THE OCCURRENCE AND RISK ASSESSMENT OF THE PESTICIDE TOXAPHENE IN FISH FROM IRISH WATERS

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ABSTRACT

The European Union project "Investigation into the monitoring, analysis and toxicity of toxaphene" (MATT), involving participants from The Netherlands, Ireland, Norway and Germany, began in 1997. Analytical methodology, concentration information and statistical interpretation of results for three indicator congeners, CHB's 26, 50 and 62, are presented. Data from 55 samples, covering 18 different fish species, from Irish waters are documented. Concentrations were lowest in shellfish and in fish species having low lipid content and were highest in medium/high lipid species. Males from a number of fish species were shown to contain significantly higher concentrations than observed in female fish. Overall no samples were shown to exceed existing German MRL or Canadian TDI recommendations.

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INTRODUCTION

Since first produced in 1945 toxaphene has been utilised mainly as an insecticide in cotton farming, but has also been used as a piscicide to control fish populations and as a miticide in farming (Saleh *et al., 1991*). Toxaphene has primarily been administered in the United States, a number of African nations and the former Soviet states, and had an estimated global usage between 1950 and 1993 of 1,330 x 10^6 kg (Voldner *et al.,* 1993*)*. Limited application has however been reported in Western Europe and its use has never been reported in Arctic regions. However, its detection in a variety of environmental compartments from remote Northern latitudes indicates the global distribution of the compound. (MacDonald *et al.,* 2003).

The lipophilic, persistent and relatively volatile nature of the compound has led to it becoming ubiquitous in the marine environment and therefore being listed as a persistent organic pollutant (POP). These properties in addition to its potential for bioaccumulation ultimately led to its use being banned by the United States Environmental Protection Agency (USEPA) in 1982 on the basis that it was a suspected human carcinogen and persistent hazardous compound with a high bioaccumulation potential (USEPA, 1982). Toxaphene has also been included as one of a dozen substances for global action in the UN Stockholm Convention 2001(UN Stockholm Convention, 2001).

In 1997 a European Union project entitled "Investigation into the Monitoring, Analysis and Toxicity of Toxaphene" (MATT) was initiated, involving participants from The Netherlands, Ireland, Norway and Germany. The main objectives of the project were to:

1) gather the most recent literature on the analysis, monitoring and toxicity of toxaphene,
2) improve and fine-tune the current analytical techniques used by the participants of the project for the measurement of toxaphene congeners,
3) gather baseline information on the levels of toxaphene residues in different fish and fishery products,
4) obtain information on the toxicity of toxaphene with emphasis on its carcinogenicity,
5) estimate the toxicological risks to the consumer of toxaphene residues in fish from European waters.

This paper reports concentration data from a number of species collected from Irish waters and describes the analytical methodology, quality assurance employed during analysis and the

possible risk to the consumer of Irish fishery products containing toxaphene residues. Other aspects of this project have been reported elsewhere (deGeus *et al.*, 1998a, McHugh *et al.*, 2000, deGeus *et al*., 1998b, de Boer *et al*., 1999, and Besselink *et al.*, 1999) and in the MATT project final report (MATT, 1998).

Properties of toxaphene

Toxaphene is a complex mixture of chlorobornanes (CHB's) with over 1000 congeners detected in technical toxaphene (Korytar *et al.,* 2003). Due to the high number of theoretically possible congeners and enantiomers, naming of individual CHB's can become problematic. A derivation of the nomenclature system proposed by Parlar, (1991) was employed during this study where three congeners (CHB's 26, 50 and 62) were numbered according to analytical column elution order preceded by the abbreviation CHB. Structures of some of the possible compound classes are presented in Figure 1.

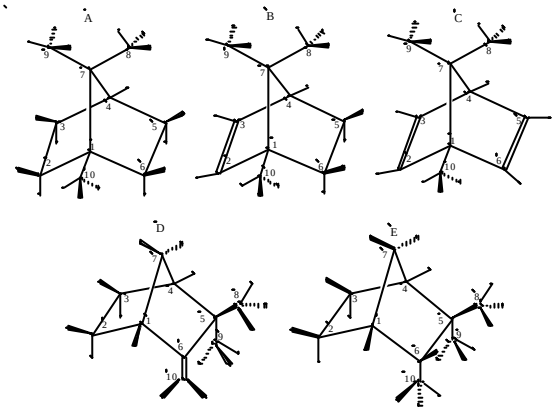

Figure 1: Carbon skeleton of bornane (A), bornene (B), bornadiene (C), camphene (D) and dihydrocamphene (E). (as proposed by Hainzl *et al.* 1995).

The physical properties of toxaphene suggest that its bioconcentration potential is high (Hooper *et al*., 1979, Wania *et al*., 1993) and it has also been suggested that toxaphene in air can condense on to particles present in the atmosphere and thus become subject to wet and dry deposition. This deposition has been reported to increase in an east/west and north/south direction along a decreasing temperature gradient (Bidleman *et al*., 1988). The ubiquitous nature of toxaphene distribution is reflected in the fact that it has been detected in diverse environmental compartments including marine sediment (Miskimmim *et al*., 1995), most commercial fish species (Alder *et al*., 1997, Glassmeyer *et al*., 1997, and Van der Valk *et al*., 1991) and mammalian milk (de Boer *et al*., 1993, Newsome *et al*., 1999) and in some cases is the most abundant organochlorine present.

Analysis of toxaphene

Chromatographic profiles in biota from European latitudes can differ from those observed in the American and Canadian continent, primarily due to the effects of weathering on the compound during atmospheric transport, in addition to the effects of metabolism and depuration within individual species. Two different approaches to the analytical determination of toxaphene have therefore been developed, that of single congener analysis and the analysis of total toxaphene, the latter being more indicative of the original contamination.

Most available data are presented in the form of total toxaphene rather than on an individual congener basis, therefore comparison of datasets can become difficult. Generally data from European countries are reported on an individual congener basis and current legislation is based on the sum of three indicator CHB's namely CHB's 26, 50 and 62. Studies have shown that these three CHB's can compose the majority of the total toxaphene detector response in biota (Alder *et al*., 1996) and are generally the congeners that persist after weathering and metabolism of toxaphene has taken place. It is therefore presumed that these "indicator congeners" will be dominant with respect to human toxaphene uptake.

Toxicology and associated legislation

Toxaphene has been shown to elicit mutagenic and carcinogenic properties in mammalian systems thereby posing a threat to humans (Hooper *et al*., 1979 and McGee *et al*., 1952). It is neurotoxic and hepatotoxic and has also been reported to be an endocrine disrupter and to have a high carcinogenic potency in rats and mice (Chaturvedi *et al*., 1991). As a persistent pollutant with high bioaccumulation potential, there is a potential risk associated with the consumption of contaminated food products. Infants may also be exposed to toxaphene from their mother's milk, as reported by deBoer (1993), however insufficient data are available to evaluate carcinogenicity risks in humans.

At present, Germany and Austria have set the maximum residue limit (MRL) for toxaphene in fish and fishery products at 0.1mg kg^{-1} wet weight, on the basis of the sum of the three indicator congeners (Anon. 1997). The German ordinance was the first national MRL for fish based on the measurement of individual congeners. Canadian authorities have taken a different approach in setting an acceptable daily intake level (ADI) of 0.2µg kg^{-1} body weight (Health Canada, 1992, Health Canada, 1995) based on technical toxaphene.

Toxaphene levels in fish from the north-east Atlantic have been shown to exceed present MRLs in some instances (Müller *et al*., 1998), thereby raising concerns about the safety for consumers of some fishery products. This paper reports the analytical methodology employed, the concentration levels of toxaphene in fish and fishery products from Irish waters and the subsequent statistical assessment of the resultant dataset. It also assesses the risk to the consumer of toxaphene in Irish fishery products.

MATERIALS AND METHODOLOGY

Sample collection and preservation

A total of 55 samples comprising 18 different marine species were collected and analysed. Samples of roughead grenadier and deepsea redfish from the Faroe Shetland Channel were also analysed and are included in the risk assessment as examples of deepwater species. Sampling locations are presented in Figure 2 and detailed information is presented in appendix 1. Where possible, samples were collected in accordance with the Oslo Paris Commission (OSPAR) Joint Assessment and Monitoring Programme Guidelines (JAMP, 1997) guidelines and were frozen at –30°C prior to analysis.

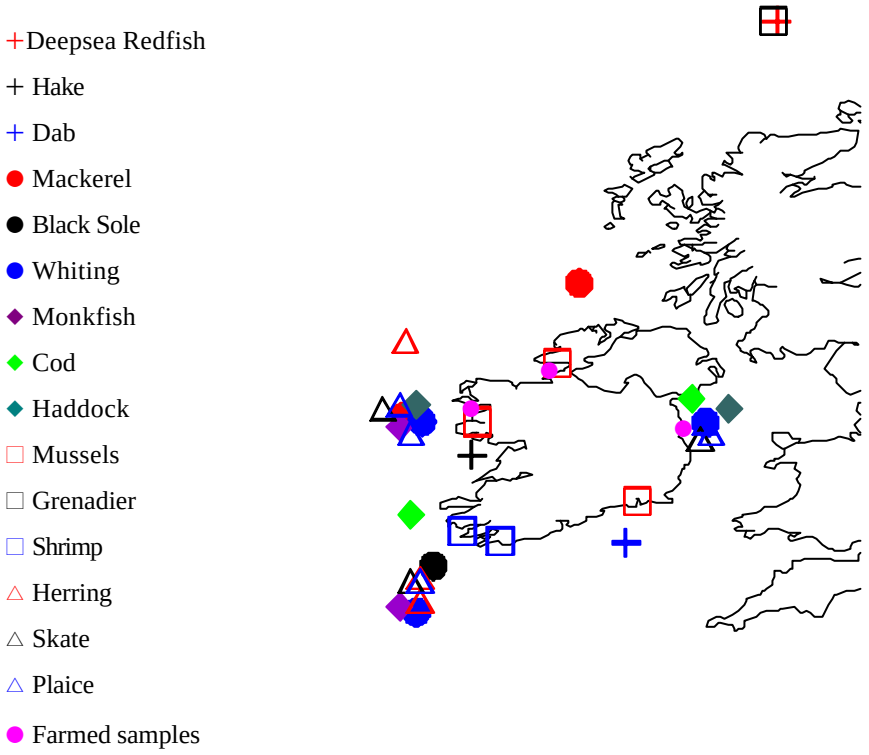

Figure 2: Sampling locations for baseline survey

The survey sampling strategy was designed in order to:

- investigate the geographical distribution of toxaphene in marine fish species,
- measure the variation in toxaphene concentration in tissues of individuals of the same species,
- assess variation in concentrations on a species wide basis.

Sampling procedures

In the case of the majority of wild finfish, samples were collected from single trawls to minimise natural variability due to differences in size/age profiles of species. Shrimp were obtained from homogeneously sub-sampled single hauls. Farmed salmon and trout were collected from aquaculture fish farms with sub-sampling being carried out in accordance with the EU Directive on residues in farmed fish (EC, 1996). Shellfish from cultivated stocks were collected from sub-tidal regions or as near to low water spring tide level as possible.

Sampling of fish took place outside of species' spawning period as during this time large variations in lipid concentrations can occur within species in all tissue types. Sample sex was recorded where possible as during spawning female fish may transfer large reserves of lipid and consequently lipid bound contaminants to their ova which may result in lower levels of these compounds being observed in females than males. The transfer of lipid to ova and the metabolic depletion of lipid reserves outside of feeding cycles can primarily account for the variation of lipid content in individual species. Selected species ranged from farmed fish, shellfish and deepwater species. Where practicable the number of individuals within a pooled sample were such that minimisation of natural variability factors such as length, weight, age and sex of individuals occurred.

Due to the bioaccumulation potential of toxaphene in the lipid of fish, age determination of a species becomes important as toxaphene concentration have been shown to increase as species' length and age increases (Alder *et al*., 1997). Age determination of fish is species dependent and was carried out by a number of means. Methods included, reading of otoliths and double frequency age-length keys, measurement of the carapace length for shrimp and measurement of total shell length for shellfish (as definitive ageing of shellfish is not possible the measurement of shell length is a good indicator of the age of the species). Individual processors confirmed the ages of farmed salmonid species.

Sample preparation

To minimise natural variation from sampling procedures, tissue removal was standardised for like species. In general fish ware sampled according to that described in figure 3 with muscle tissue removed above the lipid rich lateral line behind the dorsal fin for all fish species.

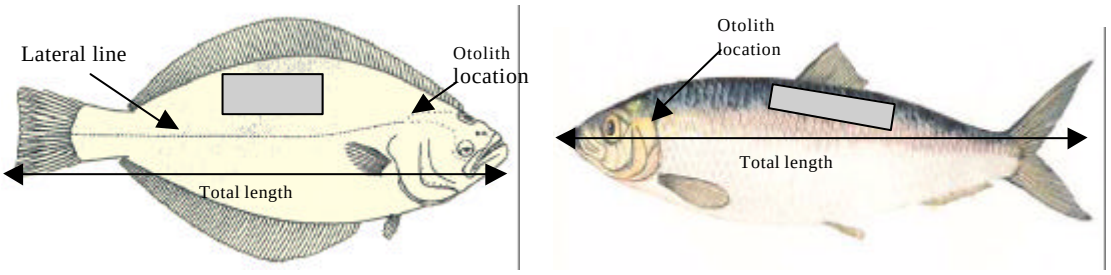

Figure 3: Muscle sample area on flatfish (indicated by shaded area)

All mussel samples were depurated overnight in approximately 5L of seawater from the sampling site. A sample size of 50 individual mussels between 4 to 6 cm in total length were selected. The soft body tissues were then pooled and homogenised before storage and analysis. Shrimp tail exoskeletons were removed and soft body tissue from 100 individuals pooled.

Analytical methodology

A combination of cold solvent extraction of lipids followed by gel permeation chromatography (GPC), silica gel group separation and sulphuric acid addition with GC-ECD analysis was selected for sample analysis. Details are reported in Annex 1.

Quality Assurance

Intercomparison exercises

Comparability of methodology between laboratories in the MATT project was assessed through a series of stepwise inter-comparison exercises and has recently been reported (deBoer et al., 2003). Additionally, a herring oil was distributed to all laboratories and analysed on a sample batch basis to assess the within and between laboratory variation. Data reported for this study were close to the mean of all participants in the exercise. Based on data from both the intercomparison and herring oil exercises, CHB's 26, 50 and 62 were selected for analyses.

QUASIMEME inter-comparison exercises

QUASIMEME is a laboratory performance study scheme set up to allow individual laboratories to assess quality management and quality measurement against laboratories and organisations on an international scale. Each individual laboratory employs their own analytical methodology and reports data on test samples directly to the coordinating office. During this study the Marine Institute participated in the determination of toxaphene congeners for a number of matrices; details of |Z| scores observed are presented in table1.

Table 1: |Z|-Scores for toxaphene congener determination in QUASIMEME exercises (QUASIMEME 1998 and QUASIMEME 1999).

Determinand	Assigned Value ($\mu g\ kg^{-1}$).	Assigned Error (%)	Result ($\mu g\ kg^{-1}$).	|Z|-Score
Round 10:QTX002SS (standard solution)				
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
Round 10:QTX003SS (standard solution)				
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
Round 14:QTX004SS (standard solution)				
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
Round 14:QTX005BT (hake liver extract)				
CHB 26	7.77	15.72	11.99	3.46
CHB 50	28.84	13.37	34.69	1.52
CHB 62	6.93	16.11	10.87	3.53
Round 14:QTX006BT (pilot whale blubber extract)				
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

Under the scheme an assigned value is provided for each determinand and a |Z|-score (bias) is calculated. A |Z| score between –2 and +2 is considered acceptable at a 95% confidence interval. Table 1 present data from QUASIMEME exercises completed during this study.

Overall the results for each of the QUASIMEME were acceptable for all analyses with the exception of two results from a liver sample. This matrix provided a number of analytical interferences resulting in an overestimation of the "assigned value". No samples of liver were however analysed during the course of this study. Differences in |Z|-scores for standard solutions and for biota give an indication of the difficulty in carrying out these analyses on actual biota.

RESULTS AND DISCUSSION

Toxaphene levels in Irish fish species

Summary data are presented in Table 2 on the distribution of toxaphene in a number of species collected from Irish waters. Data are presented in wet weight format. These wet weight data have been coupled with toxicity factors generated elsewhere (MATT, 1998) to assess the risk to the consumer and will be discussed later.

Table 2: Summary of baseline survey information.

Common name	Species	Length (mm)	std. dev. (mm)	No. of Individuals	Age (yrs)	Lipid (%)	CHB 26 (µg kg^{-1} ww)	CHB 50 (µg kg^{-1} ww)	CHB 62 (µg kg^{-1} ww)	Location
Black sole	*S solea*	260 - 420	40.9	20		0.66	<0.02	<0.02	<0.02	Celtic Sea
Cod	*G morhua*	390 - 640	78.8	10		0.56	0.10	0.10	0.11	Celtic Sea
Cod	*G morhua*	270 - 440	40.6	25	1	0.60	0.05	0.01	0.02	Irish Sea
Long rough dab	*H platessoides*	180-300	29.4	26	3	0.27	<0.02	<0.02	<0.02	Celtic Sea
Deepsea redfish	*S mentella*	350 - 490	44.8	12		2.26	0.12	0.26	0.08	Off Faroes[1]
Grenadier	*C rupestris*	190 - 335	49.3	10		0.49	0.03	0.05	0.09	Off Faroes[1]
Haddock	*M aeglefinus*	270 - 350	21.9	24	2 - 3	0.47	<0.02	<0.02	0.04	W. Ireland
Haddock	*M aeglefinus*	270 - 375	25.7	25		0.69	0.01	0.01	0.01	Irish Sea
Hake	*M merluccius*	190 - 260	17.6	24		1.18	0.05	<0.02	0.02	W. Ireland
Herring	*C harengus*	240 - 300	15.0	25	2 - 8	6.99	0.26	0.58	0.25	NW Ireland
Herring (n=2)	*C harengus*	230 - 290	13.8	25		2.11-12.8	0.27-0.33	0.46-0.47	0.10-0.24	Celtic Sea
Mackerel	*S scombrus*	250 - 360	28.2	25	0 - 4	1.93	0.04	0.06	0.01	NW Ireland
Monkfish	*L piscatorius*	230-410	66.7	10		0.52	<0.02	<0.02	<0.02	Celtic Sea
Monkfish	*L piscatorius*	300 - 490	60.3	10		0.43	0.21	0.24	0.30	West Ireland
Mussels	*M edulis*	40 - 67	5.3	50		1.03	0.06	0.01	<0.01	SW Ireland
Mussels	*M edulis*	41 - 78	8.1	50		1.05	0.02	0.02	0.06	West Ireland
Mussels (n=2)	*M edulis*	45-60	3.5	50		1.90-2.07	0.02-0.08	0.02-0.03	<0.01-0.09	West Ireland
Plaice	*P platessa*	280 - 430	44.0	20	1 - 5	0.71	0.21	0.52	0.17	Celtic Sea
Plaice (n=2)	*P platessa*	200 - 390	58.9	20	EST3	0.65-0.77	<0.02-0.01	0.01-0.02	0.03-0.05	West Ireland
Plaice (n=2)	*P platessa*	270 - 370	29.7	25	EST4	0.72-0.80	<0.02-0.07	<0.02-0.07	<0.02-0.03	Irish Sea
Salmon	*S salar*	630 - 730	28.3	10		5.97	1.67	2.42	1.96	Ireland
Shrimp	*C crangon*	15.7 - 20	0.9	50		1.19	0.03	0.07	0.10	Celtic Sea
Shrimp	*C crangon*	14 - 20	1.2	100		1.28	0.13	0.02	0.03	Celtic Sea
Skate	*Raja Sp.*	290 - 740	105	20		0.49	0.05	0.04	0.05	West Ireland
Skate	*Raja Sp.*	250 - 520	68.6	20		0.66	0.03	0.04	0.07	Celtic Sea
Skate	*Raja Sp.*	390 - 570	62.9	10	3 - 10	0.79	<0.02	0.02	0.03	Irish Sea
Trout (lake)	*O mykiss*	340-370	11.3	8		1.18	0.57	0.68	0.59	Ireland
Trout (sea)	*O mykiss*	665 - 839	62.5	10		5.86	0.62	1.18	1.26	Ireland
Whiting	*M merlangus*	280 - 440	48.3	22	1 - 4	0.59	<0.02	<0.02	<0.02	West Ireland
Whiting	*M merlangus*	230 - 350	34.5	22	0 - 3	0.67	0.02	0.03	0.07	Celtic Sea
Whiting	*M merlangus*	220 - 280	16.9	25	2 - 5	0.69	<0.02	<0.02	<0.02	Irish Sea

Notes: EST= Estimated age from age length frequency measurements.

(n=2) relates to concentration range determined in two separate samples from the same location.

1= Faroe Shetland Channel.

Overall levels were low for each of the CHB's under investigation. Levels were lowest in low lipid species and in shellfish. All species were well below the German MRL of 100 µg kg^{-1} wet weight.

Presentation in wet weight format does not allow direct comparison of concentration data from different species. Such comparisons are usually carried out on data that have undergone normalisation or transformation by one or a combination of methods, which can include:

- Logarithmic transformations to allow data be analysed by parametric and non-parametric statistics. Such transformations usually improve the homogeneity of variance between datasets (Boon *et al*., 1997 and Green *et al*., 2003)
- Normalisation to a lipid content co-factor, allowing establishment of relative trends in contaminant levels (Boon *et al*., 1997 and Green *et al*., 2003).
- Normalisation to a single reference congener, enabling the underlying contaminant pattern to be investigated (Boon *et al*., 1997).

Normalisation to a lipid content co-factor

As there is a large variation in lipid content between species it is necessary to normalise all data by converting wet weight concentrations to a lipid basis to allow comparison of all the results.

In order to further examine the dataset, 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 shrimp,
4) Mackerel and herring (medium/high lipid),
5) Farmed species.

In general concentrations of toxaphene do not differ greatly for the majority of species. However a number of samples including one sample each of farmed sea trout, herring, cod, farmed salmon, plaice and farmed trout range from 48 to 156 µg kg^{-1} lipid weight, show more elevated levels than the general data spread (see Figure 4). Concentrations determined plaice and cod samples deviate from expected levels based on the low lipid content of these species and on the levels determined for other low lipid samples during the study. Additionally a single sample

of herring shows a concentration level that is slightly higher than expected for its species and would require further investigation with a larger sample set.

Concentrations in farmed species were generally more elevated than those of all other species. Farmed fish undergo continuous feeding throughout their growth cycle, and their primary source of lipophilic contaminants, such as toxaphene, originates in their feed. These are usually composed of lipid-rich pellets where lipid may originate from fish species that may have prior exposure to environmental contaminants. Concentrations in shellfish and low lipid species were generally low (apart from individuals already discussed) and were close to the limit of quantification of the method. As phytoplankton filter-feeders, mussels are a good indicator species for the assessment of local water-borne contaminant burden, it can be determined that toxaphene levels in waters around Ireland are similar.

Figure 4 summarises concentrations measured for each grouping. Outlier values plotted with an "o" are defined as having a value greater than 1.5 times the distance between the 25th and 75th quartiles. Extreme outliers data values plotted with an "*" refer to those with values greater than 3 times this distance.

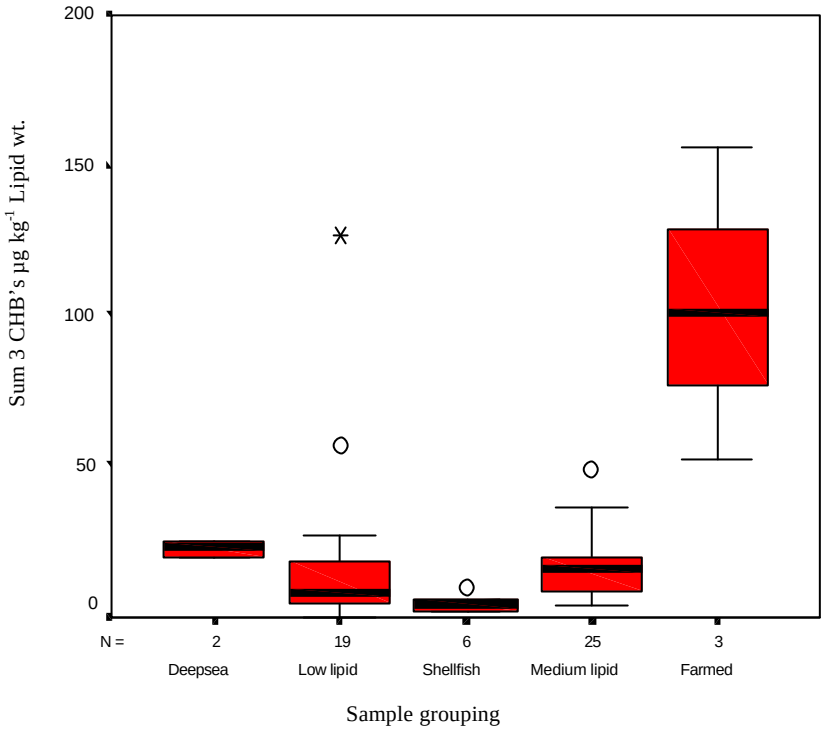

Figure 4: Boxplot of grouped samples for the sum 3 CHB's (µg kg^{-1} lipid weight).

Concentrations determined in herring from this study ranged 6.5-49 µg kg^{-1} lipid weight for the sum of (Σ) the 3 selected CHB's. Data are similar to those recorded by Alder (1997) in herring from Irish waters (7.9-11.4 µg kg^{-1} lipid wt for Σ 3 CHB's) and are lower than those from Baltic and North sea regions where concentrations ranged from 10-344 µg kg^{-1} lipid wt for Σ 3 CHB's. Concentrations reported in the MATT project (MATT, 1998) and (McHugh *et al.*, in Press) from a number of European waters are also similar to levels reported in this paper.

Toxaphene levels in mackerel in this study range 5.5-36 µg kg^{-1} lipid weight and are lower than reported by Alder (1997) in mackerel from west of the Shetland Islands (48 and 63 µg kg^{-1} lipid wt.). However levels reported from the southern North Sea (German Bay) are more comparable (15-19 µg kg^{-1} lipid wt.) Levels reported in the MATT project (1998) and McHugh *et al* (in press) from the southern North Sea of 23 and 43 µg kg^{-1} lipid wt. are also comparable to Irish data.

Concentrations reported for the Σ 3 CHB's in mussels (1.56-4.34 µg kg^{-1} lipid wt.) fall within the range of those measured in the MATT project (2-4 µg kg^{-1} lipid wt.) from North Sea and Baltic regions respectively. Levels in shrimp from the North Sea (MATT, 1998) are also similar to those from Irish waters (1.93-3.27 and 4.99-9.87 µg kg^{-1} lipid wt.) respectively.

Concentrations reported by Alder (1997) in a number of low lipid species range from not detected to 367 µg kg^{-1} lipid wt. in plaice from the North Sea and saithe from Icelandic waters respectively. Levels in Irish low lipid samples are generally low with the exception of the plaice sample which has previously been discussed. Levels in cod from Irish waters, reported as 13.9 and 56.9 µg kg^{-1} lipid wt., are within the range reported in the MATT project of 6-76 µg kg^{-1} lipid wt. from Baltic and Norwegian waters respectively. Concentrations in whiting from Irish waters range 4.97-9.67 µg kg^{-1} lipid wt (n=3) and are in agreement with values of 9.9 and 11 µg kg^{-1} lipid wt. reported from the Baltic and the North Sea respectively (MATT, 1998).

Levels in Irish farmed species reported in this study, of 56 and 156 µg kg^{-1} lipid wt. in trout and 101µg kg^{-1} lipid wt. in salmon, compare to concentrations of 72-102µg kg^{-1} lipid wt. from Norwegian aquaculture sites (MATT, 1998).

Alder (1997) reported that in general toxaphene levels are more elevated in fish with higher lipid content, which is consistent with data from this study.

Further statistical evaluation of data

Statistical evaluation by parametric means is generally regarded as being more powerful in the interpretation of nominal data than corresponding non-parametric tests. A prerequisite for the use of parametric tests is that the dataset shows a normal distribution, however environmental datasets rarely show these characteristics. The reported dataset from this Irish study was also found to have a non-normal distribution and required transformation for parametric statistical assessment. Logarithmic transformation is suitable for the assessment of organochlorine concentration data in biota as it brings about the stabilization of the variances within the data (Boon *et al*., 1997) and as such concentration data were $\log_{10}$ (Σ3CHB's)+1 transformed ensuring return of a positive number.

ANOVA and post-hoc testing

ANOVA (Analysis of variance) testing allowed the comparison of the means of the sample groupings and post-hoc least significant difference (LSD) testing further allowed differences between groupings to be quantified.

The largest difference was observed between farmed species and shellfish ($P<0.001$) followed by farmed species and low lipid fish ($P<0.001$) and thirdly farmed samples and medium lipid fish ($P<0.01$). Significant differences were also shown to exist between shellfish and deep-sea species ($P <0.05$), and between medium lipid species and shellfish ($P <0.01$). Deepsea species including redfish and grenadier from the Faroe Shetland channel were both included in data and risk assessment as they may have different exposure routes to organohalogen residues as a result of different habitats and life cycle compared to other species. In particular many deepsea species can be long lived possibly leading to an increase in bioaccumulation potential.

No significant difference was observed between deep-sea and farmed species. This was probably due to the combination of the small sample sizes for both groupings, the relatively high mean concentration of toxaphene in the deep-sea species and the high standard error between the pairings. No differences were observed between low lipid and medium lipid species, due primarily to similar means and variances between the groupings.

Investigation of the congener profile

The percentage contribution of each individual congener to the overall sum of the three congeners was calculated as a tool to examine possible species-dependent metabolism/excretion of individual congeners. CHB 50 was reported as the most abundant congener with roughly equal percentages of CHB's 26 and 62 present. Mean contributions of 28.5, 44.3 and 27.2% for CHB's 26, 50 and 62 respectively with an RSD of between 31.5 to 40.5% lie within that expected based on previous literature (deGeus *et al*., 1998b). Ratios determined are a result of the combination of the divergence expected due to differing rates of metabolism or excretion within individual species and the analytical uncertainty of the analytical technique itself. The use of much larger datasets would be required to enable further conclusions be drawn.

Investigation of natural variability within individuals of the same species

As previously discussed, sampling conditions were controlled as much as possible for the collection of all samples. However some natural variability within individuals in a pooled sample is expected, as a single haul will never contain individuals of exactly the same size, sex and age. To investigate these variations a total of 20 individual mackerel samples from a single haul were statistically evaluated to estimate the variability that may occur within a collected sample. Summary results are presented in Table 4.

Table 4: Summary statistics for various parameters in individual mackerel (n=20).

	Mean	**Median**	**Stdev**	**RSD%**	**Range**	**Interquartile range**
S 3CHB's	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

An RSD of 14.3% for the length of each individual indicates that most fish were of similar size. However RSDs of 51.1 and 63.2% for the Σ 3 CHB's and the percentage lipid respectively show that these variables are difficult to control even where care is taken to sample individuals of similar length. The observed spread is probably as low as can be expected where a sample is composed of mixed sex and variable sized individuals.

The effect of sample sex on concentration levels within a species

The individual mackerel were separated on a sex basis into 13 males and 7 females. It was observed that toxaphene levels in females were significantly lower ($P<0.05$) than in males. Females may have the capacity to excrete lipid-borne contaminant residues via their ova during

spawning periods thereby reducing their overall contaminant burden. These results are consistent with those of female cod (livers) and female herring from the MATT project (1998) and McHugh *et al*., (in press).

Other statistical analysis on mackerel individuals

Stronkhorst (1992) reported that toxaphene concentrations are dependent on the sample lipid content. However, no correlation was observed within either the male or female groupings. Alder (1997) previously reported that a correlation exists between sample length/age on the concentration levels in muscle tissue. However no relationship was observed within the individual mackerel in this study.

ASSESSMENT OF THE TOXICOLOGICAL RISKS TO THE IRISH CONSUMER OF TOXAPHENE RESIDUES IN FISHERY PRODUCTS FROM IRISH WATERS

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

Limited concentration data for herring, halibut and mackerel from the North Sea and North Atlantic have shown that levels sometimes exceed the German ordinance level of 0.1 mg kg^{-1} wet weight for fish and fish products (Anon, 1997). This could lead to serious implications for both the European fisheries industry and the consumer as human exposure to toxaphene arises mainly through consumption of contaminated fish.

Due to environmental transformation and internal metabolism within the fish, exposure is to a "weathered" mixture of technical toxaphene. Methodologies for a so-called realistic exposure procedure for toxaphene were developed in the MATT project (1998) whereby the weathered/metabolised toxaphene pattern found in fish was mimicked, therefore providing a more realistic image of the likely pattern of human exposure. Prior to this no toxicological studies based on weathered toxaphene have been reported.

Cod liver extracts from fish exposed to technical toxaphene via feed pellets showed a weathered toxaphene pattern and were used in an *in vivo* exposure study with rats and *in vitro* experiments to estimate a tolerable daily intake (TDI) for toxaphene relative to its tumour promotion potency. The cod liver exposure study was carried out in the Institute of Marine Research (IRM), Norway and toxaphene residues were isolated from oil collected from the cod livers in the Irish Marine Institute. Toxaphene residues were then used for a series of *in vitro* experiments conducted in the Wageningen Agricultural University in the Netherlands to determine TDIs for tumour promotion potency. Details of these studies are presented in the MATT report (1998) and deGeus (1998a).

An estimation of the daily intake of toxaphene for Irish consumers was derived from the levels of toxaphene found in the baseline samples in combination with fishery product consumption statistics within Ireland (BIM 1998). It was assumed for the purposes of this study that the only significant intake of toxaphene is through consumption of fishery products. This daily intake of

toxaphene was then compared with TDIs and MRLs set by Canada, US and German authorities and also to the TDI calculated in the MATT project, and allowed the potential toxicological risks to the consumer of toxaphene residues to be evaluated.

Information on TDIs and legislative limits

The MATT project (MATT, 1998) calculated a TDI for the "weathered" toxaphene residues of 4.8 mg/kg bw/week^{-1} (0.69 mg/kg bw/day^{-1}). Applying a safety factor of 100, the TDI in humans for tumour promotion potency 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 of total toxaphene per day was proposed.

USEPA reference data (USEPA 1997, 1999) suggest the maximum TDI of technical toxaphene for a person of 60 kg is 0.015 mg and 66 mg for chronic toxicity and the endpoint of carcinogenicity respectively. The TDI for carcinogenicity is much higher than the TDI estimated for tumour promotion potency in the MATT project of 0.41 mg. Several other tolerance levels and maximum residue levels in food for toxaphene have been proposed based on total toxaphene and are summarised in Table 5.

Table 5: Summary of maximum residue limits and tolerable daily intakes for toxaphene (based on a person of 60 kg).

Authority	Legislation	TDI (mg/d)	Toxicology basis	Matrix
Germany	MRL	0.1 mg kg ww	Σ 3 CHB's	Fish/fish products
	MRL	0.1 mg kg^{-1}	Total	All other animal food
Canada	TDI*	0.012 mg d^{-1}	Total	
US EPA	TDI** (Chronic toxicity)	0.015 mg d^{-1}	Total	
	TDI** (Carcinogenicity)	66 mg d^{-1}	Total	
MATT	TDI (tumour promotion potency)	0.41 mg d^{-1}	Total	

*TDI calculated from the proposed ADI . ** TDI calculated based on reference doses.

Estimated average daily intake of toxaphene

Toxaphene concentration data in 221 fishery products (excluding liver tissues) from the North Sea, Bight/Skagerrak, Irish Sea, Irish west-coast, Iceland Sea, Norwegian coast, and Barents Sea were collated (MATT, 1998). Total toxaphene was estimated in 55 samples and the three indicator congeners were determined in all samples. The ratio of the sum of total toxaphene to the three indicator congeners was calculated at 12.4, 41.6, and 24.0 for marine fish, eel and mussel respectively. 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.

Detailed fish consumption data (BIM, 1998) has shown that average annual fish consumption in Ireland amounts to 8.8 kg of fish and fish products/person (see Table 6). For the calculation of the intake of toxaphene from fishery products, data from the baseline survey reported in Table 2 were employed. Insufficient information was available to allow further breakdown of consumption statistics into constituent species classes.

For each individual sample the daily intake of toxaphene (A_{intake}) was calculated by first determining the concentration of each of the CHB's in the individual samples on a wet weight basis (C_{fish}). From these data the "total toxaphene" exposure was estimated based on application of the appropriate ratio determined above. This figure was then multiplied by the average daily consumption statistic ($D_{consumption}$) to obtain an estimate of the average daily intake of toxaphene from fishery products (A_{avg}).

$$(A_{intake}): A_{avg} = \Sigma A_{intake} / n$$

The average intake was estimated as the mean of the intake of all individual Irish samples collected (n= the total number of samples). Results are presented in summary format in Table 6.

Table 6: Estimated intake of toxaphene for the consumer of Irish fishery products.

	Average daily fish consumption (g/d) ($D_{consumption}$)	Estimated average daily intake of toxaphene from fishery products (µg) (A_{avg})
Ireland	24.1	0.30

To estimate the average daily intake of toxaphene 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 fishery products.

Consumption statistics used in this study (BIM, 1998) are similar to those from a North/South Ireland food consumption survey report (Irish Universities Nutrition Alliance, 2001) and assume that all members of the population include fish products as part of their diet; however in reality this is rarely the case. The latter report additionally determined a mean of 35g/person/day^{-1} and an upper consumption statistic of 86 g/person/day^{-1} at the 95th percentile amongst those who do include fish in their diet. It was deduced that whilst this grouping may be at higher risk than average consumers, the maximum daily intake is still within legislative values. In addition

certain preferences for the consumption of some fish species exist for individuals thereby their risk of exposure may differ from that of the average consumer. Children with a high fish diet may also be susceptible to higher risk than adults.

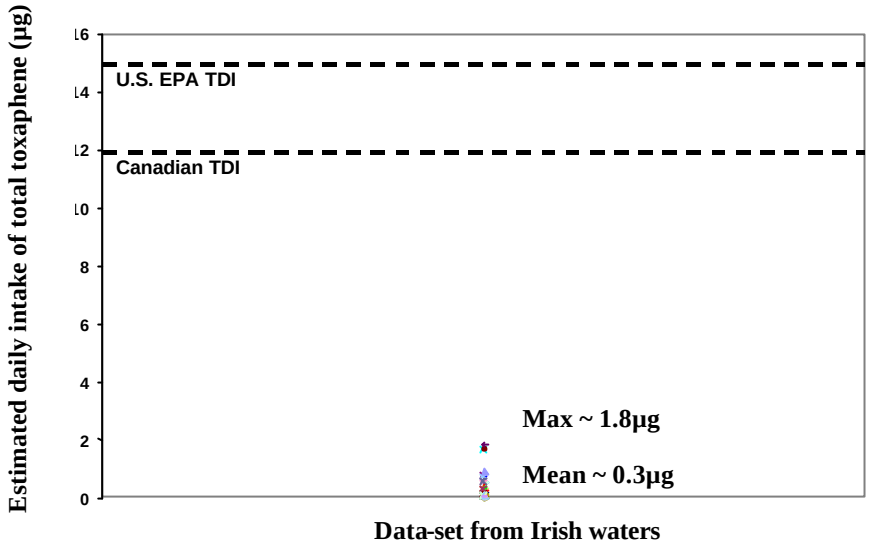

Figure 5: Estimated daily intake of total toxaphene (µg) for a person of 60 kg from all Irish samples. The Canadian and U.S. (chronic toxicity) TDIs (12 and 15 µg respectively) are shown for comparison.

A comparison of TDIs and the estimated average daily intake of toxaphene from all baseline samples are reported in Figure 5. No samples from Irish waters exceed the Canadian or USEPA TDI level for chronic toxicity or the TDI for tumour promotion (410 µg), set in the MATT project (MATT, 1998).

Comparison of toxaphene levels in Irish fish to the German MRL

All Irish baseline samples were well below the German MRL of 0.1 mg kg^{-1} wet weight for the sum of the three indicator congeners. Values of 0.03-6.05 µg kg^{-1} wet weight were observed for the Σ 3 CHB's (upperbound range). Mean and median concentrations were determined as being 0.58 and 0.14 µg kg^{-1} wet weight respectively. These values all fall well within the German legislation. 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 as is considered in the calculation of TDIs.

CONCLUSIONS

Toxaphene is detected in fish sampled from Irish waters, indicating the ubiquitous nature of the compound. Of the 55 samples tested, highest concentrations were observed in farmed fish and in deepsea species as would be expected. This reflects the bioaccumulation potential due to the high lipid content of these species and their diets, in addition to the longevity of deep-sea species. Levels were found to be lower in females compared to males, this may in part be attributable to their ability to remove lipophilic residues during spawning.

Comparison of lipid normalised values showed no sample to be elevated and additionally no differences were observed between species from the west coast, Celtic or Irish seas. Levels were all well within available MRL and TDI legislation.

Based on concentration and risk assessment data collected during this study and in comparison with legislation currently available no adverse effects are expected in the average consumer of Irish fishery products due to the presence of toxaphene residues in the fish.

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Annex 1: Summary of analytical methodology

Lipid Extraction.

Solvent extraction of lipid was carried out by ultraturrax of tissues in both acetone and acetone: hexane 3:1 mixtures. Samples were centrifuged and the solvent extracts combined. NaCl in 0.1M Orthophosphoric acid was added to aid the separation of the phases and aid removal of residual tissue to the aqueous phase. Further washing of extracts was carried out with hexane and water to remove residual lipid remaining in the aqueous phase and NaCl in the organic phases respectively.

Total lipid reported in appendix 1 was determined by the SMEDES (39) method.

Gel permeation chromatography clean-up.

Lipid extracts were separated on a size exclusion column (Polymer labs) with 100% DCM. Fractions corresponding to 175-210 ml were collected in the fraction collector and were evaporated by rotary evaporator to 1ml. Extracts were further group separated by silica gel chromatography.

Silica gel chromatographyclean-up.

Silica gel chromatography was used to further clean-up the GPC extract prior to GC separation.
5% deactivated silica columns were prepared and the GPC extract added. A fraction containing a total of 28ml n-hexane was collected for analysis of PCBs. A second fraction totalling 70ml n-hexane:diethyl-ether (3:1) was collected for examination for the majority of the indicator compounds. Extracts were evaporated to approximately 1ml prior to GC analysis.

Gas Chromatographic analysis.

Dual column chromatography with CPSIL 8 and 19 columns and electron capture detection (ECD) detection were employed. 4-5 dichloro-chlordene was used as an internal standard for quantification purposes.

Dry weight determination.

Determinations were carried out by air-drying homogenised sub-samples of tissue to a constant weight at 105^{o}C.