RECENT FINDINGS OF TOXAPHENE IN FISH – ANALYSIS AND REGULATION

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Introduction

Toxaphene is a complex mixture of chlorinated camphene derivatives containing 67 to 69 % chlorine. It has been extensively used as an insecticide e.g. for cotton and vegetables, as well as for the control of poultry and livestock ectoparasites. Toxaphene was the active agent in various products with different trademarks often applied in combinations with other organochlorine insecticides. Since 1946 the production of toxaphene is estimated to be more than one million tons[i]. Toxaphene is very persistent and has a high potential to accumulate in aquatic life[ii].
Various regulations concerning Toxaphene have been set into force. Directive 79/117/EWG prohibits the use and placing on the market of plant protection agencies containing e.g. toxaphene[iii]. Toxaphene is a listed contaminant in the UNEP Governing Council Decision 18/32 on Persistent Organic Pollutants[iv].
According to the German legislation *Rückstands-Höchstmengen-VO 2001 (RHmV)* the limit value for three toxaphene congeners (total of Parlar 26, Parlar 50 and Parlar 62 -indicator compounds) amounts 0.1 mg/kg fresh weight for fish[v]. In the beginning of the Nineties the limit value was lower by a factor of 10 (0,01 mg/kg fresh weight), but based on the results from a comprehensive study of the former German Federal Institute for Health Protection of consumers and veterinary medicine (BgVV) and the Federal Research Center for Fisheries, the limit value was increased[vi].
In the EU Directive 2002/32/EC a limit value for toxaphene for all kind of feeding stuff, which is 0.1 mg/kg related to a feeding stuff with 12 % moisture content, was set up.[vii] This EU regulation does not differentiate between single matrices like the German law does actually.

Within the frame of this paper recent findings of toxaphene congeners in fish are presented exemplarily.

Material and Methods

The samples were analysed within the routine work of the authors. 17 samples of smoked fish (mainly mackerel and salmon) and one Swedish marinated salmon were analysed by CVUA within governmental supervision work. Six samples of fresh fish (halibut and ocean perch) were investigated by ERGO within their general analytical service (samples were chosen by random selection). All samples were collected in 2003, smoked fish samples were originating from the

German market, whereas fresh fish samples were caught in the European area. For the analysis only the edible parts were used. Two different analytical methods were applied, which are described in the following:

HRGC/ECD (CVUA)
The determination of toxaphene congeners in fish was performed according to the collection of analytical methods from the German Food Law (§ 35 Lebensmittel- und Bedarfsgegenstände-gesetz, LMBG). The applied method L 00.00-34 is a revised version of the DFG multimethod S 19 for the determination of pesticides in foodstuff [viii].
Because of the high lipophilic character the analysis of the toxaphene congeners in fatty fish containing about 10 % fat and more was preferred. Fat extraction was performed with n-pentane at room temperature. For further cleanup gel permeation chromatography followed by fractionation on silica gel was used. The measurement was carried out by gas chromatography and electron capture detection (GC-ECD) simultaneously on two different capillary columns.

In brief, the homogenized fish tissue was mixed with anhydrous sodium sulphate (3+1, w/w) and extracted with n-pentane within 24h at room temperature. The lipids from about 0,8 g extracted fat were removed by gel permeation chromatography on an Autoprep 1002 A (Analytical Bio-Chemistry Laboratories, USA), fitted with a 2,5 x 60 cm column containing 60 g Bio-Beads SX-3 (Bio Rad Laboratories, 200 – 400 mesh). The mobile phase, consisting of cyclohexane/ethyl acetate (1+1, v/v), was introduced into the column at a flow rate of 5 ml/min. The toxaphene congeners eluted together with other organochlorine compounds in the range between 95 - 210 ml. After addition of 5 ml isooctane the extract was reduced to 1 ml and applied to a chromatography column (inner diameter 7,0 – 7,5 mm, length 320 mm) packed with 1 g silica gel (Merck, Germany, deactivated with 1,5 % water) and with 5 – 10 mm anhydrous sodium sulphate on top. The analytes were fractionated by successive elution with 8 ml n-hexane (eluate 0: PCB, HCB, DDE, DDT etc.), 8 ml n-hexane/toluene, 65+35, v/v, (eluate 1: toxaphene, chlordane, heptachlorepoxide, nonachlor, HCH etc.), 8 ml toluene (eluate 2: endrin, dieldrin, pyrethroids etc.) and 8 ml toluene/acetone, 95+5, v/v, (eluate 3: diazinone, pyrethroids etc.). An internal standard (ϵ-HCH) was added to each eluate.
Gas chromatography was performed using a Hewlett-Packard 6890 gas chromatograph equipped with two ^{63}Ni ECD. Nitrogen was used as ECD make-up gas. For GC-ECD verification of the analytes peak identity in the samples two capillary columns were used: HP-5 (Agilent, 30 m x 0,32 mm x 0,25 µm) and ZB-1701 (Phenomenex, 30 m x 0,32 mm x 0,25 µm). The oven temperature programme was as follows: initial temperature 100°C held for 5 min, first ramp to 150°C at 25°C/min , second ramp to 240°C at 3°C/min and third ramp to 280°C at 8°C/min held for 30 min.

HRGC/HRMS (ERGO)
The analytical procedure for toxaphene congeners in fish is based on an accredited method (EN ISO 17025) for determination of pesticides and other chlorinated contaminants in biota.
The homogenized fish tissue was mixed with anhydrous sodium sulphate (1+3, w/w). Before extraction the ^{13}C-labelled internal standard PCB 52 was added. Afterwards the homogenate was extracted with 250 ml of hexane (Merck)/ acetone (Baker) (2+1, v/v). 10 % of the extract was used for gravimetric lipid determination.
The remaining extract (90%) was introduced to a chromatography column (inner diameter 20 mm, length 400 mm) packed with 10 g of florisil (Promochem, Germany, deactivated with 5 % water)

and 5 g alumina oxide (ICN, deactivated with 6 % water) and 10 mm anhydrous sodium sulphate on top. The analytes were eluted with 300 ml hexane and 90 ml hexane/toluene (7+3, v/v). Afterwards the extract was reduced in volume first by rotary evaporator and finally by a stream of nitrogen. The final volume was about 100 µl after adding ^{13}C-labelled PCB 105 as recovery standard.
The measurement was performed by high-resolution gas chromatography/ high resolution mass spectrometry (HRGC/HRMS) on Hewlett-Packard 6890 gas chromatograph and Finnigan MAT 95 mass spectrometer. A BPX5 column (SGE, 60 m x 0,25 mm x 0,1 µm) was used for gas chromatographic separation. The oven temperature programme was as follows: initial temperature 90°C held for 3 min, first ramp to 210°C at 25°C/min , second ramp to 233°C at 3°C/min and third ramp to 300°C at 25°C/min held for 10 min. The quantification was done by using a 5-point calibration and taking into account the internal standard.

Results and Discussion

Both techniques applied (HRGC/ECD and HRGC/HRMS respectively) have been proven to be suitable for routine analysis and verification of limit values. The mean limit of detections (LODs) of both methods were similar (0.005 mg/kg, lipid based).
In accordance with the definition of official limit values, analytical results for toxaphene congeners are presented as the total of Parlar 26, Parlar 50 and Parlar 62 (indicator compounds).

describes the analytical data on a lipid base, Figure 2 shows the results referred to original sample (wet weight based). Out of 24 fish samples, concentrations of indicator compounds were below the detection limits in six samples. 18 samples were in the range between “not detected” and 0.21 mg/kg (lipid based) respectively “not detected” and 0.017 mg/kg (wet weight based). The median (for the total of Parlar 26, 50, 62) of the 24 samples analysed was found to be 0.041 mg/kg, lipid based and 0.0034 mg/kg, wet weight based respectively.

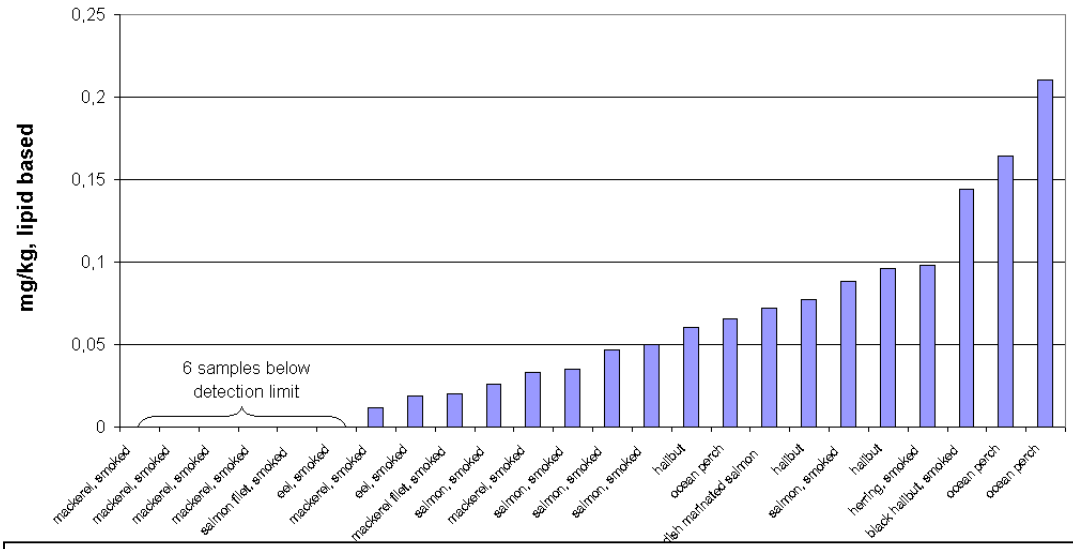

Figure 0 toxaphene congeners in 24 fish samples (total of Parlar 26, 50, 62), <u>data referred to lipids</u>

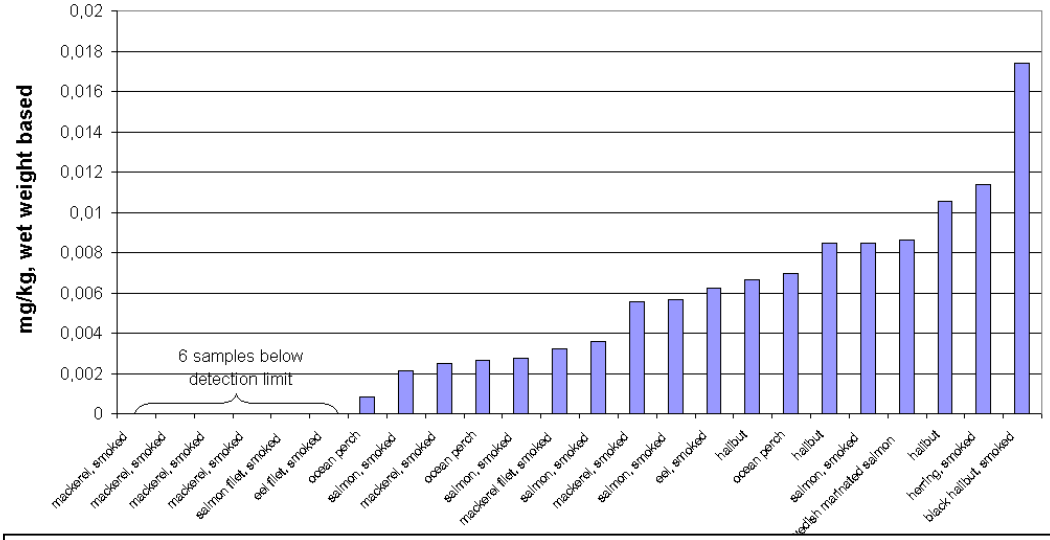

Figure 0 toxaphene congeners in 24 fish samples (total of Parlar 26, 50, 62), <u>data referred to original sample (wet weight based)</u>

Table 1 Total of Parlar 26, 50, 62 in fish - findings of other authors

Author	Findings *	Remark
Alder et al[ix]	**Herring (n=25):** 0.001- 0.035 mg/kg wet weight based **Salmon (n=5):** 0.001 – 0.022 mg/kg wet weight based **Mackerel (n=8):** 0.002 – 0.017 mg/kg wet weight based	Origin of fish samples: Various fishing areas worldwide
Fromberg et al[x]	**Various fish**: herring, salmon, eel, mackerel and cod liver **(n=18)** 0.005 – 0.1 mg/kg lipid based	Data read from bar diagram in original paper Fish samples from Danish waters
Mc Hugh et al[xi]	**Mackerel (n=24) :** 0.006– 0.04 m/kg lipid based **Golden redfish (n=24)** 0.037 – 0.18 mg/kg lipid based **Cod liver (n=25):** 0.013 – 0.045 mg/kg lipid based **Herring (n=26)** 0.008 – 0.088 µg/kg lipid based	Fish samples from European Waters

***** For better comparison with the limit value of 0.1 mg/kg the data, originally presented in µg/kg, are presented in mg/kg in the table above.

To sum up it can be stated that all samples analysed showed concentrations below 0.1 mg/kg (wet weight based). Nevertheless, 18 out of 24 samples showed detectable levels of toxaphene congeners.

References

[i] Voldner E.C., Y.F. Li, Global usage of toxaphene, Chemosphere 27 (1993), 2073-2078
[ii] http://www.epa.gov/safewater/contaminants/dw_contamfs/toxaphen.html
[iii] Council Directive 79/117/EEC of 21 December 1978 prohibiting the placing on the market and use of plant protection products containing certain active substances
[iv] Decision 18/32: Persistent Organic Pollutants, UNEP Governing Council, May 1995
[v] Verordnung über Höchstmengen an Rückständen von Pflanzenschutz- und Schädlingsbekämpfungsmitteln, Düngemitteln und sonstigen Mitteln in oder auf Lebensmitteln und Tabakerzeugnissen (Rückstands-Höchstmengenverordnung –RHmV, in der Fassung der Bekanntmachung vom 21. Oktober 1999)
[vi] Bundesgesetzblatt 1997 part I page 2370
[vii] Directive 2002/32/EC of the European Parliament and the Council of May 7, 2002, on undesirable substances in animal feed
[viii] **Amtliche Sammlung von Untersuchungsverfahren nach § 35 LMBG, L 00.00-34: Untersuchung von Lebensmitteln, Modulare Multimethode zur Bestimmung von Pflanzenschutzmittelrück-ständen in Lebensmitteln (Erweiterte Neufassung der DFG-Methode S 19), Beuth-Verlag GmbH, 2004**
[ix] Lutz Alder, Hans Beck, S. Khandker, H. Karl, I. Lehmann, Levels of toxaphene indicator compounds (chlororbornanes) in fish, Organohalogen compounds, vol 26 (1995)
[x] Arvid Fromberg, Tommy Cederberg, Gudrun Hilbert, Determinantion of toxaphene congeners in fish samples from Danish waters, Organohalogen compounds, vol 35 (1998)
[xi] Brendan Mc Hugh, Eugene Nixon, Jarle Klungsoyr, Harrie Besselink, Abraham Brouwer, Gerhard Rimkus, Pim Leonards and Jacob de Boer, Survey of toxaphene concentrations in fish from European waters, Organohalogen compounds (2000)