TOXICOLOGICAL RISKS TO HUMANS OF TOXAPHENE RESIDUES IN FISH

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Introduction

Given the concern on toxaphene concentrations in the environment, surprisingly little is known about the toxicology of this group of compounds[1,2]. Substantial uncertainties exist with regard to the carcinogenicity, toxicological risk, and tolerance levels of toxaphene in the environment, and in particular of toxaphene residues in fish[1]. Consequently, a proper risk assessment of toxaphene for the consumer of marine foodstuffs could not be made earlier. In the European research project MATT (Investigation into the Monitoring, Analysis and Toxicity of Toxaphene in Marine Foodstuffs) new information on the toxicology and risks of toxaphene was obtained through a study on the possible effects of metabolized toxaphene in cod.

Materials and methods

Tolerance levels are based on the toxicology of the technical toxaphene mixture or – in Europe - on individual chlorobornanes, 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[3]. 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 MATT study generated 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 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 inhibite gap junctional intercellular communication as a correlate to tumour promotion. A critical *in vivo* exposure study was carried out 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 MATT TDI) for toxaphene for the tumour promotion potency. The MATT TDI is compared with other proposed TDIs. The daily intake of toxaphene from fishery products for fish consumers from Germany, Ireland, Norway and The Netherlands was estimated from i) the baseline levels of toxaphene in fish and shellfish[4] and ii) the daily consumption of fishery products for the consumers of Germany, Ireland, Norway and The Netherlands. The daily intake of toxaphene was compared with TDIs set by Canada, U.S., Germany, and Austria, and the proposed MATT TDI calculated in the present study.

Results and discussion

Estimation of a tolerable daily intake (TDI) for toxaphene for tumor promotion potency

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. The Codex discussion paper[5] 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. The present studies were carried out 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 TDI from the *in vivo* toxicity studies of the study, the cod liver experiment is preferred for the calculation of the TDI 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 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. The highest dose used in the cod liver extract experiment was 4.8 mg technical toxaphene equivalents /kg bw/week, which is 0.69 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. Applying a safety factor of 100 to the NOAEL, the MATT TDI for humans for toxaphene for tumour promotion potency is 0.0069 mg/kg bw /d. This results in an MATT TDI of 0.41 mg for total toxaphene per day for a person with a body weight of 60 kg (0.0069 mg/kg bw/d x 60 kg bw = 0.41 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[6]. Either approach can be used to develop a valid and safe level for toxaphene in the food. Germany and Austria 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 and Austrian 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^{-1}, 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 x 10^{-4} mg/kg/d[7] and for carcinogenicity the EPA uses 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[8]. Based on an acceptable risk of 10^{-5} the maximum average daily dose can be estimated to approximate a reference dose for carcinogenicity, although it is not a reference dose. The chronic dose for an average body weight of a person of 60 kg for toxaphene is 0.015 mg. On a body weight (bw) 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. This provides an upper bound estimate of risk, not a reference dose. 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 (eg., x is 1 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
C_f: Toxaphene concentration in fish (mg/kg)
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.[9] who proposed that the risk assessment be revised under the 1986 US EPA cancer risk assessment guidelines. Goodman et al. proposed a lower potency factor. The Simon and Manning proposal[6] would abandon the slope factor for a margin of safety calculation. The 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. 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. They used the NOAEL from the *in vivo* rat study on cod-liver extract toxaphene[6].

Average daily and yearly intake of toxaphene

The average fish consumption in several EU countries is shown in Table 1. For the intake estimations from fishery products, recent baseline concentration data for toxaphene in fishery products were used[4]. 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. The range of estimated daily intakes of toxaphene from low contaminated fish to higher contaminated fish varied between 0.001 and 14 µg (Table 1).

Risk of toxaphene intake from fishery products

A comparison of the Canadian TDI and the estimated average daily intake of toxaphene shows that only Greenland halibut exceeded the daily intake level for total toxaphene for the Norwegian consumer. On an average basis the TDIs are not exceeded. The proposed TDI for tumour promotion (0.41 mg total toxaphene) was not exceeded by any of the individual fishery product samples. However, the maximum acceptable cancer risk of 1E-05 set by the US EPA according to the cancer slope factor approach[8] is exceeded by 1.5%, 6%, 8% of the samples for the average Dutch, German, and Irish fish consumer, respectively. About 24% of the samples exceeded this maximum risk level for the average Norwegian fish consumer, due to a higher consumption of fish than in 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, e.g. 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 this group, approximately 8% of the samples exceed the Canadian TDI, and 5% of the samples are above the US EPA TDI level for chronic toxicity. The samples that exceed the TDI are in general fatty fish: herring, mackerel, Greenland Halibut, farmed Atlantic Salmon, and eel. A large number of these samples came from the Barents Sea, which has been shown to contain elevated levels of toxaphene[4]. The maximum acceptable risk level of 1E-5 for cancer[8] was exceeded by more than 50% of the baseline samples for high fish consumers of Norway (5% of the samples exceeds the cancer risk of 1E-4).

Table 1: Estimated average daily intake of toxaphene from fishery products for the consumers of Germany, Ireland, Norway and The Netherlands.

Country	Average daily fish consumption (g/d)[1] (FC_d)	Estimated average daily intake (µg) of toxaphene by fishery products (A_{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
Netherlands	9.4	0.2	0.001-2

[1] realistic fish consumption

The 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. The use of a reference dose, as proposed by Simon and Manning[6] is preferred.

References

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