methodologies employed to determine concentration levels for a wide scope of persistent organic pollutants in marine biota and traces the flow of carbon and nitrogen isotopes through the marine food web and ultimately applies bioconcentration and biomagnification modelling techniques to these data.

Method development, baseline levels and a summary risk assessment to the consumer of toxaphene residues in fishery produce from Irish waters are presented. Contaminant trophic transfer and biological factors influencing persistent pollutant levels in blue whiting, eels and killer whales are discussed in greater detail. Finally the implications of findings for future environmental and food safety monitoring programmes are discussed. The layout and scope of the thesis are described in greater detail below.

Chapter 1 introduces and reviews the fundamental principles/models and kinetics underpinning bioconcentration, biomagnification and trophic transfer of persistent organic pollutants in biota. This chapter further reviews the physico-chemical properties and current literature on contaminant levels in marine species from Irish and surrounding waters. The principles and analytical methodologies employed to evaluate enantioselective magnification/elimination of chiral pollutants and the role of enantioselective chromatography in determining the ultimate fate of contaminants in marine species is also documented. Finally **Chapter 1** reports sampling processes and summary analytical methodologies and analytical quality control aspects of this current study.

In recent decades the detection of residues of the pesticide toxaphene in the tissues of marine species led to increased research and monitoring into the prospective detrimental effects of toxaphene in the environment but especially in relation to the potential risk to the consumer of dietary exposure to toxaphene residues via fishery produce. **Chapter 2** describes method development, sampling strategies and baseline concentrations of toxaphene in fishery produce from Irish waters. Biological and other factors influencing toxaphene levels in Irish marine species are discussed and a summary risk assessment to the consumer of Irish fishery produce is presented. The potential for trophic level biomagnification of toxaphene residues in marine biota is further addressed in **Chapter 6.**

Chapter 3 focuses on the levels of a wide range of pollutants in the gadoid fish species blue whiting *(Micromesistius poutassou)*. This species is of increasing commercial importance primarily as a result of diminishing stocks of historically important fish stocks and is of biological and chemical interest as derived fish oils have been reported to contain elevated levels of contaminant residues (especially of dioxins and furans) compared to other like species. Temporal and biological aspects thought to influence dioxin levels are discussed and the role of stable isotope techniques as a mechanism to model contaminant levels is further evaluated and discussed in the context of other marine species in **Chapters 3 and 6.**

The European eel (*Anguilla anguilla*) has long been employed as an indicator species for the purposes of monitoring contaminant levels in the environment. In recent decades eel stocks have diminished and levels of some persistent pollutants have been reported to accelerate this decline. **Chapter 4** documents the levels of dioxins, furans, PCBs, flame retardant compounds and organochlorine pesticides in eel muscle and investigates

factors influencing contaminant levels in the species. Eels primarily frequent estuarine and/or riverine habitats and have a relatively poorly understood life cycle in comparison to other invertebrate species, thereby routes of exposure to anthropogenic pollutants in eels can differ to those of other marine inhabitants. **Chapters 4 and 6** further report the relevance of stable isotope techniques as a tool to elucidate such habitats and to model contaminant uptake in eels from Irish waters.

The killer whale (*Orcinus orca*) has long been established as a top of marine food-web predator and as such they can act as a potential repository for elevated levels of marine pollutants. While contaminant levels in killer whales have been well documented in animals from Pacific regions few data exist to document pollutant levels in those resident in British and Irish waters. Transient killer whales feeding on marine mammals have been identified in other offshore waters however this is the first reported instance where stable isotope derived trophic status has been utilised to model contaminant uptake in the species in British and Irish waters. **Chapters 5 and 6** discuss biomagnification of pollutants in the killer whale food chain and report biomagnification factors for a number of PCB and PBDE congeners in the species.

Chapter 6 encompasses and summarises contaminant information from all of the above datasets and reports further data from a number of other species resident in Irish waters. The application of stable isotopes based techniques to model species trophic status, lipid content and contaminant levels are reported and the relevance of stable isotope approaches as a predictor of contaminant biomagnification are discussed. Biological and analytical considerations with respect to the use of stable isotope techniques in the design of future development of food and environmental monitoring programmes are additionally evaluated.

In summary this thesis improves the current databases on contaminant levels for a wide range of commercially important and in some cases biologically sensitive species found in Irish waters. Resulting datasets enhance our current knowledge with respect to, assessment of consumer risk from Irish fishery produce, evaluation of health status of species in Irish waters and further describes factors underlying biomagnification of pollutants in these biota.

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CHAPTER 1: ACCUMULATION OF PERSISTENT ORGANIC POLLUTANTS IN MARINE SPECIES

1. 1 Introduction

Thousands of naturally occurring and anthropogenic chemicals are now deemed to be ubiquitous in our environment. Numerous pollutant monitoring programmes/fora and research projects currently exist with goals including the identification of chemicals of concern, evaluation of the usage patterns of existing and new chemicals, assessment of the risk of these compounds on the environment, to humans, animals and to plant life and additionally to the ongoing monitoring of spatial and temporal trends of such persistent compounds, with the ultimate aim to further protect marine biodiversity, the marine environment and its inhabitants and humans from the potential adverse effects of chemical residues.

Physical and chemical properties and usage patterns ultimately deem whether the majority of these compounds pose a potential threat to the organisms that inhabit individual environmental compartments. Recent years have seen increased activity in evaluating the biomagnification and fate of these man-made chemicals within the marine ecosystem.

In order to further investigate magnification of contaminants within marine species, the definitions, underlying principles and the key limiting factors of bioconcentration, biomagnification, bioaccumulation and ecological magnification must be understood.

1.1.1 Bioconcentration and associated kinetics

Bioconcentration occurs as a result of the direct uptake by an organism of a chemical from the water phase via passive partitioning processes, this generally being expressed in terms of experimentally derived bioconcentration factors (BCFs). Numerous studies have defined BCFs as being the ratio of steady state concentration of a chemical in an

aquatic organism (C_F) to the corresponding freely dissolved chemical concentration in the surrounding water column (C_W). A number of authors report that the calculation of bioconcentration factors of compounds in water may be completed using the following equation (1-5),

Equation 1=

$$BCF = \frac{C_F}{C_W} \frac{[ng/kg^{-1}]}{[ng/L^{-1}]} = \frac{K_1}{K_2}$$

Where,

C_F and C_W relate to steady state of the chemical in organisms and concentration in water respectively.

K_1 and K_2 relate to the uptake clearance and release rate constant. K_1 and K_2 are measured as unit mass of chemical divided by time, which translates, to time^{-1} (6).

Geyer et al (7) further report that the calculated BCF of a "lipophilic" compound on a wet weight basis is dependent on the lipid content of the organism in question. Within the scope of this present study only lipophilic POPs were investigated, therefore, bioconcentration factors on a lipid basis can be described by,

Equation 2=

$$BCFL = \frac{BCFW * 100}{Lw(\%)}$$

Where,

BCFL and BCFW represent the BCF on a lipid and weight basis respectively

Lw = Percentage lipid in the organism

Passive bioconcentration of a chemical by aquatic organisms into lipid tissues is dependent on numerous abiotic factors including, the physical and chemical properties of a chemical in question, water solubility rates (log K_{ow}), temperature and flow rates of the water body, in addition to a number of biotic factors including species, sex, health status, compound elimination rates and/or the half-life of the chemical in the test species. Therefore bioconcentration kinetic modelling studies tend to be species specific, resulting in BCFs valid only within strictly controlled physico-chemical conditions.

1.1.1.1 Steady-state dynamics.

In a situation whereby steady state is observed no further accumulation or elimination of compound takes place within the organism and the above equation can be used to derive BCFs for the test chemical.

However in *in-vitro* studies it is not always possible to obtain such a state of equilibrium as additional biological factors such as continued growth of the species and metabolism of the test compound can intervene. Additionally in the case of hydrophobic (nonpolar/un-ionizable) compounds the test timeframe required to achieve steady state may be unrealistic, therefore additional parameters have to be introduced into bioconcentration equations to account for such time elements. Ultimately in a situation where it is possible to reach a steady state, the BCF of a compound can be described by,

Equation 3= $$BCF = \frac{C_F}{C_W} = \frac{K_1}{K_2 + Kg + Km}$$

Where,

Kg = specific growth rate constant

Km = metabolism rate constant

It should be noted that these equations provide species specific BCF data that are calculated under well controlled conditions, therefore their application in real-time field situations will be complicated by additional factors such as seasonal fluctuations in water temperature and pH, total organic carbon loading and biological factors such as species age, sex and/or health status. Geyer et al (8) have comprehensively reviewed these factors, therefore only factors with a high degree of influence on generation of BCFs are further discussed.

1.1.1.2 Compound uptake, elimination and biological half-life

Knowledge of uptake and elimination rates of a compound within an organism is vital to enable accurate description of bioconcentration factors to be completed. In most models uptake and elimination of a persistent chemical can be described as follows (9),

Equation 4= $$\frac{dCf}{df} = K_1 * C_w - K_2 * C_f$$

Where

K_1= uptake rate constant per day

K_2= elimination or depuration rate per day

C_w = Concentration in water

C_f= Concentration in fish

Information on a number of further kinetic modelling parameters is required to describe the processes of elimination/biological half-life within an organism. The elimination constant (K_2) of a chemical can often follow first order kinetics and be described as below,

Equation 5= $$K_2 = \frac{2.303}{t} * \log \frac{C_0}{C_t}$$

Where,

t= time

C_0 = concentration in the organism at time zero

C_t = concentration in the organism at time t.

The biological half-life ($t_{1/2}$) of a compound is often required in risk assessment processes and is defined, as the time required in reducing the concentration of a chemical in a tissue or organ by 50%.

Where elimination rate (K_2) data are available the biological half-life can be determined by,

Equation 6= $$t_{1/2} = \frac{\ln 2}{K_2} = \frac{0.693}{K_2}$$

However where hydrophobic compounds are under investigation an additional factor to account for test species growth rate has to be included in the equation as follows,

Equation 7= $$t_{1/2} = \frac{0.693}{K_2 + K_g}$$

Where,

K_g = growth rate constant

Where it is not possible to define the growth rate (K_g) it is possible to supplement this by multiplying the chemical concentration by the weight of the organism. Geyer et al

(8) further modelled the half-life of persistent pollutants in fish and other gill- breathing organisms concluding that the half-life of POPs increases as a function of the chemicals n-octanol/water partition coefficient (Log K_{ow}) and with increases in test species lipid content.

In an attempt to try to minimize variability within calculated BCFs the Organization for Economic Cooperation and Development (OECD) has proposed the use of harmonized guidelines to evaluate bioaccumulation of chemicals in fish (10). The flow-through Kinetic Fish Test is primarily applicable where organic chemicals have a log K_{ow} of between 1.5 to 6.0 but can still be employed for the testing of super-hydrophobic compounds with a log K_{ow} value >6.0 (10). This kinetic based approach requires that the uptake rate constant (K_1) and the elimination rate constant (K_2) are determined in separate experiments, with elimination in the test species usually estimated by placing the contaminated test species (e.g. mussels), in clean flowing water and determining the decrease of the concentration in the organism over time.

1.1.1.3 Other influences on bioconcentration

As previously discussed a number of additional factors can affect the bioconcentration rate of chemicals in fish and other aquatic organisms. On an individual basis each factor contributes to the overall determination of BCF, however, a number of key factors such as toxic effects, bioavailability, concentration of the chemicals in water, pH of the water, and most importantly the lipid content of the organism play key roles in the rate of bioconcentration of chemicals, a number of relevant factors are further discussed below.

1.1.1.3.1 Physico-chemical properties of POPs.

Physico-chemical properties such as the Log K_{ow}, molecular weight and molecule dimensions are fundamental to describing the bioaccumulation potential of a test chemical/contaminant. In general terms the higher the Log K_{ow} of a non-metabolised chemical the greater the potential for bioconcentration within an organism.

Within the scope of this present study the occurrence of a number of persistent organic pollutants including polychlorinated biphenyls (PCBs), dioxins (PCDDs), furans (PCDFs), Organochlorine compounds (OCPs) including toxaphene, chlordanes, 1,1,1-Trichloro-2,2-bis (*p*-chlorophenyl) ethane (p,p'DDT), hexachlorocyclohexanes (HCHs), polybrominated diphenyl ethers (PBDEs) and polybrominated biphenyls (PBBs) amongst others were investigated. Log K_{ow} data for application in later trophic transfer studies are presented in table 1.3 (page 37).

1.1.1.3.2 Bioavailability

The bioavailability (i.e. the portion of a chemical freely available/dissolved in water) greatly influences its uptake rate through biological membranes within an organism. All living organisms are separated from external media (air, water, soil) by some form of cell membranes or epithelia composed of membrane bound cells. These living membranes are further composed of lipid bilayers, protein molecules and/or water filled membrane channels. Bioavailable molecules pass through membranes and channels in either direction by passive means or via transport down physico-chemical gradients or by enzyme systems within the organism.

Bioavailable molecules generally pass through membranes by dissolving in the lipid phase of the membrane or by passing through charged channels if their molecular configuration and charge are suitable (11). As previously discussed, experimental test

conditions for the determination of BCFs can be strictly controlled, bioavailability in the field in aquatic/marine environments is much more difficult to quantify.

The bioavailability of both metals and organic compounds in the marine environment depends to varying extents on factors such as salinity, turbidity, dissolved organic matter, particulate organic matter, particle size distribution, organic content and biological activity. Additionally due to their relatively low aqueous solubility and hydrophobic nature, concentrations of POPs dissolved in water are usually low (<1 part per billion).

POPs bind strongly to particulate organic matter (POM) and ultimately can be deposited in the form of sediments; therefore where elevated POM conditions are present the fraction of the chemical truly dissolved in the water phase can be relatively small. Geyer et al (8) report that in most studies a reduction of the uptake and the consequent bioconcentration factor of chemicals in the presence of organic materials has been found.

Particulate loadings in oceanic systems are typically low (less than 1 mg/L) and total and dissolved concentrations of contaminants are generally correspondingly low. Aquatic organisms can however, often bioconcentrate low contaminant levels in water into relatively high levels in their tissues, therefore, information on the extent to which environmental pollutants are dissolved in water bodies is vital to enable the potential for detrimental biological impacts on organisms to be assessed.

Differences can become further pronounced for particle reactive metals/organic compounds in turbid coastal waters, where greater particle loads (> tens of mg/L) can

occur. The bioavailability of pollutants can be further influenced by factors such as sedimentation and re-suspension rates (e.g. oceanic vs tidal/estuarine) and/or temporal water temperature/pH/salinity fluctuations amongst others.

1.1.1.3.3 Concentration of the test chemical in the water.

As previously stated, in order for bioconcentration to take place through the gill epithelium, skin and/or gut lining of a marine species the contaminant or test chemical must be freely bioavailable to the organism. Closed or test system generation of BCFs need to take account of the Log K_{ow} and the dissociation constant (pK_a) of the test chemical in order to accurately estimate the derived BCF. At water pH values greater than the pK_a of the chemical, the ionized form of the compound will be present, the relative amount being a function of the magnitude of difference between the pH and the pK_a.

Geyer et al (7-8, 12-13) have reported that BCFs derived for a number of compounds e.g. so-called super-hydrophobic compounds with a Log K_{ow} >6.5 have been underestimated as a consequence of saturating the test water system beyond the solubility of the compound in question. As a result alterations in currently available BCFs for high log K_{ow} compounds such as octa-chlorodibenzo-p-dioxin (OCDD) in some test species may arise in the future.

In conclusion the accurate calculation of BCFs in such test systems are essential to ultimately assist in evaluating the fate of chemicals (especially those with low water solubility) in the aquatic environment and to support environmental and consumer risk assessment processes for both new and existing compounds.

1.1.2 Role of Lipids in bioconcentration and food web studies

The bioconcentration of lipophilic compounds in aquatic organisms is essentially a function of partitioning between the chemical and the lipid of the test species. Geyer et al (7-8) have demonstrated a relationship between the wet weight derived BCF_w and the lipid content of the organism for a number of POPs e.g. Lindane (γ-HCH) and Pentachlorophenol (PCP). Generally, increased lipid content will result in an increased BCF for a test species. The total lipid content of an organism usually increases throughout the life cycle of the organism therefore; the BCF_w under steady state conditions can be expected to be higher in organisms with higher body weight and/or age.

Sex differences and spawning processes within a species play a major role in determination of the BCF_w of an organism. Female fish and/or marine mammals have the possibility to transfer large percentages of their lipid and its associated contaminant burden to their oocytes and/or directly to offspring through lipid rich milk production as in the case of marine mammals than do their male counterparts. As a consequence the BCF_w decreases during such spawning and/or birth events. During the course of this present study the sex of the sampled species was recorded where possible and sampling was completed outside of spawning season to minimize the inherent variability associated with these factors.

In order to develop bioconcentration factors on a lipid basis (BCF_L) the total lipid content of the sampled organism must be determined. In cases where extractable lipid data is only available the BCF_L will be underestimated.

In the case of bioconcentration of chemicals in algal species, in addition to the lipid content the extent of the surface area in contact with the water must also be considered (14). For other large marine species the relative surface area in contact with the water decreases as a percentage of total body mass therefore surface area effects generally decrease with increases in body length/size.

Mackay et al (15) estimate that 500+ compounds are of environmental concern based on production quantities and on their physico-chemical properties. It is however impossible to estimate BCFs for each of these in all organisms therefore, modelling tools based on the log K_{ow} of the test chemical have enabled the generation of BCFs for a number of organic compounds in species including mussels (16-17) and fish (18-22). In general BCFs increase in a linear fashion according to their log K_{ow} values. This correlation the so-called Quantitative Structure-Activity Relationships (QSAR) between log BCF_W of different chemicals and their log K_{ow} have been described by a number of authors (8, 16-17, 18, 20-23). Usually a linear relationship exists for compounds with log K_{ow} <6, however where the BCF_W of super-hydrophobic compounds are to be estimated additional polynomial functionality must be added to this equation (24 –32).

A number of QSARs have been recalculated for a range of compounds once it was ensured that, the test chemical was at levels that did not saturate the test water, no adverse effects or decrease in the health status of the test species occurred throughout the test, the no test chemical metabolism was observed, the log K_{ow} was accurately determined and test conditions such as the pH of water were strictly controlled. As a result the following equation was derived (4),

Equation 8= $$\log BCF_l = 0.956 \log K_{ow} + 0.22$$

1.1.3 Bioconcentration of classes of compounds by aquatic organisms.

Physico-chemical properties and potential sources of contamination of suites of interest in this study are described in detail below. Each individual compound/class bioconcentrate to differing degrees within an organism, this primarily being driven as a function of the physico-chemical properties of the compound, the surrounding environmental conditions and the biological characteristics of the organism. Bioconcentration of compound groups relevant to this current study is further discussed below.

1.1.3.1 Bioconcentration of endocrine disrupting compounds

A number of pesticides and other chemicals can act with weak hormone like activity; thereby having the potential to alter the balance of natural endogenous hormones in organisms when their concentration exceeds certain thresholds. While a number of definitions exist regarding classification of endocrine-disrupting chemicals/compounds (EDCs) all require that the concentration or the body burden of the EDC in question exceed a threshold level in order to elicit adverse or health effects on an intact organism.

In one such definition the U.S. Environmental Protection Agency (USEPA) (33) define EDCs as exogenous agents that interfere with the synthesis, secretion, transport, binding, action, or elimination of natural hormones in the body, that are responsible for the maintenance of homeostasis, reproduction, development, and/or behaviour.

Some non-planar polychlorinated biphenyls are described as pro-endocrine-disrupting chemicals (PEDCs) i.e. compounds that are not bound to steroid receptors, which after metabolisation can be bound to the estrogen receptor and produce estrogenic effects.

Therefore in most cases their hydroxylated metabolites are responsible for endocrine (e.g. estrogenic) activity.

All natural and synthetic hormones, and many EDCs achieve their effects by binding to a receptor and/or hormone binding protein (33-35). Binding of an EDC or metabolite to the receptor is necessary to initiate activity, however this alone is insufficient to elicit an active response, this being controlled by a number of other factors but primarily by the concentration at the target tissue/receptor and the degree of chemical absorption that follows (36).

A number of authors (37-41) report EDC mediated effects by organic compounds on aquatic life including, decreased hatching success and fertility in fish/shellfish, abnormal thyroid function in fish and species (de)feminisation and (de)masculinisation of fish and gastropods.

Compounds that mimic the natural female sex hormone estrogen are often called xenoestrogens (42-43). Estrogens show multiple site activity, their effects are primarily directed towards the reproductive cycle and on the neuroendocrine system. Estrogen plays an important role especially during foetal, embryonic, and neonatal development and can elicit physiological effects at low blood concentrations; therefore, during such developmental stages the embryo/foetus are extremely sensitive to the potential effects of exogenous environmental hormones.

1.1.3.2 Bioconcentration of xenoestrogenic compounds

While pesticides such as the chiral compound *o,p'* DDT (and its metabolites *o,p'* DDE and *o,p'* DDD) in addition to dieldrin, toxaphene, and endosulfan (44-46) can be

classified as having environmental estrogenic activity. A number of other chemicals and their associated degradation products, can be metabolised in organisms especially to hydroxylated compounds which may have much more estrogenic potency than the parent compound itself, *o,p'* DDT has been shown to bind to both the estrogen receptor and to the progesterone receptor while other DDT metabolites have been shown to bind more efficiently to androgen receptors than to the estrogenic receptor (ER) (47-48).

Some polychlorinated biphenyls, especially their non-planar *para*-hydroxylated metabolites also possess estrogenic activity (44-45, 49) with the metabolite having higher estrogenic potency than the parent compound. Some co-planar polychlorinated biphenyls (PCB #77 and PCB #126) have shown *in vivo* estrogenic and anti-estrogenic properties. Other chemicals including, bisphenol-A (BPA) and tetrabromobisphenol-A have been shown to be weakly estrogenic (47-48, 50-51).

1.1.3.3 Bioconcentration of Xenoantiestrogens

Some non-steroidal chemicals possess potential to act as anti-estrogenic compounds. Such anti-estrogens are chemicals that have the ability to modify, modulate, inhibit, or antagonize the actions and effects of natural estrogens. A number of differing types exist with competitive antagonists, which have ability to bind to the estrogen receptor being the most specfic type encountered in aquatic environments.

While a number of anti-estrogens elicit their activity through the ER, an increasing number of environmental relevant chemicals are being shown to potentially cause antiestrogenic effects indirectly through the aryl hydrocarbon receptor (AhR) including polychlorinated dibenzo-*p*-dioxins (PCDDs), such as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), polychlorinated dibenzofurans (PCDFs) and a number of co-planar

polychlorinated biphenyls (PCBs), such as 3,3',4,4'-tetrachlorobiphenyl, 3,4,4',5-tetrachlorobiphenyl, 3,3',4,4',5-pentachlorobiphenyl, and 3,3',4,4',5,5'-hexachlorobiphenyl are among some anti-estrogenic chemicals which may alter estrogenic response through AhR binding (52-53).

Anti-estrogenic activity of these Ah receptor ligands is directly related to their binding affinity to the Ah receptor and associated CYP 1A and CYP 2B1 inducing potency. The structure of the Ah receptor has been shown to differ amongst species however; it is unclear whether such differences result in species specific responses to these anti-estrogenic compounds.

1.1.3.4 Bioconcentration of other POPs.

The physico-chemical properties that primarily designate a compound as a persistent organic pollutant are well described and usually require that the chemical can evaporate and condense in air, water and soil at environmental temperatures, be persistent in the environment and in biota, have a log K_{ow} >4, show relatively high potential for bioaccumulation and can be toxic and/or elicit adverse effects to life processes within living organisms. Structures, sources and general properties of POPs of interest to this current study are further described below.

Numerous difficulties are encountered in determining BCFs for some POPs, especially those with high log K_{ow} values (thereby with low solubility in water) and with a long half-life. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) is recognized as being amongst the most toxic pollutants present in the environment, however its mode of action is still poorly understood. While it is recognized that most of the toxic effects of TCDD are

mediated through the aryl hydrocarbon (Ah) receptor (53) this alone does not explain species wide differences in toxic effects towards TCDD.

Geyer et al reported a positive relationship between the acute toxicity of TCDD (54-55) (and of species susceptibility to γ-HCH) (8) and the total lipid content in the body of the organism, concluding that storage of TCDD and/or other related compounds in lipids may provide a form of a defensive mechanism to remove such chemicals from receptors, sites of action and/or other target organs such as the liver (54-56). Geyer further predicted that eels with high fat content should be resistant to the toxic effects of TCDD even though they are capable of bio-concentrating the chemical to a high degree. The influence of relative trophic status on the levels of TCDD and the potential health effects of environmental pollutants on eels will be further discussed in chapter 4 of this current study.

Further to the above, γ- and δ-hexacyclochlorohexane, dieldrin, pentachlorophenol and DDT metabolites have all been shown to have potential to inhibit the functioning of the androgen receptor and of inhibiting 17β-estradiol binding to the estrogen receptor and therefore may be capable of interrupting physiological processes regulated by these receptors (35).

Geyer (8) further reports that many hydrophobic environmental synthetic organic chemicals with endocrine activity are relatively resistant to metabolic degradation and many possess a long biological half-life, thereby persisting for extended periods within organisms especially where the total lipid content of the organism is high.

EDCs can accumulate to high concentrations in lipids of the organisms (8, 18) and can slowly be released to provide a low level in blood. Should such long-term continuous concentrations reach critical threshold levels these may be effective in stimulating certain estrogenic, anti-estrogenic, androgenic, and anti-androgenic or other hormonal responses over extended periods.

A bioconcentration factor of 2 x 10^6 has been reported by Kucklick (57) for toxaphene in Arctic cod. 2-endo,3-exo,5-endo,6-exo,8b,8c,9c10a,10c-nona-chlorobornane (CHB 50) is one of the most recalcitrant toxaphene congeners found in aquatic biota with BCF_L values of 20 X 10^6 having been recorded in zooplankton and up to 77 X 10^6 being determined in lake trout by Kidd (58). In most cases these values are higher than that predicted from the log K_{ow} this being partly explained by bioaccumulation rather than bioconcentration alone. Water solubility values of 0.4 mg l^{-1} at 298K have been reported (59).

The tumour promoting potency of uv-irradiated toxaphene (uvT), technical toxaphene (TT) and a toxaphene enriched extract from cod fed a high toxaphene diet (cod liver extract, CLE) was studied *in vivo* and *in vitro* by Besselink et al. (AI and AII). The authors completed a feed-dosing experiment in cod (*Gadus morhua*) with approximately three hundred fish being exposed to technical toxaphene via toxaphene enriched feed pellets. Fish were given time to partly metabolise and depurate the more easily degradable toxaphene compounds so that the toxaphene residue profile resembled as much as was possible that found in wild fish. Cod liver oil was extracted and cleaned up by sulphuric acid digestion, gel permeation chromatography (GPC) and silica gel chromatography with the extract being used for toxicology studies.

Besselink et al (AII) further completed *In vivo* tumour promoting potency studies using female Sprague-Dawley rats to test the potency of uvT, TT and CLE. Treatment with 2,3,7,8-TCDD (positive control) only was shown to cause liver enlargement. *In vitro* toxicity studies were also completed with inhibition of intercellular communication (IC) used as bioassay to predict the tumour promoting potency of uvT, TT, and CLE. While the potency of uvT, TT, and CLE was a factor of 10^6 lower than the TCDD positive control it was reported that toxaphene is rightly classified as a carcinogen as cod-based toxaphene residues were found to be a more potent tumour promotor than the parent mixture itself. As a consequence, consumption of toxaphene-contaminated fish was determined to be a possible human health risk factor. On the basis of these findings an extensive survey of "baseline" levels of toxaphene congeners was reported (AX and AXI) and supplemented with summary risk assessments to the consumer (AV and AVII).

1.1.4 Bioaccumulation of contaminants in food webs

While bioconcentration relates to the uptake and retention of a chemical from water alone, bioaccumulation has been described as the uptake and retention of a bioavailable chemical from any one of, or all possible sources, it being the net result of uptake, distribution and elimination of a substance in an organism due to water, food, sediment and air (60).

While bioconcentration may to a great extent be seen as an artificial process it is relatively easy to model compared to the process of bioaccumulation where a number of additional biological, temporal and trophic factors have to be further considered. The role of relative trophic status and dietary influences (as estimated by stable isotope analysis) on bioaccumulation are further discussed in chapter 6 of this current study.

In order for bioaccumulation to take place the rate of uptake of a chemical must be greater than the rate of metabolism/elimination of the compound within an organism. In the case of the uptake of the highly water soluble chemical ammonia, bioavailability in water is high, uptake through membranes is relatively rapid and elimination through diffusion, metabolic transformation and/or active transport occurs at a rate similar to that of uptake processes. As a net result bioaccumulation of such ionizable compounds will not take place to any great degree.

Bioavailable chemicals whose physico-chemical properties subject them to potential bioaccumulation will passively diffuse or will be transported across the outer membranes of an organism down a concentration or activity gradient (11). As the concentration of the chemical in the tissue increases the gradient decreases and there is a tendency for the rate of loss of the chemical (through passive diffusion or active transport) to increase. Following ongoing exposure a steady state situation may be reached where the chemical concentration/fugacity in the tissue equals that of the outside medium (61).

The equilibrium concentration is generally measured as the bioaccumulation factor (BAF), i.e. the ratio of the concentration of the chemical in the tissue to its concentration in environmental compartments in equilibrium with the organism. Farrington and Westall (62) further describe BAFs as the ratio of the sum of uptake rate constants of the chemical from all environmental compartments accessible to the organism to the sum of the release rate constants by active and passive mechanisms from the organism.

1.1.5 Biomagnification of chemical pollutants.

Marine organisms are able to bioaccumulate most forms of metals and organic contaminants from their food, this process being often referred to as trophic transfer. Biomagnification is the process whereby a chemical, as it is passed through a food chain or food web by trophic transfer, increases in concentration in each subsequent trophic level. While biomagnification processes are subject to similar physico-chemical processes as take place during bioconcentration, the process primarily takes place in the gut of the consumer. Contaminants present in the prey of the consumer are desorbed and dissolved during digestion processes into gut fluids where subsequent partitioning processes across the gut epithelia take place into tissues of the consumer.

A number of different processes can occur to enable such biomagnification take place. Lipophilic contaminants may remain associated with the dietary fat and may be incorporated with the partially digested fats into salt micelles that are absorbed directly through the intestinal mucosa (63-64). Passing through the gut epithelia in non-complexed form is more likely however, where the compounds can absorb onto lipids in the portal system and on to the liver for further processing (65). As the gut contains an abundance of compounds capable of binding with organic contaminants (decreasing their bioavailability) it is reported that the efficiency of absorption from food is lower than that observed from the water phase (66-67).

Where the efficiency of uptake is relatively high and the rate of elimination/metabolism is relatively low, contaminant levels may then increase, thereby biomagnify, through the marine food web by trophic transfer.

The subsequent biomagnification factor (BF) can be defined as the ratio of the concentration of a contaminant in the tissue of a consumer relative to its concentration in its food (65, 68-69). Equilibrium and fugacity models suggest that all water-breathing animals should be in equilibrium with bioavailable chemicals in the water (i.e. fugacities should be equal). Connell (68) suggested that the BF of a compound is only weakly dependent on log K_{ow} and that when concentrations are expressed on a lipid weight basis that the BF will be at or below unity (for compounds with log K_{ow}<6). Trophic-level differences for non-polar organic chemicals with log K_{ow} <6 can often be attributed to a general tendency for lipid concentrations in tissues of marine organisms to increase with trophic level (69). These observations will be further investigated during the course of this present study.

As previously discussed for organic compounds with a log K_{ow} >6 water solubility is low, partitioning from lipids to the aqueous phase across the gills will be slow and chemicals will be released from the animal slowly by passive means. Biomagnification of a chemical may take place if no metabolism occurs, with this primarily taking place through the gut of the consumer (65). Muir (70) reports that biomagnification of hydrophobic compounds such as organochlorine pesticides is more likely to occur in the trophic step from water-breathing prey to air-breathing consumer as the consumer will not have capacity to release the chemical by passive diffusion mechanisms.

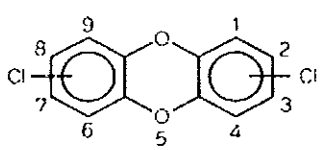
1.1.6 Persistent organic pollutants of interest.

While thousands of POP compounds have been reported to occur in marine biota this study primarily concentrated on those currently recognised within the context of long-term marine monitoring programmes and on a number of "emerging" pollutant classes. As the properties of each of these chemical classes are all well documented, only

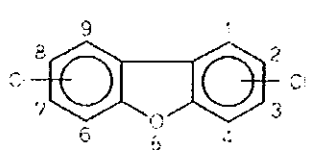
summary information on suites of interest is reported below with relevant Log K_{ow} information reported in table 1.3.

1.1.6.1 Dioxins and Furans

The term 'dioxins' covers a group of 75 polychlorinated dibenzo-p-dioxin (PCDD) and 135 polychlorinated dibenzofuran (PCDF) congeners generally formed during combustion processes or as by-products of industrial processes. The structural backbone of PCDD and PCDF contaminant groupings is illustrated in figure 1.1 below.



Dibenzo-p-dioxin



Dibenzofuran

Figure 1.1: Structural backbone of dibenzo-p-dioxin (PCDD) and dibenzo-p-furan (PCDF) congeners.

Dioxins have been identified in almost all environmental compartments and their emission to air can ultimately result in deposition in aquatic sediments, potentially allowing for uptake into the food chain. They can accumulate in tissue lipid reserves in

the primary intake species, (e.g. fish) thereby posing a potential risk to the consumer of contaminated fish and potentially to the health of the animal itself.

The potential impact of dioxin and furan congeners on the consumer of fish produce is generally measured by an internationally accepted toxic equivalency approach (TEQ), where individual congener information is multiplied by a toxicity equivalency factor (TEF) derived based on the congeners potency relative to TCDD. Similar TEF/TEQs are reported for assessing the impact of pollution on fish health (71); the relevance of the latter scheme in the context of the impact of the health of eels is further discussed in chapter 4.

1.1.6.2 Polychlorinated biphenyls

Polychlorinated biphenyls (PCBs) are extremely stable aromatic chlorinated compounds whose electrical and heat transfer properties led to their widespread use in a variety of industrial, commercial and domestic applications. Like dioxins, PCBs are relatively resistant to biological degradation and potentially can accumulate in environmental compartments and biomagnify in the food chain. Similar to a number of other POPs improper disposal of PCB containing materials and/or global atmospheric transport of PCBs can result in continued release to the environment and potentially into the human food chain.

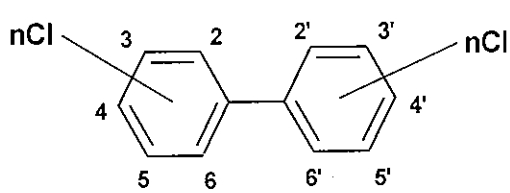


Figure 1.2: The "backbone" structure of PCB congeners

The position of the chlorine expressed in terms of its relationship to the carbon-to-carbon bond between the two aromatic rings defines whether PCBs are stated to be in the ortho, meta and para position. These positions are shown in figure 1.3 below.

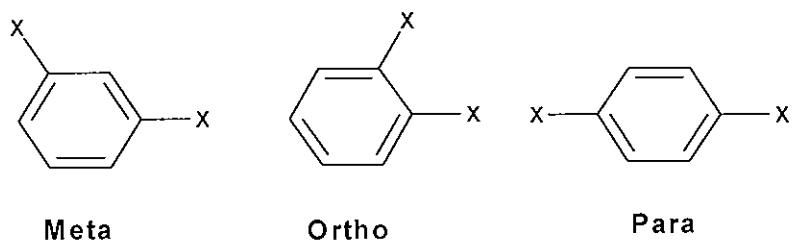


Figure 1.3: Meta, ortho and para positions on benzene ring.

Rotation of the benzene rings around the bond connecting them further define PCBs ultimate configurations as below;

1. Planar: two benzene rings in the same plane.
2. Nonplanar: benzene rings are at 90° angle to each other.

The number of chlorine substitutions in the ortho position largely determines the degree of planarity, with larger chlorine atoms forcing the benzene rings to rotate out of the planar configuration. The benzene rings of non-ortho substituted PCBs, as well as mono-ortho substituted PCBs, may assume the planar configuration and are referred to as planar or co-planar congeners. The benzene rings of other congeners cannot assume a planar or coplanar configuration and are therefore referred to as non-planar congeners. The ultimate configurations to a degree define the relative toxicity of individual congeners. Congeners with chlorines bonded to both para positions (4 and 4') and with at least two chlorines in the meta- positions (3, 5, 3', 5') are considered to be dioxin like and subsequently toxic. Single or absence of substitution in the ortho-position causes the atoms of the congener to orientate into a single plane i.e. planar or flat configuration in which form they are particularly toxic.

The ΣICES7 (PCB congeners 28, 52, 101, 118, 138, 153 and 180) and relevant OCP compounds found in a range of Irish fishery produce are presented in tables 1.1 and 1.2. Highest concentrations were found in mackerel and tuna, both of which are lipid rich fish. Higher levels are generally expected in large oily fish, as this reflects bioaccumulation of these substances due to high lipid content, diet and relative longevity of the species (72).

The level of contaminants in shellfish is a good indicator of contaminant levels present in the water column and can provide valuable information on the quality of the shellfish and the waters in which they are grown. Additionally these concentration data can provide valuable information as to the magnification potential of filter-feeding marine species. Table 1.2 presents summary literature information on the levels of PCBs and OCPs found in Irish shellfish produce

1.1.6.3 Organochlorine pesticides.

The last half-century has seen extensive global application of chlorinated pesticides (OCPs) primarily for use in a wide range of agricultural and forestry applications. Based on their lipophilic nature, environmental persistence and bioaccumulation potential, OCPs represent a threat to marine organisms in general. A number have been linked to many health problems including dysfunctions in the reproductive and immune systems in marine mammals (75-78) therefore animals having low metabolic and/or excretory capabilities for OCPs may potentially exhibit adverse physiological and behavioural effects as a result of sustained exposure (79-80).

The physico-chemical properties and sources of the pesticide toxaphene are comprehensively reviewed in Chapter 2 and in AI.

Table 1.1: Typical POP levels in finfish from Irish waters (ng g^{-1} wet weight) (73a-d)

Common name	Location	∑ 7 PCB	*pp*'DDT	*pp*'DDE	*pp*'DDD	HCB
Haddock	Dunmore East	0.36	nd	0.02	nd	0.04
Mackerel	Dunmore East	11.7	nd	1.42	0.42	0.43
Plaice	Castletownbere	0.42	nd	0.07	0.01	0.03
Mackerel	Castletownbere	1.75	0.07	0.57	0.13	0.12
Plaice	Howth	1.30	nd	0.18	0.05	0.05
Cod	Howth	1.70	nd	0.11	0.04	0.06
Plaice	Killybegs	0.69	nd	0.06	0.05	0.04
Haddock	Killybegs	0.72	nd	0.06	nd	0.19
Plaice	Rossaveal	0.66	nd	0.1	0.01	0.04
Mackerel	Rossaveal	4.97	nd	0.75	nd	0.20
Black Sole	Dunmore East	0.38	nd	0.07	0.02	0.03
Mackerel	Dunmore East	17.0	0.56	2.25	2.79	0.02
Lemon Sole	Castletownbere	0.19	0.01	0.09	0.17	0.03
Haddock	Castletownbere	0.29	<0.01	0.05	0.01	0.04
John Dory	Howth	0.32	<0.01	0.07	0.02	0.04
Anglerfish	Howth	0.63	nd	0.06	0.03	0.02
Plaice	Killybegs	0.40	0.01	0.09	0.03	0.03
Whiting	Killybegs	0.38	0.01	0.13	0.11	0.08
Prawn	Rossaveal	0.38	<0.01	0.07	0.03	0.03
Tuna	Dingle	76.3	8.09	47.7	13.0	7.62

nd =not detected

Table 1.2: Typical POP levels in shellfish from Irish waters (ng g^{-1} wet weight) (74a-d)

Common name	Location	∑ 7 PCB	*pp*'DDT	*pp*'DDE	*pp*'DDD	HCB
Pacific Oyster	Bannow Bay	3.26	0.05	2.30	0.35	0.30
Pacific Oyster	Cork Harbour	5.14	0.10	1.39	0.39	0.07
Pacific Oyster	Cork Harbour	4.12	0.34	1.30	0.31	0.03
Blue Mussel	Donegal	0.11	<0.12	0.19	0.07	0.33
Blue Mussel	Waterford Harbour.	6.48	0.17	1.58	0.72	0.22
Blue Mussel	Mulroy Bay	0.93	nd	0.13	0.08	nd
Blue Mussel	Waterford Harbour.	7.53	0.05	0.19	0.86	0.11
Blue Mussel	Lough Foyle	1.37	NA	0.35	0.20	0.03
Native Oyster	Clew Bay	1.38	0.25	0.16	0.08	0.03
Native Oyster	Cork Harbour	8.50	0.54	2.40	0.87	0.05

nd =not detected

NA = not available

1.1.6.3.1 Hexachlorocyclohexanes (HCH)

Technical HCH was manufactured in the 1940s for use as a broad-spectrum pesticide. Approximately 10 million tonnes has reportedly been applied from 1948 to 1996, with quantities peaking in the 1970s and early 1980s (81). The main isomer is α-HCH comprises 55-80% of the technical mixture but other stable HCH-isomers (β-, γ-, δ-, and ε-HCH) can also be found in environmentally relevant levels (82). Of all the HCH isomers only the α-HCH configuration is chiral. These enantiomeric properties will be further discussed below and in chapter 5. A log K_{ow} of approximately 4 (see table 1.3) has been reported for HCH compounds rendering them moderately hydrophobic. HCHs are generally more water-soluble and more volatile than other OCPs thus promoting their potential for atmospheric transport.

HCHs primarily affect the central nervous system, however, they also induce hepatic nodules and hepatocellular carcinoma in rats, the α-isomer being the most potent in this respect. β-HCH has also been shown to have estrogenic effects, (83-84). Few enantiospecific toxicity studies on α-HCH have been completed, however, Möller et al. (85) report that the (+) enantiomer was more effective than the (–) form in cytotoxic and growth stimulation bioassays of primary rat hepatocytes.

1.1.6.3.2 Chlordanes and heptachlor

The cyclodiene pesticide chlordane was first employed in the United States in 1947 (86) and was heavily used in a number of countries primarily for agricultural purposes or for use as a termiticide. The technical mixture mainly consists of *cis* (α-)-chlordane (8-13%), *trans* (γ-)-chlordane (8-15%) and *trans*-nonachlor (6-7%) (87). Other abundant components are heptachlor, *cis*-nonachlor and various chlordenes (88). Heptachlor is rapidly degraded in the environment to heptachlor-*exo*-epoxide (HEPX). Heptachlor-

endo-epoxide can also be formed but it is not environmentally stable. The degree to which these metabolites are present in eels will be further discussed in chapter 4.

Cis-chlordane, *trans*-chlordane and the nonachlordanes are ultimately degraded to form the persistent metabolite oxychlordane (89). While chlordane compounds are among the most prevalent chemicals that have been detected in tissues of living organisms, levels of chlordane compounds in Irish fishery produce historically have been low (73-74).

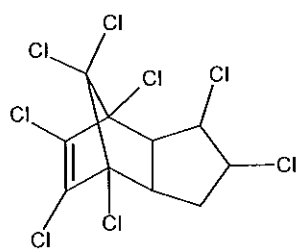


Figure 1.4: The structural configuration of chlordane.

Many of the chlordanes and chlordane-related compounds are chiral, as are some of the minor components of the technical mixture and epoxidized metabolites. The enantioselective fate of chlordane compounds comprising the killer whales diet is further discussed in chapter 5.

Generally, relatively high accumulation and biomagnification factors (BMFs) have been calculated for chlordane compounds and for the epoxide metabolites (90-91) however insufficient data are available in this present study to accurately quantify BMFs in Irish marine biota.

Many aquatic species have been affected by technical chlordane at aqueous concentrations (0.2 to 3.0 μg/L) with metabolites and photoconversion products often more toxic than their parent compounds. Miyazaki (92) found that insecticidal toxicity of several cyclodienes differed between enantiomers, and between enantiomers and racemates.

1.1.6.3.3 DDTs

DDT, or 1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane was first utilised in the United States in the 1940s in agricultural, forestry and public health programs, with optimal usage occurring around 1960. Thereafter, bans and restrictions limited its use, although some countries continue to employ it as a control for malaria. The commercial product contains about 70% *p,p'*-DDT, 15% *o,p'*-DDT and a number of other impurities. *p,p'*DDE is its most prevalent environmentally stable metabolite and can often account for 70-80% of the total DDT burden in biota. DDTs have been suspected to cause reproductive damage in wildlife (93-95). The *o,p*- DDT isomer has shown estrogenic activity (96) and is chiral. The (−) form was shown to be significantly more estrogenic than (+) enantiomer (97-98). Further developments in toxicological and analytical studies of *o,p*- DDT are hindered by the lack of commercially available enantiopure standards.

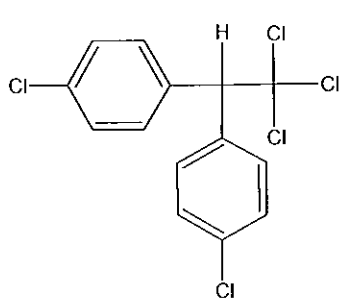


Figure 1.5: Structure of pp' –DDT.

1.1.6.3.4 Brominated flame retardants.

The term brominated flame-retardants (BFRs) is a generic classification of approximately 70+ chemicals, which, when added to materials, such as electronic equipment, furniture, construction materials or textiles inhibit, or suppress combustion processes. BFR compounds including the polybrominated diphenyl ethers hexabromocyclododecane, tetrabromobisphenol-A and polybrominated biphenyls of interest in this study are further discussed below.

Polybrominated diphenyl ethers (PBDEs) were first produced in the early 1970s. Three formulations of PBDE technical mixtures have commercially been available, penta-, octa- and decabromobiphenyl or bis-(pentabromophenyl) ethers, also known as penta-BDE, octa-BDE and deca-BDE respectively. The subsequent detection of PBDEs in the aquatic environment led to an increase in concern over their environmental persistence and potential toxicity.

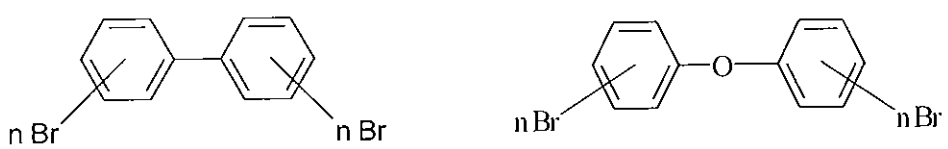


Figure 1.6: Structures of polybrominated biphenyls (PBBs) and polybrominated diphenylethers (PBDEs)

PBDEs lipophilic characteristics and their persistent nature has resulted in some congeners being considered ubiquitous environmental contaminants (99-102) prompting the European Commission to issue a proposal in 2001 to ban the production and use of

PentaBDE (103). Production of the octa- and penta- formulations has in recent years also been discontinued (104).

Hexabromocyclododecane (HBCD) has primarily been used to improve flame retardant characteristics of extruded and expanded polystyrene products. HBCD comprises three diasteroisomers (α, β and λ), with λ-HBCD contributing approximately 80% to the technical formulation (105). Tetrabromobisphenol-A (TBBPA) is the primary flame retardant used in electronic circuit boards and as such has been reported as being one of the most widely used BFRs. Although the evidence for the environmental presence of HBCD and TBBPA is small, their detection in a wide range of matrices is a potential environmental concern. Decabromodiphenyl ether (deca-BDE) and TBBPA account for approximately 50% of the world's usage of BFRs however few data are available on the environmental distribution of these two compounds. Polybrominated biphenyls (PBBs) use has in the main been limited to the USA (106) while production in Europe has been limited to decabrominated biphenyl (decaBB). Manufacture has since terminated in 2000 (106). While PBBs have been shown to have long-term effects on the balance of endocrine systems (106) few datasets exist to document the prevalence of the compound in biota.

Table 1.3: Log K_{ow} values for selected POPs reported in literature.

a Ritter (107), b Willett (83), c Mackay (108), d Simpson (109), e Mortimer and Connell (110) and f Budavari (111) Mackay (15), Rippen (112).

Compound	Log K_{ow}	Compound	Log K_{ow}	Compound	Log K_{ow}	Compound	Log K_{ow}
Aldrin	5.17a - 6.49f	PCDD	7.00a	PCBs (all)	4.30-8.26a	PBB #15	5.72f
Chlordane	6.00a - 6.16f	PCDF	5.82a	Monochlorobiphenyls	4.30-4.6a	PBB #52	6.50f
Cis Chlordane	6.10d	2,3,7,8-TCDD	6.64f	Dichlorobiphenyls	4.90-5.30a	PBB #153	7.50f
Trans Chlordane	6.22d	OctaCDD	8.60f	Trichlorobiphenyls	5.50-5.90a	BDE #28	5.47 - 5.58f
Cis-Nonachlor	6.08d	2,3,7,8-TetraCDF	6.53f	Tetrachlorobiphenyls	5.60-6.50a	BDE #47	5.87 - 6.16f
Trans nonachlor	6.35d	2,3,4,7,8-PentaCDF	6.92f	Pentachlorobiphenyls	6.20-6.50a	BDE #99	6.64 - 6.97f
oxychlordane	4.43e	1,2,3,4,6,7,8-HeptaCDF	7.92f	Hexachlorobiphenyls	6.70-7.30a	BDE #209	9.97f
Heptachlor epox	5.40c			Heptachlorobiphenyls	6.70-7.00a	TBBPa	5.21f
Dieldrin	5.40a			Octachlorobiphenyls	7.10a	Tri-BDE	5.47-5.58
Endrin	5.20a			Nonachlorobiphenyls	7.20-8.16a	Tetra-BDE	5.87-6.16
Heptachlor	4.40a			Decachlorobiphenyls	8.26a	Penta-BDE	6.64-6.97
Toxaphene	>5.00a - 5.20-7.80f			PCB 28	5.67f	Hexa-BDE	6.86-7.92
Mirex	7.50f			PCB 31	6.00f	Octa-BDE	8.35-8.90
Endosulfan (α and β)	3.83			PCB 52	6.36f	Deca-BDE	9.97
HCB	5.73f			PCB 101	6.86f		
α-HCH	3.90b			PCB 138	7.44f		
β-HCH	3.90b			PCB 153	7.23f		
γ-HCH	3.70b			PCB 180	7.36f		
p,p`-DDT	6.00c - 6.91f			PCB 81	6.4f		
o,p`-DDT	5.65c - 6.76f			PCB 77	6.63f		
p,p`-DDE	6.51 - 6.96f			PCB 126	7.2f		
o,p`-DDE	6.94f			PCB 169	7.41f		
p,p`-DDD	6.02 - 6.22f			PCB 105	6.65f		
				PCB 114	6.65f		
				PCB 156	7.18f		
				PCB 167	7.27f		
				PCB 189	7.71f		
				PCB 88	6.50e		
				PCB 136	6.70e		
				PCB 171	6.70e		

1.1.7 Role of chirality in fate of organic pollutants

Interest has grown in recent years on the significance of enantiomeric compounds in the field of environmental analysis and especially with respect to the bioaccumulation and fate of chiral persistent organic pollutants in the environment and in living organisms. As previously discussed a number of POPs can be detected in a variety of environmental compartments, a large number are suspected to cause reproductive and immunological damage in wildlife and man and they tend to bioaccumulate in living organisms. While a number of studies have reported enantiomeric ratios of POPs in marine biota few have investigated the role of the trophic status and of bioaccumulation on the fate of these POPs in biota.

There have been numerous examples in recent years of the application of chiral analysis from industrial, pharmaceutical, biological and environmental standpoints. Pharmaceutical companies need to ensure that production processes operate in an enantiomer specific manner to ensure that no harmful degradation/metabolic bi-products result from administration of a particular drug. Deviations from the racemate have been found in plasma protein binding (113-115), liver tissue (116-117) and kidney tissue/urine (118-120). However, despite considerable pharmacological research, the processes such as stereoselective active transportation through membranes (including the blood-brain barrier), stereoselective degradation and stereoselective binding are still not fully understood at the molecular level. In general, it is assumed that active sites are able to select enantiomers of matching chirality from a racemic mixture, thus giving rise to the formation of

diastereomeric complexes between the active sites of enzymes and the chiral compounds (121) as diastereomeric complexes differ in their physical properties (122).

From an environmental perspective the use of chiral analysis techniques in the investigation of industrial and point source pollution incidents is well documented (123-125). The application of chiral analysis techniques continues to be of relevance as production of chiral compounds e.g. bromocyclen an ectoparasite used in animal husbandry or synthetic musks and other pharmaceutical xenobiotics, continues.

Transportation mechanisms of a number of POP compounds can also be followed by the application of chiral analysis. By measuring the enantiomeric ratios (ERs) in environmental indicator species such as mussels, it has been possible in some instances to determine whether pollution incidents were due to more global aerial/sea/riverine transport mechanisms or as a result of local point source mechanisms.

Chiral analysis procedures have also been applied in the estimation of the health status of biota based on the biomagnification of enantiomeric compounds through the food chains of various animals. As a result environmental monitoring programs and associated risk assessments may in future years be driven by enantiomer specific analysis as toxicological and environmental databases are updated to include new enantiomer based information on chiral compounds.

1.1.7.1 Enantiomer chemistry.

In the simplest form enantiomers are compounds made up of the same atoms bonded by the same sequence of bonds but having different three-dimensional structures that are not interchangeable. They reflect the plane of linearly polarised light in opposite directions (in equal amounts). The enantiomer that rotates light to the left *levo* is indicated by a (-), and the enantiomer that rotates to the right *dextro* is indicated by a (+). Diastereomers are stereoisomers where not all asymmetric carbons are inverted. In both instances these molecules are said to be chiral. Most biological processes in nature show stereospecificity where enantiomers may differ in their toxicological or biological activity. A number of forms of chirality exist and these are further described below.

1.1.7.1.1 Asymmetric or stereogenic centre.

The most common chiral form is where a carbon has the ability to form a non-coplanar bond with four different atoms or groups. This carbon is said to be the asymmetric or stereogenic centre. Further to this, a free pair of electrons e.g. tertiary amines or sulphoxides can substitute one of the four atoms or groups. Other compounds may possess a sulphur or phosphorous as the stereogenic centre. Further impedance to chromatographic separation may lie in the presence of an "inversion barrier" where a rapid transfer between possible forms can result in separation difficulties.

1.1.7.1.2 Axial chirality

This form is most evident with the analysis of certain PCB molecules and their metabolites. Biphenyl structures with four large groups in the ortho-position cannot freely rotate about the central single bond because of steric hindrance. Molecules will then be orientated at

planes of up to 90° to each other. If either or both of these rings are symmetrical or perpendicular to each other then the molecule is said to have a plane of symmetry. PCB 96 and PCB136 are examples of this form of chirality.

Some molecules with two or three *ortho-* groups can show inhibited free rotation and then if they are suitably substituted can be chiral. From 209 possible PCB congeners some 78 are chiral but only a total of 19 are stable from racemisation at room temperature. Such PCBs that can be separated due to inhibition of the free rotation are called atropisomers.

1.1.7.1.3 Asymmetry of cyclic pollutants

It can be difficult to judge whether a compound can be presumed to be chiral. A molecule may be non-superimposable or exhibit a plane of symmetry but the classification, as whether it is chiral may still be difficult to establish. Some compounds exhibit an "alternating axis of symmetry" which can also be known as an "improper axis of rotation". To illustrate this situation, a point at the centre of the molecule is joined to any other atom or group on the molecule. The subsequent line is then extended in the opposite direction along the same plane and distance as the original to form a new "axis". This axis should contain half of the molecule in order for this condition to be observed. Therefore the fundamental symmetry condition is the absence of an improper axis to allow for optical activity.

In the case of α-Hexachlorocyclohexane, neither a plane nor a centre of alternating axis of symmetry is observed. This results in α–HCH being the only one of the eight possible isomers to satisfy the conditions as having chiral properties.

1.1.7.1.4 Additional forms of chirality.

Other forms of chirality exist but they are generally not encountered in the analysis of the majority of environmental pollutants. These forms include allele type and helical symmetry in addition to planar symmetry. Chiral pollutants with two or more stereogenic centres also exist and it is possible to calculate the number of possible stereoisomers that may be formed. This can be calculated using, 2^n where n represents the number of stereogenic centres. A further element of caution has to be observed when using this calculation, as it is possible that some molecules may exist in a *meso-* form. In this case four stereoisomers may be expected but only three actually exist. This *meso-* form may be chromatographically observed by the presence of a peak that is twice as large as any of the other stereoisomers.

In the example of the synthetic musk 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8 hexamethylcyclopenta-γ-2-benzopyran (HHCB) four stereoisomers (A, B, C and D) are formed. Each individual can only reflect one of the others e.g. A/B and C/D. It is not possible in this case to form stereoisomers A/C or B/D. These relationships define the term diastereomers. Diastereomers in general will exhibit different physical properties whereas the enantiomers will reflect identical physical and chemical properties in all but two respects.

In the presence of a chiral catalyst these enantiomers may react differently but in general these differences are small and difficult to measure. They will in the main possess identical properties in a symmetric environment but possess different ones in an asymmetric one.

This principle is the basis for chiral chromatography, where the chiral stationary phase forms the asymmetric environment resulting in enantiospecific separation under the correct conditions. By contrast, diastereomers, by virtue of the fact that they have different physical properties, usually can be separated on achiral phases.

1.1.8 Analytical determination of chiral POPs

Due to the fact that hundreds of thousands of POPs may be present in a given environmental sample, analysis of individual compounds/enantiomers can become problematic. Where a compound is chiral in nature further analytical separation and quantification difficulties can be expected, as a number of additional enantiomer/diastereomeric peaks can be present in the chromatogram that may interfere with analysis.

Separation of these chiral compounds from their constituent "mirror" images can be achieved on a compound specific basis, by coupling of appropriate stationary phases with techniques such as capillary electrophoresis or liquid chromatography. However for optimal separation of trace levels of a number of the environmentally relevant organic compounds the most appropriate technique involves the coupling of a cyclodextrin based chiral stationary phase with a gas chromatograph (GC). The coupling of the individual properties of both cyclodextrin columns and GCs allows for the optimisation of the separation potential for some of these compounds for marine biota.

Various factors need to be considered prior to the final choice of a method for enantiomeric separation. These separations generally take one of two relatively generic forms. Diastereomer derivatives may be formed by reaction of a chiral compound with a chiral reagent. The majority of these derivatives can then be separated on either a chiral or achiral stationary phase as long as there is sufficient selectivity for these individuals on the phase. Selectivity can be a reflection of any number of parameters e.g. the material used in phase manufacture or a measure of the functional groups reaction to reagents.

The second approach revolves around the more direct separation of enantiomers on a chiral column. Separation on this occasion is a function of the formation of diastereomeric association complexes between the enantiomers ultimately leading to separation on a suitable phase. These phases however do tend to show appreciable batch-to-batch variation as a result of difficulties encountered in reproducibly controlling derivatisation.

A number of different materials have been tested for their suitability as a medium for chiral separations. These have included, starch, silica, alumina and sephadex amongst others. Such work has led to a six-type classification system for these phases based on different phase properties, bond interactions, applications and modes of separation.

As previously discussed enantioselective separations are usually based on small differences in interactions between a stationary phase or chiral selector taking into consideration the number and suitability of active sites, phase selectivity in addition to parameters such as temperature regulation etc. The application of these liquid chromatography (LC) phases in the main seems to be centred towards pharmaceutical applications and to a much lesser

degree for routine monitoring of levels of persistent organic pollutants. A number of available techniques to bring about the separation of enantiomeric compounds are further discussed below.

1.1.8.1 Enantioselective high performance liquid chromatography.

Indirect and direct applications can both be a feature of enantioselective high performance liquid chromatography (eHPLC) separations. Indirect methodologies form the minority of applications especially in the analysis of POPs but usually involve the reaction of a racemic mixture with a chiral reagent to form a pair of diastereomers. Once the diastereomers have been resolved it may be possible to recover the products by reversing the derivatisation process.

Direct methodologies provide for the greater number of applications in this field and separation usually revolves around the provision of a "chiral selector" to preferably associate with individual enantiomers of a racemic mixture. This may be performed by the stationary phase itself or with the addition of a chiral compound to the mobile phase. The application of chiral stationary phases (CSP's) requires the formation of transient diastereomeric complexes between the enantiomorphs in the solute and the chiral selector in the stationary phase. Differences in the stability of these complexes result in differences in retention behaviour under appropriate separation conditions.

1.1.8.2 Capillary electrophoresis (CE)

CE is usually used in the biological and pharmaceutical sectors for the separation of proteins and ionic drugs but few studies on POPs have been reported. The principle of

operation usually involves the application of an electric field around a narrow bore capillary column containing sample and an electrophoretic medium. Sample analytes migrate towards a positive electrode on application of a current across the cell. Properties influencing separation power include viscosity of media in the capillary, current applied and the dielectric constants of the solvents. Numerous different forms of CE are possible depending on the required application e.g. based on molecular weight but their application to enantioselective separations can also be achieved. Enantiomers do not possess differences in charge and therefore the addition of a chiral selector to the mobile phase is generally the approach employed.

1.1.8.3 Enantioselective Gas Chromatography (GC)

Gil-Av (126) in 1966 brought abut the separation of N-Trifluoroacetylamine (N-TFA) amino acid by capillary GC using acetylated amino acid and di-peptide esters as chiral stationary phases thereby this was one of the first published works on the application of a chiral stationary phase to GC. In the early years of stationary phase production problems in controlling the stability of "fixing" of the stationary phase meant that the technique was restricted to a small number of applications. Through subsequent years stability of these phases improved and with the advent of capillary chromatography new approaches were initiated.

The use of cyclic glucose oligomers with 6, 7 or 8 glucose units corresponding to α, β and γ cyclodextrins respectively led to further advances in chiral phases. These torous-shaped molecules have a hydrophobic cavity of specific dimension containing 6-hydroxy groups positioned at the narrow entrance and 2 or 3-Hydroxy groups at the wider end and as a

result non-polar molecules are favoured within the cavity. These unmodified cyclodextrins by themselves are still unable to provide the stability required to enable them to provide efficient separations however in combination with various proportions of polysiloxane the production of much more stable phases is possible, these being more suitable for more widespread applications especially in the field of POP and organic pollutant analysis.

1.1.9 Enantiomeric fractions (EF) studies in air, water and soil

EFs of chiral compounds in water show little variation from the racemate and differences primarily have been explained by regional differences in microbial populations in surface sea-water (127-128). Enantioselective degradation has been found to be greater in oligotrophic Arctic and sub-Arctic waters compared with warmer and more nutrient-rich lakes in the temperate climate zone (129). Wiberg (130) report EFs for o,p'-DDT in the range 0.41 to 0.57, while EFs of chlordanes and chlordane metabolites were less variable. The variability in the enantiomeric composition of chiral pesticides in soils is still poorly understood however it is thought that processes such as deforestation, nutrient enrichment and global warming, may alter the enantioselective microbial degradation of chiral pollutants in soils. EFs in air samples above open sea or soil during warmer seasons tend to show the greatest deviation from racemic composition due to volatilization from the water or soil.

1.1.10 EF studies Biota

A number of EF studies have taken place in aquatic biota, the majority of which conclude that enantiomeric excess of non-metabolised POP enantiomers in organisms principally arises as a result of enantioselective metabolism, the degree to which this takes place being

primarily controlled by the biotransformation/metabolic capacity of individual living organisms.

Chiral POP investigations of biota have ranged from marine to terrestrial species, and from low trophic level organisms to top predators (130-133 and chapter 5). EFs were found to differ between species, within and between populations and between tissues primarily due to differences in genetics, bioaccumulation and trophic level, age, sex, health and feeding/nutrition status.

It is not the purpose of this current study to review the analytical challenges and advances in chiral POP analysis or to comprehensively review enantioselective data in the literature. Hegeman and Laane (134) and Wiberg (130) have recently provided reviews of such data, however where EFs have been determined during the course of this work reference is made to relevant literature.

With all of the above in mind the current study was designed to optimise the possibility of tracing compound fate through different trophic levels of the Irish marine web and to investigate possible ER related metabolic/excretion/degradation effects. Ultimate goals of this investigation were to provide information on,

- the levels and ERs of POPs in killer whales from British/Irish waters,
- the fate of a number of POPs in marine biota from Irish waters via bioaccumulation/biomagnification and/or metabolism/elimination processes.
- future design of environmental and/or food safety monitoring programs and potential impact of ERs for current programs.

1.2 Materials and methodology.

During the course of this study over 2000+ individual fish and shellfish comprising 24 species were sampled. The majority of fish samples were collected from either commercial or research based landings at Irish ports and at production level in the case of farmed species and commercially reared shellfish. Retail samples were collected by officers of the Food Safety Authority of Ireland (FSAI) prior to sampling in the Marine Institute (MI) laboratory.

Location details and summary biological information of each of these fish/shellfish and marine mammals sampled are presented in tables 1.4 and 1.5. A summary of sampling procedures for shellfish, round/flatfish, eels and for marine mammals is presented below and detailed in chapters 2 to 5.

1.2.1 Phytoplankton and seston collection

Collection of seston (phytoplankton and detritus) was performed at two locations on the Irish south west coast. Water samples were firstly visually inspected to remove larger particulate matter and then were pre-filtered through 200µm glass fibre filter to remove bulk zooplankton. Finally the water sample was filtered through a 30µm filter to remove phytoplankton (and detritus). This filter was then back-washed into a centrifuge tube with 5ml of sterile seawater to remove contents. The tube was further centrifuged to concentrate seston in pellet form. The pellet was further washed in 1ml of sterile sea-water, visible particulate matter and detritus was removed and the sample was freeze-dried prior to stable isotope analysis.

1.2.2 Finfish sampling

Targeted sampling of finfish landed at the major Irish fishing ports of Castletownbere, Dunmore East, Howth, Killybegs and Rossaveal took place for the purposes of this study. Depending on availability a minimum sample size of 10 individual fish of each species was sampled at each of the ports. Capture locations and retail batch origin details were collected as appropriate.

In the case of wild and farmed samples the length and where possible the sex, of each fish was recorded and a skinless portion of tissue from each of the fish was pooled to provide a sample. Subcutaneous lipid was scraped from the skin and returned to the pooled sample, which was then homogenised and sub-sampled into solvent washed glass jars. Sub-samples were stored in a freezer at –20°C prior to analysis. Retail canned fish samples were processed complete with skin, as it was not possible to remove skin tissue prior to homogenisation.

Wild tuna, wild salmon and a subset sample of herring (n=25) were sampled and analysed individually. The latter herring subset was analysed individually in order to assess inherent natural variability within a single trawl event.

1.2.3 Shellfish sampling procedures

Shellfish samples were all collected during the months of August to October, to minimize the potential effects of spawning on contaminant levels. Where possible, supporting physico-chemical and other supporting parameter information including, temperature, salinity, pH and dissolved oxygen measurements were taken *in-situ,* typically at 1m depth

using a Hydrolab®multiparameter probe (Minisonde®). At each location the water surface was visually examined for evidence of hydrocarbon contamination.

Pooled mussel samples consisted of 50-75 individuals and oysters of 25 individuals. Shellfish were depurated overnight in seawater collected from the growing area at the time of sampling. The lengths of individual shellfish were recorded before the soft tissue was removed from the shells. The percentage meat and shell weights were calculated and recorded. The pooled soft tissue was then homogenised and sub-sampled and stored as per fish samples.

1.2.4 Killer whale sampling procedures

Killer whale samples were obtained as a result of live stranding events or were collected from individuals washed ashore following death at sea. Tooth rot infection was reported to be a major contributory factor in the cause of death in three of the individuals. *Post-mortem* studies and the collection and preservation of tissue samples were conducted according to previously established protocols (135-136). Sampling details, biological and sampling information are reported in table 1.5 and illustrated in Chapter 5. The study sample comprised four females ranging in length from 525-610cm (n=3), two males ranging in length from 590-610cm (n=2) and one unsexed individual. Dorsal blubber tissue was removed with a scalpel and transferred into solvent washed glass jars, which were stored at -20°C prior to analysis.

1.2.5 Eel sampling procedures

Eels were sampled at five locations throughout Ireland with sites selected on the basis of both the ease of availability of eels and on the potential for diverse contaminant inputs. All eels were obtained from commercial fishermen between the end of October and mid-November 2005; sampling location details are presented in chapter 4.

At each site 210 eels were randomly selected from fyke nets, using a hand net with a mesh size of 3x3 mm. 100 eels were immediately anaesthetised, with chlorobutanol, and measured. These individuals were then revived in freshwater before being returned alive to the fyke nets. 10 eels were not subjected to anaesthetic and were individually bagged and frozen on return to the lab for use in contaminant analysis. Remaining eels were returned to the lab, were further anaesthetised prior to being measured and frozen for further biological examination including otolith removal to establish age profiles. It was further established that eels from the River Suir comprise of tissue from 10 resident feeding eels while the other four samples comprise tissue from migrating non-feeding eels. A number of key biological indices and parameters were determined for individual eels during this study including, length structure, age profiles (otoliths), life cycle stage, sex ratios, condition factor indices, abundance of the parasite *Anguillicolla crassus*.

Natural variation effects within individuals were inimised by the preparation of pooled eel samples. A scalpel cut was made directly behind the pectoral fin, and a parallel cut of the same length was then made approximately 3cm further down the flank, towards the tail. The skin was then peeled away to expose muscle tissue. Subcutaneous lipid was removed from skin and returned to the sample muscle tissue; samples were then aggregated with

each pooled sample containing skinless muscle tissue from 9 or 10 individual eels. Pooled samples were homogenized, sub-sampled and stored at <-18°C prior to analysis.

Table 1.4: Sampling details and analysis suite completed for fish during this study.

Common Name	Species/ecotype	Type	Length mean (mm)	Std. Dev. (mm)	N	sub-N	Location	Analysis Suites
Albacore Tuna	*T Alunga*	W			5	1	Ireland	1-3, 6, 11
Atlantic Salmon	*S salar*	W			10	1	Ireland	1-3, 6, 11
Atlantic Salmon	*S salar*	F			15	5	Ireland	1-3, 6, 11
Atlantic Salmon	*S salar*	F			10	5	Ireland	1-3, 6, 11
Black sole	*S solea*	W	260 - 420	40.9	1	20	Celtic Sea	5
Blue whiting	*M. Poutassiou*	W/R			10(4)*		Atlantic	1-6, 9,11
Cod	*G morhua*	W	390 - 640	78.8	1	10	Celtic Sea	5
Cod	*G morhua*	W	270 - 440	40.6	1	25	Irish Sea	5
Deepsea redfish	*S mentella*	W	350 - 490	44.8	1	12	Off Faroes	5
eel (silver)	*A anguilla*	W			1	9	Mayo, River Moy	1-9, 11
eel (silver)	*A anguilla*	W			1	10	Galway, River Wier	1-9, 11
eel (silver)	*A anguilla*	W			1	10	Monaghan, River Fane	1-9, 11
eel (silver)	*A anguilla*	W			1	10	Burrishoole, Co. Mayo	1-9, 11
eel (yellow)	*A anguilla*	W			1	10	Waterford, River Suir	1-9, 11
Grenadier	*C rupestris*	W	190 - 335	49.3	1	10	Off Faroes	5
Haddock	*M aeglefinus*	W	270 - 350	21.9	1	24	W. Ireland	5
Haddock	*M aeglefinus*	W	270 - 375	25.7	1	25	Irish Sea	5
Hake	*M merluccius*	W	190 - 260	17.6	1	24	W. Ireland	5
Herring	*C Harengus*	W			4	20	Ireland	1-3, 6, 11
Herring	*C Harengus*	W			2	5	Retail	1-3, 6, 11
Herring	*C harengus*	W	240 - 300	15	1	25	NW Ireland	5
Herring (M + F) a	*C harengus*	W			1	10	Dingle	10
Herring (M + F) a	*C harengus*	W			1	10	Malin	10
Herring (M + F) a	*C harengus*	W			1	10	Ballycotton	10
Herring (M + F) a	*C harengus*	W			1	10	Dunmore East	10
Herring (n=2)	*C harengus*	W	230 - 290	13.8	2	25	Celtic Sea	5
Long rough dab	*H platessoides*	W	180-300	29.4	1	26	Celtic Sea	5
Mackerel	*S scombrus*	W			5	20	Ireland	1-3, 6, 11
Mackerel	*S scombrus*	W			2	5	Retail	1-3, 6, 11
Mackerel	*S scombrus*	W	250 - 360	28.2	1	25	NW Ireland	5
Monkfish	*L piscatorius*	W	230-410	66.7	1	10	Celtic Sea	5
Monkfish	*L piscatorius*	W	300 - 490	60.3	1	10	West Ireland	5
Mussels	*M edulis*	C	40 - 67	5.3	1	50	SW Ireland	5
Mussels	*M edulis*	C	41 - 78	8.1	1	50	West Ireland	5
Mussels (n=2)	*M edulis*	C	45-60	3.5	2	50	West Ireland	5
Mussels	*M edulis*	W			1	75	Ireland (various)	2, 4, 10, 12

1 Dioxin and Furans,
2 PCBs,
3 WHO-PCB,
4 OCP
5 Toxaphene,
6 PBDE,
7 PBB,
8 TBBPA,
9 HBCD,
10 Enantiomer Ratios,
11 Stable isotopes
12 PAH.
a = incl.liver samples,
W= wild,
C= cultivated,
R=retail
F= farmed.
* Ten process line blue whiting oil samples and four retail samples

Table 1.4 cont: Sampling details and analysis suite completed for fish during this study.

Common Name	Species/ ecotype	Type	Length mean (mm)	Std. Dev. (mm)	N	sub-N	Location	Analysis Suites
Mussels	*M edulis*	W			1	75	Ireland (Various)	2, 4, 10, 12
Oysters	*C Gigas*	C			5	25	Ireland	1-3, 6, 11
Pink & Red Salmon	n.a.	R			5	5	Retail	1-3, 6, 11
Plaice	*P platessa*	W	280 - 430	44	1	20	Celtic Sea	5
Plaice (n=2)	*P platessa*	W	200 - 390	58.9	2	20	West Ireland	5
Plaice (n=2)	*P platessa*	W	270 - 370	29.7	2	25	Irish Sea	5
Salmon a	*S salar*	F	630 - 730	28.3	1	10	Ireland	5
Sardines	n.a.	R			1	5	Retail	1-3, 6, 11
Shrimp	*C crangon*	W	15.7 - 20	0.9	1	50	Celtic Sea	5
Shrimp	*C crangon*	W	14 - 20	1.2	1	100	Celtic Sea	5
Skate	*Raja Sp.*	W	290 - 740	105	1	20	West Ireland	5
Skate	*Raja Sp.*	W	250 - 520	68.6	1	20	Celtic Sea	5
Skate	*Raja Sp.*	W	390 - 570	62.9	1	10	Irish Sea	5
Skipjack Tuna	n.a.	R			5	5	Retail	1-3, 6, 11
Trout (lake)	*O mykiss*	F	340-370	11.3	1	8	Ireland	5
Trout (sea)	*O mykiss*	F	665 - 839	62.5	1	10	Ireland	5
Whiting	*M merlangus*	W	280 - 440	48.3	1	22	West Ireland	5
Whiting	*M merlangus*	W	230 - 350	34.5	1	22	Celtic Sea	5
Whiting	*M merlangus*	W	220 - 280	16.9	1	25	Irish Sea	5
Whiting	*M merlangus*	W			5	10	Irish Coastal waters	10

1 Dioxin and Furans,
2 PCBs,
3 WHO-PCB,
4 OCP
5 Toxaphene,
6 PBDE,
7 PBB,
8 TBBPA,
9 HBCD,
10 Enantiomer Ratios,
11 Stable isotopes
12 PAH.
a = incl.liver samples,
W= wild,
C= cultivated,
R=retail
F= farmed.
* Ten process line blue whiting oil samples and four retail samples
N = Number of pooled samples
Sub N= number of individuals per sample

Table 1.5: Sampling details and analysis suite completed for killer whales (*Orcinus orca*) during this study.

Sample Ref.	Sex	Age (Yrs)	Length (mm)	Blubber (mm)	Date of stranding	Location	Analysis suite
SW1995/54	F	24	525	28	26/04/1995	Kent, England	4, 10, 11
SW1994/56a	F	NA	550	NA	15/04/1994	Reay, Scotland	4, 10, 11
SW1994/169a	M	NA	610	60	16/11/1994	Shetland, Scotland	4, 10, 11
SW1994/177b1	NA	NA	NA	NA	07/12/1994	Shetland, Scotland	4, 10, 11
SW1997/135c	F	NA	610	30	16/08/1997	Western Isles, Scotland	4, 10, 11
SW2001/234	M	NA	590	40	09/10/2001	River Mersey, England	4, 10, 11
ENV-01	F	40-50	545	46	08/07/2001	Roches point, Cork, Ireland	4, 10, 11

1 Dioxin and Furans,
2 PCBs,
3 WHO-PCB,
4 OCP,
5 Toxaphene,
6 PBDE,
7 PBB,
8 TBBPA,
9 HBCD,
10 Enantiomer Ratios,
11 Stable isotopes
12 PAH.

1.2.6 Sample analysis

Numerous suites of analysis were completed on the prepared samples. Dioxins, furans WHO-PCBs, PBDE, PBB, HBCD and TBBPA and some OCP analysis was completed under sub-contract to *Eurofins/ERGO, ERGO Forschungsgesellschaft mbH,* Hamburg Germany. Stable isotope analysis was completed under sub-contract to Institute for Energy Technology (IFE) in Kjeller, Norway. The majority of toxaphene, PCB and OCP analysis and some PBDE analysis were completed by the author in the Marine Institute laboratories in Abbtostown, Ireland. Enantiomer specific separations were completed by the author onsite in the laboratories of the Norwegian Institute for Air Research (NILU), Kjeller, Norway and in the Marine Institute laboratories in Abbtostown, Ireland. The Centre for Environment, Fisheries and Aquaculture Science, CEFAS Burnham Laboratory, Burnham on Crouch, UK, (CEFAS) completed OCP and PBDE analysis in killer whales sampled in British waters. All sample preparation/work and lipid determination (according to methodology of Smedes) (137-138) was completed by the author in the Marine Institute laboratories.

1.2.6.1 Dioxins/PCBs and OCP analysis.

Dioxin and furans analysis was completed in ERGO laboratories Hamburg, Germany, according to EN ISO 17025 accredited methods GfA QMA 504-191/203/205. Analytical methodology complies with the requirements for the HRGC/HRMS confirmatory analysis of food for PCDD/Fs and PCBs as laid down by the EU directive 2002/69 as amended.

1.2.6.2 WHO-PCBs analysis.

WHO-PCBs were determined by high-resolution gas chromatography/high resolution mass spectrometry (HRGC/HRMS) following sample extraction and clean-up on a carbon/glass-fibre column. Separation and quantification were carried out on a VG-AutoSpec and/or Finnigan MAT 95 XL with DB-5 capillary columns with chromatographic responses for two isotope masses measured for each compound. Quantification was completed by the use of internal/external standard mixtures (isotope dilution method). Toxicity equivalents (TEQs), utilizing WHO-TEFs (See table 1.6) were calculated for each of the samples. Marker-PCBs and OCPs were determined by HRGC/HRMS on a DB-5 capillary column following solvent extraction and clean-up on an alumina/silica column. For each substance two isotope masses were measured. Quantification was carried out with the use of internal/external standard mixtures.

1.2.6.3 OCP analysis.

Due to the lipophilic nature of OCPs, lipid was firstly extracted from homogenised tissue samples utilizing either Soxhlet apparatus or the method developed by Smedes (137-138). Lipids were removed from the solvent extract by alumina column chromatography followed by separation of PCBs from the chlorinated pesticides using silica gel column chromatography. OCP concentrations were determined by dual column Gas Chromatography with Electron Capture Detection (GC-ECD) using Varian 3800 or HP6890 instrumentation on fused silica capillary columns HT8, (J & W Scientific, Inc.) and CP-SIL 8CB and CP-SIL 19CB, (Chrompack, Varian Inc) and DB5 (J & W Scientific, Inc.) (73a-d, 139) OCP analysis was completed in Marine Institute, CEFAS and ERGO laboratories.

1.2.6.4 Brominated Flame Retardant analysis.

Analysis of brominated flame retardant compounds was completed in both ERGO Gfa and CEFAS, UK laboratories. Tissue samples were extracted with n-hexane and solvent extracts were further treated on an H_2SO_4/SiO_2 clean-up column. PBDEs, PBBs and TBBPA were quantified by HRGC/HRMS as per WHO PCBs by means of internal / external standards (isotope dilution). It should be noted that HRGC/HRMS methodologies only provide data for the total of the three HBCD isomers. Liquid chromatography mass spectrometry (LC/MS) can be used to selectively monitor and quantify on an individual isomer basis, however sensitivity is generally lower than with HRGC/HRMS.

1.2.6.5 Stable isotope analysis.

Stable isotope analysis was completed under subcontract to IFE laboratories, Kjeller, Norway. The presence of lipid in biotic tissue samples can affect isotopic ratio determinations (140) therefore total lipid was removed from all tissue samples by soxhlet extraction of muscle prior to stable isotope analysis. Lipid free tissues were then freeze-dried and approximately 1 mg of sample was transferred to a 9x15 mm tin capsule before combustion in the presence of O_2 and Cr_2O_3 at 1700° in a Carlo Erba NCS 2500 element analyser. Reduction of NO_x to N_2 was then performed in a Cu oven at 650 °C. H_2O was removed in a $KMnO_4$ chemical trap before separation of N_2 and CO_2 on a 3 m Poraplot Q GC column prior to on-line detection of $\delta^{15}N$, $\delta^{13}C$ and $\delta^{34}S$ on a Micromass Optima, Isotope Ratio Mass Spectrometer.

Differences in stable isotope abundances are expressed by (δ) notation as the deviation from standards in parts per thousand (‰) by,

Equation 9= $$\delta X = [(R_{sample} / R_{Standard}) - 1] \times 1000$$

Where,

X relates to ^{13}C or ^{15}N and R is the corresponding ratio $^{13}C/^{12}C$, $^{15}N/^{14}N$ or $^{34}S/^{35}S$. $R_{standard}$ for ^{13}C and ^{15}N relate to Pee Dee Belemnite and atmospheric N_2 standards respectively.

1.2.6.6 Enantiomer specific separations.

These analyses were completed by the author in both the Marine Institute and onsite in NIVA laboratories. Cleaned up sample extracts were analysed on a Varian 1200 Triple Quad by Gas Chromatrography Mass Spectrometry (GC-MS) utilizing a BGB-172 (20% *tert*-butyldimethylsilylated β-cyclodextrin dissolved in BGB-25) chiral column (BGB Analytik AG, Adiswil, Switzerland), dimensions (30m x 0.25mm id x 0.18μm film thickness) with two characteristic ions used for individual compound identification. GC-EI-MS was utilized for identification of enantiomerically separated o, p'-DDT, while chemical ionization (CI) techniques were used for detection of all other OCPs. The enantiomeric composition was expressed on an enantiomer fraction (EF) basis as per Kallenborn and Hühnerfuss (141) and deGeus (142), calculated from peak areas of enantiomeric pairs by the following formula,

Equation 10 = $$EF = \frac{(-)A}{(-)A + (+)A}$$

where A= peak area of the (+) and (-) enantiomer respectively. EFs < 0.5 or >0.5 indicate selective depletion/enrichment of the (+) and (-) enantiomers respectively.

1.2.6.7 Total lipid determination

The Smedes tri-phasic solvent and water extraction is internationally recognised as being suitable for the determination of total lipid content of marine samples (137-138). A total of 16ml isopropanol and 20ml cyclohexane were added to an accurately weighed sub-sample of the defrosted and homogenized sample. The sample is then homogenised and centrifuged. The organic layer is then carefully removed. Water added and the procedure repeated and organic layers pooled. The sample is then evaporated to dryness and the lipid content determined gravimetrically. All Smedes lipid determinations were completed in the Marine Institute laboratories in Abbotstown, Dublin, Ireland.

1.2.6.8 Analytical assessment tools

While concentration data alone is sufficient for spatial and/or temporal trend assessment purposes, toxic equivalency factors (TEFs) and associated toxicity equivalencies (TEQs) are internationally recognized as an additional assessment tool to further evaluate the toxicological significance of concentration data for food safety purposes.

1.2.6.8.1 Toxic equivalency factors.

A total of 17 dioxins and dioxin-like PCB congeners are of toxicological concern with the most toxic dioxin congener 2, 3, 7, 8-tetrachlordibenzo-p-dioxin (TCDD) being classified by a number of international organisations as a known human carcinogen, by analogy other dioxins are therefore considered as presumed carcinogens. The toxic equivalency approach (TEQ) is an Internationally recognized tool utilized for the assessment of the relative toxicity of biological samples, this TEQ approach is further described below.

The toxicity of PCDD, PCDF and the dioxin-like PCB congeners are expressed using toxic equivalence factors (TEFs) (see Table 1.6) representing the relative toxicity of the compound relative to the most toxic dioxin congener, TCDD. This mechanism in turn reflects the relative strength of binding of each compound to the Ah receptor. It should be noted however that the toxicity of many of these substances (both dioxins and PCBs), have not been extensively evaluated.

Several different TEF schemes have been proposed, the so-called International TEFs (I-TEFs) for PCDDs and PCDFs (NATO/CCMS), and the WHO-ECEH (European Centre for Environment and Health of the World Health Organization) scheme for PCBs which is now the most commonly used methodology (71).

WHO-TEFs (table 1.6) were utilized in this current study. WHO-ECEH have assigned an arbitrary TEF of 1 to TCDD, thereby, multiplying the analytically determined sample concentrations of individual congeners by its corresponding TEF, individual TEQs can be determined. Summing of the contribution from each congener allows calculation of the total TEQ as follows:

Equation 11= $TEQ = (PCDD^{i} \times TEF^{ii}) + (PCDF^{i} \times TEF^{ii}) + (\textit{dioxin-like}\ PCB^{i} \times TEF^{ii})$

Where,

(i) = concentration in sample and (ii)= relevant TEF

Table 1.6: Toxic equivalency factors for Dioxins,Furans and Dioxin like PCBs (71).

PCDDs and PCDFs	WHO-TEF	PCB (congener number)	WHO-TEF
2,3,7,8-TCDD	1	3,3',4,4'-TCB (77)	0.0001
1,2,3,7,8-PnCDD	1	3,4,4',5-TCB (81)	0.0001
1,2,3,4,7,8-HxCDD	0.1	3,3',4,4',5-PnCB (126)	0.1
1,2,3,6,7,8-HxCDD	0.1	3,3',4,4',5,5'-HxCB (169)	0.01
1,2,3,7,8,9-HxCDD	0.1	2,3,3',4,4'-PnCB (105)	0.0001
1,2,3,4,6,7,8-HpCDD	0.01	2,3,4,4',5-PnCB (114)	0.0005
OCDD	0.0001	2,3',4,4',5-PnCB (118)	0.0001
2,3,7,8-TCDF	0.1	2,3,4,4'5-PnCB (123)	0.0001
1,2,3,7,8-PnCDF	0.05	2,3,3',4,4',5-HxCB (156)	0.0005
2,3,4,7,8-PnCDF	0.5	2,3,3',4,4',5'-HxCB (157)	0.0005
1,2,3,4,7,8-HxCDF	0.1	2,3',4,4',5,5'-HxCB (167)	0.00001
1,2,3,6,7,8-HxCDF	0.1	2,3,3',4,4',5,5'-HpCB (189)	0.0001
1,2,3,7,8,9-HxCDF	0.1	2,2',3,3',4,4',5-HpCB (170)	0.0001
2,3,4,6,7,8-HxCDF	0.1	2,2',3,4,4',5,5'-HpCB (180)	0.00001
1,2,3,4,6,7,8-HpCDF	0.01		
1,2,3,4,7,8,9-HpCDF	0.01		
OCDF	0.0001		

Abbreviations: PnCDD/F, pentachlorodibenzo-p-dioxin/furan; HxCDD/F, hexachlorodibenzo-p-dioxin/furan; HpCDD/F, heptachlorodibenzo-p-dioxin/furan; OCDD/F, octachlorodibenzo-p-dioxin/furan; TCB, tetrachlorobiphenyl; PnCB, pentachlorobiphenyl; HxCB, hexachlorobiphenyl; HpCB, heptachlorobiphenyl.

1.2.6.9 Analytical quality control

Although proficiency exercises related to analysis of OCPs in marine mammals are not currently available in Europe, both CEFAS and the Marine Institute laboratories show continued successful participation in the Quality Assurance of Information for Marine Environmental Monitoring in Europe (QUASIMEME) laboratory proficiency scheme for the analysis of OCPs in other marine biological tissue. Full analytical quality control protocols involving the use of internal standards and the analysis of blanks and within batch laboratory reference materials were utilised.

Stable isotope analysis was completed in the Institute for Energy Technology (IFE) in Kjeller, Norway. Accuracy and precision of $\delta^{15}N$ and $\delta^{13}C$ analyses was evaluated against

International Atomic Energy Agency (IAEA-N-1, IAEA-N-2) and US Geological Survey (USGS-24) standards in addition to an internal IFE trout standard material.

Enantiomerically pure standards of α-HCH, (Dr. Ehrenstorfer, GmbH, Hamburg Germany) were analysed on a batch basis to ensure correct enantiomeric assignment. Enantiomerically pure standards for *o,p,*-DDT and two toxaphene congeners (CHB26 and CHB50) are not currently commercially available, therefore a racemic mixture of each was employed for identification purposes. Enantiomers were then assigned nomenclature EN1 and EN2 (first and second eluting respectively) for the purposes of this study.

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CHAPTER 2: TOXAPHENE: DISTRIBUTION IN MARINE BIOTA AND A SUMMARY RISK ASSESSMENT TO THE CONSUMER OF IRISH FISHERY PRODUCE.

2.1 Introduction

The Hercules Company of the United States (U.S.) first produced toxaphene, a complex mixture of polychlorinated camphenes in 1945. The ensuing years led to the mass production of the compound (and especially that of toxaphene 3965) for use as an insecticide mainly for application in cotton farming. Use of the compound was not restricted to that of an insecticide, as in subsequent years it was also applied as a piscicide to control fish populations and as a miticide in farming (1). The lipophilic, persistent and volatile nature of toxaphene has led to it becoming ubiquitous in the marine environment. These properties in addition to its bioaccumulation potential ultimately led to its use being banned by the US environmental protection agency (USEPA) in 1982 on the basis that it was a suspected human carcinogen and persistent hazardous compound to non-target organisms.

It was not until the 1990s when the presence of toxaphene in marine fish species was detected that interest in the analytical and toxicological nature of toxaphene developed. In 1997 a European Union project entitled "Investigation into the monitoring, analysis and toxicity of toxaphene" (MATT) was initiated.

The main objectives of this project were;

1) To gather information on the analysis, monitoring and toxicity of toxaphene from available literature (Literature review).
2) To improve methodologies for the determination of toxaphene and to tune participants analytical techniques (Tuning of analytical techniques).

3) To gather information on the background levels of toxaphene residues in different fish and fishery products (Baseline survey).
4) To obtain more information on the toxicity of toxaphene with emphasis on its carcinogenicity (Toxicology).
5) To estimate the toxicological risks to the consumer of toxaphene residues in fish from European waters (Toxicological risk to fish consumer).

This current work sets out to investigate the levels of toxaphene in fishery products from Irish waters and to assess the risk to the consumer of fish from Irish waters.

Summary information on toxaphene physico-chemical properties, sources, occurrence and toxicology are presented below. It is not the purpose of this work however, to critically review and update available literature as comprehensive reviews have been published by Saleh (1) and by deGeus et al (A1).

2.1.1 Physical and chemical properties of toxaphene.

Toxaphene (CAS No. 8001-35-2) was one of the main products produced by the Hercules Company in the USA (1). The process consisted of the extraction of crude α-pinene from pine stumps, using methylisobutylketone, heat and pressure. Isomerisation of the α-pinene produced camphene, bornylene and α-terpineol. The camphene was subsequently chlorinated under UV light to produce technical toxaphene. Structures of the bornane, bornene and other main components of toxaphene are shown in figure 2.1.

Toxaphene is a yellow, waxy solid with a mild terpene odour, softening in the range of 343 to 363 K with average chlorine content of 67-69%. While readily soluble in most organic solvents, it is more soluble in aromatic than aliphatic hydrocarbons and it has an average elemental composition of $C_{10}H_{10}Cl_8$ (1). Fingerling (2) states that toxaphene consists of at least 180-190 components mostly with the formula $C_{10}H_{18-n}Cl_n$ or $C_{10}H_{16-n}Cl_n$ where n is 6-10 (see table 2.1). Buser (3) reports that polychlorobornanes ($C_{10}H_{18-n}Cl_n$ where n =5-12) are formed as the main components in a Wagner-Meerwin type rearrangement reaction.

The commercial product is relatively stable, but may be degraded by losing HCl or Cl_2 on prolonged exposure to sunlight, alkali, or temperatures above 393 K (4). Saleh (1) states that technical toxaphene does not undergo a serious change when exposed to normal sunlight. Saleh and Casida (5) and Parlar (6) reported that irradiation at wavelengths below 290 nm results in reductive dechlorination and dehydrochlorination. Radiation above 290 nm does not seem to affect toxaphene composition. When adsorbed on silica, however, technical toxaphene is completely mineralised to CO_2 and HCl at 230 nm (7).

A specific gravity of 1.6 kg g^{-1} has been reported for technical toxaphene (8). The vapour pressure and the log octanol-water partition coefficient (K_{ow}) value have been estimated to be comparable to that of hexachlorobenzene (HCB), 1.73 x 10^{-3} Pa at 298 K (9), and a log K_{ow} of 5.5 (10). Howard (11) and Sullivan and Armstrong (12) have recorded K_{ow} values of 4.82 to 6.4, respectively while a log K_{ow} value of 6.44 was recorded by Hooper (13), this being somewhat lower than that of technical polychlorinated biphenyl (PCB) mixtures but higher than those of p,p'-DDT and its metabolites, suggesting that the bioconcentration potential of toxaphene is high. These data are difficult to compare due to the variation of

mixtures used. Bioconcentration factors of 2 x 10^6 have been observed for toxaphene in Arctic cod by Kucklick (14). This value being higher than that predicted from the log K_{ow}. Based on their vapour pressure calculations Wania and Mackay (15) have suggested that toxaphene changes its characteristic of being a chemical, which is mostly in the gas-phase, to one, which is largely aerosol-adsorbed within the range of global environmental temperatures. At 298 K less than 10% is adsorbed to aerosols, at 253 K almost 90% is adsorbed. This implies that with this change of temperature, almost all of the toxaphene in air condenses onto particles present in the atmosphere and thus becomes subject to wet and dry deposition. Therefore at low temperatures toxaphene is more rapidly transferred from the atmosphere to soil and water.

The water solubility of toxaphene has been reported with an equally broad spectrum of values, ranging from 0.4 mg l^{-1} at 298 K (16), and 0.55 to 3.3 mg kg^{-1} at 293 to 298 K (12).

The most important factor determining the flux between the air-water interface is the Henry's law constant (H). Murphy (17) measured H for a technical mixture of toxaphene congeners as being 0.62 Pa m^3 mol^{-1} at 293 K. Using fugacity-based equations (10,18), the direction and magnitude of the flux was calculated by McConnell (19), assuming that the temperature slope of Tateya (20) for PCBs was also valid for toxaphene. With the insertion of the measured H, a toxaphene specific intercept was determined and from that a temperature "corrected" Henry's law constant was obtained. This value allows the direction of the flux to be calculated. Such calculations have suggested that up to 2 kg of material will be deposited on Lake Baikal per month by gas exchange, this process being further

enhanced by the low water temperature of the lake (20). More accurate congener specific H values are required to improve such estimations. Bidleman (21) has also reported this flux direction of air to water, while, Hoff (22) reports that additional inputs via precipitation and particle deposition are likely to be ten to twenty times smaller than those from gas absorption.

Most chlorinated bornanes contain at least one chlorine atom at the C2 and C10 positions, while the bridging carbons, C1 and C4, are non-chlorinated (23). As technical toxaphene is synthesized by photoinduction it will have a high percentage of components containing a dichloro group in the C2 position (24).

Toxaphene congeners demonstrate differing stability to UV light, acid and alkaline treatment. Fingerling (2, 24) demonstrated that in soil 2,2,5-endo,6-exo,8,9,10-heptachlorobornane, 2,2,5-endo,6-exo,8,8,9,10-octachlorobornane, 2,2,5-endo,6-exo,8,9,9,10-octachlorobornane, 2,2,3-exo,5-endo,6-exo,8,9,9,10-nonachloro-bornane, 2,2,5-endo,6-exo,8,8,9,10,10-nonachlorobornane and 2,2,5-endo,6-exo,- 8,9,9,10,10-nonachlorobornane (B[30012]-(111), B[30012]-(211), B[30012]-(121), B[32012]-(121), B[30012]-(212) and B[30012]-(122)) (nomenclature according to Wester (25)) were all dechlorinated by reductive removal of one chlorine atom from each geminal dichloro group, beginning with that in the C2 position which is the most labile under anaerobic conditions. The authors suggest that dechlorination also occurs during photodegradation and that the dechlorination rate is nonachlorobornanes > octachlorobornane > heptachlorobornanes.

Fingerling (2) also found that during irradiation in solvents, the bornane structure is generally preserved and that photolability seems to depend on the presence of a geminal dichloro group in C2-position, the dechlorination rate being enhanced by an additional chlorine atom in the C3-position but not by a dichloro group in C5-position. Components with only a single chlorine atom at each secondary ring atom in alternating orientation, such as 2-endo,3-exo,5-endo,6-exo,8,8,10,10-octachloro-bornane, 2-endo,3-exo,5-endo,6-exo,8,8,9,10,10-nonachlorobornane, or 2-endo,3-exo,5-endo,6-exo,8,9,9,10,10-nonachlorobornane (B[12012]-(202), B[12012]-(212), or B[12012]-(212), (according to Wester (25)), have been found to be extremely photostable.

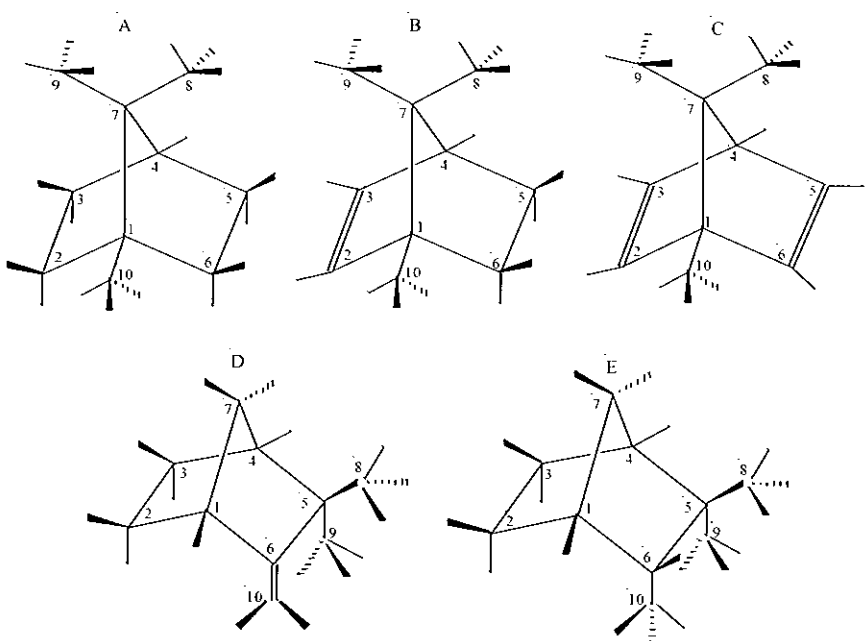

Figure 2.1: Carbon skeleton of bornane (A), bornene (B), bornadiene (C), camphene (D) and dihydrocamphene (E)

Note: (D and E as proposed by Hainzl (26).)

2.1.2 Toxaphene nomenclature systems.

As presented in table 2.2 various compound classes are to be found in technical toxaphene. Within each group a large number of congeners are theoretically possible. Other classes such as, compounds of technical toxaphene (CTTs) (27), polychlorinated camphene (PCCs) (28) and chlorinated bornanes (CHBs) (29) also exist. These group names may alone only account for a single congener in the technical mixture.

The large number of congeners and compound groups can therefore lead to confusion with the systematic naming and nomenclature of individual congeners, this being further complicated where the congener is enantiomeric.

Table 2.1: Compound classes present in technical toxaphene.

Class of compound	General formula	Number of congeners
Chlorinated bornanes	$C_{10}H_{18-n}Cl_n$	32,767
Chlorinated bornenes	$C_{10}H_{16-n}Cl_n$	8.191
Chlorinated bornadienes	$C_{10}H_{14-n}Cl_n$	2.047
Chlorinated camphenes	$C_{10}H_{16-n}Cl_n$	16.383
Chlorinated dihydrocamphenes	$C_{10}H_{18-n}Cl_n$	65,534

Many nomenclature systems exist, including those proposed by Burhenne (30) and Hainzl (31) naming the congeners based on gas chromatographic retention on a particular stationary phase. This naming method is inadequate in that co-elutions are likely to occur and it will not be universally standard due to analytical differences between stationary phases. Nikiforov (32) proposed a system of assigning a 13 digit binary code based on the locations at which chlorination can occur. The code can then with the aid of a computer be shortened to a 4-digit number to describe the structure. Oehme and Kallenborn (33) also

proposed a system similar to that of Nikiforov, these systems are however cumbersome and do not account for all the congeners possible.

A simpler system proposed by Wester (25) (See table 2.2) yields a code in two parts that allows easier visualisation of the compound. Digits in part 1 reflect the degree of chlorination on carbons C2-C6 and the second part the degree of chlorination on carbons C8-C10. The system takes into account the various groups of toxaphene that exist in addition to further structural information such as *endo* or *exo* positioning of the chlorine and the enantiomeric form of the compound. This nomenclature system was also extended to include bornenes and bornadienes.

Table 2.2: Codes indicating chlorination of C2 - C6 atoms of chlorinated bornanes. According to Wester (25).

endo	exo	code
0	0	0
1	0	1
0	1	2
1	1	3

A system proposed by Parlar (34) also suggests that congeners be numbered in elution order on a particular stationary phase. A derivation of the system of Parlar was employed on this project whereby environmentally and toxicologically relevant congeners were numbered as per Parlar but were preceded by CHB (chlorobornane). This notation allowed simplification of the analysis due to the small number of congeners under investigation.

2.1.3 Sources of toxaphene.

Since its introduction toxaphene has been employed for a variety of uses. Voldner and Smith (35) and Rapaport and Eisenreich (36) report that by 1986 toxaphene usage had dropped from 45 x 10^6 kg $year^{-1}$ to 7.20 x 10^6 kg $year^{-1}$.

In 1989 there were 168 registered uses in the USA (37), and more than 277 worldwide for agricultural commodities and crops to control 167 major insect pests. Its use in livestock dips as a miticide, and in lakes as a piscicide to control undesired fish populations have all been reported (1). The interpolated total global use between 1950-1993 was 1,330 x 10^6 kg and from 1970 to 1993 was 670 x 10^6 kg (38). This estimation was based on literature data and contacts with international agencies and researchers; its quality varies and shows large spatial and temporal gaps.

The USA (39), the Central American states, and the former Soviet states have had the highest recorded usage. This may be because more detailed information on usage was received from these countries whereas information is not kept or is confidential in others (38). El-Sebae (16) reports that toxaphene continues to be used in African countries especially Ethiopia, Sudan, Tanzania and Uganda where field run-off eventually leads to the Nile and ultimately to the Mediterranean Sea. These could act as an additional reservoir for future contamination. Information is however lacking for other African countries.

Toxaphene is currently banned in many countries worldwide and it was only used in small quantities in Sweden and has been banned since 1956 (40). In 1971 the Soviet government restricted the use of toxaphene, it is thought to be still in use as an insecticide for sugar

beet, peas, potatoes, mustard, rape and perennial herbs during sprout stage (41). Voldner and Li (38) state that 1 x 10^8 kg has been used since 1970 in the former Soviet Union.

It was previously thought that chlorohydrocarbons were produced in chloro-bleaching from the residual monoterpenes in the wood pulp industry. However, no indication of compounds identical to the main congeners in commercial toxaphene has been reported providing evidence that toxaphene in fish may not have originated from chlorobleaching of pulp (42). However, chlorinated camphenes are present in pulp mill runoff (43) and chlorine bleaching of wood pulp produces chlorinated compounds that are similar in composition to toxaphene but with lower chlorine content (44).

It has been suggested that toxaphene found in marine species from areas such as the Arctic where it was never used is due to long-range atmospheric transport (45). Wania and Mackay (15) suggest that toxaphene changes its chemical characteristics from gas phase to largely aerosol absorbed within the range of global environmental temperatures. This suggests that changes in air temperatures will bring about wet and dry deposition of toxaphene, as it will condense onto particles in the air.

2.1.4 Toxicology of toxaphene.

A summary of some available literature on toxicological aspects of toxaphene is reported below.

2.1.4.1 Absorption and distribution.

Toxaphene and several of its individual congeners have been shown to be absorbed from the gastrointestinal tract of mice (46), rats (47), guinea fowl, hamsters, rabbits, monkeys and chickens (1,48). This has been evidenced by the finding of toxaphene residues in fat

and various tissues and by the excretion of toxaphene in urine and faeces. The distribution in the fat of all these species also resembled that of the administered technical toxaphene. Experiments by Mohammed, showed an initial accumulation in the adrenal cortex followed by brown fat, lung, brain, kidney and ovaria in female mice injected with ^{14}C labelled toxaphene. A gradual redistribution of radioactivity to white fat was seen after 4 hours (49).

2.1.4.2 Biotransformation and excretion.

The major metabolite degradation mechanisms for toxaphene in all organisms from bacteria to primates seems to be reductive dechlorination, reductive dehydrochlorination and oxidative dechlorination to produce hydroxyl derivatives, acids or ketones (1).

2.1.4.3 In-Vitro toxicology.

Saleh and Casida (5) studied the metabolism of ^{14}C labelled toxaphene and its toxic components, toxicants B and C, using rat liver preparations. Results showed that the microsomal monooxygenases and to a lesser extent glutathione *s*-transferases were involved in metabolism processes. On the addition of NADPH, toxaphene was metabolised aerobically to form an additional 49 metabolites. *In vitro* studies by Chandurkar and Matsumura (50) concluded that oxidative metabolism might play an important role in the metabolism of toxaphene.

2.1.4.4 In-Vivo toxicology.

Ohsawa (51) in addition to Turner (52) showed radioactivity in urine and faeces of rats on oral administration of ^{14}C labelled toxaphene. Metabolites included acidic materials and expired ^{14}C labelled carbon dioxide. Dose dependent toxaphene excretion in the milk of cows has also been reported (53). For these studies it appears that the biological half-life of

commercial toxaphene is in the order of a few days. Some of the compounds are more persistent however as evidenced by the finding of residues in fat tissues 2-3 weeks after administration of a single dose.

2.1.4.5 Reproduction and developmental effects.

No effects on fertility have been reported in either rats or mice although lower dose levels have been shown to affect the behaviour of rat offspring and suppress immune response in mouse offspring (46).

2.1.4.6 Genotoxicity.

Toxaphene has been shown to be genotoxic *in-vitro* (54) but with the limited database it is unknown whether it is genotoxic *in-vivo*.

2.1.4.7 Carcinogenicity.

Long term studies in mice have shown induction of hepatocellular carcinomas (55). Carcinogenicity may well be promoted due to the genotoxic properties of toxaphene as it inhibits intra-cellular communication. It may also interfere with the carcinogenicity of other compounds through induction or depression of enzyme activities.

2.1.4.8 Human toxicological data.

Human exposure is mainly through diet and to a lesser extent inhalation of ambient air (56,57). Inhalation is low compared to dietary exposure with values of 0.4-3.3 ng/day reported (74). Munn discovered that occupational exposure via dermal and inhalation routes was high during the manufacture of technical toxaphene. In a single incident seven persons suffered convulsions after eating vegetables sprayed with toxaphene three days previously

(58). A human toxicology study on exposure to toxaphene in the air over a 10-day period did not reveal any toxicity in blood or urine (59).

Acute lethal doses have been estimated at 2-7g^{-1} person where poisoning symptoms appear within four hours of ingestion with death reported within four days. Recovery from poisoning has also been reported to be rapid in similar cases (60). Infants may also be exposed to toxaphene from their mother's milk as reported by deBoer (61).

Toxicokinetic studies on toxaphene are to be treated with caution, as differences between formulations of technical toxaphene used in experiments will result in data, which is not fully compatible between studies. There are however insufficient data to evaluate the carcinogenicity of toxaphene to humans. Further more expansive detail on the toxicology of toxaphene and novel toxicological aspects to this current work are reported in (AII).

2.1.5 Legislation related to toxaphene

Germany has set the maximum residue limit (MRL) for toxaphene on the basis of the sum of the three indicator congeners for fish and fishery products at 0.1mg kg^{-1} wet weight (62). Canada has taken a different approach of having set an acceptable daily intake level (ADI) of 0.2µg kg^{-1} body weight. Toxaphene levels have recently been shown to exceed MRLs set by some European countries raising concern over the safety of the consumer from some fishery products and hence initiation of this work program.

2.1.6 Analytical methods.

The analytical determination of toxaphene is considered more difficult than for most other organochlorine pesticides for a number of reasons;

1) toxaphene levels are much lower than those for PCBs and organochlorine pestcides (OCPs). As a result chromatogram peaks for toxaphene congeners can be swamped by those from other organochlorines,
2) the response of toxaphene is lower on most detection systems than PCBs resulting in higher limits of detection,
3) standards and extracted samples do not always have the same response,
4) purchased analytical standards are not always 100% pure.

It is therefore vital to optimise extraction, pre-separation, clean-up and detection procedures to ensure maximum resolution from possible interfering compounds.

2.1.6.1 Extraction.

Little attention has been paid to extraction procedures in the literature. However, it is thought that procedures suitable for the extraction of related compounds such as PCBs, DDT and chlordanes could also be used for toxaphene compounds due to similarity in their lipophilic and structural properties (29).

2.1.6.2 Pre-separation and clean-up.

Pre-separation and clean-up are of major importance in the analysis of toxaphene, as efficient lipid extraction and residue clean-up are essential to allow accurate analysis to take place. Many stationary phases have been employed in the preparation of samples for analysis namely aluminium oxide (63), gel permeation chromatography (GPC) on size

exclusion polymer compounds (64) for lipid removal and florosil (42, 64-65) and silica gel (50, 56, 66) for residue clean-up.

Reversed phase HPLC has also been utilised in the pre-separation of interfering compounds from toxaphene congeners (66). Silica gel with varying percentages of deactivation and differing solvent systems has also been used in the separation of PCBs and other organochlorines from toxaphene (61).

2.1.6.3 Chromatographic separation.

Due to the large number of possible congeners and the highly chlorinated nature of toxaphene a separation methodology with a high resolution potential in combination with specific detection systems is preferred. This can readily be achieved with GC using a variety of stationary phases when coupled with electron capture detection (ECD) or mass spectrometry (MS).

The relatively non-polar stationary phase, 5% diphenyl, 95% dimethylpolysiloxane (DB-5, Sil-8, Ultra-2) column with lengths of 30 to 60 m and internal diameters of 0.15 to 0.32 mm are frequently used. However, more polar columns are often used to validate results e.g., 14% cyanopropylphenyl 86% dimethylpolysiloxane (DB-1701, Sil-19) (67), or 6% cyanopropylphenyl 94% dimethylpolysiloxane (DB-1301) (68).

Relatively good separation using a very non-polar Sil-2 stationary phase has been obtained by Krock (69) with the same elution order as on the more polar DB-5 columns being observed (70). The CP-Sil 2 phase was successfully used up to a temperature as high as 563

K, and no alteration of retention times was observed after several hundred analyses on this phase (71). It was also observed that compounds with a single chlorine atom on both C8 and C9 elute much later from this phase than compounds with two chlorines on either of these carbons.

Heart-cut multidimensional gas chromatography (MDGC) (72) offers a possibility to overcome co-elution problems caused by the large number of congeners present in a sample. Transferring heart-cuts from a separation performed on a DB-5 type phase to a DX-4 phase, in addition to a Rtx-2330 phase for further separation (73), it was observed that a large number of peaks were to be found in the secondary chromatograms. This shows that the resolution offered by one single column can be non-specific and can lead to false-positive results.

2.1.6.4 Detection.

With the use of non-specific ECD detection good pre-separation of PCBs and other organochlorine compounds present in environmental samples is required as they can interfere in the chromatographic identification of toxaphene.

Negative chemical ionization (NCI) is the most widely used MS detection technique but this has the drawback of being relatively insensitive to some congeners. One study (74) showed a total of 25 additional peaks in a sample analysed in electron impact (EI) mode compared to that in NCI mode. Flame ionization detection (FID) has also been used for toxaphene analysis, but results are similar to those obtained in EI/MS mode. NCI detection has however been reported to show a positive bias in response factors between individual

congeners (75), a bias that is not observed with ECD detection. ECD detection is considered sufficient for the detection of environmentally relevant toxaphene congeners in most matrices.

2.1.7 Indicator compounds and total toxaphene.

Toxaphene chromatogram profiles in biota sampled in northern latitudes do not readily resemble those from American or Canadian samples, whose profile more reflects that of technical toxaphene. Differences in profile can primarily be attributed to weathering effects on the compound during aerial transport in addition to the effects of metabolisation and depuration in the contaminated species. As a result two approaches to the analytical determination of toxaphene have developed. The first refers to the measurement of total toxaphene, which is used, where the pattern resembles that of the technical mixture. The second measures the "indicator congeners", these congeners are generally those that persist in the environment after weathering effects. It is therefore presumed that these indicators will be dominant in regard to human toxaphene uptake. Studies have shown that these indicator compounds can compose up to 60% of the total toxaphene concentration in a sample (1).

The accurate measurement of total toxaphene in biota from European waters is more difficult to carry out due to the effects of metabolisation and weathering on expected congener profiles. The concentration of CHBs (other than indicator compounds) is generally much lower than for the indicator compounds themselves. As these concentrations are low, analytical errors can increase due to co-elution problems with compounds such as PCBs that have higher response factors than toxaphene when ECD

detection is employed. In addition existing legislation is based on the sum of the three indicator CHBs.

Biota from areas close to where toxaphene was used will have a profile more consistent with that of the technical mixture, as weathering effects are lessened. As a consequence the measurement of total toxaphene is more indicative of the full extent of contamination.

Total toxaphene refers to the sum of all the measured congeners in a sample. As most of the available data is presented in the form of "total toxaphene" it is difficult to compare it to data in the form of the sum of the three indicator compounds. AI, AV and AX compiled during this study summarise some of the datasets documenting total and indicator compound concentrations in marine species.

2.1.8 Summary

Toxaphene has been found in biota including zooplankton, most commercial fish species, bird eggs and mammalian milk. In some cases toxaphene has been found to be the major organochlorine contaminant present in samples. Animals occupying the highest trophic levels have also been shown to have high levels in tissue indicating the high bioaccumulation potential of toxaphene. Toxaphene has been shown to be a persistent pollutant with bioaccumulation potential. It is neurotoxic, hepatotoxic and an endocrine toxin and has also been reported to be highly carcinogenic in rats and mice, leading to the assumption that it is a possible risk to the consumer. As such this project sets out to develop and validate analytical methodology, to determine the baseline levels of toxaphene and to estimate the possible risk to the consumer of fish and fishery products from Irish waters.

2.2 Materials and methodology.

The optimisation and validation of a combination of a number of individual analytical techniques allows accurate toxaphene concentrations in marine biota be determined. Gas chromatographic (GC) analysis, sample clean-up, separation of interfering compounds, and lipid extraction completed during this study are further discussed below.

2.2.1 Gas chromatographic analysis-separation, detection and quantification.

GC brings about the separation of mixtures down to picogram quantities by passage of a vaporised sample in a gas stream through a column containing a stationary liquid or solid phase. Components migrate towards the end of the column and detector at a rate determined by differences in their boiling point, solubility or adsorption. GC utilises partition via sorption processes on a high boiling point liquid stationary phase bonded to the walls of a narrow bore capillary column. When vapourised in the injector the volume of the sample is much greater than in its liquid state.

2.2.1.1 Electron capture detection (ECD).

The ECD contains ^{63}Ni a radioactive isotope emitting high-energy electrons (β-particles) which undergo repeated collisions with the molecules in the carrier gas to produce about a hundred-fold increase in secondary electrons for each initial β-particle. Further collisions bring about a reduction of the energy of these particles into the thermal range. These low energy electrons are "captured" by suitable sample molecules thereby reducing the total electron population within the cell. Uncaptured electrons are collected periodically by applying short-term voltage pulses to cell electrodes. The cell current is measured and compared to a reference current, the pulse interval is then adjusted to maintain constant cell

current. Therefore pulse rate (frequency) rises when an electron-capturing compound passes through the cell. The pulse rate is converted to a voltage linearly related to the amount of electron-capturing material in the cell. Thus, the ECD responds to compounds having an affinity for electrons (see table 2.3).

Table 2.3: Relative sensitivity of various compound classes on ECD (76).

Chemical type	Relative sensitivity
Hydrocarbons	1
Mono-Cl and Mono-F compounds	100
Mono-Br and Di-Cl compounds	1000
Tri-Br and Poly-Cl compounds	10^6

When electro-negative compounds especially chlorinated, fluorinated or brominated molecules, such as toxaphene, PCBs or pesticides such as DDT enter the cell, they immediately combine with some of the free electrons, temporarily reducing the number remaining in the electron cloud. ECD detection is however dependent on the degree and the position of chlorination on the analyte, therefore each congener may have a different response factor making analysis and quantitation more difficult.

PCBs are more sensitive than toxaphene on ECD and will usually mask toxaphene peaks in the chromatogram. For this reason the correct stationary phase, column dimensions and carrier gas are important to optimise the separation of toxaphene from other organochlorines.

2.2.1.2 Stationary phase selection.

The choice of stationary phase for analysis is largely dictated by the nature of the compounds of interest. The phase has to be non-volatile and thermally stable at the operating temperature to prevent "bleeding" and noisy or drifting baselines. The column has to be chemically stable and physically inert towards the samples to ensure reliable results. In general the most suitable stationary phase is one that is chemically similar to the analyte of interest.

Traditionally CPSIL8 (5% phenyl 95% dimethylpolysiloxane) and CPSIL19 (14% cyanopropyl-phenyl 86% dimethylpolysiloxane) stationary phases have been shown to be suitable for the separation of PCBs and other organochlorine compounds (77). These two columns were chosen for the purposes of this work.

The choice of these two columns allowed for the setting up of a dual system where two columns were attached to two separate ECD detectors, the sample administered via a Y splitter and retention gap, resulting in two chromatograms with differing retention times being obtained. This allowed dual column confirmation of the presence of an analyte to be established.

2.2.1.3 Optimisation of injection temperature.

A commercially available analytical standard containing CHBs 26,50 and 62 was injected onto a Hewlett Packard (HP) 5890 Series II GC with ECD detection. Peak height, width and symmetry were measured between (200°C and 300°C) to investigate the effect of injection temperature on the compounds chromatographic properties. Based on these data in

conjuction with the fact that CHB 62 is potentially thermolabile where the injection temperature is above 240°C, it was concluded that 240°C was appropriate to ensure minimal CHB degradation occurred within the injection port. This temperature was shown to provide peak height, width and symmetry parameters at or close to the optimum for the 3 CHBs. Fully optimised GC conditions are detailed in table 2.4.

Table 2.4: Conditions employed in the analysis of toxaphene in biota.

Instrument	Hewlett Packard 5890 Series II GC	
Analytical separation	CPSIL8 Column	
	Length (m)	50
	Internal diameter (mm)	0.15
	Film thickness (µm)	0.25
	CPSIL19 Column	
	Length (m)	50
	Internal diameter (mm)	0.15
	Film thickness (µm)	0.25
	Retention gap (m)	5
	Hydrogen Carrier Gas	
	Nitrogen Make up Gas	
Injection parameters	Splitless	
	Temperature (°C)	240
	Double gooseneck liner	
	Injection volume (µl)	2
Detector parameters	Detector temperature(s) (°C)	330
Temperature programmes	Initial temperature (°C)	80
	Initial time (mins)	1
	Ramp 1 (°C)	25
	Final temperature (°C)	250
	Hold time (mins)	55
	Ramp 2 (°C)	10
	Final temperature (°C)	280
	Hold time (mins)	17
Internal standard	4-5 dichloro-chlordene	

2.2.2 Extraction of lipid for toxaphene analysis.

Due to the lipophilic nature of organochlorine compounds they accumulate in both bound and free lipid fractions of tissue. An efficient extraction system for organochlorine compounds will consequently be one that extracts both of these lipid classes as lipids in tissue can range from non-polar to highly polar. Some are deemed bound lipids and will bind with proteins and carbohydrates while others make up the so-called free lipids.

Generally speaking an extraction system using a more polar solvent will result in higher organochlorine concentrations than observed for a non-polar extraction (78). This too is tissue dependent, and the importance of the polarity of a solvent becomes greater for a lipid rich compared to lipid lean tissue. In some species depot or bound lipids consisting mainly of phospholipids can contribute up to 5g per kg of the total lipid content. Non depot or free lipids making up the remainder of the fat content generally consist of neutral lipids such as cholesterol ethers or wax ethers. Non-polar solvents will extract the free lipids but not all of the bound forms, which in the case of lean fish make up a considerable portion of the total lipid content.

A cold solvent extraction technique using a mixture of polar and non-polar solvents was utilised during this work. This selected solvent mixture ensured that most of the important lipid classes are removed from tissue and in addition cause less damage to the organochlorines and thermolabile compounds in the lipid (78).

2.2.2.1 Methodology and findings on lipid removal.

Tissue and solvents (hexane: acetone 3:1) were mixed with the Ultraturrax and centrifugation at 2000 RPM X 2 followed. Two further repeat extractions were carried out on the tissue.

All of the solvent extracts were combined for cleanup. 60ml of 0.2M NaCl in 0.1M orthophosphoric acid was added to aid separation of the phases and help removal of residual tissue to the aqueous phase.

Water phases and hexane phase collected in separate flasks. Water phase returned to separating funnel and re-extracted with 10 ml hexane. The organic phase was collected and added to the previously collected organic phase and the water phase was discarded.

The combined hexane extracts were returned to the separating funnel and washed with 20-ml water, to remove residual salts from the organic phase. Hexane phases were collected and the volume reduced to 4-5ml in rotary evaporator.

Lipid recovery was estimated to be 98.2% ± 5.3% for a series of cold solvent extractions (n=8) on a QUASIMEME proficiency study herring tissue (QOR058 BT) sample (79). Based on the results of these experiments it was concluded that cold solvent extraction was acceptable for the purposes of this study.

2.2.3 Lipid clean-up procedures

Gel permeation (GPC) was selected for the removal of lipid from the tissue extracts. GPC generally refers to the separation of materials according to molecular size and shape by passage of a solution through a column or across a surface consisting of a polymeric gel. The pores of the gel exclude molecules greater than a certain critical size whilst smaller molecules can permeate the gel structure by diffusion. Excluded molecules pass through the system more rapidly than smaller ones that can diffuse into the gel. Diffusion within the gel also varies with molecular size and shape because pores of different dimensions are distributed throughout the gel structure in a random manner. Smaller molecules are eluted at rates dependent on their degree of permeation into the gel, and components of a mixture therefore elute in order of decreasing size or molecular weight.

2.2.3.1 Methodology and findings for lipid removal by GPC.

The degree of separation of lipid from organochlorine residues was evaluated under a number of experimental conditions.

Corn oil containing no pesticide residue was spiked with the three indicator CHBs and a total of 51 PCB congeners. The elution pattern for lipid was determined by running a known aliquot of the mixture on the GPC system, evaporating resultant fractions to dryness and determining the lipid content gravimetrically in pre-weighed sample tubes. This allowed an estimate to be made as to the end of lipid elution and the start of organochlorine elution.

The remaining aliquot was run in a similar way to the first with fractions between 35-42 min. collected; dried to a known volume and analysed by GC-ECD to determine which compounds were present in the individual fractions.

Optimal run conditions similar to those reported by Rimkus el al (80) were observed as being 5ml/min 100% DCM. Resolution of lipid and toxaphene residues was deemed to be insufficient at higher flow rates. Recoveries ranged from 85.3% for CHB26, 89.8% for CHB50 to 94.3% for CHB62 in DCM. It was observed under the conditions described above that the separation between lipid and pesticide elution was sufficient to allow collection of the residue without lipid interference. Separations achieved under optimal GPC conditions are presented in figure 2.2 below.

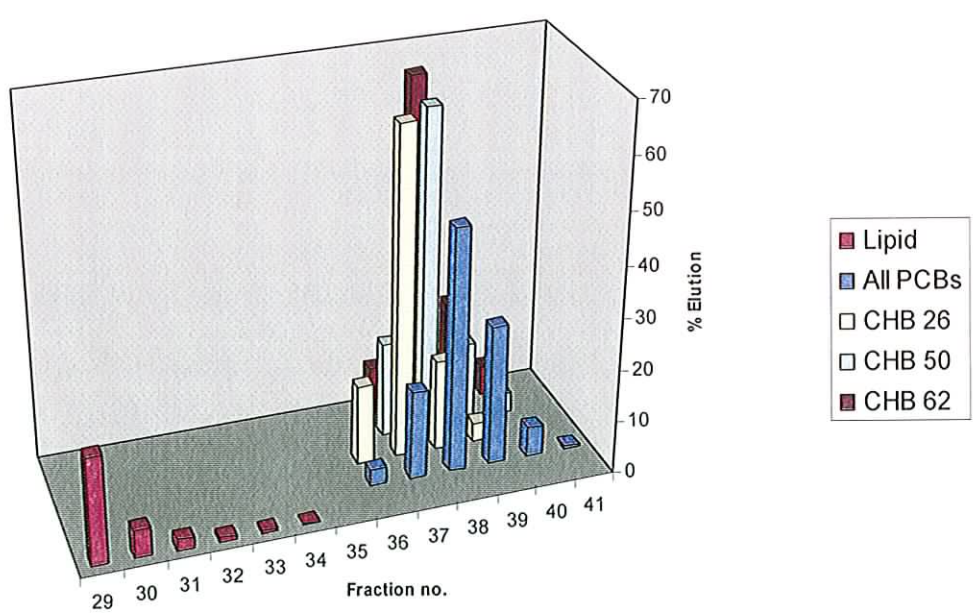

Figure 2.2: Elution order of lipid, PCBs and CHBs on GPC with DCM as eluant.

2.2.4 Separation of interfering compounds in analytical samples.

A separation in which surface adsorption is the main sorption mechanism requires polarity differences between solute molecules. Generally non-polar molecules comprise of atoms that are highly symmetrical with similar electronegativities; the addition of functional groups to the system leads to an increase in polarity. Molecules that are highly polar will adsorb to a stronger degree on to a polar surface. Competition takes place between solute molecules and those from the mobile phase in the separation process. These molecules are continuously being adsorbed and desorbed with competition taking place for adsorption sites as the mobile phase flows through the system. Low polarity solutes have a greater affinity for the mobile phase than highly polar solutes resulting in separation of mixtures based on increasing polarities.

Materials that are commonly used in order of decreasing polarity include alumina, charcoal, silica gel and cellulose. For toxaphene analysis silica gel an "active" adsorbent which separates based on the presence of oxygen atoms and silanol groups that readily form hydrogen bonds with polar molecules was used. The activity of the gel was controlled with the addition of water to block some of the adsorption sites. This process helps to minimise batch-to-batch variation in supplies of silica.

2.2.4.1 The separation potential of silica gel chromatography.

The use of silica gel has been reported by deBoer (81) and by Krock (82) for the separation of toxaphene congeners from other organochlorine residues in various fish tissues. These "interfering" organochlorine compounds can affect the analytical determination of toxaphene as their presence may swamp the appearance of a toxaphene congener in GC

separation therefore it is desirable to remove as many of these compounds as possible prior to analysis. The procedure of Krock (82) was investigated to assess the separation capacity of silica gel.

2.2.4.1.1 Methodology and results of silica gel optimisation.

Heating to 400°C for 4 hours activated silica gel. 5%-deionised water was then added to the gel in a dropwise manner to deactivate the gel. 8g of 5% of deactivated silica gel were slowly added to a column with 50ml reservoir. The column was slowly filled with hexane and approximately 25-30ml allowed to flow through to waste.

A solution containing technical toxaphene, 51 PCB congeners and 15 organochlorine pesticides in iso-octane was prepared as the test mixture. The sample was applied with a Pasteur pipette.

Seven fractions (totalling 28ml hexane) were collected for separation of PCBs. Approximately 2ml of iso-octane was added to each vial. Sixteen further fractions were collected using the second elution solvent hexane: diethyl-ether (3:1) (totalling of 70 ml) was collected for examination for the majority of the indicator compounds. Approximately 2ml of iso-octane was then added to each vial and contents were concentrated under a stream of nitrogen to approximately 1ml and samples were analysed by GC-ECD.

In summary, analytical evaluation of the first 28 ml hexane making up fractions (1-7) demonstrated that these fractions contained the majority of PCBs (>90%) and some organo-chlorine pesticides (>30%). The remaining fractions (8-23) composed of 70 ml hexane:

diethyl ether (3:1) contained the majority of the toxaphene residue (>97%), see figure 2.3. This information allowed a cut–off point of 28ml hexane (PCB fraction) and 70 ml hexane:diethyl ether (3:1) (toxaphene fraction) to be established as being optimal for the separation of PCBs and toxaphene.

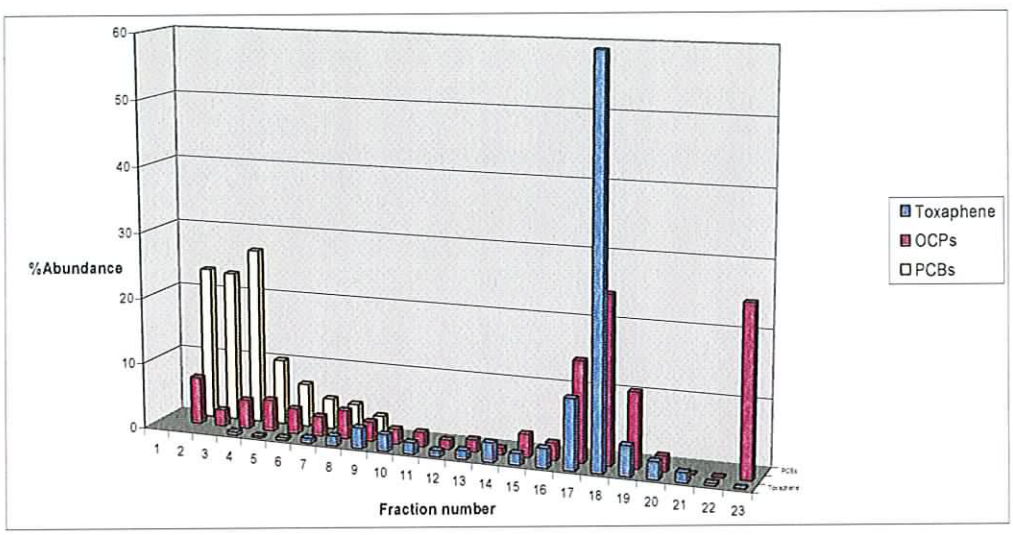

Figure 2.3: Separation of PCB's and OCP's from technical toxaphene on silica gel.

This cut-off point did not however allow all of CHB26 to be collected in the toxaphene fraction. It was observed that approximately 30% of this congener eluted in the first PCB fraction and as a result both fractions would have to be analysed for its presence. GC-ECD conditions were optimised so that no major interfering peaks eluted close to the congeners of interest. The silica separation was suitable to allow analysis to proceed.

2.2.5 Overall method Summary.

In conclusion a combination of cold solvent extraction followed by GPC, silica gel separation and addition of 0.5-1ml of sulphuric acid to the sample with subsequent GC-ECD analysis was selected for sample analysis (see figure 2.4).

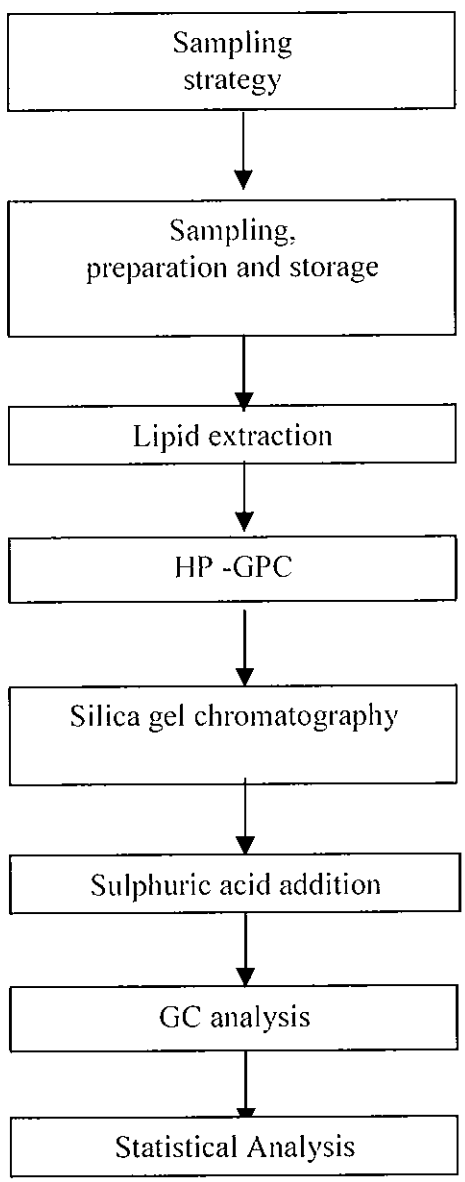

Figure 2.4: Flow chart of analytical methodology employed for toxaphene analysis.

2.3 Validation of toxaphene methodology.

Method validation provides an assurance of reliability and is sometime referred to as "the process of providing documented evidence that the method does what it is intended to do". Method validation was completed to ensure that an analytical methodology is accurate, precise, specific, reproducible, and linear over specified ranges ensuring that only the analyte will be analysed.

No certified reference materials for toxaphene analysis were available for use in evaluating analytical methodology during the time scale in which this work was completed. A combination of participation in inter-laboratory comparison exercises and the analysis of fortified corn oils allowed the validation process to be completed. A detailed overview of these studies is presented in AVI, therefore detailed descriptions are not further described here. Selected validation parameters including, accuracy, precision, specificity, linearity, limit of detection and quantification are further described below.

2.3.1 Accuracy

Accuracy is the measure of exactness of an analytical method (83). It measures the closeness of agreement between the value accepted either as a conventional true value or a reference value and the result obtained from a particular method. Accuracy can be calculated by comparison of observed results from fortified samples to expected values.

Accuracy was determined by fortification of a blank corn oil at three concentration levels covering the range 0.23-121 $\mu g\ kg^{-1}$, followed by removal of lipid by GPC, fractionation on silica gel followed by GC analysis. Results are reported in table 2.5.

CHB 62 is more thermolabile than either CHB 50 or 26 and as such recoveries were slightly lower. In general as the concentration of spike increased the percentage recovery increased probably due to less matrix interference being observed at the higher concentration ranges. Over a concentration range of 0.23-121 $\mu g\ kg^{-1}$ (at a level of 95% confidence for the method) the accuracy of analysis of CHBs 26, 50 and 62 was determined as being (87.4 ± 2.79%), (85.0 ± 3.99%) and (76.8 ± 5.16%) respectively with all values falling within 95% confidence intervals for the exercise.

Table 2.5: Accuracy (%) of toxaphene method for 3 congeners of toxaphene based on spiked corn oil. (n=3 for all determinations).

	Conc.	Recovery Range (%)			Mean	RSD(%)	UCL[1]	LCL[2]
	($\mu g\ kg^{-1}$)	1	2	3				
CHB 26	0.23	77.7	89.5	86.9	85.1	7.3	91.7	77.7
	5.57	86.8	93.8	88.9	89.9	4.00	93.9	85.8
	121	83.9	92.6	85.2	87.2	5.35	92.5	81.9
CHB 50	0.23	76.9	88.7	82.1	82.8	7.16	89.1	76.0
	5.57	83.5	97.5	85.7	88.9	8.47	97.4	80.4
	121	81.7	84.7	83.9	83.4	1.88	85.2	81.7
CHB 62	0.23	66.8	77.3	73.4	72.4	7.30	78.6	66.4
	5.57	74.9	86.2	71.7	77.6	9.82	86.3	69.0
	121	77.6	84.4	78.1	80.2	5.00	84.7	75.7

1 UCL= Upper confidence limit.

2 LCL= Lower confidence limit.

As no certified reference material is available for the analysis of toxaphene in fish tissue an acceptable recovery range is difficult to quantify. With respect to the MATT project (84) inter-comparison studies showed that recoveries of between 70-110% would be acceptable for the purposes of the study, all figures obtained fell within these criteria. These data are reported in AIV.

The accuracy of GC analysis was completed during a QUASIMEME inter-comparison study yielding means of 180, 195 and 174 µg/kg for CHB's 26, 50 and 62 respectively in a standard solution compared to expected values of 181 µg kg^{-1} for each of the congeners. For these determinations GC analysis can therefore be shown to have an accuracy of 99.4, 108 and 96.1% respectively for CHBs 26, 50 and 62.

2.3.2 Precision

Precision can be defined as the measure of the amount of scatter of data points around the mean and can be determined by multiple analysis of a homogeneous sample. It is a measure of the degree of repeatability of an analytical method under normal operation and is normally expressed as the percent relative standard deviation (RSD). Analytical conditions were as for the accuracy determination with corn oil being spiked at a concentration of 0.23 µg kg^{-1} with the exercise being performed on 8 individual determinations of the homogeneous sample.

Table 2.6: Repeatability of analysis for 3 toxaphene congeners charting recovery, range, standard deviation (Stdev), relative standard deviation (RSD%) and upper (UCL) and lower (LCL) 95% confidence intervals (n=8 in all determinations).

CHB	Recovery (%)	Range(%)	Stdev (%)	RSD(%)	UCL(%)	LCL(%)
26	86.7	79.6-95.9	5.94	6.86	90.8	82.5
50	87.7	79.6-97.3	6.41	7.31	92.2	83.3
62	88.3	81.0-98.2	7.19	8.15	93.3	83.3

For each of the analytes tested, the mean falls inside the statistical 95% confidence interval for the method but the range fell outside these limits (see table 2.6). This however is to be expected as the low spiking concentration of 0.221 µg kg^{-1} would be expected to yield a

wider spread of results than that of a higher fortification. RSD values of 6.86, 7.31 and 8.15% were deemed acceptable for these spiking concentrations.

2.3.3 Specificity.

Specificity is the ability to measure accurately and specifically the analyte of interest in the presence of other components that may be expected to be present in the sample matrix (83). It is a measure of the degree of interference from active ingredients, impurities or degradation products amongst others, ensuring that a peak response is due to a single component only i.e. that co-elution does not occur. The chemical nature of toxaphene does not allow specificity to be measured easily with an ECD detector. Matrix effects are sample dependent; therefore compounds that could cause interference in analyte peak identification will change depending on the sample type. To resolve these problems an analytical system whereby the injected sample was split equally between two columns (dual column chromatography) of differing polarity was set up.

Identification of each sample analyte peak required that the relative retention time of the analyte to a specified internal standard be similar to the relative retention time observed with an analytical standard. This would not be deemed sufficient for specificity based on one column alone therefore the same criteria had to be observed on the second differing polarity column also. On both columns the internal standard eluted at a retention time that was clear from all possible interfering peaks ensuring that it could be identified specifically. Once the analyte peak had been specifically identified, the observed concentrations on both columns could then be evaluated and the level of agreement between them quantified.

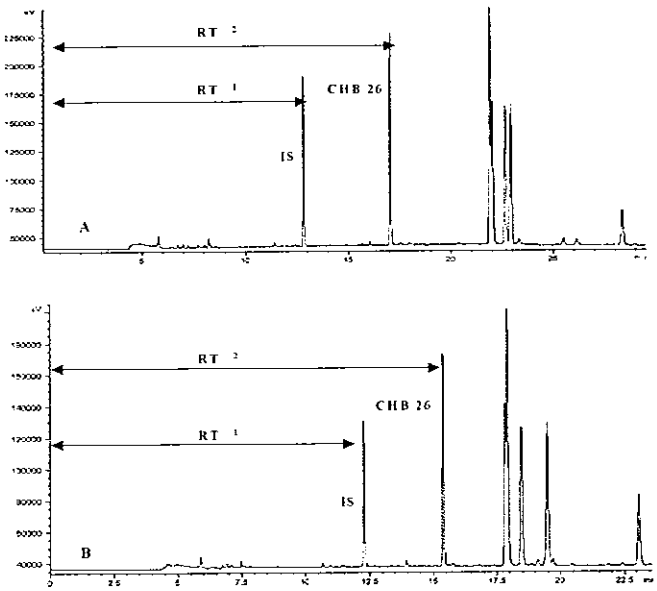

Figure 2.5: Calculation of relative retention times (RT_1/RT_2) for CHB 26 on a CPSIL19 column (A) and on a CPSIL8 column (B) using 4-5 dichloro-chlordene as an internal standard (IS).

Relative retention time (mins) ±1.5% based on calibration standards had to be observed on both columns to ensure positive congener specific identification (see figure 2.5). Once these criteria were met the method was deemed specific. Relative retention times were calculated by dividing the retention time of the internal standard by the retention time recorded for the analyte peak.

2.3.4 Linearity.

Linearity shows the ability of the method to produce results that are directly proportional to analyte concentration within a given range. Linearity is generally reported as the RSD of the variance to the slope of the regression line. Range is the interval between the upper and

lower levels of analyte (inclusive) that has been demonstrated to be determined with precision, accuracy and linearity using the method as written (see table 2.7).

Table 2.7: Linearity statistics for calibration curves corrected for internal standard expressed as a percentage of variance (RSD) of the regression line.

CHB	Points in regression	Range (µg kg^{-1})	Regression slope	Variance	Variance %RSD	R^2
26	8	0.027-132	0.546	0.0019	0.35	0.9999
50	8	0.027-132	0.383	0.0017	0.46	0.9998
62	8	0.027-132	0.196	0.0007	0.37	0.9999

An analysis of variance (ANOVA) (85) test showed that for all three congeners that the regression lines show that a highly significant relationship exists between all of the individual points in the series. The linear range of the method was determined to lie between 0.28 and 132 µg kg^{-1} for each of the CHBs 26, 50 and 62. Regression slopes were found to decrease in order of congener elution.

2.3.5 Limit of detection (LOD).

The LOD can be defined as the lowest concentration of an analyte in a sample that can be detected, but that is not quantified. It is a limit test that specifies whether or not an analyte is above or below a certain value.

In this work the LOD was expressed as the analyte concentration corresponding to a mean sample blank value +3SD. Limits of detection were calculated as being 0.001µg/kg for all samples with the exception being that of mussels which had a limit of detection of 0.002µg kg^{-1}. For all matrices the LOD was deemed to be sufficiently low to enable congener concentrations be calculated at environmentally relevant levels.

2.3.6 The Limit of Quantitation (LOQ).

The LOQ can be defined as the lowest concentration of an analyte in a sample that can be determined with acceptable precision and accuracy under the stated operational conditions of the method. Validation of the LOQ for this method was expressed as the analyte concentration corresponding to a mean sample blank value +10SD.

All LOQs reported in this section are matrix dependent, and values change according to the individual tissue type. LOQs were calculated as being 0.005µg kg^{-1} for all samples with the exception being, that of mussels which had a limit of detection of 0.01µg kg^{-1} for all 3 CHBs. For all matrices the LOQ was sufficiently low to allow accurate sample concentrations be calculated.

2.3.7 QUASIMEME inter-comparison exercises.

Quality Assurance of Information for Marine Environmental Monitoring In Europe (QUASIMEME) is an internationally recognised laboratory performance study scheme set up to allow laboratories assess quality management and quality measurement against laboratories and organisations on an international scale. Individual laboratories use their own analytical methodology and then report results to the QUASIMEME office for the generation of |Z| score statistics related to the performance of their methodology.

Generated |Z| Scores can be assessed as follows;

$\|Z\| \leq 2$	Satisfactory	ca. 95% of values produced by data under control
$\|Z\| < 3$	Questionable	ca. 5% of values produced by data under control
$\|Z\| > 3$	Unsatisfactory	ca. 0.3% of values produced by data under control

Results from all QUASIMEME exercises completed (rounds 10 and 14) during this study are presented below (see tables 2.8 –2.12). During the timescale of this project various matrices were analysed under this scheme supporting method development and validation. Details on assignment of |Z| scores are reported by QUASIMEME (86-87).

Table 2.8: Results and |Z|-Scores for toxaphene congener determination in standard solution QTX002SS (μg kg^{-1}) in Round 10.

Determinand	Assigned Value (μg kg^{-1})	Assigned Error (%)	Result (μg kg^{-1})	|Z|-Score
CHB 32	145.00	6.17	151.07	0.68
CHB 26	116.00	6.22	115.51	-0.07
CHB 50	101.00	6.25	100.51	-0.08
CHB 62	188.00	6.13	201.13	1.14

Table 2.9: Results and |Z|-Scores for toxaphene congener determination in standard solution QTX003SS (μg kg^{-1}) in Round 10.

Determinand	Assigned Value (μg kg^{-1})	Assigned Error (%)	Result (μg kg^{-1})	|Z|-Score
CHB 32	116.00	6.22	126.32	1.43
CHB 26	101.00	6.25	111.30	1.63
CHB 50	145.00	6.17	147.94	0.33
CHB 62	188.00	6.13	212.16	2.09

Table 2.10: Results and |Z|-Scores for toxaphene congener determination in standard solution QTX004SS in Round 14.

Determinand	Assigned Value (μg kg^{-1})	Assigned Error (%)	Result (μg kg^{-1})	|Z|-Score
CHB 26	160.92	6.16	160.74	-0.02
CHB 50	142.40	6.18	143.57	0.13
CHB 62	112.63	6.22	117.47	0.69

Table 2.11: Results and |Z|-Scores for toxaphene congener determination hake liver extract QTX005BT in Round 14. Values in () relate to |Z| scores returned after repeat analysis.

Determinand	Assigned Value ($\mu g\ kg^{-1}$)	Assigned Error (%)	Result ($\mu g\ kg^{-1}$)	|Z|-Score
CHB 26	7.77	15.72	11.99	3.46 (1.67)
CHB 50	28.8	13.37	34.69	1.52
CHB 62	6.93	16.11	10.87	3.53 (1.21)

Table 2.12: Results and |Z|-Scores for toxaphene congener determination pilot whale blubber extract QTX006BT in Round 14.

Determinand	Assigned Value ($\mu g\ kg^{-1}$)	Assigned Error (%)	Result ($\mu g\ kg^{-1}$)	|Z|-Score
CHB 26	84.07	12.80	102.06	1.67
CHB 50	187.98	12.63	178.94	-0.38
CHB 62	45.87	13.05	53.78	1.32

The results for both of the standard solutions in rounds 10 and 14 all fell within the expected |Z| score ranges. Results for all the other matrices were also acceptable with the exception of those in hake (round 14) where CHB 26 and CHB 62 fell outside the acceptable range of values. This was due to interfering peaks eluting close to the CHB peaks of interest due to a shortening of the GC run time. The sample was re-extracted in triplicate and analysed using a longer run time resulting in values falling within the set limits.

Overall the results for each of the QUASIMEME exercises were acceptable for all analyses, however the differences in |Z|-scores for standard solutions and for biota show the difficulty in carrying out these analyses on actual biota. Actual |Z|-scores fitted well within the range of the |Z|-scores from all participants.

2.3.8 Summary discussion on validation of toxaphene methodology.

Results showed that the current state-of-the-art for inter-laboratory performance was not lower than 20% and usually between 20 and 30% and that despite some separation problems, a CPSil8 column is preferred for the analysis of CHBs 26, 50 and 62. Reproducibly of CHB 62 analysis was more difficult to control than that of CHB 26 and 50 and a three step clean-up method is recommended to minimise interferences especially where ECD is employed.

2.4 Sampling strategies for POP analysis.

Sampling procedures were carried out where possible in accordance with the Oslo and Paris commission Joint Assessment and Monitoring Programme (JAMP) guidelines for monitoring contaminants in food (88). These guidelines set out technical details relating to sampling, analysis, quality assurance and reporting of results. The main purposes of monitoring contaminants in food according to JAMP are,

1) to assess the effectiveness of measures taken for the reduction of marine contamination (temporal trend monitoring).
2) to assess the existing level of marine contamination (spatial distribution monitoring). Monitoring contaminant concentration can be used to indicate large-scale regional differences in contamination.
3) to assess harm to living resources and marine life.

Selection of the target population requires that the organism,

1) reflects changes in contaminant concentrations in the surrounding environment;
2) for a given species, have similar bioconcentration factors throughout the maritime area;
3) accumulate the contaminant without being seriously affected by the concentrations encountered in the marine environment;
4) be representative of the study area;
5) be of reasonable size for all analyses and be easy to sample.

2.4.1 Species selection for spatial distribution monitoring.

In the case of this study a wide range of species were selected, enabling contamination levels in most of the commercially viable species to be evaluated. Species properties ranged from those having less pronounced migration properties making them more likely to represent the area of capture, to those of deepwater species living near the seabed, and to species that occur in shallow waters along coasts making them suitable for monitoring inshore waters. Where practicable the number of individuals within a pooled sample was such that minimisation of natural variability factors within the species occurred, including length, weight, age and sex of individuals. Procedures employed are discussed below.

2.4.1.1 Sampling of Fin fish.

The majority of marine finfish and shrimp were collected from a single trawl to minimise sample variability. Where this was not possible catches from two or more trawls from the same and/or adjacent International Commission for the Exploration of the Sea (ICES) (89) rectangles were combined.

2.4.1.2 Sampling of salmon and Trout.

These derived from aquaculture fish farms with sub-sampling being carried out in accordance with the EU directive on Residues in farmed fish (90).

2.4.1.3 Sampling of shellfish.

Shellfish samples were collected from sub-tidal regions or as near to low water spring tide level and at the same depth and exposure to reduce variability in contaminant uptake. Where natural populations were unsuitable or unavailable cultivated species sufficed.

2.4.1.4 Effects of sampling period.

The sampling of fish preferably occurred when the sample was in a stable physiological state, and in any case outside of species spawning period as during this time large variations in lipid concentrations occur within species in all tissue types. This variation in lipid is due in most cases to the transfer of lipid in females to ova, which may also have the net result of contaminant transfer to ova. Sampling of shellfish outside of spawning period has the additional importance that during the spawning period up to 50% of the soft tissue weight of the organism may be lost due to factors such as reduced feeding and metabolic use of stored lipid reserves.

2.4.1.5 Determination of length for individual species.

Length measurements allow an estimation of the age of the species to be established where otoliths or other age determination techniques are not available or cannot be applied. The measurement of length for individual species groupings are described in 2.4.3.

2.4.1.6 Determination of weight for individual species.

Weight measurements can allow age estimations be established where other aging procedures are not possible, however weight measurements of species are dependent on the sampling time and location and as such its use as an aging tool is lessened.

2.4.1.7 Determination of age for individual species.

The determination of the age of fish is species dependent and was carried out where possible as described below.

2.4.1.7.1 Reading of otoliths

Otoliths are small, white bone-like structures found in the head of all fish other than sharks, rays and lampreys. They provide a sense of balance to fish and also aid in hearing. Growth rings (annuli) similar to those on a tree record the age and growth of a fish from the date of hatch to the time of death. Counting the number of rings allows an estimation of the age of the species be determined (91) (see figure 2.6).

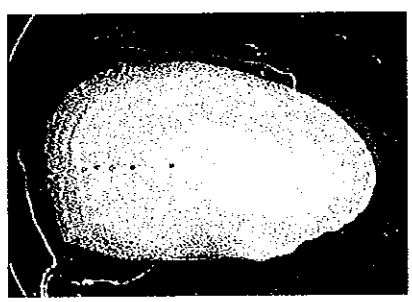

Figure 2.6: Plaice otolith (annual growth rings are indicated by green dots)

2.4.1.7.2 Reading from age-length keys.

These are double frequency tables, with the number of individuals from a trawl of a certain age (as determined by otolith evaluation or otherwise), in the columns and the individual length in the rows (92). Fish aged by this procedure must be taken from the same stock, during the same season and using the same gear as that used to catch fish employed for the construction of the length frequency tables.

2.4.1.7.3 Measurement of the nephrops carapace.

This procedure can only estimate the age of a species (93) and in any case shrimp are not expected to live more than 2 years (see figure 2.12). A female of this age will grow to around 5 cm in carapace length and male of similar age will be a little smaller. Sexing of the species is required to estimate of the age of the sample.

2.4.1.7.4 Measurement of total shell length for shellfish.

As definitive ageing of mussels is not possible the measurement of shell length (see figure 2.14) is a good indicator of the age of a species, however such measurements are location dependent.

2.4.1.7.5 Interpretation of scales from salmonid species.

Growth rings similar to those present in the otoliths of finfish are present on the scales of salmonid species. Interpretation of these rings can allow the age of species to be estimated (94). These procedures do not work accurately for farmed fish due to the fact that distinct rings are not as visible on their scales. This occurs because feeding is more continuous throughout the year on farmed individuals unlike the situation for wild species where feeding slows at certain times of the year e.g. spawning time. This slow down in feeding (and consequently growth) results in more distinct bands forming on the scales. In any event farmed salmon are generally processed at approximately 2 years.

Due to the bioaccumulation potential of toxaphene in the lipid of fish, age determination of a species becomes important as in general toxaphene concentration has been shown to increase as species length or age increases (95).

2.4.1.6 Determination of the sex of individual species.

Where possible the sex of all samples was recorded. Procedures ranged from visual identification in some species to examination of the gonads in others. The determination of the sex of a species becomes important in relation to the bioacculation potential of a

particular sample. During spawning periods female fish can transfer large reserves of lipid and consequently lipid bound toxaphene to their ova.

2.4.2 Preparation of marine biota for toxaphene analysis.

In total 17 species were sampled in addition to one sample containing 20 individual fish resulting in 55 individual samples being analysed. Sampling procedures are further described in 2.4.3. Species sampled are presented below in table 2.13 and sampling locations are graphically illustrated in figure 2.7.

Table 2.13: Summary of species sampled for toxaphene analysis.

Species	No. of samples analysed.
Cod	2
Herring	3
Mackerel	1
Plaice	5
Mussels	4
Sea Trout	2
Redfish	1
Sole	1
Haddock	2
Hake	1
Monkfish	2
Shrimp	2
Skate	3
Whiting	3
Atlantic Salmon	1
Dab	1
Grenadier	1
Individual species (n=20)	
Mackerel	1

Note: The number of pooled individuals in each sample is reported in annex 1.

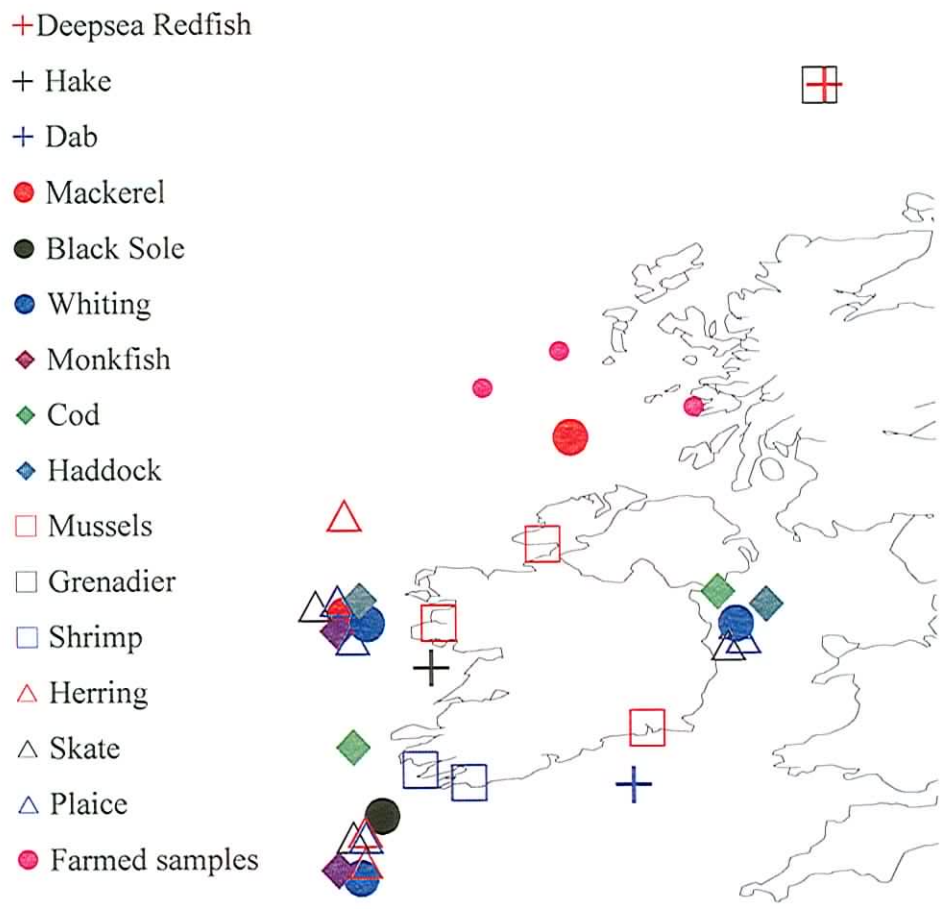

Figure 2.7: Sampling location for each of the species indicated.

2.4.3 Quality assurance of sampling and supporting parameters.

To minimise the risk of cross-contamination or the loss of determinands during sampling, storage, and pre-treatment the following procedures were developed to ensure that like species were treated in a similar fashion. Due to physical differences within species sampling procedures were standardised for like species, procedures are described below. All samples of pooled individuals were homogenised prior to storage and freezing at –18°C.

2.4.3.1 Flatfish sampling

Flatfish species including dab, black sole, monkfish and plaice were sampled as follows:

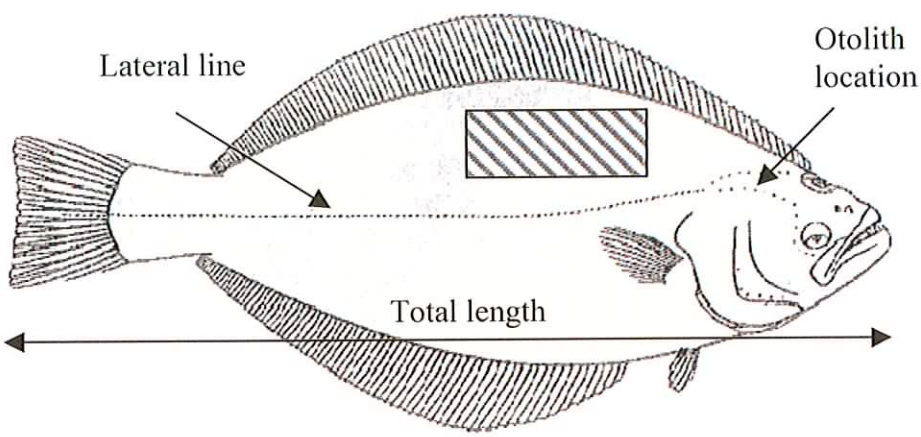

Figure 2.8: Muscle sampling area on flatfish 

Sampling took place above the lipid rich lateral line behind the dorsal fin on all flatfish species. The laterel line has the function of aiding balance in the fish. Due to the lipid rich properties of the line this area was avoided during sampling.

2.4.3.2 Sampling of deepsea species.

Total fish length was measured in deepsea redfish however the tail of roughhead grenadier is brittle therefore anal rather than total length was measured. In all cases the sample was taken from the left side of the fish (see figures 2.9-2.10). In large individuals tissue was taken below the dorsal fin and above the lateral line, on smaller fish the whole left side above the lateral line was sampled. Length and weight measurements were taken, otoliths removed and the sex determined of individual fish in the pooled sample.

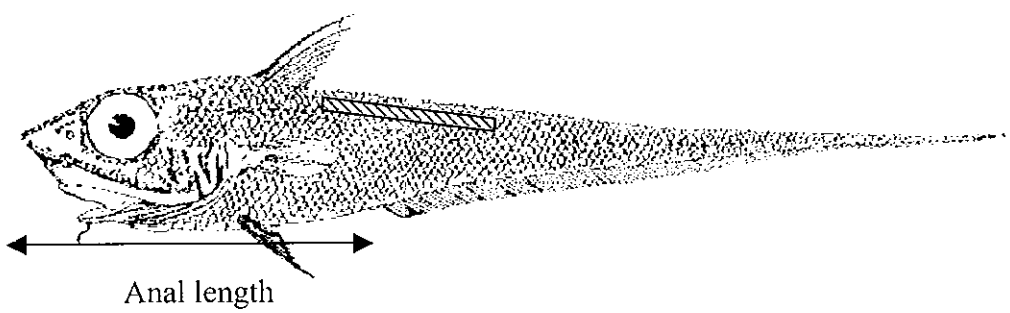

Figure 2.9: Muscle sampling area on roughhead grenadier

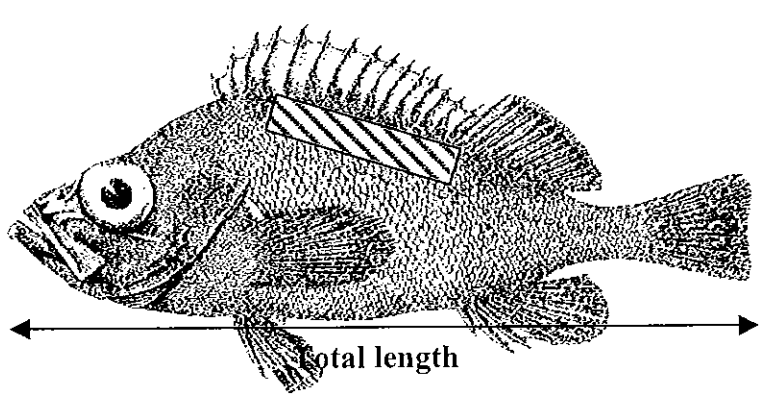

Figure 2.10: Muscle sampling area on deepsea redfish -

2.4.3.3 Sampling of demersal and pelagic species

Sampling of herring, mackerel, hake, whiting, cod and haddock took place in a similar way to that for the deepwater species with the sample being removed from the left-hand side of the fish above the lateral line. Length and sex determinations were made where possible for all individuals within a species (see figure 2.11).

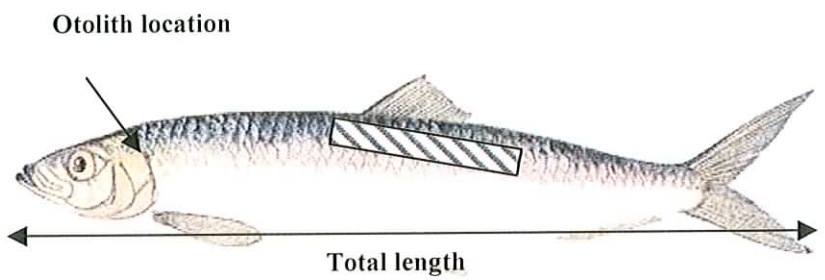

Figure 2.11: Muscle sample area of the demersal and pelagic species

2.4.3.4 Sampling of nephrops species.

Measurement of the carapace length is usually carried out on Nephrops species (incl. Shrimp) (see figure 2.12). This is due to the fact that the tail is made up of interlocking plates that are difficult to fully extend, leading to possible measurement error. The tail portion individual exoskeletons were removed and the soft body tissue pooled.

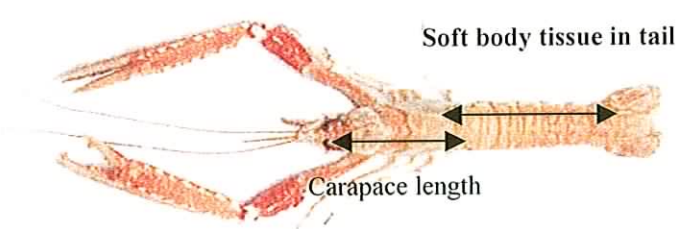

Figure 2.12: Sampling of nephrops species for length and soft body tissue.

2.4.3.5 Sampling of salmonids

All salmon and sea-trout were obtained from aquaculture farms and were sampled as follows. The left side of the fish (see figure 2.13) was removed with a solvent washed knife and placed on solvent washed aluminium foil. A scalpel was then used to remove a representative sample from the fillet. Approximately 10g was removed from each of 10 individual fish, the samples were pooled and homogenised with a hand blender. The final sample was stored in a similar fashion to all of the other samples prior to analysis.

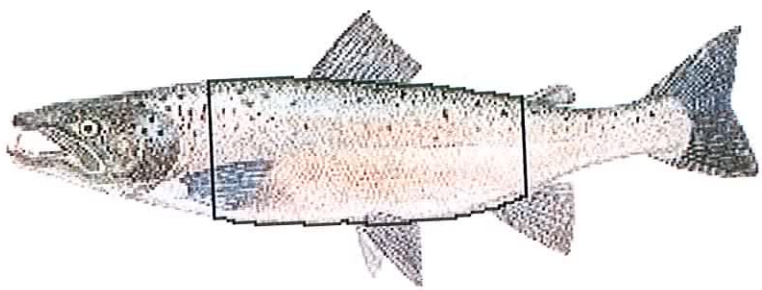

Figure 2.13: Sampling of Salmon and Seatrout.

2.4.3.6 Sampling of shellfish.

All mussel samples were derived from cultivated stocks. Mussels were depurated overnight in approximately 5L of seawater from the sampling site. A sample of soft body tissue from 50 individual mussels between 4-6 cm in total length was then pooled for analysis.

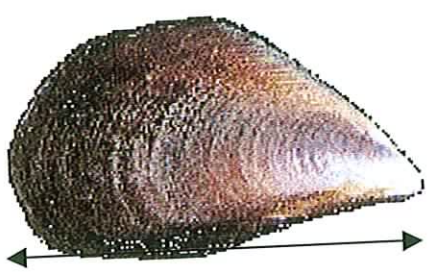

Figure 2.14: Sampling of mussels.

2.4.3.7 Quality assurance of lipid determination in marine biota.

Both the solvent extractable lipid and total lipid according to Smedes (96) were determined for all pooled samples. With the aid of these determinations normalisation of results over all species was carried out.

Suitable matrix matched QUASIMEME inter-laboratory proficiency study samples were analysed on a batch basis to ensure quality control aspects to lipid determination were maintained.

2.4.3.8 Dry weight determination.

These determinations were carried out by air-drying homogenised sub-samples of the material to be analysed to a constant weight at 105°C. Such information can be of benefit for normalisation processes during food safety based assessments. The

$$\left(\frac{(\text{Weight of sample wet - weight of sample dry})}{\text{Weight of sample wet}}\right) X\,100$$

percentage moisture in the sample was calculated as follows.

As per lipid determination, suitable matrix matched QUASIMEME inter-laboratory proficiency study samples were analysed on a batch basis to ensure quality control aspects were maintained.

2.5 Results and discussion.

In order to assess the baseline survey dataset the procedures below were carried out allowing all aspects of the information collected to be interpreted in a stepwise manner.

- Variation study on concentration levels on all data.
- Group comparisons.
- Analysis of individual species.
- Geographical distribution.
- Investigation of within sample congener profiles.
- Investigation of natural variability within individuals of the same species.
- The effect of sample sex on concentration levels.
- Investigation of the effect of sample length/ age and lipid on concentration levels in an individual species.
- Congener profiling techniques
- Information on the toxicological risk to the consumer of fishery products from Irish waters.

2.5.1 Concentration level variation in all species.

The purpose of this section was to investigate the range in concentration of the sum of the 3 CHBs on a lipid weight basis for all samples. As there is a large variation in lipid content between different species it is necessary to normalise all data by converting wet weight concentrations to a lipid basis to enable comparison of all concentration data between species/groups of species and to other literature data.

It can be observed from Table 2.14 that in general lipid normalised concentration ranges do not differ greatly from each other for the majority of species. However individual farmed sea trout, herring, cod, farmed salmon, plaice and farmed trout having concentration levels of 48.8, 52.2, 56.9, 101, 127 and 156 μg kg^{-1} (lipid weight) respectively are much higher than the general data spread.

Table 2.14: Toxaphene congener concentration ranges in biota (μg kg^{-1} lipid weight).

Species	n=	CHB 26	CHB 50	CHB 62	Σ3CHBs
Whiting	3	1.60-2.55	1.96-4.90	1.13-2.22	4.97-9.67
Shrimp	2	1.03-2.89	1.48-5.73	1.24-2.48	4.99-9.87
Mussels	4	0.55-1.94	0.90-1.80	0.10-0.60	1.56-4.34
Plaice	5	0.09-29.6	0.14-72.8	0.14-24.4	0.37-127
Herring	3	2.10-15.4	3.61-22.1	0.80-11.2	6.51-48.8
Farmed trout	2	10.7-47.9	20.1-57.3	21.4-50.2	52.2-156
Farmed salmon	1	28.0	40.5	32.8	101
Monkfish	1	0.85	2.22	1.92	4.99
Cod	2	8.68-18.1	1.92-18.7	3.33-20	13.9-56.9
Mackerel	22	1.90-10.4	1.91-16.2	0.67-10.9	5.47-36.3
Sole	1	0.15	2.19	1.52	3.86
Hake	1	3.90	0.63	1.70	6.22
Deepsea redfish	1	5.17	11.5	3.61	20.3
Skate	3	1.79-9.66	1.97-7.33	3.70-10.3	7.46-27.3
Haddock	2	0.75-2.84	0.81-3.12	1.45-2.75	3.01-8.71
Grenadier	1	6.98	10.5	7.96	25.5
Dab	1	2.65	7.81	3.72	14.2

From Table 2.14 it can be observed that a single sample of plaice has a concentration of 127μg kg^{-1} for the sum of 3 CHBs, which is, relatively elevated compared to other species. It can also be observed that in general the concentration range in similar low lipid species is much lower than observed for this single sample. In addition single samples of cod and herring have also concentration levels that are slightly higher than expected for their species. These results can partly be explained by the fact that the extractable lipid content for plaice, cod and herring are all slightly lower than may be

expected for their species. This may be a result of lower than expected recovery of lipid from the sample due to extraction errors.

It is also possible that the plaice sample was taken after spawning has occurred and the lipid content in the tissue would be expected to be lower than normal due to the transfer of some lipid, and consequently toxaphene to ova. This in turn has a multiplier effect on the calculation of the concentration on a lipid basis. Concentration levels on a lipid basis obtained for the cod and the plaice individuals are much higher than would be expected for lean fish and would require further investigation with a larger sample set.

A total of 51% of samples were determined to have a concentration range less than 10 $\mu g\ kg^{-1}$, 73% of samples falling below the mean value with a total of 9% recording concentrations in excess of 100 $\mu g\ kg^{-1}$ lipid weight.

Concentration levels observed in farmed species are also much higher that those from wild samples. This may partly be explained by the fact that farmed fish are fed lipid rich food pellets for sustained periods during their growth, where the oil for these pellets would normally be derived from the livers of fish previously exposed to toxaphene residues. This bioaccumulative effect would therefore be expected to give rise to higher organochlorine levels in these species. Elevated POP levels in marine species are discussed in chapter 6.

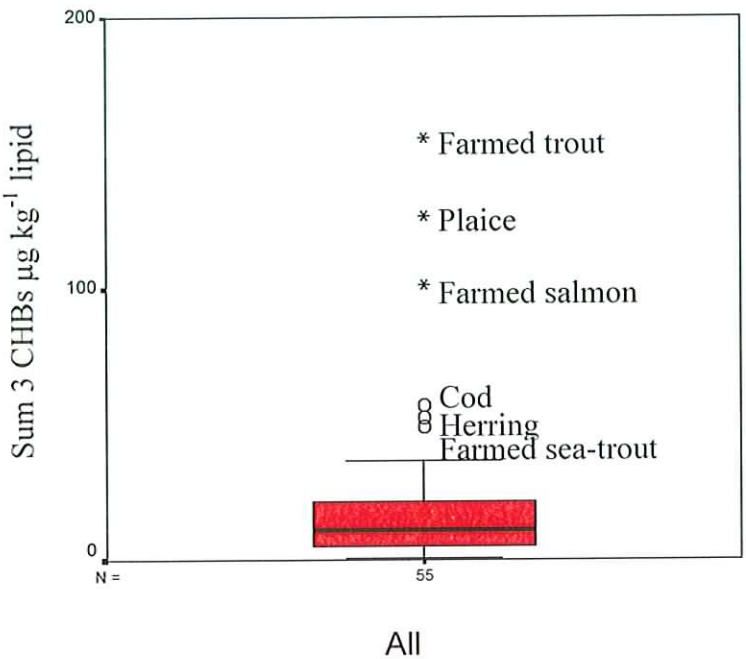

Figure 2.15: Boxplot of the sum 3 congeners CHBs 26, 50 and 62 (µg kg^{-1} lipid weight).

The production of a boxplot (see figure 2.15) charts the differences between these 6 samples and the general body of data. Boxplots display and compare the distribution of a variable hiding individual sample value details to show more summary information.

A boxplot is comprised of several parts with the box depicting the central half of the data between the 25th and 75th percentiles with the line across the box displaying the median value. Whiskers extending from the top and the bottom of the box to depict the extent of the main body of the data. Outlier values are plotted with an "o", extreme outliers data values are plotted with an "*". Outliers are values greater than 1.5 times the distance between the 25th and 75th quartiles. Extreme outliers are those that have a value of greater than 3 times this distance.

Outliers presented in figure 2.15 correspond to the six samples previously referred to and with the exception of the farmed species a larger data-set is required to statistically assess the relevance of levels in the plaice, cod and herring samples.

In order to assess the data-set on a species basis, further grouping of samples is required. These groupings are discussed in section 2.5.2.

2.5.2 Group comparisons.

All samples were placed into groups on the basis of like species and/or like lipid content. These groupings were determined as follows;

1) Deepsea species,
2) Whitefish (low lipid),
3) Shellfish and nephrop species,
4) Mackerel and herring (medium/high lipid),
5) Farmed species.

A summary of the concentration ranges within these groupings is presented in Table 2.15.

Statistical evaluation was carried out on the groups to determine whether concentration levels within a particular group were significantly different to those in any of the other groups. Prior to statistical analysis the production of a box-plot (see figure 2.16) allowed the spread of data within the groups be visually assessed.

Table 2.15: Concentration range of toxaphene congeners in biota (μg kg^{-1} lipid weight).

Deepsea species	CHB 26	CHB 50	CHB 62	Σ3CHBs
Redfish	5.17	11.5	3.61	20.3
Grenadier	6.98	10.5	7.86	25.5

Low lipid species	CHB 26	CHB 50	CHB 62	Σ3CHBs
Whiting	1.60-2.55	1.96-4.90	1.13-2.22	4.97-9.67
Haddock	0.75-2.84	0.81-3.12	1.45-2.75	3.01-8.71
Skate	1.79-9.66	1.97-7.33	3.70-10.3	7.46-27.3
Plaice	0.09-29.6	0.14-72.8	0.14-24.4	0.37-127
Monkfish	0.85	2.22	1.92	4.99
Cod	8.68-18.1	1.92-18.7	3.33-20	13.9-56.9
Sole	0.15	2.19	1.52	3.86
Hake	3.90	0.63	1.70	6.22
Dab	2.65	7.81	3.72	14.2

Shellfish species	CHB 26	CHB 50	CHB 62	Σ3CHBs
Shrimp	1.03-2.89	1.48-5.73	1.24-2.48	4.99-9.87
Mussels	0.55-1.94	0.90-1.80	0.10-0.60	1.56-4.34

Medium. lipid Species	CHB 26	CHB 50	CHB 62	Σ3CHBs
Herring	2.10-15.4	3.61-22.1	0.80-11.2	6.51-48.8
Mackerel	1.90-10.4	1.91-16.22	0.67-10.9	5.47-36.3

Farmed species	CHB 26	CHB 50	CHB 62	Σ3CHBs
Farmed trout	10.7-47.9	20.1-57.3	21.4-50.2	52.2-156
Farmed salmon	28.0	40.5	32.8	101

To date one of the most comprehensive studies of toxaphene levels in biota is that reported by Alder (97). Levels of the 3 indicator CHBs in herring from the Irish coast reported by Alder ranged 87-110 μg kg^{-1} (lipid weight), levels reported in this current work for three samples of herring were 6.5, 15 and 49 μg kg^{-1} (lipid weight). Concentrations of toxaphene in Irish herring are also much lower than those reported from Baltic regions where values have ranged from 132-344 μg kg^{-1} lipid weight. Toxaphene concentrations in North Sea herring as reported in the MATT project ranged 10-69 μg kg^{-1} lipid weight (98).

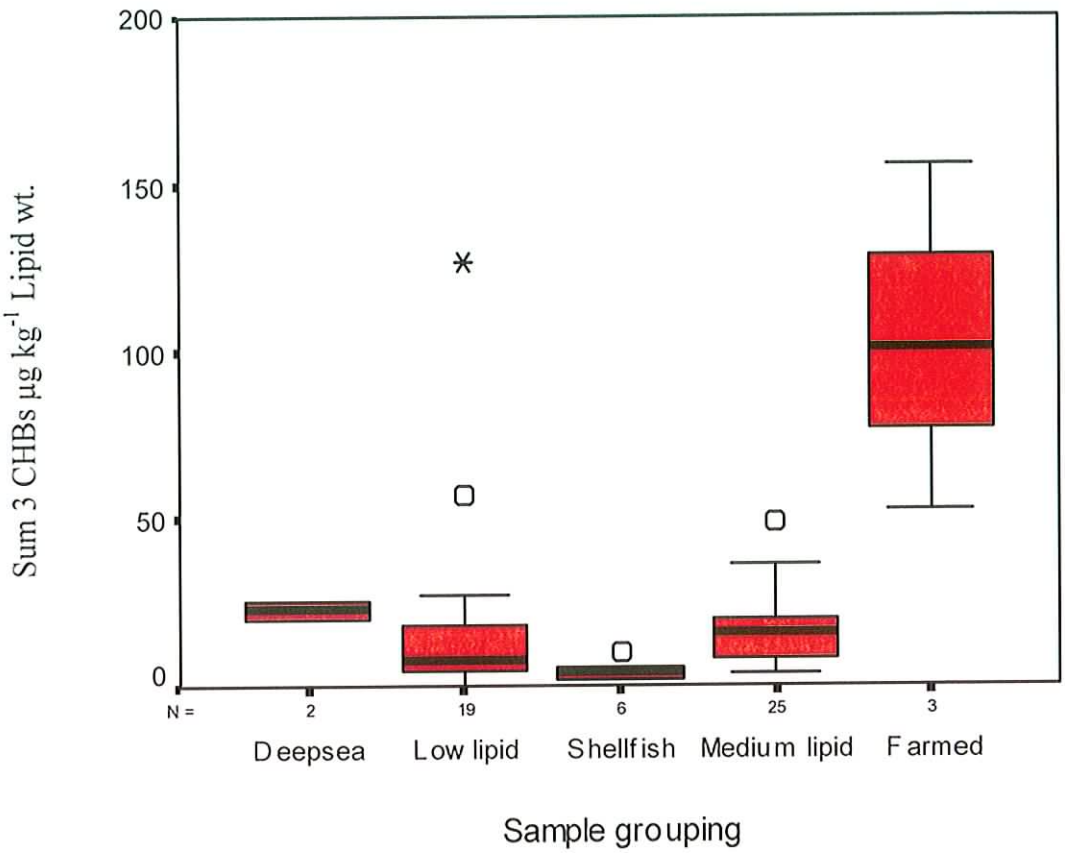

Figure 2.16: Boxplot of grouped samples for the sum 3 CHBs (µg kg^{-1} lipid weight).

Toxaphene levels in mackerel from Irish waters in this study ranged from 5.50-36 µg kg^{-1} (lipid weight), which are lower than has been reported by Alder (97) in mackerel from west of the Shetland Islands (48 and 63 µg kg^{-1} lipid weight). However levels reported from the southern North Sea (German Bay) are comparable (15-19 µg kg^{-1} lipid weight) to Irish data. Levels reported in southern North Sea mackerel the MATT project (98) of 23 and 43 µg kg^{-1} (lipid weight) are also comparable to Irish data. The stock of mackerel to the west of the British Isles feed from late summer to the end of winter in the northern North Sea. If the level of toxaphene in this area is elevated then

mackerel from this source could be expected to contain higher levels of toxaphene than other less transient species caught in Irish waters.

The concentration of the 3 CHBs reported in mussels in the MATT report (98) showed levels of 3-4 μg kg^{-1} (lipid weight) in Baltic regions to 2-3 μg kg^{-1} (lipid weight) in the North Sea in comparison to 1.56-4.34 μg kg^{-1} (lipid weight) reported in Irish samples. Levels in shrimp from the North Sea reported in the MATT project are lower that those in the Irish waters (1.93-3.27 and 4.99-9.87 μg kg^{-1} (lipid weight)) respectively. Insufficent sample numbers are present to assess whether these differences are statistically significant.

Alder (97) additionally reported concentrations in the range from not detected (n.d.) in plaice from the North Sea, to 367μg kg^{-1} lipid weight for saithe from Icelandic waters. Levels in Irish whitefish are generally low with the exception of a single sample of plaice with a concentration of 127 μg kg^{-1} (lipid weight). Levels in cod from Irish waters are reported as 13.9 and 56.9 μg kg^{-1} (lipid weight) and are within the range of data reported in the MATT project of 6-76 μg kg^{-1} (lipid weight) from Baltic and Norwegian waters respectively. Results from Irish whiting ranging 4.97-9.67 μg kg^{-1} (lipid weight) (n=3) are in agreement with values of 9.9 and 11μg kg^{-1} (lipid weight) reported from the Baltic and the North Sea respectively.

Levels in Irish farmed species reported in this study are 56 and 156 μg kg^{-1} (lipid weight) in trout and 101μg kg^{-1} (lipid weight) in salmon. These compare to concentrations of 72-102μg/kg (lipid weight) from Norwegian aquaculture sites as reported in MATT (98). Additionally Alder (97) reported that in general toxaphene

levels are higher in fish with higher lipid content, this is consistent with work carried out for this study.

2.5.2.1 Statistical evaluation of data

Further statistical evaluation of the data was carried out by parametric analysis, as this form of analyses of analytical data is generally regarded as being more powerful in the interpretation of nominal data than corresponding non-parametric tests. In this study a comparison of means and variances between species/lipid groupings was examined. A prerequisite for the use of parametric tests is that the data is normally distributed. All original sum 3 congener data was checked for its fit to the normal distribution (see table 2.16).

Table 2.16: Summary statistics of the sum 3 CHBs for the complete data-set (μg kg^{-1} lipid weight).

	Sum 3 CHBs	% RSD	% Data within ± 1SD
Mean	20.6	142	90.91
Standard deviation (STDEV)	29.3		
Variance	860		
Mean + 1 SD	49.9		
Mean - 1 SD	-8.67		

Although >90% of the data falls within ± 1SD the data set tends to have a higher frequency at lower concentration levels and therefore it was found to have a non-normal distribution. A large variance was also observed for all the data.

2.5.2.1.1 Transformation of contaminant data.

Transformation is the process whereby data that may be "skewed" or not normally distributed can be "normalised" to allow parametric testing. This transformation process

can bring about the stabilisation of the variances within data where groups or populations may have large means. As a result corresponding large differences in variances would also be expected. Such logarithmic transformations of organochlorine concentration data have previously been reported by Stronkhorst (99).

Transformation can be carried out in a number of ways including logarithmic or square root transformations. In the case of these data set all data was log (sum 3 CHBs+1) transformed, ensuring that stabilisation of variances was achieved and no values were returned having a negative value, allowing further parametric testing in the form of an analysis of variance (ANOVA) test to be carried out as reported by Fowler (100).

Table 2.17: Summary statistics of the Log (sum3 CHBs+1) transformed data-set.

	Sum 3 CHBs	% RSD	% Data within ± 1SD
Mean	2.04	23.7	72.7
STDEV	0.48		
Variance	0.23		
Mean + 1 SD	2.53		
Mean - 1 SD	1.56		

2.5.2.1.2 Analysis of variance testing.

Analysis of variance (ANOVA) testing is a technique that can be employed to allow the comparison of the means of more than two samples as long as the within group variances are similar. As the number of groupings increase with some statistical tests there is a good chance that at least one false conclusion will be drawn, thus the risk of committing such a "Type 1" error i.e. rejecting H_o when it should not be rejected increases the chance of making a "Type 2" error i.e. not rejecting a value that should be omitted. The use of an ANOVA can overcome such data issues by allowing comparisons to be made between any number of sample means in a single test.

Used in this way to compare the means of several samples this process is described as a one-way ANOVA. As a result generation of ANOVA statistics allow for the comparison of all of the species groupings to be completed.

Before proceeding with an ANOVA it is necessary to check that sample variances are similar to each other. A homogeneity of variance test according to Fowler (100) was carried out on the samples by dividing the highest variance within the groupings by the lowest and comparing to the F_{max} tables.

2.5.2.1.2 Homogeneity of variance testing

The F_{max} test is carried out by computing the variances of Log (sum 3 CHBs+1) from all groups and dividing the highest variance by the lowest. It is assumed that if this difference between these two groupings falls within set criteria then it was not possible for the variance of the other groups to be significantly different from each other.

The variance of each of the groups was computed and an F_{max} of was 64.08 recorded. As the F_{max} of 64.08 is less than the $F_{critical}$ of 142 we conclude that the variances are homogeneous and proceed with the ANOVA. Summary statistics of the F_{max} test are presented in table 2.18.

Once the homogeneity of variance was established using the F_{max} test, ANOVA and post-hoc statistics were completed on transformed data to check for statistically relevant differences within the groups.

Table 2.18: Summary statistics of F_{max} test for all transformed sample data.

	Sample grouping				
	Deepsea	**Low lipid**	**Shellfish**	**Med. lipid**	**Farmed**
No. samples	2	19	6	25	3
Mean	2.36	1.92	1.55	2.13	2.97
STDEV	0.07	0.55	0.29	0.29	0.24
Variance	0.005	0.31	0.09	0.08	0.06
		F_{max}	64.08	$F_{critical}$	142

Completion of the ANOVA results in the generation of a F_{ratio} and associated probability statistics. The F_{ratio} is a measure of the mean square between groups and the mean square within groups. As the F_{ratio} is greater than the $F_{critical}$ we reject the null hypothesis and conclude that the variation in the mean mass of the samples was significantly different. Summary statistics are presented in table 2.19.

2.5.2.1.3 Least significant difference post-hoc testing

In order to quantify these differences a post-hoc least significant difference (LSD) test was carried out. This test compared each pair of regions delivering statistics on the differences between them (see tables 2.20-2.21).

Greatest differences were observed between groups including farmed finfish samples. The largest of these differences occurring between farmed species and shellfish, followed by farmed species and low lipid fish and thirdly between farmed samples and medium lipid fish, these differences being significant at $P < 0.001$, $P < 0.001$ and $P < 0.01$ respectively.

Table 2.19: Summary statistics for ANOVA on transformed and grouped data.

Source of Variation	Sum Square	Degrees Freedom	Mean Square	F	Probability	F critical
Between Groups	4.70	4	1.18	7.29	0.0001	2.56
Within Groups	8.06	50	0.16			
Total	12.7	54				

Table 2.20: LSD post-hoc statistics on transformed/grouped data.

Grouping	Difference	Std. error	Probability	Significance
5-3	1.42	0.28	$1 X10^{-5}$	<0.001
5-2	1.05	0.25	$1 X10^{-4}$	<0.001
5-4	0.85	0.25	0.001	<0.01
3-1	0.81	0.33	0.017	<0.05
5-1	0.61	0.37	0.099	No sig. diff.
4-3	0.58	0.18	0.003	<0.01
2-1	0.44	0.30	0.146	No sig. diff.
3-2	0.37	0.19	0.053	No sig. diff.
4-1	0.23	0.29	0.433	No sig. diff.
4-2	0.21	0.12	0.096	No sig. diff.

Groupings 1= deepsea, 2= low lipid, 3= shellfish, 4= medium lipid and 5= farmed species.

Significant differences were also determined between shellfish and deep-sea species ($P < 0.05$) and medium lipid and shellfish ($P < 0.01$). No significant difference was observed between deep-sea and farmed species, this was probably due to the fact that the mean concentration in the deep-sea species was the second highest of all groupings after farmed fish. Additionally, the reported standard error (0.366) was the highest between all pairings making any statistical assessment between them more difficult to quantify. The small sample sizes for both groupings make a significant difference between the groups harder to quantify.

No statistical difference was observed between low lipid and medium lipid species this being backed up by the fact that the smallest difference between group means was observed between these samples in addition to them having the smallest standard error.

2.5.3 Analysis of individual species.

As significant differences were observed between the different groups, further statistical analysis on an individual species basis was carried out where sufficient sample datasets containing data from at least three pooled samples were available. Data was log (Sum 3 CHBs +1) transformed, the variance of groups was computed and an F_{max} of 31.7 was recorded. As the F_{max} is less than the $F_{critical}$ of 50.7 it was concluded that the variances were homogeneous and an ANOVA was completed (see table 2.21).

Table 2.21: Summary statistics of transformed individual species groupings.

	Sample grouping				
	Plaice	**Mussels**	**Herring**	**Whiting**	**Skate**
N=	5	4	3	3	3
Mean	1.81	1.40	2.23	1.79	2.21
STDEV	0.95	0.20	0.43	0.17	0.29
Variance	0.91	0.04	0.19	0.03	0.09
		F_{max}	31.7	$F_{critical}$	50.7

The production of a boxplot (see figure 2.17) allows the spread of the dataset to be visualised after completion of the ANOVA.

Table 2.22: Summary statistics for ANOVA on transformed and grouped data.

Source of Variation	**Sum Square**	**Degrees Freedom**	**Mean Square**	**F value**	**Probability**	**$F_{crit.}$**
Between Groups	1.65	4	0.41	1.22	0.35	3.18
Within Groups	4.40	13	0.34			
Total	6.04	17				

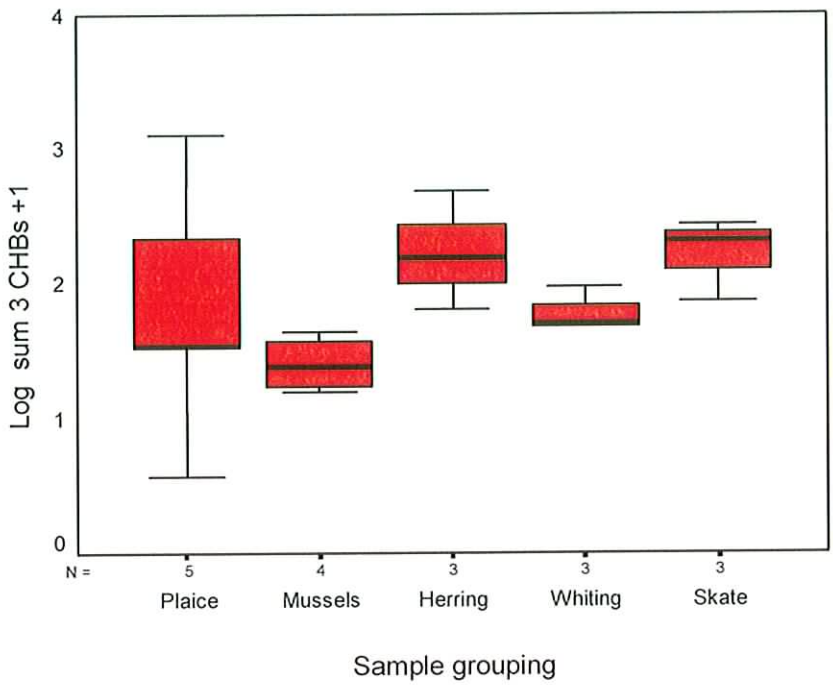

Figure 2.17: Boxplot of transformed individual species data.

As the F_{ratio} is less than the $F_{critical}$ (see table 2.22) no significant difference was shown to exist between these species groupings.

2.5.4 Investigation of within sample congener profiles

The percentage contribution of each of the individual CHBs 26, 50 and 62 to the overall sum of the three was investigated. This process was carried out to investigate whether a distinctive pattern emerged within a grouping or within individual species. As in the case of the geographical distribution study all farmed samples were omitted.

From the boxplot in Figure 2.18 it can be deduced that the extreme outlier "*" for CHB 26 refers to a hake sample whereas outliers "o" correspond to samples of black sole, and two mackerel samples. Outliers for CHB 50 correspond to a black sole and hake sample whereas the outlier for CHB 62 corresponds to a cod sample.

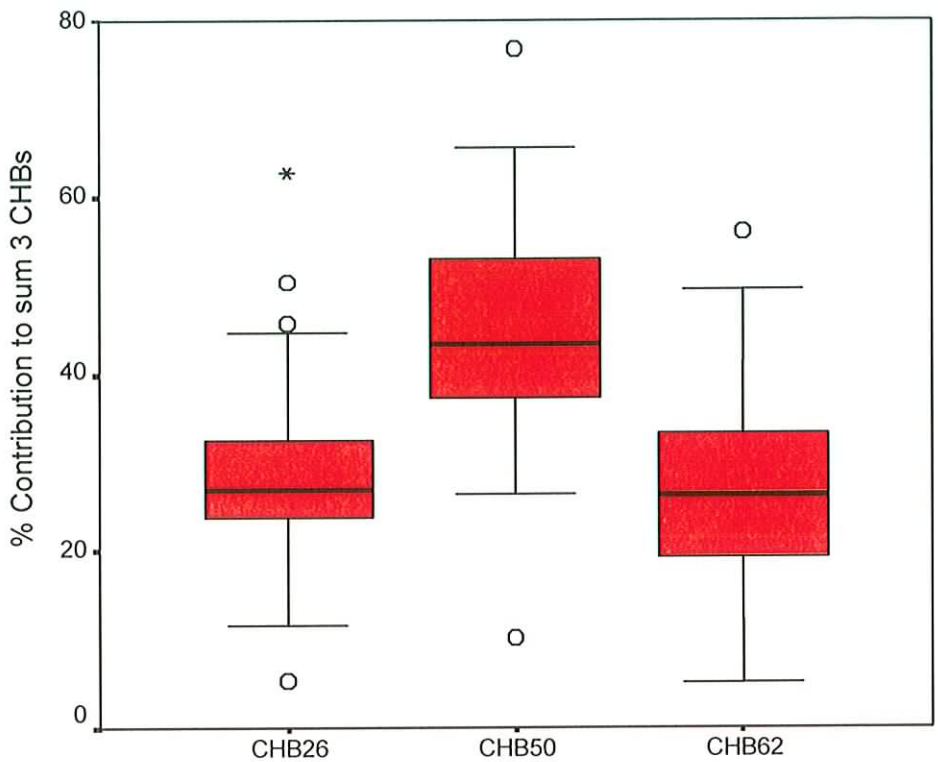

Figure 2.18: Boxplot of the percentage contribution of the 3 congeners to the sum of CHBs.

As has occurred in black sole and hake where one congener has a value that is outside the expected range it will follow that one of the other congeners will also be disproportionately high or low. These outliers may not necessarily be due to analytical problems but may represent an actual distribution pattern in that particular sample.

It is however unlikely in the case of black sole that CHB 26 would only account for 5.3% of the overall sum of 3 CHBs and that CHB 50, the most abundant CHB in most samples would account for only 10.1% of the total in hake. As only one of each of these species was analysed it cannot be determined whether this is a true reflection of the

profile within the species or whether this is due to analytical difficulties. Summary statistics on the % contribution of each of the congeners to the total are presented in table 2.23.

Table 2.23: Summary of the contribution (%) of each congener to the sum 3 CHBs.

							Percentile	
CHB	Mean	STDEV	%RSD	Min	Max	Median	25th %	75th %
26	28.5	8.98	31.5	5.34	62.7	26.9	23.8	32.4
50	44.3	11.7	26.4	10.1	76.8	43.3	37.5	53.0
62	27.3	11.0	40.5	5.15	56.1	26.2	19.3	33.3

Data from table 2.23 above shows that CHB 50 was the most abundant congener, while roughly equal percentages of CHBs 26 and 62 were found to be present with overall contributions of 28.5, 44.3 and 27.3% for CHBs 26, 50 and 62 respectively similar to those of previous literature reports (101). RSDs in the range 31.5 to 40.5% for CHBs 26 and 62 respectively were observed, primarily as a result of divergence expected due to differing rates of metabolism or excretion of individual congeners within individual species in addition to inherent difficulties associated with analytical methodology.

2.5.5 Variability within individuals of the same species.

In order to estimate the level of natural variability within individuals of the same species a total of 20 individual mackerel samples from a single haul were each analysed and statistically evaluated. Summary results for length, percentage lipid and the sum 3 CHBs in 20 mackerel individuals are presented in table 2.24.

A single haul of fish will not generally be comprised of individuals of the same size, sex and age therefore minimisation of sampling variance is difficult to control in the field. The recorded RSD of 14.3% for the length of individual fish show that most were of

similar size. However RSDs of 51.1 and 63.2% for the sum 3 CHBs and the percentage lipid respectively show that these variables cannot be minimised even where care is taken to sample individuals of similar length.

Table 2.24: Summary statistics for length (mm), lipid (%) and the sum 3 CHBs (lipid weight) in individual mackerel (n=20).

	Mean	Median	Stdev	RSD%	Range	Interquartile range
Σ3CHBs	16.6	16.5	8.46	51.1	5.69-36.3	9.03-20.2
Length	255	250	36.5	14.3	210 – 310	219- 290
Lipid	9.5	7.46	6.0	63.2	2.2- 22.5	4.60 - 15.3

A wide range of concentrations was observed, 55% of which were less than the mean of 16.6 μg kg^{-1} lipid weight. A total of 75% of samples fell within the concentration range of 5-20 μg kg^{-1} lipid weight. The spread of this concentration data is therefore probably as low as can be expected form a sample composed of mixed sex and variable sized individuals.

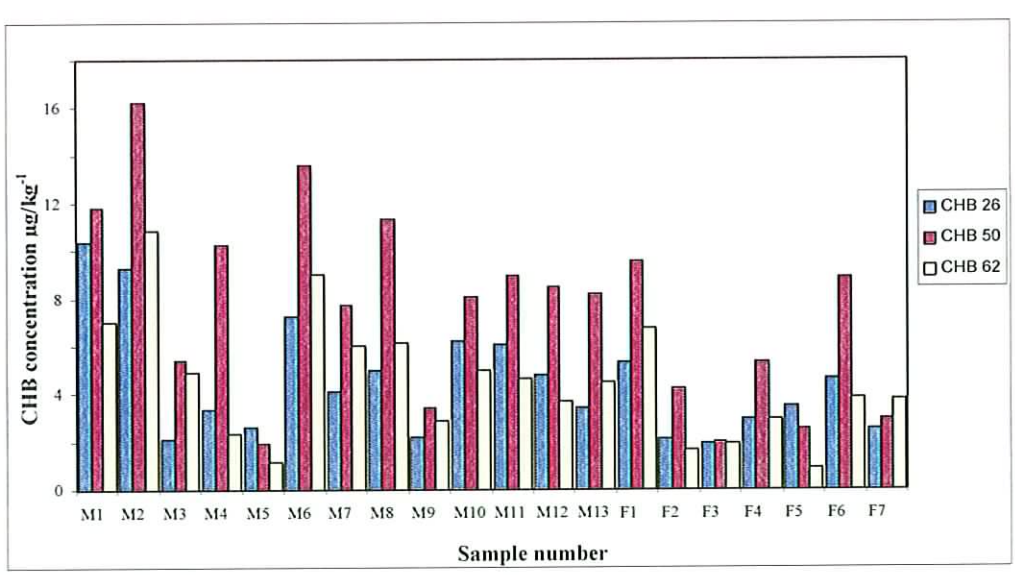

Figure 2.19: Concentration range in individual mackerel samples (μg kg^{-1} lipid weight).

M1-13 and F1-7 represent 13 and 7 individual males and females respectively.
In almost all cases CHB 50 is the predominant congener, which follows the trend observed from the overall data set. In some cases CHB 26 or CHB 62 are predominant but this occurs only where the sample concentration levels are low with respect to the others. Figure 2.19 presents the range of concentration data recorded among the 20 mackerel individuals.

2.5.5.1 Congener profiling within individual mackerel samples

Due to the variation in concentration levels described in 2.3.5 above, the possibility of variability in the percentage contribution of each congener to the sum 3 CHBs was also investigated. Figure 2.20 presents the relative contribution of individual congeners to the sum 3 CHB burden. Such congener profiling data can often provide information into possible sources of contamination and also, to an extent, on the physiological response mechanisms to contaminant burden within a species. The utilisation of such congener profiling techniques is further discussed in chapter 6.

Summary statistics are presented in tables 2.25 and 2.26 for the percentage contribution of each congener to the sum CHBs in both male and female samples respectively. The RSD of the contribution of each congener to the sum CHBs varies to a much lesser extent than the RSD of the observed concentrations in the samples, consequently while concentration levels of toxaphene may vary to a large extent, the actual chromatographic profile within the samples can often remain very similar. The profile is similar in both male and female samples, but the %RSD for CHB 62 is slightly greater in female fish.

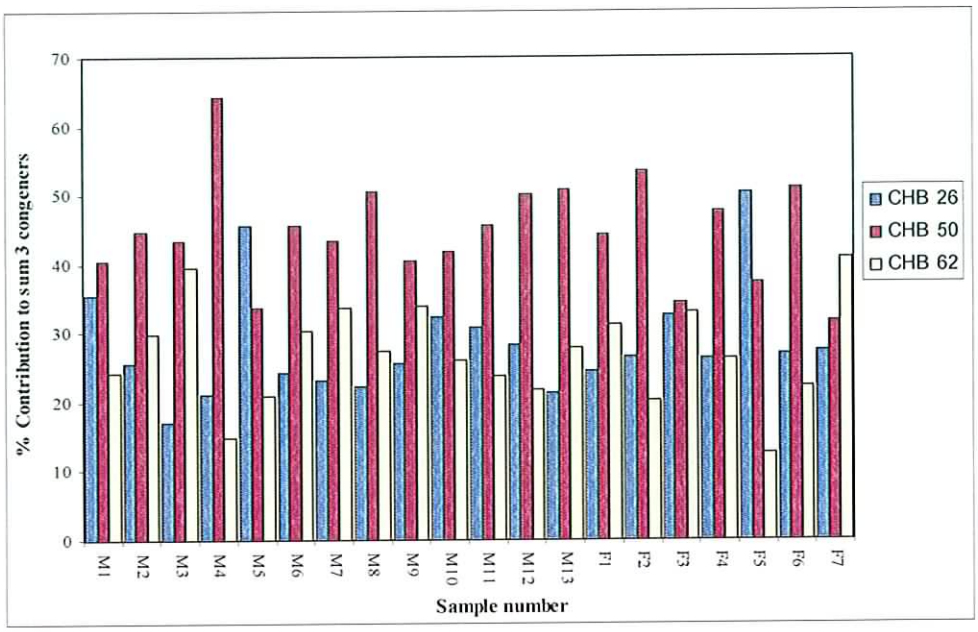

Figure 2.20: Percentage contribution of each CHB to the total 3 CHBs .

M1-13 represents all male data and F1-7 represents female data.

Table 2.25: Percentage contribution of each CHB to the sum CHBs in male mackerel.

	Mean	Stdev	%RSD	Median	Min	Max
CHB 26	27.1	7.51	27.7	25.5	17.1	45.6
CHB 50	45.7	7.31	16.0	44.6	33.6	64.2
CHB 62	27.2	6.48	23.9	27.4	14.7	39.5

Table 2.26: Percentage contribution of each CHB to the sum CHBs in female mackerel.

	Mean	Stdev	%RSD	Median	Min	Max
CHB 26	30.6	9.03	29.5	26.9	24.5	50.3
CHB 50	42.8	8.47	19.8	44.3	31.6	53.4
CHB 62	26.6	9.35	35.2	26.3	12.6	40.9

Overall the congener profile was similar between individual mackerel. Differences may be explained by the inherent variability that exists (even where targeted sampling is completed) within a sampling population and on the fact that it is not possible to ensure that individuals are derived from a single stock/source, such mixed stock fish may have

been exposed to differeng degrees of toxaphene contamination. It is also possible that these individuals (or stocks) may show different physiological responses to the pesticide and/or they may exhibit additive or synergistic responses to a cocktail of different pollutants. This could result in different amounts of each CHB being present in individuals in the pooled sample. Such variations have previously been reported to occur in marine mammals (102), however fish and their lower trophic level prey generally only show limited ability for preferential metabolic activity, therefore analytical issues are a more likely source of observed variation.

2.5.6 The influence of sample sex on toxaphene levels.

It can be visually observed from figure 2.21 that concentrations in male samples seem to be more elevated than those in females and in order to statistically investigate this an ANOVA was carried out on the sample data-set. As with all previous ANOVA calculations, data were log (sum 3+1) transformed.

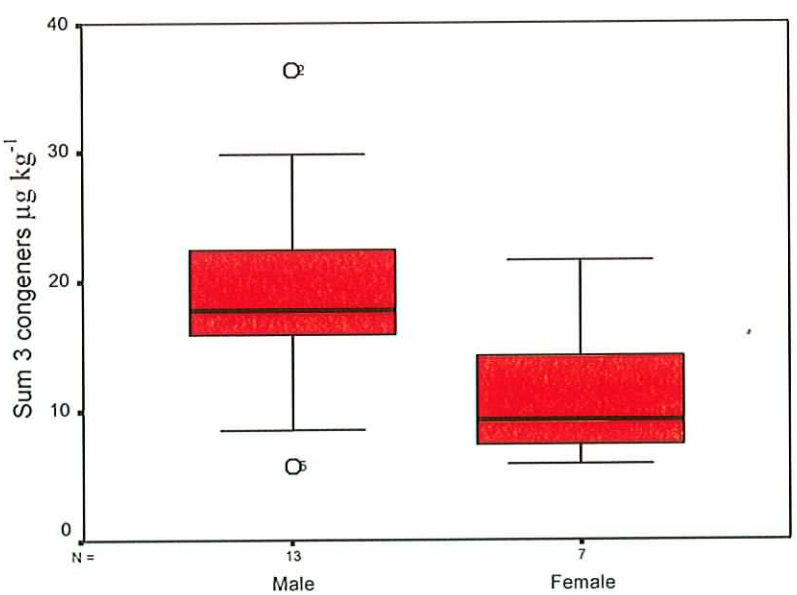

Figure 2.21: Sum 3 congeners for mackerel individuals (µg kg^{-1} lipid weight).

Table 2.27: Homogeneity of variance on all data log (Sum 3 +1) lipid weight.

	Male (n=13)	Female (n=7)	F_{max}	$F_{critical}$
No. samples	13	7	1.05	4.99
Mean	2.24	2.01		
STDEV	0.22	0.21		

2 and 7 degrees of freedom

As the F_{max} was less than the $F_{critical}$ then homogeneity of variance was assumed and the ANOVA was completed. Summary statistics are presented in table 2.28.

Table 2.28: Summary statistics for ANOVA on transformed mackerel data.

Source of Variation	Sum Square	Degrees freedom	Mean Square	F	Probability	F crit
Between Groups	0.23	1	0.23	4.89	0.04	4.41
Within Groups	0.85	18	0.47			
Total	1.08	19				

As the F_{ratio} is greater than the $F_{critical}$ we reject the null hypothesis and conclude that the variation in the mean mass of the samples is significantly different.

The ANOVA determined that a significant difference exists between the male and female groupings ($P<0.05$). The concentration in male species can be said to be significantly higher than in females from the same catch. While these samples were collected outside of the spawning period it is possible however, that females may have eliminated toxaphene residues in lipid reserve transfer to ova during previous spawning events thereby potentially reducing their overall contaminant burden. Additional information on the mobilisation of lipid reserves during spawning is described in chapter 4.

2.5.7 Assessment of risk to the consumer.

It is essential that both the quality of the aquatic environment, and consumers of marine foodstuffs be protected from possible risk arising from exposure to toxaphene residues. Given the concern worldwide over the environmental effect of toxaphene, surprisingly little is known about the toxicology of this group of compounds.

Germany and Austria have a consumer tolerance standard for toxaphene of 0.1 mg kg^{-1} wet weight for fish and fish products on the basis of the sum of the three indicator congeners (62). Limited concentration data from herring, halibut, mackerel and hake liver from the North Sea and North Atlantic showed that levels (0.001 to 0.2 mg kg^{-1} wet weight) sometimes exceed this tolerance level (103), possibly leading to serious implications for both the consumer of contaminated fish and/or the European fisheries industry.

A large number of uncertainties exist in the data with regard to the analysis, baseline levels, carcinogenicity, toxicological risk, tolerance levels and fate of toxaphene in the environment (103). These uncertainties have led to difficulties in the measurement of the risk to the consumer of toxaphene in fishery products.

Tolerance levels are usually based on the toxicology of technical toxaphene mixture. It has been reported that substantial differences exist in the number and pattern of congeners in environmental samples compared to original technical mixtures (1).

The primary route of exposure to toxaphene arises mainly through consumption of contaminated fish. As a consequence of atmospheric transport, uptake in the fish and

potential metabolism/excretion of the original technical mixtures, human exposure, therefore, is to a weathered mixture of technical toxaphene. Only a limited number of studies on the carcinogenicity of toxaphene (104-105) and no studies at all on weathered toxaphene have been reported in the literature.

In the MATT project (98) new methodology for a so-called 'realistic exposure' procedure for toxaphene was developed. The weathered/metabolised toxaphene pattern found in fish was mimicked and should therefore provide a more realistic image of the human exposure situation.

The procedure involved exposing fish (cod), to technical toxaphene in their food. Toxaphene residues were then extracted from the liver of the exposed fish (see AII). These showed a more "weathered" pattern compared to that of the technical mixure and were used in *in vivo* exposure study with rats and *in vitro* experiments to obtain information about the tumour promotion potency of toxaphene residues. The effect levels obtained in the *in vivo* studies were then utilised in order to estimate a tolerable daily intake (TDI) for toxaphene relative to its tumour promotion potency.

An estimation of the average consumer daily daily intake of toxaphene from fishery products from Irish waters was then completed, thus allowing for the potential toxicological risks to the consumer of toxaphene residues to be evaluated. The estimation of the daily intake of toxaphene was based on the levels of toxaphene found in the baseline samples in combination with an estimate of the daily consumption of fishery products in Ireland. Furthermore, the daily intake of toxaphene was compared with the TDI values set by Canada and the US, MRL legislation in Germany and the

TDI calculated in the MATT project, to obtain information on the potential toxicological risks of toxaphene for the consumers.

In general, the main objectives of this current work were to:

- estimate the daily intake of toxaphene residues from Irish fishery products.
- provide information on the toxicological risks to the consumer of toxaphene residues from fishery products from Irish waters.
- compare concentration levels in Irish products to available toxicological information.

2.5.7.1 Estimation of a tolerable daily intake (TDI) for toxaphene.

To derive a TDI for humans, normally toxicity data from mammals are used in combination with a relevant safety factor, with the TDI being defined as the daily intake of a contaminant (in this case toxaphene), which should not result in adverse health effects. Normally for the extrapolation of an effect level based on animal experiments to humans a safety factor of 100 is used.

The World Health Organisation set a safety factor of a 100-fold to obtain a zero-risk effect for humans for the transformation of a no observable adverse effect level (NOAEL) in animals to humans (106), therefore the use of an extrapolation factor of 100 in this study should cover uncertainties in the extrapolation procedure itself, as well as variation between species and within the human species.

With respect to the calculation of a TDI from the *in vivo* toxicity studies of the MATT project (98) the highest dose used in the cod liver extract experiment was 4.8 mg kg^{-1} body weight (bw)/$week^{-1}$ (0.69 mg/kg bw /day^{-1}). This level was used as the NOAEL.

Applying the safety factor of 100 to the NOAEL, the TDI for the tumour promotion potency of toxaphene in humans was estimated at 0.0069 mg/kg bw/d^{-1}. Based on an average body weight of 60 kg a maximum TDI of 0.41 mg for total toxaphene per day was proposed (see table 2.29).

Table 2.29: Overview of effect levels and parameters used to derive a tolerable daily intake (TDI) for toxaphene from the MATT *in vivo* toxicity studies (98).

	Level
Lowest observed adverse effect dose of weathered extract in rat	4.8 mg/kg bw /$week^{-1}$
	0.69 mg/kg bw/d^{-1}
Safety factor for extrapolation from rat to human	100
Tolerable daily intake per kg body weight for humans	0.0069 mg/kg bw/d^{-1}
Proposed maximum TDI for a person of 60 kg	0.41 mg

2.5.7.2 Comparison of data to current legislation.

Several tolerance levels and maximum residue levels in food for toxaphene have been proposed based on total toxaphene or on the sum of three indicator congeners. Germany (62) use an MRL of 0.1 mg kg^{-1} (wet weight) on the basis of the sum of the three indicator congeners (CHBs 26, 50 and 62) for fish and fish products, with the MRL for all other food of animal origin having been set at 0.1 mg kg^{-1} on the basis of total toxaphene. Canada use an allowable daily intake (ADI) of 0.2 µg/kg/bw d^{-1} (equivalent to a tolerable daily intake (TDI) of 0.012 mg for a person of 60 kg) to assess potential risk to the consumer.

The USEPA utilise two health benchmarks for toxaphene, a chronic toxicity reference dose of 2.5 x 10^{-4} mg/kg/d^{-1} (107) and 1.1 mg/kg/d^{-1} for carcinogenicity calculated on a maximum acceptable cancer risk level of 10^{-5} over a 70-year lifetime (108). Based on these levels and on an average body weight of 60 kg both health marks can be converted to TDI's. For chronic toxicity the maximum tolerable daily intake for a person of 60 kg for toxaphene is 0.015 mg, and 66 mg for the endpoint of carcinogenicity. The TDI for carcinogenicity is much higher than the TDI estimated for tumour promotion potency in the MATT project, which was determined to be 0.41 mg.

Table 2.30: MRLs and TDIs of toxaphene for a person of 60 kg.

		Total or CHB specific	Matrix
Germany			
MRL	0.1 mg kg^{-1} ww	sum of 3 CHBs	Fish/fish products
MRL	0.1 mg kg^{-1}	total toxaphene	all other animal food
Canada			
TDI*	0.012 mg d^{-1}	total toxaphene	
US (EPA)			
TDI**, Chronic toxicity	0.015 mg d^{-1}	total toxaphene	
TDI**, Carcinogenicity	66 mg d^{-1}	total toxaphene	
MATT			
TDI, tumour promotion potency	0.41 mg d^{-1}	total toxaphene	
FAO/WHO			
First indication of TDI***	0.012 mg d^{-1}	total toxaphene	

*TDI calculated from the proposed ADI .

** TDI calculated based on the reference doses.

*** Still in discussion

FAO = Food and Agriculture Association

2.5.7.3 Estimated average daily intake of toxaphene.

To estimate the average daily intake of toxaphene from fishery products, consumption statistics are required. Eurostat Statistical Office of the European Communities in Luxembourg provides information on the fish production of European countries (18). However, limited information provided by Eurostat and FAO are insufficient for the purposes of this study.

The Irish Fisheries Board- Bord Iascaigh Mhára (BIM) (20) report that average annual fish consumption in Ireland amounts to 8.8 kg of fish and fish products/person. This figure being much less than that reported by Eurostat and FAO data (see table 2.31).

Table 2.31: Consumption of fishery products (kg/year) in Ireland from various sources (19, 110, 111).

	BIM (kg/person/year)	Eurostat (kg/person/year)	FAO (kg/person/year)
Ireland	8.8	18	20.6

For the calculation of the consumer intake of toxaphene from fishery produce, the current indicator congener dataset from fish fillet tissue (excluding farmed species) and shellfish samples were used.

2.5.7.3.1 Derivation of "total" toxaphene levels.

Data from the MATT project (98) on the concentrations of toxaphene in 221 edible parts of fishery products from the North Sea, Bight/Skagerrak, Irish Sea, Irish west-coast, Iceland Sea, Norwegian coast, and Barents Sea, as well in farmed fish samples were collated.

Levels of total toxaphene and the three indicator congeners were estimated in 55 samples. The total indicator congener/total toxaphene ratio was calculated as 12.4, 41.6, and 24.0 in marine fish, eel and mussel respectively (98). These ratios were then used to estimate the average daily intake of total toxaphene from the sum of the three indicator congeners. These ratios were then used to calculate the estimated concentration of total toxaphene in Irish products based on the sum of the three indicator congeners.

2.5.7.3.2 "Total" toxaphene intake from Irish fishery produce.

For each individual sample the daily intake of toxaphene (A_{intake}) was calculated by multiplying the toxaphene concentration in individual wet weight concentration data (C_{fish}) from baseline survey samples from this study with the average daily consumption of fishery products ($D_{consumption}$) (table 2.31).

$$A_{intake} = C_{fish} \times D_{consumption}$$

To estimate the average daily intake of toxaphene (A_{avg}) the following assumptions were made:

- All persons had access to all fishery products
- All fishery products were eaten in equal amounts
- The baseline survey samples are a good representation of commercial products.

The average intake (A_{avg}) was estimated as the mean of the intake of all individual Irish samples (A_{intake}): where n= total number of samples.

$$A_{avg} = \Sigma A_{intake} / n$$

Table 2.32: Estimated average daily intake of toxaphene from fishery products for the consumer of Irish fishery products (μg).

	Average daily fish consumption (g/d^{-1})* ($D_{consumption}$)	Estimated average daily intake (μg) of toxaphene by fishery products (A_{avg})	Estimated range of daily intake (μg) of toxaphene by fishery products
Ireland	24.1	0.3	0.002-1.8

*Data from BIM 1998 (111).

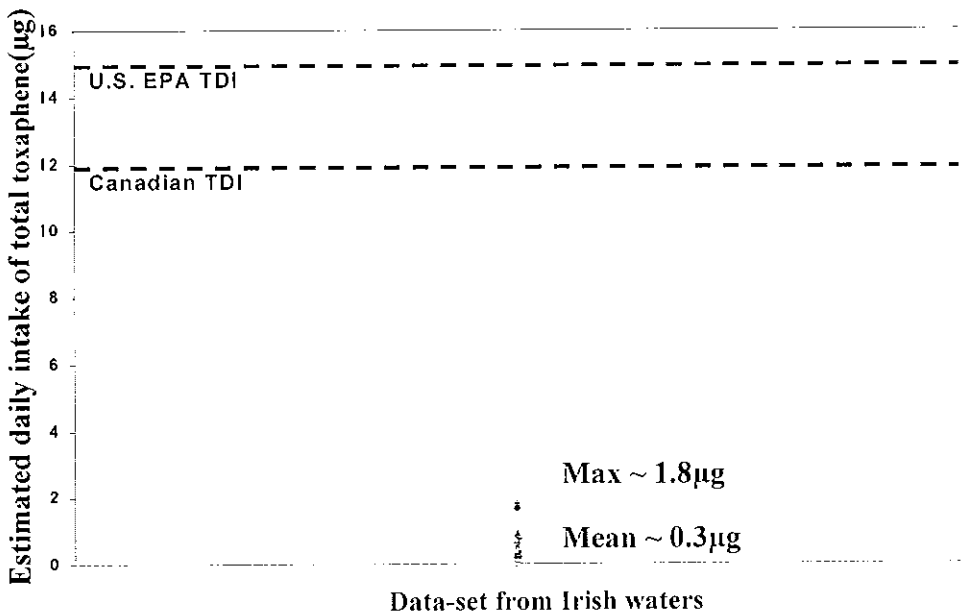

Figure 2.23: Estimated daily intake of total toxaphene (μg) from all Irish samples.

While BIM fishery consumption data was used in this study in reality preferences for the consumption of some fish species exists for individuals therefore intakes will depend on the extent of such preferences within individuals.

A comparison of TDIs and the estimated average daily intake of toxaphene from all baseline samples are shown in Figure 2.23. No samples from Ireland were found to exceed the Canadian TDI or U.S-EPA TDI level for chronic toxicity.

TDI levels from Canada and the U.S. for chronic toxicity (15 µg) are shown (107-108). The TDI for tumour promotion (410 µg), which was set in the MATT project (98), was not exceeded by any of the Irish fishery samples.

2.5.7.4 Comparison of Irish data to German MRL.

With regard to the MRL set by Germany and Austria, all Irish baseline samples were below the threshold level of 0.1 mg kg^{-1} wet weight for the sum of the three indicator congeners in fish and fish products (see Figure 2.24). It is important to note however, that the MRL is based on the toxaphene concentration in the fish product and is not related to the amount of fish consumption.

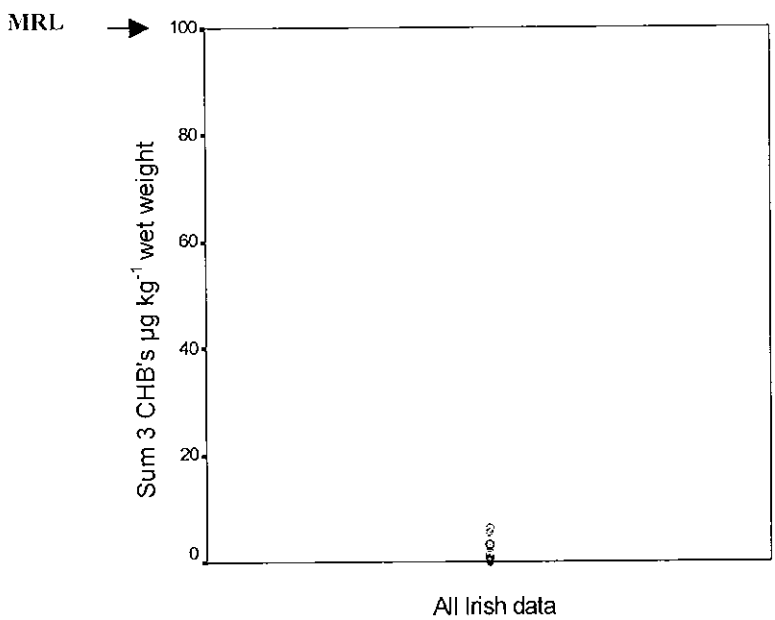

Figure 2.24: Concentration of the sum of the three indicator toxaphene congeners in all Irish samples compared to an MRL of 100 µg kg^{-1} (wet weight).

2.5.8 Overall conclusions

Elevated levels of toxaphene were observed in farmed species (sea-trout, salmon and lake-trout) and in individual samples of plaice, cod and herring. Concentrations in farmed species were significantly higher that in most other sample groupings with the exception of those of deepsea species (large sample variance in the deepsea grouping was a contributing factor). Elevated levels in individual cod, plaice and herring samples do not correspond with general data for their particular species grouping, this probably being due to ineffectual lipid extraction from these samples. Elevated levels in farmed species may be related to fish oils present in feed pellets. These oils are generally derived from fish species, which would have had prior exposure to toxaphene residues before oil is harvested.

Concentrations in mussels are lower than all other sample groupings, this may be a result of a feeding mechanism involving filtration of large volumes of water to feed on phytoplankton. Levels of toxaphene in phytoplankton would be expected to be lower than in other marine species as they are the lowest step on the marine food web.

Even though focused sampling to minimise sample variation with respect to size and/or age was completed during this current work, large variability can be expected to occur in individuals within a pooled sample. In the case of mackerel individuals a mixture of both males and females of differing ages and degrees of maturity can be expected within a single shoal.

Overall, based on the generation of summary risk assessment data, no adverse effects to the consumer of Irish fishery produce is currently expected.

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CHAPTER 3: INVESTIGATION IN THE LEVELS AND INFLUENCING FACTORS OF PERSISTENT ORGANIC POLLUTANTS IN BLUE WHITING.

3.1 Introduction.

Blue Whiting (*Micromesistius poutassou)(Risso 1810)(1)*, a member of the Cod family (Gadidae) is a widely distributed and commercially important fish species accessible in Irish waters in early spring. In recent years it has been reported that dioxin levels in blue whiting derived fish oil can exceed EU maximum limits. It has been suggested that levels are more elevated during the early months of the year (2), this is the period when blue whiting are commercially attractive to Irish fleets due to their proximity to Irish coastal waters. Although blue whiting has limited suitability as a direct consumer product, the use of blue whiting fish oils in animal feeds may be considered as a potential indirect risk to the consumer. This study reports the levels of a number of POP groupings in blue whiting derived fish oils and fillets and investigates the factors influencing them.

Current fishery management practices treat blue whiting as a single stock of which Ireland had a share of 75,893 tonnes in 2005 (3). Killybegs is the primary landing port for the processing of blue whiting fillets and of fish oil in Ireland. In February 2005, the Marine Institute and Irish Agricultural Wholesale Suppliers (IAWS), Killybegs, prepared a joint work programme to contribute to a European project focused on determining the levels of a suite of Persistent Organic Pollutants (POP's) in blue whiting derived fish oils. Representative blue whiting sub-sampling was additionally completed to provide biological characterisation information to support these analyses.

3.1.1 General biology of blue whiting

Blue whiting occur throughout the North East Atlantic from Spain and the NW coast of Africa to Iceland and east to Spitzbergen, but are most abundant during the spring

spawning period in deep waters to the west of Scotland and Ireland and along the Faeroe-Shetland channel (3). On a European context it is reported that changes in fishing patterns have resulted in much greater numbers of younger fish in blue whiting catches than observed in recent years and that the stock largely misses fish greater than 6 years old. In 2005 blue whiting landings in Irish waters comprised of fish of approximately 10 years old or less. (see figure 3.1).

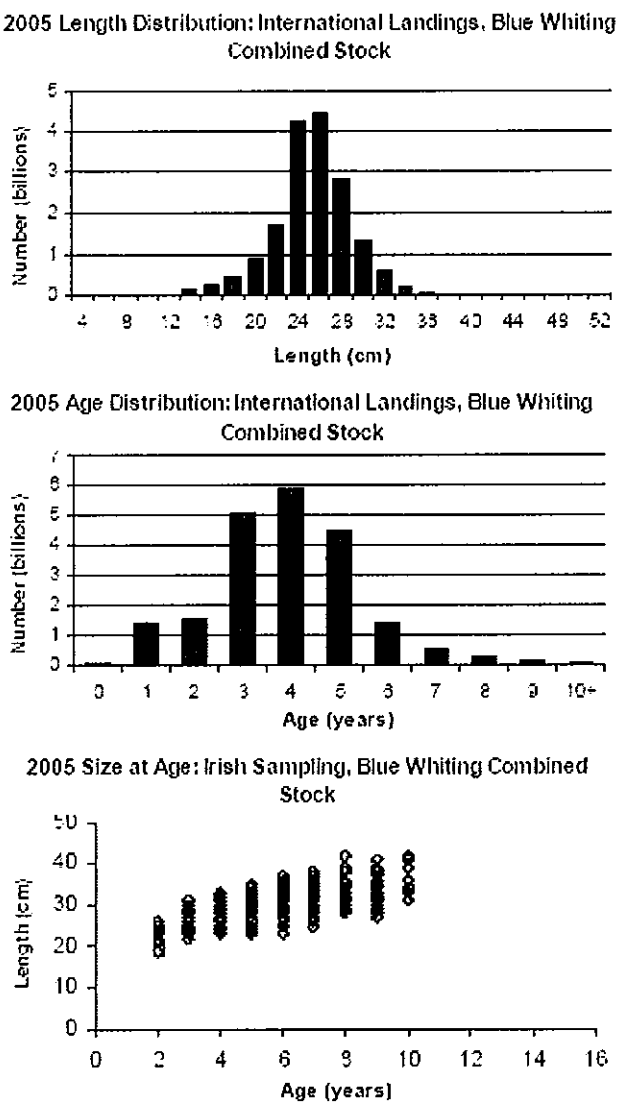

Figure 3.1: Length-frequency distribution for combined blue whiting landings 2005 and age profile of Irish landed fish (3).

3.1.1.1 Blue whiting diet and reproduction.

The main feeding period for blue whiting tends to occur in late spring and the early summer, while little feeding occurs during the spawning season (February to April/May) (4). Blue whiting have a varied diet, however meso-pelagic crustaceans, cephalopods, small fish and fish larvae feature prominently (5). Small blue whiting are known to feed on larger crustaceans and small fish as they grow to full size (4).

The spawning stock is dominated by young fish, and above average recruitment is believed to be an important factor in maintaining the current spawning stock biomass (4). Ryan et al (6) report evidence of heterogeneity in population structure in the Hebrides-Porcupine bank blue whiting stock but, to date, blue whiting in European waters has been assessed as a single stock, extending from Spain to Norway with important spawning and nursery areas located off the Irish coast, with peak spawning times ranging from February in the Celtic Sea to May in the Southern Faeroe, as the species move northwards (4).

Eggs and larvae move slowly towards the surface as they develop, and drift away from the spawning grounds. Many young fish, up to 2 years old, remain in the upper 100m of the open ocean in the north-east Atlantic and Norwegian Sea until they mature and join the breeding population. Some adult post-spawning fish are reported to migrate northwards as far as Bear Island and Spitzbergen to feed before returning to the spawning area in the autumn via Jan Mayen and the east of Iceland, but the full extent of feeding migration is unclear. Figure 3.2 illustrates current knowledge of migration routes (7).

3.1.1.2 Blue whiting commercial products.

The majority of blue whiting landed in Ireland is reduced to fish oil however limited processing to graded frozen blocks is completed in Killybegs. Research initiatives are currently underway towards the development of other potential markets. However exploitation of blue whiting as whole fish or as fillets is compromised due to their small size, relatively rapid discoloration due to autolysis and the potential presence of parasites which all weigh against them in competition with other well established white fish species.

It is reported that some blue whiting reach maturity in their third year, but that recruitment to the spawning stock is incomplete until most fish reach 7 to 8 years old. Blue whiting individuals range from approximately 17cm in length at 2 years old to approximately 30cm at 7 to 8 years old. Males mature at an earlier age and reach their maximum length faster than females, but females eventually grow slightly larger than the males (7).

3.1.1.3 Contaminant uptake in marine biota.

The biological processes of contaminant bioconcentration, bioaccumulation, biomagnification and/or biotransformation in marine species are governed by a variety of physico-chemical, biological, spatial and/or temporal factors with POP uptake and elimination a function of, uptake and loss via gills, uptake via lower trophic food sources, metabolism and/or excretion (see chapter 1).

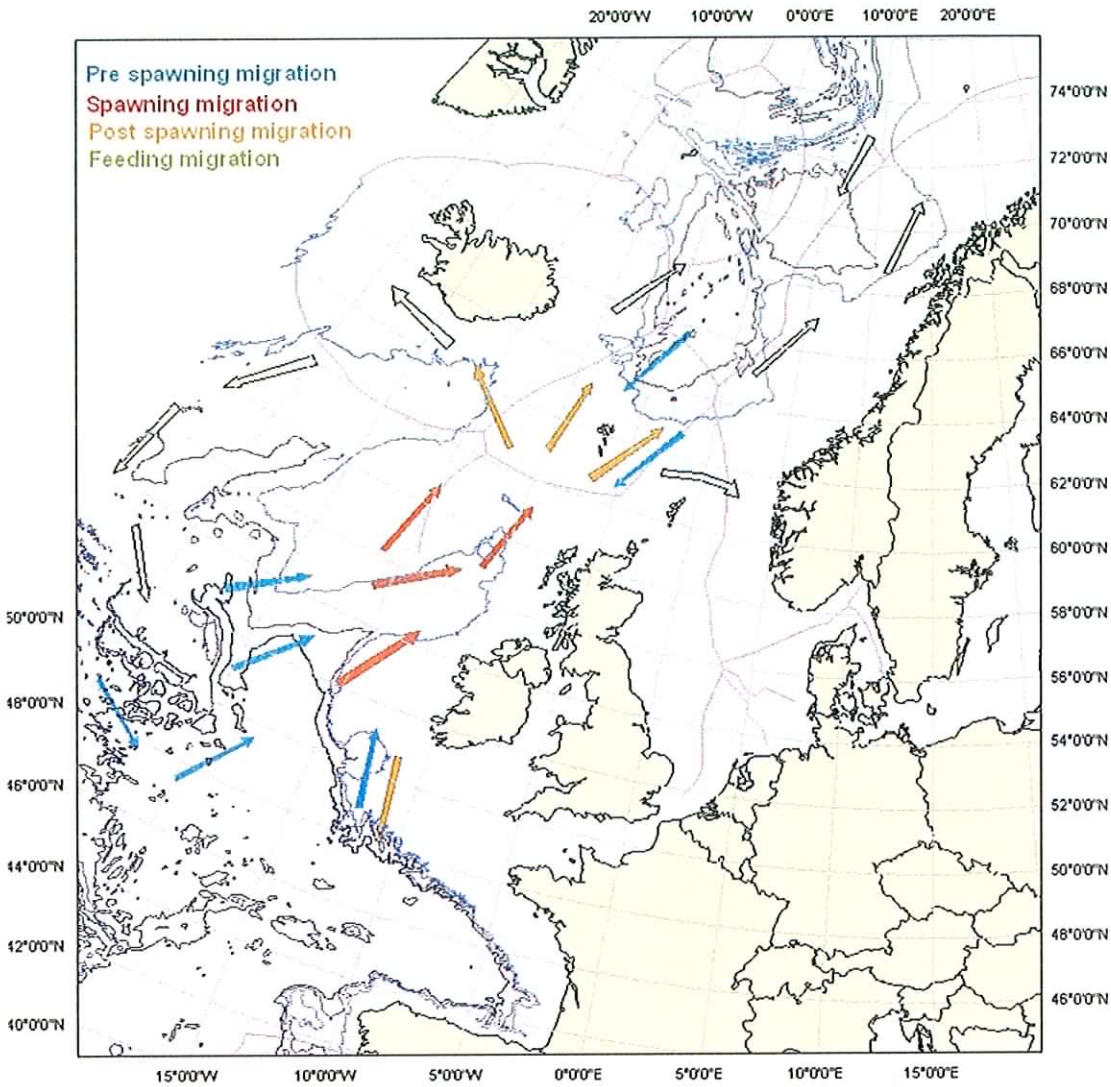

Figure 3.2: Proposed spawning and migration routes for blue whiting (7).

3.1.2 Persistent Organic Pollutants (POPs).

The term POPs describe a range of widespread pollutants whose distribution is primarily the result of long-range atmospheric transport and/or emission, often distant from point of application. The physico-chemical properties of a number of POPs can predispose them to accumulation in fish and especially within lipid rich species and can result in biomagnification through the food chain. Biological factors including, lipid

levels, longevity, diet, trophic status amongst others, can pre-dispose fish species to elevated POP levels (12).

A number of lipophilic POPs including Polychlorinated dibenzo-p-dioxin (PCDD), polychlorinated dibenzo-p-furans (PCDF), polychlorinated biphenyls (PCB), polybrominated biphenyl-ethers (PBDEs) and organochlorine pesticides (OCPs) are considered pollutants of priority concern, primarily on the basis of their environmental persistence, tendency towards bioaccumulation and toxicological characteristics.

3.1.3 Legislation related to fish and associated produce.

EU legislation has the ultimate aim of protecting the consumer against exposure to contaminated foodstuffs and the TEQ approach is used as an analytical tool in order to determine compliance with agreed legislative thresholds. There are three pillars to the dioxin legislation

- The establishment of maximum levels (MLs), taking into account the current background contamination, at a strict but feasible level.
- The establishment of action levels acting as a tool for 'early warning', which triggers a proactive approach from competent authorities and operators.
- The establishment of target levels to be achieved in order to reduce exposure of the large majority of the European population to contaminants.

It should be noted that the European Union (EU) wide pesticide residue limits are not currently applicable to produce of marine origin, even where such produce are used in animal feeds.

3.1.3.1 Legislation related to dioxins/furans and dioxin-like PCBs in foodstuffs and feeds .

Commission directive 2006/13/EC of 3 February 2006 amends Annexes I and II to Directive 2002/32/EC of the European Parliament and of the Council on undesirable substances in animal feed as regards dioxins and dioxin-like PCBs. This directive sets new combined maximum levels for dioxin and dioxin-like PCB levels in farmed animal and fish feed applicable from 4 November 2006. (28)

EU Commission Regulation 199/2006 (29) (amending regulation 466/2001) sets combined maximum levels for dioxin/furans and DL-PCB levels in foodstuffs for human consumption and are applicable from 4 November 2006 (see tables 3.1 and 3.2). New combined maximum limits are in addition to the current maximum limits (MLs) for dioxins only.

Table 3.1: Current and new legislative limits for PCDD/Fs in fish and animal feeds (pg g^{-1} TEQ).

Product	Current limit	Product	New limit
Fish oil	6.0 pg g^{-1} product	Fish oil	24 pg g^{-1} (18 DL-PCBs and 6 PCDD/F)
Fish meal	1.25 pg g^{-1} product	Fish meal	4.5 pg g^{-1} (3.25 DL-PCBs and 1.25 PCDD/F).
Compounded fish feed	2.25 pg g^{-1} product	Feedingstuffs for fish and pet food	7 pg g^{-1} (4.75 DL-PCBs and 2.25 PCDD/F)

Table 3.2: Current and new legislative limits for PCDD/Fs in fish and animal feeds pg g^{-1} TEQ

Product	Current limit	Product	New limit
Fish	4pg WHO-PCDD/F-TEQ/g fresh weight	Fish (excl. eel)	8 pg g^{-1} (4 DL-PCBs and 4 PCDD/F)
Fish oil	2pg WHO-PCDD/F-TEQ/g fat	Marine oil incl. fish body oil and liver oil	10 pg g^{-1} (8 DL-PCBs and 2 PCDD/F)
		Eel	12 pg g^{-1} (8 DL-PCBs and 4 PCDD/F).

From November 2006, any food or feed exceeding the maximum levels for dioxins or dioxin-like PCBs will not be marketable in the EU. Maximum limits for dioxins in food of animal origin and animal feed have been in force since July 2002 but a lack of scientific information available at the time meant that no limits were set on dioxin-like PCBs. The new legislation lays down mandatory limits for the combined level of dioxins and dioxin-like PCBs and will apply in conjunction with the existing limits on dioxins.

3.1.3.2 Action limits related to PCDD/Fs and DL-PCBs.

"Action limits" to trigger management procedures to trace and eliminate sources of contamination are also suggested to EU Member States (see tables 3.3 and 3.4). It should be noted that these recommendations are not legally binding on Member States. Key recommendations with respect to action limits are as follows:

- In cases of non-compliance Member States should investigate the source of contamination, check for dioxin-like PCBs and take measures to reduce or eliminate contamination.
- Member States with high background levels should set National action levels for domestic feed production. 5% of the monitoring results should be followed up with action to identify sources of contamination.

Table 3.3: Current and new Action limits for PCDD/Fs and DL-PCBs in fish and fish oils for animal feeds (pg g^{-1} TEQ).

Product	Current Action limit	Product	New Action limit
Fish oil	4.5 pg g^{-1} PCDD/F product	Fish oil	14 pg g^{-1} (9 DL-PCBs and 5 PCDD/F)
Fish meal	1.00 pg g^{-1} PCDD/F product	Fish meal	2.5 pg g^{-1} (1.5 DL-PCBs and 1 PCDD/F).
Compounded fish feed	1.50 pg g^{-1} PCDD/F product	Feedingstuffs for fish and pet food	3.50 pg g^{-1} (1.75 DL-PCBs and 1.75 PCDD/F)

Table 3.4: Current and new Action limits for PCDD/Fs and DL-PCBs in fish and fish oils for human consumption (pg g^{-1} TEQ).

Product	Current Action limit	Product	New Action limit
Fish	3 pg WHO-PCDD/F-TEQ/g fresh weight	Fish (excl. eel)	6 pg g^{-1} (3 DL-PCBs and 3 PCDD/F)
Fish oil	1.50 pg WHO-PCDD/F-TEQ/g fat	Marine oil incl. fish body oil and liver oil	7.5 pg g^{-1} (6 DL-PCBs and 1 PCDD/F)
		Eel	12 pg g^{-1} (8 DL-PCBs and 4 PCDD/F).

The primary objectives of this current study were to,

- determine the levels of a number of suites of persistent organic pollutants in commercially processed blue whiting fish oil,
- assess the role of a variety of biological and/or spatial factors/indicators on the levels of dioxins/furans and other POPs in blue whiting fish oil.
- collect and assess biological characterization data from representative whole fish sub-samples of blue whiting used for the generation of the fish oil product.
- assess the levels of dioxins and furans in blue whiting fillet tissue
- compare PCDD/F levels in blue whiting muscle fillet to consumer safety legislation.

3.2 Materials and methodology

Sampling procedures and location details are further described below.

3.2.1 Blue whiting sampling procedures.

A total of 10 fish oil and corresponding blue whiting biological sampling events were collected between February and April 2005. Representative sub-sampling of blue whiting was completed in accordance with established Marine Institute protocols. Procedures are further described below and details of sampling dates and locations are presented in table 3.5 and in figures 3.3 and 3.4. The process of fish oil production is further detailed in Annex 3.1.

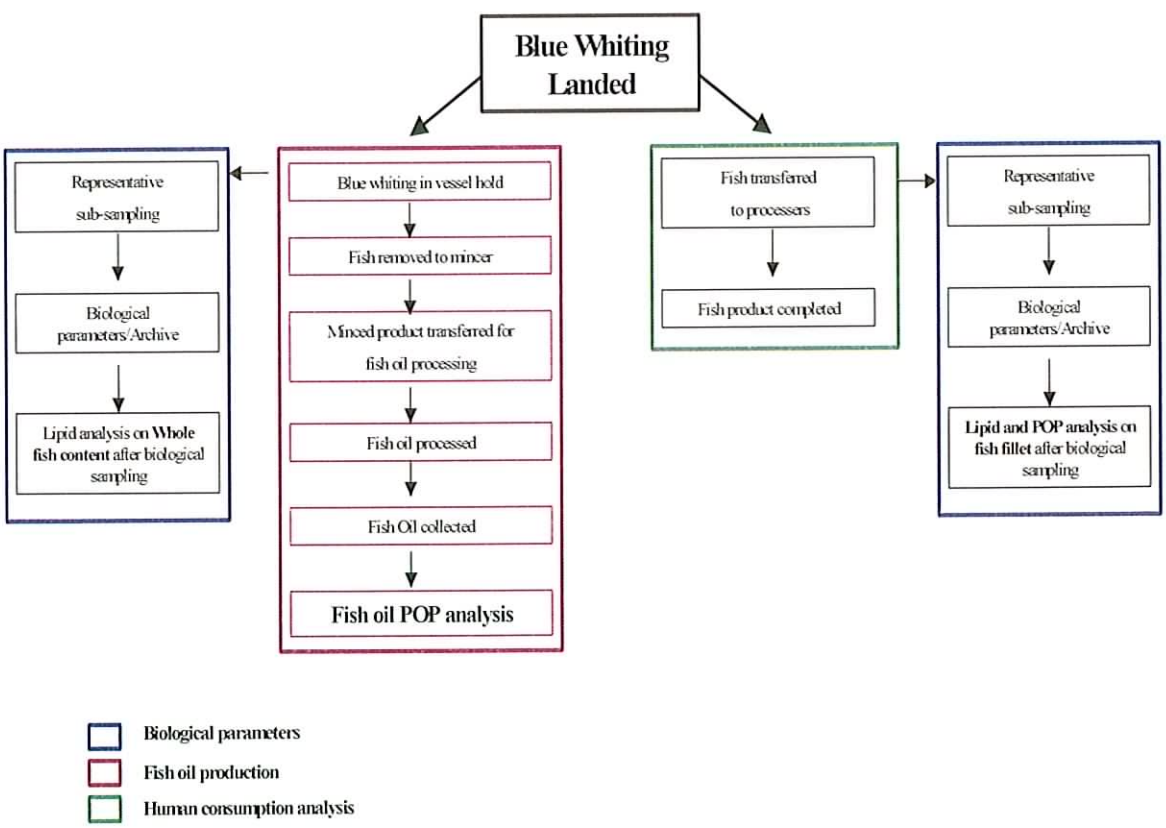

Figure 3.3: Blue whiting biological and fish oil collection procedures.

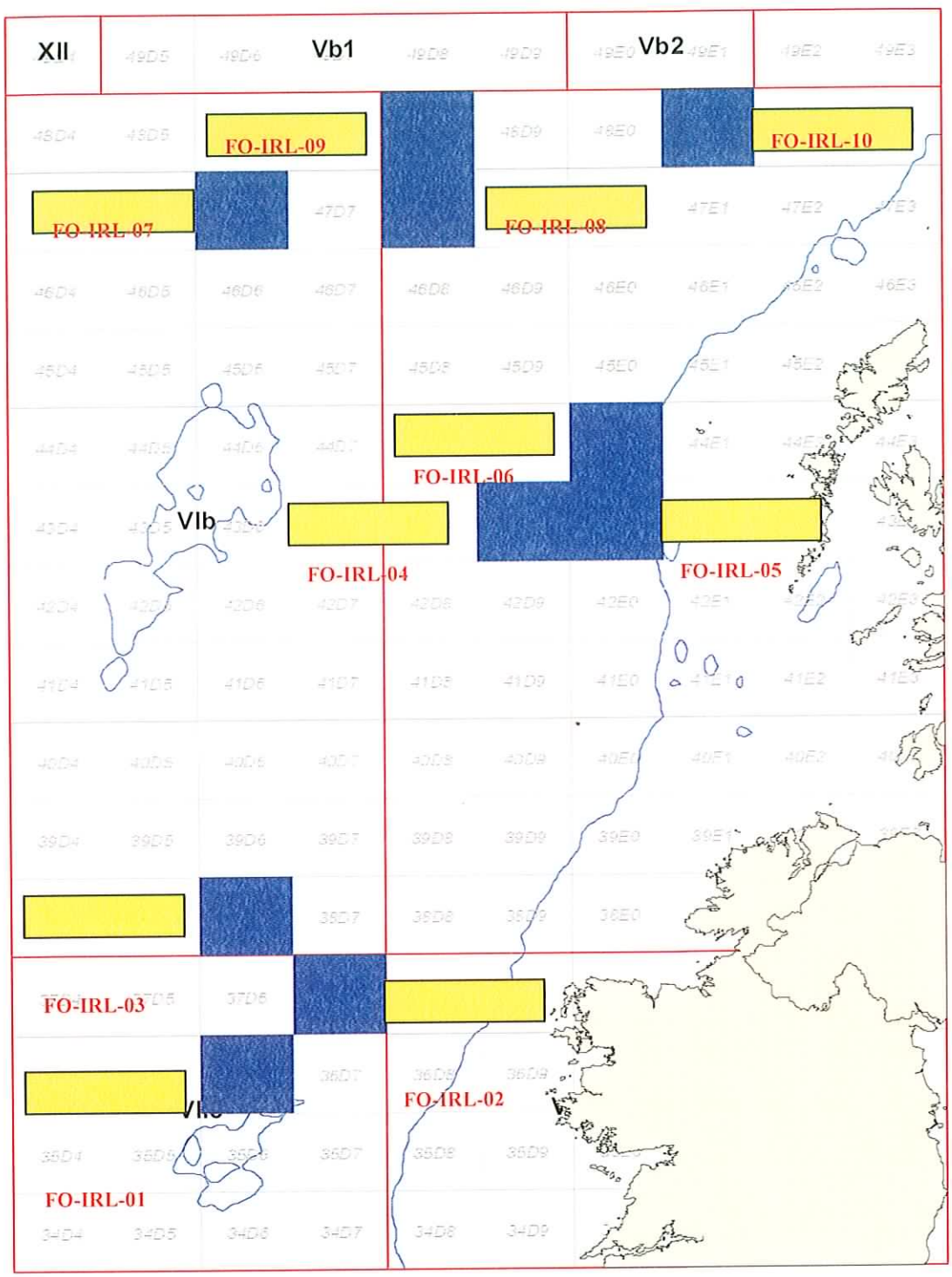

Figure 3.4: Blue whiting capture locations for fish oil samples indicated by blue squares.

3.2.1.1 Collection of blue whiting fish oil for contaminants analysis

Landed blue whiting were directly transferred from fishing vessels to the processing plant for oil production according to the process outlined in Appendix 3.1. Pure blue whiting fish oil was collected in solvent rinsed glass containers as it emerged from production process. On each occasion approximately 100-200ml of pure blue whiting process-line fish oil was collected and chilled aliquots were forwarded to ERGO laboratories, Hamburg, Germany for analysis of a suite of contaminants including PCDD/Fs, PCBs, PBDEs and OCPs

Table 3.5: Sampling location information for blue whiting fish oils (FO) and human consumption (HC) samples.

Sample ID	Sampling Date	Fishing ground	Location
FO-IRL-01	12-Feb-05	36D6/35D6[1]	Porcupine bank
FO-IRL-02	27-Feb-05	37D7	North Porcupine bank
FO-IRL-03	21-Mar-05	38D6	Rockall trough
FO-IRL-04	23-Mar-05	43D9	West of St. Kilda
FO-IRL-05	29-Mar-05	43EO	West of St. Kilda
FO-IRL-06	08-Apr-05	44EO	West of St. Kilda
FO-IRL-07	07-Apr-05	47D6	North of Rockall
FO-IRL-08	08-Apr-05	47D8	Bailey bank
FO-IRL-09	14-Apr-05	48D8	Bailey bank
FO-IRL-10	21-Apr-05	48E1	West Wyville ridge
HC IRL-01	08-Mar-05	VIIb	NE of Porcupine bank
HC IRL-02	09-Mar-05	VIIb	West of Achill
HC IRL-03	14-Mar-05	VIa	NorthWest of Donegal
HC IRL-04	15-Mar-05	VIIc	North Porcupine bank

FO=Fish oil samples

HC=Human consumption samples.

3.2.1.1.1 Biological assessment of blue whiting for fish oil production.

Individual whole blue whiting, representative of those used in production of pure fish oil were collected onboard commercial fishing vessels prior to fish oil processing. 100-110 whole undamaged individual fish representative of those for oil production were collected, individually weighed, had length and sex determined and had otoliths removed for ageing purposes. A further 100-110 fish were removed for archive purposes.

3.2.1.2 Total lipid analysis of representative fish.

Once biological testing was completed the whole fish contents were pooled, coarsely minced and stored in a large solvent washed container with an aluminium foil lined lid. Total lipid content in whole fish homogenates was completed according to the method of Smedes (30, 31).

3.2.1.3 Biological assessment of blue whiting for human consumption.

Representative individual whole blue whiting were collected at the process line prior to the start of processing of fish for human consumption. 100-110 whole undamaged individual fish were collected, individually weighed, had length and sex determined and had otoliths removed for ageing purposes. A further 100-110 fish were removed for archive purposes.

3.2.1.3.1 Total lipid analysis in human consumption samples

Once biological sampling had been completed the remaining fish fillet muscle was pooled and was coarsely minced and stored in a solvent washed glass container with an aluminium foil lined lid for the purposes of total lipid content determination and for

archive purposes. Total lipid content in fillet fish homogenates was completed according to the method of Smedes (30, 31).

3.2.2 Contaminants analysis-Analytical procedures

Analysis of a number of POP suites including PCDDs, PCDFs, (DL-)PCBs, PBDEs and OCPs was completed by ERGO Forschungsgesellschaft mbH, Hamburg, Germany. Analytical procedures are further described below.

3.2.2.1 Dioxin, furan and DL-PCB analysis.

Within the scope of this study 7 PCDD congeners were determined as follows, 2,3,7,8-TCDD, 1,2,3,7,8-PentaCDD, 1,2,3,4,7,8-HexaCDD , 1,2,3,6,7,8-HexaCDD, 1,2,3,7,8,9-HexaCDD, 1,2,3,4,6,7,8-HeptaCDD and OctaCDD. Additionally 10 PCDF congeners, 2,3,7,8-TetraCDF, 1,2,3,7,8-PentaCDF, 2,3,4,7,8-PentaCDF, 1,2,3,4,7,8-HexaCDF, 1,2,3,6,7,8-HexaCDF, 1,2,3,7,8,9-HexaCDF, 2,3,4,6,7,8-HexaCDF, 1,2,3,4,6,7,8-HeptaCDF, 1,2,3,4,7,8,9-HeptaCDF and OctaCDF were quantified. A total of four Non-ortho PCBs 81, 77, 126 and 169 and eight mono-ortho substituted PCBs 105, 114, 118, 123, 156, 157, 167 and 189 were analysed.

Fish oils were cleaned-up on a carbon-glass-fibre multi-column system. Analytical separation was performed on DB-5 capillary columns using VG-AutoSpec and/or Finnigan MAT 95 XL GC instrumentation. Analytical detection was carried out using high-resolution gas chromatography together with high-resolution mass spectrometry (HRGC/HRMS). Two characteristic isotope masses were measured for each congener and quantification was carried out utilising internal/external standard mixtures based on isotope dilution principles. Details of internal standards employed are reported in Annex 3.2.

3.2.2.2 Marker PCB and OCP analysis

Within the scope of this project the sum of the ICES 7 PCBs 28, 52, 101, 118, 138, 153 and 180 and the following organochlorine pesticides (OCPs), α-, β-, γ-HCH and δ-HCH, o,p'-DDT, p,p'-DDT, o,p'-DDD, p,p'-DDD, o,p'-DDE, p,p'-DDE, Hexachlorobenzene, Heptachlor, Aldrin, Dieldrin, Endrin, Endosulphane sulphate, α-Endosulphane, β-Endosulphane , γ-Chlordane, α-Chlordane, Oxychlordane, cis Heptachlor epoxide, trans Heptachlor epoxide, Toxaphene congeners CHBs 26, 50, 62 and Octachlorstyrene were determined in blue whiting oils. No dioxin-like PCBs, marker PCBs or OCP analysis was completed on human consumption samples.

3.2.2.3 PBDE congener analysis

PBDE congeners 28, 47, 66, 99, 100, 153, 154 and 183 were all analysed during the course of this current project. Prior to sample extraction appropriate ^{13}C-UL-labeled internal standards (See Annex 3.2) were added to the fish oil sample. After spiking, samples were cleaned-up by adsorption chromatography techniques. Analytical determination was carried out by high-resolution gas chromatography and mass spectrometry (HRGC/MS) using a DB-5 capillary column. For each substance 2 isotope masses were measured and quantification was carried out with the use of internal/external standard mixtures (mass resolution 0.7 amu).

3.2.2.4 Total lipid determination

Total lipid content was determined by the Smedes method (30, 31) in representative fish fillet samples in the case of human consumption samples and in whole fish homogenates in the case of fish representative of fish oil production. This cold solvent-based extraction technique utilises a tri-phasic extraction procedure with water,

cyclohexane and isopropanol in order to extract total lipid content from tissue. Total lipid content was then gravimetrically determined following drying of the solvent extracted lipid under a stream of nitrogen.

3.2.3 Quality Assurance procedures

Spiking of the blue whiting muscle tissue and fish oils and of a pooled laboratory reference oil with appropriate isotopically labelled standards was completed prior to analysis of samples. Summary analytical recoveries on a contaminant suite basis and a listing of internal spike standards utilised during this study are reported in tables 3.6 and details are reported in Annex 3.2.

The majority of recovery data lie within limits deemed acceptable for the purposes of this study. Greater variation was observed however for PDBE spiking data with PBDE 47 and PBDE 183 showing recoveries of 275% and 37% respectively in the laboratory pooled oil sample and from 38.3-439% for PBDEs 154 and 47 respectively in the spiked fish oil samples.

Table 3.6: Recoveries from internal standard spiking of blue whiting fish oils and from of laboratory reference pooled fish oil (%).

Analyte group	Spiking recoveries	Pooled fish oil recoveries
PCDD	63.2-119	73.0 -107
PCDF	48.7.-137	84.0 -102
Non-ortho PCBs	60.4-97.0	89.0-102
Mono-ortho PCBs	73.5-111	83.0-102
Marker PCBs (ΣICES 7)	65.4-92.5	74.0-82.0
PBDEs	38.3-439	37.0-275
OCPs	52.0-112	52.0-91.0

3.3 Results and discussion

Results of both biological and analytical components of this study are documented below and assessments are completed on,

- biological characterisation of representative blue whiting,
- concentration data for PCDDs, PCDFs, PCBs, PBDEs and OCPs,
- an overall assessment of factors potentially influencing levels of contaminants in blue whiting,
- consumer and food safety considerations.

3.3.1 Biological sampling aspects.

Tables 3.7-3.9 presents summary data related to the length, weight, age and maturity indices of blue whiting collected during this study. It was determined that the mean age profile of both female and male fish increased as the study progressed (see figure 3.5). It was also determined that the mean age profile of female fish in 9 out of 10 sampling events was greater than that of their male counterparts.

Optimal maturity indices were approached at approximately similar timeframes (see figure 3.6). Additionally the ratio of females to males shows wide variance with older female fish dominating in most batches. In general the total lipid content of fish homogenates decreases throughout the sampling season. Such decreases may be a function of increased spawning activity and the subsequent transfer of lipid reserves to ova during this period.

It should be noted that in all instances where percentage lipid data are reported, they reflect the total lipid content in whole fish homogenates (Smedes methodology) and are not reflective of the percentage lipid derived from fish oil processing.

Table 3.7: Frequency of age distribution of blue whiting representative of fish oil batches.

Age Females	FO-IRL-01	FO-IRL-02	FO-IRL-03	FO-IRL-04	FO-IRL-05	FO-IRL-06	FO-IRL-07	FO-IRL-08	FO-IRL-09	FO-IRL-10
1	0	0	0	0	0	0	0	0	0	0
2	2	2	0	0	2	0	0	0	0	0
3	11	7	7	6	8	10	2	5	1	0
4	20	15	14	5	16	14	17	10	4	6
5	12	24	20	13	15	19	15	19	11	13
6	2	6	11	5	5	14	11	10	6	9
7	1	7	4	4	1	6	7	4	2	3
8	0	1	0	1	3	1	6	0	0	2
>8	0	1	1	1	0	0	4	0	1	2

Age Males	FO-IRL-01	FO-IRL-02	FO-IRL-03	FO-IRL-04	FO-IRL-05	FO-IRL-06	FO-IRL-07	FO-IRL-08	FO-IRL-09	FO-IRL-10
1	0	0	0	0	0	0	0	0	0	0
2	3	0	3	0	2	0	0	1	0	0
3	14	11	7	7	7	11	4	8	14	3
4	25	12	13	21	12	12	11	14	29	31
5	8	10	13	23	22	9	13	22	21	20
6	2	1	5	6	3	1	6	4	8	9
7	0	2	2	5	3	3	1	2	3	2
8	0	0	0	2	1	0	1	0	0	0
>8	0	0	0	1	0	0	2	1	0	0

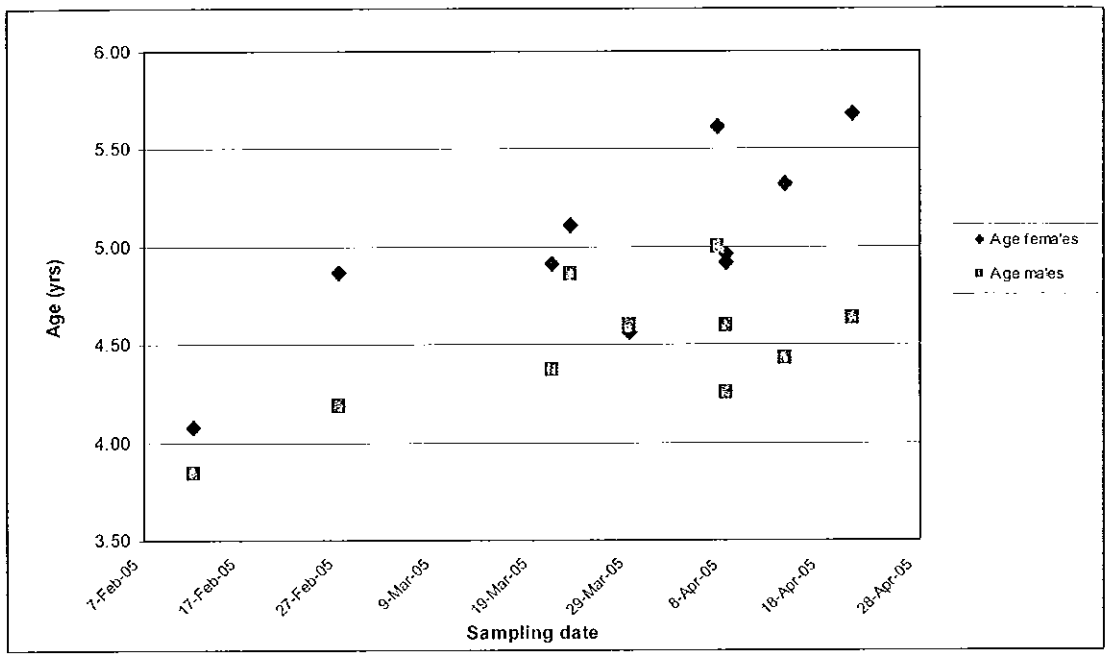

Figure 3.5: Mean age profiles (yrs) of male and female blue whiting representative of fish oil production.

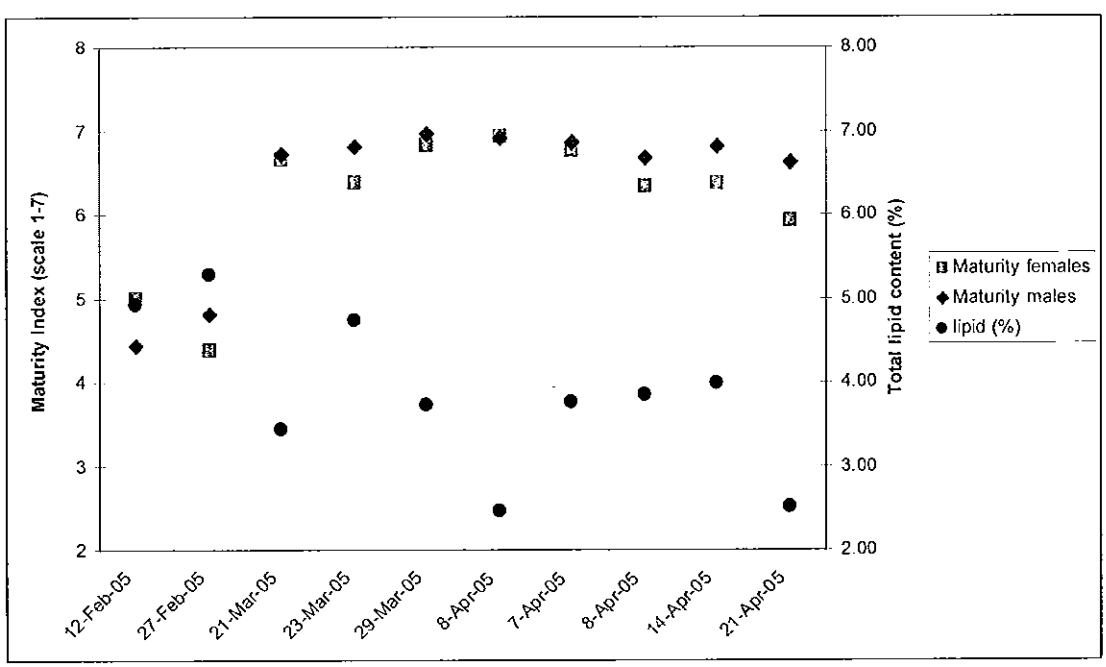

Figure 3.6: Mean maturity indices (scale 1-7) for male and female blue whiting and composite sample total lipid content (%).

Table 3.8: Summary biological parameters for blue whiting representative of fish oil production. (n= total of 100 in all cases). For all parameters the minimum and maximum values are reported in ().

		FO-IRL-01	FO-IRL-02	FO-IRL-03	FO-IRL-04	FO-IRL-05	FO-IRL-06	FO-IRL-07	FO-IRL-08	FO-IRL-09	FO-IRL-10
Females	Length (mean)	27.3 (24.5-31.5)	28.7 (25.0-35.0)	29.5 (25.0-38.0)	29.1 (23.5-41.5)	28.2 (23.0-38.0)	28.9 (24.0-38.0)	30.1 (24.0-39.5)	28.5 (25.0-36.0)	29.0 (24.5-32.0)	30.3 (25.0-36.5)
	Length (mode)	26.0	28.0	28.5	28.5	27.5	29.0	30.5	29.5	30.5	29.5
	Weight	106 (68-180)	132 (85-247)	150 (72-308)	127 (56-346)	93.9 (48.0-195)	105 (56.0-274)	133 (59.0-362)	119 (65.0-266)	119 (73.0-191)	149 (88.0-266)
	Maturity (mean)	5.00 (3-6)	4.38 (3-7)	6.65 (3-7)	6.37 (6-7)	6.82 (6-7)	6.92 (6-7)	6.75 (6-7)	6.33 (6-7)	6.26 (6-7)	5.91 (4-7)
	Maturity (mode)	5 (3-6)	4 (3-7)	7 (3-7)	6 (6-7)	7 (6-7)	7 (6-7)	7 (6-7)	6 (6-7)	6 (6-7)	7 (4-7)
	Age (mean)	4.08 (2-7	4.87 (2-9)	4.91 (3-9)	5.11 (3-10)	4.56 (2-8)	4.92 (6-7)	5.61 (6-7)	4.96 (3-7)	5.32 (3-9)	5.68 (4-10)
	Age (mode)	4 (2-7)	5 (2-9)	5 (3-9)	5 (3-10)	4 (2-8)	5 (3-8)	4 (3-9)	5 (3-7)	5 (3-9)	5 (4-10)
Males	Length (mean)	25.7 (19.5-28.5)	26.0 (23.0-38.0)	26.7 (19.5-31.0)	27.0 (24.0-31.5)	26.8 (23.0-36.5)	26.2 (23.0-30.5)	27.3 (24.0-31.5)	26.2 (22.5-30.0)	27.1 (23.5-32.0)	27.2 (24.5-31.5)
	Length (mode)	26.0	25.0	25.5	25.5	26.5	25.5	28.5	26.5	28.0	27.0
	Weight	87.9 (42.0-133)	100 (64.0-334)	97.4 (36.0-179)	99.5 (62.0-183)	86.2 (51.0-282)	81.6 (53.0-129)	104 (62.0-173)	92.6 (54.0-150)	98.2 (58.0-168)	99.9 (64.0-172)
	Maturity (mean)	4.42 (3-5)	4.81 (3-7)	6.72 (1-7)	6.8 (6-7)	6.96 (6-7)	6.91 (6-7)	6.86 (6-7)	6.67 (6-7)	6.80 (5-7)	6.62 (5-7)
	Maturity (mode)	5 (3-5)	5 (3-7)	7 (1-7)	7 (6-7)	7 (6-7)	7 (6-7)	7 (6-7)	7 (6-7)	7 (5-7)	7 (5-7)
	Age (mean)	3.84 (2-6)	4.19 (3-7)	4.37 (2-7)	4.86 (3-9)	4.60 (2-8)	4.25 (3-7)	5.00 (3-9)	4.60 (2-9)	4.43 (3-7)	4.63 (3-7)
	Age (mode)	4 (2-6)	4 (3-7)	4 (2-7)	5 (3-9)	5 (2-8)	4 (3-7)	5 (3-9)	5 (2-9)	4 (3-7)	4 (3-7)
Combined	Ratio M/F	0.92	1.75	1.33	0.54	1.00	1.78	1.63	0.92	0.33	0.5
	% lipid	4.93	5.28	3.44	4.74	3.72	2.47	3.76	3.85	3.98	2.51
	Length (mean)	26.5	27.8	28.3	27.8	27.5	27.9	29.0	27.3	27.6	28.2
	Length (mode)	26.0	28.0	28.5	26.0	27.5	29.0	29.0	26.5	28.5	28.0
	Weight	96.7	121	116	109	90.0	96.8	121	105	108	114
	Maturity (mean)	4.70	4.54	6.68	6.65	6.89	6.92	6.80	6.51	6.60	6.37
	Maturity (mode)	5	5	7	7	7	7	7	7	7	7
	Age (mean)	3.96	4.63	4.68	4.95	4.58	4.68	5.38	4.77	4.65	5.00
	Age (mode)	4	5	5	5	5	5	4	5	4	4

Table 3.9: Summary biological parameters for blue whiting representative of human consumption samples production. (n= total of 100 in all cases). For all parameters the minimum and maximum values are reported in ().

		HC-1	HC-2	HC-3	HC-4
Females	Length (mean)	28.7 (23.0-42.0)	28.3 (24.0-32.0)	28.8 (24.5-41)	28.8 (24.5-36.0)
	Length (mode)	28.0	27.5	29.5	29.5
	Weight	129 (61.0-440)	120 (64.0-193)	124 (67.0-417)	110 (59.0-231)
	Maturity (mean)	4.97 (4-7)	5.84 (4-7)	5.00 (3-7)	6.56 (3-70
	Maturity (mode)	5	7	7	7
	Age (mean)	5.16 (2-9)	4.84 (3-9)	5.07 (2-9)	4.89 (3-8)
	Age (mode)	5	5	5	5
Males	Length (mean)	27.0 (23.5-33.0)	25.9 (22.5-31.0)	26.2 (23.0-32.0)	26.3 (23.0-31.5)
	Length (mode)	27.5	26.0	25.0	25.0
	Weight	105 (64.0-207)	88.7 (51.0-136)	88.3 (53.0-161)	87.2 (51.0-145)
	Maturity (mean)	5.11 (3-7)	4.92 (3-7)	5.67 (3-7)	5.96 (1-7)
	Maturity (mode)	5	5	5	7
	Age (mean)	4.89 (2-9)	4.38 (3-6)	4.18 (2-7)	4.57 (3-7)
	Age (mode)	4	4	4	5
Combined	Ratio M/F	1.77	0.50	1.56	0.49
	% lipid	0.47	0.62	0.60	0.60
	Length (mean)	28.1	27.1	27.2	27.9
	Length (mode)	27.5	28.0	26.5	25.0
	Weight	120	104	102	102
	Maturity (mean)	5.02	5.38	5.41	6.37
	Maturity (mode)	5	5	5	7
	Age (mean)	5.06	4.56	4.53	4.79
	Age (mode)	5	5	5	5

3.3.2 PCDDs, PCDFs and PCBs in blue whiting fish oil.

Concentrations and upper-bound TEQs (lipid basis) for dioxins, furans, DL-PCBs and marker PCBs are presented in tables 3.10a to 3.11b. The upperbound approach requires that where individual congeners are reported as being "not detected" (n.d.) that the appropriate LOD be used for the calculation of individual TEQ values. Concentration data in parenthesis indicate the appropriate detection limit.

Total PCDD/PCDF–TEQs in blue whiting fish oil ranged from 5.08 to 12.2 ng kg^{-1} TEQ thereby EU maximum limits (6 pg g^{-1} TEQ) for fish oil was exceeded in 9 out of 10 blue whiting fish oils analysed. The ratio of PCDD-TEQ to PCDF-TEQ between samples was ranged from 0.38 to 0.45. Non-ortho PCB-TEQ values ranged from 10.8 to 36.1 ng kg^{-1} lipid weight, while mono-ortho PCB-TEQs ranged from 3.84 to 12.3 ng kg^{-1} lipid weight. The ratio of non-ortho to mono-ortho PCB-TEQ ranged from 2.81 to 3.16 (R=0.99) also indicative of a degree of homogeneity in the source of these PCBs. Figure 3.7 graphically illustrates the relative contribution of each of the contaminant groupings to the overall TEQ.

The ratio of ΣPCDD/F-TEQ to ΣPCB-TEQ ranged from 0.35 to 0.22 throughout the study period (R^2=0.91). These data suggest that blue whiting may bioaccumulate/eliminate PCBs and PCDD/Fs from their lipid reserves at differing rates throughout the year. An inverse correlation (R^2-0.59) between the ΣPCDD/F-TEQ to ΣPCB-TEQ ratio and the lipid content in representative whole fish homogenates was also determined.

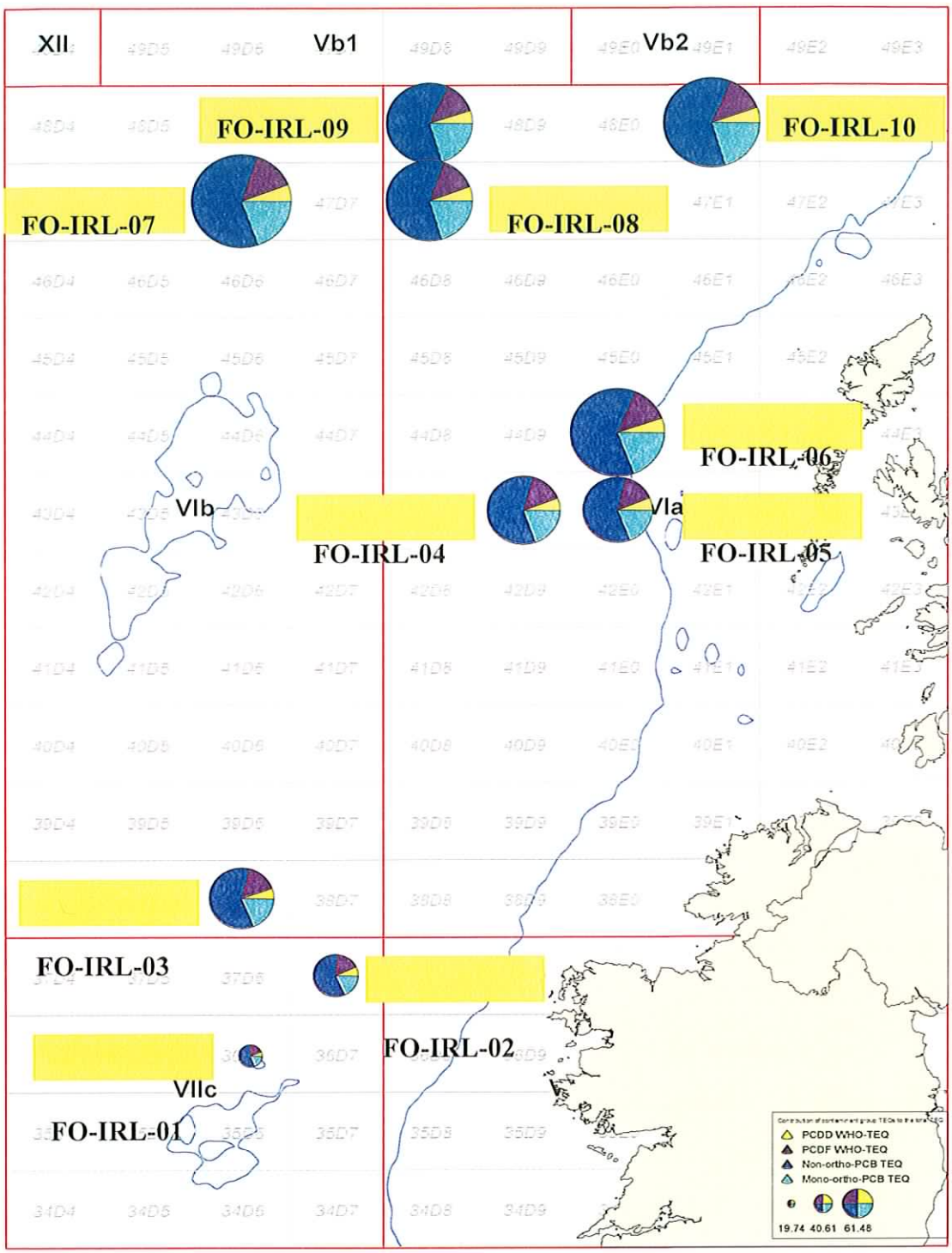

Figure 3.7: The relative contributions of contaminant groups to the total TEQ in blue whiting fish oil

Table 3.10a: Concentrations of PCDD, PCDF, DL-PCBs (pg g^{-1}) in blue whiting fish oil.

	FO-IRL-01	FO-IRL-02	FO-IRL-03	FO-IRL-04	FO-IRL-05	FO-IRL-06	FO-IRL-07	FO-IRL-08	FO-IRL-09	FO-IRL-10
2.3.7.8-Tetra-CDD	0.78	1.16	1.32	1.45	1.55	1.8	2.01	1.82	1.43	1.96
1.2.3.7.8-Penta-CDD	0.52	0.78	0.70	0.94	0.90	0.86	1.01	0.81	0.92	0.86
1.2.3.4.7.8-Hexa-CDD	n.d.(0.1)	0.15	n.d.(0.1)	n.d.(0.1)	n.d.(0.2)	n.d.(0.1)	n.d.(0.1)	n.d.(0.1)	n.d.(0.1)	n.d.(0.1)
1.2.3.6.7.8-Hexa-CDD	1.76	2.87	3.52	3.73	3.31	4.48	5.44	4.94	4.50	4.34
1.2.3.7.8.9-Hexa-CDD	0.42	0.73	0.84	0.92	0.90	1.22	1.32	1.07	1.11	1.18
1.2.3.4.6.7.8-Hepta-CDD	1.33	1.98	2.74	2.98	3.31	3.48	4.26	3.1	3.09	3.53
OCDD	n.d.(0.8)	n.d.(0.8)	1.38	1.10	2.77	1.28	2.20	1.72	1.37	2.07
2.3.7.8-Tetra-CDF	23.6	35.1	47.3	49.4	44.6	55.9	60.8	53.1	46.5	54.1
1.2.3.7.8-Penta-CDF	3.56	5.91	8.12	8.58	8.21	10.4	11.0	9.37	9.21	10.2
2.3.4.7.8-Penta-CDF	1.12	1.42	1.24	1.58	2.05	1.43	1.41	1.44	1.64	1.67
1.2.3.4.7.8-Hexa-CDF	1.07	1.73	1.95	2.17	2.45	2.61	2.77	2.41	2.24	2.33
1.2.3.6.7.8-Hexa-CDF	0.99	1.55	1.90	2.16	2.10	2.67	2.74	2.36	2.35	2.52
1.2.3.7.8.9-Hexa-CDF	n.d.(0.1)	n.d.(0.1)	n.d.(0.09)	n.d.(0.10)	n.d.(0.1)	n.d.(0.1)	n.d.(0.2)	n.d.(0.1)	n.d.(0.1)	n.d.(0.10)
2.3.4.6.7.8-Hexa-CDF	2.03	3.13	4.05	4.34	3.93	4.87	5.49	4.89	4.88	5.37
1.2.3.4.6.7.8-Hepta-CDF	0.86	1.41	1.76	1.85	1.58	2.30	2.32	1.91	1.92	2.1
1.2.3.4.7.8.9-Hepta-CDF	0.41	0.32	0.45	0.79	0.54	0.52	0.68	0.49	0.55	0.46
OCDF	n.d.(0.2)	n.d.(0.2)	n.d.(0.2)	0.40	0.76	n.d.(0.3)	n.d.(0.3)	n.d.(0.3)	n.d.(0.3)	n.d.(0.2)
PCB 77	143	191	231	243	254	289	303	277	271	285
PCB 81	5.93	8.12	8.48	9.08	8.3	9.74	9.53	9.76	10.6	10.4
PCB 126	105	177	246	269	258	349	360	312	321	349
PCB 169	28.0	48.4	70.5	78.9	77.0	101	109	95.1	96.9	109
PCB 105	6733	9431	12673	14875	12256	20728	19723	14206	15360	16997
PCB 114	324	561	866	949	813	1153	1282	1082	1043	1209
PCB 118	18454	28631	39805	47647	46232	57555	60035	57551	57392	62770
PCB 123	354	487	588	654	521	883	1226	937	863	1044
PCB 156	1772	2733	3653	3749	3434	4367	4866	5230	5105	5363
PCB 157	424	590	906	1115	1144	1227	1652	1404	1232	1600
PCB 167	1327	2069	2743	2971	2783	3404	3899	3164	3974	4184

n.d = not detected (LOD)

It should be noted that while legislative maxima exist for PCDD/Fs in fish oils, the majority (65 to 78%) of the total TEQ in the fish oils was provided by the WHO-PCBs.

Table 3.10b: WHO-TEQs (pg g^{-1}) for PCDD, PCDF and DL-PCBs in blue whiting fish oil.

	FO-HRI-01	FO-HRI-02	FO-HRI-03	FO-HRI-04	FO-HRI-05	FO-HRI-06	FO-HRI-07	FO-HRI-08	FO-HRI-09	FO-HRI-10
ΣPCDD-WHO-TEQ	1.55	2.34	2.50	2.90	2.92	3.28	3.75	3.27	2.95	3.42
ΣPCDF-WHO-TEQ	3.53	5.19	6.58	7.06	6.77	7.88	8.49	7.49	6.91	7.81
Σ TEQ-PCDD/F	5.08	7.53	9.07	9.97	9.7	11.2	12.2	10.8	9.86	11.2
Ratio PCDD TEQ/PCDF TEQs	0.44	0.45	0.38	0.41	0.43	0.42	0.44	0.44	0.43	0.44
Σnon-ortho-PCB	10.8	18.3	25.3	27.7	26.6	35.9	37.1	32.2	33.1	36.1
Σmono-ortho-PCB	3.84	5.85	8.08	9.29	8.67	11.4	12.1	11.2	11.1	12.3
Ratio non/mono-ortho TEQs	2.81	3.12	3.13	2.98	3.06	3.16	3.07	2.87	2.97	2.94
Σ TEQ-mono/non-ortho PCBs	14.6	24.2	33.4	37.0	35.3	47.3	49.2	43.4	44.2	48.4
Ratio PCDD/F to Σ PCBs	0.35	0.31	0.27	0.27	0.28	0.24	0.25	0.25	0.22	0.23
Total TEQ	19.7	31.6	42.5	47.0	44.9	58.4	61.5	54.2	54.1	59.6

3.3.2.1 Influences on PCDD/F and PCB levels.

As previously discussed a number of biological parameters including, fish length, weight and age in addition to degree of maturity and total lipid content were determined during this study and their influence on the overall fish oil TEQ levels are further assessed below.

3.3.2.1.1 Spatial aspects and PCDD/F and PCB TEQs

Total-TEQ values in blue whiting fish oils are higher at more northern latitudes than those in oils derived from blue whiting from more southern waters (figure 3.7). Global atmospheric transport and deposition of persistent pollutants tends to increase towards more northern latitudes (32), therefore, while contaminant exposure may increase towards more northerly latitudes the full extent to which such spatial factors may contribute to more elevated contaminant levels in these blue whiting oils cannot be confirmed with this current dataset, primarily because, as sampling progressed in a northerly direction the age classification of fish landed also increased. This increase in

age profile can result in increased bioaccumulation of POPs compared to levels in younger fish. As a result, further sampling and analysis of similar size/age classification fish and greater geographical coverage is required in order to fully clarify the extent to which spatial factors influence contaminant levels.

3.3.2.1.2 Maturity indices and sex ratios as indicators of TEQ levels.

As the sampling season progressed the maturity index of blue whiting increased. While correlations between the mean maturity index and TEQ values for the individual contaminant groupings and/or total TEQ (R=0.81) are evident only two relatively distinct mean maturity scale ranges (4.50 to 4.68 and 6.37 to 6.89) were determined during this study.

It has been reported that male fish can contain contaminant levels that are higher than those observed in females (33), primarily as a function of the fact that females have the ability to transfer some of their burden to offspring during spawning processes. Segregated male and female sample analysis was not completed during this study therefore potential sex dependent influences on contaminant levels are not currently possible to evaluate.

3.3.2.1.3 The influence of fish age on PCDD/F and PCB levels

Increased levels of a number of contaminant groups can often be expressed as a function of specimen age; thereby reflecting continued bioaccumulation of such compounds over time. In general the mean age of fish sampled increased throughout the sampling period. While longevity acts as a likely indicator of more elevated contaminant levels further sampling and contaminants analysis would allow for more detailed age based assessments to be completed.

One comprehensive study by the Nordisk Atlantsamarbejde (NORA) (34) on the levels of PCDD/Fs and dioxins in four commercially important pelagic species found that PCDD/F and WHO-PCB levels were inversely proportional to the level of lipid in the fish and increased with the age of the fish. The authors completed age division analysis of blue whiting also finding that there was a greater tendency to accumulate WHO-PCBs as opposed to PCDD/Fs (34). The results of this current study show good agreement with the findings of the NORA study.

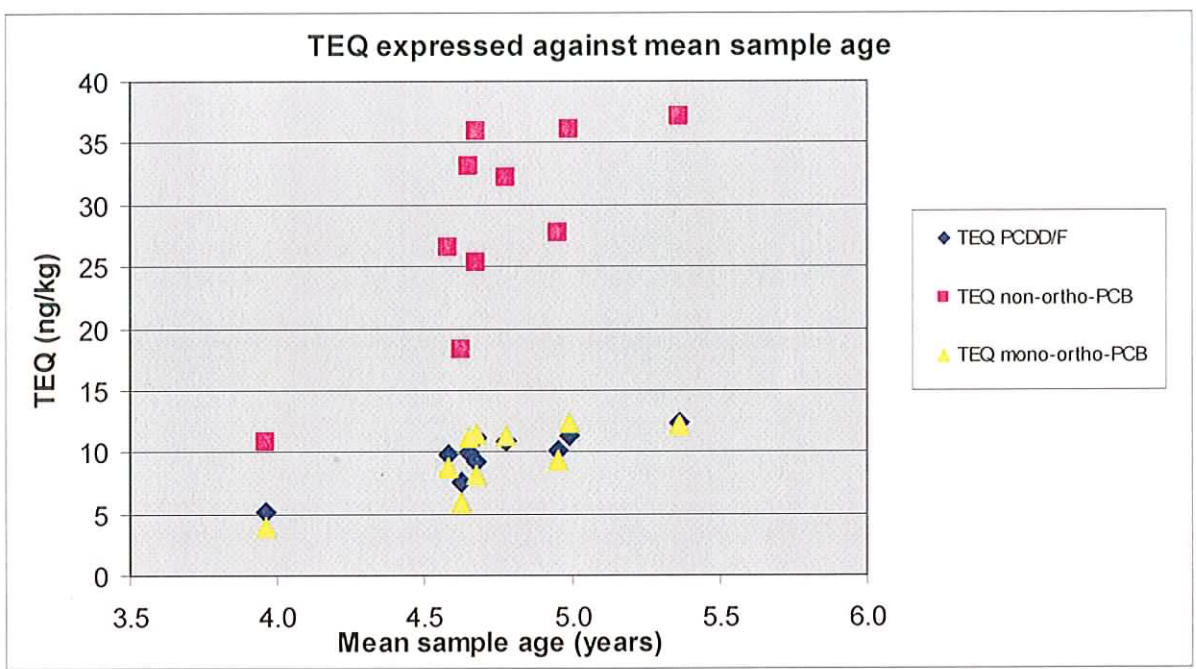

Figure 3.8: Relationship of mean age (yrs) of representative fish and PCDD/F and PCB-TEQ levels (ng kg^{-1}) in blue whiting fish oil

3.3.2.2 Contaminant profiling techniques

As demonstrated in figure 3.8 above, TEQ values for each of the contaminant groupings increase as a function of mean sample age. It was determined that the relative contributions of mono-ortho TEQ to the total TEQ appears to increase over the study period (see figure 3.8). Within the mono-ortho PCB grouping the relative contribution

of PCB 105 to the Σmono-ortho-TEQ decreased while the relative contribution of PCB 118 increased as the study progressed.

Boon (35) suggests that a number of species (including fish) are incapable of metabolising PCB118 therefore PCB118 as a more recalcitrant PCB may accumulate in blue whiting oils at a greater rate than a number of other PCBs. The relative contribution of a number of PCDD and PCDF congeners to their respective TEQ burden were also found to vary throughout the study period. Blue whiting store the majority of their lipid reserves in the liver, as opposed to species such as capelin and/or herring that store their fat in adipose tissue. Blue whiting therefore will retain the majority of its lipophilic contaminant burden in the liver tissue where congener specific contaminant transformation processes may take place. Other factors such as, the potential for differential source inputs and exposure patterns at more northerly latitudes in both the water column and/or via blue whiting dietary influences must be considered in making such assessments. The NORA (34) report concluded that migration area has probably a direct effect on the levels of PCDD/Fs and WHO-PCB as blue whiting are a migratory species they may spend a considerable portion of their lives in an area of the NE Atlantic, distant from area of capture.

3.3.2.3 Influence of lipid content on PCDD/F and PCB levels.

It was demonstrated that TEQ values increase while whole fish total lipid levels decrease (figure) throughout the study period. The relationship between total lipid content and contaminant TEQ in blue whiting oils are further illustrated in figure 3.9. Correlation coefficients (R) of 0.74, 0.72 and 0.68 were determined between whole fish lipid content and non-ortho PCBs, mono-ortho PCBs and PCDD/Fs TEQs respectively.

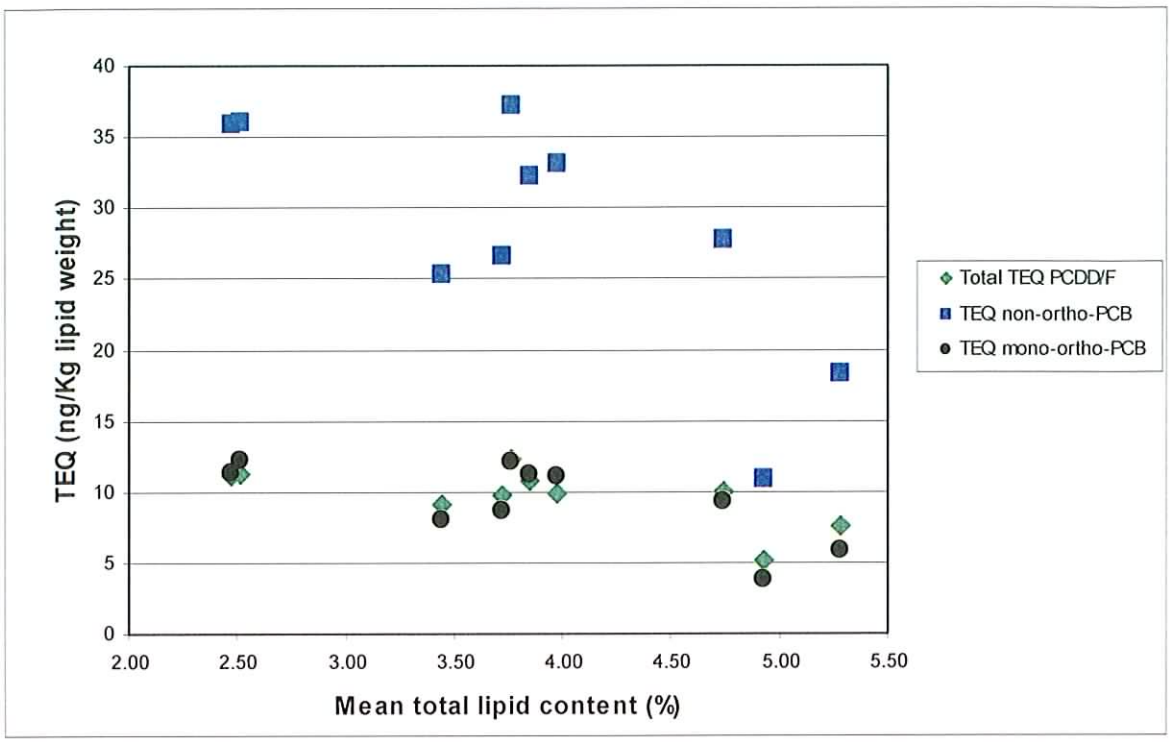

Figure 3.9: Mean total lipid content in representative sample whole fish and contaminant group TEQ (ng kg^{-1}) in process fish oil.

As the percentage lipid increases the overall TEQ decreases. Such lipid dilution effects have been reported to be potentially useful in "locking up" lipophilic contaminants such as PCDD/Fs thereby affording fish a level of protection from potentially harmful contaminant related effects (36).

3.3.3 PCDDs and PCDFs in blue whiting for human consumption.

Table 3.11 presents concentration and TEQ data from four blue whiting fillet tissue samples collected for human consumption purposes. Concentrations and consequently total PCDD/F- TEQs are low ranging from 0.73 to 0.92 pg/g TEQ-wet weight. Total TEQ values for PCDD/F are well within current EU legislative limits for fish muscle for

human consumption (4pg/g WHO-TEQ). It should be noted that WHO-PCBs were not analysed in these samples therefore the total PCDD/PCDF and dioxin-like TEQ value cannot be calculated.

Table 3.11: PCDD and PCDF concentrations and (upperbound TEQs) in blue whiting fillet tissue prepared for human consumption purposes pg g^{-1} and (pg g^{-1} -TEQ) wet weight.

	1081-ORII	1080-ORII	1079-ORII	1082-ORII
2.3.7.8-Tetra-CDD	0.02 (0.02)	0.01 (0.01)	0.01 (0.01)	0.01 (0.01)
1.2.3.7.8-Penta-CDD	<0.01 (0.01)	<0.01 (0.01)	<0.01 (0.01)	<0.01 (0.01)
1.2.3.4.7.8-Hexa-CDD	<0.01 (0.001)	<0.01 (0.001)	< 0.01 (0.001)	<0.01 (0.001)
1.2.3.6.7.8-Hexa-CDD	0.03 (0.003)	0.02 (0.002)	0.02 (0.002)	0.02 (0.002)
1.2.3.7.8.9-Hexa-CDD	0.01 (0.001)	<0.01 (0.001)	<0.01 (0.001)	<0.01 (0.001)
1.2.3.4.6.7.8-Hepta-CDD	0.05 (0.0005)	0.04 (0.0004)	0.03 (0.0003)	0.05 (0.0005)
OCDD	0.2 (0.00002)	0.16 (0.000016)	0.14 (0.000014)	0.24 (0.000024)
Total PCDD upperbound	0.33 (0.036)	0.26 (0.024)	0.23 (0.024)	0.35 (0.025)
2.3.7.8-Tetra-CDF	0.34 (0.034)	0.3 (0.03)	0.31 (0.031)	0.24 (0.024)
1.2.3.7.8-Penta-CDF	0.07 (0.0035)	0.05 (0.0025)	0.06 (0.003)	0.06 (0.003)
2.3.4.7.8-Penta-CDF	0.01 (0.005)	0.01 (0.005)	<0.01 (0.005)	<0.01 (0.005)
1.2.3.4.7.8-Hexa-CDF	0.03 (0.003)	0.02 (0.002)	0.02 (0.002)	0.02 (0.002)
1.2.3.6.7.8-Hexa-CDF	0.02 (0.002)	0.02 (0.002)	0.01 (0.001)	0.02 (0.002)
1.2.3.7.8.9-Hexa-CDF	<0.01 (0.001)	<0.01 (0.001)	<0.01 (0.001)	<0.01 (0.001)
2.3.4.6.7.8-Hexa-CDF	0.02 (0.002)	0.02 (0.002)	0.02 (0.002)	0.03 (0.003)
1.2.3.4.6.7.8-Hepta-CDF	0.06 (0.0006)	0.06 (0.0006)	0.04 (0.0004)	0.07 (0.0007)
1.2.3.4.7.8.9-Hepta-CDF	<0.01 (0.0001)	<0.01 (0.0001)	<0.01 (0.0001)	<0.01 (0.0001)
OCDF	0.02 (0.000002)	0.03 (0.000003)	0.01 (0.000001)	0.03 (0.000003)
Total PCDF upperbound	0.59 (0.051)	0.53 (0.045)	0.5 (0.045)	0.5 (0.041)
Total PCDD/F Upperbound	0.92 (0.0867)	0.79 (0.0696)	0.73 (0.0698)	0.85 (0.0653)

3.3.4 Marker PCB levels in blue whiting fish oil.

Concentration data for marker PCBs in the 10 fish oil samples are presented in Table 3.12. As occurred in the case of PCDD/Fs and WHO-PCBs, the sum of 7 marker PCBs (Σ7PCBs) increased throughout the course of the study period ranging from 127 to 385 ng g^{-1} lipid weight.

The contribution of PCBs 28, 52 and 101 to the overall Σ7PCBs burden was found to decrease over the study period while the relative contribution of PCB 118 increased. The relative contribution of PCB 153 to Σ7PCBs remained steady throughout the study period demonstrative of the reported recalcitrant nature of this PCB in marine biota. Further normalisation of individual PCB concentration data to the concentration of PCB 153 in samples further suggests preferential temporal elimination of PCBs 28, 52 and 101.

Table 3.12: Concentrations of marker PCBs in blue whiting fish oils (ng g^{-1})

	FO-IRL-01	FO-IRL-02	FO-IRL-03	FO-IRL-04	FO-IRL-05	FO-IRL-06	FO-IRL-07	FO-IRL-08	FO-IRL-09	FO-IRL-10
PCB 28	2.90	4.30	5.10	5.00	5.00	6.00	5.90	5.90	5.10	6.50
PCB 52	10.0	13.0	18.0	20.0	18.0	24.0	27.0	21.0	22.0	23.0
PCB 101	21.0	28.0	41.0	43.0	44.0	54.0	51.0	50.0	49.0	53.0
PCB 118	18.5	28.6	39.8	47.6	46.2	57.6	60.0	57.6	57.4	62.8
PCB 138	31.0	45.0	69.0	68.0	74.0	96.0	101	81.0	90.0	100
PCB 153	31.0	47.0	72.0	70.0	71.0	99.0	98.0	80.0	88.0	99.0
PCB 180	13.0	17.0	29.0	28.0	30.0	37.0	41.0	33.0	35.0	41.0
ΣSum 7 PCBs	127	183	274	282	288	374	384	328	346	385

Positive correlations (R= 0.71 to 0.83) were observed between individual marker PCBs throughout the study period. Concentrations of individual PCBs were shown to be inversely proportional (R= 0.69 to 0.81) to the mean lipid content in the sample. These data may suggest that marker PCB bioaccumulation may occur at a different rate to

those of some of the other contaminant groupings. It should be noted however that the potential for spatial differences and/or potential differences in exposure to contaminants must be further considered for future assessments.

No EU legislation currently exists with respect to levels of marker PCBs in fish oils, however country specific guidelines can be applicable.

3.3.5 Organochlorine pesticides in blue whiting fish oil

A suite of 27 organochlorine pesticides were analysed in blue whiting fish oil. Individual compound data are presented in table 3.13 and grouped compound information is presented in table 3.14. Dichloro-diphenyl-trichloroethane (DDT) and its associated metabolites followed by the sum of the three-toxaphene congeners (CHBs 26, 50 and 62) were the most prevalent contaminants in the samples ranging from 157 to 489 ng g^{-1} and from 132 to 452 ng g^{-1} respectively. Levels of endosulphane compounds were low ranging from not detected to 4.50 ng g^{-1} lipid weight.

Inverse correlations between the DDTs, Heptachlors, hexachlorobenzene and chlordanes and total lipid concentrations in representative whole fish were observed (R=-0.75, -0.71, -0.72 and -0.78 respectively). No correlation was evident between levels of HCH compounds in fish oils and the overall whole body total lipid content. HCHs physico-chemical properties do not predispose them to a high degree of bioaccumulation, fish are reported to have sufficient metabolic capacity to eliminate HCH residues from their bodies at rates that ensures that a high degree of bioaccumulation does not take place.

Table 3.13: Concentrations of OCPs in blue whiting fish oils (ng g^{-1}).

	FO-IRL-01	FO-IRL-02	FO-IRL-03	FO-IRL-04	FO-IRL-05	FO-IRL-06	FO-IRL-07	FO-IRL-08	FO-IRL-09	FO-IRL-10
α-HCH	2.50	2.60	2.30	2.50	2.70	2.20	2.40	2.40	2.90	2.80
β-HCH	0.77	0.88	0.79	1.00	0.85	0.65	0.93	0.89	1.10	0.65
γ-HCH	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
δ-HCH	n.d.	n.d.	0.24	n.d.	0.21	n.d.	n.d.	n.d.	n.d.	n.d.
o,p-DDT	11.0	7.8	28.0	29.0	16.0	33.0	34.0	18.0	37.0	41.0
p,p'-DDT	19.0	11.0	50.0	54.0	27.0	69.0	67.0	31.0	71.0	76.0
o,p-DDD	13.0	23.0	29.0	24.0	28.0	28.0	31.0	34.0	27.0	28.0
p,p'-DDD	37.0	66.0	97.0	84.0	80.0	100	109	108	95.0	96.0
o,p-DDE	5.10	6.9	14.0	11.0	10.0	13.0	14.0	12.0	13.0	14.0
p,p'-DDE	72.0	107	155	171	152	209	221	184	222	234
Hexachlorobenzene	28.0	36.0	47.0	49.0	47.0	57.0	62.0	56.0	58.0	61.0
Heptachlor	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
cis Heptachlorepoxide	5.80	6.30	7.00	6.90	6.50	8.50	8.70	7.50	7.50	7.80
trans Heptachlorepoxide	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Aldrin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
CHB 26	45.0	49.0	91.0	90.0	64.0	106	114	80.0	101	65.0
CHB 50	73.0	67.0	153	165	73.0	194	218	116	166	86
CHB 62	37.0	16.0	85.0	95.0	24.0	120	120	45.0	102	49.0
Octachlorstyrene	1.20	2.20	2.80	2.80	2.80	3.70	3.40	3.10	3.20	3.70
Dieldrin	35.0	39.0	45.0	53.0	49.0	56.0	65.0	60.0	59.0	59.0
Endrin	7.70	7.40	10.0	11.0	13.0	11.0	15.0	16.0	34.0	14.0
Endosulphane sulfate	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
α-Endosulphane	n.d.	n.d.	n.d.	n.d.	1.30	2.20	4.50	n.d.	n.d.	n.d.
β-Endosulphane	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
γ-Chlordane	8.00	9.40	14.0	14.0	13.0	17.0	19.0	15.0	18.0	19.0
α-Chlordane	33.0	37.0	56.0	58.0	51.0	74.0	80.0	63.0	73.0	89.0
Oxychlordane	7.70	9.10	13.0	14.0	14.0	19.0	19.0	14.0	16.0	21.0

n.d. = not detected

Table 3.14: Summary concentration data for organochlorine groups in blue whiting fish oil (ng g^{-1}).

	FO-IRL-01	FO-IRL-02	FO-IRL-03	FO-IRL-04	FO-IRL-05	FO-IRL-06	FO-IRL-07	FO-IRL-08	FO-IRL-09	FO-IRL-10
ΣHCHs	3.27	3.48	3.33	3.50	3.76	2.85	3.33	3.29	4.00	3.45
ΣDDTs	157	221	373	373	313	452	476	387	465	489
ΣHeptachlors	5.80	6.30	7.00	6.90	6.50	8.50	8.70	7.50	7.50	7.80
Hexachlorobenzene	28.0	36.0	47.0	49.0	47.0	57.0	62.0	56.0	58.0	61.0
ΣToxaphenes	155	132	329	350	161	420	452	241	369	200
ΣEndosulphanes[1]	n.d.	n.d.	n.d.	n.d.	1.30	2.20	4.50	n.d.	n.d.	n.d.
ΣChlordanes	48.7	55.5	83.0	86.0	78.0	110	118	92.0	107	129
ΣOther cyclodienes[2]	42.7	46.4	55.0	64.0	62.0	67.0	80.0	76.0	93.0	73.0

[1] Sum of α and β endosulphane + endosulphane sulphate

[2] Sum of aldrin, dieldrin and endrin.

n.d = not detected

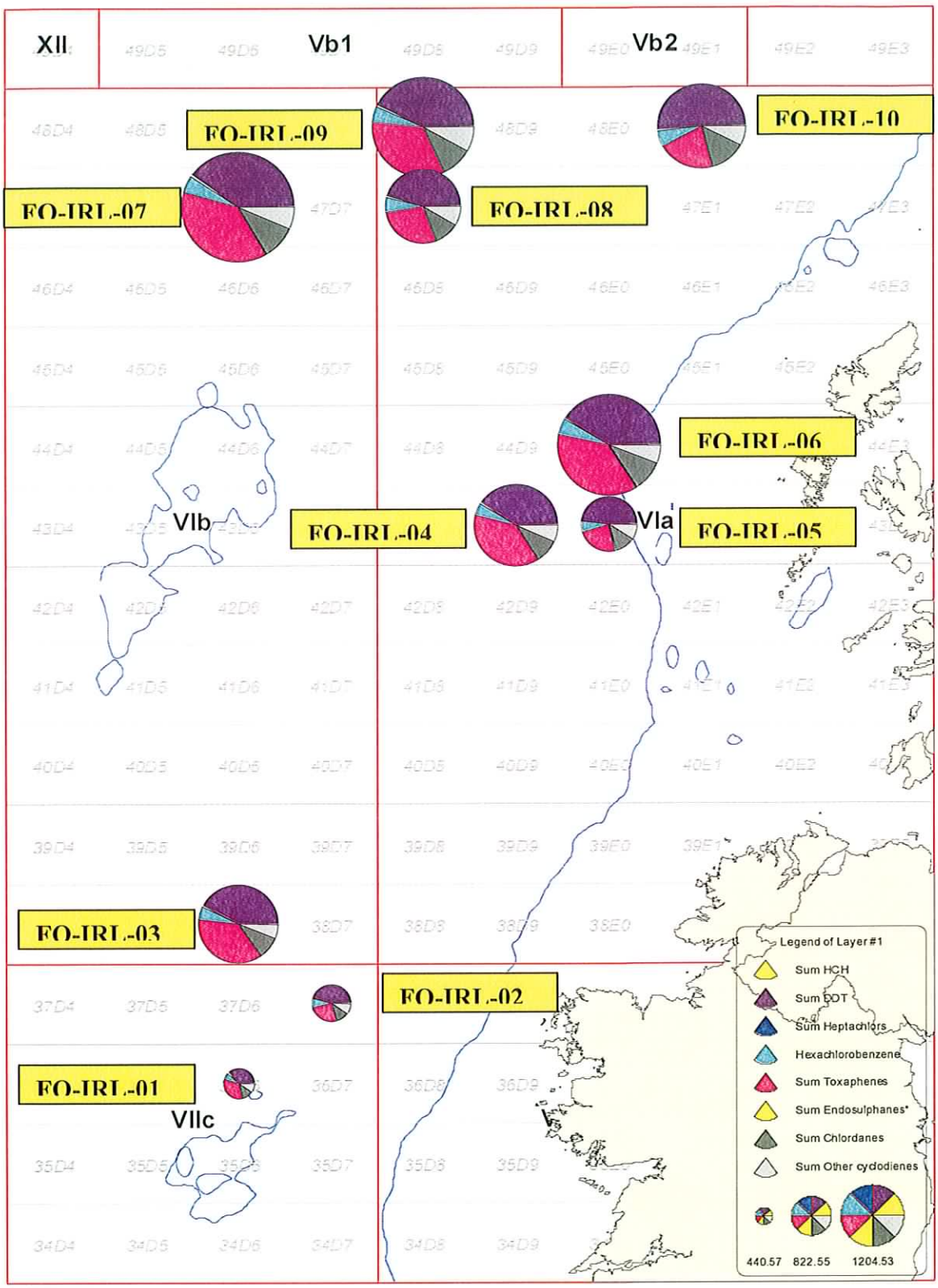

Figure 3.10: The relative contribution of individual OCPs to the total OCP burden.

3.3.6 PBDEs in blue whiting fish oil.

Analysis of 8 PBDE (ΣPBDEs) congeners was completed on the fish oils (see table 3.14). Upperbound ΣPBDEs concentrations ranged from 17.6 to 53.9 ng g^{-1} lipid weight. PBDE 47 was found to be the most prevalent congener in all samples, comprising approximately 52.9 to 54.9% of the total PBDE burden. No EU legislative controls currently exist for PBDE compounds in fish oils.

The ΣPBDE congeners were found to increase as a function of mean fish age (R=0.80). However as older fish were captured in more northerly latitudes the relative contribution (R=0.96) of both spatial and age effects cannot be easily separated with this current dataset.

While the overall concentration of the ΣPBDE congeners increased throughout the sampling period, the relative contribution of each of the congeners to the total PBDE burden remained similar between all samples. This is suggestive that little congener specific elimination/metabolism seems to take place within blue whiting.

Table 3.14: Upperbound concentrations of PBDEs in blue whiting fish oil (ng g^{-1}).

	FO-IRL-01	FO-IRL-02	FO-IRL-03	FO-IRL-04	FO-IRL-05	FO-IRL-06	FO-IRL-07	FO-IRL-08	FO-IRL-09	FO-IRL-10
PBDE 28	0.53	0.74	1.05	1.11	1.10	1.46	1.49	1.26	1.28	1.44
PBDE 47	9.50	14.6	19.2	21.7	21.6	26.8	28.7	24.8	24.5	28.5
PBDE 66	0.54	0.77	1.04	1.16	1.02	1.43	1.49	1.21	1.23	1.28
PBDE 99	2.83	4.31	5.49	5.85	6.01	7.36	7.93	6.44	6.80	8.14
PBDE 100	1.98	3.35	4.51	4.51	4.77	5.61	6.52	5.35	5.53	6.66
PBDE 153	0.49	0.75	0.40	1.21	1.11	1.49	1.60	1.31	1.45	1.62
PBDE 154	1.69	2.39	3.67	3.97	4.49	5.61	5.94	4.96	5.47	6.17
PBDE 183	<0.03	<0.03	0.05	0.06	0.05	0.07	0.08	0.06	0.07	0.07
Upperbound Sum	17.6	26.9	35.4	39.6	40.1	49.9	53.7	45.4	46.3	53.9

3.4 Conclusions.

- 9 out of 10 fish oil samples exceeded the PCDD/F legislative value for fish oil product for use in animal/fish feeds.
- The percentage lipid shows an apparent decrease throughout the duration of the sampling period.
- Blue whiting seem to mature as the fishing season progresses reaching full maturity in mid March to end April.
- There are no apparent relationships in the ratio of males to females in representative samples throughout the sampling period.
- Based on current data there are apparent increasing correlations in the TEQs of PCDD/F, mono-ortho and non-ortho PCBs over the sampling period. However these have to be considered carefully as there are potential stock differences and age profiles to be further considered.
- Lipid normalisation procedures seem to suggest that contaminant burden continues to increase for marker PCBs, OCPs and Dioxin-like PCBs at a greater rate than is observed for dioxins and furans. TEQ values normalised against Smedes total lipid content in representative fresh fish samples suggest that the rate of TEQ increase with respect to contaminants is shown to be greater than the apparent rate of lipid metabolism/excretion/transfer to ova etc within the species.
- The percentage contribution of non-ortho and mono-ortho PCBs remains relatively steady throughout the study period, however the contribution of PCDD/F seems to decrease over the sampling period. The overall influence of differences in stock identities and age related uptake should be further investigated.

- A number of possibilities may contribute to this apparent decrease, some may relate to analytical issues and others may be representative of biological processes within blue whiting that may enable preferential metabolism/excretion of dioxin compounds compared to PCB compounds. Additionally lipid classes containing dioxins (and possibly more specifically tetra substituted forms) may possibly be preferentially excreted/removed etc. The overall influence of differences in stock identities and species age have to be further investigated.
- Tetra-substituted compounds have been assigned the highest TEF of 1.0, which may indicate a higher degree of toxicity to fish than some of the other classes. No such structural dependencies are evident for furan compounds. It should be noted that while individual structural classes may show a downward trend the overall Dioxin related TEQ still increases (R^2=0.94) over the sampling period. The overall influence of differences in stock identities has to be further investigated.
- Increases in TEQ over the sampling period may relate to diet changes throughout the short sampling period. The possibility of whether blue whiting could possibly change diet to one containing higher dioxin/pcb contents would need to be further investigated. There is no apparent change in fish size or weight throughout the sampling period although large amounts of energy are involved in the spawning process.
- While in the majority of cases simple linear models are utilized to investigate potential relationships, in a number of cases other modelling expressions may better describe the relationship. An example of this would be that fish length and age may not be expected to occur in a linear fashion throughout the lifetime of the species. Thereby other statistical models may describe potential trends in a clearer manner.

- The potential for contamination sources within processing/production plant should also be further evaluated.
- Differences in fish age profiles dependent on where and when caught. Over the course of the study the age increased as the sampling location moved more North Easterly.
- Over the course of sampling there was a general increase in contaminant burden, however it is unclear whether this is reflective of differences in location and exposure, age profiles of the samples, lipid content or other factors as all of the above are interlinked.

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3.6 Glossary:

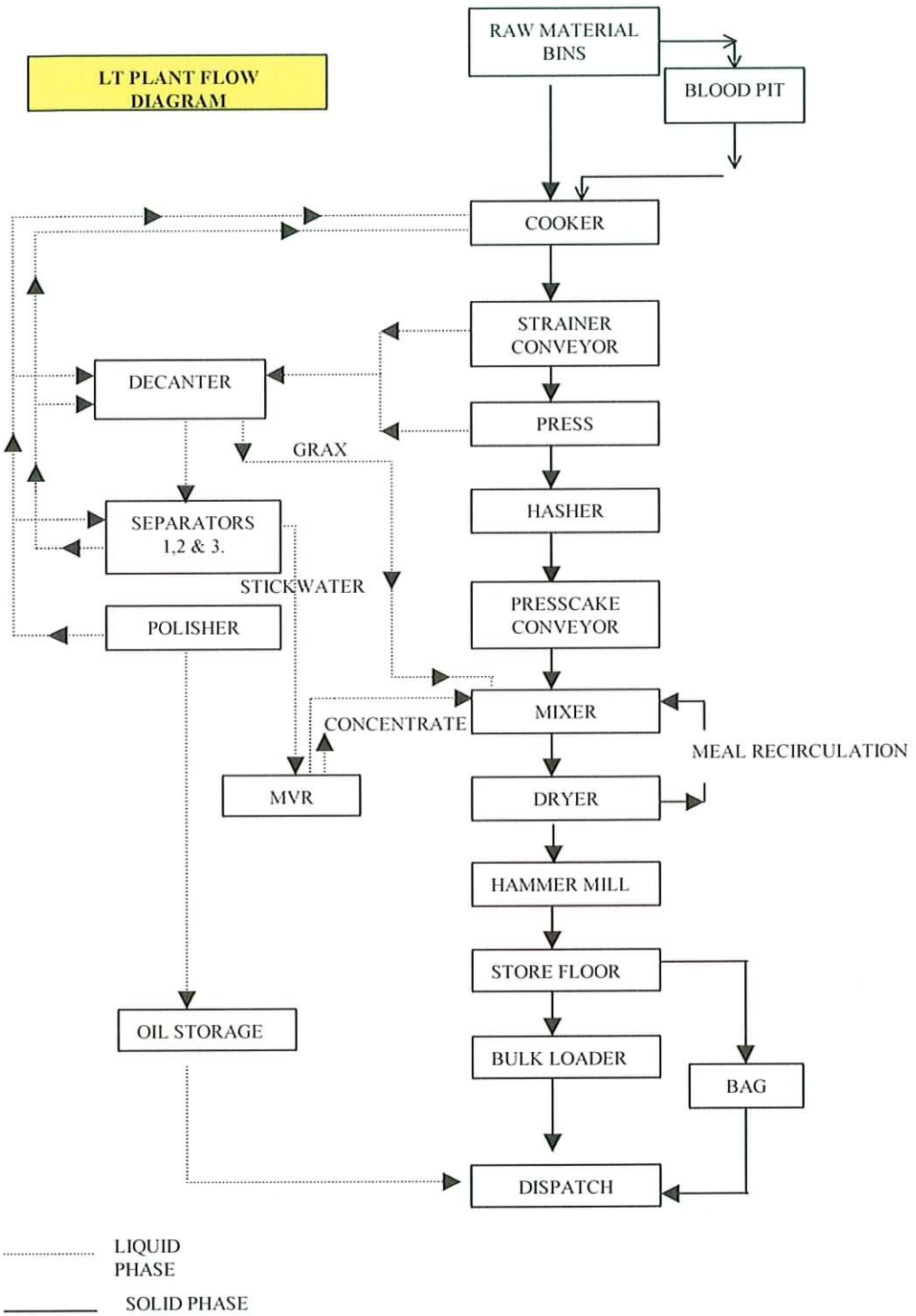
Bioaccumulation: The accumulation of a substance within the tissues of an organism. This includes 'bioconcentration' and uptake via the food chain (OSPAR QSR)(37).

Bioconcentration: Occurs as a result of the direct uptake by an organism of a chemical from the water phase, this generally being expressed in terms bioconcentration factors (BCFs). Factors such as toxic effects, bioavailability, concentration of the chemicals in water, pH of the water, physico-chemical characteristics of the chemical and the lipid content of the organisms are key in describing bioconcentration in marine species (9).

Biomagnification: The process whereby concentrations of certain substances increase with each step in the food chain. (OSPAR QSR)(37).

Biotransformation: Often described as the rate at which contaminants are degraded/structurally altered, metabolised and/or eliminated in an organism often as a result of POP-responsive induction of appropriate enzymes. Increase in contaminant levels may increase biotransformation rates and high proportions of metabolites may indicate high biotransformation capacity within an organism (11).

Annex 3.1: Flow diagram of blue whiting fish oil production process.



Annex 3.2: Percentage recovery of spiked recovery standards in fish oils and in reference fish oil.

Sample Code	FO-IRL-01	FO-IRL-02	FO-IRL-03	FO-IRL-04	FO-IRL-05	FO-IRL-06	FO-IRL-07	FO-IRL-08	FO-IRL-09	FO-IRL-10	Reference
2.3.7.8-Tetra-CDD	69	73	81	77	83	80	76	63	81	81	73
1.2.3.7.8-Penta-CDD	88	95	98	90	94	93	89	79	98	100	102
1.2.3.4.7.8-Hexa-CDD	93	106	92	93	77	91	97	85	102	80	106
1.2.3.6.7.8-Hexa-CDD	89	94	103	104	111	102	91	71	95	103	107
1.2.3.7.8.9-Hexa-CDD	82	84	91	93	94	90	86	71	91	92	91
1.2.3.4.6.7.8-Hepta-CDD	93	102	102	99	95	97	95	81	103	102	100
OCDD	103	111	116	113	107	112	109	91	117	119	93
2.3.7.8-Tetra-CDF	78	85	92	89	96	93	87	75	95	94	84
1.2.3.7.8-Penta-CDF	85	95	101	91	98	91	93	80	103	106	92
2.3.4.7.8-Penta-CDF	89	97	103	94	97	96	93	49	105	89	102
1.2.3.4.7.8-Hexa-CDF	84	92	94	93	83	98	95	81	108	96	101
1.2.3.6.7.8-Hexa-CDF	77	83	88	89	94	85	83	70	90	99	86
1.2.3.7.8.9-Hexa-CDF	85	89	99	100	109	101	95	84	104	108	88
2.3.4.6.7.8-Hexa-CDF	88	96	100	99	96	100	98	80	106	99	102
1.2.3.4.6.7.8-Hepta-CDF	76	84	89	88	87	89	88	73	91	91	86
1.2.3.4.7.8.9-Hepta-CDF	80	92	90	86	97	88	88	79	96	105	83
OCDF	116	122	137	128	120	132	128	110	133	136	98
3.3'.4.4'-Tetra-CB 77	80	88	92	91	65	92	89	76	96	74	91
3.4.4'.5-Tetra-CB 81	82	91	90	94	60	95	94	75	97	64	89
3.3'.4.4'.5-Penta-CB 126	82	87	95	92	68	93	90	75	96	78	92
3.3'.4.4'.5.5'-Hexa-CB 169	88	95	97	90	77	90	88	69	95	85	102
2.3.3'.4.4'-Penta-CB 105	81	90	85	83	96	76	90	101	109	95	91
2.3.4.4'.5-Penta-CB 114	92	99	82	79	100	91	94	91	112	96	95
2.3'.4.4'.5-Penta-CB 118	104	105	92	88	97	96	97	89	102	92	95
2'.3.4.4'.5-Penta-CB 123	93	89	80	83	92	89	90	79	110	97	102
2.3.3'.4.4'.5.-Hexa-CB 156	87	85	85	91	96	100	97	79	97	90	83
2.3.3'.4.4'.5'-Hexa-CB 157	96	105	95	83	79	94	80	83	111	80	92
2.3'.4.4'.5.5'-Hexa-CB 167	87	84	82	83	87	91	94	95	89	83	84
2.3.3'.4.4'.5.5'-Hepta-CB 189	88	82	77	85	79	86	95	86	82	74	86
PCB #28	78	78	69	69	76	77	80	74	81	79	78
PCB #52	80	86	74	72	76	78	76	84	90	83	77
PCB #101	79	88	74	76	75	77	92	79	92	84	82
PCB #138	78	82	67	78	77	69	76	80	83	74	74
PCB #153	84	89	72	83	75	74	77	82	87	75	82
PCB #180	76	93	65	76	68	74	73	77	86	73	75
BDE #28	78	82	120	49	80	51	64	73	92	95	66
BDE #47	120	150	190	80	147	83	295	109	440	142	275
BDE #100	121	71	141	70	120	74	52	95	80	121	70
BDE #99	97	49	128	59	77	60	41	73	61	72	58
BDE #154	112	38	232	92	124	96	83	124	106	122	73
BDE #153	101	72	231	89	122	94	76	115	102	118	61
BDE #183	69	71	136	60	73	63	51	75	64	78	37
BDE #209	335	80	92	58	164	110	9	18	49	170	51
β-HCH	52	52	64	66	72	74	68	75	85	99	52
γ-HCH	53	55	64	70	76	72	72	81	89	102	55
p.p'-DDT	86	74	82	87	76	112	104	101	108	107	91
p.p'-DDE	67	68	57	75	81	76	83	87	95	100	87
Hexachlorobenzene	52	58	64	70	78	71	72	82	90	98	53
PCB #52	52	57	66	71	78	76	75	85	91	103	58
Dieldrin	70	72	84	78	79	84	82	84	87	92	90
PCB #101	67	77	79	84	92	83	90	100	105	107	81
Phenanthrene	48	52	47	52	41	37	49	45	46	48	
Anthracene	43	48	43	46	37	36	44	42	42	44	
Fluoranthene	58	55	56	62	57	48	62	53	56	69	
Pyrene	57	49	53	62	52	45	61	52	53	64	
Benzo[b]naphtho [2,1-d] thiophene	54	50	51	53	48	44	53	47	52	55	
Benzo[c]phenanthrene	57	62	55	60	56	51	55	52	57	62	
Benzo[a]anthracene	57	62	55	60	56	51	55	52	57	62	
Chrysene - Triphenylene	59	59	56	61	54	52	68	54	57	64	
Benzo[ghi]fluoranthene	65	72	61	67	65	63	67	63	63	67	
Benzo[b+j+k]fluoranthene	65	72	61	67	65	63	67	63	63	67	
Benzo[e]pyrene	69	73	66	73	71	67	68	70	60	68	
Benzo[a]pyrene	69	73	66	73	71	67	68	70	60	68	
Indeno[1.2.3-cd]pyrene	70	95	64	69	77	74	71	76	69	62	
Benzo[ghi]perylene	69	69	69	79	77	76	71	79	62	70	
Anthanthrene	69	69	69	79	77	76	71	79	62	70	
Dibenz[ah]anthracene	62	76	70	77	71	70	69	78	60	65	
Coronene	46	65	69	73	75	75	60	63	54	57	

CHAPTER 4:THE OCCURRENCE OF DIOXINS, FURANS, POLYCHLORINATED BIPHENYLS AND BROMINATED FLAME RETARDANTS IN THE EUROPEAN EEL *(ANGUILLA ANGUILLA)* FROM IRISH WATERS.

4.1 Introduction

The European eel *Anguilla anguilla* is widely utilized by a number of national and international monitoring programmes as a bio-monitor of pollution. Concerns related to eel population declines have widely been reported across the animals natural European range (1) and the number of young eels joining existing depleted stocks has in some cases has been estimated to be as low as 1% of historic levels. Such declines have led to calls for management action to reverse these declines (2-4) ultimately leading to the European Commission recently proposing mandatory seasonal closure of eel fisheries until such time as Member States have in place national plans to ensure recovery of stocks (5).

Throughout Europe the numbers of glass eels returning from sea crashed in the early 1980s, to 10% or less of the historical mean of the 1960s and 1970s (6). While the cause of the eel stock collapse remains unidentified, it is likely to include a combination of anthropogenic mortality along with environmental degradation. The role of oceanic factors in spawning success and juvenile survival also remains unknown. ICES (2006) (7) suggest that spawner quality issues (i.e. parasites, disease, contaminants) are highly likely to impact on migration and spawning success. Lack of reliable data over the wide geographical range of the eel make it difficult to assess the impact of these quality issues on the global spawning stock of European Eel and further field data will be required to allow the impact of such factors be evaluated.

Robinet and Feunteun (8) suggest that there is strong evidence to suggest that compounds commonly used in agriculture, industry or found in sewerage, affect the biological cycle of eels. Previous studies have suggested that the European eel has a low sensitivity to chemical pollution, while Knights (9) concluded that organochlorine

contaminants, were not a major causative factor in recruitment declines, however the author states that given the substantial information gaps caution must be observed in interpreting such data

4.1.1 Eel biology summary

Eels spend a large proportion of their lives in estuarine and/or freshwater systems which when combined with their long lifecycle, relatively localised habitat and diverse feeding patterns (crustaceans, worms, snails, insect larvae and on occasion small fish), can result in the eel accumulating substantial contaminant body burdens (10).

While our understanding of the biology of the European eel still contains a number information gaps it is well documented that the eel progresses through five principal life cycle stages namely, the leptocephalus, glass eel, elver, yellow and silver eel stages (11). The leptocephali metamorphose into glass eels and a proportion migrate upstream as elvers. Pigmented elvers develop into the feeding yellow eel stage, which continue to feed and grow in a wide range of habitats in marine and freshwater, before completing their life cycle and metamorphosing to the silver eel stage for migration to the spawning grounds of the Sargasso Sea (12). This ability for eels to inhabit such a diverse range of marine and freshwater environments, along with a wide variety of diets, may subject eels to potentially different routes (sources) of exposure to environmental contaminants.

4.1.2 Persistent organic pollutants summary

Within the scope of this current chapter, PCDDs, PCDFs, WHO-PCBs, Marker PCBs, OCPs and PBDEs were analysed in eel muscle tissue. Physico-chemical properties, sources and associated toxicological information are documented in chapters 1 and 3. In addition to PBDEs, Hexabromocyclododecane (HBCD), Tetrabromobisphenol-A

(TBBPA) and Polybrominated Bipenyls (PBBs) were analysed during this work, their properties, sources and toxicological risks are further described below.

Hexabromocyclododecane (HBCD) has primarily been used to improve the flame retardant characteristics of extruded and expanded polystyrene products. HBCD comprises three diasteroisomers (α, β and γ), with γ-HBCD contributing approximately 80% to the technical formulation (13). TBBPA is the primary flame retardant used in electronic circuit boards and as such has been reported as being one of the most widely used BFRs. Although evidence for the environmental presence of HBCD and TBBPA is limited, their detection in a wide range of matrices is a potential environmental concern. Decabromodiphenyl ether (deca-BDE) and TBBPA account for approximately 50% of the world's usage of BFRs however relatively few data are available on the environmental distribution of these two compounds.

Polybrominated biphenyl (PBB) usage has in the main been limited to within the USA (14), while production in Europe has been limited to that of decabrominated biphenyl (decaBB), the manufacture of which terminated in 2000 (14-15). While PBBs have been shown to have long-term effects on the balance of endocrine systems (14) few datasets exist to document the prevalence of the compound in marine biota.

4.1.3 Stable isotopes in dietary and contaminant studies

The measurement of stable isotopes of nitrogen and carbon has been employed in various studies to assess relative trophic level status in biotic systems and to assess dietary carbon sources in aquatic biota. As such stable isotope analysis has been shown to provide a quantitative, continuous variable to study biomagnification of contaminants and to demonstrate trophic interactions within complex food webs (16-17). Changes in

the isotopic ratio ($\delta^{15}N$) between ^{14}N and ^{15}N of a consumer can typically be enriched by 3–4‰ relative to its prey thereby potentially providing a measure of trophic status relative to animals primary prey (18-20),.

Ratios of carbon ^{13}C and ^{12}C isotopes ($\delta^{13}C$) change little as carbon moves through the food web (21-23); therefore ($\delta^{13}C$) measurements can typically be used in evaluation of the ultimate carbon sources of an organism. The use of stable isotope based approaches in combination with traditional methodologies such as gut contents analysis can account for dietary assimilation over extended feeding periods (18) and can provide data related to the overall feeding ecology, potentially allowing for routes of contaminant uptake within a species or food web to be further elucidated.

4.1.4 Contaminant toxicology and health effects in eels and marine biota

Geyer et al report that increased bioaccumulation of a number of persistent pollutants can in part be described as a function of the physical and chemical properties of the individual compounds and of species lipid content. Tesch (23) reports high lipidosomatic ratios in eels (up to 31%) compared to other teleost fishes, thereby potentially subjecting them to higher contaminant exposure, while Rasmussen (24) and Thomann (25) further suggest that high trophic level fish are at increased risk of pollutant accumulation.

A number of studies suggest that organochlorine compounds having an octanol-water coefficient (log K_{ow}) of >5 can easily be transferred to predator organism via their prey. With the exception of molecules such as Octachlorodibenzo-*p*-dioxin (OCDD) bioaccumulation of molecules with a Log K_{ow}>6.5 tend to decrease, probably as a result of molecules becoming too large to cross cell membranes. Thomann and Connolly (26)

and Castonguay (27) have both validated this theory in the case of PCBS, additionally Thomann (28) further suggests that the excretion rate of hydrophobic molecules is inversely proportional to its Log K_{ow}.

Once present within consumer lipid reserves, top of food web species/predators can be subject to high degree of bioaccumulation of POP compounds. deBoer has reported half-life in eels of 480 days for lindane and between 380 and 1450 days for PCBs dependent on the level of halogenation on the molecule (29).

Corsi (30) suggest an absence of detoxification/metabolism processes for OCPs and PCBs in eels potentially affecting spawning success of the species. Exposure to chlorinated compounds; particularly dioxins and furans (31-32) have been shown to decrease reproductive success in fish. Lindane and some PCBs have been shown to disturb lipid storage mechanisms (by affecting thyroid function) and ovarian development in fish (33). Recently Palstra (34) demonstrated an inverse relationship between the TEQ level and the survival period of the fertilised eel eggs strongly suggesting that levels of dioxin-like compounds presently found in some countries, seriously impair the reproduction of the European eel.

Limited fish toxicity data (35-36) for brominated flame retardants are currently available however PBDEs have in some species been shown to exhibit weak dioxin-like and endocrine disruptor activity. Morris (37) demonstrated HBCD bioaccumulation at the trophic level with increased biomagnification ascending through the aquatic food chain with such observations justifying risk assessment studies on an ecosystem level. While HBCDs chemical properties deem it to be relatively insoluble in water, some

studies indicate that its aquatic toxicity is high, with LC_{50} concentrations at ppb ($\mu g\ l^{-1}$) level for some invertebrate and fish species having been reported (38).

Feunteun (1) and Versonnen (10) report that while contaminant lethal effects in adult eels generally only occurs at very high exposure levels, sub-lethal effects on physiology and on spawning success on reaching sexual maturity cannot be discounted. Boetius and Boetius (39) suggest that in order to generate sufficient energy reserves to complete migration, gamete production and spawning, total stored lipid reserves must exceed 20% of the eel's body weight. The "mobilization" of these lipid reserves during the transition from yellow to silver stages, prior to migration may release substantial quantities of persistent organic pollutants and heavy metals into the circulatory system, potentially impacting on gonad function during spawning and on the subsequent return of healthy larvae and young eels (8).

4.1.5 Human and other toxicological and health effects.

Non-ortho (PCBs 77, 81, 126 and 169) and mono-ortho (PCBs 105, 114, 118, 123, 156, 157, 167 and 189) PCBs are assumed to have essentially the same toxicity profile as the dioxins and furans, since they bind to the same Ah- receptor as dioxins. Other PCBs (non-dioxin-like PCBs) do not exert their toxicological effects via binding to the Ah receptor but have been shown to exert a number of toxic responses including developmental effects, immuno- and neurotoxicity, endocrine disrupting effects and tumour promotion.

BFR acute toxicity is considered low but chronic exposure to certain PBDEs has been linked to abnormal brain and skeletal development in animals with potential for long-term impacts on memory, learning and behavior (40-41), such data has leading to

concerns that similar effects may be of relevance to humans (42). PBDEs have also been demonstrated to exhibit endocrine disrupting effects with parent compounds or metabolites interacting with both estrogen and thyroid hormone systems (43-45) while effects mediated on the immune system have also been reported (36, 40).

Lindstrom (46), Meneses (47), Strandman (48) and She (49) all report PBDEs as common contaminants in humans. Lower brominated PBDEs have been reported in blood and breast milk, particularly in individuals living in areas where Penta-PBDEs are still in use (13, 50-51). Harrad reports that the key exposure route to PBDEs generally occurs through a combination of food contamination, direct exposure via consumer products and/or contaminated dusts (52).

Chronic liver toxicity to HBCD has been reported to occur at only relatively high doses, while impacts on blood thyroid hormones have been reported at lower levels, again suggesting that HBCD may potentially possess endocrine disrupting properties (36). Neurological and behavioural responses associated with impacts on nervous system receptors have been reported in mice exposed to HBCD, concurrent exposure to elevated PCB levels further increased overall impacts (41). Reistad reported *in vitro* neurological damage concluding that HBCD yielded the most potent response of all brominated flame-retardants tested (53).

Santillo (54) recently reported low levels of BFRs in eels from two rural, non-industrialised catchments in the west of Ireland. However, few such contaminant data exist on the occurrence of persistent organic pollutants in Irish eels, therefore this current study describes concentration data for a number of persistent organic pollutants (POPs) including dioxins, PCBs, pesticides and brominated flame retardant compounds in eels sampled at five locations around Ireland. This report further assesses the

potential for contaminant mediated adverse effects to the consumer and on the health status of eel itself.

On the basis of such concerns, improvement of the current database on the levels of POPs in eels from Irish waters is merited. The determination of PCBs, dioxin-like PCBs, PCDD/Fs, organochlorine pesticides and a number of BFRs in this current study substantially adds to this current, and especially to the available data for eels from Irish waters. The measurement of stable isotopes attempts to further elucidate habitat and dietary preferences of these eels and allows for the further investigation of the potential role of relative trophic status on contaminant levels.

4.2 Materials and methodology

As part of this initial survey of contaminant levels in eels from Irish waters, samples were collected at five locations throughout Ireland with sites selected to give broad geographical coverage as an initial survey. Eels were obtained between the end of October and mid-November 2005 from commercial fishermen or from fish monitoring traps in the case of those from the Burrishoole catchment. The restricted sampling period additionally minimised the potential for seasonal variation in diet between individual sampling events. Sampling location details are presented in table 4.1 and in figure 4.1.

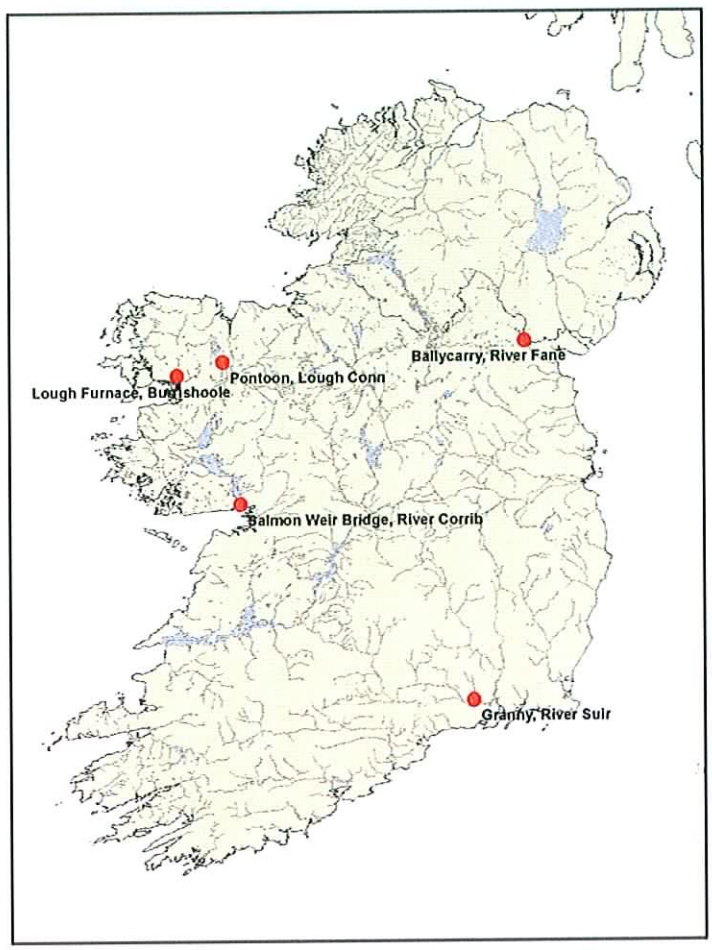


Figure 4.1: Sampling location map for eels collected for contaminants analysis.

Table 4.1: Sampling details for eels collected for biological and contaminants analysis.

MI Reference	Ecotype	Location	Longitude	Latitude	Date Fished	Individuals (n)
MSC/05/1119	Yellow eel	Waterford, River Suir	07° 10.0	52° 16.6	NA	10
MSC/05/1120	Silver eel	Mayo, L. Conn	09° 11.8	53° 59.3	11/08/2005	9
MSC/05/1121	Silver eel	Galway, River Wier	09° 31.0	53° 16.5	10/27/2005	10
MSC/05/1122	Silver eel	Monaghan, River Fane	06° 39.8	54° 04.0	11/04/2005	10
MSC/05/1140	Silver eel	Burrishoole, Co. Mayo	09° 35.0	53° 55.1	11/29/2005	10

4.2.1 Sampling methodology – eel biology

At each site at least 210 eels were randomly selected from each catch. At four of the five sites downstream migrating silver eels were captured by use of a hand net (mesh size 3x3 mm) while at the River Suir, eel-pots in tidal waters were employed to capture yellow eels. 100 eels were immediately anaesthetised, with chlorobutanol, measured (± 0.1 cm) and frozen for further biological examination and for subsequent removal of otoliths for ageing purposes. The remaining eels were also anaesthetised, measured then revived in freshwater before being returned alive to the water. 10 eels, which were not subjected to anaesthetic, were individually bagged and frozen on return to the lab for use in contaminant analysis.

Eels were sexed macroscopically by dissection and ageing analysis was carried out utilising otoliths prepared by burning and cracking (56-58), followed by reading under x100 magnification by two independent readers. Where discrepancies were reported, a third reading was taken and all three readings were then averaged.

4.2.2 Sampling methodology – contaminants analysis

In order to minimise potential effects of natural variation within individuals, pooled eel samples were prepared. A scalpel cut was made directly behind the pectoral fin, and a parallel cut of the same length was then made approximately 3cm further down the flank, towards the tail. The skin was then peeled away to expose muscle tissue. Subcutaneous lipid was removed from skin and returned to the sample muscle tissue; samples were then aggregated with each pooled sample containing skinless muscle tissue from 9 or 10 individual eels (see table 4.1). Pooled samples were then homogenized, sub-sampled and stored at <-18°C prior to analysis for a range of

contaminant suites flame retardants (incl. Dioxins/Furans, PCBs, OCPs, PBDEs, PBBs, TBBPA and total HBCD (sum of α-, β-, γ- diastereomers) by Eurofins Europe.

4.2.3 Sample analysis

Samples were analysed by Eurofins/ERGO, ERGO Forschungsgesellschaft mbH, Hamburg Germany according to EN ISO 17025 accredited methods GfA QMA 504-191/203/205. Analytical methodology complies with the requirements for the HRGC/HRMS confirmatory analysis of food for PCDD/Fs and PCBs as laid down by the EU directive 2002/69 as amended.

For the analysis of brominated flame retardant compounds, a GfA-established GC/MS method was used.

4.2.3.1 Dioxins/PCBs and OCP analysis.

WHO-PCBs were determined by high-resolution gas chromatography/high resolution mass spectrometry (HRGC/HRMS) following sample extraction and clean-up on a carbon/glass-fibre column. Separation and quantification were carried out on a VG-AutoSpec and/or Finnigan MAT 95 XL with DB-5 capillary columns with chromatographic responses for two isotope masses measured for each compound. Quantification was completed by the use of internal/external standard mixtures (isotope dilution method). Toxicity equivalents (TEQs), utilizing WHO-TEFs (See table 4.2) were calculated for each of the samples.

Marker-PCBs and OCPs were determined by HRGC/HRMS on a DB-5 capillary column following solvent extraction and clean-up on an alumina/silica column. For each substance two isotope masses were measured. Quantification was carried out with the use of internal/external standard mixtures.

4.2.3.2 Brominated Flame Retardant analysis

Tissue samples were extracted with n-hexane and solvent extracts were further treated on an H_2SO_4/SiO_2 clean-up column. PBDEs, PBBs, total HBCD and TBBPA were quantified by HRGC/HRMS as per WHO PCBs by means of internal / external standards (isotope dilution).

4.2.3.3 Stable isotope analysis.

The presence of lipid in biotic tissue samples can affect isotopic ratio determinations (58) therefore lipid was removed from all eel tissue samples according to the method of Smedes (59-60), prior to stable isotope analysis. Lipid free tissues were then freeze-dried and approximately 1 mg of sample was transferred to a 9x15 mm tin capsule before combustion in the presence of O_2 and Cr_2O_3 at 1700° in a Carlo Erba NCS 2500 element analyser. Reduction of NO_x to N_2 was then performed in a Cu oven at 650 °C. H_2O was removed in a $KMnO_4$ chemical trap before separation of N_2 and CO_2 on a 3 m Poraplot Q GC column prior to on-line detection of $\delta^{15}N$, $\delta^{13}C$ and $\delta^{34}S$ on a Micromass Optima, Isotope Ratio Mass Spectrometer.

Differences in stable isotope abundances are expressed by (δ) notation as the deviation from standards in parts per thousand (‰) by,

EQUATION 12= $\delta X = [(R_{sample} / R_{Standard}) - 1] \times 1000$

Where,

X relates to ^{13}C or ^{15}N and R is the corresponding ratio $^{13}C/^{12}C$, $^{15}N/^{14}N$ or $^{34}S/^{35}S$. $R_{standard}$ for ^{13}C and ^{15}N relate to Pee Dee Belemnite standard and atmospheric N_2, values respectively.

Isotopic $\delta^{15}N$ and $\delta^{13}C$ ratios in marine web predator tissues are primarily compared to the isotopic composition of baseline phytoplankton and zooplankton sources in the particulate organic matter (POM) of surrounding waters. In the case of marine species where POM data are unavailable, a mean $\delta^{15}N$ value of 5% has been widely utilized as a measure of offshore POM, however inherent variation between offshore and coastal POM values render the use of such estimations unsafe, especially where an animal's native habitat is unclear. Eels in this present study come from a diverse range of habitats (freshwater, coastal/estuarine) therefore, due to the lack of such primary and/or secondary trophic level prey data; direct ($\delta^{15}N$) data were used as a measure of the relative trophic status of these eels.

4.2.3.4 WHO- and Fish- Toxic Equivalency Factors.

The toxic equivalency approach (61) is previously documented and is not expanded on in this current work. Additionally current EU legislative controls are previously discussed.

4.2.4 Analytical quality control

A number of quality controls steps were incorporated into the analysis of each of the contaminants groupings, details are presented below.

4.2.4.1 Stable isotope analysis.

The accuracy and precision of the $\delta^{15}N$, $\delta^{13}C$ and $\delta^{34}S$ analyses were checked, using an International reference material and an internal laboratory reference trout standard. Analysis results (Mean ± 1 Std dev.) for the International standard from 2000 to present are presented in table 4.2 below:

Table 4.2: Quality control data for stable isotope International trout standard and laboratory reference material.

Standard	IFE results	International values	Laboratory Reference material
IAEA-N-1 ($\delta^{15}N_{AIR}$):	0.53‰ ± 0.22	0.538‰ ± 0.186	$\delta^{15}N_{AIR}$: 11.56‰ ± 0.14
IAEA-N-2 ($\delta^{15}N_{AIR}$):	20.38‰ ± 0.30	20.343‰ ± 0.473	
USGS-24 ($\delta^{13}C_{VPDB}$):	-16.02‰ ± 0.17	-15.994‰ ± 0.105	$\delta^{13}C_{VPDB}$:-20.14‰ ± 0.09
NBS 127 ($\delta^{34}S_{CDT}$)	20.38‰ ± 0.27	20.315‰ ± 0.357	$\delta^{34}S_{CDT}$: 16.13‰ ±0.53

The laboratory trout standard was prepared by Soxhlet extraction with CH_2Cl_2 : 7 % CH_3OH for approximately 2 hours, cleansed with 2N HCl for approximately 5 minutes and rinsed with distilled water to neutral pH. The $\delta^{15}N$ and $\delta^{13}C$ composition of laboratory trout has been calibrated against IAEA-N-1 and IAEA-N-2.

These data are all well within quality control limits demonstrating that a high level of quality control was achieved during the course of this present study.

4.2.4.2 Contaminants analysis quality control.

A combination of appropriate certified and/or laboratory reference materials were analysed with samples during this study. Additionally prior to sample extraction and clean-up procedures, representative ^{13}C labeled standards were spiked onto each of the eel samples as follows, PCBs 77,81 126 and 169 (Non-Ortho), PCBs, 105, 118, 123, 156, 157 and 167 189 (Mono-ortho) and PCBs 28, 52, 101, 118, 138, 153 and 180 (Marker PCBs), p,p'-DDT, p,p'-DDE, β-HCH, γ-HCH, pentachlorobenzene, hexachlorobenzene, dieldrin (OCPs), PBDEs 3, 15, 28, 47, 99, 153, 154, 197, 207, 209 (PBDEs), PBBs 52 and 153 in addition to ^{13}C TBBPA and ^{13}C α –HBCD.

Recoveries for Non-ortho, mono-ortho and marker PCBs ranged from 62 to 105%, 70 to 110% and 77 to 118% respectively. Recoveries of PBDEs were of the order 83 to 135%, PBB recoveries ranged 60 to 100% while HBCD and TBPPA recoveries were lower between 41 to 70 % and 25 to 50% respectively.

4.3 Results and Discussion

A number of different techniques were employed in order to analyse both biological and contaminants data, details are presented below.

4.3.1 Biological characteristics of pooled contaminant samples.

As previously described a total of 10 eels were collected at each sampling site for the purpose of analytical analysis. Student-t and F-test statistics were calculated to ascertain whether eels sampled for contaminants analysis were of similar size (and consequently age) as those of the overall population at each sampling site.

It was determined that variances in eel length (cm) between the pooled individuals for contaminants analysis (n=10) and corresponding biological sample population (n>=210) were found to be homogeneous ($P<0.05$) for four of the five locations sampled. In the case of the Burrishoole sample (MSC/05/1140), the overall sample population variance was found to be greater than that of the pooled contaminant sample. Additionally the mean age (32.3 yrs) of pooled individuals in the Burrishoole sample is greater than the average age of eels from other sampling sites (16.0 to 19.8 years) (see table 4.3).

In a number of fish studies a degree of correlation between average length of pooled samples and analytical concentrations of PBDEs, HBCD and PCBs can often be demonstrated, no such relationship was evident in this present study.

While POP levels are relatively low in all eel muscle samples analysed, the potential for factors such as localised contaminant events, and/or potential mobilization of lipid reserves to other organs (as a response to beginning of migration) need to be considered when evaluating such contaminant data.

Table 4.3: Summary length (cm) and age (yrs) statistics for both the overall sample population and the pooled analytical sample for eels collected during this study. Pooled analytical sample data are reported in parenthesis.

Sample ID	MSC-05/1122	MSC-05/1119	MSC-05/1140	MSC-05/1120	MSC-05/1121
Location	River Fane	River Suir	Burrishoole	Pontoon/Moy	River Corrib
Mean Length (cm)	45.7(43.7)	40.1 (46.9)	48.8 (52.8)	47.5 (53.1)	46.4 (52.2)
Std. Dev. (cm)	11.6(9.62)	9.92 (10.7)	9.69 (4.29)	8.73 (6.17)	9.54 (6.97)
RSD (%)	25.3 (22.1)	24.8 (22.8)	19.8 (8.14)	18.4 (11.6)	20.6 (13.4)
95thile	65.1(58.6)	60.0 (61.5)	59.0 (57.6)	60.4 (62.6)	61.8 (59.5)
Variance	134 (92.7)	98.4 (113)	93.9 (18.4)	76.2 (38.1)	90.9 (48.6)
Mean Age (yrs)[1]	17.9	16.0	32.3	19.8	19.4

[1] Mean Age determined in sample for analytical analysis only.

4.3.2 Stable isotopes analysis

The role of stable isotope analysis in describing food web derived carbon and nitrogen sources has been discussed by a number of authors with Hobson (62) reporting that $\delta^{13}C$ is often a more useful indicator of the origin of consumer carbon sources than it is as an indicator of trophic status. Table 4.4 presents stable isotope data for each of the three isotopes determined in eels from this study.

Table 4.4: Muscle tissue stable isotope ratios (‰) in eels from Irish waters.

MI Reference	Ecotype	δ^{13}CVPDB	δ^{15}NAIR	δ^{34}SCDT
MSC/05/1119	Yellow eel	-21.1	15.2	16.8
MSC/05/1120	Silver eel	-29.6	13.4	16.6
MSC/05/1121	Silver eel	-25.8	13.9	10.2
MSC/05/1122	Silver eel	-29.4	14.6	3.27 (0.23)[1]
MSC/05/1140	Silver eel	-28.6	11.1	12.4

[1] Mean and (Std dev) derived from triplicate analysis

4.3.2.1 Carbon and Nitrogen isotope ratio analysis

$\delta^{13}C$ values for the four silver eels in this study were in the range –25.8 to –29.6‰ are indicative of the influence of freshwater or terrigenous organic matter on the diet of these animals and potentially indicating a modification in diet compared to that of the yellow eel from the River Suir. Enriched $\delta^{13}C$ data (–21.1‰) in eels from the river Suir

system may be a reflection of the estuarine and/or planktonic/oceanic influences on these animals' diets and/or the fact that they were actively feeding at time of capture unlike the silver eels from the other four sampling locations who at the time of capture are suspected to have ceased feeding. Figure 4.2 graphically illustrates $\delta^{13}C$ and $\delta^{15}N$ isotopic ratio data.

Hobson (62) and Das (63) reported that the general pattern of inshore, benthos-linked food webs tends to be enriched in $\delta^{13}C$ compared to offshore, pelagic webs. Bardonnet detailing dietary preferences of eels report $\delta^{13}C$ and $\delta^{15}N$ values of -24.0 +/-1.6 ‰ and 11.6 +/- 0.9‰, respectively in river-pigmented eels with data indicative of eels belonging to a food chain based on terrigenous organic matter sources (64).

The authors further report that the main sources of organic matter into marine waters from freshwater environments are characterized by their $\delta^{15}N$ and $\delta^{13}C$ values with differences providing a good indication of the influence of different water bodies on the diet of the species, with $\delta^{13}C$ values in the range -20.5 to –22 indicative of animals that have primarily fed on a diet of oceanic (planktonic) origin. As the negative $\delta^{13}C$ values increase, this provides a further indication of the increase of freshwater influence on the eel diet. Vizzini further reports that heavier $\delta^{13}C$ data are observed where a marine influence is present in animal's dietary preferences (65).

Further to this $\delta^{15}N$ data suggest that eels from the River Suir eels are of a slightly higher relative trophic position compared to others in the study. Caution is however advisable as the relative trophic position of "non-feeding" silver eels should not be compared directly to that of feeding resident yellow eels. Additional factors such as the

potential influence of industrial or agricultural processes or nutrient rich fluxes in the vicinity of sampling sites should also need to be considered when interpreting such data.

The majority of silver eels in this study have a 15+ year feeding history and all would have ceased feeding in the days/weeks/months prior to migration, however it is not possible to put an exact timeframe on when such "fasting" begins. It should be noted that $\delta^{13}C$ differences in muscle tissue at the silver stage may be further influenced by fasting and/or mobilisation of lipid reserves to other organs, rather than being as a direct result of modification of diet. Few data related to nutrient assimilation rates in eels are currently available to confirm such observations.

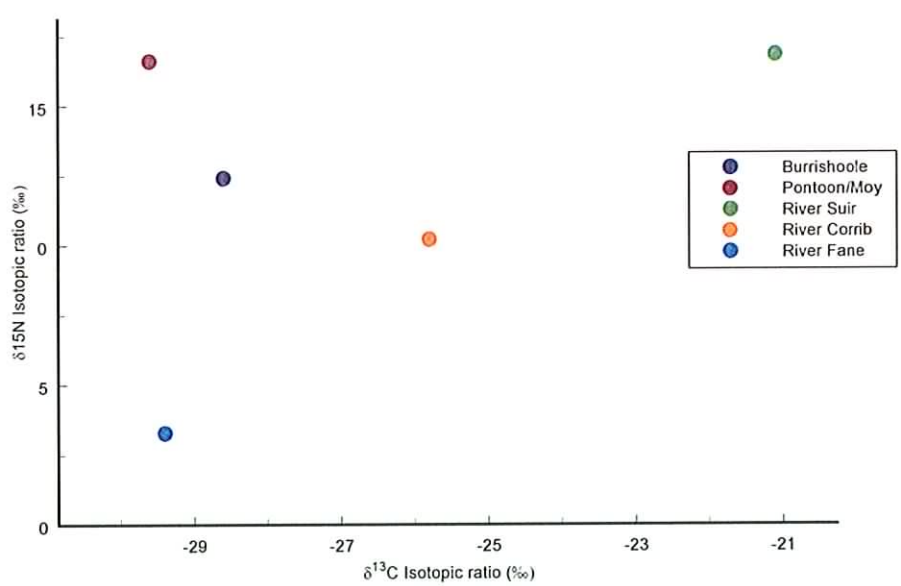


Figure 4.2: Scatterplot of $\delta^{15}N$ and $\delta^{13}C$ isotopic ratios from eel muscle.

4.3.2.2 Sulphur isotopes analysis

Substantial $\delta^{34}S$ differences were observed in eels from the river Fane compared to those from other locations. It is reported that $\delta^{34}S$ is closely related to a few essential amino acids that occasionally can give rise to decoupling between $\delta^{34}S$ and other isotopes. At this current time it is not possible to confirm whether changes in muscle tissue amino acid levels may have taken place in these eels, therefore, differences in $\delta^{34}S$ ratios between the samples require further investigation.

4.3.2.3 Stable isotope ratios and lipid content

Eels from the river Suir showed lower lipid total content than were observed in the other samples. It is suspected that eels from the river Suir were not preparing to migrate at the time of sampling therefore lower lipid levels would be expected in these animals compared to migrating silver eels.

A strong correlation (R=-0.88) was observed between $\delta^{15}N$ derived trophic status and eel muscle extractable lipid content suggesting that as the relative trophic status of the eel increases the corresponding extractable lipid content in muscle tissue decreases (see figure 8.3). A slightly lower correlation (R=-0.789) (see figure 4.3) was observed between muscle total lipid content and $\delta^{13}C$ values suggesting that dietary preferences may have lesser influence on the overall lipid levels than does trophic status of the animal. No such correlations were observed between $\delta^{34}S$ isotopic ratios and total lipid content.

In brackish ecosystems, the trophic web structure depends on environmental and seasonal factors; consequently dietary influences and potentially $\delta^{13}C$ values and muscle lipid content may vary throughout the year. Such observations further highlight the

necessity for ensuring that standardized sampling procedures and timeframes are observed during such studies.

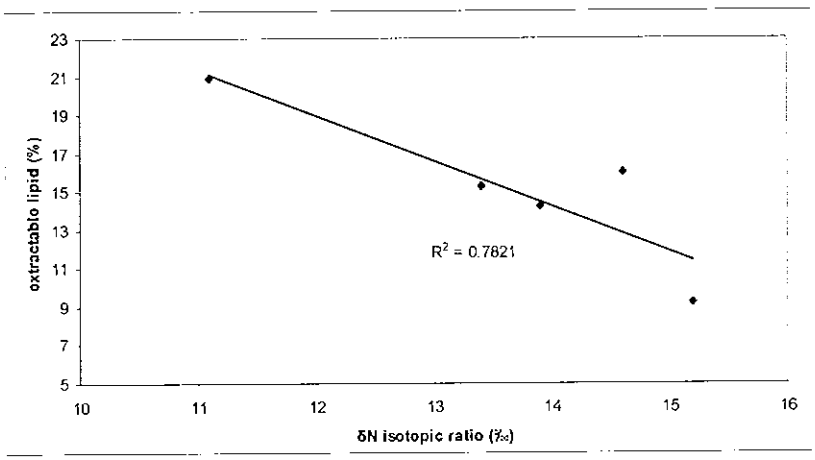


Figure 4.3: Relationship between $\delta^{15}N$ derived trophic status and extractable lipid (%) in eels from Irish waters.

4.3.3 Levels of contaminants in eel muscle

Details of the levels of each of the contaminants classes are presented below, in each case data relate to levels in eel muscle only.

4.3.3.1 Dioxin and Furan levels in eel samples

Current consumer safety legislation is designed to protect the consumer from dioxin/furan and dioxin-like-PCBs in foodstuffs and using the additive TEQs approach (61), total PCDD/PCDF-TEQs in the range 0.18 to 0.26 pg Total-TEQ (wet weight) were calculated for four of the samples in this study, while in the sample from the Burrishoole catchment a much more elevated TEQ of 4.4 pg Total-TEQ wet weight was recorded. Table 4.5 summarises individual congener concentration data and the calculated TEQs for each of the pooled eel samples in this study.

In the Burrishoole sample PCDDs (4.27 pg TEQ/g^{-1} wet weight) contribute the majority of the total TEQ in eels at this location, such dioxin levels thereby exceeding EU legislative limits for dioxins in eel muscle. While similar PCDD-TEQ/PCDF-TEQ ratios (1.75-2.45) were calculated at each of the four sampling sites with low total TEQ, the PCDD-TEQ/PCDF-TEQ ratio of 32.3 calculated in eels from the Burrishoole catchment shows much more elevated dioxin levels in these animals strongly suggesting point source contamination at this location.

OCDD was not detected in four of the five samples however it was detected at 42.0 pg g^{-1} wet weight in the sample from the Burrishoole catchment. OCDD belongs to the group of "super-hydrophobic" or "super-lipophilic" compounds with an octanol/water partitioning coefficient (log K_{ow}) of 8.6 and water solubility of 74 pg l^{-1}. Unlike the majority of such super-hydrophobic compounds a high lipid bioaccumulation factor of 85 X 10^6 has been reported by Geyer (66) for OCDD.

While elevated dioxin/OCDD levels have been determined in eels from the Burrishoole sampling site, no point source input is clearly identifiable within the catchment, meriting further investigation. Geyer (66) reports that OCDD is often the most prevalent polychlorinated dibenzo-p-dioxin congener found in pentachlorophenol (PCP) a highly persistent halogenated compound, which has primarily been utilized in the timber processing industry (67). No information related to the application of PCP or any other dioxin-like compound are documented within the Burrishoole catchment, therefore further follow up studies will be initiated in eels from within the catchment.

Table 4.5: Concentrations (pg g^{-1} wet weight) and TEQs of Dioxins, Furans and dioxin like PCB levels in eel muscle from Irish waters (pg g^{-1} wet weight)

PCDDs/PCDFs	MSC/05/1119	MSC/05/1120	MSC/05/1121	MSC/05/1122	MSC/05/1140
2.3.7.8-Tetra-CDD	0.042	0.029	0.023	0.026	0.055
1.2.3.7.8-Penta-CDD	0.100	0.083	0.091	0.085	2.0
1.2.3.4.7.8-Hexa-CDD	0.032	0.081	0.035	0.062	5.3
1.2.3.6.7.8-Hexa-CDD	0.16	0.17	0.10	0.23	13
1.2.3.7.8.9-Hexa-CDD	0.044	0.037	n.d.(0.02)	0.031	2.2
1.2.3.4.6.7.8-Hepta-CDD	0.10	0.11	0.048	0.080	13
OCDD	n.d.(0.3)	n.d.(0.3)	n.d.(0.3)	n.d.(0.3)	42
2.3.7.8-Tetra-CDF	n.d.(0.03)	n.d.(0.02)	n.d.(0.03)	n.d.(0.04)	n.d.(0.02)
1.2.3.7.8-Penta-CDF	0.024	0.014	0.016	0.017	0.085
2.3.4.7.8-Penta-CDF	0.15	0.10	0.064	0.086	0.12
1.2.3.4.7.8-Hexa-CDF	0.053	0.059	0.032	0.062	0.22
1.2.3.6.7.8-Hexa-CDF	0.033	0.035	0.024	0.033	0.12
1.2.3.7.8.9-Hexa-CDF	n.d.(0.02)	n.d.(0.02)	n.d.(0.03)	n.d.(0.02)	n.d.(0.02)
2.3.4.6.7.8-Hexa-CDF	0.085	0.086	0.077	0.066	0.25
1.2.3.4.6.7.8-Hepta-CDF	0.041	n.d.(0.04)	n.d.(0.04)	n.d.(0.04)	0.088
1.2.3.4.7.8.9-Hepta-CDF	n.d.(0.03)	n.d.(0.02)	n.d.(0.04)	n.d.(0.05)	n.d.(0.02)
OCDF	n.d.(0.05)	n.d.(0.05)	n.d.(0.07)	n.d.(0.05)	0.067
Total 2.3.7.8-PCDD	0.49	0.51	0.30	0.51	78
Total 2.3.7.8-PCDF	0.38	0.30	0.21	0.26	0.96
Total 2.3.7.8-PCDD/PCDF	0.87	0.81	0.52	0.78	79
Total WHO-TEQ 2.3.7.8-PCDD	0.17	0.14	0.13	0.14	4.27
Total WHO-TEQ 2.3.7.8-PCDF	0.097	0.075	0.053	0.067	0.13
Total WHO-TEQ 2.3.7.8-PCDD/PCDF	0.26	0.22	0.18	0.21	4.40
Total WHO-TEQ non-ortho PCB	0.65	0.18	0.18	0.25	0.30
Total WHO-TEQ mono-ortho PCB	0.59	0.13	0.067	0.32	0.26
Total WHO-TEQ WHO-PCB	1.24	0.31	0.25	0.57	0.56
Ratio PCDD TEQ/PCDF TEQ	1.75	1.87	2.45	2.09	32.3
Ratio non-ortho TEQ/ mono-ortho TEQ	1.10	1.38	2.69	0.78	1.15
Total PCDD/PCDF FISH TEQ	**0.26**	**0.23**	**0.18**	**0.21**	**5.00**
Total DL-PCB FISH TEQ	**0.05**	**0.01**	**0.01**	**0.02**	**0.02**
Σ PCDD/PCDF+ DL PCB FISH TEQ	**0.31**	**0.24**	**0.19**	**0.23**	**5.02**

n.d. = not detectable, detection limits in ()

Lassiter and Hallam (68) report that animals with higher body fat/lipid content (such as eels) can show greater resistance to the toxic effects of lipophilic compounds than organisms with lower lipid content. Geyer suggest that storage of TCDD and related compounds in fish/animal lipid can provide a detoxifying mechanism by which the chemicals are removed from receptors and target organs such as the liver and other sites of action. Geyer further predicts that eels with high fat content should be resistant to the

toxic effect of TCDD although they are capable of bio-concentrating the chemical to a high degree (69-71).

Total furan levels in all five samples were low, ranging (0.05 to 0.13) and (0.37 to 1.1) WHO-TEQ- PCDF on a wet weight and lipid weight basis respectively. Concentrations and consequentially PCDF-TEQs in the Burrishoole being much less elevated than those observed for dioxins. The sum of penta- and hexa-substituted Furan congeners provided greater than 90% of the furan derived TEQ for all samples, thereby suggesting a degree of homogeneity in the source of furan contamination.

Strong relationships were determined between extractable lipid content and penta- (decreasing levels) and hexa- (increasing levels) substituted furan congeners (see figure 4.4). As the lipid percentage in muscle tissue increases, the relative contribution of hexa-substituted PCDF congeners increases (R= 0.91), correspondingly the levels of penta-substituted congeners decrease (R= -0.90). It should be noted that other factors such as differences in exposure patterns, bio-transformation of furan congeners at lower stages in the eels food web also need to be considered when evaluating such relationships.

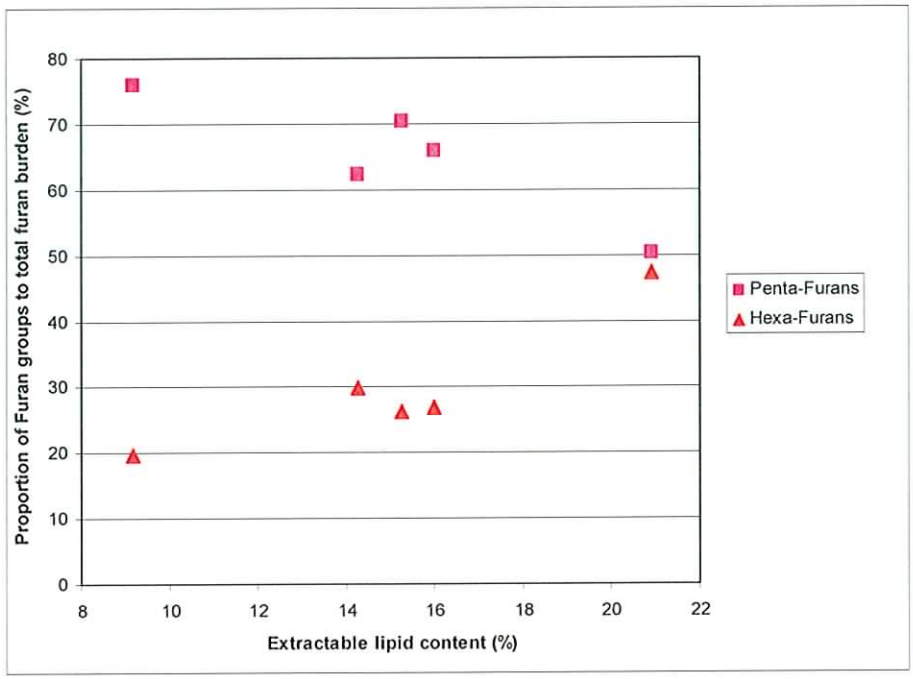


Figure 4.4: Relationship between extractable lipid content (%) and the contribution of penta- and hexa- substituted furan congeners to the total furan contaminant burden

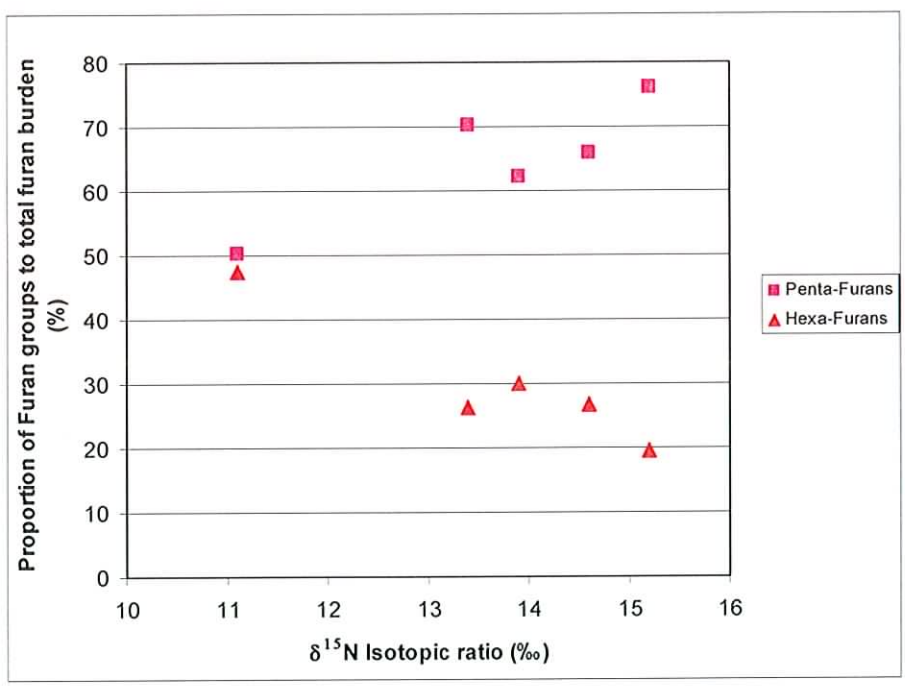


Figure 4.5: Relationship between $\delta^{15}N$ derived trophic position and the contribution of penta- and hexa- substituted furan congeners to the total furan contaminant burden

Additionally strong relationships exist between relative trophic position as derived by $\delta^{15}N$ stable isotopes and the contribution of penta- (increase) (R=0.88) and hexa- (decrease) (R=-0.95) substituted furan congeners to the total furan contaminant burden (see figure 4.5). While penta- and hexa- substitution groupings are the most toxicologically relevant in terms of TEFs, it is unclear whether data demonstrate trophic status based ability to biotransform hexa-substituted congeners or to transfer lipid (and potentially any associated hexa-substituted furan congeners) from muscle tissue to other organs. It should be noted that,

- sample size in this study was limited to five composite samples thereby reducing the statistical power of this current study,
- furan concentration data are not available from liver or other tissues therefore such trophic status driven observations need to be further examined,
- any potential dioxin input to the Burrishoole sample may conceivably contain additional furan contamination requiring consideration for future assessments.

4.3.3.2 WHO-PCBs in eel muscle

Overall levels of WHO-PCBs were low with the non-ortho PCBs 77 and 81 not detected in any of the five samples. Concentrations of PCB 126 ranged from not detected to 6.3 pg g^{-1} wet weight. PCB 118 was the most dominant mono-ortho PCB present with levels ranging from 282 to 2783 pg g^{-1} wet weight. The total mono-ortho PCB burden ranged 465 to 4351 pg g^{-1} (wet weight) while the combined mono-ortho and non-ortho WHO-PCB TEQ ranged from 0.25 to 1.24 pg g^{-1} WHO TEQ/g wet weight. These values are well below the current EU legislative maximum level for WHO-PCBs in eel.

No relationship between the contribution of individual non-ortho PCBs to the total non-ortho PCB burden and trophic status was determined, however strong downward

correlations were observed between the contribution of congeners PCB 118 and PCB 156 (R=0.83 and 0.98 respectively) to the mono-ortho contaminant burden and $\delta^{15}N$ derived trophic status. These data may suggest an increased capacity within higher trophic status eels to eliminate PCB 118 and/or 156 PCB from muscle tissue. No further correlations were observed between other mono-ortho PCBs and trophic status.

Table 4.6: Concentrations (pg g^{-1} wet weight) and TEQs of Non-and mono-ortho PCB and marker PCBs in eel muscle.

Sample Code	MSC/05/1119	MSC/05/1120	MSC/05/1121	MSC/05/1122	MSC/05/1140
WHO-PCBs (pg g^{-1})					
3,3',4,4'-Tetra-CB 77	n.d.(4.0)	n.d.(2)	n.d.(3)	n.d.(3)	n.d.(2)
3,4,4',5-Tetra-CB 81	n.d.(0.14)	n.d.(0.08)	n.d.(0.08)	n.d.(0.1)	n.d.(0.06)
3,3',4,4',5-Penta-CB 126	6.3	n.d.(2)	n.d.(2)	2.3	2.8
3,3',4,4',5,5'-Hexa-CB 169	2.0	1.2	1.3	1.3	1.8
Total non-ortho PCB	**8.3**	**1.2**	**1.3**	**3.6**	**4.6**
2,3,3',4,4'-Penta-CB 105	853	188	75	429	381
2,3,4,4',5-Penta-CB 114	43	10	8.4	36	22
2,3',4,4',5-Penta-CB 118	2783	564	282	1496	1077
2',3,4,4',5-Penta-CB 123	43	12	n.d.(9)	26	n.d.(10)
2,3,3',4,4',5,-Hexa-CB 156	311	75	37	170	168
2,3,3',4,4',5'-Hexa-CB 157	72	13	12	42	33
2,3',4,4',5,5'-Hexa-CB 167	215	46	38	84	94
2,3,3',4,4',5,5'-Hepta-CB 189	32	11	13	19	16
Total mono-ortho PCB	**4351**	**919**	**465**	**2302**	**1792**
Total WHO-TEQ non-ortho PCB	**0.65**	**0.18**	**0.18**	**0.25**	**0.30**
Total WHO-TEQ mono-ortho PCB	**0.59**	**0.13**	**0.067**	**0.32**	**0.26**
Total WHO-TEQ WHO-PCB	**1.24**	**0.31**	**0.25**	**0.57**	**0.56**
Marker-PCBs (ng g^{-1})					
PCB #28	0.58	0.03	0.03	0.35	0.03
PCB #52	1.40	0.13	0.06	0.90	0.21
PCB #101	1.55	0.20	0.11	0.69	0.27
PCB #118	2.78	0.56	0.28	1.50	1.08
PCB #138	4.11	1.01	0.56	1.83	2.13
PCB #153	5.30	1.19	0.64	1.62	2.09
PCB #180	2.33	0.51	0.26	0.75	0.96
Σ Marker-PCBs	**18.1**	**3.63**	**1.95**	**7.63**	**6.76**
Lipid content %	9.18	15.3	14.3	16.0	20.9

n.d. = not detectable, detection limits in ()

4.3.3.3 Levels of marker PCBs in eels

Levels of marker PCBs in this current study ranged 1.95 to 18.1 ng g^{-1} wet weight and from 13.7 to 197 ng g^{-1} (ppb) lipid weight. Data are comparable to those reported by Santillo (54) for the ΣICES 7 PCBs ranging from 32 ppb and 114 ppb lipid weight in Irish samples from the Owengarve stream and Lough Furnace (a brackish lake in the Burrishoole catchment) respectively, further to this levels of 30.3 ppb wet weight (146 ppb lipid weight) have been recorded in eel samples from the river Nore, Ireland (Marine Institute unpublished data).

Concentration of marker PCBs reported by Santillo (54) in these Irish samples levels were amongst the lowest determined in the study, with marker PCB concentrations of up to 1512 ppb wet weight (9947 ppb lipid weight) reported in a composite sample from the Netherlands. One further study by deBoer (29) reported lipid normalized concentrations ranging from 274±176 ppb to 14400±9700 ppb (lipid weight) in 142 samples from the Netherlands.

Higher trophic status eels as estimated by nitrogen stable isotope data, seem to have a greater proportion of PCB101 in their overall contaminant burden than lower status eels. The ratio of PCB138 to the total marker PCB burden shows a strong downward correlation with trophic status (R= -0.92) with lower ratios of this congener being observed in higher trophic status animals. No correlations were observed between $\delta^{13}C$ or $\delta^{34}S$ isotopic ratio data and any of the individual marker PCB and/or congener groupings.

4.3.3.4 PBDE levels in eel muscle

Table 4.7 presents concentration data for a range of BFR compounds in the five composite eels samples. Upperbound levels of the Σ11 PBDE congeners (excluding BDE 209) ranged from 0.89 ng g^{-1} wet weight in the Burrishoole sample to 7.05 ng g^{-1} wet weight in eels from the River Suir. These data are comparable to those of a recent survey by the UK food standards (FSA) agency, which determined an upperbound total of 4.88 ng g^{-1} wet weight for the Σ11 PBDE congeners in a composite eel sample (72).

Table 4.7: Levels of Brominated flame retardants in eels from Irish waters (ng g^{-1} wet weight).

PBBs	MSC/05/1119	MSC/05/1120	MSC/05/1121	MSC/05/1122	MSC/05/1140
PBB #15	0.0015	n.d.(0.002)	n.d.(0.001)	n.d.(0.002)	n.d.(0.002)
PBB #49	n.d.(0.0009)	n.d.(0.002)	n.d.(0.001)	n.d.(0.002)	n.d.(0.002)
PBB #52	0.0031	n.d.(0.002)	n.d.(0.001)	n.d.(0.002)	n.d.(0.002)
PBB #101	0.0011	0.0018	n.d.(0.001)	n.d.(0.002)	0.0040
PBB #153	n.d.(0.001)	n.d.(0.002)	n.d.(0.001)	n.d.(0.002)	0.0027
Total PBBs (Upper Bound)					
PBDEs					
BDE #17	0.011	0.0016	n.d.(0.001)	0.0042	n.d.(0.002)
BDE #28	0.065	0.017	0.0070	0.038	0.0061
BDE #47	5.2	1.7	0.77	1.8	0.52
BDE #66	0.099	0.053	0.015	0.064	0.017
BDE #85	n.d.(0.0009)	n.d.(0.002)	n.d.(0.001)	n.d.(0.002)	n.d.(0.002)
BDE #99	0.16	0.025	0.017	0.10	0.024
BDE #100	1.3	0.33	0.20	0.40	0.16
BDE #138	n.d.(0.002)	n.d.(0.002)	n.d.(0.002)	n.d.(0.002)	n.d.(0.002)
BDE #153	0.072	0.055	0.031	0.12	0.052
BDE #154	0.20	0.13	0.090	0.20	0.095
BDE #183	n.d.(0.002)	0.0038	0.0019	0.022	0.0076
Total PBDEs (lower bound)	**7.0**	**2.3**	**1.1**	**2.8**	**1.0**
Total PBDEs (upper bound)	**7.1**	**2.3**	**1.2**	**2.8**	**1.0**
TBBPA	n.d.(0.03)	n.d.(0.05)	n.d.(0.04)	n.d.(0.05)	n.d.(0.2)

n.d. = not detectable, detection limits in ()

PBDE 47 was the most prevalent congener determined in all samples, with concentrations ranging from 0.52 to 5.2 ng g^{-1} wet weight in the Burrishoole and River Suir samples respectively. The majority of the remaining BDE burden is accounted for by BDEs 100, 153 and 154.

Santillo (54) reports that levels of BDE 47 were low in the Irish samples (not detected to 0.2 ng/g wet weight) compared to those determined in eels from other European countries. Eels from Germany in the range 7.9 to 17.0 ng g^{-1} wet weight, while one composite sample from the UK showed BDE47 levels of 46.0 ng g^{-1} wet weight. It must be noted however that these elevated results were derived from much larger eels that were sampled in areas subject to greater contamination sources than were the Irish eels. For further comparison Lepom reports PBDE 47 levels of 4.50 ng g^{-1} lipid weight in eels from German waters (73).

BDE 183 concentrations were low (not detected to 0.02 ng g^{-1} wet weight) indicating that eels in Irish waters are primarily exposed to penta-mixtures than to octa-formulations.

4.3.3.5 HBCD levels in eel muscle

Within the scope of this present study, a screening exercise for the levels of total HBCD isomers was completed for all five samples with concentrations between 1.2-15 ng g^{-1} wet weight total HBCD were determined, corresponding to 7.4 to 166 ng g^{-1} lipid weight total HBCD. The two samples at the upper end of the range were further re-analysed on an isomer specific basis by a second independent laboratory. Results of both the screening exercise and isomer specific analysis are presented in table 4.8.

The UK food standards agency report levels of 5.11 ng g^{-1} wet weight for α–HBCD been in eel, with these results being the highest value recorded in 48 diverse fisheries products analysed (72). Santillo report eel tissues concentrations of HBCD of the order (<1 - >50 ng g^{-1} wet weight) for a range of composite eel samples in European waters. Irish eels showed no detectable residues in one sample (Lake Furnace) and a level of 3 ng g^{-1} in a further sample from the River Owengarve (54).

Table 4.8: Isomer specific and total HBCD levels in eels from Irish waters (ng g^{-1} wet weight)

	MSC/05/1119	MSC/05/1120	MSC/05/1121	MSC/05/1122	MSC/05/1140
total HBCD	15	2.2	1.2	15	1.6
α-HBCD	8.9	NA	NA	6.0	NA
β-HBCD	0.48	NA	NA	0.18	NA
γ-HBCD	0.58	NA	NA	0.20	NA

Note: isomer specific confirmation analysis completed by CSL York England.

NA = Not analysed

Morris (37) reports that concentrations of HBCD in yellow eels from the Scheldt and River Leie at Oeselgem reflect the spatial distribution of HBCD found in sediments in the area. The author suggests that the study provides evidence of HBCD bioaccumulation at the trophic level and biomagnification in the ascending aquatic food chain, justifying risk assessment studies at the ecosystem level.

4.3.3.6 TBBPA levels in eels

TBBPA was not detected in any of the five samples analysed in this study. Recoveries of TBPPA in this current study were low (see appendix 4.1) ranging from 25-50 % between the five samples, this may be as a result of TBBPA binding to other endogenous compounds in muscle tissue such as proteins (74).

Relatively low concentrations of TBBPA tend to occur in aquatic biota for a number of reasons. TBBPA is generally chemically bound to the polymer matrix of the end product thereby; potential emissions of TBBPA are likely to be limited compared to other BFR compounds (54). TBBPA has lower bioaccumulation potential than HBCD due to low partition rates to the particulate and organic carbon compartments of sediments and structural phenolic groups have been reported to allow direct phase-II biotransformation (75). Further to this the polar nature of TBBPA can subject it to metabolism and elimination from the organism.

Morris provides one of the most comprehensive reviews of the levels of TBBPA in biota, reporting poor correlation between TBBPA residues in eel tissues and those found in sediments from the Scheldt basin. TBBPA in eel samples from the Scheldt basin were generally low with concentrations from <0.1 to 13 µg/kg lipid weight recorded while eels from a number of rivers in the Netherlands showed concentrations in the range <0.1 to 1.3 µg/kg lipid weight (37).

TBPPA was also analysed as part of the UK food standards agency study but residues were not detected above the limit of detection in 48 fisheries products (72). Tetrabromobisphenol-A residues were not detected in any of the 20 pooled samples (detection limit 3-5 ppb) reported by Santillo (54), while deBoer (76-77) reported that concentrations appear to be similar or even lower in other freshwater and marine species compared to those determined in eels.

4.3.3.7 PBB levels in eels

PBBs were only detected in three of the five samples ranging 0.0018 to 0.0067 ng g^{-1} wet weight suggesting low usage/transport of PBBs to environments in which these eels

were sampled (see table 4.7). Levels of PBBs were similarly low in the UK food standards survey of 48 fisheries products, reporting levels of 0.016 ng g^{-1} wet weight for the Σ6PBBs in one composite eel sample (72).

4.3.3.8 Organochlorine pesticides in eels.

Overall levels of OCPs in eels were low, p,p'DDE was found to the most abundant compound in the samples followed by dieldrin, p,p'DDD, p,p'DDT and/or *cis*-heptachlor-epoxide. Toxaphene and endrin residues were only detected in one sample MSC/05/1140 from the Burrishoole catchment.

α-Chlordane, γ-chlordane, trans-nonachlor and oxy-chlordane in addition to heptachlor and associated epoxides were determined in this present study. Trans-nonachlor was found to be the most abundant of these, contributing (42-56%) of the total chlordane contaminant burden. While α-chlordane and γ-chlordane isomers form a major proportion of technical chlordane, transformation to trans-nonachlor leading to the formation of oxy-chlordane (has been reported to take place in rats), with the γ-chlordane isomer being shown to oxidise more rapidly than α-chlordane (78). α-chlordane was found in higher concentrations than the γ-isomer in each of the eel samples in this present study

In this current study a strong downward correlation (R= -0.89) was found between α-chlordane and trans-nonachlor levels, while a weaker correlation (R=0.60) was observed between γ-chlordane and trans-nonachlor suggesting potential capacity within eels to convert α-chlordane to its metabolite trans-nonachlor at higher trophic levels. While trans-nonachlor is metabolised to oxychlordane, the low percentage of oxy-

chlordane found in eels from this study may suggest that eels (and/or lower trophic species in eel food web) show relatively low capacity to metabolise trans-nonachlor. Low capacity in eels to biotransform OCPs has previously been reported (30).

Strong downward correlations (R> -0.97) were observed between $\delta^{15}N$ derived trophic status and Σchlordane and Σheptachlor-epoxides in eel tissue, with higher trophic status eels seemingly having lower levels of these compound groups in their muscle tissue. Heptachlor epoxide, the primary oxidation metabolite of heptachlor with the epoxide form being more persistent than the parent heptachlor, was not detected in eel samples.

Table 4.9: Concentrations of Organochlorine compounds in eel muscle from Irish waters (ng g^{-1} wet weight).

	MSC/05/1119	MSC/05/1120	MSC/05/1121	MSC/05/1122	MSC/05/1140
β-HCH	n.d.(0.10)	0.14	0.061	0.20	0.31
α-HCH	n.d.(0.1)	0.21	0.22	0.19	0.29
γ-HCH	0.21	0.16	n.d.(0.2)	0.29	0.45
δ-HCH	n.d.(0.2)	n.d.(0.2)	n.d.(0.2)	n.d.(0.2)	n.d.(0.2)
o,p-DDT	0.038	0.041	0.037	n.d.(0.04)	0.072
p,p'-DDT	0.31	0.27	0.14	0.17	0.55
o,p-DDD	0.093	n.d.(0.04)	0.059	0.045	n.d.(0.04)
p,p'-DDD	2.70	0.55	0.21	1.90	0.67
o,p-DDE	0.047	n.d.(0.03)	n.d.(0.04)	n.d.(0.04)	n.d.(0.04)
p,p'-DDE	7.10	3.20	1.60	5.00	3.10
Hexachlorobenzene	n.d.(0.5)	n.d.(1)	n.d.(0.8)	n.d.(0.9)	n.d.(2)
Heptachlor	n.d.(0.2)	n.d.(0.2)	n.d.(0.2)	n.d.(0.2)	n.d.(0.2)
cis Heptachlorepoxide	0.11	0.45	0.32	0.19	0.88
trans Heptachlorepoxide	n.d.(0.7)	n.d.(0.7)	n.d.(0.8)	n.d.(0.7)	n.d.(0.8)
Aldrin	n.d.(0.1)	n.d.(0.10)	n.d.(0.1)	n.d.(0.1)	n.d.(0.1)
Toxaphene 26	n.d.(0.3)	n.d.(0.3)	n.d.(0.4)	n.d.(0.2)	0.86
Toxaphene 50	n.d.(0.3)	n.d.(0.4)	n.d.(0.4)	n.d.(0.3)	1.2
Toxaphene 62	n.d.(1)	n.d.(1)	n.d.(1)	n.d.(1)	n.d.(1)
Octachlorstyrene	n.d.(0.08)	0.071	n.d.(0.08)	n.d.(0.07)	0.14
Dieldrin	2.00	1.40	2.20	2.10	3.50
Endrin	n.d.(0.1)	n.d.(0.10)	n.d.(0.1)	n.d.(0.1)	0.16
Mirex	n.d.(0.01)	0.050	0.016	n.d.(0.02)	0.20
Endosulphane sulphate	n.d.(0.3)	n.d.(0.2)	n.d.(0.2)	n.d.(0.2)	n.d.(0.2)
α-Endosulphane	n.d.(0.9)	n.d.(0.8)	n.d.(0.9)	n.d.(0.9)	n.d.(1.0)
β-Endosulphane	n.d.(3)	n.d.(2)	n.d.(3)	n.d.(3)	n.d.(2)
γ-Chlordane	n.d.(0.05)	0.084	0.11	0.058	0.096
α-Chlordane	0.081	0.28	0.15	0.095	0.86
Oxychlordane	n.d.(0.2)	n.d.(0.2)	n.d.(0.2)	n.d.(0.2)	0.29
Transnonachlor	0.26	0.72	0.48	0.28	1.60

n.d. =not detected, detection limit in parenthesis.

γ-HCH levels ranged from not detected to 0.45 ng g^{-1} wet weight (2.1 ng g^{-1} lipid weight) and are lower than concentrations reported by Corsi (30) (6.33 to 707 ng g^{-1} lipid weight) in eels from the Orbetello lagoon in Italy. β-HCH was detected in four samples in the range 0.06 to 0.31 ng g^{-1} wet weight while the δ-HCH isomer was not detected in any of the five samples.

Upperbound ΣDDTs levels 2.12 to 10.2 ng g^{-1} wet weight (13.6 to 111 ng g^{-1} lipid weight) were similar to those reported by Corsi (30) (22.9 to 98.4 ng g^{-1} lipid weight) in Italian eels. Upperbound p,p'DDE levels (11 to 77 ng g^{-1} lipid weight) were also similar to those of the Corsi study (17.1 to 94.6 ng g^{-1} lipid weight).

The bioaccumulation of ΣDDT and associated metabolic products did not show correlation with relative trophic status, however, the proportion of p,p'DDT to the overall ΣDDT burden was shown to decrease with increasing relative trophic status.

A number of contaminant studies determine the *p, p'*-DDE/Total DDT ratio in order to assess the chronology of DDT inputs. Aguilar (79), and Tanabe (78) state that a ratio > 0.6 is indicative of a stable system with no new DDT inputs. In the current study, *p, p'*-DDE/Total DDT ratios determined in eels muscle tissue ranged from 0.69 to 0.78, suggesting that eel DDT residues are derived from historic contamination incidents.

4.3.4 Contaminant related effects on eel health

The majority of authors suggest that assessment of chemical data alone is insufficient in determining the health status of eel populations and must be assessed in close consideration with other environmental and biological variables and that the presence of

a particular chemical does not necessarily imply a measurable decrease in the health of an ecosystem or a toxicological effect on biological components (30).

Corsi (30) additionally reported that the presence of persistent organochlorine contaminants, including pesticides and PCBs, in eels sampled from the Orbetello lagoon in Tuscany (Italy) may, in combination with other types of manmade contaminants, act to reduce fitness and ultimate reproductive success in those populations.

Feunteun (1) recommended that a number of wider ecological considerations including, pathways of contaminants into the environment, transfer via groundwater to the aquatic environment, processes of contaminant assimilation into eels, implications for survival and overall the effects on breeding success, ability to achieve transoceanic migration, spawning, egg development, and survival of larvae, all must be considered as fundamental parameters alongside contaminant levels themselves when assessing the relative health status of eel populations. Additionally the quality of spawners, potential changes in ocean current and variations in oceanic secondary productivity must be considered when assessing relative health status.

Palstra recently suggested that current environmental levels of dioxin-like contaminants may potentially assist in the decline of eel stocks, based on their effects on development and survival of eel embryos (34). During maturation of female European silver eels, approximately 60 g lipid per kg is incorporated in eel oocytes. Persistent organic pollutants such as dioxin-like polychlorinated biphenyls (PCBs) may subsequently be incorporated into these oocytes. Palstra calculated the total PCDD/PCDF and dioxin-like PCB (TEQ) of the individual eel gonad batches using an *in vitro* reporter gene assay. The observed differences in development and survival showed a significant

negative correlation with the Total-TEQ levels in the gonads, at levels far below the maximal allowable level for fish consumption, i.e., 4 ng TEQ/kg fish (34).

The authors conclude that Dioxin-like PCBs can contribute up to 86% of the Total-TEQ therefore the inverse relationship between the Dioxin-like TEQ level and the survival period of the fertilised eggs strongly suggests that the current levels of these compounds may seriously impair reproduction of the European eel. Additionally Palstra states that the peak of the environmental levels of dioxin-like PCBs and the decline of eel populations coincide worldwide, further suggesting that, in addition to other threats, these DL-PCBs contributed significantly to the current collapse in eel populations (34).

Versonnen suggested that while the European "yellow" eel appears to remain insensitive to the potential effects of POPs on their hormone systems, few data are available to determine whether such sensitivity alters on mobilization of lipid reserves (10). Yamaguchi further discusses that while "yellow eels" may tolerate elevated POP levels, these may be deleterious to eel predators, such as aquatic mammals and/or birds (81).

The inverse relationship demonstrated by Palstra between gonad-Total-TEQ levels and the survival period of fertilised eggs, may suggest that the current levels of dioxin-like compounds seriously impair the reproduction of the European eel (34). The relatively low WHO-PCB-TEQs in this present study (0.25 to 1.24 TEQ) were derived in eel muscle tissue, therefore direct comparison to gonad-TEQ levels as per the Palstra study should not be made.

4.4 Conclusions

With the exception of dioxin levels in one sample from the Burrishoole region POP levels in general are low in eels from Irish waters compared to those in other countries. Further investigations into potential point source influences are merited. Stable isotope data provide a useful tool to further describe habitat/dietary influences of eels from Irish waters. Such habitat information is especially relevant where eels are collected for environmental monitoring purposes.

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Appendix 4.1: Quality Assurance data of recoveries for PCBs and Dl-PCBs (%)

	MSC/05/1119	MSC/05/1120	MSC/05/1121	MSC/05/1122	MSC/05/1140
Non-ortho PCBs					
PCB #81	95	68	92	81	62
PCB #77	93	68	89	79	64
PCB #126	89	73	90	93	80
PCB #169	101	83	97	105	85
Mono-ortho PCBs					
PCB #105	83	89	80	109	84
PCB #114	96	106	78	95	91
PCB #118	90	96	84	90	92
PCB #123	87	101	78	96	82
PCB #156	91	110	104	97	92
PCB #157	75	104	70	95	96
PCB #167	70	102	78	84	92
PCB #189	82	99	78	89	105
Marker-PCBs					
PCB #28	81	81	77	96	80
PCB #52	90	108	93	105	103
PCB #101	94	112	99	109	118
PCB #153	83	108	91	94	105
PCB #138	86	112	92	100	100
PCB #180	77	96	86	92	103
BFRs					
BDE #28	85	112	109	91	84
BDE #47	98	123	112	107	83
BDE #100	97	119	113	106	88
BDE #99	102	125	117	107	93
BDE #154	86	103	101	101	79
BDE #153	93	109	100	87	92
BDE #183	111	135	122	129	96
PBB #52	64	74	81	83	60
PBB #153	84	100	91	96	73
HBCD	45	70	50	51	41
TBBPA	50	33	29	25	28
Organochlorine compounds					
β-HCH ^{13}C	60	69	71	69	68
γ-HCH ^{13}C	62	76	69	71	72
pp-DDT ^{13}C	60	71	64	67	59
pp-DDE ^{13}C	65	77	72	72	69
Hexachlorobenzene ^{13}C	70	81	73	77	71
PCB 52 ^{13}C	65	76	70	73	72
Dieldrin ^{13}C	70	84	77	79	75
PCB 101 ^{13}C	66	75	65	77	74

CHAPTER 5: BIOACCUMULATION AND ENANTIOMERIC PROFILING OF ORGANOCHLORINE PESTICIDES AND PERSISTENT ORGANIC POLLUTANTS IN THE KILLER WHALE (*ORCINUS ORCA*) FROM BRITISH AND IRISH WATERS.

5.1 Introduction

The killer whale (*Orcinus orca*) is a widely distributed, and intensely social marine mammal living in stable pods consisting of up to 50 individuals, in localised resident populations, or in highly migratory groups (1). Killer whales have been shown to prey on fish and squid, and on occasion on blue, gray and sperm whales, seals and other cetacean species (2-13), and represent the top predator in many marine food webs. In the North Atlantic, herring, mackerel, salmon, cod, and halibut are among the main fish species consumed by killer whales (14-16). North-east Atlantic mature males attain sexual maturity at approximately 16 years old (5.2-6.5m in length) and females at approximately 10 years old (4.6-5.4m in length (17-18). Geographic variations in size at sexual maturity have been noted, with animals inhabitating North-Eastern Atlantic waters maturing at the lower end of the size range scale, while Antarctic killer whales mature at the upper end (17-19). In British and Irish waters, the killer whale has been observed along the Atlantic seaboard and in the northern North Sea (20). Sightings are rare in the English Channel and in the southern North Sea (21), although one animal was reported stranded at Sandwich, Kent in 1995 (22).

As a consequence of their lipophilic nature, environmental persistence and bioaccumulation potential, OCPs represent a threat to marine organisms in general, but especially to those occupying the top of the marine food web, including marine mammals (23-27). Marine mammals have low metabolic and/or excretory capabilities for these pollutants and so may exhibit adverse physiological and behavioural effects as a result of sustained exposure (28-34).

Chiral OCP compounds may have identical physico-chemical properties but as a consequence of molecular configuration differences, selective binding to structure-

sensitive biological receptors may result in different degradation/accumulation rates in biota and ultimately may lead to differential toxicological effects in animals (35-36), however few data are available related to such metabolic processes in high trophic level marine mammals and especially in killer whales.

The role of stable isotope techniques in elucidation of habitat/dietary preferences of the European eel (*Anguilla anguilla*) have previously been reported in this thesis (chapter 4) and their application on an ecosystem/food web basis are further described in chapter 6. In the case of Killer whales and/or other marine mammals the use of stable isotope based approaches in combination with traditional methodologies such as stomach contents analysis can account for an organisms dietary assimilation over extended feeding periods and can provide valuable information related to the overall species feeding ecology (37-41).

As such primary objectives of this current work were to investigate the influence of stable isotope derived trophic status on the levels, bioaccumulation and enantioselective profile of selected OCPs in killer whales from British and Irish waters.

5.2 Materials and methodology

Obtaining suitable killer whale tissues for biological and/or contaminants analysis tends to occur (preferably) as a consequence of targeted biopsy sampling or as in the case of the majority of studies as a result of mammal stranding events. Details of sampling and contaminants analysis procedures relevant to this work are presented below.

5.2.1 Killer whale sampling procedures

Killer whale are relatively uncommon in British and Irish waters compared to numbers found in Pacific regions and as such opportunistic sampling form the basis for the majority of studies completed. These current samples were obtained as a result of live stranding events or were collected from individuals washed ashore following death at sea. Tooth rot infection was reported to be a major contributory factor in the death of three individuals.

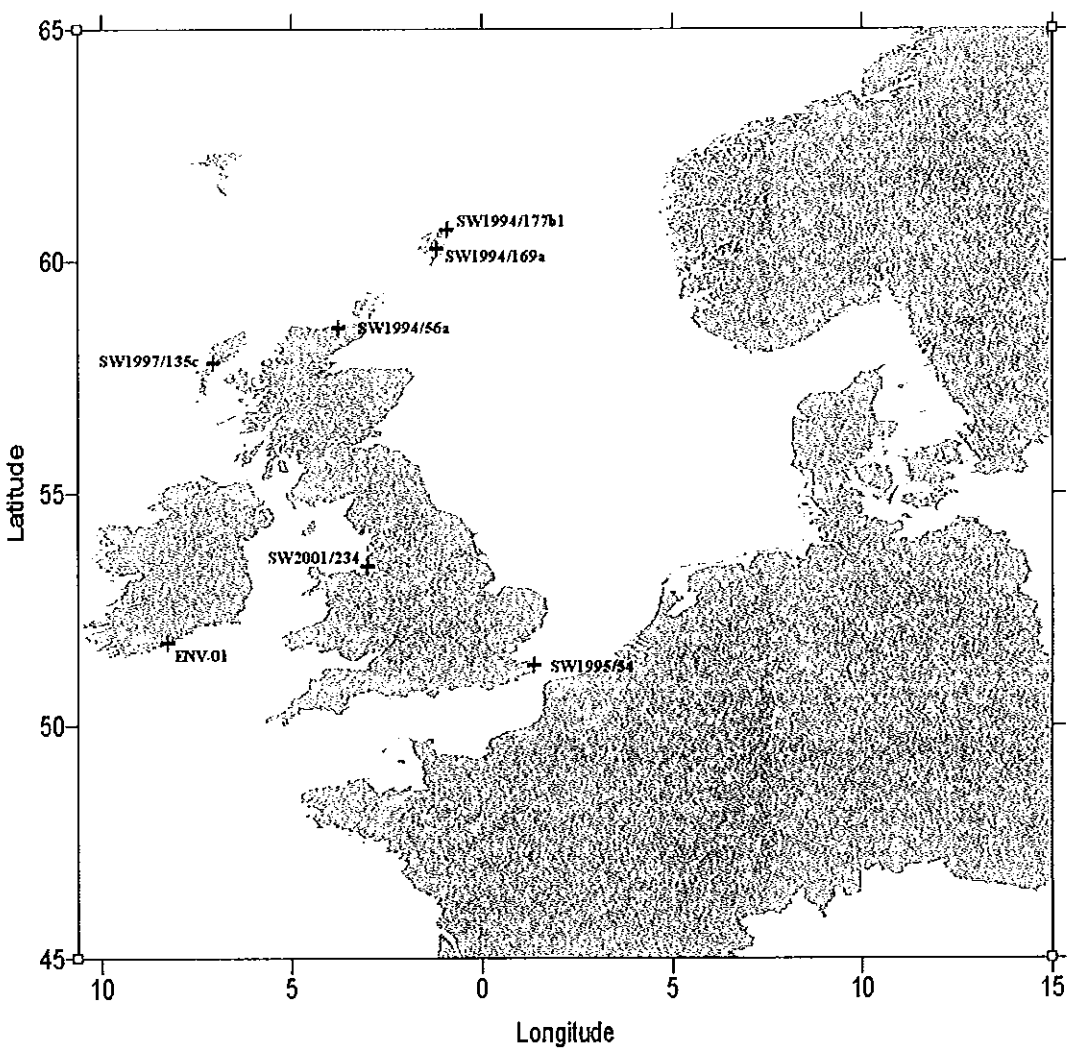

Figure 5.1: Geographic distribution of killer whale sampling locations.

Table 5.1: Killer whale sampling and biological information.

Reference.	Sex	Age (Yrs)	Length (mm)	Date	Location	Latitude Longitude	Cause of death
British Waters							
SW1995/54	F	24	525	26-04-95	Pegwell Bay, Kent	51° 19.00' 1° 22.00'	starvation
SW1994/56a	F	NA	550	15-04-94	Sandside Bay, Reay	58° 34.10' 3° 46.57'	tooth rot infection
SW1994/169a	M	NA	610	16-11-94	Catfirth Voe, Shetland	60° 16.15' 1° 11.73'	live stranding
SW1994/177b1	NA	NA	NA	07-12-94	Uyeasound, Shetland	60° 41.31' 0° 55.17'	no information
SW1997/135c	F	NA	610	16-08-97	Harris, Western Isles	57° 48.30' 7° 04.90'	old age/worn teeth & tooth rot infection
SW2001/234	M	NA	590	09-10-01	River Mersey	53° 26.02' 3° 00.97'	starvation
Irish Waters							
ENV-01	F	40-50	545	08/07/01	Roches point, Cork	*51° 47.00 8°15.30'*	Worn teeth causing *Staphylococcus aureus* septicaemia

NA: not available

Post-mortem studies and the collection and preservation of tissue samples were conducted according to previously established protocols (42-43) Sampling details and biological information are reported in Table 5.1 and location information is illustrated in Figure 5.1. Study samples comprised blubber tissue from four females ranging in length from 525-610cm (n=3), two males ranging in length from 590-610cm (n=2) and one unsexed individual. Further details on storage of samples are provided in chapter 1.

5.2.2 OCP analysis in killer whale blubber

Due to the lipophilic nature of OCPs, homogenised tissue samples were extracted by soxhlet apparatus or the method developed by Smedes (44-45). Lipids were removed from the solvent extract by alumina column chromatography followed by separation of PCBs from the chlorinated pesticides using silica gel column chromatography. OCP concentrations in Irish killer whales were determined by dual column Gas Chromatography with Electron Capture Detection (GC-ECD) using a Varian 3800 GC using HT8 (J & W Scientific, Inc.) and CP-SIL 19 (Chrompack, Varian Inc) columns according to Glynn (46). British killer whale samples were analysed in the CEFAS

Burnham-on-Crouch laboratories in the UK, on a HP6890 GC with a DB5 (J & W Scientific, Inc.) column according to Allchin (47).

5.2.3 Enantiomeric separation

Cleaned up sample extracts were analysed by the author on a Varian 1200 Triple Quadripole GC-MS utilizing a BGB-172 chiral column (BGB Analytik AG, Adiswil, Switzerland), dimensions (30m x 0.25mm id x 0.18μm film thickness) with two characteristic ions used for individual compound identification. GC-EI-MS was utilized for identification of enantiomerically separated o, p'-DDT, while negative chemical ionization (NCI) techniques were used for detection of all other OCPs. Enantiomeric compositions were then determined as per chapter 1 (section 1.2.6.6). Additionally chapter 1 further details the application of enantioselective analytical techniques in environmental analysis.

5.2.4 Stable isotope analysis

As lipids in biotic tissue samples can affect isotopic ratio determinations (48), total lipid free tissue samples were prepared according to Smedes (44-45) prior to stable isotope analysis. Lipid free, freeze-dried tissues were then analysed according to the procedures documented in chapter 1. These analyses were completed under sub-contract to the Institute for Energy Technology (IFE) in Kjeller, Norway.

5.2.5 Analytical quality control

Although proficiency exercises related to analysis of OCPs in marine mammals are not currently available in Europe, both laboratories show continued successful participation in the Quality Assurance of Information for Marine Environmental Monitoring in Europe (QUASIMEME) laboratory proficiency scheme for the analysis of OCPs in

other marine biological tissue. Full analytical quality control protocols involving the use of internal standards and the analysis of blanks and within batch laboratory reference materials were utilised.

Accuracy and precision of $\delta^{15}N$ and $\delta^{13}C$ analyses was evaluated against International Atomic Energy Agency (IAEA-N-1, IAEA-N-2) and US Geological Survey (USGS-24) standards in addition to an internal IFE trout standard material.

Enantiomerically pure standards +αHCH and -αHCH (Dr. Ehrenstorfer, GmbH, Hamburg Germany) were analysed on a batch basis to ensure correct enantiomeric assignment. Enantiomerically pure standards for *o,p,*-DDT and two toxaphene congeners (CHB 26 and CHB50) are not currently commercially available, therefore a racemic mixture of each was employed for identification purposes. Enantiomers were then assigned nomenclature EN1 and EN2 (first and second eluting enantiomer respectively) for the purposes of this study.

5.3 Results and discussion

Lipid content, animal age, gender, habitat and trophic position of species sampled are known to influence OCP concentrations in marine organisms. Additionally dietary assimilation of primary prey and biotransformation potential within a species are dominant factors in influencing contaminant levels in marine mammals (49) This current dataset is of insufficient sample size to statistically investigate potential influences of whale length/age, gender or sampling location. However, a number of other factors are discussed.

5.3.1 Trophic status evaluation/Stable isotope analysis

Isotopic $\delta^{15}N$ and $\delta^{13}C$ ratios in marine web predator tissues are primarily determined by the isotopic composition of baseline phytoplankton and zooplankton sources in the particulate organic matter of surrounding waters. Where POM data are unavailable, a mean $\delta^{15}N$ value of 5% has been widely utilized as a measure of offshore POM (50), however inherent variation between offshore and coastal POM values (51-52) render the use of such estimations unsafe, especially where an animal's native habitat is unclear. As a result a portion of the $\delta^{15}N$ variation in marine mammals may be related to the coastal versus offshore $\delta^{15}N$ signature of the primary producers (53) (see chapter 6).

While this current study is concerned with individual killer whales, few conclusive data are available related to each individual's dietary sources of nitrogen and carbon. Salmon fish bones were found in the stomach of the Irish killer whale, however no dietary information are available for any of the other individuals. Therefore, due to the lack of such primary and/or secondary trophic level prey data, direct ($\delta^{15}N$) measurements were utilised to estimate killer whale relative trophic status.

Nitrogen isotopic ratios ($\delta^{15}N$) in individual killer whale blubber samples were determined to lie in the range 14.5 to 17.3 ‰ (see table 5.2 and figure 5.2) suggesting that different ($\delta^{15}N$ derived) trophic status levels may exist in the killer whales sampled (39,54-55).

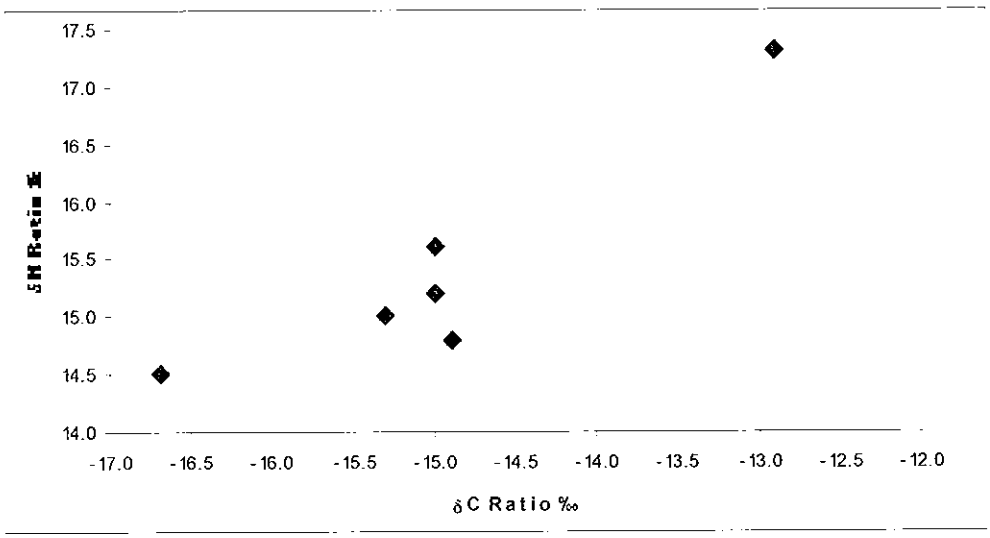

Figure 5.2: Stable isotope ($\delta^{15}N$ and $\delta^{13}C$) ratios in killer whales from British and Irish waters.

Table 5.2: Carbon and Nitrogen isotopic ratios (‰) and enantiomeric factors (EFs) for chiral OCPs in killer whale blubber samples.

Reference no.	δ C	δ N	α-HCH	o, p'-DDT	CHB26	CHB50
SW1994/56a	-14.9	14.8	0.17	0.49	0.52	0.47
SW1994/169a	-15.3	15	0.40	0.49	0.51	0.45
SW1994/177b4	-16.7	14.5	0.44	0.52	0.49	0.51
SW1997/135c	-12.9	17.3	0.14	0.41	0.52	0.54
SW2001/234	-15	15.6	NA	0.52	0.49	0.49
ENV-01-DB	-15	15.2	0.49	0.55	0.52	0.50

The highest $\delta^{15}N$ and lowest $\delta^{13}C$ ratios were observed in the killer whale SW1997/135c from Harris, in the Western Isles, Scotland. Higher $\delta^{15}N$ ratios suggest that this individual may be of higher trophic status than the other individuals while lower $\delta^{13}C$ results suggest that this individual may have derived its primary carbon intake from an

offshore higher trophic status source than those of the other animals (56). Das (56) and Hobson (57) report that the general pattern of inshore, benthos-linked food webs tends to be more enriched in $\delta^{13}C$ compared to offshore, pelagic webs. This hypothesis may support the findings of this current work, where killer whale SW1997/135c $\delta^{13}C$ data suggest that this whale may have derived a proportion of its prey from an offshore marine-mammal based source.

5.3.2 OCP levels in Killer whales

OCP levels were more elevated in one individual (SW1997/135c) than in the other whales analysed (see table 5.3). As previously discussed, stable isotope analysis also suggests that this individual may have had a different feeding ecology compared to the other individuals, and been of a higher trophic status. This interpretation is supported by Ylitalo who suggested that higher contaminant concentrations found in transient killer whales (as against residents) can be attributed to dietary differences in the two ecotypes, i.e. transient killer whales feed largely on marine mammals, with elevated POP levels, while resident animals are primarily piscivores (58). Additionally Herman reported the unambiguous presence of three "ecotypes" for North Pacific killer whales; in addition to transient and resident killer whales the authors assigned an additional offshore ecotype (59).

Within the North East Atlantic, killer whales are known to feed predominately on fish species, however killer whales have been reported to feed on seals (21). While the killer whale SW1997/135c stable isotope and contaminant data may suggest marine mammal based dietary influences, it is still not known to what extent killer whales feed on seals or possibly other marine mammals in European waters, and if a 'transient like' marine mammal consuming 'ecotype' exists.

OCP levels were highest in lipid rich blubber tissues compared to muscle and kidney tissue. Wet weight OCP levels in dorsal blubber were found to be higher than those in mid-blubber while lipid based normalisation procedures reversed this observation. Krahn *et al* have previously reported such variations and suggested that OCP concentrations may be more dependent on blubber sampling depth rather than the tissue sampling location (60). Data from this current work further stress the importance in adopting standardised procedures (where possible) for the collection of blubber samples from marine mammals.

Table 5.3: Concentrations of selected OCPs in killer whales (*Orcinus orca*) from British and Irish coastal waters (mg kg^{-1}) wet weight.

	SW1995/54	SW1994/56a	SW1994/169a	SW1994/177b4	SW1997/135c	SW2001/234	ENV-01-DB	ENV-01-MB	ENV-01-KD	ENV-01-MU	ENV-01-VB
Tissue	B	B	B	B	B	B	DB	MB	K	M	VB
%Lipid[1]	49.0	65.0	71.0	44.0	68.0	46.0	54.0	22.8	NA	NA	NA
α-HCH	0.07	0.30	< 0.001	0.03	0.20	0.02	0.14	0.08	0.01	< 0.001	0.13
γ-HCH	0.05	0.07	< 0.001	0.02	0.18	0.03	0.02	0.01	< 0.001	0.001	0.02
HCB	0.74	1.80	0.27	0.07	5.80	0.38	0.18	0.10	<0.03	<0.03	<0.03
***p, p'*-DDE**	18.0	46.0	18.0	2.60	386	79.0	36.3	11.8	0.04	0.14	0.72
***p, p'*-DDD**	2.90	4.70	0.81	0.39	16.0	1.70	2.29	1.27	0.09	0.03	2.48
***p, p'*-DDT**	3.60	2.60	0.62	0.49	6.4	1.80	1.45	0.95	0.04	0.02	1.58
Dieldrin	2.40	5.60	0.82	0.49	50.0	1.20	NA	NA	NA	NA	NA
CB153	2.90	8.30	3.70	1.30	141	19.0	19.2	13.8	0.02	0.07	NA
Σ7PCBs[2]	9.54	23.5	11.6	3.31	365	55.3	47.0	34.1	0.04	0.16	NA
Trans-chlordane	NA	NA	NA	NA	NA	NA	0.05	0.02	<0.02	0.08	NA
Cis-chlordane	NA	NA	NA	NA	NA	NA	0.05	0.13	<0.01	<0.02	<0.02
aldrin	NA	NA	NA	NA	NA	NA	<0.02	<0.02	n.d.	<0.02	<0.03
isodrin	NA	NA	NA	NA	NA	NA	<0.02	<0.02	NA	<0.04	<0.03

[1] Hexane extractable lipid (%HEL) for British waters samples.
[2] The sum of PCBs 28, 52, 101, 118, 138, 153 and 180.
NA= Not available.
n.d.= Not detected.
NA= Not analysed
B= Blubber
DB= Dorsal blubber
MB= Mid blubber
K= Kidney
M= Muscle
VB= Ventral blubber

5.3.2.1 HCH and HCB in killer whales

Technical grade hexachlorocyclohexane (HCH) consists of a number of isomers, but generally only the α-, β- and γ- isomers are detected in biological samples. Concentrations of α-HCH in individual blubber tissues in this study were low, ranging < 0.001 to 0.3 mg kg^{-1}wet weight suggesting that killer whales in common with a number of other marine species may have the capacity to regulate α-HCH levels within their metabolic system. DeBruijn (61) and Isnard & Lambert (62) report log K_{ow} values ranging from 3.69 to 4.0 for α-HCH and associated isomers; therefore HCH isomers would be expected to show relatively low levels of bioaccumulation. Buser reported that, of the HCH isomers, only α-HCH is enantiomeric and while each of the isomers and enantiomers has identical physical and chemical properties and abiotic degradation rates, they may have different biotransformation rates (63). Moisey further suggests that the ability of an organism to degrade α-HCH increases with trophic level (64).

Ratios of α-HCH to γ-HCH in samples from British waters ranged from 0.64 to 4.22 (mean 1.76) while the ratio recorded in 3 separate blubber tissues from the Irish killer whale ranged from 6.5 to 7.36 (mean 6.67). Variations in α-HCH to γ-HCH ratios may suggest dietary preferences such as fish, zooplankton, squid or other marine mammal sources within individuals, or may relate to the capacity within animals to preferentially bio-transform/metabolise α-HCH.

For comparison purposes shellfish from Irish coastal waters show greater contribution of low log K_{ow} compounds such as α-HCH and HCB (see table 5.2) in their overall contaminant burden compared to that observed in the Irish killer whale Glynn (46). The primary bioconcentration route for low log K_{ow} compounds in shellfish and or fish is via uptake through gills from their surrounding waters, while killer whales have a smaller

percentage of respiratory surface in contact with water relative to their body mass therefore bio-concentration of contaminants occurs primarily though their diet. Additionally killer whales may have a much greater capacity to metabolise and eliminate such compounds.

Thomann suggested that low biomagnification of compounds with log Kow < 5.0 is due to decreased uptake and increased excretion (65). Clark and Mackay suggest that HCB may be metabolized in fish and that significant bioaccumulation is unlikely in fish because fugacity never reaches that of prey due to rapid elimination through gill water and excretion. As fish predominate the diet of North East Atlantic killer whales, low bioaccumulation of HCB would be expected (66).

5.3.2.2 DDT and PCBs in killer whales.

The log K_{ow} values for DDT, *p, p'*-DDE and PCB congeners in combination with their relatively low water solubility, can render these contaminant groups difficult to eliminate from within organisms ultimately leading to bioaccumulation in lipid rich tissues such as blubber. This study found *p, p'*-DDE followed by (Σ)7 PCBs to be the predominant contaminants determined in all samples. In one female individual (SW1997/135c) from Scottish waters levels of *p, p'*-DDE reached 386 mg kg^{-1} wet weight, between 5 to 148 times higher on a wet weight basis than those determined in any of the other individuals. On an additional note, elevated levels of PBDE congeners have also been reported in this individual compared to other killer whales from UK waters (67).

A number of contaminant studies in marine mammals have determined the *p, p'*-DDE/Total DDT ratio in order to assess the chronology of DDT inputs. Aguilar (68),

and Tanabe (69) stated that a ratio > 0.6 is indicative of a stable system with no new DDT inputs. In the current study, *p, p'*-DDE/Total DDT ratios determined in killer whales blubber samples ranged from 0.73 to 0.96, suggesting that killer whale DDT residues are derived from historic contamination. A degree of caution must be observed when interpreting such ratios though as several studies in delphinid species (68, 70-71) have shown higher transfer rates of *p, p'*-DDE from mother to calf during lactation than was observed for *p,p'*-DDT or total DDT, potentially altering the observed ratio.

The female killer whale from Irish waters was found to be sexually mature, with numerous corpora scars on her ovaries, indicating previous reproduction, however, insufficient data are currently available on whether the other females in this study had previously given birth. Consequential disproportionate *p, p'*-DDE/Total DDT transfer therefore cannot be eliminated in these whales. Ultimately the measurement of *p, p'*-DDE/Total DDT ratios in mature male animals may provide a better indication of the origin of DDT inputs.

5.3.3 Trophic transfer and biomagnification in killer whales.

Strong correlations were observed between Log (X+1) normalised OCP data and $\delta^{15}N$ derived trophic status for ΣDDT (R=0.78), p, p'-DDE (R=0.77) and p, p'-DDD (R=0.69). Positive correlations were also observed between Log (X+1) normalised PCB congeners CB101, CB118, CB153, CB105, CB138 and CB180 and the ΣICES7CBs (R=0.93). Weaker correlations were observed for dieldrin and HCB, while no correlation was observed between $\delta^{15}N$ derived trophic status and α-, β–, or-γ- HCH isomers (See figure 5.3). These results suggest that bioconcentration through the food chain is the primary exposure route for cetaceans and marine mammals for a number of organochlorine compounds.

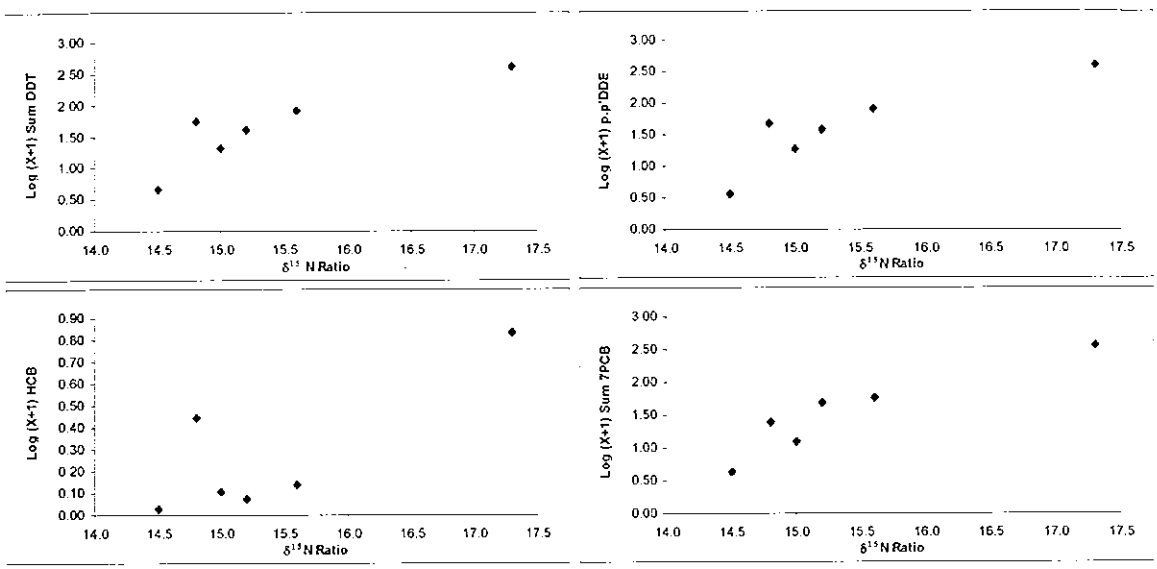

Figure 5.3: Correlations between $\delta^{15}N$ derived trophic status and log (x+1) normalised wet weight contaminant data. A=ΣDDT, B= *p,p'*DDE. C=HCB and D=Σ7PCBs

A weak correlation between $\delta^{15}N$ derived trophic status and Log (X+1) normalised CB52 concentrations was observed, while no correlation was observed for CB28, CB31 or CB156, each of which were only present in low concentrations. Mackay reported log K_{ow} values of between 5 and 8 for PCB congeners, with the log K_{ow} value rising with an increase in the degree of chlorination (72), supporting the findings of this current study where trophic transfer/bioaccumulation is less pronounced for lower chlorinated congeners with lower log K_{ow} values.

Fisk reported that food web magnification factors where diets contain only fish and invertebrates, tend to be lower that those which also contain marine mammals (37). Both, Fisk (37) and Ruus (38), suggest that marine mammals in a food web tend to show higher potential for biomagnification of OCPs than fish or invertebrate species. Ruus further suggest that this may be attributed to the greater energy requirements and feeding rate of such homeotherms (38).

5.3.4 Enantiomeric profiling

Structurally sensitive biological receptors in animals can differentiate between the subtle differences in molecular and enantiomeric configurations of chiral compounds, allowing enantioselective degradation or metabolism to take place (73), thereby potentially providing further information on the fate, dynamics and toxicological relevance of POPs. Such enantioselective processes have been reported to occur at different rates between (and often within) species and between different organisms at different trophic levels (74).

Enantiomeric fractions were calculated as per Kallenborn and Hühnerfuss (35) and deGeus (75) for four compounds namely o, *p*'-DDT, α-HCH and toxaphene congeners CHB 26 and CHB50 were determined in each of the individual killer whales. α-HCH showed some enantioselective enrichment with EFs of 0.14 to 0.49 being determined. EFs of 0.41-0.55, 0.49-0.52 and 0.45-0.54 were observed for o, *p*'-DDT, CHB26 and CHB50 respectively (see table 5.3), these values showing little deviation from that of the racemate. No clear correlations between enantioselective enrichment, ($\delta^{13}C$) derived carbon intake, ($\delta^{15}N$) derived trophic status and individual animal length or sex were observed.

5.4 Conclusions

Elevated contaminant levels, enriched nitrogen and depleted carbon isotopic ratios were determined in one individual whale from the Scottish Western Isles compared to the other killer whales suggesting marine mammal dietary influences in this animal. Enantioselective enrichment of α-HCH enantiomers and accumulation was demonstrated, providing a potential mechanism to trace energy flows through complex food webs and to track primary productivity.

The potential application of isotopic ratios in routine monitoring studies to model contaminant uptake, enantioselective enrichment and accumulation was demonstrated. Data was presented providing information on enantioselective enrichment factors (EFs) for o, *p*'-DDT, α-HCH and toxaphene congeners CHB26 and CHB 50.

This dataset further improves the current database on the levels of a number of contaminants and provides additional background information on potential metabolic processes of killer whales from British and Irish waters.

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CHAPTER 6: THE ROLE OF STABLE ISOTOPES ANALYSIS IN MODELLING TROPHIC TRANSFER OF PERSISTENT ORGANIC POLLUTANTS

6.1 Introduction

The technique of stable isotope fractionation is routinely utilised in a range of diverse environmental and biological applications including, geochemical tracing of oil and other pollutant spill sources, tracking of atmospheric sulphur deposition, monitoring of bacterial degradation in remediation studies and in tracing the history of eutrophication processes.

One of the most significant applications of the technique however lies in the measurement of the degree of fractionation of nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) in marine species to assess relative trophic level status in biotic systems and to assess dietary carbon sources in aquatic biota (1-6) where trophic level status (TS) can simply be expressed as the relative position of constituent organisms within an ecosystem to each other.

As such stable isotope techniques have been shown to provide a quantitative, continuous variable to study biomagnification of contaminants and to demonstrate trophic interactions within complex food webs (7-8).

This current chapter further describes the scientific principles underpinning the use of stable isotope approaches and discusses the role that stable isotopes play in modelling both the relative trophic level status and of contaminant uptake in marine species resident in Irish and surrounding waters.

6.1.1 Ecological considerations for the use of stable isotope techniques.

Stable isotope based techniques assume that during the ingestion of food and excretion of waste products, that a stepwise enrichment of the heavier isotope (i.e., ^{13}C or ^{15}N)

occurs relative to the lighter isotopes ^{12}C and ^{14}N in a process known as fractionation. Predators preferentially excrete the lighter isotopes therefore becoming enriched with the heavier isotope, the process often being reported to take place in a stepwise manner through the food web from primary producer to consumer (5, 9-12).

When completing dietary assessments within organisms, stable isotope data provide some advantages compared to conventional techniques such as stomach content analysis (SCA). SCA can be subject to greater temporal changes depending on feeding/fasting habits of the species of interest, and may also be dependent on the availability of prey species during feeding cycles. Additionally fish have been known to regurgitate stomach contents on capture (13).

While stable isotopes of nitrogen and carbon have become useful tools in tracing feeding ecology in fish species, care has to be taken when interpreting isotopic data. In fasting (enforced or otherwise) animals, $\delta^{15}N$ values can often be enriched due to the catabolic breakdown of protein reserves and in such instances SCA can complement isotopic data. Stable isotope analysis is preferable however in examination of temporal diet patterns compared to traditional methods such as SCA.

A further factor that can influence isotopic ratios is the actual turnover rate of nutrients in individual tissues of an organism. MacNeil et al (14) report that it may be advisable to select multiple tissue types when designing trophic status based experiments as the turnover rates of nutrients can differ between tissues. Such advice needs to be considered especially where an animals diet is not homogeneous or where feeding is sporadic throughout its lifetime. Large consumers, such as fish, have tissue turnover

rates ranging from months to years and their isotopic signature is representative of their diet over long periods of time (15).

Tieszen et al (16) found that more metabolically active tissues have more rapid isotopic turnover rates than other less metabolically active tissues, such as scale and/or bones; therefore, muscle tissue is generally preferable for stable isotope analysis purposes. The rate of isotopic turnover in muscle is less than would be observed in liver tissue enabling the animals' longer term feeding habits to be examined and as the liver has a faster isotope turnover rate than muscle tissue, isotopic ratios will better reflect an integrated diet over shorter periods than those of muscle tissue (17).

6.1.1.1 Biotic fractionation of nitrogen isotopes

Changes in the isotopic ratio ($\delta^{15}N$) between ^{14}N and ^{15}N of a consumer can typically be enriched by 3–4‰ for each stepwise increase in trophic status relative to its prey (11, 18-19) thereby potentially providing a measure of trophic status relative to animals primary prey.

Excreted nitrogen is typically depleted in ^{15}N relative to a consumers diet, therefore greater trophic shifts for N can be observed in animals whose diet contains the highest N content. As the ratio of excreted to assimilated N is much greater during periods where an animal does not feed, greater $\delta^{15}N$ ratios will be observed in such starved animals (20). While the form of excretion and rates may affect $\delta^{15}N$ ratios, the full extent to which these affect overall ratios is still unclear.

6.1.1.2 Biotic fractionation of carbon isotopes

Ratios of carbon ^{13}C and ^{12}C isotopes ($\delta^{13}C$) change little as carbon moves through the food web (19, 21-22); therefore ($\delta^{13}C$) measurements can typically be used in evaluation of the ultimate carbon sources of an organism. The use of stable isotope based approaches in combination with traditional methodologies such as gut/stomach contents analysis can account for dietary assimilation over extended feeding periods and can provide valuable information related to the overall feeding ecology within a species or food web (11).

It must be noted that carbon can enter the body of an organism via food assimilation, thereby contributing to organism growth through anabolic pathways or it may be lost via excretion and/or respiration. As respiratory CO_2 is isotopically lighter than assimilated carbon, animals are generally slightly enriched relative to their diet. As a consequence any observed trophic shift will be greatest in animals with the highest rates of respiration relative to growth and as a result $\delta^{13}C$ should be greater in birds and mammals compared to fish and invertebrates. Additionally as uric acid and urea contain carbon and ammonia does not, the form of excreted waste can also affect isotopic fractionation, especially in animals with high excretion rates (23).

6.1.2 Stable isotopes as a quantification tool.

As previously discussed stable isotope techniques can provide a continuous measure of trophic position integrating the assimilation of energy or mass flow through different trophic pathways and to track energy or mass flow through ecological communities (19, 24-25).

Generation of stable isotope data $\delta^{15}N$ is a useful mechanism for the estimation of the "relative" trophic status of members of a particular food web, however such data are generally not sufficient to further infer trophic position or carbon source without an appropriate isotopic baseline. Post (26) reports that the combination of stable isotope data from two primary consumers (such as surface-grazing snails and filter feeding mussels) can capture the temporal and spatial variation at the base of the littoral and pelagic food chains in lake ecosystems respectively. As such, data from primary consumers can be employed where trophic level estimates of secondary or higher-level consumers are required.

$\delta^{13}C$ has been used to differentiate between diets based on plants with different photosynthetic pathways (19, 27), while in lake ecosystems $\delta^{13}C$ has been reported to allow differentiation between two major sources of available energy, littoral production (algae and detritus), and pelagic production from phytoplankton. In such studies the baseline $\delta^{13}C$ of the littoral food web tends to be enriched in ^{13}C (less negative $\delta^{13}C$) relative to that at base of the pelagic food web (28).

As a result stable isotope data collected from differing ecosystems and/or locations should not be directly compared without additional information on the variation of $\delta^{15}N$ and $\delta^{13}C$ of primary producers at the base $\delta^{15}N_{base}$ and $\delta^{13}C_{base}$ of the ecosystem under investigation. Without such information there is no reliable way to determine whether $\delta^{15}N$ and $\delta^{13}C$ changes within an organism reflect changes in food web structure or carbon flow or whether they reflect a true shift in the baseline (δN_{base} and δC_{base}) of the ecosystem.

A number of difficulties are encountered in defining such a baseline in marine and terrestrial ecosystems, namely the selected organism must capture temporal (and spatial) variations in $\delta^{15}N$ of primary producers and theoretically should also integrate the isotopic signature on a time-scale comparable to that of other constituent members of the web, there is little evidence however to show that long lived organisms provide such information.

One model proposed for estimating the trophic position of a secondary consumer is:

Equation 13= Trophic level $= \lambda + (\delta^{15}N_{\text{secondary consumer}} - \delta^{15}N_{\text{base}})/\triangle N$

Where

λ = trophic position of the organism estimating $\delta^{15}N_{\text{base}}$ e.g. 1 for primary producers.

$\triangle N$ = enrichment in $\delta^{15}N$ per trophic level (‰)

While $\triangle N$ above, is generally assumed to fall between 3 to 4‰, $\delta^{15}N_{\text{secondary consumer}}$ and $\delta^{15}N_{\text{base}}$ need to be analytically measured in order for the trophic status to be determined (19, 26).

Further difficulties arise in trophic level assessment where the consumer derives nutrient sources from a combination of sources, these are built in as follows,

Equation 14= $TL = \lambda + (\delta^{15}N_{\text{secondary consumer}} - ([\delta^{15}N_{\text{base}} * \alpha] + [\delta^{15}N_{\text{base2}} * (1-\alpha)])/\triangle N$

Where :

α= proportion of nitrogen in the consumer derived from the base of food web one. A value of 3.4 is generally used to depict an increase in trophic level (26)

Further mixing models as reported by Fry and Sherr (29) are not further discussed in this report. While Post (26) primarily discusses lake ecosystems similar principles as those above apply to marine and other aquatic ecosystems.

In this present study the soft body tissue of the primary consumer the blue mussel (*Mytilis edulis*) was collected as it reflects the isotopic signature of their diets. Shells of mussels are a biologically mediated, carbon-based precipitate that can better reflect the isotopic signature of the inorganic environment and as such shells were not utilised in this current study (30). Zohary (31) and delGiorgio and France (32) report that seston may contain recalcitrant carbon that is not assimilated by organisms, seston collected in this present study was utilised to present indicative information on the isotopic signature of material available to primary consumers and were not further employed when assigning trophic level status to marine web constituents.

6.1.3 Trophic level data from Irish marine species

While extensive research has been reported for fish species from Pacific and western Atlantic regions (33), relatively few data are available for marine species from the waters surrounding Ireland (5, 34-35). Tables 6.1 and 6.2 present selected stable isotope data currently available in the literature for fish and marine mammals from waters surrounding Ireland. It should be noted for comparison purposes that Pinnegar et al (5) did not carry out lipid removal from tissues prior to stable isotope analysis. As such this current study provides further baseline stable isotopic information for species from Irish coastal waters.

Satterfield (33) reported trophic level hierarchy in pacific salmon from chinook to coho, sockeye and pink salmon. Chum salmon were seen to be part of a different food web than the other species. These results have implications for this present study as tinned salmon on the Irish marketplace may originate in pacific regions and would thereby potentially show different contaminant residue levels and/or patterns than those of Irish origin.

These spatial differences can result in the requirement for additional isotope information for base primary producers such as phytoplankton. Variation in the isotopic composition in phytoplankton can in part be a direct result of a number of factors including species assemblage differences, carbon source differences, nitrogen assimilation and usage, nutrient cycling processes and differences in growth rates. Variation can occur where different preparation steps have been employed prior to the determination of isotopic ratio data. Fish condition data should also be collected during sampling to minimise effects disease or fasting may have on muscle tissue (33).

Table 6.1: Selected mean and (median) stable isotope data ($\delta^{15}N$ and $\delta^{13}C$) in marine mammal species from Irish waters as per Das (35).

Common name	Species	$\delta^{15}N$	$\delta^{13}C$ mean ± SD	number
Striped dolphin	*Stenella coeruleoalba*	10.8 (11.0) ± 0.6	-17.5 (-17.5) ± 0.1	n=3
Common dolphin	*Delphinus delphis*	12.2 (12.4) ± 1.0	-17.1 (-17.1) ± 0.4	n=14
Atlantic white-sided dolphin	*Lagenorhynchus acutus*	12.7 (12.6) ± 0.5	-17.0 (-17.2) ± 0.5	n=4
Harbour porpoise	*Phocoena phocoena*	14.1 (13.9) ± 1.6	-16.5 (-16.4) ± 0.7	n=7
White-beaked dolphin	*Lagenorhynchus albirostris*	15.8 (16.4) ± 2.3	-16.3 (-16.4) ± 0.3	n=3

Table 6.2: Selected stable isotope data ($\delta^{15}N$ and $\delta^{13}C$) and derived trophic status in fish species from Irish waters, TS calculated as per Pinnegar (5) and Domi (34).

Common name	Species	$\delta^{15}N$ mean ± SD	Trophic Status mean ± SD
Lesser spotted dogfish	*Scyliorhinus canicula*	15·0 ± 0·91	4·29 ± 0·27
Tope	*Galeorhinus galeus*	17·0 ± 0·82	4·88 ± 0·24
Spurdog	*Squalus acanthias*	12·0 ± 0·57	3·41 ± 0·17
Spotted ray	*Raja montagui*	13·7 ± 0·93	3·91 ± 0·27
Cuckoo ray	*Leucoraja naevus*	13·6 ± 0·44	3·88 ± 0·13
Herring	*Clupea harengus*	13·3 ± 0·23	3·79 ± 0·07
Sprat	*Sprattus sprattus*	14·3 ± 1·26	4·09 ± 0·37
Anchovy	*Engraulis encrasicolus*	14·6 ± 0·95	4·17 ± 0·28
Anglerfish	*Lophius piscatorius*	14·3 ± 0·72	4·09 ± 0·21
Cod	*Gadus morhua*	15·2 ± 0·77	4·35 ± 0·23
Haddock	*Melanogrammus aeglefinus*	12·6 ± 0·85	3·59 ± 0·25
Blue whiting	*Micromesistius poutassou*	11·1 ± 0·64	3·14 ± 0·19
Saithe	*Pollachius virens*	14·4 ± 0·60	4·11 ± 0·18
Norway pout	*Trisopterus esmarki*	13·7 ± 1·03	3·91 ± 0·30
Poor cod	*Trisopterus minutus*	12·9 ± 1·12	3·67 ± 0·33
Hake	*Merluccius merluccius*	13·5 ± 0·29	3·85 ± 0·09
John dory	*Zeus faber*	14·7 ± 0·74	4·20 ± 0·22
Red gurnard	*Aspitrigla cuculus*	13·2 ± 0·11	3·76 ± 0·03
Grey gurnard	*Eutrigla gurnardus*	12·8 ± 0·23	3·64 ± 0·07
Red mullet	*Mullus surmuletus*	15·3 ± 0·82	4·38 ± 0·24
Megrim	*Lepidorhombus whiffiagonis*	12·5 ± 0·74	3·56 ± 0·22
Witch	*Glyptocephalus cyanoglossus*	13·6 ± 0·51	3·88 ± 0·15
Long rough dab	*Hippoglossoides platessoides*	14·1 ± 0·16	4·03 ± 0·05
Dab	*Limanda limanda*	14·7 ± 1·66	4·20 ± 0·49
Lemon sole	*Microstomus kitt*	12·9 ± 2·00	3·67 ± 0·59
Flounder	*Platichthys flesus*	13·5 ± 1·17	3·85 ± 0·34
Plaice	*Pleuronectes platessa*	12·9 ± 0·97	3·67 ± 0·29
Sole	*Solea solea*	14·6 ± 0·71	4·17 ± 0·21
Whiting	*M. merlangus*	17.2 ± 1.24	
Horse Mackerel	*T. trachurus*	13.8 ± 0.41	
Mackerel	*S scombrus*	12.7 ± 1.26	

Note: $\delta^{15}N$ and $\delta^{13}C$ data reported without lipid removal for Jennings

Hobson (36) has shown that isotopic differences exist between farmed species and their wild counterparts. These differences are reported to be as a result of differences in diet where fish feed is usually composed of waste fish and other terrestrial compounds. These observations will be further examined as part of this current study and the potential for the utilisation of stable isotope based approaches for the differentiation of wild and farmed/non-indigenous species are further discussed.

6.1.4 Trophic level accumulation of contaminants in biota.

As previously discussed bioaccumulation of organochlorine residues is often observed to increase through the food web, this usually being attributable to biomagnification processes. Biomagnification assumes more efficient food chain transfer of contaminant residues compared to the transfer of energy, ultimately resulting in higher POP levels as trophic status increases.

This phenomenon requires that the food conversion co-efficient (gross growth efficiency) (α) is lower than the POP assimilation co-efficient (γ), resulting in the net trophic transfer from lower to higher levels and has been shown to be positively related to $\delta^{15}N$ in both freshwater and marine food webs (37-42).

Broman (43) modelled such a trophic linkage for the uptake of PCDD and PCDF congeners in two different food chains. Stable isotope analysis ($\delta^{15}N$) was used to identify different trophic level changes between phytoplankton to eider ducks in one grouping and phytoplankton to cod in a second food web. An inverse relationship between $\delta^{15}N$ derived trophic status and concentrations in 2,3,7,8-substituted PCDDs and PCDFs that also show 1,4 and/or 6,9 chlorine substitution was observed. OCDD

and OCDF were not accumulated to a great degree and their concentrations decreased with increasing trophic status. The authors suggest that these congeners are metabolised more efficiently as they have larger cross-sectional areas (0.95nm) than congeners with other substitution characteristics.

A further study by Hargrave (44) showed different biomagnification rates in some species in the Canadian polar marine food chain. Unlike bioconcentration studies where conditions are controlled, biomagnification of POPs in marine environment settings can be more difficult to predict, as a result of additional temporal factors and/or consumer dietary influences.

While this process may often be shown to be linear, care has to be taken when assessing resultant datasets as there are often major differences other than diet alone, some factors of importance include species size, age, sex and lipid composition. The issue of consumer omnivory must also be considered so that corrections can be made for species that do not prey on specific single populations thereby resulting in potential for error introduction (45). Where shellfish $\delta^{15}N$ has been determined sampling proximity to potential nutrient sources must be investigated as these can result in elevated ratios being observed (33).

In recent years the use of stable isotope techniques has aided the elucidation of some key trophic status and marine food web dietary linkages and has enabled scientists continue to clarify contaminant related metabolic pathways and fate in marine species.

6.2 Materials and methodology

Biological details, sampling location information in addition to analytical methodologies and associated quality assurance employed for all samples analysed during this current work are presented in chapter 1. Detailed sampling and analytical data for blue whiting, eels and killer whales are additionally documented in chapters 3-5. Concentration and isotopic ratio data for each of the samples are documented in table 6.3 and in annexes 2 to 7.

6.2.1 Evaluation tools employed in stable isotope studies

In order to demonstrate biomagnification of POPs through the full marine web, biomagnification factors (BMFs), food web specific magnification factors (FWMFs) and species/group magnification factors (SFFs) were calculated as follows.

6.2.1.1 Biomagnification factors (BMFs).

The process of direct biomagnification of contaminants from prey to predator/consumer was calculated in the food web of the killer whale as primary consumer with other pelagic fish species as its potential prey. This food web interaction is based on the findings of post-mortem stomach contents data for one killer whale, which was found to have salmon bones in its stomach at time of death. Additional to this Simila (46) report the importance of herring and salmon in the diet of resident killer whales from Norwegian waters. Contaminant data in killer whales and fish species were subjected to a two-sample z-test to test the hypotheses that the means of contaminant concentrations significantly differ; thereby differences can be considered to be a result of prey to predator biomagnification. Therefore biomagnification of POP groupings in this Killer whale/fish web was estimated by the calculation of the bio-magnification factor (BMF) as follows,

Equation 14=

$$BMF = \frac{POPpredator}{POPprey} / \frac{TLpredator}{TLprey}$$

where,

$POP_{predator/prey}$ = the lipid normalised log_{10} normalised data for individual contaminants

$Tl_{predator/prey}$ = trophic level of predator/prey as per Wada and Minigawa.

6.2.1.2 Species/group specific and food web magnification factors.

FWMF and SMFs were estimated by initially calculating the slopes of the lines derived from plotting the mean $\delta^{15}N$ derived trophic status against the mean log_{10} transformed contaminant data for all constituent species in the web under study. By further calculating the FWMF= 10^b (where b is the slope of the line) this provides an indication of the magnitude of biomagnification that occurs in this particular food web (47-49). These FWMF data can then be compared to previous studies from other marine ecosystems.

While it was only possible to generate BMF data for the killer whale to fish food web, in order to further evaluate biomagnification/elimination processes on a species-specific basis, a species/group specific magnification factor (SMF) was generated for each of the species investigated.

While the BMF generated on the basis of mean log_{10}-transformed concentration and trophic status data provides an indication of the magnitude of biomagnification between two species, an element of the within species variance in both trophic status and contaminant concentrations can be lost in such calculations. The generation of SMFs allows for contaminant biomagnification/elimination processes within individual species or groupings (e.g. all pelagic fish or all shellfish) to be further investigated.

Eel data were removed from FWMF calculations as their heavier $\delta^{13}C$ isotopic signature suggests a benthos-based feeding regime compared to the other constituent web members, however their position relative to other species in the marine web can be established by the generation of SMFs thereby providing further insights into biomagnification/elimination within the species.

6.2.1.3 Data considerations for FWMFs, BMFs and SMFs.

Data were treated differently for the generation of food web and species magnification factors. Where data below the LOQ and/or the limit of detection were recorded these were not used for the generation of food web or species magnification factors.

Should such data be included there is a potential for the calculated FWMFs, BMFs and/or SMFs to be over estimated as a result of being a function of the limit of quantitation of the analytical method. Therefore generation of FWMFs and SMFs based on "true" values ensured that false magnification factors were not generated.

Where FWMFs, BMFs and SMFs were generated for the sum (Σ) of contaminant groups, the number of constituent congeners at levels greater than the LOQ used to generate the factor is also reported. In some cases data from only two or three congeners may have been used in the generation of the relevant magnification factor.

Additionally where samples have been identified as being statistical or other outliers from the main body of data, details on these omissions are also reported. In some cases (e.g. oysters/eels) potential point source inputs identified in concentration box plots may result in their omission from further statistical analysis.

6.3 Results and discussion

As the detailed biomagnification/bioaccumulation of POPs and the role stable isotope derived trophic position plays in contaminant levels/profiles has not been previously been studied in constituent species in Irish waters, the following work packages were completed during this current work,

- Completion of an isotopic ratio based evaluation of the structure of the marine web for selected species (6.3.1).
- Derivation of $\delta^{15}N$ and $\delta^{13}C$ baseline information for the primary consumer blue mussels (*Mytilis edulis*) from Irish waters (6.3.2).
- Assigning relative trophic status to constituent members of the web (6.3.3).
- Investigation of the potential application of $\delta^{15}N$ and $\delta^{13}C$ isotope ratios in modelling lipid levels in marine organisms (6.3.4).
- Investigation of the potential role of $\delta^{15}N$ and $\delta^{13}C$ to model accumulation/metabolism of selected POPs in marine species (6.3.5).
- Investigation of the role of stable isotope analysis as a potential tool for identification of wild/farmed species (6.3.1.4).

6.3.1 Isotopic structure of the study marine web.

The current study determined stable isotope ratios in individual muscle/blubber tissue samples covering a variety of marine species and/or retail fishery produce. With the exception of 5 killer whale samples from Scottish waters, and all tinned samples, all samples originate in the waters around Ireland.

The utilization of $\delta^{15}N$ vs. $\delta^{13}C$ scatter-plots to further illustrate the spread of $\delta^{15}N$ and $\delta^{13}C$ isotopic ratios within a particular marine web has been well documented, however

this is the first reported study to present data from a wide range of trophic levels in species from Irish waters. Tables 6.3 and 6.4 present all $\delta^{15}N$ and $\delta^{13}C$ stable isotope data collected during this study. Tables 6.4 and 6.5 present summary statistics for wild species and marine mammals and farmed species and other retail fishery produce respectively while figures 6.1 and 6.2 graphically present the spread of $\delta^{15}N$ and $\delta^{13}C$ isotopic ratio data in wild species and marine mammals and in farmed species and other retail fishery produce respectively.

Table 6.3: Stable isotope $\delta^{15}N$ and ($\delta^{13}C$) ratio data (‰) in farmed fish and retail collected fish produce.

	Kipper (Tin)	Mackerel (Tin)	Sardines (Tin)	Tuna (Tin)	Salmon (Smoked)	Salmon (Tin)	Salmon (Farmed)
Sample 1	13.2 (-17.6)	11.8 (-19.2)	10.3 (-15.7)	13.3 (-17.8)	13.1 (-18.8)	10.9 (-20.0)	14.3 (-19.1)
Sample 2	13.9 (-17.4)	12.2 (-19.2)		13.2 (-17.4)	13.3 (-20.4)	11.4 (-20.6)	15.6 (-19.5)
Sample 3				14.0 (-17.1)	13.2 (-20.4)	10.7 (-20.7)	13.8 (-18.7)
Sample 4				13.4 (-16.7)	14.5 (-19.8)	11.1 (-21.1)	15.2 (-18.8)
Sample 5				13.0 (-17.2)	13.5 (-20.0)	11.4 (-21.3)	14.3 (-18.6)
Sample 6					13.8 (-20.1)		15.2 (-19.1)
Sample 7					13.6 (-20.0)		13.9 (-19.6)
Sample 8					13.8 (-19.0)		14.3 (-19.0)
Sample 9					14.4 (-19.9)		13.3 (-20.1)
Sample 10					13.5 (-20.7)		13.6 (-19.6)
Sample 11					13.8 (-20.2)		15.3 (-18.8)
Sample 12							13.4 (-19.6)
Sample 13							16.0 (-17.9)
Sample 14							15.2 (-18.2)
Mean	13.6 (-17.5)	12 (-19.2)	10.3 (-15.7)	13.4 (-17.2)	13.7 (-19.9)	11.1 (-20.7)	14.5 (-19)
STDEV	0.49 (0.14)	0.28 (NA)		0.38 (0.40)	0.45 (0.57)	0.31 (0.50)	0.88 (0.60)
CV (%)	3.65 (0.81)	2.36 (NA)		2.82 (2.34)	3.28 (2.88)	2.78 (2.43)	6.03 (3.16)

NA = Not applicable

Table 6.4: Stable isotope ratios, percentage lipid and calculated trophic status in wild, farmed and retail fishery produce collected during this study.

Sample Ref	Species	Tissue	% lipid	$\delta^{13}C_{VPDB}$	$\delta^{15}N_{AIR}$	Trophic status [1]	Trophic status [2]	Sample Ref	Species	Tissue	% lipid	$\delta^{13}C_{VPDB}$	$\delta^{15}N_{AIR}$	$\delta^{34}S_{VCDT}$	Trophic status [1]	Trophic status [2]
MSC 04/1040	Salmon wild	MU	11.7	-19.0	14.2	3.41	3.26	MSC 05/1119	Eel	MU	9.18	-21.1	15.2	16.8	3.71	3.53
MSC 04/1041	Salmon wild	MU	12.6	-18.0	12.7	2.97	2.87	MSC 05/1120	Eel	MU	15.3	-29.6	13.4	16.6	3.18	3.05
MSC 04/1042	Salmon wild	MU	9.72	-18.7	12.0	2.76	2.68	MSC 05/1121	Eel	MU	14.3	-25.8	13.9	10.2	3.32	3.18
MSC 04/1043	Salmon wild	MU	12.7	-18.2	10.9	2.44	2.39	MSC 05/1122	Eel	MU	16.0	-29.4	14.6	3.27	3.53	3.37
MSC 04/1044	Salmon wild	MU	8.99	-18.4	12.0	2.76	2.68	MSC 05/1140	Eel	MU	20.9	-28.6	11.1	12.4	2.50	2.45
MSC 04/1045	Salmon wild	MU	12.7	-18.1	11.7	2.68	2.61	MSC/2005/1053	Blue Whiting	WF	4.93	-20.5	12.4	NA	2.88	2.79
MSC 04/1046	Salmon wild	MU	12.2	-18.1	11.5	2.62	2.55	MSC/2005/1054	Blue Whiting	WF	5.28	-19.8	12.5	NA	2.91	2.82
MSC 04/1047	Salmon wild	MU	11.8	-18.1	12.1	2.79	2.71	MSC/2005/1079	Blue Whiting	MU	0.47	-19.0	12.9	NA	3.03	2.92
MSC 04/1119	Salmon tin	MU	4.77	-20.0	10.9	2.44	2.39	MSC/2005/1080	Blue Whiting	MU	0.62	-19.8	12.7	NA	2.97	2.87
MSC 04/1120	Salmon tin	MU	4.67	-20.6	11.4	2.59	2.53	MSC/2005/1081	Blue Whiting	MU	0.60	-19.8	12.4	NA	2.88	2.79
MSC 04/1121	Salmon tin	MU	8.00	-20.7	10.7	2.58	2.34	MSC 2005/1082	Blue Whiting	MU	0.60	-19.5	13.1	NA	3.09	2.97
MSC 04/1122	Salmon tin	MU	7.89	-21.3	11.1	2.50	2.45	ENV/2004 055-001	Mussels	SB	1.69	-20.5	12.1	NA	2.79	2.71
MSC 04/1123	Salmon tin	MU	7.91	-21.3	11.4	2.59	2.53	ENV/2004 055-002	Mussels	SB	1.70	-20.5	12.1	NA	2.79	2.71
MSC 04/1124	Tuna tin	MU	0.70	-17.8	13.3	3.15	3.03	ENV/2004 055-003	Mussels	SB	1.61	-20.5	12.1	NA	2.79	2.71
MSC 04/1125	Tuna tin	MU	12.5	-17.4	13.2	3.12	3.00	ENV/2004 069-001	Mussels	SB	1.98	-19.3	11.0	NA	2.47	2.42
MSC 04/1126	Tuna tin	MU	10.9	-17.1	14.0	3.35	3.21	ENV 2004 069-002	Mussels	SB	1.73	-19.3	11.0	NA	2.47	2.42
MSC 04/1127	Tuna tin	MU	0.82	-16.7	13.4	3.18	3.05	ENV 2004 069-003	Mussels	SB	1.87	-19.3	11.0	NA	2.47	2.42
MSC 04/1128	Tuna tin	MU	1.62	-17.2	13.0	3.06	2.95	ENV/2004 071-001	Mussels	SB	2.22	-17.1	10.3	NA	2.26	2.24
MSC 04/1172	Albacore	MU	5.61	-18.1	12.6	2.94	2.84	ENV 2004 071-002	Mussels	SB	2.39	-17.1	10.3	NA	2.26	2.24
MSC 04/1173	Albacore	MU	15.2	-18.8	11.6	2.65	2.58	ENV 2004 071-003	Mussels	SB	2.28	-17.1	10.3	NA	2.26	2.24
MSC 04/1174	Albacore	MU	15.8	-17.6	12.0	2.76	2.68	ENV/2004 078-001	Mussels	SB	2.81	-20.1	11.0	NA	2.47	2.42
MSC 04/1175	Albacore	MU	8.09	-18.1	11.8	2.71	2.63	ENV 2004 078-002	Mussels	SB	2.69	-20.1	11.0	NA	2.47	2.42
MSC 04/1176	Albacore	MU	5.75	-17.6	12.5	2.91	2.82	ENV 2004 078-003	Mussels	SB	2.92	-20.1	11.0	NA	2.47	2.42
MSC 04/1177	Herring	MU	13.9	-17.7	13.4	3.18	3.05	MSC 2005/1086	Killer whale [3]	BL	65.0	-14.9	14.8	NA	3.59	3.42
MSC 04/1178	Herring	MU	14.4	-17.8	13.6	3.24	3.11	MSC 2005/1087	Killer whale [3]	BL	71.0	-15.3	15.0	NA	3.65	3.47
MSC 04/1179	Herring	MU	12.0	-17.7	13.5	3.21	3.08	MSC 2005/1088	Killer whale [3]	BL	44.0	-16.7	14.5	NA	3.50	3.34
MSC 04/1180	Herring	MU	11.5	-18.1	13.0	3.06	2.95	MSC 2005/1089	Killer whale [3]	BL	68.0	-12.9	17.3	NA	4.32	4.08
MSC 04/1182	Mackerel	MU	9.04	-18.1	14.3	3.44	3.29	MSC 2005/1090	Killer whale [3]	BL	46.0	-15.0	15.6	NA	3.82	3.63
MSC 04/1183	Mackerel	MU	9.87	-18.6	14.3	3.44	3.29	MSC 2005/1091-D	Killer whale	BL	54.0	-15.0	15.2	NA	3.71	3.53
MSC 04/1184	Mackerel	MU	6.96	-18.4	13.2	3.12	3.00	MSC 2005/1092-V	Killer whale	BL	NA	-16.2	15.2	NA	3.71	3.53
MSC 04/1185	Mackerel	MU	11.4	-17.9	14.2	3.41	3.26	23ENV03 157-9	Mussels	SB	2.36	-17.2	9.00	NA	1.88	1.89
MSC 04/1186	Mackerel	MU	13.0	-17.9	14.3	3.44	3.29	24ENV03 170-2	Mussels	SB	1.97	-18.7	9.70	NA	2.09	2.08
MSC 05 0001	Sardines tin	MU	29.5	-15.7	10.3	2.26	2.24	25ENV03 146-8	Mussels	SB	1.87	-22.6	12.1	NA	2.79	2.71
MSC 05.0002	Mackerel tin	MU	28.6	-19.2	11.8	2.71	2.63	28ENV03 164-6	Mussels	SB	1.86	-21.4	9.50	NA	2.03	2.03
MSC 05.0003	Mackerel tin	MU	31.6	-19.2	12.2	2.82	2.74	MSC/2005/1053	Blue Whiting [4]	WF	4.93	-20.5	12.4	NA	2.88	2.79
MSC 05.0004	Kipper tin	MU	15.1	-17.6	13.2	3.12	3.00	MSC/2005/1054	Blue Whiting [4]	WF	5.28	-19.8	12.5	NA	2.91	2.82
MSC 05.0005	Kipper tin	MU	16.9	-17.4	13.9	3.32	3.18	MSC 2005/1055	Blue Whiting [4]	WF	3.44	-19.8	12.5	NA	2.91	2.82
ENV.04 0050	Oysters	MU	2.06	-19.7	12.8	3.00	2.89	MSC/2005/1056	Blue Whiting [4]	WF	4.74	-20.2	12.5	NA	2.91	2.82
ENV 04 0053	Oysters	MU	3.10	-18.8	12.2	2.82	2.74	MSC 2005/1057	Blue Whiting [4]	WF	3.72	-19.8	12.5	NA	2.91	2.82
ENV 04 0056	Oysters	MU	2.04	-21.3	15.4	3.76	3.58	MSC 2005/1058	Blue Whiting [4]	WF	2.47	-19.6	12.6	NA	2.94	2.84
ENV.04 0057	Oysters	MU	3.27	-21.6	14.6	3.53	3.37	MSC 2005/1059	Blue Whiting [4]	WF	3.76	-19.8	12.7	NA	2.97	2.87
ENV.04 0062	Oysters	MU	2.02	-20.5	10.7	2.38	2.34	MSC 2005/1060	Blue Whiting [4]	WF	3.85	-19.1	12.2	NA	2.82	2.74
MSC 04/1129	Salmon farmed	MU	10.9	-19.1	14.3	3.44	3.29	MSC 2005/1061	Blue Whiting [4]	WF	3.98	-20.4	12.1	NA	2.79	2.71
MSC 04/1130	Salmon farmed	MU	18.4	-19.5	15.6	3.82	3.63	MSC 2005/1062	Blue Whiting [4]	WF	2.51	-20.2	12.6	NA	2.94	2.84
MSC 04/1131	Salmon farmed	MU	15.3	-18.7	13.8	3.29	3.16	3Ballycotton F	Herring	MU	3.36	-17.4	13.7	NA	3.26	3.13
MSC 04/1132	Salmon farmed	MU	14.5	-18.8	15.2	3.71	3.53	4Dun East F	Herring	MU	3.03	-17.4	13.5	NA	3.21	3.08
MSC 04/1133	Salmon farmed	MU	12.4	-18.6	14.3	3.44	3.29	7Dingle F	Herring	LI	NA	-18.3	12.9	NA	3.03	2.92
MSC 04/1134	Salmon farmed	MU	17.2	-19.1	15.2	3.71	3.53	8Malin F	Herring	LI	NA	-18.3	12.2	NA	2.82	2.74
MSC 04/1135	Salmon farmed	MU	14.4	-19.6	13.9	3.32	3.18	9Dingle M	Herring	LI	NA	-17.8	12.2	NA	2.82	2.74
MSC 04/1136	Salmon farmed	MU	12.3	-19.0	14.3	3.44	3.29	12Malin M	Herring	LI	NA	-18.1	11.9	NA	2.74	2.66
MSC 04/1137	Salmon farmed	MU	16.3	-20.1	13.3	3.15	3.03	13Dun East M	Herring	MU	3.69	-17.4	13.4	NA	3.18	3.05
MSC 04/1138	Salmon farmed	MU	15.5	-19.6	13.6	3.24	3.11	14Dingle M	Herring	MU	3.03	-17.4	13.0	NA	3.06	2.95
MSC 04/1139	Salmon farmed	MU	16.5	-18.8	15.3	3.74	3.55	17-2002 087	Whiting	MU	0.47	-17.6	14.8	NA	3.59	3.42
MSC 04/1141	Salmon farmed	MU	15.1	-19.6	13.4	3.18	3.05	19-2002-049	Whiting	MU	0.05	-17.7	14.1	NA	3.38	3.24
MSC 04/1142	Salmon farmed	MU	17.0	-17.9	16.0	3.94	3.74	20-2003/120	Whiting	MU	0.52	-17.0	14.4	NA	3.47	3.32
MSC 04/1143	Salmon farmed	MU	11.6	-18.2	15.2	3.71	3.53	22-2002-019	Whiting	MU	0.52	-17.1	15.2	NA	3.71	3.53
MSC 04/1145	Salmon smoked	MU	9.20	-18.8	13.1	3.09	2.97	29-HP 2-02	Harbour Perpoise	BL	NA	-23.0	13.9	NA	3.32	3.18
MSC 04/1146	Salmon smoked	MU	9.58	-20.4	13.3	3.15	3.03	30-HP 4-01	Harbour Perpoise	BL	NA	-21.3	15.2	NA	3.71	3.53
MSC 04/1147	Salmon smoked	MU	10.3	-20.4	13.2	3.12	3.00	31-HP 3-99	Harbour Perpoise	BL	NA	-22.8	12.5	NA	2.91	2.82
MSC 04/1148	Salmon smoked	MU	11.6	-19.8	14.5	3.50	3.34	32-HP 4-00	Harbour Perpoise	BL	NA	-20.2	15.2	NA	3.71	3.53
MSC 04/1149	Salmon smoked	MU	10.7	-20.0	13.5	3.21	3.08	34-HP 1-02	Harbour Perpoise	BL	NA	-20.0	16.6	NA	4.12	3.89
MSC 04/1150	Salmon smoked	MU	10.4	-20.1	13.8	3.29	3.16	36-HP 8-00	Harbour Perpoise	BL	NA	-21.5	15.2	NA	3.71	3.53
MSC 04/1151	Salmon smoked	MU	11.5	-20.0	13.6	3.24	3.11	MSC/2006/1039	Plankton/seston	NA	NA	-25.7	3.10	NA	NA	NA
MSC 04/1152	Salmon smoked	MU	9.83	-19.0	13.8	3.29	3.16	MSC/2006/1040	Plankton/seston	NA	NA	-25.5	3.60	NA	NA	NA
MSC 04/1153	Salmon smoked	MU	10.1	-19.9	14.4	3.47	3.32									
MSC 04/1154	Salmon smoked	MU	9.5	-20.7	13.5	3.21	3.08									
MSC 04/1155	Salmon smoked	MU	10.7	-20.2	13.8	3.29	3.16									

1 Trophic status as per Minegawa and Wada (18)
2 Trophic status as per Fisk (48)
3 Lipid by hexane extraction
4 Stable isotope and lipid data from whole blue whiting.
NA = Not applicable
MU= Muscle
WF= Whole fish
SB = Soft body
Oil = Fish oil
LI = Liver
BL = Blubber

Table 6.5: Summary table isotope $\delta^{15}N$ and ($\delta^{13}C$) ratio data (‰) in "wild" shellfish, fish and marine mammal species.

	Albacore	Blue Whiting	Eel	Harbour Porpoise	Herring	Killer Whale	Mackerel	Oysters	Mussels	Whiting	Salmon
Sample 1	12.6 (-18.1)	12.4 (-20.5)	15.2 (-21.1)	13.9 (-23.0)	13.4 (-17.7)	14.8 (-14.9)	14.3 (-18.1)	12.8 (-19.7)	9.00 (-17.2)	14.8 (-17.6)	14.2 (-19.0)
Sample 2	11.6 (-18.8)	12.5 (-19.8)	13.4 (-29.6)	15.2 (-21.3)	13.6 (-17.8)	15.0 (-15.3)	14.3 (-18.6)	12.2 (-18.8)	9.70 (-18.7)	14.1 (-17.7)	12.7 (-18.0)
Sample 3	12.0 (-17.6)	12.5 (-19.8)	13.9 (-25.8)	12.5 (-22.8)	13.5 (-17.7)	14.5 (-16.7)	13.2 (-18.4)	15.4 (-21.3)	12.1 (-22.6)	14.4 (-17.0)	12.0 (-18.7)
Sample 4	11.8 (-18.1)	12.5 (-20.2)	14.6 (-29.4)	15.2 (-20.2)	13.0 (-18.1)	17.3 (-12.9)	14.2 (-17.9)	14.6 (-21.6)	9.50 (-21.4)	15.2 (-17.1)	10.9 (-18.2)
Sample 5	12.5 (-17.6)	12.5 (-19.8)	11.1 (-28.6)	16.6 (-20.0)	13.7 (-17.4)	15.6 (-15.0)	14.3 (-17.9)	10.7 (-20.5)			12.0 (-18.4)
Sample 6		12.6 (-19.6)		15.2 (-21.5)	13.5 (-17.4)	15.2 (-15.0)					11.7 (-18.1)
Sample 7		12.7 (-19.8)			12.9 (-18.3)	15.2 (-16.2)					11.5 (-18.1)
Sample 8		12.2 (-19.1)			12.2 (-18.3)						12.1 (-18.1)
Sample 9		12.1 (-20.4)			12.2 (-17.8)						
Sample 10		12.6 (-20.2)			11.9 (-18.1)						
Sample 11		12.9 (-19.0)			13.4 (-17.4)						
Sample 12		12.7 (-19.8)			13.0 (-17.4)						
Sample 13		12.4 (-19.8)									
Sample 14		13.1 (-19.5)									
Mean	12.1 (-18.0)	12.6(-19.8)	13.6 (-26.9)	14.8 (-21.5)	13.0 (-17.8)	15.4 (-15.1)	14.1 (-18.2)	13.1 (-20.4)	10 (-20.0)	14.6 (-17.4)	12.1 (-18.3)
STDEV	0.43 (0.49)	0.25 (0.43)	1.57 (3.58)	1.40 (1.25)	0.61 (0.34)	0.91 (1.2)	0.48 (0.31)	1.88 (1.15)	1.38 (2.46)	0.47 (0.35)	0.98 (0.35)
CV(%)	3.60 (2.73)	2.04 (2.18)	11.6 (13.3)	9.48 (5.86)	4.72 (1.96)	5.97 (7.95)	3.43 (1.71)	14.3 (5.65)	13.7 (12.3)	3.27 (2.02)	8.08 (1.92)

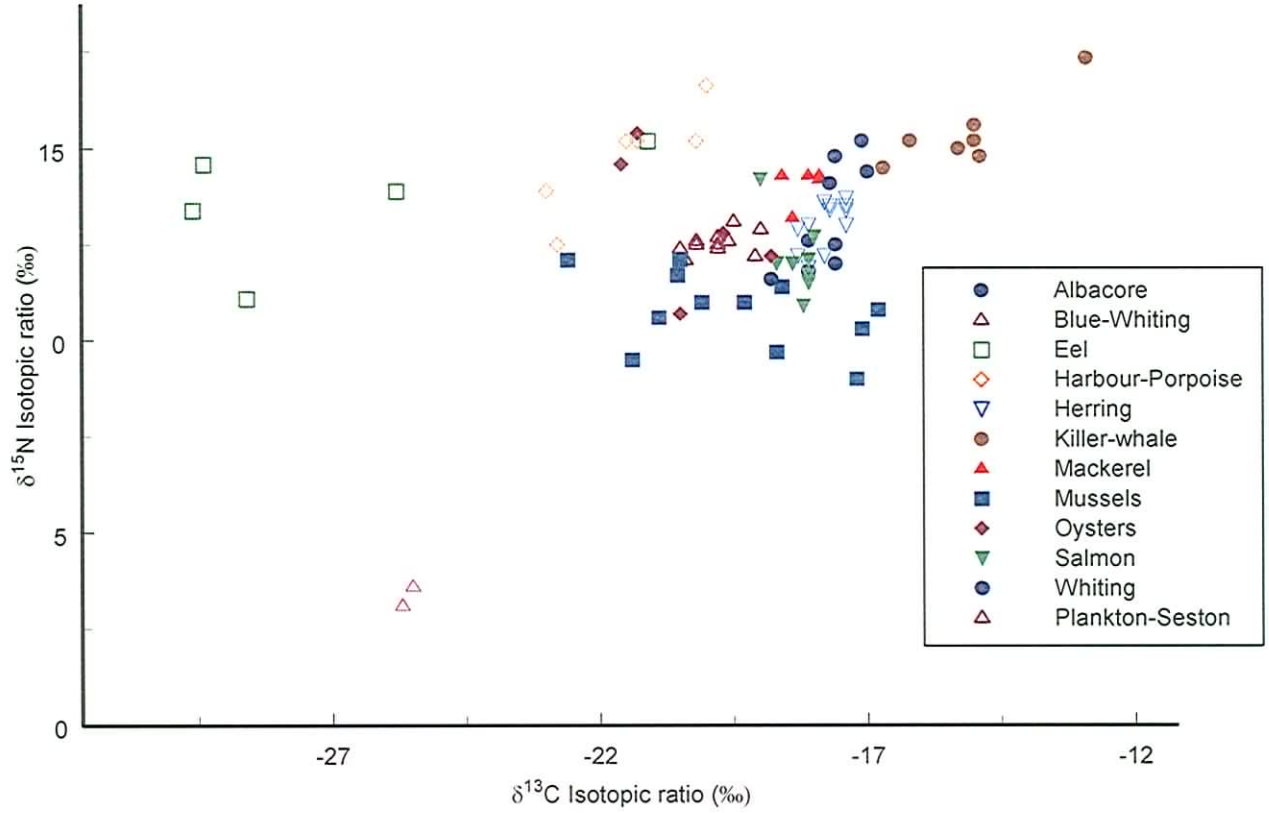

Figure 6.1: Scatterplot of $\delta^{15}N$ vs. $\delta^{13}C$ in lipid free muscle tissue for all "wild" species from Irish and surrounding waters.

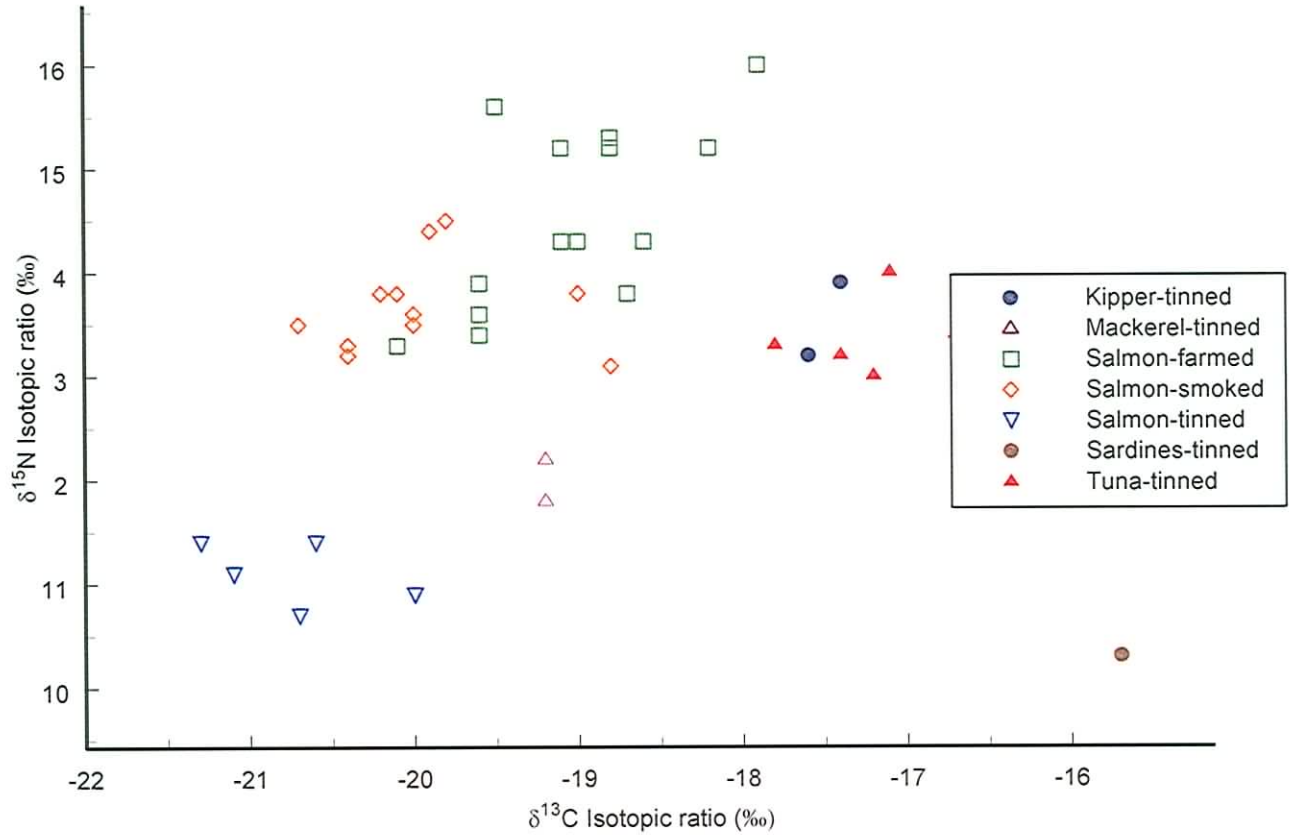

Figure 6.2: Scatterplot of $\delta^{15}N$ vs. $\delta^{13}C$ in lipid free muscle tissue of "farmed" Irish species and retail produce on the Irish marketplace.

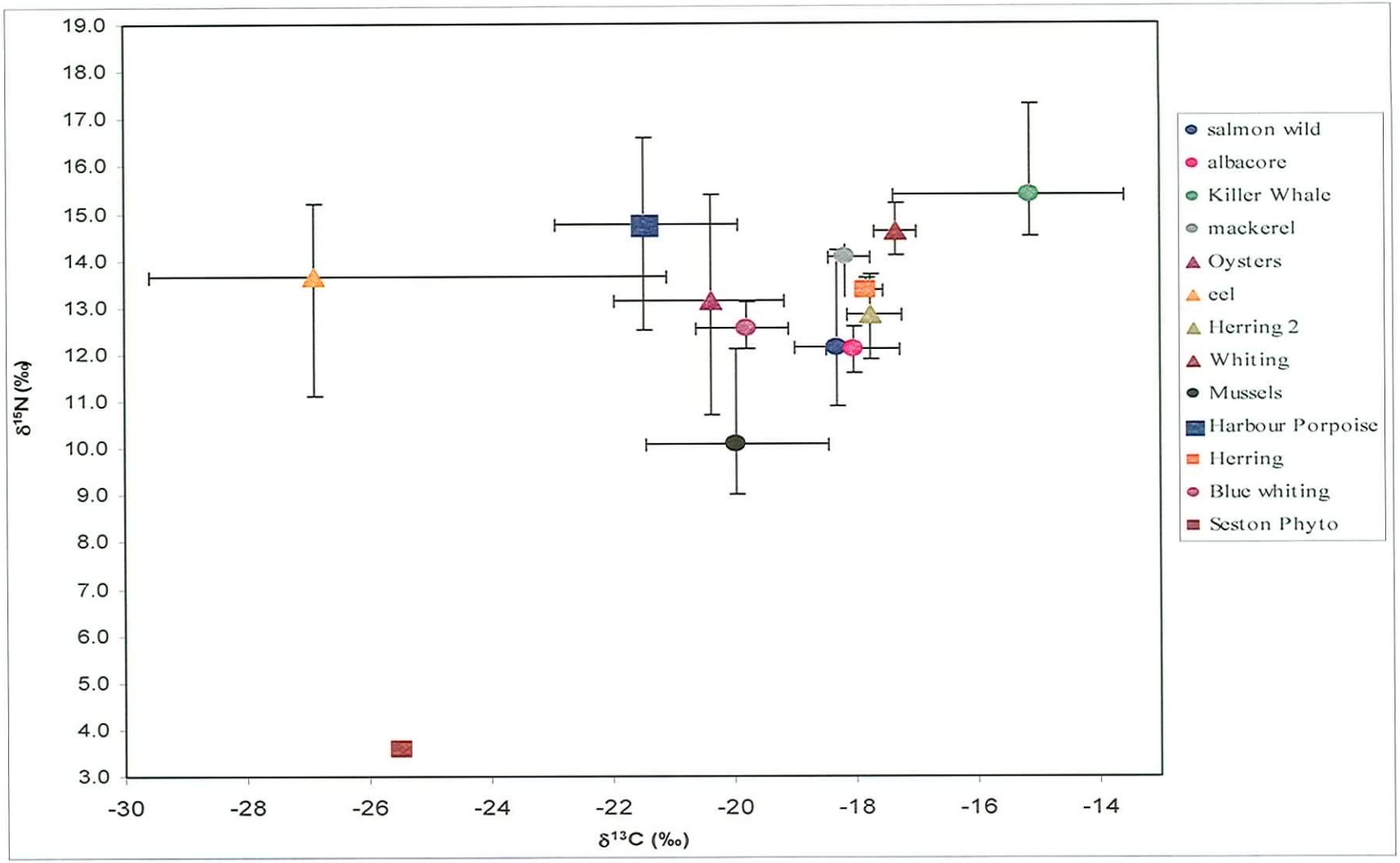

Figure 6.3: Mean $\delta^{13}C$ and $\delta^{15}N$ (‰) ratios in wild species. Bars indicate minimum and maximum values observed.

Phytoplankton/seston samples were found to show the least enriched $\delta^{15}N$ and $\delta^{13}C$ ratios, this being indicative of their position at the foot of the marine web. Only two phytoplankton/seston samples were available for analysis therefore insufficient data were available to conclusively derive a "baseline" as primary producers based on these limited data. As a consequence isotopic baseline information currently reported in literature were utilised during this study. These data are further discussed in section 6.3.2.

A number of species datasets were found to have strong relationships between $\delta^{15}N$ and $\delta^{13}C$ isotopic ratio data (see table 6.6).

Table 6.6: Regression and correlation statistics for $\delta^{15}N$ vs. $\delta^{13}C$ relationships in various marine species.

Species/grouping	Regression Co-efficient $=R^2$	Correlation Co-efficient =R	Direction of slope
Killer Whale	0.76	0.87	Upward
Eel	0.28	0.53	Upward
Whiting	0.17	0.41	NA
Blue Whiting	0.002	0.04	NA
Oysters	0.37	-0.61	Downward
Herring	0.73	0.85	Upward
Mackerel	0.13	0.36	NA
Albacore	0.34	0.58	Upward
Harbour Porpoise	0.75	0.87	Upward
Mussels	0.61	-0.78	Downward
Tuna (Tin)	0.07	0.26	NA
Salmon (Wild)	0.41	-0.64	Downward
Salmon (Tin)	0.22	0.47	NA
Salmon (Smoked)	0.001	0.03	NA
Salmon (Farmed)	0.43	0.66	Upward

Note: Indicative direction not assigned where R =<±0.50.

Upward relationships were observed for both marine mammal species, these data indicate that the relative trophic status as determined by $\delta^{15}N$ increases with modification of diet to species with a more enriched $\delta^{15}N$ profile.

Harbour porpoise and killer whale $\delta^{13}C$ isotopic data show a relatively wide spread compared to fish species indicating that these species feed on a wide variety of different marine species.

It should be noted that only three downward relationships between $\delta^{15}N$ and $\delta^{13}C$ were observed, significantly both mussels and oyster groupings exhibited this trend, thereby as the $\delta^{13}C$ isotopic ratio becomes more enriched (less negative) the relative trophic status (as determined by $\delta^{15}N$) decreases. It is unclear whether the observed variation within species groupings are as a result of spatial, temporal and/or are truly related to the isotopic profiles of phytoplankton/seston food sources ingested by mussels and oysters.

6.3.1.1 $\delta^{15}N$ isotopic ratios in "wild" species

Overall killer whales showed the highest $\delta^{15}N$ ratios (mean 15.4‰) compared to other "wild marine species", with one killer whale from Scottish waters having a $\delta^{15}N$ ratio of 17.3‰. Higher $\delta^{15}N$ ratios suggest that this individual may be of higher trophic status than the other individuals.

Harbour porpoise demonstrated similar $\delta^{15}N$ ratios to those of the killer whales (mean 14.8‰). Of the fish species under investigation, whiting showed $\delta^{15}N$ ratios (mean 14.6‰) similar to those of harbour porpoise.

Herring muscle samples showed $\delta^{15}N$ ratios in the range of 13.0 to 13.7‰ while herring liver tissue samples showed lower enrichment than was observed in muscle tissue ranging 11.9 to 12.9‰. No relationship between $\delta^{15}N$ ratios and fish lengths was

observed. Both males and females were collected at three sampling locations; $\delta^{15}N$ ratios observed between the different sexes were well conserved at each sampling station.

A total of 14 separate blue whiting sampling events took place within a 4 month time period, with a mean $\delta^{15}N$ ratio of 12.6‰ being observed. The coefficient of variation between all $\delta^{15}N$ results was 2.04% indicating that the species trophic status shows little temporal change throughout the sampling period.

$\delta^{15}N$ data for eels suggest a slightly higher relative trophic position for the river Suir eels compared to others in the study. However it should be noted that the relative trophic position of non-feeding silver eels should not be compared directly to that of feeding resident yellow eels. Additional factors such as the potential influence of industrial or agricultural processes or nutrient rich fluxes in the vicinity of sampling sites should also be considered when interpreting such data.

As expected blue mussels (*Mytilus edulis*) showed the lowest $\delta^{15}N$ ratios of the primary consumers investigated (mean 10.1‰). Pacific oysters (*Crassostrea gigas*) $\delta^{15}N$ ratios were much more enriched in two samples from the mid west of Ireland (Clarinbridge and Aughinish) compared to the other three samples analysed.

The observed variation in $\delta^{15}N$ ratios for shellfish (14.3% for oysters and 13.7% for mussels) may reflect potential localised nutrient inputs close to the respective sampling location and highlights the difficulties encountered in selecting suitable species and locations for derivation of a trophic baseline. Variations observed in shellfish were the

highest observed among all species analysed. Additionally the variation in $\delta^{15}N$ ratios highlights that caution must be observed during sampling site selection, especially where localised inputs may influence isotopic ratios and/or contaminant levels within an organism.

6.3.1.2 $\delta^{13}C$ isotopic ratios in "wild" species.

Eels showed the least enriched $\delta^{13}C$ isotopic ratio profile with a mean of –26.9‰ and a range of –21.1 to –29.6‰ determined. It can be observed from figure 6.1 and 6.3 that the $\delta^{13}C$ isotopic ratios in silver and yellow eels are distinct from any other species grouping analysed. In this present study $\delta^{13}C$ values for the four silver eels in this study were in the range –25.8 to –29.6‰ while the $\delta^{13}C$ value for a pooled yellow eel sample was –21.1‰.

Such data can be indicative of a modification in diet in the yellow eel compared to that of the silver eels. Data suggest an increased freshwater or terrigenous organic matter influence on the diet of the silver eels compared to that of the yellow eels from the river Suir system where there may be estuarine and/or oceanic influences on diet. Zooplankton generally elicit a $\delta^{13}C$ isotopic signature of approximately -20‰ (50) therefore eels with similar $\delta^{13}C$ values may derive the majority of their dietary carbon via zooplankton. Other eels with less enriched profiles show influences of a benthic-based or mixed benthic/zooplankton diet.

However it should be noted that such $\delta^{13}C$ differences and associated stable isotope turnaround time may be influenced by "fasting" in silver eels rather than being a direct consequence of modification of the silver eels diet and that eels from the river Suir were

actively feeding at time of capture unlike the four pooled silver eel samples. Few data are currently available related to the rates of assimilation of nutrients in eels to confirm these observations. Details of the structure of the eel food web are reported in chapter 4.

Oysters (mean –20.4‰) and Mussels (mean –20.0‰) showed similar enrichment profiles indicative of communality in dietary carbon influences, these being consistent with a carbon source originating with phytoplankton/zooplankton.

Killer whale $\delta^{13}C$ isotopic ratios (mean –15.1‰) were the most enriched of all species sampled with one individual having a significantly different $\delta^{13}C$ isotopic profile (-12.9‰) to that of all other killer whales. Data suggest that this individual may have derived its primary carbon intake from an higher trophic status source than those of the other animals (50).

Das (51) and Hobson (52) report that the general pattern of inshore, benthos-linked food webs tends to be more enriched in $\delta^{13}C$ compared to offshore, pelagic webs. This hypothesis may support the findings of this current study, where killer whale SW1997/135c $\delta^{13}C$ data suggests that this whale may have derived a proportion of its prey from an offshore marine-mammal based source. This individual was additionally found to have a greater contaminant burden than was determined in other killer whales; this will be further discussed below. Other killer whale $\delta^{13}C$ isotopic ratios were more consistent with a diet primarily based on fish; salmon bones in the stomach of one killer whale further corroborate this. Details are further discussed in chapter 5.

In general the majority of marine fish species showed similar $\delta^{13}C$ isotopic ratios, with species means in the range of –17.4‰ (Whiting) to –19.8‰ (Blue whiting) being

determined. $\delta^{13}C$ isotopic ratio data were well conserved within the fish species groupings (n=6 groups) with coefficient of variance (CV) less than 2.73% calculated, indicating that while small temporal and spatial differences exist between individual sampling events and within a species grouping, these have only a small influence on the overall $\delta^{13}C$ isotopic ratio for individual fish species. Inherent species variability may be a result of resource partitioning, a common mechanism by which substantial differences in resource use occur between co-existing species (52). Partitioning occurs as a result of high trophic adaptability in fish feeding behaviour and represents a major factor regulating the development and structure of fish assemblages in aquatic ecosystems (54).

6.3.1.4 $\delta^{13}C$ and $\delta^{15}N$ ratios in farmed and retail salmon produce.

Stable isotope ratio data ($\delta^{13}C$ and $\delta^{15}N$) were collected in a number of salmon products available on the Irish marketplace. Wild, farmed and smoked salmon all originated in Irish waters but location data are not available as to the origin of tinned samples. Plotting $\delta^{13}C$ and $\delta^{15}N$ (see figure 6.4) it is generally possible to separate the samples into distinct groupings with a small degree of overlap. $\delta^{13}C$ data are much more conserved between the four groups with a CV of between 1.92 to 3.16% having been determined for wild and farmed salmon respectively. $\delta^{15}N$ data are much more varied with CVs of 2.78 to 8.08% observed for tinned and wild salmon respectively.

Mean $\delta^{13}C$ data for tinned salmon (-20.7‰) are less enriched than for the Irish wild salmon (-18.3‰) therefore it can be deduced that they are unlikely to have originated from Irish waters as their $\delta^{13}C$ deduced dietary influences are dissimilar. While mean $\delta^{15}N$ ratios for tinned salmon (11.1‰) are similar to those of wild Irish salmon (12.1‰)

it is not possible to conclusively deduce whether the tinned produce is of wild or farmed origin.

Irish farmed and smoked samples show mean $\delta^{13}C$ values of –19.0 and –19.9‰ respectively with a high degree of overlap observed between individual pooled samples. When $\delta^{13}C$ data are combined with their respective $\delta^{15}N$ ratios is can be deduced that smoked salmon are more likely to be of farmed origin rather than from wild stocks as data suggests similarity in carbon and nitrogen sources.

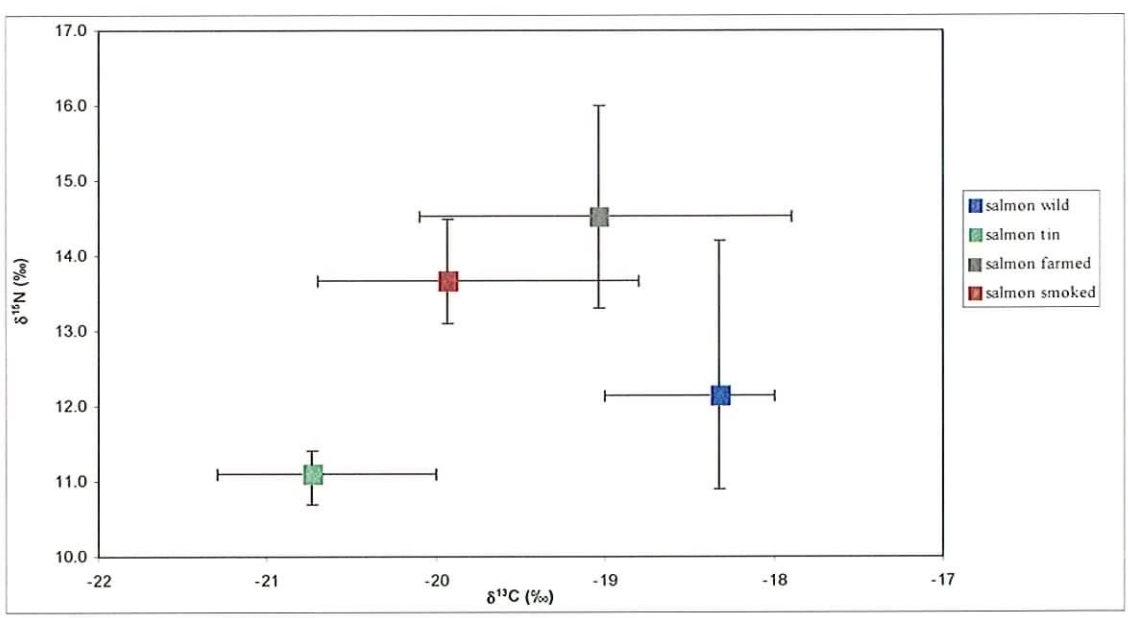

Figure 6.4: Stable isotope ratios for $\delta^{13}C$ and $\delta^{15}N$ (‰) in salmon produce available on the Irish marketplace. X and Y bars represent minimum and maximum values for respective species.

Of greater interest is the relative trophic status of the wild, farmed and smoked produce (see figure 6.4). Mean $\delta^{15}N$ ratios for wild Irish salmon (12.1‰) are 2.4‰ and 1.6‰

lower than the mean $\delta^{15}N$ ratios for their farmed and smoked counterparts respectively, placing the farmed fish on a higher trophic level relative to the wild variety. Wild salmon will generally feed on an opportunistic basis on krill and/or other similar species while farmed fish regularly receive lipid rich feed pellets. While differences in feeding mechanisms are well reported, few stable isotope study data exist documenting the effects of such feeding regimes on the trophic status of farmed species and as such these data are valuable. Additionally the resolution power of stable isotope based techniques for the identification of wild and farmed species is of importance.

6.3.2 Derivation of a trophic baseline.

Ponsard and Averbuch report that fractionation of nitrogen occurs during the assimilation and subsequent elimination of nitrogenous excretory products and that trophic fractionation is a result of the combination of these two processes (47).

While the accuracy of stable isotope models is hindered by the fact that it is not possible to replicate environmental food web conditions in the laboratory, enrichment models as proposed by Minagawa and Wada (18) and Fisk (48) have commonly been applied in food-web studies. These models were also utilised in this study in order to generate trophic level estimates from $\delta^{15}N$ stable isotope data.

Based on the findings of Minagawa and Wada (18), Fisk (48) and Post (26), the definition of a suitable marine web baseline is critical prior to completion of relative trophic level calculations. While calanoid copepods (49, 52) and the scallop *Pectin maximus* (55) have been utilised in correcting for a trophic level baseline, for this study it was assumed that blue mussels occupied the secondary trophic position (TL=2) and as such the isotopically derived trophic levels for all species were determined using blue

mussels as the isotopic baseline. As previously discussed phytoplankton/seston isotope ratio data are reported in figure 6.1, however at present only two data-points are available, therefore data are insufficient for use in this study

A baseline $\delta^{15}N$ ratio of 9.40‰ was calculated from mussel data available during this study, one sample showed a more elevated $\delta^{15}N$ ratio (12.4‰) than of other mussels investigated and as such was removed from baseline trophic level calculations.

Further to the requirement of a trophic baseline, calculations require the use of a trophic level isotopic enrichment factor ($\triangle N$). This enrichment factor is generally accepted to fall between 3-4‰ between trophic levels, however site/food-web specific spatial and temporal differences can result in variation from the above. Trophic enrichment factors of 3.4‰ as per Minagawa and Wada (18) and 3.8‰ as per Fisk et al (48) were both used during this study as was the $\delta^{15}N_{base}$ of 7.21‰ as used by Pinnegar et al, the latter to allow comparison to available literature from Celtic sea fish species (5). Utilising the equation as below, the trophic levels of all samples was determined,

Equation 13= $\text{Trophic level} = \lambda + (\delta^{15}N_{\text{secondary consumer}} - \delta^{15}N_{base})/\triangle N$

Where,

λ = trophic position of the organism used to estimate $\delta^{15}N_{base}$

$\triangle N$ = enrichment in $\delta^{15}N$ per trophic level (‰).

Table 6.7: Summary of mean stable isotope ratios $\delta^{13}C_{VPDB}$ and $\delta^{15}N_{AIR}$ and mean trophic status in shellfish, wild species and marine mammals.

	Mean $\delta^{13}C_{VPDB}$	Mean $\delta^{15}N_{AIR}$	Mean TL[2]	Mean TL[3]	Mean TL[4]
Eel	-21.1	13.6	3.12	3.25	3.89
Blue Whiting	-19.8	12.6	2.83	2.93	3.57
Killer Whale	-15.1	15.4	3.57	3.76	4.40
Albacore	-18.0	12.1	2.71	2.79	3.44
Salmon	-18.3	12.1	2.72	2.81	3.45
Herring [1]	-17.8	13.4	3.05	3.17	3.81
Mackerel	-18.2	14.1	3.23	3.37	4.01
Oysters	-20.4	13.1	2.98	3.10	3.74
Herring [1]	-17.8	12.9	2.91	3.01	3.66
Whiting	-17.4	14.6	3.38	3.54	4.18
Mussels	-20.0	10.1	2.18	2.20	2.84
Harbour Porpoise	-21.5	14.8	3.41	3.58	4.22

1 = Two distinct herring sampling events

2= Use a trophic enrichment factor of 3.8 as per Fisk et al (47)

3= Use of a trophic enrichment factor of 3.4 as per Minagawa and Wada (18)

4= Use of trophic enrichment factor of 3.4 as per Minagawa and Wada (18) and baseline of 7.21‰ from *Pectin maximus*

Table 6.8: Summary of mean stable isotope ratios $\delta^{13}C$ and $\delta^{15}N$ and mean trophic status in farmed and retail samples.

	Mean $\delta^{13}C_{VPDB}$	Mean $\delta^{15}N_{AIR}$	Mean TL[2]	Mean TL[3]	Mean TL[4]
Salmon (tin)	-20.7	11.1	2.45	2.50	3.14
Tuna (tin)	-17.2	13.4	3.05	3.17	3.81
Salmon (smoked)[1]	-19.9	13.7	3.13	3.26	3.90
Salmon (farmed)	-19.0	14.5	3.35	3.51	4.15
Sardines (tin)	-15.7	10.3	2.24	2.26	2.91
Mackerel (tin)	-19.2	12.0	2.68	2.76	3.41
Kippers (tin)	-17.5	13.6	3.09	3.22	3.86

1 =Smoked salmon derived from farmed fish

2 = Use of a trophic enrichment factor of 3.8 as per Fisk et al (48)

3= Use of a trophic enrichment factor of 3.4 as per Minagawa and Wada (18)

4= Use of trophic enrichment factor of 3.4 as per Minagawa and Wada (18) and baseline of 7.21‰ from *Pectin maximus*

Trophic level data from each of the three methodologies employed are presented in table 6.7 for "wild" and marine mammal species and in table 6.8 for farmed and retail samples. Data are subsequently plotted in figures 6.5 and 6.6.

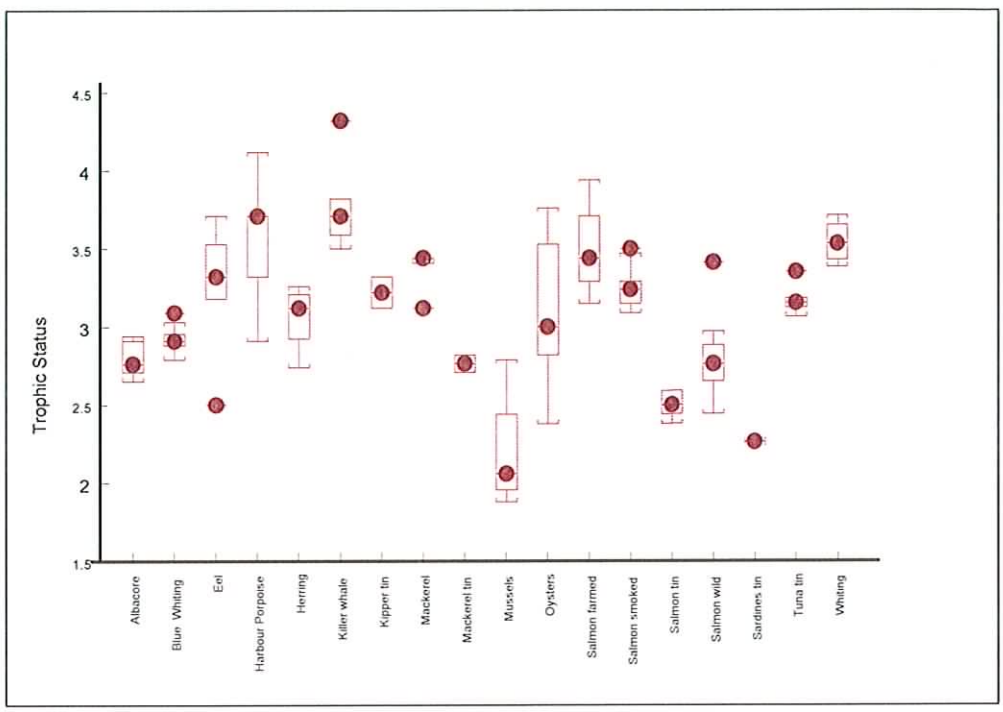

Figure 6.5: Trophic status of samples as per Minagawa and Wada (18)

One of the major drawbacks of conversion of $\delta^{15}N$ data into trophic status, lies in the fact that a great degree of uncertainty exists related to both enrichment factors and suitable baseline isotopic reference values. While each of the three TL generation mechanisms used placed killer whales on the highest trophic level, the mean TL positions generated differed greatly from each other ranging from 3.57 to 4.40 (See table 6.7).

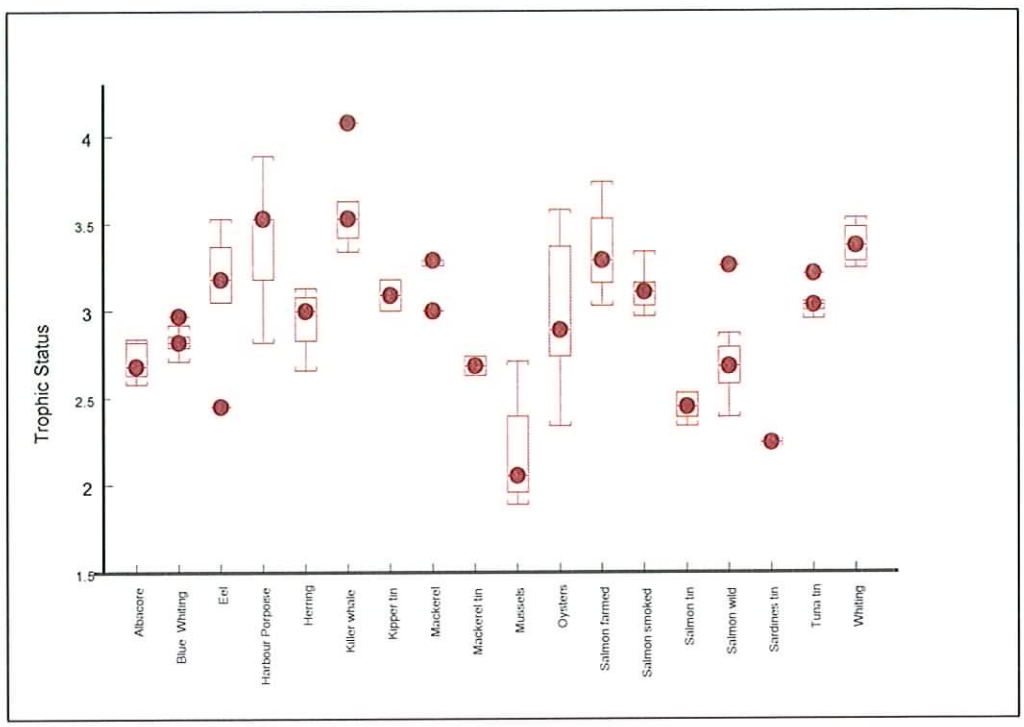

Figure 6.6: Trophic status of samples as per model proposed by Fisk (48).

Pinnegar et al (5) report some of the only stable isotope data available from species resident in Irish waters, mean TL values of 3.79 ± 0.07, 4.94 ± 0.36 and 3.61 ± 0.37 are detailed for herring, whiting and mackerel respectively. This present study reports TL data of 3.81 and 3.66 for herring, 4.18 for whiting and 4.01 for mackerel (see table 6.7) using the same $\delta^{15}N_{base}$ (7.21‰) as per Pinnegar et al (5).

While differences are observed between whiting and mackerel data, TL data for herring are very similar. It should be noted that while Pinnegar et al (5) also used freeze dried muscle tissue for their TL estimations, lipid was not removed from the samples prior to analysis. While differences of opinion exist as to the overall influence of tissue lipid

content on stable isotope data, several authors have reported TL differences as a result of the presence of total lipid.

The Gadoid family include blue whiting which is primarily planktivorous compared with the piscivorous cod and hake species, consequently blue whiting are placed on a relatively low trophic level compared to other fish species. Similarly the relatively low position of salmon (mean TL 2.72) is indicative of a pelagic amphipod or primary producer based diet. With the exception of these species, fish tend to occupy TL>3.0 indicative of the increase in relevance of invertebrates on the diet of these species.

6.3.4 The role of $\delta^{15}N$ and $\delta^{13}C$ in modelling lipid levels in marine biota.

Establishing the trophic position or more precisely the "relative" trophic position of an organism is fundamental to understanding food-web structures. Further to this, investigation bioaccumulation/biomagnification of persistent organic pollutants and especially that of lipophilic compounds must take account of such marine web structures.

As previously discussed stable isotope approaches can provide estimates of the trophic status of individual members of food webs, which, when combined with analytical, physico-chemical and biological measurements can further our current understanding of the processes of bioaccumulation/biomagnification.

Bioconcentration of chemicals is generally considered to be a partitioning process between the lipids of aquatic organisms and water, this being controlled by the relative solubility of the chemical in the lipid reserves of the organism and in the water column. As discussed in chapter 1, higher lipid content in an organism can result in higher

potential for bioaccumulation of lipophilic compounds additionally lipid content generally increases with an increase in trophic status, this is further discussed below.

Table 6.9 below describes the correlation between the percentage lipid content in tissue and their respective $\delta^{13}C$ and $\delta^{15}N$ isotopic ratios. Clear correlation was demonstrated between lipid content and $\delta^{13}C$ for eels (R=0.79), salmon (R=0.49), herring (R=0.54) and mussels (R=0.79), tinned salmon (R=0.78) lipid content was also shown to be correlated with $\delta^{13}C$. The lipid content in samples was correlated to $\delta^{15}N$ in eel (R=0.88), albacore (R=0.74), herring (R=0.70) and mackerel (R=0.73).

Table 6.10 summarises the mean lipid content, isotopic ratios and trophic level (as per Minigawa and Wada (18) of each of the constituent species groups in the marine web. These data are utilised in order to further investigate potential correlation between stable isotope ratios (and corresponding relative trophic status) and organism lipid content. Figure 6.7 subsequently presents mean trophic status of each species vs. log (x+1) normalised lipid content in all "wild" species where data were available.

Table 6.9: Correlation (R) and regression (R^2) statistics between $\delta^{13}C$ and $\delta^{15}N$ and tissue lipid (%) for all study samples

	$\delta^{13}C$ R^2	$\delta^{13}C$ R	$\delta^{15}N$ R^2	$\delta^{15}N$ R
Eel	0.63	0.79	0.78	0.88
Blue Whiting	0.07	0.26	0.08	0.28
Killer Whale	0.01	0.10	0.05	0.22
Salmon (tin)	0.61	0.78	0.03	0.17
Salmon	0.24	0.49	0.01	0.09
Salmon (smoked)	0.003	0.05	0.26	0.51
Salmom (farmed)	0.06	0.24	0.07	0.26
Albacore	0.09	0.29	0.55	0.74
Tuna (tin)	0.001	0.03	0.19	0.44
Herring	0.29	0.54	0.49	0.70
Mackerel	0.04	0.20	0.54	0.73
Oysters	0.004	0.06	0.03	0.17
Mussels	0.62	0.79	0.19	0.44

A correlation statistic R=0.76 was determined for the log (x+1) lipid content and the mean trophic status of individual species. The removal of the oysters dataset improves the overall correlation statistic to R=0.87. While all oyster samples were collected at similar times there may be a site-specific temporal and/or spatial aspect to raised $\delta^{15}N$ isotopic ratio data. The potential for anthropogenic nutrient input at one or more sampling locations cannot be discounted, as indeed can the potential for differences in the isotopic signature of phytoplankton (e.g. dinoflagellates vs.. diatoms) may account for the wide range of $\delta^{15}N$ data observed.

Table 6.10: Summary statistics of lipid content (%), isotopic ratios (‰) and trophic status (18). Figures in parenthesis indicate the standard deviation of result sets.

	Lipid (%)	Log (x+1) lipid	$\delta^{13}C$	$\delta^{15}N$	TL[1]
Salmon wild	11.5 (1.41)	1.10	-18.3 (0.35)	12.1 (0.98)	2.72 (0.25)
Albacore	10.1 (5.03)	1.04	-18.0 (0.49)	12.1 (0.43)	2.71 (0.11)
Herring	12.9 (1.43)	1.14	-17.8 (0.18)	13.4 (0.26)	3.04 (0.06)
Mackerel	10.1 (2.29)	1.04	-18.2 (0.31)	14.1 (0.48)	3.22 (0.12)
Oysters	2.49 (0.62)	0.54	-20.4 (1.15)	13.1 (1.88)	2.98 (0.49)
Eel	15.1 (4.19)	1.21	-26.9 (3.58)	13.6 (1.57)	3.11 (0.41)
Blue Whiting	3.86 (0.93)	0.69	-20.0 (0.41)	12.5 (0.16)	2.80 (0.04)
Killer whale	58.0 (11.6)	1.77	-15.0 (1.21)	15.4 (1.00)	3.57 (0.26)
Mussels	2.03 (0.22)	0.48	-20.0 (2.46)	10.1 (1.38)	2.17 (0.36)

It should also be noted that literature tends to suggest that long-lived and slow growing species generally assimilate and eliminate carbon and nitrogen into and out of their muscle tissues at slower rates than short-lived and fast growing species (15, 56). Therefore while isotopic ratios (and associated trophic level status) provide good indicators of time integrated feeding processes within a species, the potential for error associated with longer nutrient turnover rates and consequential changes in high trophic level consumer isotopic signatures must be accounted for in food-web model design.

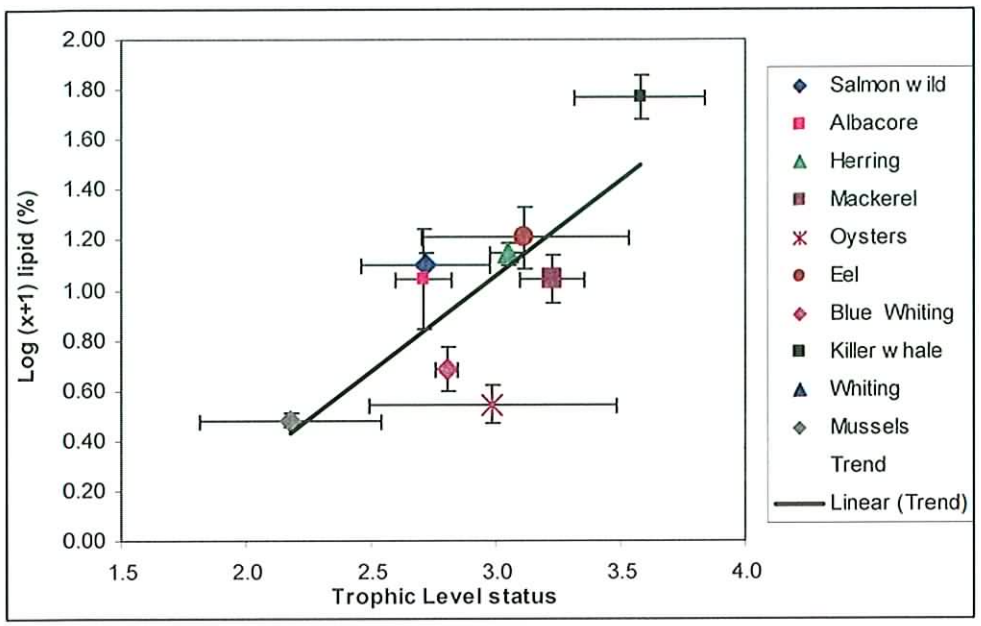

Figure 6.7: Mean trophic status vs. mean log (x+1) lipid (%) in "wild" species (R^2= 0.58). Data as per table 6.10.

6.3.5 Investigation of the potential role of $\delta^{15}N$ and $\delta^{13}C$ on modelling accumulation/metabolism of selected POPs for a number of marine species

Chapter 1 documents mechanisms by which persistent organic pollutants can accumulate from the water column into tissues of marine species from where pollutants can then biomagnify with increasing trophic position as a result of dietary exposure (57). As the accumulation, biotransformation and excretion of POPs in lower trophic level biota influences the concentration of contaminants in higher trophic level species (7) it is important that factors influencing bio-magnification processes are understood in order to protect marine web constituent species in addition to protecting the consumer of fish produce available on the marketplace.

When contaminant concentration and stable isotope data from marine biota are combined with an understanding of biological processes in marine organisms, they provide a powerful tool to further elucidate the pathways involved in the biomagnification of POPs in the marine food web. While the biomagnification of POPs in lower trophic level species and top of food web predators in Arctic regions have been completed (8, 26) few such assessments are available in species from Irish waters (35). In any case these have not fully utilised stable isotope techniques as a mechanism to assess relative trophic status and/or to evaluate consumer/prey interactions.

As such this section aims to further describe POP biomagnification in marine species from Irish and surrounding waters by completion of the following,

- Discuss the relevance/importance of normalisation of contaminants data.
- Evaluation of biomagnification of contaminant groups in the marine web, including where appropriate,
    - Food web biomagnification factors (FWMFs).

- Calculation of biomagnification factors (BMFs)
- Investigate Species/group specific magnification factors (SMFs)

- Congener profiling techniques.

6.3.5.1 Normalisation and statistical assessment of POP data.

A number of contaminant groups were studied as part of the investigation into trophic transfer of contaminants, including dioxins, furans, PCBs and PBDEs. Datasets were of insufficient statistical size to further evaluate trophic enrichment for a number of OCP groups and for toxaphene residues and as such these are currently omitted.

Contaminant data do not generally show a normal distribution therefore non-parametric statistical analysis can only be completed on such datasets. As wet weight POP levels can generally be correlated with lipid content, lipid normalisation techniques can reduce possible effects of inter-species variation in lipid content. While normalisation of tissue contaminant data may not fully account for inter-tissue contaminant differences and differences between lipid classification between and within organisms, it is generally not practical to complete analysis on total body tissues of large animals.

As demonstrated in table 6.11, completion of lipid normalisation followed by $\log_{10}$ transformation greatly reduces the skewness and kurtosis associated with data for each contaminant grouping. Kurtosis characterises the relative flatness of a distribution compared with the normal distribution. Positive kurtosis indicates a distribution having a peak while negative kurtosis indicates a relatively flat distribution. Skewness characterises the degree of asymmetry of a distribution around its mean. Positive skewness indicates a distribution with an asymmetric tail extending toward more

positive values. Negative skewness indicates a distribution with an asymmetric tail extending toward more negative values.

These log_{10} transformed lipid weight data are subsequently used for assessment of the various biomagnification factors discussed below.

6.3.5.1.1 Statistical treatment of concentration data.

Wet weight, lipid weight and log_{10} transformed data from a large number of individual compounds and congeners were collected during this study. Species-specific boxplots to present wet weight and lipid weight concentrations of individual congeners and Σ congeners data were constructed as follows.

Table 6.11: Summary statistics of skewness and kurtosis for contaminant groupings

	Σ Group WW		Σ Group LW		Σ Group Log_{10}	
	Skewness	Kurtosis	Skewness	Kurtosis	Skewness	Kurtosis
Dioxins	2.43	5.50	1.79	2.03	1.05	-0.39
Furans	2.80	9.28	1.63	2.10	-0.37	0.19
Mono-ortho PCBs	6.58	45.1	6.14	39.8	1.76	3.28
PCB118	6.69	46.7	6.24	41.1	1.87	3.68
Non-Ortho PCBs	2.57	5.56	3.17	9.40	0.28	0.13
Marker PCBs	7.02	50.4	6.76	47.5	2.07	4.08
PBDEs	6.21	39.2	6.04	37.6	1.56	1.82

6.3.5.1.2 Concentration boxplots

In general the levels of POPs in biota in Irish waters can be relatively low compared to levels reported from more industrialised regions of Europe. As such for a number of contaminant groups the determined tissue concentrations can often be very low, occurring at or below the limits of quantitation (LOQ) or limits of detection (LOD) for the compound. Datapoints greater than 2.5 times the distance between the 25^{th} and 75^{th} percentiles are deemed as potential statistical outliers and are indicated by closed circles "●". Outlier data were however included in statistical assessments for this study.

For each contaminant grouping where the wet weight concentration was determined to be above the detection limit but below the LOQ, the sample LOQ was reported. Where the wet weight concentration was determined as not detected (nd), congener concentrations were not included in boxplot generation.

This upperbound (UB) approach is generally applied in food safety studies where it reduces the likelihood of erroneously excluding potentially toxic concentration data from risk assessment studies when concentrations are below the limit of quantification for a method/sample.

6.3.5.2 Biomagnification processes in the marine food web.

As previously discussed biomagnification has often been viewed to be similar to a lipid-water partitioning process, estimated by the octanol-water partitioning co-efficient. Connolly and Pedersen (58) reported that the fugacity in fish was higher than that in water, therefore chemicals will diffuse via partial pressure mechanisms from high to low fugacity, eventually reaching equilibrium when the fugacity in each phase equalise. Gobas et al (59-60) further demonstrated that food digestion and absorption provide a mechanism whereby the chemical fugacity becomes elevated in the consumer, thus providing an explanation for the biomagnification process.

A number of different magnification processes were evaluated from the contaminant and isotopic ratio data collected during the course of this study, these included

- Food web biomagnification factors (FWMFs).
- Biomagnification factors (BMFs)
- Species/group specific magnification factors (SMFs)

Details on generation of each of these factors are discussed below but it should be noted that only "wild" species were selected for the generation of these statistics. Tables 6.12 and 6.13 (and annexes 2-4) provide summary data utilised for the purposes of generation of the above factors. It should be noted that species/group specific biomagnification factors are an extension of BMFs and were generated in order to further investigate whether accumulation of contaminants within a species (and or like species) occurs in a homogeneous manner.

Table 6.12: Summary data of mean lipid (%), relative trophic status, marker PCBs and PBDEs (ng g^{-1}) and dioxins, furans and DL-PCBs (pg g^{-1}) in wild marine species.

Species	Salmon wild	Albacore	Herring	Mackerel	Oysters	Killer whale	Eel	Mussels [3]	Mussels [3]	Blue Whiting
Lipid (%)	11.5 ± 1.53	10.1 ± 5.03	12.9 ± 1.43	10.1 ± 2.29	2.34 ± 0.61	58.0 ± 11.6	13.7 ± 3.08	2.14 ± 0.55	2.10 ± 0.24	0.56 ± 0.06
Trophic status [1]	2.71 ± 0.16	2.79 ± 0.12	3.16 ± 0.07	3.37 ± 0.14	3.16 ± 0.61	3.76 ± 0.29	3.43 ± 0.23	0.50 ± 1.22	0.49 ± 0.92	2.99 ± 0.08
Trophic status [2]	2.64 ± 0.14	2.71 ± 0.11	3.04 ± 0.06	3.22 ± 0.12	3.04 ± 0.54	3.57 ± 0.26	3.28 ± 0.20	0.53 ± 1.15	0.49 ± 0.92	2.88 ± 0.07
Dioxins	2.84 ± 0.79	4.02 ± 1.22	4.01 ± 0.30	9.02 ± 8.50	36.4 ± 21.7	NA	3.45 ± 1.38	NA	NA	46.2 ± 8.37
Furans	12.8 ± 6.77	8.51 ± 1.51	11.9 ± 0.98	42.8 ± 26.1	48.2 ± 21.3	NA	2.31 ± 1.24	NA	NA	25.0 ± 3.09
Mean Mon-ortho	9.99 ± 4.06	17.1 ± 11.9	11.6 ± 1.21	39.8 ± 36.4	10.2 ± 6.83	8348 ± 12458	17.8 ± 20.3	29.3 ± 8.48	113 ± 56.9	NA
Mean PCB 118	6.53 ± 2.68	10.5 ± 7.28	7.29 ± 0.69	25.7 ± 24.1	6.16 ± 4.03	6845 ± 10206	11.3 ± 13.0	16.2 ± 5.55	67.0 ± 31.9	NA
Mean Non-ortho	102 ± 44.2	199± 75.1	180 ± 19.0	1105 ± 916	380 ± 193	NA	32.8 ± 39.4	1128 ± 676	7905 ± 5236	NA
Mean Marker PCB	49.1 ± 18.1	113 ± 77.8	59.7 ± 6.73	173 ± 131	50.1 ± 32.0	1279901 ± 204249	70.5 ± 85.4	117 ± 44.8	399 ± 209	NA
Mean PBDEs	6.55 ± 2.10	10.0 ± 6.88	9.53 ± 0.94	10.9 ± 0.80	79.8 ± 69.2	6455 ± 9905	29.1 ± 31.7	93.1 ± 9.68	351 ± 75.2	NA

[1] Trophic status as per Minigawa and Wada (18).

[2] Trophic status as per Fisk (46).

[3] Two distinct mussel samples, one collected from areas of relatively low input potential and the latter from relatively industrialised areas.

[4] Fish oil generated originates from mechanised processing of blue whiting. Total lipid content was measured in representative fish.

Table 6.13: Summary statistical data for mean lipid (%) trophic status, marker PCBs, PBDEs (ng g^{-1}) and dioxins, furans (pg g^{-1}) in retail samples.

	Salmon tin	Tuna tin	Sardines tin	Mackerel tin	Kipper tin	Salmon farmed	Salmon smoked
Lipid (%)	6.64 ± 1.75	5.30 ± 5.86	29.6	30.1 ± 2.11	16.0 ± 1.31	14.5 ± 2.47	10.3 ± 0.79
Trophic status [1]	2.50 ± 0.09	3.17 ± 0.11	2.26	2.76 ± 0.08	3.22 ± 0.14	3.27 ± 0.93	3.25 ± 0.13
Trophic status [2]	2.44 ± 0.08	3.04 ± 0.09	2.24	2.68 ± 0.07	3.09 ± 0.13	3.12 ± 0.89	3.12 ± 0.11
Dioxins	3.01 ± 0.48	10.8 ± 8.42	2.16	2.20 ± 0.30	2.55 ± 0.12	3.34 ± 0.79	2.99 ± 0.40
Furans	6.92 ± 3.24	13.7 ± 11.7	10.9	6.80 ± 0.32	9.13 ± 0.43	17.8 ± 5.62	13.6 ± 3.37
Mean Mon-ortho	4.07 ± 3.18	1.35 ± 1.16	1.21	3.25 ± 0.2	6.03 ± 1.04	24.0 ± 7.17	19.8 ± 3.13
Mean PCB 118	2.79 ± 2.21	0.62 ± 0.53	0.71	2.07 ± 0.12	3.84 ± 0.67	15.4 ± 4.53	12.7 ± 2.11
Mean Non-ortho	88.5 ± 52.39	139 ± 120	262	116 ± 8.89	89.6 ± 0.66	350 ± 92.5	292 ± 43.4
Mean Marker PCB	20.5 ± 13.5	4.95 ± 4.40	9.6	19.5 ± 0.89	32.5 ± 3.38	122 ± 36.5	102 ± 16.9
Mean PBDEs	4.08 ± 1.11	15.8 ± 14.3	0.88	4.28 ± 0.92	7.00 ± 0.79	22.5 ± 6.09	19.6 ± 4.94

[1] Trophic status as per Minigawa and Wada (18)

[2] Trophic status as per Fisk (46).

6.3.5.2 Accumulation of dioxins in marine species.

Lowest upperbound wet weight Σ7 dioxin concentrations were found in blue whiting (0.19 pg g^{-1}) with the highest recorded in mackerel (1.55 pg g^{-1}). Figure 6.8 summarises Σ7 dioxin wet weight data in seven wild species where concentrations were determined.

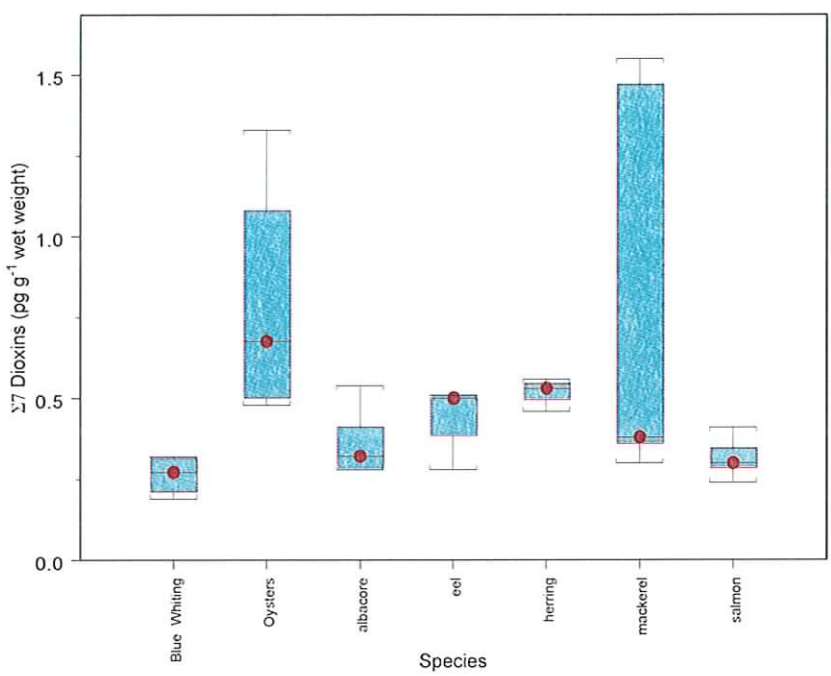

Figure 6.8: Boxplot of Σ7 dioxin congeners (pg g^{-1}wet weight) in all wild species.

Levels appear similarly elevated in both oysters and mackerel, which is surprising as their feeding mechanisms differ. Oysters would be expected to accumulate dioxin from the water column via a filter-feeding route, while mackerel generally consume vertebrate species.

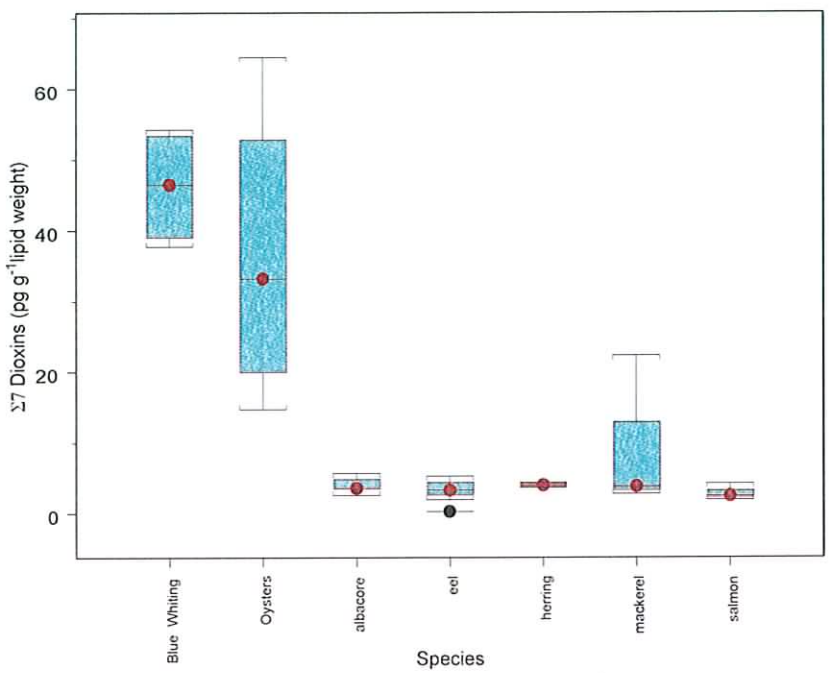

Figure 6.9: Boxplot of upperbound Σ7dioxin congeners (pg g^{-1} lipid weight) in wild species.

Figure 6.9 presents lipid normalised concentration data for the seven species. It can be observed that lipid normalised levels of blue whiting are much more elevated that those of other fish species. Such elevated levels in the lipid of blue whiting are of concern regarding the continued harvesting of blue whiting oil for use in animal/fish-feed and for inclusion in fish oil based produce.

Dioxin levels in blue whiting oils have been shown to exceed legislative values for dioxins in fish oil, thereby raising concerns over the ultimate safety of human end-users of constituent blue whiting oil produce. However; blue whiting muscle tissue contains low

levels of lipid, human (and/or other fish species) therefore consumption of blue whiting fillets will generally result in exposure to low levels of lipophilic dioxin congeners and consequently low TEQs. Further temporal and/or biological factors such as stage of maturity and or lipid percentage can affect the levels of dioxins in blue whiting fish oils, these factors and the role of stable isotope analysis in modelling dioxin uptake are summarised below and are discussed in greater detail in Chapter 3.

Closer examination of the wet and lipid weight box shapes and of individual congener data determined that elevated OctaCDD determined in one oyster and in two mackerel samples were much more elevated than observed in other like species. OctaCDD has been allotted a low level of relative importance in terms of it potential toxicity (TEF= 0.001 compared to that of PCDD TEF=1) therefore the use of toxicity equivalency approaches alone does not enable elevated levels of individual congeners to be easily identified.

6.3.5.2.1 The role of stable isotopes in modelling dioxin magnification.

Figure 6.10 presents log (X+1) (lipid weight) transformed Σ3 dioxin congeners (2,3,7,8-TetraCDD, 1,2,3,7,8-PentaCDD and 1,2,3,6,7,8-HexaCDD) against sample trophic status.

The primary observation from the data is that for a number of species dioxin levels increase with increases in trophic status. Such trophic levels/dioxin concentration increases are evident in the case of salmon, blue whiting, albacore and eels. It can also be observed that wide $\delta^{15}N$ derived trophic status ranges have been found for a number of species. Strongest relationships were demonstrated in albacore (R^2= 0.67) and eel (R^2=0.72) with biomagnification factors of 1.62 and 1.29 being determined respectively.

This is especially evident in the case of oysters where two samples are shown to have a trophic status in excess of 3.5 and for the other two samples transformed dioxin concentrations were among the most elevated in all samples tested. It has not been fully elucidated why elevated dioxin levels and enriched $\delta^{15}N$ isotopic ratios are found in these samples and while further temporal and/or spatial investigation is merited, results may be a reflection of the influence of potential localised nutrient or other anthropogenic inputs and/or be a result of elevated levels of particulate organic matter (POM) in the water column. As dioxins and other hydrophobic organic pollutants preferentially absorb onto particulate matter, filter feeding oysters and mussels may potentially be subjected to increased concentrations of POPs via elevated POM levels in the surrounding water column.

While no data are available to evaluate dioxin magnification through a full marine food web, data are available to estimate magnification processes within individual fish species but also over a range of fish species.

Weaker dioxin level/trophic status relationships were demonstrated in other species, indicating that while trophic status plays a significant role in dioxin biomagnification additional factors play a major role in this process in other fish species.

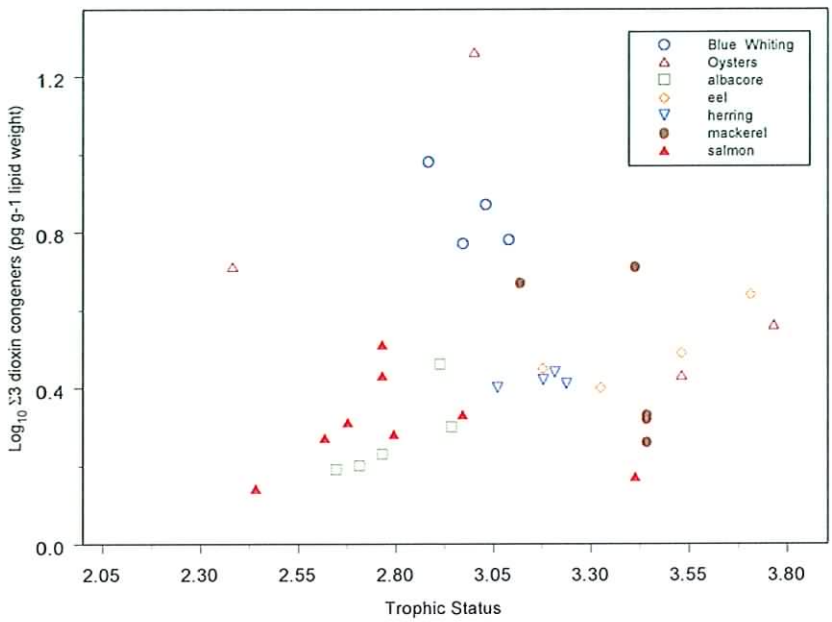

Figure 6.10: Scatterplot of trophic status vs. Σ3 Dioxin congeners in wild species.

Plotting mean Σ3 dioxin concentrations vs. mean trophic status for herring, mackerel, salmon and albacore returned a regression statistic of R^2=0.89. While these fish show a narrow trophic status range (means 2.72 to 3.37 for salmon and mackerel respectively) they also have similar dietary preferences. The model therefore demonstrates that it may be possible to predict dioxin levels in these four species based on stable isotope ratio information alone.

Table 6.14 reports a weak relationship (R^2 = 0.02) between mean log (x+1) Σ3 dioxin congeners (lipid weight) and mean species $\delta^{13}C$ isotopic ratio data for four fish species, to an extent confirming the observations of Rüüs et al (8) that $\delta^{15}N$ derived trophic status is a more powerful indicator of trophic magnification of contaminants than $\delta^{13}C$.

Table 6.14: Summary regression statistics and species magnification factors between $\delta^{15}N$ derived trophic status and Σ3 dioxin congeners and $\delta^{13}C$ and Log(X+1)Σ3 dioxin congeners in marine biota.

	R^2 TS	Slope TS	SMF*	R^2 $\delta^{13}C$	Slope $\delta^{13}C$	N
Fish (means)[1]	0.89	0.07	1.17	0.02	0.06	4
Oysters	0.18	-0.07	NA	0.93	0.41	4
Wild salmon	0.33	0.12	NA	0.52	-0.36	7
Herring	0.35	0.04	NA	0.59	0.06	4
Albacore	0.67	0.21	1.62	0.36	0.14	5
Eel	0.72	0.11	1.29	0.47	0.02	4
Mackerel	0.39	-0.27	NA	0.01	0.03	5
Blue Whiting	0.51	-0.22	NA	0.01	-0.01	4

[1]= Regression and slope between mean values for herring, mackerel, salmon and albacore.

*= Species magnification factor determined by 10^b where b is the slope of the regression line between mean species trophic status and mean log (x+1) normalised concentration data. SMF generated for regression coefficients R^2=>0.65

N= number of pooled samples/species in the regression

R^2 TS = Regression coefficient between dioxin concentrations and $\delta^{15}N$ derived trophic status.

Slope TS= Slope of regression line.

R^2 $\delta^{13}C$= Regression coefficient between dioxin concentrations and $\delta^{13}C$.

However when the relationship between log (x+1) Σ3 dioxin congeners and individual species $\delta^{13}C$ isotopic ratio were further evaluated $\delta^{13}C$ isotope ratio data can describe a significant proportion of Σ3 dioxin congener data. R^2 values of 0.36, 0.47 and 0.59 were determined between log (X+1) transformed Σ3 dioxin congener levels and $\delta^{13}C$ isotope ratio data for albacore, eel and herring respectively demonstrating that $\delta^{13}C$ ratio data can be significant in describing biomagnification of dioxins in some species.

It is in describing the concentration of dioxins in filter feeding oysters that $\delta^{13}C$ ratio data were found to be most powerful. An R^2 of 0.93 between log (X+1) transformed Σ3 dioxin congener lipid weight levels and $\delta^{13}C$ isotope ratio data indicative of the importance of carbon source influences on dioxin biomagnification for these primary consumers.

6.3.5.2.2 Dioxin congener profiling

The relative contribution of each of the three dioxin congeners to the overall Σ3 dioxin congeners was also investigated. PentaCDD provided the majority contribution of the total in fish and oysters however the 1,2,3,6,7,8-HexaCDD congener provided the majority of the dioxin contaminant burden in eels. Such differences may partly be attributable to dietary influences in eels compared to other species.

Eels may spend a significant portion of their life cycle in riverine and/or estuarine environments and $\delta^{13}C$ data suggests a terrigenous and/or benthic influence in eel's carbon sources. Additionally terrestrial based POM may differ in contaminant profile from POM derived from open ocean sources.

The contribution of lipid normalised PentaCDD was strongly related (R^2= 0.80) with $\delta^{13}C$ isotope ratio data. The importance of dietary carbon source on dioxin levels in eels is also demonstrated by the strong relationship (R^2=0.80) between 2,3,7,8 TCDD and $\delta^{13}C$. It should be noted that the relative contributions of individual congeners to the total dioxin burden in oysters and eels show much greater variance than was observed in fish species, indicative of greater potential for localised input and particulate matter influences on these species.

As dioxin levels were found to be low in the majority of species limited data (only three congeners > LOQ) were available to further assess relative dioxin congener trends therefore further conclusions were not drawn from the data.

6.3.5.3 Accumulation of furans in marine species.

Lowest upperbound wet weight Σ10 furan concentration were recorded in blue whiting (0.10 to 0.17 pg g^{-1}) with the highest recorded in mackerel (1.58 to 7.74 pg g^{-1}). Figure 6.11 summarises Σ furan (sum of 10 congeners) wet weight data in seven species where furans were determined.

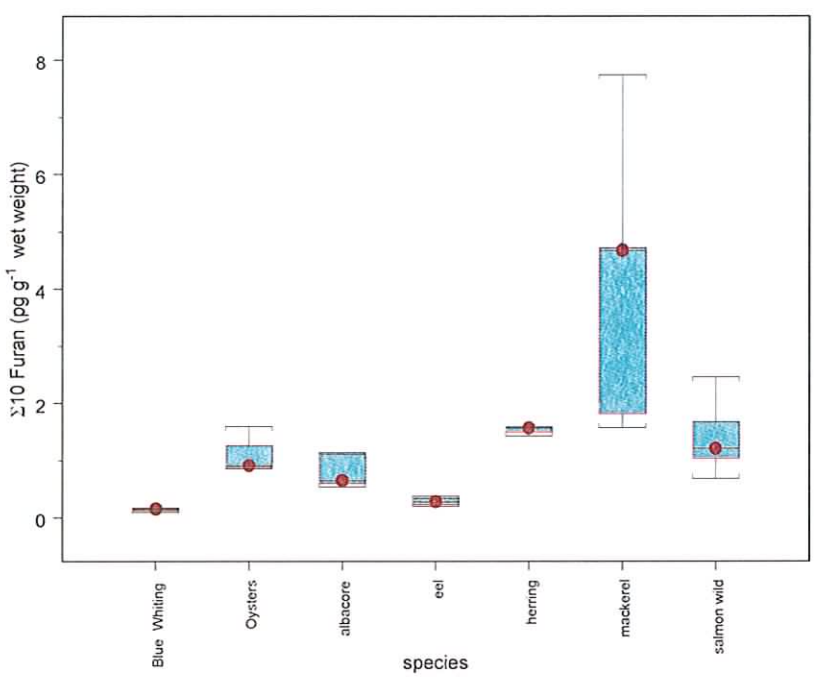

Figure 6.11: Boxplot of Σ10 furan congeners (pg g^{-1}wet weight) in all wild species.

Normalisation to a lipid basis results in mackerel samples continuing to show the most elevated levels. Oysters were also shown to have relatively high Σ furan levels, however for a number of congeners levels were below the limit of quantification for the method. Limits of quantification for Hexa-, Hepta- and Octa- substituted congeners were greater than were

observed for the majority of other species, therefore this upperbound Σ furan approach may result in an overestimation of the true levels in the samples.

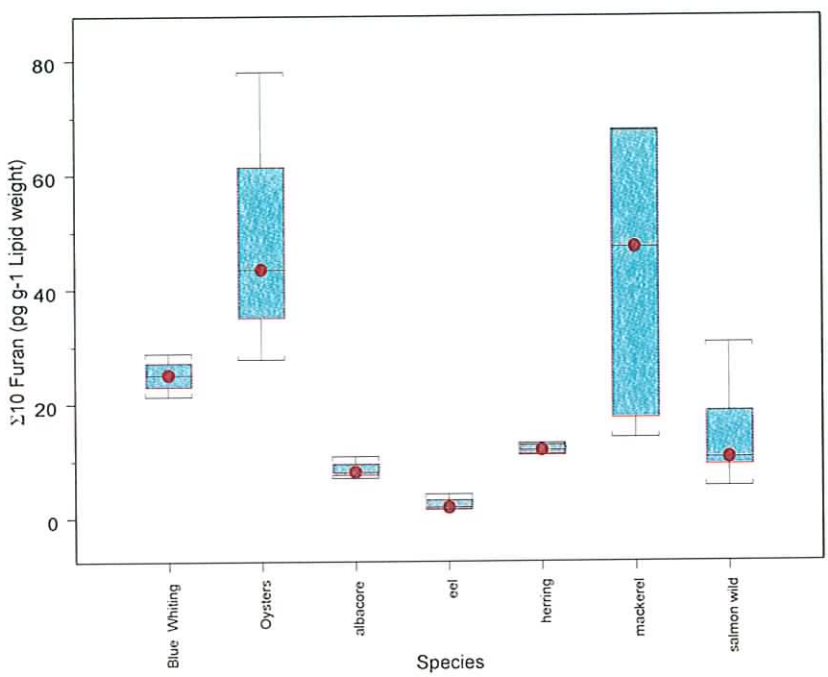

Figure 6.12: Boxplot of upperbound Σ10 furan congeners (pg g^{-1} lipid weight) in wild species.

6.3.5.3.1 The role of stable isotopes in modelling furan magnification.

Figure 6.13 presents Σ6 most prevelant furan congeners vs. sample trophic status. Furan levels increase with increases in trophic status in the case of salmon and albacore. Regression statistics in table 6.15 document the relationship between these two variables in all species.

As per the dioxin study no data are available to evaluate furan magnification through a full marine food web, however, data are available to estimate magnification processes within individual fish species and over a number of fish species.

A strong relationship was demonstrated between log (X+1) (lipid weight) transformed Σ furan congeners and trophic status in albacore (R^2=0.98) and eel (R^2=0.46) with a species magnification factor of 1.48 generated from the slopes of regression lines for albacore. Weaker furan concentration/trophic status relationships were demonstrated in other species, again indicating that while trophic status plays a significant role in furan biomagnification additional factors play a major role in this process in other fish species.

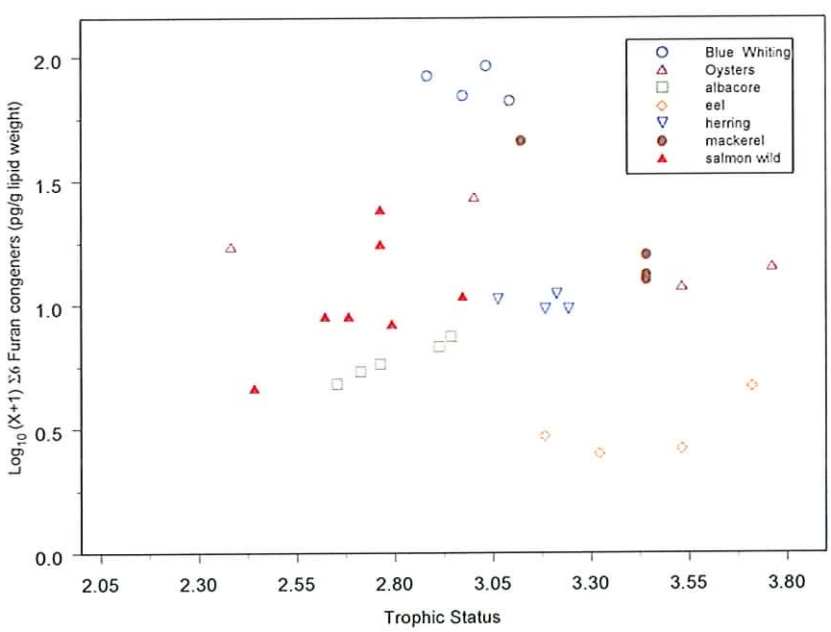

Figure 6.13: Scatterplot of trophic status vs. Σ6 furan congeners in wild species.

As per the dioxin study a strong relationship (R^2=0.60) was observed between mean log (X+1) Σ6 furan congeners and mean species trophic status in the four fish species (herring, mackerel, salmon and albacore). A species/group biomagnification factor of 1.51 for the Σ6 furans was recorded for this species grouping; the model therefore demonstrates that it may be possible to predict furan levels in muscle tissue on stable isotope ratio information alone.

A low level relationship (R^2=0.22) was found to exist between $\delta^{15}N$ derived trophic status and levels of furans in oysters, while the relationship between furan levels and $\delta^{13}C$ was found to be stronger (R^2=0.96). This again suggests that POM levels in surrounding water column may influence bioaccumulation of furans in filter-feeding oysters. A regression coefficient (R^2=0.63) was observed between furan levels and $\delta^{13}C$ in eels, possibly indicative of the importance of terrigenous POM as a carbon source in the diet of eels sampled. The dietary influences and habitats of eels are further discussed in chapter 4.

Table 6.15: Summary regression statistics and species magnification factors between trophic status and Σ6 furan congeners and $\delta^{13}C$ and log (X+1) Σ6 furan congeners in biota.

	R^2 TS	Slope TS	SMF[2]	R^2 $\delta^{13}C$	Slope $\delta^{13}C$	N
Fish (means) [1]	0.60	0.72	1.51	0.14	-0.50	
Oysters	0.22	-0.04	NA	0.96	0.18	4
Wild salmon	0.56	0.43	NA	0.65	-0.85	7
Herring	0.06	-0.03	NA	0.03	-0.02	4
Albacore	0.98	0.17	1.48	0.32	0.08	5
Eel	0.46	0.11	NA	0.63	0.02	4
Mackerel	0.35	-0.38	NA	0.02	0.16	5
Blue Whiting	0.19	-0.09	NA	0.23	0.08	4

1= Regression and slope between mean values for herring, mackerel, salmon and albacore.

2= species magnification factor determined by 10^b where b is the slope of the regression line between mean species trophic status and mean log(x+1) normalised concentration data. SMF generated for regression coefficients R^2=>0.70

N= number of pooled samples/species in the regression

R^2 TS = Regression coefficient between dioxin concentrations and $\delta^{15}N$ derived trophic status.

Slope TS= Slope of regression line.

R^2 $\delta^{13}C$= Regression coefficient between dioxin concentrations and $\delta^{13}C$.

6.3.5.3.2 Furan congener profiling.

The relative contribution of each of the six-furan congeners to the overall Σfuran contamination was further studied using congener profiling techniques. 2,3,7,8-TetraCDF provided the majority contribution of the total furan burden in all species with the exception of eels where this congener was not detected. Mean 2,3,7,8-TetraCDF levels contributed approximately 80.3% of the measurable furan burden in mackerel while the congener provided a mean of 51.8% of the furan burden in herring. In total for fish species and oysters the sum of 2,3,7,8-TetraCDF, 1,2,3,7,8-PentaCDF and 2,3,4,7,8-PentaCDF congeners contributes greater than 90% of the contaminant burden in these species.

It is currently unclear whether the non-detection of 2,3,7,8-TetraCDF in eels is a function of the detection limit of the method (0.02 to 0.04 pg g^{-1} wet weight) or is a reflection of other biological and or other spatial aspects within the animals.

A decrease in relative 1,2,3,4,7,8-HexaCDF, 1,2,3,7,8,9-HexaCDF and 2,3,4,6,7,8-HexaCDF levels in salmon with respect to trophic status was observed. A similar relationship (R^2=0.81) between relative 1,2,3,4,7,8-HexaCDF levels and trophic status was observed in herring. $\delta^{13}C$ isotopic ratios was also found to be correlated with the relative contribution of this congener as $\delta^{13}C$ enriched samples tended to have lower levels of the congener present in their muscle tissue.

While the 1,2,3,4,7,8-HexaCDF congener only makes up a small percentage of the total contaminant burden however any reduction of levels in fish muscle are of significance to the consumer and potentially to the overall health of the species.

The above assessment was based on the relative proportion of 1,2,3,4,7,8-HexaCDF to the overall Σ6 furan burden, therefore; in order to eliminate the potential that such relative reductions in 2,3,4,7,8-HexaCDF levels are not merely an artefact of an increase in the concentrations of other congeners a further normalisation process was completed as below.

Each of the lipid weight congener concentrations were normalised relative to the concentration of the most prevalent congener 2,3,7,8-TetraCDF, thus increased 2,3,7,8-TetraCDF via food and/or bioconcentration routes (e.g. in herring muscle tissue) will theoretically result in proportional increases in the levels of other congeners. It was determined that hexa-congeners with a 1,2,3 substitution decreased at a greater rate than was observed for the 2,3,4- substituted forms (R^2=0.55 and 0.78) for 1,2,3,6,7,8 and 1,2,3,4,7,8-substitutions respectively. Similar patterns were observed in salmon samples, 1,2,3,4,7,8 (R^2=0.61) and 1,2,3,6,7,8 (R^2=0.69) substituted congener regression coefficients were greater than those of the 2,3,4,6,7,8 substituted congener (R^2= 0.33).

It is not possible to clarify based on current data whether these congeners are eliminated from the body of the fish or whether they are "mobilised" via transfer of lipid to other tissues within the animals. Chapter 3 further discusses such mobilisation processes in greater detail in the case of blue whiting. No significant trophic status related decreases in hexa-substituted congeners were observed in albacore, mackerel, blue whiting or oysters.

A similar approach with respect to the relationship of hexa-substituted congeners and $\delta^{13}C$ ratios in herring was completed. It was determined that samples with more enriched $\delta^{13}C$

ratios (less negative) tended to have lower overall ratios of hexa-substituted congeners present with the 1,2,3,4,7,8 (R^2= 0.97) and 1,2,3,6,7,8 (R^2= 0.84) substituted congeners showing stronger agreement with $\delta^{13}C$ than the 2,3,4,6,7,8 hexa- congener (R^2= 0.34).

Similar strong relationships between $\delta^{13}C$ ratios and hexa- substituted congeners were determined in mackerel. The 1,2,3,4,7,8 (R^2= 0.77) and 1,2,3,6,7,8 (R^2= 0.91) substituted congeners showing strong agreement with $\delta^{13}C$ ratios with 2,3,4,6,7,8 hexa- (R^2= 0.92) congener levels reduction also strongly correlated with $\delta^{13}C$ ratios.

France and Peters (22) suggest that ($\delta^{13}C$) measurements can typically be used in evaluation of the ultimate carbon sources of an organism and can provide valuable information related to the overall feeding ecology within a species or food web. While weaker correlations were found in other species, it is unclear whether these data suggest that as herring/mackerel consume prey with increasingly enriched $\delta^{13}C$ ratios, that this prey will be more depleted in hexa- substituted congeners or whether such $\delta^{13}C$ enriched prey can confer higher $\delta^{15}N$ and consequently higher trophic status in herring. On the basis of mean $\delta^{13}C$ ratios (-17.8‰ and –18.2‰ in herring and mackerel respectively) prey would be expected to be similar for both species thereby both species would be exposed to similar contaminant profiles.

6.3.5.4 Accumulation of Mono-ortho PCBs in marine species.

Lowest wet weight Σ mono-ortho concentrations were found in oysters. Data for PCBs 105 and 118 only were available for Killer whales who exhibited the greatest concentrations of mono-ortho PCBs for the sum of two congeners. With the exception of mussels for PCB114 and PCB189 and for PCB123 in two eel samples, mono-ortho PCB residues were detected in all samples. Levels were lowest in oysters where a number of congeners were below the LOQ in all samples. Figure 6.14 presents Log (X+1) (wet weight) transformed data wild species analysed.

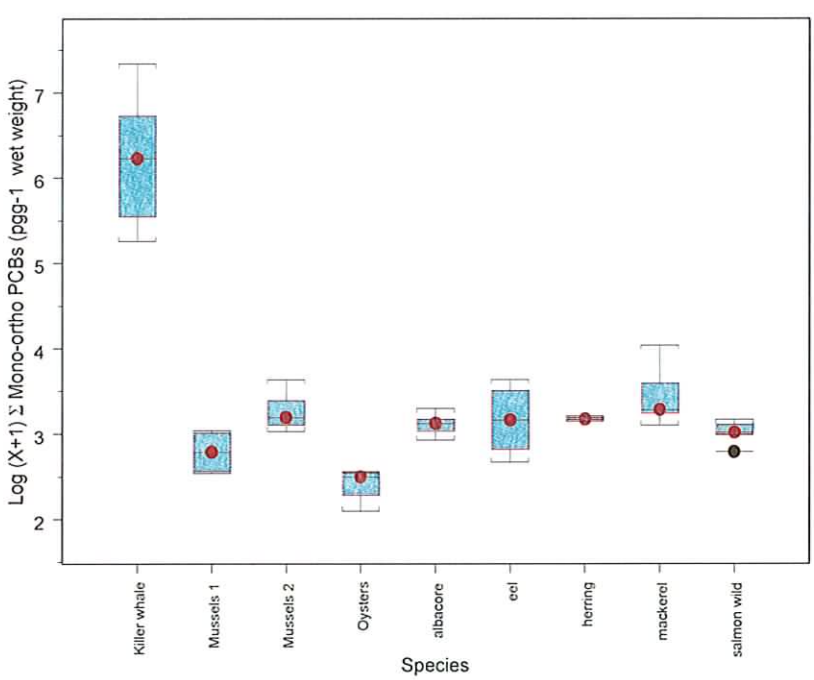

Figure 6.14: Boxplot of log (X+1) transformed Σ8 mono-ortho PCBs (pg g^{-1} wet weight) in all wild species.

Normalisation to a lipid basis still results in killer whale PCB levels being much more elevated that for all other species. For the purposes of this study results from mussels were

collated into two distinct groups (mussels 1 and 2), with mussels 1 originating in sampling locations relatively free from source inputs and the latter grouping originating from sites in the vicinity of industrialised cities of Dublin and Cork. Levels in the mussels 2 grouping were shown to be much more elevated than those of mussel group 1 indicative of the industrialised nature of the areas surrounding sampling sites. Figure 6.15 presents lipid based log (X+1) transformed data for the species sampled.

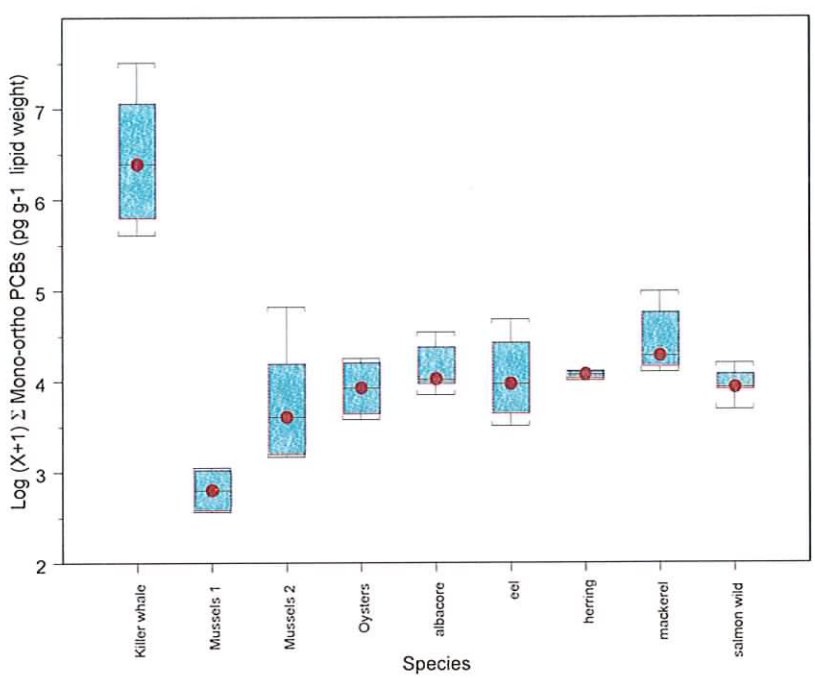

Figure 6.15: Boxplot of log (X+1) transformed Σ 8 mono-ortho PCBs (pg g^{-1} lipid weight) in all wild species.

Figure 6.16 presents the mean percentage contribution of each of the mono-ortho congeners to the overall mono-ortho burden. Only the relative proportion of PCB123 in mussels differs to any great extent from the other species. The mean percentage of PCB114 in fish species was found to decrease with increasing trophic status (R^2=0.84) and with enriched

$\delta^{13}C$ isotopic ratios (R^2=0.85) however no other significant relationships to trophic status were observed.

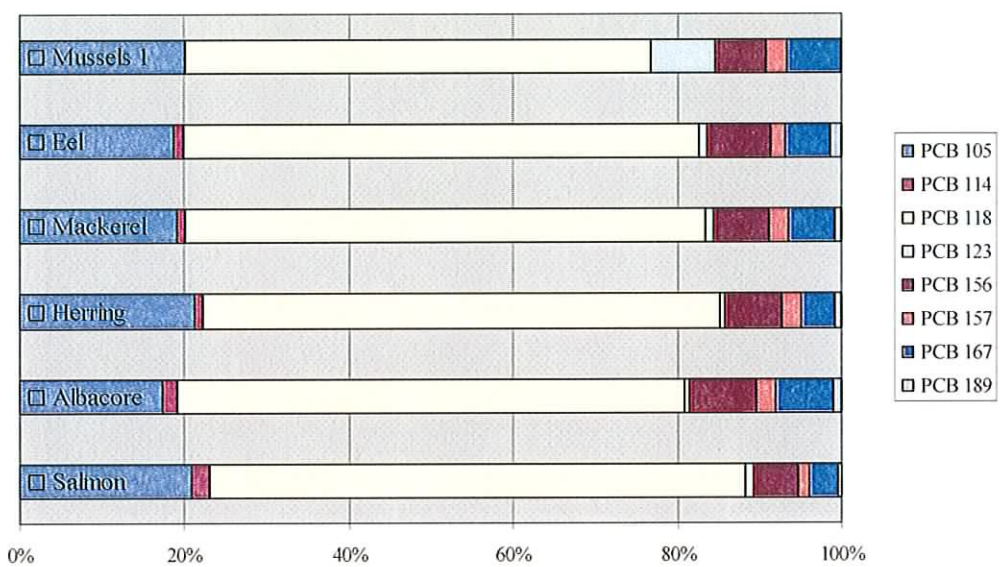

Figure 6.16: Mean percentage contribution of individual mono-ortho congeners to the Σ8 congeners in six marine species.

6.3.5.4.1 The role of stable isotopes in modelling mono-ortho PCB magnification.

Figure 6.17 presents log (X+1) transformed Σ mono-ortho congeners (PCBs 118 and 105 only for killer whale) vs. sample trophic status. Regression statistics presented in table 6.16 document the relationship between these two variables in all species.

In addition to the generation of species magnification factors (SMFs) to evaluate the flow of carbon and nitrogen within a species, sufficient mono-ortho congener information are

available from killer whale through to salmon and herring and as such it is possible to evaluate the biomagnification factor (BMF) for PCB118 and PCB105 in these species, results are further discussed below.

Strong relationships between both trophic status (R^2 = 0.91) and $\delta^{13}C$ isotopic ratios (R^2= 0.53) were determined in the combined mussel grouping. Increasing trophic status resulting in a decrease in Σmono-ortho levels in the organism.

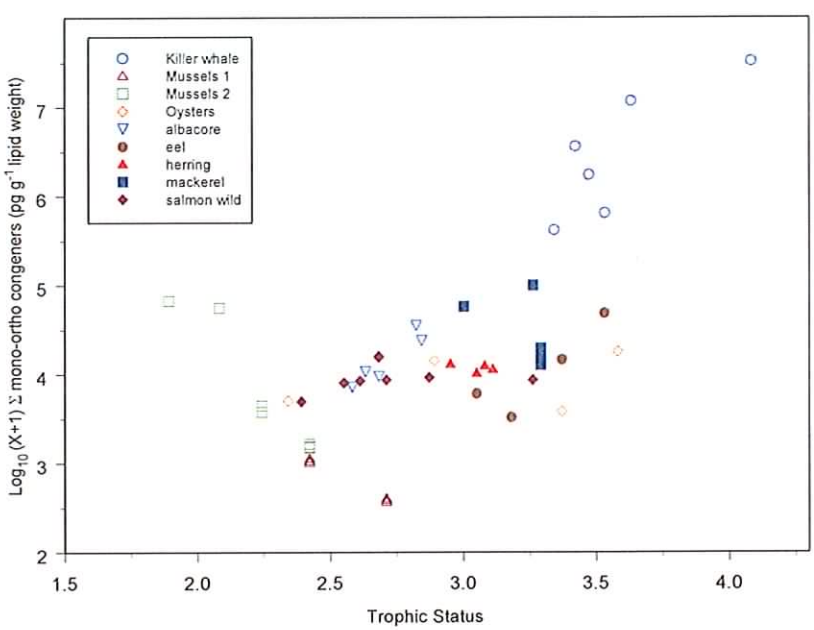

Figure 6.17: Scatterplot of trophic status vs. Σ8 mono-ortho congeners in wild species.

Note: Killer whale only PCBs 118 and 105 data available.

Similarly a strong relationship between trophic status and eels was observed, however an increase in trophic status results in increased contaminant burden. A regression coefficient (R^2=0.69) was determined between trophic status and contaminant levels in killer whales;

the slope of this line yielding a species magnification factor of 199 for the sum of the mono-ortho PCB118 and PCB105.

Table 6.16: Summary regression statistics and species magnification factors for log (X+1) Σ8 mono-ortho PCB congeners, trophic status and $\delta^{13}C$.

	R^2 TS	Slope TS	SMF*	R^2 $\delta^{13}C$	Slope $\delta^{13}C$	N
Oysters	NA	NA	NA	0.12	0.13	4
Wild salmon	0.34	0.18	NA	0.45	-0.49	7
Herring	0.28	-0.09	NA	0.43	-0.15	5
Albacore	0.87	0.62	NA	0.30	0.32	5
Eel	0.78	0.56	3.63	0.34	0.07	4
Mackerel	0.25	-0.40	NA	0.07	0.35	5
Killer whale[2]	0.69	2.30	199	0.70	0.50	6
Mussels (Groups 1+2)	0.91	-0.75	0.09	0.53	0.37	4

1= Regression and slope between mean values for herring, mackerel, salmon and albacore.

2= Congeners PCB105 and 118 only

*= Species magnification factor determined by 10^b where b is the slope of the regression line between mean species trophic status and mean log (x+1) normalised concentration data. SMF generated for regression coefficients R^2=>0.70

N= number of pooled samples/species in the regression

R^2 TS = Regression coefficient between dioxin concentrations and $\delta^{15}N$ derived trophic status.

Slope TS= Slope of regression line.

R^2 $\delta^{13}C$= Regression coefficient between dioxin concentrations and $\delta^{13}C$.

In order to investigate the potential for species specific accumulation/elimination of PCB residues within biota the ratio of PCB 118 and PCB 105 was plotted against trophic status, figure 6.18 presents these data. It was determined that in general increased trophic status resulted in an increased PCB118/PCB105 ratio. The controlling mechanisms remain however unclear.

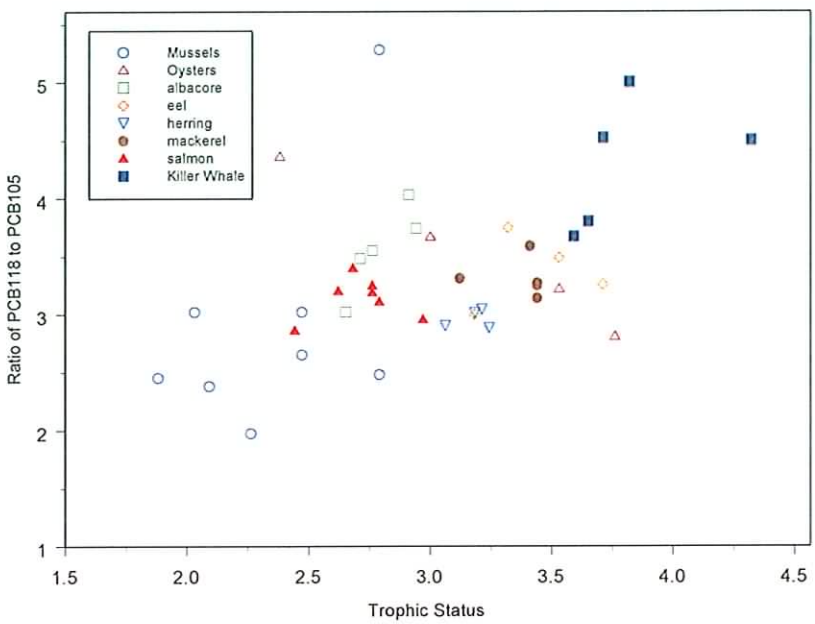

Figure 6.18: Trophic status against the ratio of PCB118 to PCB105.

6.3.5.4.2 Biomagnification factors for PCBs 118 and 105

As previously discussed the killer whale diet has been reported to comprise of both salmon and herring. Additionally post-mortem studies found salmon bones in the stomach of one of the killer whales in this study. Killer whale omnivory was assumed for calculation purposes therefore PCB concentrations and trophic status information for prey were based on mean data from both herring and salmon.

Additionally as $\delta^{13}C$ isotopic ratios suggest that one individual killer whale (from the west of Scotland) shows dietary preferences towards marine mammals, therefore for the purposes of this study trophic status and POP levels in this individual were removed from the calculation of the BMF as below.

Equation 14=

$$BMF = \frac{POPpredator}{POPprey} / \frac{TLpredator}{TLprey}$$

where,

$POP_{predator/prey}$ = the lipid normalised log_{10} normalised data for individual contaminants

$TI_{predator/prey}$ = trophic level of predator/prey as per Wada and Minigawa.

Using mean contaminant levels and mean trophic status, BMFs of 204 and 97 were calculated for PCBs 105 and 118 respectively for the Killer whale/Salmon/Herring food web.

6.3.5.5 Accumulation of non-ortho PCBs in marine species.

Lowest wet weight Σ non-ortho concentrations were found in eels (mean 3.60 pg g^{-1}) while highest were observed in mussels from industrialised areas (mean 174 pg g^{-1}). Figure 6.19 presents Log (X+1) (wet weight) transformed data of wild species analysed.

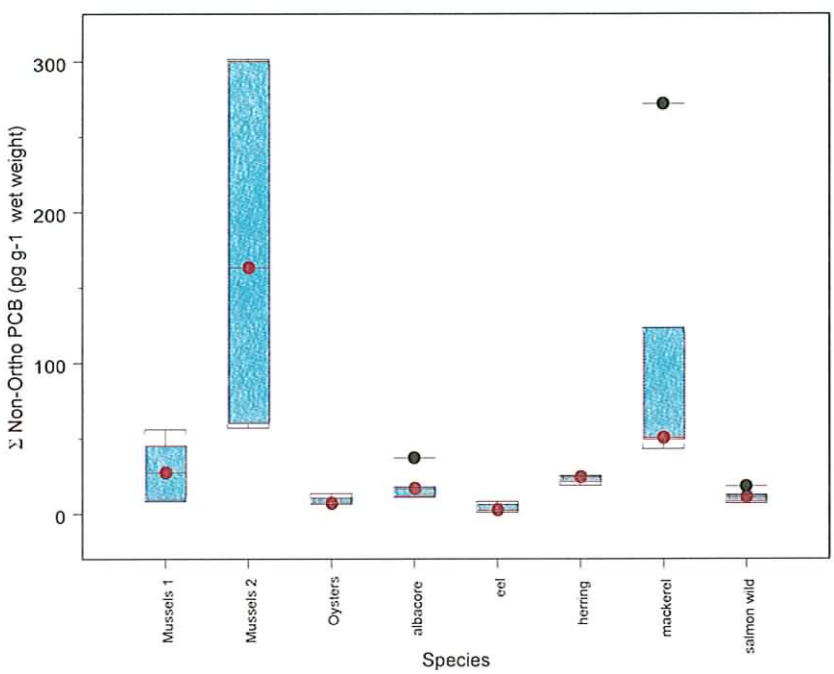

Figure 6.19: Boxplot of Σ4 non-ortho PCBs (pg g^{-1} wet weight) in all wild species.

Normalisation to a lipid basis still results in mussels from industrialised regions being much more elevated that for all other species. Figure 6.20 presents lipid based log (X+1) transformed data from the 8 species sampled. Levels of non-ortho PCBs in fish species were relatively conserved, indicative of a degree of communality in source of pollutant exposure.

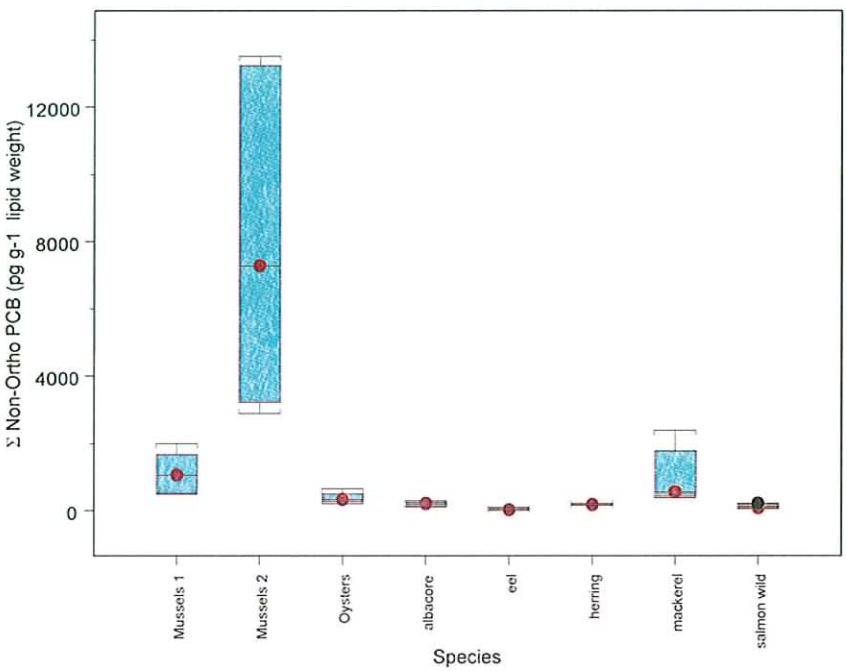

Figure 6.20: Boxplot of Σ4 non-ortho PCBs (pg g^{-1} lipid weight) in all wild species.

Table 6.17: Summary regression statistics and species magnification factors for log (X+1) Σ non-ortho PCB congeners, trophic status and $\delta^{13}C$.

	R^2 TS	Slope TS	SMF	R^2 $\delta^{13}C$	Slope $\delta^{13}C$	N
Fish (means)[1]	0.69	1.18	NA	0.07	0.20	4
Oysters	0.09	-0.10	NA	0.84	0.22	4
Wild salmon	0.01	-0.063	NA	0.01	-0.06	7
Herring	0.33	0.33	NA	0.32	0.13	5
Albacore	0.15	0.52	NA	0.25	-0.17	5
Eel	0.33	-1.49	NA	0.14	0.17	4
Mackerel	0.15	-0.15	NA	0.02	0.16	5
Mussels (Group 1)	0.98	-1.60	-39.8	0.89	0.18	4
Mussels (Group 2)	0.99	-2.80	-631	0.99	0.27	4

1= Regression and slope between mean values for herring, mackerel, salmon and albacore.

*= Species magnification factor determined by 10^b where b is the slope of the regression line between mean species trophic status and mean log (x+1) normalised concentration data. SMF generated for regression coefficients R^2=>0.70

N= number of pooled samples/species in the regression

R^2 TS = Regression coefficient between dioxin concentrations and $\delta^{15}N$ derived trophic status.

Slope TS= Slope of regression line.

R^2 $\delta^{13}C$= Regression coefficient between dioxin concentrations and $\delta^{13}C$.

Strong relationships between both trophic status (R^2 = 0.98 and 0.99) and $\delta^{13}C$ isotopic ratios (R^2= 0.89 and 0.99) were determined in both mussel groupings. Increasing trophic status resulting in a decrease in Σnon-ortho PCB levels in the organism. See figure 6.21 and table 6.17 for graphical representation of $\delta^{15}N$ derived trophic status and $\delta^{13}C$ isotopic ratios vs. log (X+1) transformed lipid weight data respectively. Few other strong relationships exist between log (X+1) transformed lipid weight data and either trophic status and/or $\delta^{13}C$ isotopic ratios.

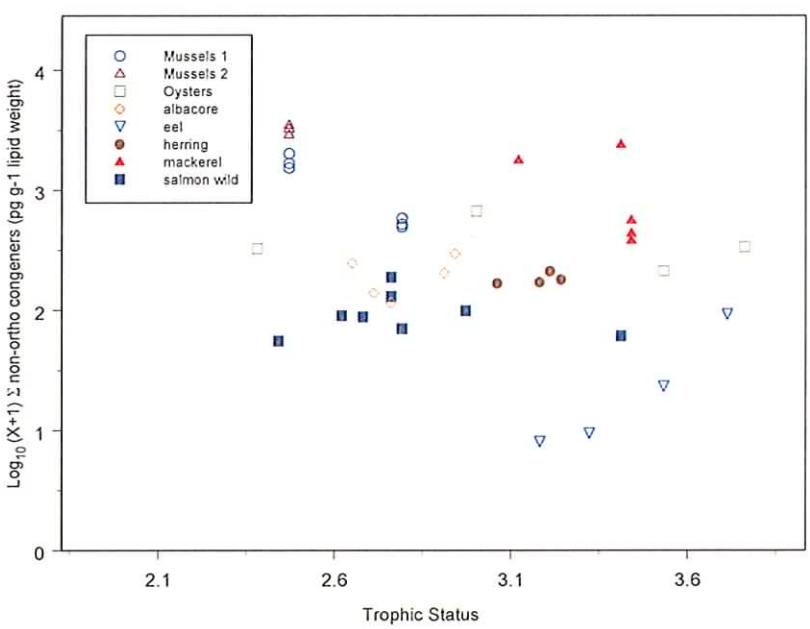

Figure 6.21: Scatterplot of trophic status vs. log (X+1) transformed non-ortho PCB congeners in wild species.

6.3.5.6 Accumulation of marker PCBs in marine species.

Lowest wet weight Σ marker PCB concentrations were found in oysters while highest levels were observed in killer whales. As the levels in killer whales are much more elevated than in other species Log (X+1) (wet weight) transformed data are presented in figure 6.22.

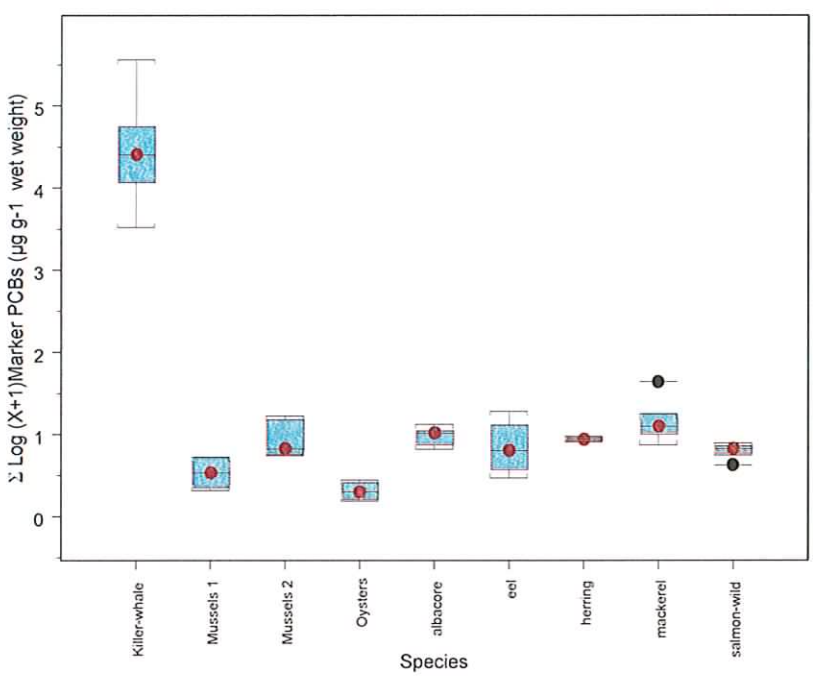

Figure 6.22: Boxplot of log (X+1) transformed Σ Marker PCBs (ng g^{-1} wet weight) in all wild species.

Normalisation to a lipid basis (see figure 6.23) still results in killer whale levels being higher than for all other species. As killer whale levels are more elevated than those of other species, figure 6.23 is plotted on a log (X+1) transformed scale for graphical purposes. Lipid weight levels of marker PCBs in fish species were relatively conserved,

whilst mussels from relatively industrialised sampling sites were more elevated than those from areas having lesser input pressures.

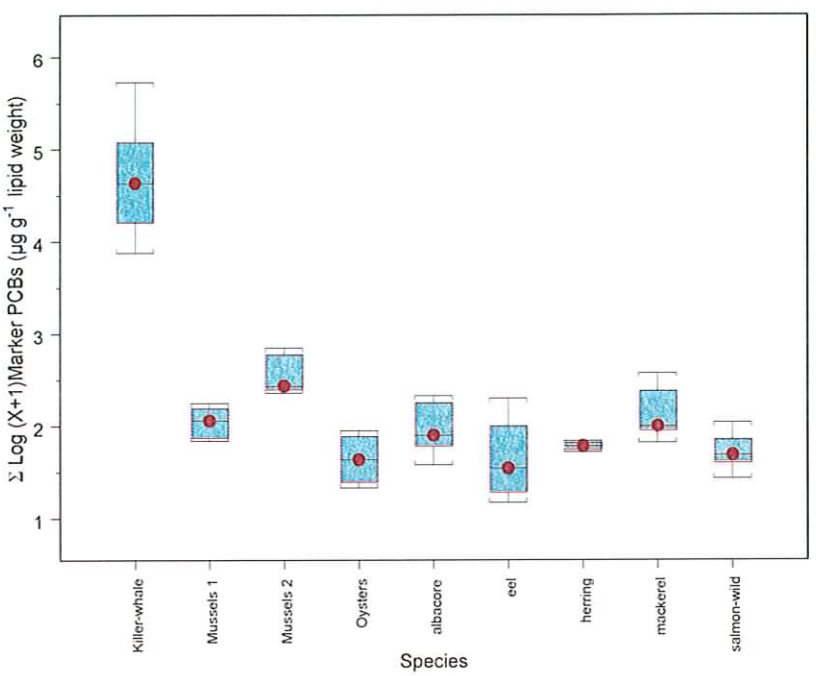

Figure 6.23: Boxplot of log (X+1) transformed Σ marker PCBs (ng g^{-1} lipid weight) in all wild species.

Table 6.18: Summary regression statistics and species magnification factors for log (X+1) Σ marker PCB congeners, trophic status and $\delta^{13}C$.

	R^2 TS	Slope TS	SMF	R^2 $\delta^{13}C$	Slope $\delta^{13}C$	N
Killer whale	0.87	2.06	115	0.87	0.50	6
Oysters	0.13	0.16	NA	0.09	0.10	4
Wild salmon	0.07	0.14	NA	0.11	-0.14	7
Herring	0.34	-0.36	NA	0.61	-0.20	5
Albacore	0.88	2.32	209	0.33	0.37	5
Eel	0.74	1.85	70.8	0.43	0.005	4
Mackerel	0.24	-0.46	NA	0.12	0.36	5
Mussels (Group 1)	0.83	-0.46	0.35	0.03	0.02	4
Mussels (Group 2)	0.01	0.10	NA	0.48	0.12	4

1= Regression and slope between mean values for herring, mackerel, salmon and albacore.

*= Species magnification factor determined by 10^b where b is the slope of the regression line between mean species trophic status and mean log (x+1) normalised concentration data. SMF generated for regression coefficients R^2=>0.70

N= number of pooled samples/species in the regression

R^2 TS = Regression coefficient between dioxin concentrations and $\delta^{15}N$ derived trophic status.

Slope TS= Slope of regression line.

R^2 $\delta^{13}C$= Regression coefficient between dioxin concentrations and $\delta^{13}C$.

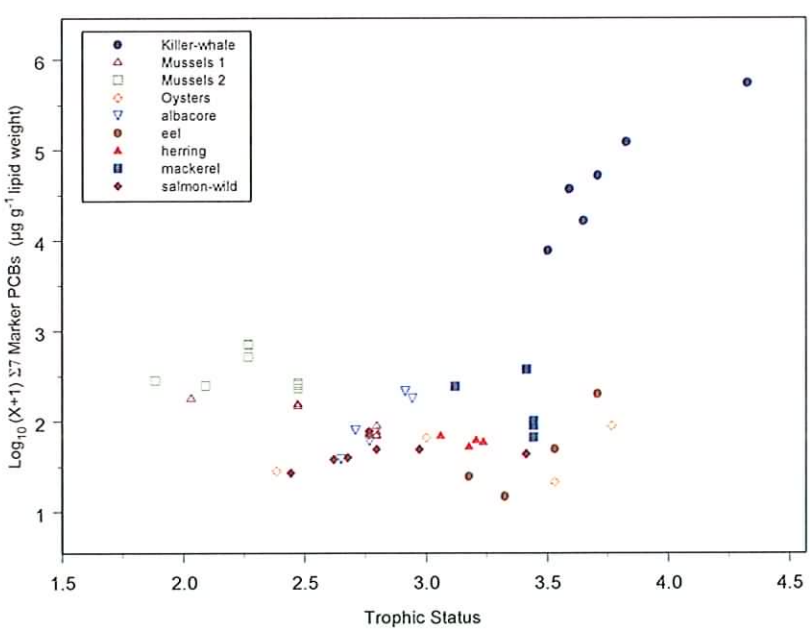

Figure 6.24: Scatterplot of trophic status vs. log (X+1) transformed Σ marker PCBs (ng g^{-1} lipid weight) in wild species.

Strong relationships between both trophic status (R^2 = 0.88, 0.87 and 0.74) and marker PCB levels were determined for Albacore (tuna), killer whales and eels respectively. Killer whales and herring returned regression coefficients (R^2 =0.87 and 0.61) between $\delta^{13}C$ isotopic ratios and log (X+1) Σ marker PCB congeners respectively.

Increasing trophic status resulted in an increase in Σ marker PCB levels in the killer whales and this is further discussed in chapter 5. See figure 6.24 and table 6.18 for graphical representation of $\delta^{15}N$ derived trophic status and $\delta^{13}C$ isotopic ratios vs. log (X+1) transformed lipid weight data respectively. Few other strong relationships exist between log (X+1) transformed lipid weight data and either trophic status and/or $\delta^{13}C$ isotopic ratios.

6.3.5.7 Accumulation of PBDEs in marine species.

Lowest wet weight Σ10 PBDE (congeners 28, 47, 66, 85, 99, 100, 138, 153, 154 and 183) levels were found in albacore while highest were observed in mussels from industrialised locations. Levels in eels varied, this being indicative of differences of habitat for the animals sampled.

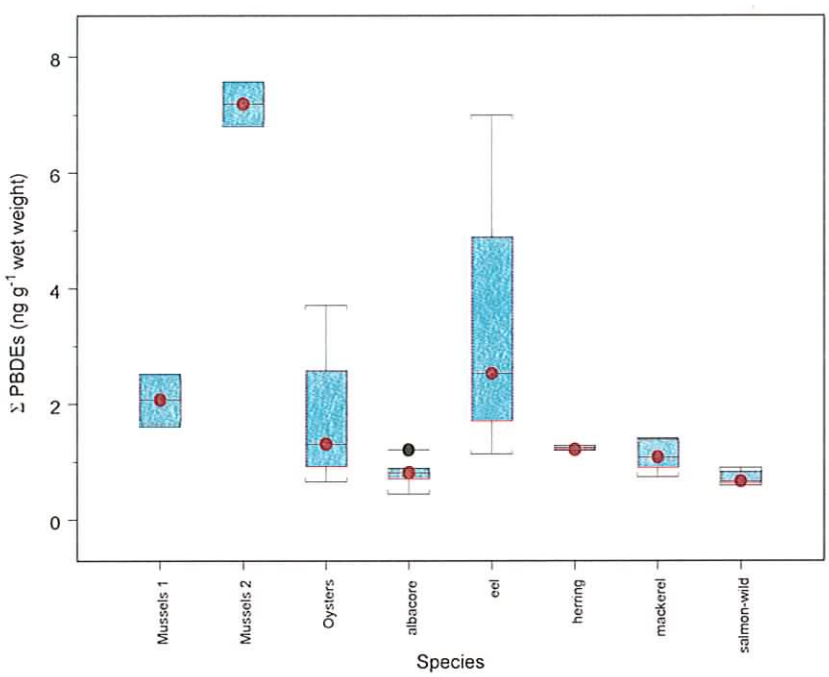

Figure 6.25: Boxplot of Σ10 PBDEs (ng g^{-1} wet weight) in all wild species.

Normalisation to a lipid basis (see figure 6.26) still results in mussels from industrialised regions being more elevated in PBDEs than for all other species. Again lipid weight levels of PBDEs in fish species were relatively conserved.

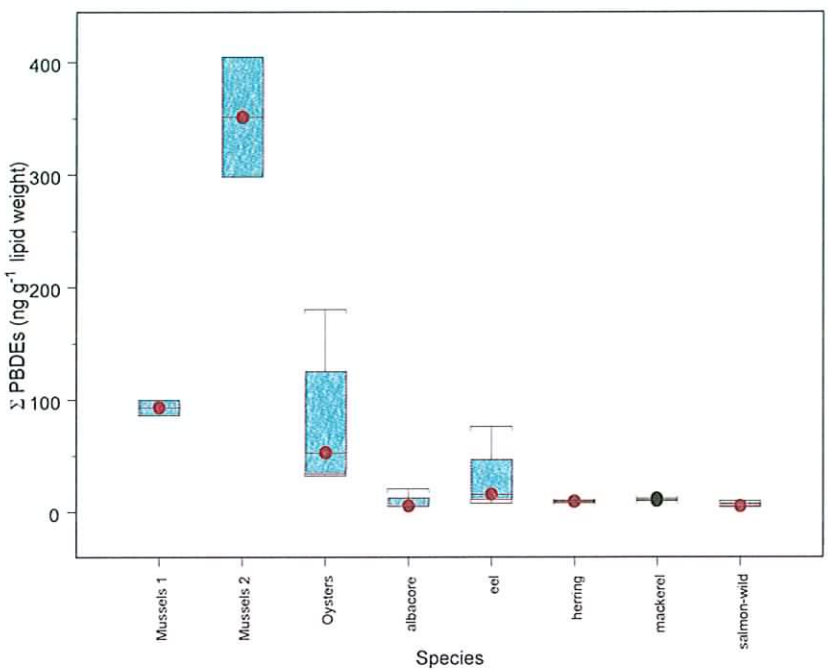

Figure 6.26: Boxplot of Σ PBDEs (ng g^{-1} lipid weight) in all wild species.

Table 6.19: Summary regression statistics and species magnification factors for log (X+1) Σ10 PBDE congeners, trophic status and $\delta^{13}C$.

	R^2 TS	Slope TS	SMF	R^2 $\delta^{13}C$	Slope $\delta^{13}C$	N
Oysters	NA	NA	NA	0.45	0.26	4
Wild salmon	NA	NA	NA	0.13	-0.10	7
Herring	0.60	-0.39	NA	0.26	-0.10	5
Albacore	0.78	1.68	47.9	0.22	0.23	5
Eel	0.63	1.36	NA	0.49	0.07	4
Mackerel	NA	NA	NA	NA	NA	5
Mussels (Group 1)	NA	NA	NA	NA	NA	4
Mussels (Group 2)	NA	NA	NA	NA	NA	4

1= Regression and slope between mean values for herring, mackerel, salmon and albacore.

N= number of pooled samples/species in the regression

*= Species magnification factor determined by 10^b where b is the slope of the regression line between mean species trophic status and mean log (x+1) normalised concentration data. SMF generated for regression coefficients R^2=>0.70

R^2 TS = Regression coefficient between dioxin concentrations and $\delta^{15}N$ derived trophic status.

Slope TS= Slope of regression line.

R^2 $\delta^{13}C$= Regression coefficient between dioxin concentrations and $\delta^{13}C$.

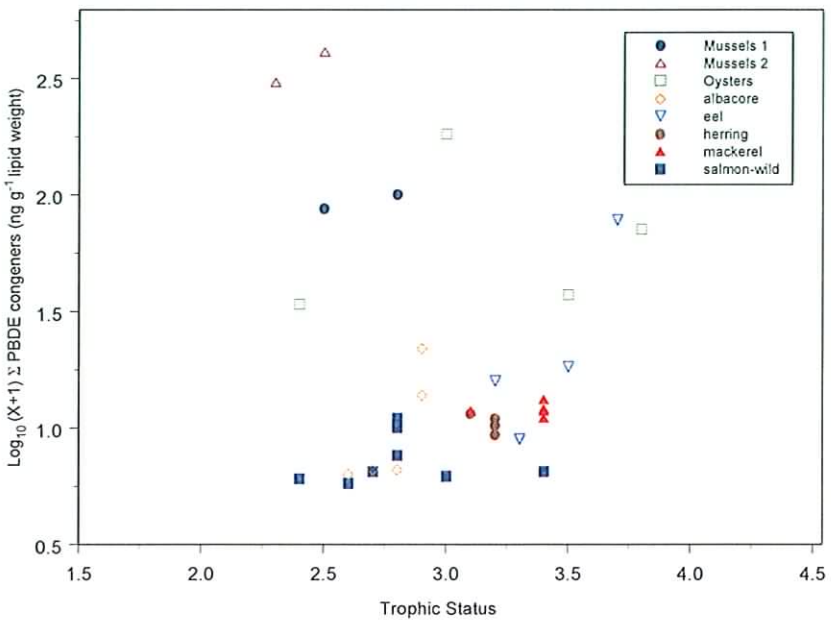

Figure 6.27: Scatterplot of trophic status vs. log (X+1) transformed Σ10 PBDE(ng g^{-1} lipid weight)in wild species

Strong relationships between both trophic status (R^2 = 0.78, 0.63 and 0.60) were determined for Albacore (tuna), eel and herring respectively. Increasing trophic status resulting in an increase in Σ PBDE levels in eels, this is further discussed in chapter 4. See figure 6.27 and table 6.20 for graphical representation of $\delta^{15}N$ derived trophic status and $\delta^{13}C$ isotopic ratios vs. log (X+1) transformed lipid weight data respectively. Few other strong relationships exist between log (X+1) transformed lipid weight data and either trophic status and/or $\delta^{13}C$ isotopic ratios.

6.4 Stable isotopes discussion and conclusions

Determination of the stable isotope ratios of nitrogen while vital to our understanding of the mechanisms of trophic status and food-web structure should be used cautiously when investigating accumulation of pollutants. When used in combination with other techniques such as gut contents analysis (GCA) they can act as a powerful tool to describe food web structures and interactions. Trophic level estimates resulting from stable isotope analysis largely corroborate steady-state modelling techniques and/or gut contents analysis as techniques such as GCA alone cannot always fully account for the dietary influence of material such as plankton and/or zooplankton species or additionally other factors such as fasting/unavailability of food cannot readily be evaluated.

Temporal changes in isotopic signature of an organism need to be investigated/assessed as the inherent variation can reflect changes in feeding conditions such as availability and or changes in food sources, feeding rates, enforced or other fasting, spawning or unavailability of food. Other factors such as health status of test organism also need to be considered in food web studies.

Stable isotope analysis while allowing for tracking of time integrated nutrient assimilation must also account for turnover rates of nutrients within organisms, it has generally been found that longer lived and slow growing species assimilate/eliminate carbon and nitrogen into and out of their muscle tissues at slower rates than short-lived and fast growing species. An understanding of such biological mechanisms must be developed in order to accurately interpret stable isotope data on a temporal scale.

Temporal changes in climatic conditions such as colder water temperatures in winter or the presence/absence of thermoclines can affect the levels of nutrients in the water column resulting in differences in the available species of phytoplankton. Dinoflagellates will have a different isotopic profile to that of diatoms and as a consequence the isotopic profile of primary consumers such as mussels will change to reflect this. Where primary producers show a change in isotopic signature this must be accounted for where determinations of the trophic status are required.

Trophic level concepts are limited to use of discrete trophic levels which are limited by the techniques inability to capture complex interactions and trophic omnivory prevalent in many ecosystems. Food webs capture the complexity of trophic interactions but hold all trophic linkages of equal importance thereby making them ineffectual in tracking energy and mass flow through an ecosystem. Stable isotope techniques provide a mechanism that allows such flows be evaluated.

The use of direct δ15N measurements without correction for trophic baseline can often be a better mechanism for comparing data between locations, as common species will not be subjected to different correction factors. Additionally choosing a suitable baseline can be problematic especially when describing an open ocean food web as estuarine primary producers can be subject to anthropogenic inputs that may not be a factor for open ocean organisms.

This work demonstrates that stable isotope based techniques can be utilised to confer relative trophic status on marine organisms and can be utilised to model contaminant flow

through the marine web. Additionally the power of stable isotope approaches as tools to differentiate between farmed and wild fish species was demonstrated.

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CHAPTER 7: OVERALL CONCLUSIONS AND APPLICATIONS

7.1 Overall project conclusions.

This thesis documents the occurrence of a number of persistent pollutants and the utilisation of stable isotope techniques in the modelling of bioaccumulation of selected contaminants in a wide variety of marine species. These are further discussed below.

7.1.1 Provision of information on "baseline" levels of contaminants in marine species surrounding Ireland and summary risk assessment to the consumer Irish fishery produce.

Such baseline data are essential in order to ensure continued protection of the consumer of contaminants in Irish fishery produce. Additionally these data are of importance in furthering our understanding of the mechanisms by which POPs accumulate in marine species and are of assistance in deriving information related to the health status of species themselves.

Elevated levels of toxaphene were observed in farmed species (sea-trout, salmon and lake-trout) and these were significantly higher that in most other sample groupings. Such data are of relevance with respect to ensuring the ongoing safety of the consumer in addition to allowing aquaculture producers assess the effects of changes in their onsite feeding management practices.

The population of the European eel is currently in decline, with levels of POPs often linked to this decrease. There are currently few data related to the levels of POP compounds in eels from Irish waters and this dataset provides a "snap-shot" of contaminant levels in a

number of these long lived species. The relevance of habitat on contaminant levels in eel populations was also identified.

Elevated levels and enantiospecific enrichement of OCP compounds are reported in Killer whales resident in the waters around Britain and Ireland. Sampling of killer whales should ideally be completed on animals in their natural environment, however as such sampling opportunities (and consequently baseline contaminant data) are relatively uncommon in these waters, the determination of OCPs in stranded individuals provides a mechanism by which contaminant bioaccumulation in these top of food web predators can be assessed.

This thesis further investigated the baseline levels of a variety of pollutants in blue whiting, concluding that POP levels can be much greater in the oils in this species than in other pelagic fish. Most elevated levels are detected at the height of the spawning season when fish have migrated further north. These data are relevant with respect to exceedences of EU food safety legislative limits for fish oils for human and/or anaimal consumption. Contaminant profiling techniques established that preferential accumulation/metabolism/excretion of specifice compound classes may be possible in blue whiting. Such complex biological and spatial interactions need to be further investigated in order to further elucidate contaminant bioaccumulation pathways.

A high degree of emphasis is usually placed on the analytical analysis of extracted samples, however improper sampling of a test species will result in incorrect test data being obtained. With respect to overall sampling procedures it was concluded that inherent natural variability is difficult to control in field situations and that mixtures of both males

and females of differing ages and degrees of maturity can be expected within a single shoal of sampled fish. Species specific standard sampling procedures were developed during this work in order to minimise such variability and are fundamental to accurate analysis.

The reported baseline data provide both "spot-check" information related to one-off sampling programmes. Such data further assist environmental/food safety managers in determining whether ongoing trend monitoring programs or more extensive surveys are necessary to monitor the pollutant levels in a particular location and/or species (eg. elevated levels of OCDD in a sample of eels from the west of Ireland).

Overall this thesis (and associated publications) report a number of new baseline datasets of contaminant levels in Irish marine species. Summary risk assessments utilising these data show that no adverse effects to the consumer of a number of POP residues (toxaphene, PBDEs and HBCD) in Irish fishery produce are currently expected. These consumer risk assessments are based on an individual contaminant group basis; there is however a lack of information on potential effects of mixtures of pollutants on the health risks to both marine species. The additive nature of toxicity of mixtures and the need for further chronic bioassay measurements require further research.

7.1.2 The role of stable isotopes in environmental modelling applications.

The measurement of ratios of stable isotopes in a number of marine species was demonstrated in this work to;

- enable modelling of the bioaccumulation of a number of lipophilic POPs in marine species to be completed,

- provide valuable information related to these species dietary preferences.
- provide data related to the habitat (and on potential anthropogenic inluences) for a number of marine species,
- be a suitable tool to assist in determiming suitability of individual species and/or locations for environmental monitoring purposes.
- provide a technique that allows for the differentiation between farmed and wild fish species.

Determination of the stable isotope ratios while vital to our understanding of the mechanisms of trophic status and food-web structure must be used cautiously when investigating accumulation of pollutants. When utilised in combination with other techniques such as gut contents analysis, stable isotope data can be a powerful tool in describing food web structures and interactions. A number of areas need to be considered when utilising this powerful tool, including,

- temporal changes in isotopic signature of an organism need to be investigated/assessed as the inherent variation can reflect changes in feeding conditions such as availability and or changes in food sources,
- feeding rates, enforced or other fasting, spawning or unavailability of food and/or other factors such as health status of test organism etc also need to be considered in food web studies,
- information related to the baseline isotopic signal of the relevant food web is required, to allow for "correction" of inherent background ratios. Dinoflagellates will have a different isotopic profile to that of diatoms and as a consequence the isotopic profile of primary consumers such as mussels will change to reflect this.

Where primary producers show a change in isotopic signature these differences must be accounted for when determining a trophic status index,

- an understanding of biological mechanisms such as temporal nutrient turnover rates and/or the effect of colder water temperatures is required in order to accurately interpret stable isotope data,
- an understanding of potential geographical influences (eg. open ocean food web vs. estuarine primary producers) is also a requirement, as individual food web constituent members may be subject to anthropogenic inputs that may not be a factor for open ocean organisms.

Overall the use of trophic level index concepts are limited to use of discrete trophic levels which are restricted by the techniques inability to capture complex interactions and trophic omnivory prevalent in many ecosystems. Food webs capture the complexity of trophic interactions but hold all trophic linkages of equal importance thereby making them ineffectual in tracking energy and mass flow through an ecosystem. Stable isotope techniques provide a mechanism that allows such flows be evaluated.

Application of stable isotope principles with respect to OCP bioaccumulation in killer whales was demonstrated with a number of OCPs found to increase in level with respect to trophic status. The potential application of isotopic ratios in routine monitoring studies to model contaminant uptake, enantioselective enrichment and accumulation was demonstrated. Data was presented providing information on enantioselective enrichment factors (EFs) for o, *p'*-DDT, α-HCH and toxaphene congeners CHB26 and CHB 50. Additionally, based on killer whale stable isotope signatures, it is proposed that elevated

contaminant levels one individual whale from Scotish waters are influenced by a marine mammal dietary preferences. In this case this is the first known instance in British/Irish waters where stable isotope techniques have been utilised to suggest marine mammal influences in killer whale dietary preferences. This dataset further improves current information on potential metabolic processes within the species.

In conclusion, techniques described during the course of this work demonstrate the power of stable isotope based techniques in environmental and future environmental and food safety monitoring programmes and the necessity to complete routine analysis programs in order to protect both the consumer of marine produce, marine species and marine biodiversity.

Annex 1: Biological, sampling and analytical information (wet weight) in species sampled during this study.

		Length statistics (mm)				Weight statistics (g)					Age statistics (yrs)					Lipid (%)	CHB concs. (µg kg^{-1}) Wet Weight				Latitude		Longitude	
Species	individs	min.	max.	mean	std dev	min	max	mean	stddev	Sex	min	max	mean	Tissue	Dry wt.	Total	26	50	62	Sum 3	deg.	min	deg	min
Redfish	12	350	490	407	44.8	479	1755	906	368	X				MU	20.6	2.26	0.12	0.26	0.08	0.46	60	32	-4	29
Dab	26	180	300	242	29.4	65	327	186	74.8	X	2	6	3	MU	20	0.27	0.01	0.02	0.01	0.04	51	30	-7	10
Black sole	20	260	420	328	40.9	160	532	410	74.7	X				MU	20.5	0.66	0	0.01	0	0.02	50	50	-10	50
Hake	24	190	260	227	17.6	130	311	200	38.9	X				MU	22.9	1.18	0.05	0.01	0.02	0.07	53	1	-9	55
Haddock	25	270	375	297	25.7	201	472	266	80.1	X				MU	20	0.69	0.01	0.01	0.01	0.02	53	50	-5	20
Haddock	24	270	350	305	21.9	186	501	287	89.7	X	2	3	3	MU	21	0.47	0.01	0.01	0.01	0.04	53	50	-11	0
Grenadier	10	190	335	264	49.3	722	3691	2138	964	X				MU	19.5	0.49	0.03	0.05	0.04	0.12	60	32	-4	29
Mackerel	20	215	310	262	35.1	59	206	122	50.1	X			0	MU	28	7.05	0.22	0.22	0.15	0.59	53	50	-11	0
Mackerel	25	250	360	282	28.2	124	342	185	60.3	X	0	4	1	MU	24.3	1.93	0.04	0.06	0.01	0.11	56	0	-8	0
Monkfish	10	230	410	320	66.7	129	511	351	168	X				MU	17.2	0.52	0	0.01	0.01	0.03	50	50	-10	50
Cod	25	270	440	308	40.6	234	1076	398	193	X	1	1	1	MU	20.2	0.6	0	0.01	0.02	0.04	53	40	-6	0
Cod	10	390	640	537	78.8	194	1525	992	337	X				MU	20.7	0.56	0.1	0.1	0.11	0.32	50	50	-10	50
Shrimp	50	15.7	20	18	0.9					X				TM	22	1.19	0.03	0.07	0.01	0.12	51	32	-9	25
Shrimp	100	13.5	19.6	17	1.2					X				TM	23	1.28	0.01	0.02	0.03	0.06	51	42	-10	5
Herring	25	230	290	251	13.8	90	143	112	13.8	X				MU	23.9	2.11	0.33	0.47	0.24	1.03	50	50	-10	50
Herring	25	240	300	270	15	123	201	166	19.5	X	2	8	4	MU	23.9	6.99	0.26	0.58	0.25	1.09	55	0	-9	0
Herring	25	230	280	268	10.3	88	198	172	25.2	X				MU	31.4	12.7	0.27	0.46	0.1	0.82	50	50	-10	50
Whiting	22	280	440	345	48.3					X	1	4	3	MU	23.1	0.59	0.01	0.01	0.01	0.03	53	50	-11	0
Whiting	22	230	350	286	34.5					X	0	3	1	MU	19.9	0.67	0.02	0.03	0.01	0.06	50	50	-10	50
Whiting	25	220	280	248	16.9	92	195	132	28.7	X	2	5	2	MU	19.9	0.69	0.01	0.01	0.01	0.03	53	35	-5	45
Skate	20	290	740	445	105	120	2310	575	472	X				MU	22	0.49	0.05	0.04	0.05	0.13	53	50	-11	0
Skate	20	250	520	378	68.6	74	731	385	174	X				MU	17.4	0.66	0.03	0.04	0.07	0.14	50	50	-10	50
Skate	10	390	570	464	62.9	357	1275	683	295	X	3	10	6	MU	24.6	0.79	0.01	0.02	0.03	0.06	53	35	-5	45
Lake trout	8	340	370	352	11.3	414	577	487	49	X				MU	22.3	1.18	0.57	0.68	0.59	1.83	0	0	0	0
Sea trout	10	665	839	744	62.5									MU	27.8	5.86	0.62	1.18	1.26	3.06	0	0	0	0
Salmon	10	630	730	689	28.3									MU	29.3	5.97	1.67	2.42	1.96	6.05	0	0	0	0

Annex 1 cont.: Biological, sampling and analytical information (wet weight) in species sampled during this study.

		Length statistics (mm)				Weight statistics (g)					Age statistics (yrs)					Lipid (%)	CHB concs. (µg kg^{-1}) Wet Weight				Latitude		Longitude	
Species	individs	min.	max.	mean	std dev	min	max	mean	stdev	Sex	min	max	mean	Tissue	Dry wt.	Total	26	50	62	Sum 3	deg.	min	deg	min
Plaice	20	200	380	274	58.9	85	593	252	140	X			EST3	MU	19.8	0.77	0.01	0.02	0.01	0.03	53	50	-11	0
Plaice	20	280	430	368	44	305	904	576	186	X	1	5	2	MU	19.9	0.71	0.21	0.52	0.17	0.9	50	50	-10	50
Plaice	25	270	370	314	29.7	210	617	359	114	X			EST4	MU	16.9	0.72	0	0	0	0	53	35	-5	45
Plaice	25	270	370	318	29.1	254	697	402	142	X			EST 4	MU	20.5	0.8	0.07	0.07	0.03	0.18	53	35	-5	45
Plaice	20	250	390	295	40.6	129	477	242	83.7	X				MU	20.2	0.65	0.01	0.01	0	0.02	53	50	-11	0
Mussels	50	45	60	53	3.5									SB	20.3	1.9	0.01	0.02	0	0.03	53	36	-9	49
Mussels	50	49.9	60	54	3									SB	20.8	2.07	0.02	0.03	0.01	0.07	53	36	-9	49
Mussels	50	40.4	66.6	51	5.3									SB	22.5	1.03	0.01	0.01	0	0.02	52	14	-6	58
Mussels	50	41	77.7	62	8.1									SB	24.7	1.05	0.02	0.02	0.01	0.05	54	37	-8	23
Mackerel	1			310				201		M			1	MU	38.4	18.7	1.94	2.2	1.32	5.46	53	50	-11	0
Mackerel	1			290				155		F			0	MU	35.3	11.3	0.6	1.08	0.76	2.44	53	50	-11	0
Mackerel	1			285				156		M			1	MU	35.8	15.3	1.42	2.49	1.66	5.57	53	50	-11	0
Mackerel	1			285				159		F			1	MU	36.9	22.5	0.47	0.96	0.36	1.79	53	50	-11	0
Mackerel	1			305				175		M			1	MU	32.1	17	0.65	0.91	0.83	2.39	53	50	-11	0
Mackerel	1			300				206		F			0	MU	29.5	16.6	0.31	0.33	0.32	0.96	53	50	-11	0
Mackerel	1			280				147		F			1	MU	35.2	15.4	0.45	0.82	0.45	1.72	53	50	-11	0
Mackerel	1			290				151		M			1	MU	31.2	10.4	0.35	1.06	0.24	1.65	53	50	-11	0
Mackerel	1			295				166		M			3	MU	34.8	18	0.47	0.34	0.21	1.03	53	50	-11	0
Mackerel	1			270				126		M			3	MU	31.8	7.87	0.57	1.07	0.71	2.35	53	50	-11	0
Mackerel	1			290				156		M			3	MU	32.8	9.02	0.37	0.69	0.54	1.6	53	50	-11	0
Mackerel	1			235				93		F			1	MU	25.3	4.63	0.16	0.12	0.04	0.32	53	50	-11	0
Mackerel	1			215				63		M			0	MU	25.6	4.44	0.22	0.5	0.27	1	53	50	-11	0
Mackerel	1			215				61		F			0	MU	24.9	3.29	0.15	0.29	0.13	0.57	53	50	-11	0
Mackerel	1			225				69		M			1	MU	23.5	2.82	0.06	0.1	0.08	0.24	53	50	-11	0
Mackerel	1			265				88		M			1	MU	24.3	2.16	0.13	0.17	0.11	0.41	53	50	-11	0
Mackerel	1			230				77		M			1	MU	25.3	3.93	0.24	0.35	0.18	0.77	53	50	-11	0
Mackerel	1			215				59		F			0	MU	24.7	5.84	0.15	0.17	0.22	0.54	53	50	-11	0
Mackerel	1			215				63		M			1	MU	25.5	4.69	0.22	0.4	0.17	0.79	53	50	-11	0
Mackerel	1			230				78		M			1	MU	27.1	10.6	0.36	0.86	0.47	1.7	53	50	-11	0

Note: MU, TM and SB =muscle tissue, tail muscle and soft body tissue respectively

Annex 2: Concentrations of PCDD and PCDF congeners in wild species (pg g^{-1} wet weight).

	Reference	2378-TetraCDD	12378-PentaCDD	123478-HexaCDD	123678-HexaCDD	123789-HexaCDD	1234678-HeptaCDD	OctaCDD	2378-TetraCDF	12378-PentaCDF	23478-PentaCDF	123478-HexaCDF	123678-HexaCDF	123789-HexaCDF	234678-HexaCDF	1234678-HeptaCDF	1234789-HeptaCDF	OctaCDF
Salmon wild	NA	0.02	0.05	< 0.007	0.02	< 0.007	< 0.047	< 0.117	0.53	0.04	0.23	0.01	< 0.007	< 0.007	0.01	< 0.047	< 0.047	< 0.117
Salmon wild	NA	0.04	0.08	0.01	0.03	< 0.006	< 0.04	< 0.1	1.03	0.10	0.39	0.02	0.02	< 0.006	0.02	< 0.04	< 0.04	< 0.1
Salmon wild	MSC/04/1040	0.02	0.03	< 0.008	0.01	< 0.008	< 0.055	< 0.138	0.40	0.04	0.19	0.01	< 0.008	< 0.008	< 0.008	< 0.055	< 0.055	< 0.138
Salmon wild	MSC/04/1041	0.04	0.08	0.01	0.02	< 0.009	< 0.059	< 0.147	0.75	0.07	0.37	< 0.009	0.01	< 0.009	0.01	< 0.059	< 0.059	< 0.147
Salmon wild	MSC/04/1042	0.05	0.13	0.01	0.04	< 0.007	< 0.05	< 0.124	1.51	0.14	0.51	0.02	0.02	< 0.007	0.03	< 0.05	< 0.05	< 0.124
Salmon wild	MSC/04/1043	0.01	0.03	< 0.008	< 0.008	< 0.008	< 0.051	< 0.126	0.30	0.02	0.11	< 0.008	< 0.008	< 0.008	< 0.008	< 0.051	< 0.051	< 0.126
Salmon wild	MSC/04/1044	0.04	0.08	0.01	0.03	0.01	< 0.042	< 0.104	0.98	0.08	0.38	0.01	0.01	< 0.006	0.01	< 0.042	< 0.042	< 0.104
Salmon wild	MSC/04/1045	0.05	0.07	< 0.007	0.02	< 0.007	< 0.045	< 0.111	0.62	0.07	0.28	0.01	0.01	< 0.007	0.02	< 0.045	< 0.045	< 0.111
Salmon wild	MSC/04/1046	0.03	0.06	< 0.008	0.02	< 0.008	< 0.051	< 0.128	0.57	0.06	0.30	0.01	0.01	< 0.008	0.01	< 0.051	< 0.051	< 0.128
Salmon wild	MSC/04/1047	0.02	0.07	< 0.008	0.01	< 0.008	< 0.05	< 0.126	0.47	0.05	0.31	0.01	0.01	< 0.008	0.01	< 0.05	< 0.05	< 0.126
Albacore	MSC/04/1172	0.02	0.03	< 0.008	0.01	0.01	< 0.052	< 0.195	0.23	0.04	0.07	0.01	0.01	< 0.008	< 0.008	< 0.052	< 0.052	< 0.129
Albacore	MSC/04/1173	0.02	0.04	< 0.018	< 0.018	< 0.018	< 0.12	< 0.301	0.34	0.04	0.15	< 0.018	< 0.018	< 0.018	< 0.018	< 0.12	< 0.12	< 0.301
Albacore	MSC/04/1174	0.02	0.07	< 0.012	0.02	< 0.012	< 0.077	< 0.193	0.46	0.07	0.17	0.02	0.03	< 0.012	0.01	< 0.077	< 0.077	< 0.193
Albacore	MSC/04/1175	< 0.009	0.03	< 0.01	0.01	< 0.01	< 0.062	< 0.154	0.20	0.04	0.09	0.01	< 0.01	< 0.01	< 0.011	< 0.062	< 0.062	< 0.154
Albacore	MSC/04/1176	0.03	0.05	< 0.007	0.02	< 0.007	< 0.044	< 0.11	0.14	0.06	0.10	0.01	0.01	< 0.007	0.01	< 0.044	< 0.044	< 0.11
Herring	MSC/04/1177	0.03	0.12	0.02	0.08	0.02	< 0.082	< 0.205	0.64	0.09	0.37	0.03	0.03	< 0.012	0.03	< 0.082	< 0.082	< 0.205
Herring	MSC/04/1178	0.03	0.11	0.03	0.09	0.02	< 0.074	< 0.185	0.64	0.11	0.41	0.03	0.03	< 0.011	0.04	< 0.074	< 0.074	< 0.185
Herring	MSC/04/1179	0.02	0.11	0.02	0.08	0.02	< 0.079	< 0.199	0.63	0.08	0.41	0.03	0.02	< 0.012	0.03	< 0.079	< 0.079	< 0.199
Herring	MSC/04/1180	0.02	0.09	0.03	0.06	< 0.011	0.06	0.19	0.54	0.09	0.37	0.03	0.03	< 0.01	0.03	< 0.065	< 0.06	< 0.209
Mackerel	MSC/04/1182	0.04	0.05	< 0.008	0.01	< 0.008	< 0.053	< 0.133	1.10	0.03	0.17	< 0.008	< 0.008	< 0.009	< 0.008	< 0.053	< 0.053	< 0.133
Mackerel	MSC/04/1183	0.04	0.06	< 0.009	0.01	< 0.009	< 0.06	0.19	0.88	0.03	0.18	0.05	0.03	0.02	0.03	0.41	0.13	2.92
Mackerel	MSC/04/1184	0.07	0.14	< 0.051	< 0.051	< 0.051	< 0.342	< 0.855	2.55	0.09	0.33	< 0.051	< 0.051	< 0.051	< 0.051	< 0.342	< 0.342	< 0.855
Mackerel	MSC/04/1185	0.14	0.28	< 0.039	0.05	< 0.039	< 0.263	< 0.658	5.22	0.17	1.01	< 0.039	< 0.039	< 0.039	< 0.039	< 0.263	< 0.263	< 0.658
Mackerel	MSC/04/1186	0.04	0.06	< 0.01	0.01	< 0.01	< 0.067	< 0.167	1.26	0.03	0.19	< 0.01	< 0.011	< 0.01	< 0.01	< 0.067	< 0.067	< 0.167
Oysters	ENV/04/0050	0.06	0.23	0.05	0.06	0.04	< 0.216	0.67	0.31	0.04	0.14	< 0.032	< 0.032	< 0.032	0.05	< 0.216	< 0.216	< 0.54
Oysters	ENV/04/0053	0.03	0.09	0.03	0.11	0.11	0.42	3.70	0.37	0.05	0.14	< 0.022	< 0.022	< 0.022	0.04	< 0.149	< 0.149	< 0.372
Oysters	ENV/04/0056	< 0.006	0.02	< 0.018	0.03	0.02	< 0.121	< 0.302	0.15	0.02	0.07	< 0.018	< 0.018	< 0.018	0.03	< 0.121	< 0.121	< 0.302
Oysters	ENV/04/0057	0.03	0.01	< 0.017	< 0.017	< 0.017	< 0.112	< 0.28	0.23	0.03	0.07	< 0.017	< 0.017	< 0.017	0.02	< 0.112	< 0.112	< 0.28
Oysters	ENV/04/0062	< 0.009	0.04	< 0.018	0.03	0.02	0.14	0.58	0.20	0.02	0.09	< 0.018	< 0.018	< 0.018	0.02	< 0.117	< 0.117	< 0.293
Eel	MSC/05/1119	0.04	0.10	0.03	0.16	0.04	0.10	n.d	n.d	0.02	0.15	0.05	0.03	n.d	0.08	0.04	n.d	n.d
Eel	MSC/05/1120	0.03	0.08	0.08	0.17	0.04	0.11	n.d	n.d	0.01	0.10	0.06	0.03	n.d	0.09	n.d	n.d	n.d
Eel	MSC/05/1121	0.02	0.09	0.04	0.10	n.d	0.05	n.d	n.d	0.02	0.06	0.03	0.02	n.d	0.08	n.d	n.d	n.d
Eel	MSC/05/1122	0.03	0.08	0.06	0.23	0.03	0.08	n.d	n.d	0.02	0.09	0.06	0.03	n.d	0.07	n.d	n.d	n.d
Blue whiting	MSC/2005/1079	0.01	n d	n d	0.02	n.d	0.03	0.13	0.31	0.06	n d	0.02	0.01	n.d	0.02	0.04	n.d	0.01
Blue whiting	MSC/2005/1080	0.01	n d	n.d	0.02	n.d	0.04	0.16	0.3	0.05	0.01	0.02	0.02	n.d	0.02	0.06	n.d	0.03
Blue whiting	MSC/2005/1081	0.02	n d	n.d	0.03	0.01	0.05	0.2	0.34	0.07	0.01	0.03	0.02	n.d	0 02	0.06	n.d	0.02
Blue whiting	MSC/2005/1082	0.01	n.d	n.d	0.02	n d	0.05	0.24	0.24	0.06	0.01	0.02	0.02	n.d	0 03	0.07	n.d	0.03

n.d. =not detected

Annex 3 : Concentrations of PCDD and PCDF congeners in farmed and retail samples (pg g^{-1} wet weight).

	Reference	2378-TetraCDD	12378-PentaCDD	123478-HexaCDD	123678-HexaCDD	123789-HexaCDD	1234678-HeptaCDD	OctaCDD	2378-TetraCDF	12378-PentaCDF	23478-PentaCDF	123478-HexaCDF	123678-HexaCDF	123789-HexaCDF	234678-HexaCDF	1234678-HeptaCDF	1234789-HeptaCDF	OctaCDF
Sardines tin	MSC/05/0001	0.09	0.08	< 0.018	< 0.018	< 0.018	< 0.119	< 0.297	2.23	0.07	0.31	< 0.018	< 0.018	< 0.018	< 0.018	< 0.119	< 0.119	< 0.297
Mackerel tin	MSC/05/0002	0.04	0.05	< 0.023	< 0.023	< 0.023	< 0.154	< 0.385	1.02	0.03	0.17	< 0.023	< 0.023	< 0.023	< 0.023	< 0.154	< 0.154	< 0.385
Mackerel tin	MSC/05/0003	0.04	0.06	< 0.02	< 0.02	< 0.02	< 0.137	< 0.341	1.12	0.05	0.22	< 0.02	< 0.02	< 0.02	< 0.02	< 0.137	< 0.137	< 0.341
Kipper tin	MSC/05/0004	0.02	0.10	0.01	0.05	0.01	< 0.059	< 0.147	0.65	0.08	0.35	0.02	0.02	< 0.009	0.02	< 0.059	< 0.059	< 0.147
Kipper tin	MSC/05/0005	0.02	0.10	0.01	0.06	0.02	< 0.061	< 0.152	0.71	0.09	0.34	0.03	0.02	< 0.009	0.03	< 0.061	< 0.061	< 0.152
Salmon tin	MSC/04/1119	< 0.002	0.01	< 0.005	< 0.005	< 0.005	< 0.035	< 0.088	0.04	< 0.004	0.01	< 0.005	< 0.005	< 0.005	< 0.005	< 0.035	< 0.035	< 0.088
Salmon tin	MSC/04/1120	< 0.002	< 0.003	< 0.006	< 0.006	< 0.006	< 0.038	< 0.095	0.05	< 0.004	0.01	< 0.006	< 0.006	< 0.006	< 0.006	< 0.038	< 0.038	< 0.095
Salmon tin	MSC/04/1121	0.01	0.03	< 0.008	0.02	< 0.008	< 0.053	< 0.13	0.24	0.06	0.12	0.01	0.01	< 0.008	0.02	< 0.053	< 0.053	< 0.13
Salmon tin	MSC/04/1122	< 0.005	< 0.005	< 0.006	< 0.006	< 0.006	< 0.041	< 0.103	0.07	0.01	0.02	< 0.006	< 0.006	< 0.006	< 0.006	< 0.041	< 0.041	< 0.103
Salmon tin	MSC/04/1123	0.02	0.06	0.01	0.03	0.01	< 0.04	< 0.101	0.36	0.10	0.22	0.02	0.02	< 0.006	0.03	< 0.04	< 0.04	< 0.101
Tuna tin	MSC/04/1124	< 0.002	0.01	< 0.005	< 0.005	< 0.005	< 0.035	< 0.088	< 0.008	< 0.004	< 0.004	< 0.005	< 0.005	< 0.005	< 0.005	< 0.035	< 0.035	< 0.088
Tuna tin	MSC/04/1125	< 0.003	< 0.004	< 0.008	< 0.008	< 0.008	< 0.051	0.17	< 0.012	< 0.005	< 0.006	< 0.008	< 0.008	< 0.008	< 0.008	< 0.051	< 0.051	< 0.128
Tuna tin	MSC/04/1126	< 0.002	< 0.004	< 0.007	< 0.007	< 0.007	< 0.049	0.37	< 0.011	< 0.007	< 0.005	< 0.007	< 0.007	< 0.007	< 0.007	< 0.049	< 0.049	< 0.122
Tuna tin	MSC/04/1127	< 0.002	< 0.003	< 0.005	< 0.005	< 0.005	< 0.035	< 0.089	< 0.008	< 0.004	< 0.004	< 0.005	< 0.005	< 0.005	< 0.005	< 0.035	< 0.035	< 0.089
Tuna tin	MSC/04/1128	< 0.002	< 0.003	< 0.005	< 0.005	< 0.005	< 0.036	< 0.089	< 0.008	< 0.004	< 0.004	< 0.005	< 0.005	< 0.005	< 0.005	< 0.036	< 0.036	< 0.089
Salmon farmed	MSC/04/1129	0.05	0.09	0.01	0.04	0.01	< 0.042	< 0.106	1.47	0.10	0.32	0.01	0.02	< 0.006	0.02	< 0.042	< 0.042	< 0.106
Salmon farmed	MSC/04/1130	0.03	0.04	< 0.011	0.03	< 0.011	< 0.077	< 0.192	0.90	0.06	0.15	< 0.011	< 0.011	< 0.011	< 0.011	< 0.077	< 0.077	< 0.192
Salmon farmed	MSC/04/1131	0.06	0.14	< 0.012	0.04	0.02	< 0.077	< 0.194	1.68	0.13	0.44	0.02	0.03	< 0.012	0.03	< 0.077	< 0.077	< 0.194
Salmon farmed	MSC/04/1132	0.09	0.16	0.02	0.06	0.01	< 0.062	< 0.154	2.20	0.18	0.44	0.02	0.02	< 0.009	0.03	< 0.062	< 0.062	< 0.154
Salmon farmed	MSC/04/1133	0.10	0.17	0.02	0.08	0.02	< 0.071	< 0.177	2.27	0.19	0.56	0.02	0.04	< 0.011	0.04	< 0.071	< 0.071	< 0.177
Salmon farmed	MSC/04/1134	0.07	0.16	0.02	0.07	0.01	< 0.063	< 0.157	2.03	0.18	0.49	0.02	0.03	< 0.009	0.03	< 0.063	< 0.063	< 0.157
Salmon farmed	MSC/04/1135	0.06	0.07	0.01	0.04	0.01	< 0.068	< 0.17	1.28	0.11	0.30	0.02	0.02	< 0.01	0.02	< 0.068	< 0.068	< 0.17
Salmon farmed	MSC/04/1136	0.09	0.15	0.01	0.05	0.01	< 0.049	< 0.123	1.97	0.16	0.39	0.02	0.04	< 0.007	0.03	< 0.049	< 0.049	< 0.123
Salmon farmed	MSC/04/1137	0.05	0.06	< 0.009	0.03	0.01	< 0.061	< 0.152	1.35	0.10	0.34	0.02	0.02	< 0.009	0.02	< 0.061	< 0.061	< 0.152
Salmon farmed	MSC/04/1138	0.04	0.06	< 0.012	0.02	< 0.012	< 0.083	< 0.208	1.14	0.09	0.27	0.01	0.02	< 0.012	0.02	< 0.083	< 0.083	< 0.208
Salmon farmed	MSC/04/1139	0.05	0.08	< 0.011	0.05	< 0.011	< 0.076	< 0.19	1.32	0.09	0.29	0.01	0.01	< 0.011	0.02	< 0.076	< 0.076	< 0.19
Salmon farmed	NA	0.07	0.09	< 0.008	0.05	0.01	< 0.056	< 0.14	1.54	0.13	0.30	0.02	0.02	< 0.008	0.03	< 0.056	< 0.056	< 0.14
Salmon farmed	MSC/04/1141	0.03	0.05	< 0.01	0.03	< 0.01	< 0.07	< 0.174	0.99	0.09	0.24	0.02	0.02	< 0.01	0.02	< 0.07	< 0.07	< 0.174
Salmon farmed	MSC/04/1142	0.09	0.16	0.01	0.07	< 0.013	< 0.09	< 0.224	2.35	0.18	0.51	0.02	0.02	< 0.013	0.03	< 0.09	< 0.09	< 0.224
Salmon farmed	MSC/04/1143	0.09	0.10	0.01	0.06	0.02	< 0.052	< 0.13	2.07	0.16	0.32	0.01	0.03	< 0.008	0.03	< 0.052	< 0.052	< 0.13
Salmon smoked	MSC/04/1145	0.03	0.06	< 0.007	0.04	< 0.007	< 0.045	< 0.112	1.11	0.09	0.21	0.01	0.02	< 0.007	0.02	< 0.045	< 0.045	< 0.112
Salmon smoked	MSC/04/1146	0.02	0.03	< 0.007	0.02	< 0.007	< 0.047	< 0.116	0.58	0.05	0.14	0.01	0.01	< 0.007	0.01	< 0.047	< 0.047	< 0.116
Salmon smoked	MSC/04/1147	0.03	0.05	< 0.007	0.03	0.01	< 0.049	< 0.122	0.90	0.07	0.16	0.01	0.02	< 0.007	0.02	< 0.049	< 0.049	< 0.122
Salmon smoked	MSC/04/1148	0.04	0.06	< 0.008	0.03	< 0.008	< 0.053	< 0.132	0.95	0.09	0.23	0.02	0.01	< 0.008	0.02	< 0.053	< 0.053	< 0.132
Salmon smoked	MSC/04/1149	0.03	0.05	0.03	0.02	< 0.011	< 0.051	< 0.128	0.79	0.07	0.16	0.01	0.02	< 0.008	0.02	< 0.051	< 0.051	< 0.128
Salmon smoked	MSC/04/1150	0.03	0.04	< 0.008	0.02	< 0.008	< 0.053	< 0.131	0.63	0.06	0.16	0.01	0.01	< 0.008	0.01	< 0.048	< 0.048	< 0.131
Salmon smoked	MSC/04/1151	0.04	0.04	< 0.011	0.02	< 0.011	< 0.073	< 0.182	0.71	0.06	0.18	0.02	0.01	< 0.011	< 0.011	< 0.073	< 0.073	< 0.182
Salmon smoked	MSC/04/1152	0.05	0.11	0.01	0.05	0.01	< 0.049	< 0.123	1.45	0.11	0.29	0.01	0.02	< 0.007	0.02	< 0.049	< 0.049	< 0.123
Salmon smoked	MSC/04/1153	0.03	0.05	< 0.008	0.02	< 0.008	< 0.051	< 0.126	0.78	0.07	0.19	0.01	0.01	< 0.008	0.01	< 0.051	< 0.051	< 0.126
Salmon smoked	MSC/04/1154	0.03	0.05	< 0.007	0.02	< 0.007	< 0.047	< 0.118	0.67	0.05	0.16	0.01	0.01	< 0.007	0.02	< 0.047	< 0.047	< 0.118
Salmon smoked	MSC/04/1155	0.03	0.06	< 0.007	0.03	< 0.007	< 0.048	< 0.12	0.82	0.06	0.21	0.01	0.02	< 0.007	0.02	< 0.048	< 0.048	< 0.12

n.d. =not detected

Annex 4: Concentrations of DL-PCBs (pg g^{-1} wet weight) and PCB 118 marker PCBs (ng g^{-1} wet weight) in wild species.

		Non-Ortho PCBs				Mono-Ortho PCBs								Marker PCBS				
Species	Reference	PCB77	PCB81	PCB126	PCB169	PCB105	PCB114	PCB118	PCB123	PCB156	PCB157	PCB167	PCB189	PCB28	PCB52	PCB101	PCB138	PCB153
salmon wild	NA	4.71	< 0.586	2.53	0.89	207	11.6	757	9.09	36.4	14.1	47.9	5.23	105	474	917	1280	1760
salmon wild	NA	7.03	< 0.588	3.95	1.15	252	22.4	859	13.1	69.1	23.3	48.5	6.42	124	449	955	1430	1870
salmon wild	MSC/04-1040	3.40	< 0.691	2.10	0.87	195	19.2	654	10.9	62.6	18.1	34.6	3.84	75.8	290	783	1030	1500
salmon wild	MSC/04-1041	7.28	< 0.733	3.14	0.99	250	23.1	739	11.9	66.6	17.7	42.6	3.51	121	469	1100	1230	1710
salmon wild	MSC/04-1042	11.50	< 0.889	4.74	1.14	303	41.7	986	15.4	78.9	16.4	45.3	4.66	157	624	1060	1330	2160
salmon wild	MSC/04-1043	4.79	< 0.631	1.90	< 0.631	138	16.2	595	6.92	29.8	6.40	22.1	2.61	75.1	218	482	707	1160
salmon wild	MSC/04-1044	6.08	< 0.524	3.72	1.15	291	27.0	927	14.3	77.9	24.7	55.9	5.25	139	455	1070	1420	2060
salmon wild	MSC/04-1045	6.26	< 0.557	3.31	0.86	210	19.6	713	9.07	57.2	12.2	35.0	2.86	119	328	790	968	1580
salmon wild	MSC/04-1046	6.75	< 0.640	2.80	0.84	198	17.6	643	10.7	50.4	13.7	37.3	2.85	88	361	891	916	1270
salmon wild	MSC/04-1047	4.13	< 0.629	2.52	0.92	207	21.2	613	11.1	58.1	14.0	41.3	3.27	119	405	944	1200	1860
albacore	MSC/04-1172	7.08	0.66	6.78	1.36	214	12.2	800	6.75	116	46.2	110	15.8	63.7	236	1050	2240	3730
albacore	MSC/04-1173	22.3	5.72	3.66	5.64	214	23.7	646	10.6	75.4	24.4	63.3	7.33	55.2	180	710	1360	1980
albacore	MSC/04-1174	9.91	< 0.960	5.92	1.23	267	15.6	948	7.32	106	42.0	101	10.3	79.5	326	1120	2050	3460
albacore	MSC/04-1175	5.78	< 0.772	4.74	< 0.772	151	13.5	521	5.03	71.3	16.5	62.0	8.23	48.7	180	808	1600	2385
albacore	MSC/04-1176	2.65	< 0.552	6.36	2.04	308	41.3	1240	15.7	199	41.0	146	18.9	35.1	205	968	3310	4640
herring	MSC/04-1177	17.9	< .103	3.82	< 1.03	300	14.8	904	10.3	89.9	28.6	56.0	8.67	252	407	951	1550	2450
herring	MSC/04-1178	19.1	< 0.925	4.06	1.36	347	15.2	1000	6.91	122	42.5	87.3	14.0	259	377	953	2010	2960
herring	MSC/04-1179	18.7	< 0.993	4.12	1.03	307	14.7	933	11.5	98.0	33.4	59.3	9.84	248	407	972	1650	2460
herring	MSC/04-1180	13.7	< 0.745	3.37	1.08	316	12.6	915	5.41	107	39.1	70.9	13.2	141	322	1046	1785	2725
mackerel	MSC/04-1182	39.9	1.88	8.04	0.73	436	17.4	1100	14.1	110	40.3	108	12.6	134	418	1150	1950	4500
mackerel	MSC/04-1183	34.9	1.54	5.77	< 0.749	250	10.3	784	10.5	82.5	32.5	76.6	9.47	151	296	814	1450	2340
mackerel	MSC/04-1184	96.3	< 4.27	18.5	< 4.27	744	45.0	2460	47.0	271	80.0	213	28.5	422	901	2490	5330	5270
mackerel	MSC/04-1185	217	6.78	44.1	4.15	2030	65.8	7290	82.9	791	242	494	72.1	605	1690	5790	9290	13900
mackerel	MSC/04-1186	36.5	2.03	9.86	< 0.868	360	16.1	1170	12.1	151	42.0	121	13.3	128	350	1575	2740	4470
Oysters	ENV/04/0050	6.71	< 2.70	1.38	< 2.70	47.4	< 5.40	174	< 5.40	< 16.1	< 14.8	15.5	< 8.54	< 54.0	61.9	135	339	517
Oysters	ENV/04/0051	12.5	< 1.86	1.98	< 1.86	71.9	4.74	216	4.17	12.3	4.46	21.4	< 3.72	19.1	64.8	204	306	542
Oysters	ENV/04/0056	< 3.02	< 1.51	< 0.755	< 1.51	77.6	4.39	218	4.60	26.6	9.55	20.6	< 3.02	< 30.2	39.7	215	479	775
Oysters	ENV/04/0057	3.27	< 1.40	< 0.701	< 1.40	24.3	< 2.80	78.2	< 2.80	3.66	2.82	7.38	< 2.80	< 28.0	35.6	86.7	122	289
Oysters	ENV/04/0062	< 2.93	< 1.46	< 0.732	< 1.46	< 14.6	< 2.93	63.7	< 2.93	5.01	< 2.93	6.20	< 2.93	< 29.3	< 29.3	61.3	85.0	241
eel	MSC/05/1119	n.d	n.d	6.31	2.04	853	42.5	2783	42.5	311	72.2	215	41.9	579	1404	1547	4113	5302
eel	MSC/05/1120	n.d	n.d	n.d	1.21	188	10.2	564	12.0	74.9	14.2	45.6	11.0	29.3	135	198	1014	1185
eel	MSC/05/1121	n.d	n.d	n.d	1.31	75.1	8.38	282	n.d	37.5	11.6	48.2	12.6	29.3	64.1	112	558	635
eel	MSC/05/1122	n.d	n.d	2.15	1.30	429	35.8	1496	26.2	170	42.1	86.1	19.1	639	896	600	1845	1618
eel	MSC/05/1140	n.d	n.d	2.77	1.79	381	22.5	1077	n.d	168	33.3	94.0	16.4	35.0	206	265	2131	2094
Mussels	ENV/2004/055-001	5.87	0.35	1.63	0.44	42.4	n.d	187	43.72	35.2	19.7	46.2	n.d	100	500	200	483	815
Mussels	ENV/2004/055-002	6.41	0.32	1.57	0.44	35.5	n.d	211	48.05	28.9	15.0	40.3	n.d	100	500	200	481	552
Mussels	ENV/2004/055-003	6.90	0.34	1.70	0.34	33.2	n.d	189	42.47	30.8	16.6	32.1	n.d	100	500	200	424	480
Mussels	ENV/2004/069-001	48.6	2.06	5.77	0.65	292	n.d	870	76.77	91.5	34.0	93.7	n.d	200	241	674	1354	1275
Mussels	ENV/2004/069-002	51.5	2.06	5.75	0.65	352	n.d	866	77.16	89.4	33.8	93.1	n.d	200	241	673	1407	1294
Mussels	ENV/2004/069-003	51.4	2.31	5.89	0.70	352	n.d	907	97.45	83.8	39.7	105	n.d	200	228	699	1405	1117
Mussels	ENV/2004/071-001	278	14.1	7.65	0.50	1267	n.d	2456	168.11	181	56.7	151	n.d	1903	2798	2781	2214	1651
Mussels	ENV/2004/071-002	247	12.2	6.46	0.43	1119	n.d	2132	129.81	168	46.2	107	n.d	2903	2083	2307	1641	1242
Mussels	ENV/2004/071-003	279	14.0	7.42	0.48	1244	n.d	2574	157.56	174	58.3	129	n.d	1865	2796	2908	2044	1541
Mussels	ENV/2004/078-001	48.4	1.96	5.09	0.77	192	n.d	621	107.01	71.2	26.0	82.3	n.d	244	350	676	1165	1173
Mussels	ENV/2004/078-002	58.4	1.85	4.22	0.72	191	n.d	568	109.75	56.2	21.8	72.3	n.d	166	359	660	1120	1176
Mussels	ENV/2004/078-003	38.4	1.52	4.08	0.71	196	n.d	557	98.19	50.4	18.0	62.5	n.d	229	360	707	1148	1170
Killer whale	MSC/2005/1086					4900000		18000000						160	17000	18000	67000	84000
Killer whale	MSC/2005/1087					2500000		9500000						1100	10000	9600	39000	37000
Killer whale	MSC/2005/1088					1000		180000						1100	1300	2100	8600	13000
Killer whale	MSC/2005/1089					4000000		13000000						5800	240000	220000	1060000	1410000
Killer whale	MSC/2005/1090					8800000		44000000						1100	17000	5100	170000	190000
Killer whale	MSC/2005/1091-D					62361		282017						6.84	480	2922	13313	2254
Killer whale	MSC/2005/1092-V																	
Mussels	24ENV03 157-9					434		1063		81.7				896	1057	1098	1092	1349
Mussels	24ENV03 170-2					298		710		69.3				219	462	718	1101	1383
Mussels	25ENV03 160-8					72.4		180		24.1				41.1	122	194	398	657
Mussels	28ENV03 164-6					142		427		39.4				118	245	458	756	1167

n.d. =not detected

Annex 5: Concentrations of DL-PCBs (pg g^{-1} wet weight) and marker PCBs and PCB 118 (ng g^{-1} wet weight) in farmed and retail samples.

		Non-Ortho PCBs				Mono-Ortho PCBs								Marker PCBS				
Species	Reference	PCB 77	PCB 81	PCB 126	PCB 169	PCB 105	PCB 114	PCB 118	PCB 123	PCB 156	PCB 157	PCB 167	PCB 189	PCB 28	PCB 52	PCB 101	PCB 138	PCB 153
Sardines tin	MSC/05/0001	57.8	3.15	14.9	< 1.48	53.4	4.58	210	< 2.97	40.2	6.82	32.8	5.72	36.7	114	132	670	1180
Mackerel tin	MSC/05/0002	25.3	< 1.92	5.77	< 1.92	195	14.9	621	5.21	58.4	16.6	55.1	5.35	136	511	1040	1360	1710
Mackerel tin	MSC/05/0003	25.4	< 1.71	5.77	< 1.71	204	11.4	628	7.41	56.8	16.9	54.2	< 3.41	184	571	1040	1410	1720
Kipper tin	MSC/05/0004	8.86	< 0.733	2.92	1.04	217	14.8	651	3.58	69.1	18.8	38.3	8.10	160	518	793	1220	1500
Kipper tin	MSC/05/0005	10.4	< 0.761	2.99	0.92	187	8.57	569	7.66	60.0	21.3	37.0	5.64	173	436	797	1100	1590
salmon tin	MSC/04/1119	1.52	< 0.442	0.31	< 0.442	17	3.07	64	1.65	4.32	1.78	3.29	< 0.883	46.5	87.4	116	84.2	138
salmon tin	MSC/04/1120	1.63	< 0.476	0.37	< 0.476	20	1.95	75	1.66	4.34	1.50	3.56	< 0.951	48.4	89.0	135	111	153
salmon tin	MSC/04/1121	5.53	< 0.649	1.49	< 0.649	76	5.28	271	3.03	15.4	3.92	11.4	1.34	142	275	434	304	463
salmon tin	MSC/04/1122	2.09	< 0.515	0.43	< 0.515	29	3.83	96	1.60	6.59	2.47	4.67	< 1.03	66.4	117	133	139	207
salmon tin	MSC/04/1123	9.78	0.68	2.66	0.59	138	18.4	508	13.40	29.2	10.0	19.90	2.17	241	438	706	458	822
tuna tin	MSC/04/1124	< 0.884	< 0.442	< 0.221	< 0.442	< 4.42	< 0.884	< 8.84	< 0.884	< 0.884	< 0.884	< 0.884	< 0.884	< 8.84	< 8.84	< 9.44	< 11.5	< 21.8
tuna tin	MSC/04/1125	< 1.28	< 0.639	< 0.320	< 0.639	< 6.39	< 1.28	< 12.8	< 1.28	< 1.28	< 1.28	< 1.28	< 1.28	< 12.8	< 12.8	< 12.8	< 12.8	15.0
tuna tin	MSC/04/1126	< 1.22	< 0.612	< 0.306	< 0.612	< 6.12	< 1.22	14	< 1.22	2.05	< 1.22	1.32	< 1.22	< 12.2	< 12.2	12.90	17.70	35.1
tuna tin	MSC/04/1127	< 0.886	< 0.443	< 0.222	< 0.443	< 4.43	< 0.886	< 8.86	< 0.886	< 0.971	< 0.944	< 0.960	< 2.23	< 8.86	< 8.86	< 8.86	< 8.86	< 8.86
tuna tin	MSC/04/1128	< 0.889	< 0.445	< 0.222	< 0.445	< 4.45	< 0.889	< 8.89	< 0.939	< 1.07	< 0.988	< 1.03	< 0.953	11.1	12.2	< 8.89	< 8.89	11.40
salmon farmed	MSC/04/1129	27.2	1.28	9.17	1.47	566	55.9	1650	25.8	197	50.4	116	14.8	374	1060	2120	2610	5080
salmon farmed	MSC/04/1130	27.7	1.06	7.28	< 0.958	319	21.1	1120	13.8	111	26.8	83.6	11.3	152	532	1150	1640	3110
salmon farmed	MSC/04/1131	33.5	1.47	10.40	1.77	641	55.6	1990	30.3	188	63.9	140	24.0	353	947	2270	3210	6460
salmon farmed	MSC/04/1132	40.7	1.92	14.20	2.19	787	39.5	2600	36.1	268	72.8	162	26.5	362	1230	2870	4250	5890
salmon farmed	MSC/04/1133	46.4	1.86	14.40	2.47	947	66.3	2690	47.2	244	83.7	183	35.6	585	1320	3250	4660	9460
salmon farmed	MSC/04/1134	37.9	1.60	11.90	2.06	614	43.4	2130	39.0	214	62.4	156	25.7	388	906	2290	3730	7140
salmon farmed	MSC/04/1135	32.0	1.11	11.00	1.63	526	39.3	1930	30.0	172	47.2	136	15.2	378	968	2550	3310	5380
salmon farmed	MSC/04/1136	38.5	1.70	13.10	2.04	750	89.7	2530	37.3	254	65.2	178	25.1	435	1300	2610	3970	6620
salmon farmed	MSC/04/1137	35.0	1.22	12.00	2.01	735	69.8	2340	29.9	207	52.1	172	22.2	378	1110	2480	3540	6420
salmon farmed	MSC/04/1138	29.5	< 1.04	10.40	1.83	687	40.0	2140	23.0	179	45.6	141	12.5	454	1190	2850	2850	5760
salmon farmed	MSC/04/1139	32.0	1.23	9.94	1.69	560	55.9	1850	25.3	201	52.2	132	16.7	455	1510	2130	3070	5040
salmon farmed	NA	33.3	1.32	12.10	1.95	728	48.7	2270	31.5	222	48.1	147	15.8	362	1060	2840	3330	5660
salmon farmed	MSC/04/1141	25.2	< 0.870	8.95	1.56	594	67.6	1880	23.9	166	35.6	120	12.3	312	924	2330	2600	4910
salmon farmed	MSC/04/1142	42.4	1.77	14.10	2.37	928	56.0	2650	31.9	251	53.7	179	25.1	505	1450	3500	3790	8060
salmon farmed	MSC/04/1143	35.8	1.67	13.00	1.99	849	47.8	2500	34.1	239	54.8	148	16.9	335	1180	3060	3570	6910
salmon smoked	MSC/04/1145	15.4	0.64	6.08	1.21	340	26.8	1150	16.5	107	27.1	64.3	9.76	248	758	1180	1810	2620
salmon smoked	MSC/04/1146	17.3	0.59	5.59	0.96	307	29.7	965	15.0	109	30.9	85.4	9.85	202	647	1070	1440	1970
salmon smoked	MSC/04/1147	20.9	0.76	7.06	1.13	348	29.9	1010	14.0	94.6	28.4	83.6	13.7	231	566	1170	1800	3170
salmon smoked	MSC/04/1148	23.1	0.88	7.94	1.44	423	45.3	1490	23.2	121	43.2	107	18.5	278	682	1930	2680	5130
salmon smoked	MSC/04/1149	22.6	0.80	7.31	1.30	448	55.6	1350	22.1	145	39.5	115	24.0	249	692	1850	2530	4850
salmon smoked	MSC/04/1150	18.2	0.70	6.11	1.08	373	37.3	1115	15.4	113	34.5	82.1	12.5	215	610	1500	2030	3205
salmon smoked	MSC/04/1151	21.9	< 0.912	7.34	1.28	428	21.3	1480	16.7	146	40.9	98.7	15.1	327	765	1780	2470	3940
salmon smoked	MSC/04/1152	28.1	1.12	9.22	1.64	563	22.3	1700	13.3	164	47.1	132	18.7	258	892	1740	2690	4490
salmon smoked	MSC/04/1153	22.1	0.81	7.05	1.20	440	19.9	1480	10.5	127	36.1	105	13.1	202	692	2030	2180	3210
salmon smoked	MSC/04/1154	18.9	0.68	6.25	1.08	412	39.2	1250	15.5	134	33.5	81.9	13.0	183	514	1480	2080	3160
salmon smoked	MSC/04/1155	22.8	0.79	7.49	1.34	437	42.4	1420	20.1	169	36.4	94.7	13.0	291	1040	2030	2370	4310

Annex 6: Concentrations of PBDEs (ng g^{-1} wet weight) in wild species.

Species	Reference	BDE #17	BDE #28	BDE #47	BDE #49	BDE #66	BDE #71	BDE #77	BDE #85	BDE #99	BDE #100	BDE #119	BDE #126	BDE #138	BDE #153	BDE #154	BDE #183
salmon wild	NA	0.01	0.02	0.41	0.05	0.02	0.01	0.01	0.02	0.14	0.07	0.02	0.02	0.03	0.03	0.07	0.05
salmon wild	NA	0.01	0.02	0.4	0.05	0.02	0.01	0.01	0.02	0.12	0.06	0.02	0.02	0.03	0.03	0.09	0.05
salmon wild	MSC/04/1040	0.01	0.01	0.29	0.03	0.02	0.01	0.01	0.02	0.07	0.05	0.02	0.02	0.03	0.03	0.07	0.05
salmon wild	MSC/04/1041	0.01	0.02	0.29	0.04	0.02	0.01	0.01	0.02	0.05	0.05	0.02	0.02	0.03	0.03	0.08	0.05
salmon wild	MSC/04/1042	0.01	0.02	0.41	0.06	0.03	0.01	0.01	0.02	0.13	0.06	0.02	0.02	0.03	0.03	0.09	0.05
salmon wild	MSC/04/1043	0.01	0.01	0.26	0.03	0.02	0.01	0.01	0.02	0.12	0.05	0.02	0.02	0.03	0.03	0.04	0.05
salmon wild	MSC/04/1044	0.01	0.02	0.41	0.04	0.02	0.01	0.01	0.02	0.14	0.07	0.02	0.02	0.03	0.03	0.11	0.05
salmon wild	MSC/04/1045	0.01	0.02	0.31	0.04	0.02	0.01	0.01	0.02	0.1	0.05	0.02	0.02	0.03	0.03	0.06	0.05
salmon wild	MSC/04/1046	0.01	0.02	0.26	0.04	0.01	0.01	0.01	0.02	0.07	0.04	0.02	0.02	0.03	0.03	0.06	0.05
salmon wild	MSC/04/1047	0.01	0.02	0.36	0.04	0.02	0.01	0.01	0.02	0.11	0.07	0.02	0.02	0.03	0.03	0.07	0.05
albacore	MSC/04/1172	0.01	0.03	0.31	0.08	0.04	0.01	0.01	0.02	0.05	0.08	0.02	0.02	0.03	0.03	0.07	0.05
albacore	MSC/04/1173	0.01	0.02	0.35	0.09	0.05	0.01	0.01	0.02	0.05	0.11	0.02	0.02	0.03	0.03	0.1	0.05
albacore	MSC/04/1174	0.01	0.03	0.39	0.09	0.05	0.01	0.01	0.02	0.05	0.13	0.02	0.02	0.03	0.03	0.11	0.05
albacore	MSC/04/1175	0.01	0.01	0.19	0.05	0.02	0.01	0.01	0.02	0.03	0.04	0.02	0.02	0.03	0.03	0.03	0.05
albacore	MSC/04/1176	0.01	0.03	0.56	0.08	0.07	0.01	0.01	0.02	0.06	0.19	0.02	0.02	0.03	0.03	0.17	0.05
herring	MSC/04/1177	0.01	0.03	0.77	0.42	0.04	0.01	0.01	0.02	0.12	0.14	0.02	0.02	0.03	0.03	0.05	0.05
herring	MSC/04/1178	0.01	0.03	0.71	0.33	0.03	0.01	0.01	0.02	0.1	0.16	0.02	0.02	0.03	0.03	0.05	0.05
herring	MSC/04/1179	0.01	0.03	0.7	0.37	0.03	0.01	0.01	0.02	0.11	0.15	0.02	0.02	0.03	0.03	0.05	0.05
herring	MSC/04/1180	0.01	0.02	0.72	0.37	0.03	0.01	0.01	0.02	0.1	0.16	0.02	0.02	0.03	0.03	0.05	0.05
mackerel	MSC/04/1182	0.01	0.03	0.42	0.14	0.04	0.01	0.01	0.02	0.16	0.08	0.02	0.02	0.03	0.03	0.04	0.05
mackerel	MSC/04/1183	0.01	0.03	0.53	0.18	0.04	0.01	0.01	0.02	0.19	0.11	0.02	0.02	0.03	0.03	0.05	0.05
mackerel	MSC/04/1184	0.01	0.02	0.34	0.12	0.03	0.01	0.01	0.02	0.12	0.07	0.02	0.02	0.03	0.03	0.03	0.05
mackerel	MSC/04/1185	0.01	0.04	0.7	0.23	0.05	0.01	0.01	0.02	0.24	0.16	0.02	0.02	0.03	0.03	0.07	0.05
mackerel	MSC/04/1186	0.01	0.04	0.7	0.22	0.07	0.01	0.01	0.02	0.26	0.16	0.02	0.02	0.03	0.03	0.05	0.05
Oysters	ENV/04/0050	0.1	0.06	1.56	0.33	0.07	0.02	0.01	0.04	1.04	0.65	0.02	0.02	0.03	0.05	0.16	0.05
Oysters	ENV/04/0053	0.03	0.01	0.58	0.11	0.03	0.01	0.01	0.02	0.39	0.23	0.02	0.02	0.03	0.03	0.06	0.05
Oysters	ENV/04/0056	0.04	0.02	0.4	0.17	0.02	0.01	0.01	0.02	0.29	0.14	0.02	0.02	0.03	0.03	0.18	0.05
Oysters	ENV/04/0057	0.03	0.01	0.27	0.04	0.01	0.01	0.01	0.02	0.13	0.08	0.02	0.02	0.03	0.03	0.03	0.05
Oysters	ENV/04/0062	0.03	0.01	0.18	0.07	0.01	0.01	0.01	0.02	0.11	0.06	0.02	0.02	0.03	0.03	0.03	0.05
eel	MSC/05/1119	<0.01	0.06	5.15	NA	0.10	NA	NA	n.d	0.16	1.26	NA	NA	n.d.	0.07	0.20	n.d.
eel	MSC/05/1120	<0.001	0.02	1.67	NA	0.05	NA	NA	n.d	0.02	0.33	NA	NA	n.d.	0.06	0.13	0.004
eel	MSC/05/1121	n.d	0.01	0.77	NA	0.01	NA	NA	n.d	0.02	0.20	NA	NA	n.d.	0.03	0.09	0.002
eel	MSC/05/1122	<0.004	0.04	1.83	NA	0.06	NA	NA	n.d	0.10	0.40	NA	NA	n.d.	0.12	0.20	0.02
eel	MSC/05/1140	n.d	0.01	0.52	NA	0.02	NA	NA	n.d	0.02	0.16	NA	NA	n.d.	0.05	0.10	0.01
Mussels	ENV/2004/055-003	0.04	0.02	0.61	0.24	0.03	n.d.	n.d.	0.02	0.42	0.20	n.d	n.d	0.01	0.03	0.26	0.01
Mussels	ENV/2004/069-003	0.17	0.09	3.64	0.65	0.14	n.d.	n.d.	0.13	2.12	1.17	n.d	n.d	0.01	0.08	0.18	n.d.
Mussels	ENV/2004/071-003	0.10	0.09	3.04	0.39	0.09	n.d.	n.d.	0.13	2.32	0.80	n.d	n.d	0.01	0.14	0.12	0.06
Mussels	ENV/2004/078-003	0.08	0.06	1.23	0.38	0.05	n.d.	n.d.	0.04	0.72	0.34	n.d	n.d	n.d.	0.03	0.05	n.d.
Killer whale	MSC/2005/1086	NA	0.03	NA	NA	0.09	NA	NA	< 0.02	0.12	0.13	NA	NA	0.11	0.03	0.12	< 0.02
Killer whale	MSC/2005/1087	NA	< 0.005	NA	NA	0.01	NA	NA	< 0.02	0.14	0.05	NA	NA	< 0.02	< 0.005	< 0.005	< 0.02
Killer whale	MSC/2005/1088	NA	0.02	NA	NA	0.02	NA	NA	< 0.02	0.14	0.05	NA	NA	0.03	0.03	0.03	< 0.05
Killer whale	MSC/2005/1089	NA	< 0.02	NA	NA	< 0.02	NA	NA	< 0.02	3.7	2	NA	NA	< 0.02	0.47	0.52	< 0.05
Killer whale	MSC/2005/1090	NA	< 0.005	NA	NA	< 0.005	NA	NA	0.006	0.64	0.47	NA	NA	0.24	0.064	0.12	< 0.005

n.d. = not detected

Annex 7: Concentrations of PBDEs (ng g^{-1} wet weight) in farmed and retail samples.

Species	Reference	BDE #17	BDE #28	BDE #47	BDE #49	BDE #66	BDE #71	BDE #77	BDE #85	BDE #99	BDE #100	BDE #119	BDE #126	BDE #138	BDE #153	BDE #154	BDE #183
salmon tin	MSC/04/1119	0.01	0.01	0.03	0.01	0.01	0.01	0.01	0.02	0.02	0.02	0.02	0.02	0.03	0.03	0.03	0.05
salmon tin	MSC/04/1120	0.01	0.01	0.03	0.01	0.01	0.01	0.01	0.02	0.02	0.02	0.02	0.02	0.03	0.03	0.03	0.05
salmon tin	MSC/04/1121	0.01	0.01	0.03	0.01	0.01	0.01	0.01	0.02	0.02	0.02	0.02	0.02	0.03	0.03	0.03	0.05
salmon tin	MSC/04/1122	0.01	0.01	0.04	0.01	0.01	0.01	0.01	0.02	0.02	0.02	0.02	0.02	0.03	0.03	0.03	0.05
salmon tin	MSC/04/1123	0.01	0.01	0.05	0.01	0.01	0.01	0.01	0.02	0.02	0.02	0.02	0.02	0.03	0.03	0.03	0.05
tuna tin	MSC/04/1124	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.02	0.02	0.02	0.02	0.03	0.03	0.03	0.05
tuna tin	MSC/04/1125	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.02	0.02	0.02	0.02	0.03	0.03	0.03	0.05
tuna tin	MSC/04/1126	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.02	0.02	0.02	0.02	0.03	0.03	0.03	0.05
tuna tin	MSC/04/1127	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.02	0.02	0.02	0.02	0.03	0.03	0.03	0.05
tuna tin	MSC/04/1128	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.02	0.02	0.02	0.02	0.03	0.03	0.03	0.05
Sardines tin	MSC/05/0001	0.01	0.01	0.04	0.01	0.01	0.01	0.01	0.02	0.02	0.02	0.02	0.02	0.03	0.03	0.03	0.05
Mackerel tin	MSC/05/0002	0.01	0.04	0.45	0.15	0.05	0.01	0.01	0.02	0.21	0.11	0.02	0.02	0.03	0.03	0.05	0.05
Mackerel tin	MSC/05/0003	0.01	0.05	0.67	0.23	0.08	0.01	0.01	0.02	0.35	0.19	0.02	0.02	0.03	0.04	0.08	0.05
Kipper tin	MSC/05/0004	0.01	0.03	0.65	0.32	0.03	0.01	0.01	0.02	0.09	0.16	0.02	0.02	0.03	0.03	0.05	0.05
Kipper tin	MSC/05/0005	0.01	0.03	0.6	0.3	0.03	0.01	0.01	0.02	0.13	0.13	0.02	0.02	0.03	0.03	0.04	0.05
salmon farmed	MSC/04/1129	0.01	0.06	1.59	0.39	0.12	0.01	0.01	0.02	0.42	0.32	0.02	0.02	0.03	0.06	0.15	0.05
salmon farmed	MSC/04/1130	0.01	0.04	1.78	0.22	0.1	0.01	0.01	0.02	1.99	0.43	0.02	0.02	0.03	0.15	0.17	0.05
salmon farmed	MSC/04/1131	0.01	0.08	1.88	0.56	0.13	0.01	0.01	0.02	0.48	0.39	0.02	0.02	0.03	0.07	0.16	0.05
salmon farmed	MSC/04/1132	0.01	0.09	2.13	0.51	0.16	0.01	0.01	0.02	0.59	0.4	0.02	0.02	0.03	0.08	0.18	0.05
salmon farmed	MSC/04/1133	0.01	0.1	2.26	0.73	0.17	0.01	0.01	0.02	0.57	0.44	0.03	0.02	0.03	0.09	0.21	0.05
salmon farmed	MSC/04/1134	0.01	0.08	1.86	0.59	0.12	0.01	0.01	0.02	0.49	0.37	0.02	0.02	0.03	0.07	0.16	0.05
salmon farmed	MSC/04/1135	0.01	0.07	1.41	0.33	0.07	0.01	0.01	0.02	0.35	0.25	0.02	0.02	0.03	0.05	0.11	0.05
salmon farmed	MSC/04/1136	0.01	0.09	1.92	0.51	0.13	0.01	0.01	0.02	0.61	0.41	0.03	0.02	0.03	0.1	0.21	0.05
salmon farmed	MSC/04/1137	0.01	0.08	1.57	0.44	0.08	0.01	0.01	0.02	0.4	0.29	0.02	0.02	0.03	0.05	0.12	0.05
salmon farmed	MSC/04/1138	0.01	0.07	1.46	0.42	0.07	0.01	0.01	0.02	0.37	0.27	0.02	0.02	0.03	0.05	0.12	0.05
salmon farmed	MSC/04/1139	0.01	0.07	1.48	0.45	0.08	0.01	0.01	0.02	0.4	0.29	0.02	0.02	0.03	0.05	0.13	0.05
salmon farmed	NA	0.01	0.08	1.55	0.37	0.09	0.01	0.01	0.02	0.45	0.31	0.02	0.02	0.03	0.06	0.13	0.05
salmon farmed	MSC/04/1141	0.01	0.1	2.15	0.6	0.14	0.01	0.01	0.02	0.62	0.43	0.03	0.02	0.03	0.09	0.19	0.05
salmon farmed	MSC/04/1142	0.01	0.05	1.2	0.3	0.05	0.01	0.01	0.02	0.3	0.22	0.02	0.02	0.03	0.04	0.09	0.05
salmon farmed	MSC/04/1143	0.02	0.09	1.92	0.45	0.13	0.01	0.01	0.02	0.59	0.39	0.02	0.02	0.03	0.08	0.2	0.05
salmon smoked	MSC/04/1145	0.01	0.04	0.8	0.18	0.05	0.01	0.01	0.02	0.2	0.14	0.02	0.02	0.03	0.03	0.08	0.05
salmon smoked	MSC/04/1146	0.01	0.04	0.81	0.25	0.05	0.01	0.01	0.02	0.23	0.15	0.02	0.02	0.03	0.03	0.07	0.05
salmon smoked	MSC/04/1147	0.01	0.04	1.35	0.23	0.09	0.01	0.01	0.02	1.12	0.32	0.02	0.02	0.03	0.11	0.15	0.05
salmon smoked	MSC/04/1148	0.01	0.06	1.26	0.37	0.07	0.01	0.01	0.02	0.37	0.25	0.02	0.02	0.03	0.05	0.12	0.05
salmon smoked	MSC/04/1149	0.01	0.05	1.13	0.33	0.06	0.01	0.01	0.02	0.33	0.23	0.02	0.02	0.03	0.04	0.1	0.05
salmon smoked	MSC/04/1150	0.01	0.04	0.9	0.26	0.05	0.01	0.01	0.02	0.26	0.18	0.02	0.02	0.03	0.05	0.09	0.05
salmon smoked	MSC/04/1151	0.01	0.05	1	0.3	0.05	0.01	0.01	0.02	0.29	0.2	0.02	0.02	0.03	0.04	0.09	0.05
salmon smoked	MSC/04/1152	0.01	0.07	1.31	0.41	0.09	0.01	0.01	0.02	0.36	0.27	0.02	0.02	0.03	0.06	0.15	0.05
salmon smoked	MSC/04/1153	0.01	0.06	1.18	0.37	0.07	0.01	0.01	0.02	0.35	0.23	0.02	0.02	0.03	0.05	0.1	0.05
salmon smoked	MSC/04/1154	0.01	0.04	0.89	0.27	0.05	0.01	0.01	0.02	0.26	0.17	0.02	0.02	0.03	0.03	0.08	0.05
salmon smoked	MSC/04/1155	0.01	0.06	1.12	0.33	0.06	0.01	0.01	0.02	0.34	0.22	0.02	0.02	0.03	0.05	0.1	0.05

n.d. = not detected

Annex 8: List of associated publications and reports by this author.

AI de Geus H-J, Besselink H, Brouwer A, Klungsøyr J, **McHugh B,** Nixon E, Rimkus G, Wester P, deBoer J. Environment Occurrence, Analysis, Toxicology of Toxaphene Compounds. Environmental Health Perspectives, Vol 107, Supplement 1. (1999).

AII Besselink, H, Nixon E, **McHugh B,** Klungsøyr J, and Brouwer A. *In vitro* and *in vivo* tumour promoting potency of technical toxaphene, uv-irradiated toxaphene, and biotransformed toxaphene. Organohalogen compounds 47, 113-116 (2000).

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Annex 9: Glossary

AhR	Aryl Hydrocarbon receptor
ANOVA	Analysis of Variance
BAF	Bioaccumulation Factor
BAF	Bioaccumulation factor
BCF	Bioconcentration factor
BF	Biomagnification factor
BFR	Brominated Flame Retardant
BIM	Bord Iascaigh Mhara (Irish Sea Fisheries Board)
Bioaccumulation	The accumulation of a substance within the tissues of an organism. This includes 'bioconcentration' and uptake via the food chain
Bioconcentration	The result of the direct uptake by an organism of a chemical from the water phase, this generally being expressed in terms bioconcentration factors (BCFs).
Bioconcentration	Occurs as a result of the direct uptake by an organism of a chemical from the water phase, this generally being expressed in terms bioconcentration factors (BCFs).
Biomagnification	The process whereby concentrations of certain substances increase with each step in the food chain.
Biomagnification	The process whereby concentrations of certain substances increase with each step in the food chain.

Biotransformation	The rate at which contaminants are degraded/structurally altered, metabolised and/or eliminated in an organism often as a result of POP-responsive induction of appropriate enzymes.
BPA	Bis-phenol A
CE	Capillary Electrophoresis
CEFAS	Centre for Environment, Fisheries and Aquaculture Science, CEFAS Burnham Laboratory, Burnham on Crouch, UK.
CHB	Chlorobornane
CHB	Chlorinated Bornane
CLE	Cod Liver Extract
CSP	Chiral Stationary Phase
CTT	Compounds of Technical Toxaphene
CYP1A/CYP2B1	Cytochrome P450 activity on dioxin like/non dioxin like compounds
$\delta^{15}N/\delta^{13}C$	Stable Isotopes ratios of Nitrogen and Carbon respectively
DBDE	Deca Bromo Diphenyl Ether
DDT	Dichloro-diphenyl-trichloroethane
DL-PCB	Dioxin Like Polychlorinated Biphenyls
EDCs	Endocrine disrupting chemical
EF	Enantiomeric fractions
eHPLC	Enantoselective High Performance Liquid Chromatography.
EN1/EN2	First and second eluting Enantiomers respectively

ERGO	ERGO Forschungsgesellschaft mbH, Hamburg, Germany.
ERs	Enantiomeric Ratios
EU	European Union
FAO	Food and Agriclture Organisation
FID	Flame Ionisation Detector
FSAI	Food Safety Authority of Ireland
FWMF	Food Web Magnification Factor
GC	Gas chromatography
GC-CIMS	Gas Chromatography Chemical Ionisation Mass Spectrometry
GC-ECD	Gas Chromatography Electron Capture Detector
GC-EIMS	Gas Chromatography Electron Impact Mass Spectrometry
γHCH	Lindane
GPC	Gel Permeation Chromatography
HBCD	Hexa Bromo Cyclododecane
HCB	Hexachlorobenzene
HCH	Hexachlorocyclohexane
HCH	Hexachlorocyclohexanes
HEPX	Heptachlor-*epoxide*
HRGC/HRMS	High Resolution Gas Chromatography/High Resolution Mass Spectrometry
IAWS	Irish Agricultural Wholesale Supplies

IC	Intercellular Communication
IFE	Institute for Energy Technology, Kjeller, Norway
I-TEF	International Toxic Equivalency Factor
LC	Liquid Chromatography
LOD	Liit of Detection
$LogK_{ow}$	Octanol water partition coefficient
LOQ	Limit of Quantification
MATT	Monitoring Analysis and Toxicity of Toxaphene
MDGC	Multi-Dimensional Gas Chromatography
MI	Marine Institute, Ireland
MRL	Maximum Residue Limit
nd	Not detected
NIVA	Norwegian Institute for Air Research
NOAEL	No Observable Adverse Effect Level
OBDE	Octa Bromo Diphenyl Ether
OCP	Organochlorine Pesticides
OCP	Organochlorine Pesticide
OECD	Organization for Economic Cooperation and Development
PBB	Poly Bromo Biphenyl
PBDEs	Poly Brominated Diphenyl Ethers
PCB	Poly Chlorinated Biphenyl

PCC	Polychlorinated Camphene
PCDD	polychlorinated dibenzo-*p*-dioxin
PCDF	polychlorinated dibenzo-*p*-furan
PCP	Pentachlorophenol
PEDC	Pro Endocrine Disrupting Chemical/Compound
pKa	Dissociation constant
POM	Particulate Organic Matter
POM	Particulate Organic Matter
POP	Persistent Organic Pollutant
ppb	parts per billion (equal to ng/g or µg/kg)
QSAR	Quantitative structure activity relationships
QUASIMEME	Quality Assurance of Information for Marine Environmental Monitoring in Europe
RSD	Relative Standard Deviation
SFF	Spcies/Group Magnification Factor
ΣICES7	The sum of the seven PCB congeners PCBs 28, 52, 101, 118, 138, 153 and 180. Recognised by the International Council for the Exploration of the Sea (ICES) as being among the most prevalent congeners found in the environment.
ΣIndicator CHBs	Sum Chlorobornanes #26,#50 and #62
TBBPA	Tetrabromobis-phenol A
TCDD	Tetrachlorobiphenyl

TDI	Tolerable Daily Intake
TEF	Toxic Equivalency Factor
TEQ	Toxic Equivalency approach
TT	Technical toxaphene
USEPA	United States Environmental Protection Agency
uvT	UV irradiated toxaphene
WHO-ECEH	European Centre for Environment and Health of the World Health Organisation.
WHO-PCB	World Health Organisation Polychlorinated Biphenyls.