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Toxicological risks to humans of toxaphene residues in fish

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ABSTRACT

A revised risk assessment for toxaphene was developed, based on the assumption that fish consumers are only exposed to toxaphene residues that differ substantially from technical toxaphene due to environmental degradation and metabolism. In vitro studies confirmed that both technical toxaphene and degraded toxaphene inhibit gap junctional intercellular communication that correlates with the mechanistic potential to cause tumour promotion. In vivo rat studies established the NOAEL for degraded and technical toxaphene at the highest dose tested in the bioassay. Toxaphene residue intakes from European fishery products were estimated and compared to the provisional tolerable daily intakes (TDIs) from various regulatory agencies including Canada, the United States, Germany. The estimated intake was also compared to a new calculated provisional MATT pTDI. The MATT pTDI is based upon new toxicological information (*in vivo* rat studies) developed on a model for environmental toxaphene residues rather than technical toxaphene. A MATT pTDI (1.08 mg total toxaphene for a person of 60 kg) for tumour promotion potency was adopted for use in Europe and is hitherto referred to as the MATT pTDI. These new data result in a better estimate of safety and a higher TDI than previously used. Based on realistic fish consumption data and recent baseline concentration data of toxaphene in European fishery products the toxaphene intake for the consumers of Germany, Ireland, Norway and The Netherlands was estimated. For an average adult fish consumer the average daily intake of toxaphene was estimated to be 1.2 µg, and 0.4, 0.5, and 0.2 µg for the consumers of Norway, Germany, Ireland, and The Netherlands, respectively. The toxaphene intake of these average fish consumers was far below the MATT pTDI of 1.08 mg/60 kg body weight. In conclusion, based on the most relevant toxicological studies and the most realistic estimates of fish consumption and recent concentrations of toxaphene in European fishery products, adverse health effects are unlikely for the average European consumer of fishery products. In no case is the MATT pTDI exceeded.

Keywords: Toxicological risk, Toxaphene, Fish, Human, European fish consumption

INTRODUCTION

Given the concern for the worldwide environmental effect of toxaphene, surprisingly little is known about the toxicology of this group of compounds (de Geus et al 1999; Swackhamer et al 1993; Arnold et al 2001; Bryce et al 2011). It is essential that the quality of the aquatic environment and consumers of marine foodstuffs be protected. However, a large number of uncertainties exist in the data or data are even absent with regard to the analysis, baseline levels, carcinogenicity, toxicological risk, tolerance levels and fate of toxaphene in the environment (de Geus et al 1999). Consequently, a proper risk assessment of toxaphene for the consumer of marine foodstuffs could not be made earlier. In the European project MATT (Investigation into the Monitoring, Analysis and Toxicity of Toxaphene in Marine Foodstuffs) new information on the monitoring, analysis, toxicology and risks of toxaphene was obtained (McHugh et al 2004; Besselink et al 2008).

Tolerance levels are based on the toxicology of technical toxaphene mixture, but the number and pattern of congeners in environmental samples are substantially different, as a result of environmental and metabolic modification, from the technical toxaphene mixture. Human exposure is mainly through consumption of toxaphene-contaminated fish (Berti et al 1998). The composition of toxaphene mixtures changes from the original technical toxaphene mixtures through environmental transformation and internal metabolism in the fish. Human exposure, therefore, is to a weathered mixture of technical toxaphene. However, the toxic and carcinogenic properties of toxaphene residues in fish were unknown. No carcinogenicity studies at all on weathered toxaphene have been reported in the literature. The study of Buranatrevedh (2004) showed that toxaphene might have a carcinogenic risk for humans based on a four step risk assessment, however, also this risk assessment used data from toxicity studies using technical toxaphene. Besselink et al. (2008) developed new toxicology data using a more realistic exposure of test animals to degraded toxaphene. The

toxicology test mimics the weathered toxaphene pattern found in fish, and should provide a more realistic model of the human exposure situation. The procedure exposed fish (cod) to technical toxaphene mixture. Toxaphene residues were then extracted from the liver of the exposed fish, which showed the weathered toxaphene pattern. The extracted toxaphene residues were used in *in vitro* experiments to demonstrate the plausibility that technical toxaphene and degraded toxaphene inhibited gap junctional intercellular communication as a correlate to tumour promotion. They also ran a critical *in vivo* exposure study with rats to determine the tumour promotion potency of technical and weathered toxaphene residues. In addition, uv-irradiated toxaphene was tested in *in vivo* and *in vitro* studies. The no observed adverse effect levels (NOAELs) in the *in vivo* studies are used to set a new tolerable daily intake (referred to as the provisional MATT TDI) for toxaphene for the tumour promotion potency. The MATT TDI is compared with other proposed TDIs.

The objectives of the present study were:

- to estimate a TDI for weathered toxaphene for tumour promotion based on the new toxicological data,
- to estimate the daily intake of toxaphene residues from European fishery products for the consumers of Germany, Ireland, Norway and The Netherlands, and
- to provide information on the toxicological risks to consumers of toxaphene residues from fishery products from European waters.

The daily intake of toxaphene was estimated from i) the baseline levels of toxaphene in fish and shellfish (McHugh et al 2004) and ii) the daily consumption of fishery products for the consumers of Germany, Ireland, Norway and The Netherlands. The daily consumer intake of toxaphene was

compared with TDIs set by Canada, U.S., and Germany, and the provisional MATT TDI calculated in our study based on a new tumour promotion potency study (Besselink et al 2008).

ESTIMATION OF A TOLERABLE DAILY INTAKE (TDI) FOR TOXAPHENE FOR TUMOUR PROMOTION POTENCY

To derive a TDI for humans, toxicity data from mammals are used in combination with a safety factor. The TDI is defined as the daily intake of a contaminant, in this case toxaphene, which should not result in adverse health effects. Normally, one applies a safety factor of 100, 10 for the extrapolation of an effect level from animal experiments to humans and 10 to account for variability amongst humans. In the 1950s, the Joint Expert Committee on Food Additives (JECFA) set a safety factor of a 100-fold to protect humans based upon a NOAEL in animals (factor 10 for species differences and a factor 10 to allow for inter-individual differences). The Codex discussion paper (2000) advised applying a safety factor of 1000 for toxaphene. The extra safety factor of 10 for toxaphene was supported by the observed variation in toxaphene patterns between the technical toxaphene mixture and the patterns found in the environment, and because most toxicity studies have been performed with technical toxaphene. Toxicological studies were carried out by Besselink et al. (2008) on three toxaphene mixtures including technical toxaphene (TT), uv-irradiated toxaphene (uvT), and toxaphene residues extracted from cod liver (CLE). As a consequence of the additional information from these experiments the extra safety factor of 10 is considered no longer necessary.

With respect to the calculation of a MATT pTDI from the *in vivo* toxicity studies of the study of Besselink et al. (2008), the cod liver experiment is preferred for the calculation of the MATT pTDI because this extract mimics the toxaphene pattern found in fish and, therefore, provides a more realistic human exposure situation. We note that the cod liver extract (CLE) showed a weathered toxaphene pattern, however, the residue samples were less altered than expected based upon residues

typically found in marine fish. The most likely reason for that observation is that the major changes in the technical toxaphene pattern take place prior to the uptake by fish while toxaphene is in the environment where it is exposed to UV light, evaporation, etc. However, the chromatograms show that also in fish some changes in the technical toxaphene have taken place (Besselink et al 2008). The present data indicate that the highest exposure concentration for the cod liver extract should serve as a NOAEL for tumour promotion in female Sprague-Dawley rats (Besselink et al 2008). The highest dose used in the cod liver extract experiment was 12.5 mg technical toxaphene equivalents /kg bw/week, which is 1.8 mg/kg bw /day. This level is the NOAEL. The MATT established a safety factor of 100 considering the uncertainties of intra- and interspecies differences. Although an extra factor of 10 was proposed by the Nordic Council, the MATT group determined that it was not required, because the prior uncertainty was addressed by the Besselink et al. studies on the two forms of degraded toxaphene (uvT and CLE). Applying a safety factor of 100 to the NOAEL, the MATT pTDI for humans for toxaphene for tumour promotion potency is 0.018 mg/kg bw/d. This results in an MATT pTDI of 1.08 mg for total toxaphene per day for a person with a body weight of 60 kg (0.018 mg/kg bw/d x 60 kg bw = 1.08 mg/d).

MAXIMUM RESIDUE LEVEL (MRL) AND TOLERABLE DAILY INTAKES (TDI)

Several tolerance levels and maximum residue levels in food for toxaphene have been proposed based on total toxaphene or on the sum of three persistent indicator congeners (Simon and Manning 2006). Either approach can be used to develop a valid and safe level for toxaphene in the food. Germany use a maximum residue level (MRL) of 0.1 mg/kg ww on the basis of the sum of the three indicator congeners (CHBs 26, 50 and 62) for fish and fish products. The German MRLs for all other food of animal origin were set at 0.1 mg/kg ww on the basis of total toxaphene. Canada also uses

total toxaphene residues to set an allowable daily intake (ADI) of 0.2 µg/kg bw/d, which is equivalent to a tolerable daily intake (TDI) of 0.012 mg for a person of 60 kg.

The US EPA set two health benchmarks for toxaphene; a chronic toxicity reference dose of 2.5×10^{-4} mg/kg/d (US EPA 1997) and, for carcinogenicity, the upper bound (95% confidence limit) cancer slope factor (CSF) which is 1.1 $(mg/kg/d)^{-1}$ with a maximum acceptable upper bound cancer risk level of 10^{-5} (1 in 100,000) over a 70-year lifetime (US EPA 1999). Based on an acceptable risk of 10^{-5} the maximum average daily dose can be estimated to approximate a reference dose for carcinogenicity. The chronic dose for an average body weight of a person of 60 kg for toxaphene is 0.015 mg. On a body weight basis, the dose is 0.015/60 or 0.00025 mg/kg/d. For carcinogenicity, the upper bound risk of toxaphene in fisheries products can be estimated by multiplying CSF with the concentration of toxaphene in fisheries products (C_f), the average yearly fish consumption (FC_{yr}), and the exposure duration (30 years). This average lifetime intake should be divided by body weight (BW) and an average lifetime of 70-years, see formula 1. The risk is expressed in terms of an upper bound incidence, for example a certain exposure would result in an estimate of risk that has a 95% probability of being no greater than x (e.g., x is1 in a million or 1 in 100,000) and could be as low as zero.

$$\text{Risk} = \text{CSF} \ \frac{C_f \cdot FC_{yr} \cdot ED}{BW \cdot L} \qquad [1]$$

CSF: Cancer slope factor, 1.1 per $mg/kg/day^{-1}$

C_f: Toxaphene concentration in fish (mg/kg), data from McHugh et al. (2004)

FC_{yr}: Average yearly fish consumption, kg/year

ED: exposure duration, 30 years

BW: body weight, kg

L: Lifetime, 25550 days = 70 years

The cancer slope factor approach was reviewed by Goodman et al. (2000). They proposed the risk assessment be revised under the 1986 US EPA cancer risk assessment guidelines. Now that US EPA has published new risk assessment guidelines, even further revision is appropriate. Goodman et al. proposed a lower potency factor. The Simon and Manning (2006) proposal would abandon the slope factor for a margin of safety calculation.

The most recent proposal from Simon and Manning creates a reference dose (essentially the same as a TDI) using 3 persistent congeners as the measure of toxaphene in the environment. The 3 persistent congeners (congeners p-26, p-50, and p-62) represent the entire group of toxaphene congeners, so the values are lower than total toxaphene numbers. They propose the reference dose at 2E-05 mg/kg/day of the 3 persistent congeners based upon the same toxicology data that are relied upon in this risk assessment. Simon and Manning used the NOAEL from the *in vivo* rat study on cod-liver extract toxaphene (Simon and Manning 2006; Besselink et al 2008).

AVERAGE DAILY AND YEARLY INTAKE OF TOXAPHENE

To estimate the average daily intake of toxaphene from fishery products for the consumers of Germany, Ireland, Norway and The Netherlands, information on the consumption of fishery products is needed. The Statistical Office of the European Communities in Luxembourg (Eurostat) provides information on the fish production of European countries, see Table 3 (Eurostat, 2000). However, the Eurostat data accounts for the fish production without exports and some other factors. The data are based on the whole fish weight and not just the edible portion of the fish, and therefore the fish

consumption will be overestimated. The FAO database also includes information on the world-fish production (Table 3). Detailed fish consumption data in The Netherlands have shown, however, that a large difference exists in the amount of fish production and the amount of fish consumption (edible part of the fish) (Temminghoff et al 1999). For 1998, the average fish consumption in The Netherlands was 9.4 g/day, which is 3.4 kg/year and more than 4-fold lower than the amount of fish production set by Eurostat or FAO. The Irish Sea Fisheries Board (BIM) has statistics on average fish consumption in Ireland, and also these data (8.8 kg of fish and fish products per person) show that the consumption is lower than that based on Eurostat and FAO data. For Germany fish consumption data from the Deutschen Gessellschaft für Ernährung (DGE) showed an average of 14.9 kg/year for whole fish. We assume that the real consumption of fish is 50% of the whole fish, 7.5 kg/year. For Norway the Statens Næringsmiddeltilsyn (SNT) Institute provided a realistic fish consumption of 21.9 kg/year. For both Germany and Norway the realistic fish consumption data are lower than the Eurostat and the FAO data. Therefore, the most realistic fish consumption data were used for the calculations of the intake of toxaphene from fishery products.

For the intake estimations from fishery products, recent baseline concentration data for toxaphene in fishery products from the North-East Atlantic (North Sea, German Bight / Skagerak, Baltic Sea, Irish Sea, Irish west-coast, Norwegian coast, and Barents Sea) were used (McHugh et al 2004). Liver samples were removed from the dataset, and only fillet or shellfish samples were included. In the study of McHugh et al (2004) concentrations of toxaphene were determined in 221 fillet samples of fishery products from the North-East Atlantic, as well in farmed fish samples. In all samples the three indicator congeners (CHB 26, 50 and 62) as well as total toxaphene were determined in 55 samples. Based on the ratio of the sum of the three indicator congeners and total toxaphene, the total toxaphene concentration in the other samples was estimated. The ratios for marine fish, eel and

mussel were 12%, 42%, and 24%, respectively. This dataset was used to estimate the average daily intake of total toxaphene and the intake of the sum of the three indicator congeners.

The daily intake of toxaphene (I_{intake}) was calculated (table 4) by multiplying the toxaphene concentration of each individual sample on a wet weight basis (C_{fish}) with the average daily consumption of fishery products (FC_d):

$$I_{intake} = C_{fish} \cdot FC_d$$

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

- All people 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.

The average daily intake (I_{avg}) was estimated as the mean of the intake of all individual samples (I_{intake}):

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

in which n is the total number of samples. In reality preferences for the consumption of some fish species exists in countries, e.g. a Scandinavian penchant for herring. Yet, detailed information on the fish consumption (species frequency and amounts) was not available for the countries and, therefore, was not used in this study.

The highest estimated average daily intake of total toxaphene (1.2 µg) was found for Norway, and 0.4, 0.5, and 0.2 µg for Germany, Ireland, and The Netherlands, respectively. However, people in Iceland eat on average even more fish than Norway and an intake of 2.6 µg per day is estimated. In estimated intake of Toxaphene from fish for people from Greenland is estimated to vary from 0.03 to 6.7 µg/day (Johansen et al 2004). The estimated daily intakes of total toxaphene are in agreement with the daily intakes reported by Alder et al (1997) for Germany, 2.8-5.6 ng/kg body weight, which is 0.2-0.3 µg for a person of 60 kg per day. A similar daily intake has been reported by Brüschweiler et al (2004), based on fish, meat, milk and plant samples, of 25 ng total toxaphene/kg b.w, which is equivalent to 1.5 µg for a person of 60 kg. The range of estimated daily intakes of toxaphene from low contaminated fish to higher contaminated fish varied between 0.001 and 14 µg (Table 4).

RISK OF TOXAPHENE INTAKE FROM FISHERY PRODUCTS

A comparison of TDIs and the estimated average daily intake of toxaphene from all baseline samples of McHugh et al (2004) is shown in Figure 1. This figure shows that only one baseline sample (Greenland halibut) exceeded the Canadian TDI for the Norwegian consumer. On an average basis the TDIs are not exceeded. The proposed MATT TDI for tumour promotion (1.08 mg total toxaphene) was not exceeded by any of the individual fishery samples. The risk of cancer based on the cancer slope factor and a lifetime intake of toxaphene by fishery products is shown in Figure 2. The maximum acceptable cancer risk of 1E-05 set by the US EPA (US EPA 1999) is marked. About 1.5%, 6%, 8% of the baseline samples exceeded the maximum cancer risk of 1E-5 for an average Dutch, German, and Irish fish consumer, respectively. For an average Norwegian fish consumer about 24% of the samples exceeded the maximum risk level, due to a higher consumption of fish than the consumers of the above three countries.

These conclusions are based on an average consumption of fishery products and an adult person of 60 kg. It is known that specific groups of people, for instance fishermen, eat more fish than average. For high fish consumers of Norway (184 g fish/day, 67 kg/year) the estimated daily intake was 3.7µg instead of 1.2 µg for an average Norwegian fish consumer (60g fish/day). For the high Norwegian fish consumers approximately 8% of the total number of fish samples exceeds the Canadian TDI, and 5% of the samples are above the US EPA TDI level for chronic toxicity (Figure 3). The samples that exceed the TDI are in general fatty fish; four herring, five mackerel, two Greenland Halibut, four farmed Atlantic Salmon, and one eel. A large number of these samples came from the Barents Sea, which has been shown to contain elevated levels of toxaphene (McHugh et al 2004). The maximum acceptable risk level of 1E-5 for cancer was exceeded by 24% of the samples for an average Norwegian fish consumer and by more than 50% of the baseline samples for high fish consumers of Norway.

In addition to the differences in fish consumption between groups of people, also regional differences within a country exist. For instance, in Germany a large variation in fish consumption is present between the northern and southern part. Figure 4 shows the estimated average daily intake of toxaphene for three German regions.

With regard to the maximum residue level (MRL) set by Germany, all baseline samples were below the threshold level of 0.1 mg/kg ww for the sum of the three indicator congeners in fish and fish products (Figure 5). Important to note, the MRL is based on the toxaphene concentration in the fish product and is not related to the amount of fish consumption.

CONCLUSIONS

In the past, toxaphene risks were based upon toxicology data on technical toxaphene. The adverse effect driving the risk assessment was tumour promotion. *In vitro* studies have confirmed that TT,

CLE and uvT all show biologically plausible ability to inhibit gap junctional intercellular communication. *In vivo* studies have established the NOAEL for each of these, and the CLE-generated NOAEL is proposed for use in the risk assessment. These new risk data based on toxaphene residues in fish, established in the MATT project show that the risks associated with fish consumption in Europe as regards toxaphene concentrations are negligible and in the worst case scenario limited to high fish consumers in Norway and possibly Iceland. However, when using the cancer slope factor approach of US EPA, a substantially higher risk is predicted. The cancer slope factor approach may, however, be too conservative, and the MATT data on tumour promotion do not support this approach. The new toxicological data from the MATT project show that Norwegian fish consumers are not exposed to serious risks due to toxaphene.

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FIGURE LIST LEGENDS

Figure 1: Frequency distributions of estimated intake of total toxaphene (µg) in 221 fish and shellfish samples from the study of McHugh et al. (2004) and realistic average fish consumption data of Germany, Ireland, Norway, and The Netherlands. The TDI thresholds for Canada (12 µg) and the U.S. EPA for chronic toxicity (15 µg) are shown.

Figure 2: Frequency distributions of cancer risk estimated from the lifetime intake of total toxaphene from fish and shellfish samples (n=221) from the study of McHugh et al. (2004) and realistic average fish consumption data of Germany (A), Ireland (B), Norway (C) and The Netherlands (D), and high fish consumption for the Norwegian consumer (E). The number of samples per frequency class is shown at the top of the bars. The U.S. EPA cancer slope factor of 1.1 per mg/kg-day (U.S. EPA, 1999) with a maximum acceptable risk level of 1E-05 was used.

Figure 3: Frequency distributions of estimated intake of total toxaphene (µg) in 221 fish and shellfish samples from the study of McHugh et al. (2004) and average and high fish consumption for the Norwegian consumer. The TDI thresholds for Canada (12 µg) and the U.S. EPA for chronic toxicity (15 µg) are shown.

Figure 4: Frequency distributions of estimated daily intake of total toxaphene (µg) based on toxaphene levels in 221 fish and shellfish samples from the study of McHugh et al. (2004) and average consumption of fishery products from three regions in Germany. Average fish consumption for Schleswig-Holstein, Hamburg and the northern part of Lower Saxony are 41.1 g/person per day,

for Nordrhein-Westfalen 15.1 g/person per day, and for Bavaria and Baden-Württeberg 6.2 g/person per day. TDI thresholds for Canada (12 µg) and the U.S. EPA for chronic toxicity (15 µg) are shown.

Figure 5: Concentration of the sum of the three indicator toxaphene congeners in fishery products from McHugh et al. (2004) compared to the maximum residue limit (MRL) set in Germany of 0.1 mg/kg ww.

Table 1: Overview of effect levels and parameters used to derive a tolerable daily intake (TDI) for toxaphene. No observed adverse effect dose was taken from the Besselink et al., (2008).

	Level
No observed effect level dose of cod liver extract for rat	12.5 mg/kg bw /week
No observed effect level dose of cod liver extract for rat adjusted for daily intake	1.8 mg/kg bw/d
Safety factor for extrapolation from rat to human	100
Tolerable daily intake per kg body weight for humans	0.018 mg/kg bw/d
Proposed TDI for a person of 60 kg	1.08 mg/d

Table 2: Overview of maximum tolerable daily intake (TDI) values of toxaphene for a person of 60 kg and maximum tolerable levels (MRL) in fish and fish products.

Tolerable daily intakes (TDI)	Value
Canada, pTDI*	0.012 mg/d
US EPA* *Chronic toxicity*	0.015 mg/d
*For acceptable upper bound risk of 1 in 100,000***	0.00025 mg /d
Simon and Manning, 2006 proposed RfD	0.012 mg/d***
This study, *tumour promotion potency*	1.08 mg/d

Maximum tolerable level (MRL)	Value
Germany	0.1 mg/kg ww***

* TDI calculated from the proposed ADI based on a person of 60 kg.

** Based upon a cancer slope factor of 1.1 $(mg/kg/d)^{-1}$

*** The acceptable level was converted to total toxaphene assuming a toxaphene mixture contains 10% ∑3PC (p26+p50+p62)

Table 3: Consumption of fishery products (kg/year) per person for Germany, Ireland, Norway and The Netherlands from Eurostat for 1998 and FAO for 1997.

	Realistic consumption (kg/person/year)	Eurostat (kg/person/year)	FAO (kg/person/year)
Germany	7.5[a]	12	15.6
Ireland	8.8	18	20.6
Norway	21.9	46	69.1
The Netherlands	3.4	12	14.6

[a] Calculated on the basis of 50% consumption of whole fish (14.9 kg/year).

Table 4: Estimated average daily intake (µg) of toxaphene from fishery products for the consumers of Germany, Ireland, Norway and The Netherlands.

Country	Average daily fish consumption (g/d)[a] (FC_d)	Estimated average daily intake (µg) of toxaphene by fishery products (I_{avg})	Estimated range of daily intake (µg) of toxaphene by fishery products for low and high contaminated fish
Germany	20.4	0.4	0.001-5
Ireland	24.1	0.5	0.002-6
Norway	60.0	1.2	0.004-14
The Netherlands	9.4	0.2	0.001-2

[a] Realistic fish consumption

Fig. 1

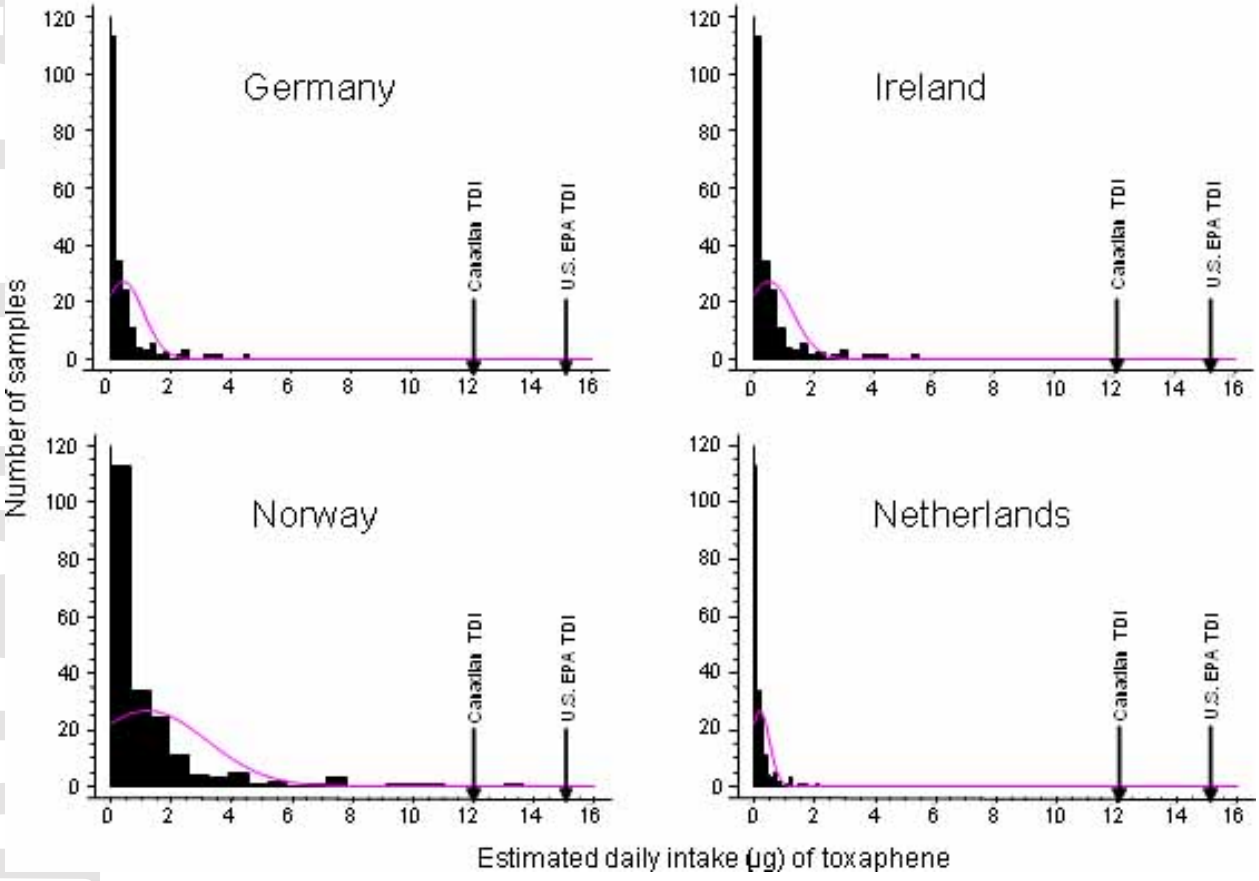

Fig. 2

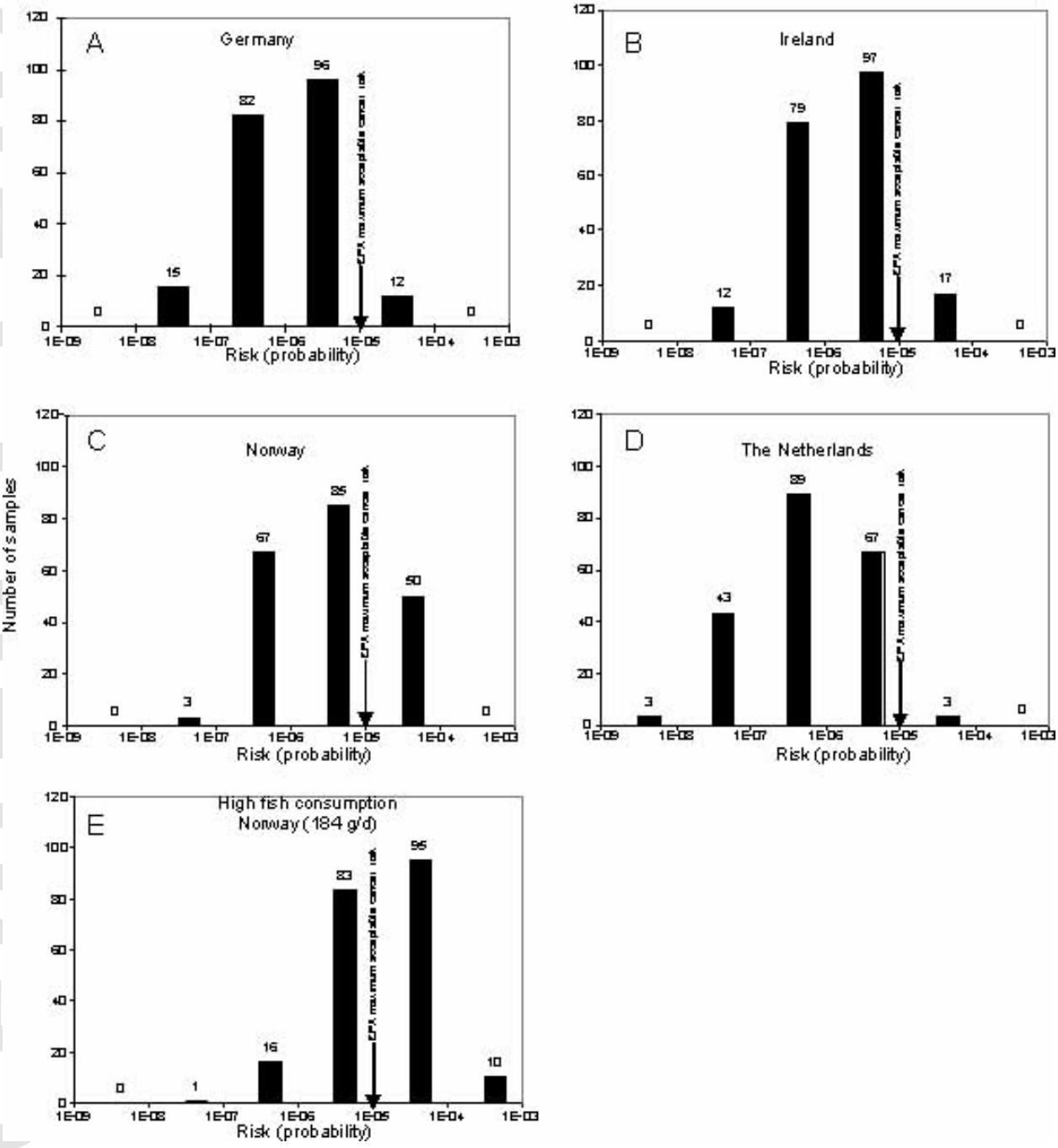

Fig. 3

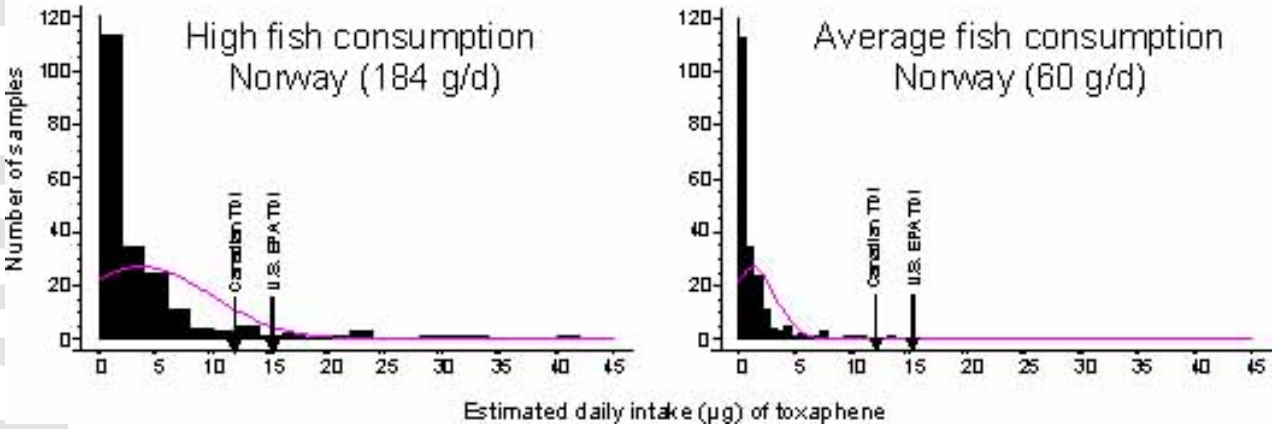

Fig 4

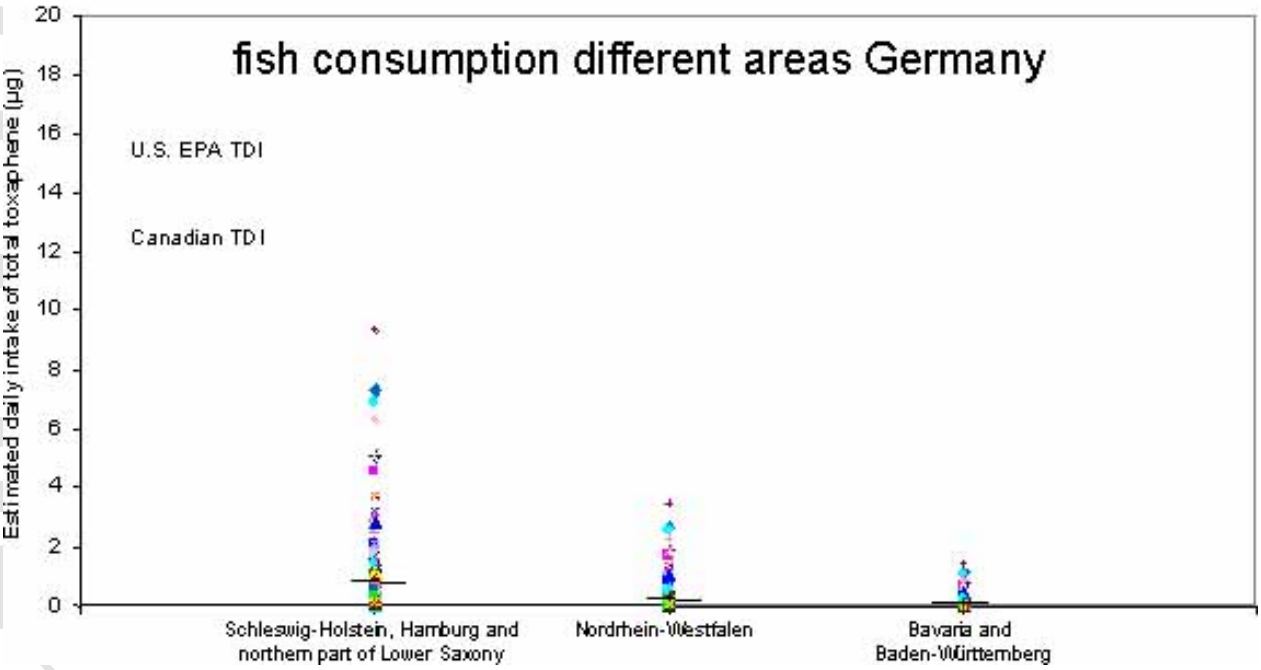

Fig. 5