Collaborative study on Toxaphene Indicator Compounds (Chlorobornanes) in Fish Oil

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1. Introduction

Determination of toxaphene (camphechlor) residues in environmental matrices and food is very difficult. Most problems arise from the complex composition of toxaphene and the substantial differences in peak patterns in environmental samples as compared to the technical product. Based on earlier preparations of pure toxaphene congeners, we identified three chlorobornanes (CHBs) which are present in marine fish in relativly high concentrations. These CHBs **1** - **3** are proposed as indicator compounds for toxaphene residues[1]. A CHB **4** is under discussion as an indicator compound for certain applications. Additionally, these congeners are proposed for the establishment of MRLs.

Indicator compound **1**: 2-exo,3-endo,5-exo,6-endo,8b, 8c,10a,10b-octachlorobornane
Indicator compound **2**: 2-exo,3-endo,5-exo,6-endo,8b, 8c,9c,10a,10b-nonachlorobornane
Indicator compound **3**: 2,2,5,5,8b,8c,9c,10a,10b-nonachlorobornane
Indicator compound **4**: 2,2,5-endo,6-exo,8b,9c,10a-heptachlorobornane

Using these individual CHBs as standards, a simple and reproducible determination of toxaphene residues in samples is possible without the need of special cleanup procedures and conventions according to standards, GC methods or quantitation procedures.

To check whether the congeners can be reliably detected and quantitated, a method validation study was conducted on the determination of these toxaphene indicator compounds by a multipesticide cleanup combined with GC/ECD detection.

2. Method used for cleanup and GC/ECD detection

The lipids from 0,5g oil sample were removed by gel permeation chromatograph, fitted with a 2.5 × 40 cm column containing 50 g Bio-Beads SX-3 (Bio Rad Laboratories, 200 - 400 mesh). The mobile phase, consisting of cyclohexane/ethylacetate (1:1), was introduced into the column at a flow rate of 5 ml/min. The camphechlor congeners eluted together with PCBs, other chlorinated pesticides such as chlordane components or DDT and metabolites in the range of 95 - 150 ml.

After the removal of solvent, the sample was dissolved in isooctane and then applied to a 0.7 × 23 cm chromatography column packed with 1 g silica gel (Merck No. 7734, deactivated with 1.5 % water) and with a layer of 1 cm dried sodium sulfate on the top. The toxaphene components, other organochlorine pesticides and PCBs were fractionated by consecutive elution with 8 ml hexane/toluene (65:35 v/v, fraction 1) and 8 ml toluene (fraction 2). In the case of cod liver oil samples, the first eluate contained DDE, DDD, DDT, chlordane components and the PCBs, in additon to toxaphene components.

Following the protocol of the study, gas chromatography was started in each case by using a SE-54 type capillary column of at least 30m length. 65% of participants used 60m capillaries. Generally, columns with an internal diameter of 0.25mm or 0.32mm were used. In most cases, the results were validated with capillaries of higher polarity (DB-1301/DB-1701). Hydrogen or helium were used as carrier gases. The temperature of the splitless injector was in the range of 220 to 250°C with a median of 230°C.

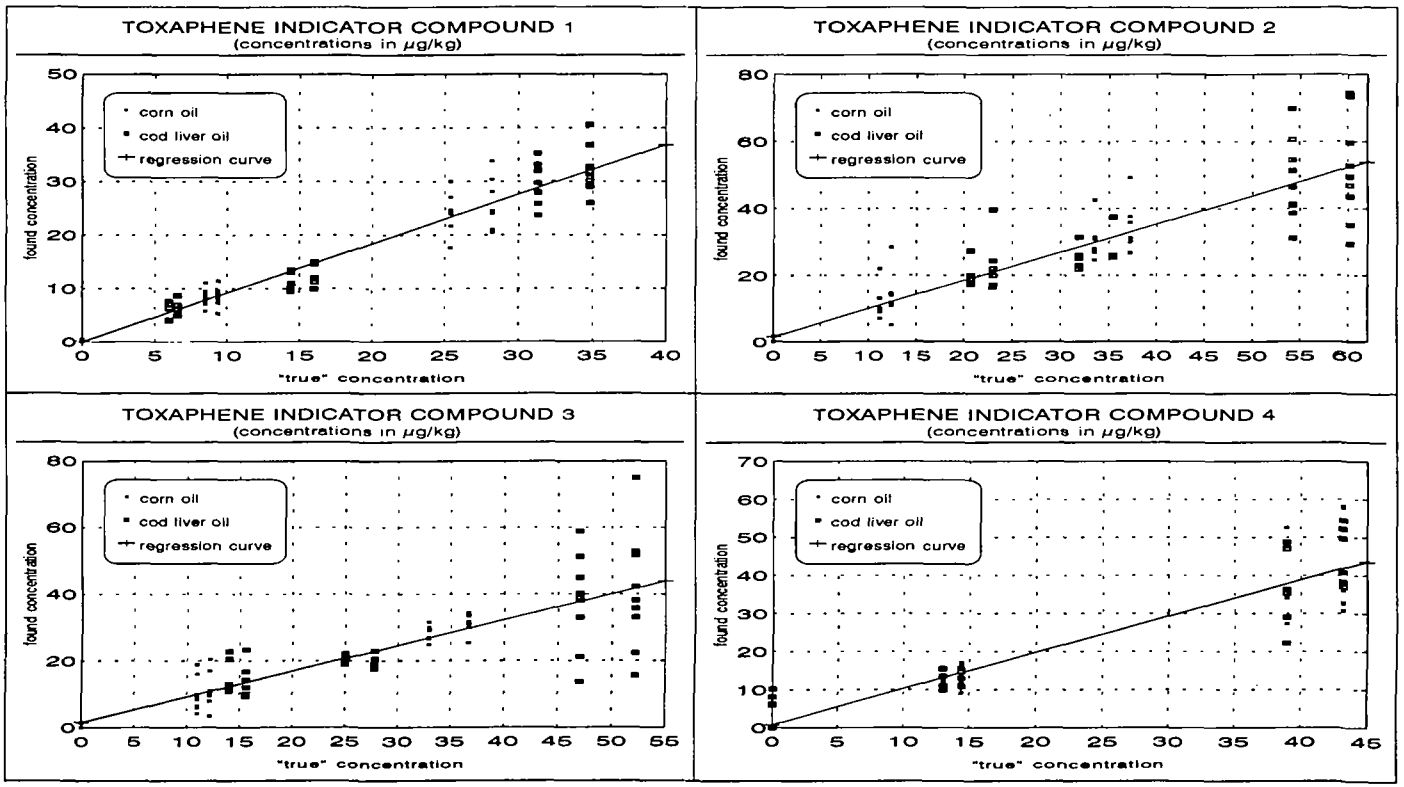
3. Design of the study

The study was based on the nonreplicate split level design for collaborative tests (Youden pairs)[2]. For the preparation of test samples two starting materials were used. The first material, a corn oil without any toxaphene residues was spiked with the four CHBs at four concentration levels, prepared as two Youden pairs with concentration differences of 10%. Additionally, the unspiked corn oil was used as blank.
As a more realistic sample, a commercial cod liver oil with organochlorine residues typically found in marine fish samples (inclusive of toxaphene) was used as the second starting material. Aliquots of that cod liver oil were spiked at two concentration levels. 180ml of both spiked cod liver oils and 180 ml of the unspiked material were diluted with 20ml corn oil. The three undiluted materials (100% level) and their corresponding diluted samples (90% level) were used as three Youden pairs. In total, eleven concentration levels, prepared as 5 Youden pairs and a blank sample were obtained by this procedure. The concentrations of indicator CHBs **1** - **4** were in the range from 6µg/kg to 60,2 µg/kg (Tables 1-4).
Each participating laboratory received 6 randomly coded sample ampoules (three Youden pairs, or two Youden pairs and one pair of blanks), one CHB calibration standard concentrate and additionally three standard mixtures with PCBs, chlordane components and other chlorinated pesticides found in the cod liver oil. A special cod liver oil with specified concentrations of indicator compounds 1 - 4 was added to the test material for training purposes.
All 15 participants received a complete description of the method to be used for the analysis. Individual deviations from the operating procedure were not allowed.

4. Treatment of data

The resulting data were first inspected for major discrepancies. "Less than" and "not detected" values were considered as zero. Outliers and method performance values were calculated from raw data using the AOAC guidelines[2]. Grubbs' (single and pair value) and Dixon's tests were considered to detect outliers. Cochran's tests was used to check the homogeneity of data. From a total of 318 concentration data submitted by 14 laboratories, only 2 had been rejected. Outlier tests and summary statistics (repeatability and reproducibility standard deviations s_r/s_R and their relative values) were calculated by a computer program according to the AOAC statistics for split level design, where repeatability (r) and reproducibility limits (R) are simple multiples of the above measures of precision expressed as standard deviations (a factor of 2.8 based on a statistical probability of 95% was used).

Figures 1-4: Concentrations found versus true concentrations for indicator compounds 1-4 in all materials



Tables 1 and 2: GC/ECD determination of toxaphene indicator compounds 1 and 2 - Interlaboratory method performance (concentrations in µg/kg)

Toxaphene indicator compound 1	No. of labs with valid results	No. of valid results	'True' conc. at 100% level	'True' conc. at 90% level	Calc. 'true' conc. (mean of both levels)	Conc. found (mean of both levels)	Mean recovery	Repeat-ability standard deviation	Repeat-ability limit	Repeat-ability relative standard deviation	Repro-ducibility standard deviation	Repro-ducibility limit	Repro-ducibility relative standard deviation	Highest acceptable reproducibility relative standard deviation
			x_1	x_2	$(x_1+x_2)/2$	$\bar{x}$		S_r	$r = 2{,}8 \cdot S_r$	$S_r / \bar{x}$	S_R	$R = 2{,}8 \cdot S_R$	$S_R / \bar{x}$	$2 \cdot 2^{(1-0{,}5 \log c)}$
Corn oil, not spiked	6	12	0,0	0,0	0,0	0,1								
Corn oil, low spiked	9	18	9,4	8,5	8,9	8,5	95%	0,83	2,32	9,8%	1,62	4,54	19,2%	65,6%
Corn oil, high spiked	7	14	28,2	25,4	26,8	24,9	93%	1,4	3,92	5,6%	4,50	12,60	18,1%	55,8%
Cod liver oil, not spiked	6	12	6,6	6,0	6,3	6,3		0,43	1,20	6,8%	1,59	4,45	25,2%	68,6%
Cod liver oil, low spiked	5	10	16,0	14,4	15,2	12,0	79%	0,42	1,18	3,5%	1,97	5,52	16,4%	62,3%
Cod liver oil, high spiked	9	18	34,8	31,3	33,1	30,9	93%	2,55	7,14	8,3%	4,08	11,42	13,2%	54,0%

Toxaphene indicator compound 2	No. of labs with valid results	No. of valid results	'True' conc. at 100% level	'True' conc. at 90% level	Calc. 'true' conc. (mean of both levels)	Conc. found (mean of both levels)	Mean recovery	Repeat-ability standard deviation	Repeat-ability limit	Repeat-ability relative standard deviation	Repro-ducibility standard deviation	Repro-ducibility limit	Repro-ducibility relative standard deviation	Highest acceptable reproducibility relative standard deviation
			x_1	x_2	$(x_1+x_2)/2$	$\bar{x}$		S_r	$r = 2{,}8 \cdot S_r$	$S_r / \bar{x}$	S_R	$R = 2{,}8 \cdot S_R$	$S_R / \bar{x}$	$2 \cdot 2^{(1-0{,}5 \log c)}$
Corn oil, not spiked	6	12	0,0	0,0	0,0	0,3								
Corn oil, low spiked	8	16	12,4	11,2	11,8	10,7	91%	1,47	4,12	13,7%	2,50	7,00	23,4%	63,4%
Corn oil, high spiked	7	14	37,2	33,5	35,3	32,1	91%	1,95	5,46	6,1%	6,96	19,49	21,7%	53,7%
Cod liver oil, not spiked	6	12	23,0	20,7	21,9	21,9		3,58	10,02	16,3%	6,53	18,28	29,8%	56,9%
Cod liver oil, low spiked	5	10	35,4	31,9	33,6	26,8	80%	1,68	4,70	6,3%	4,48	12,54	16,7%	55,2%
Cod liver oil, high spiked	9	18	60,2	54,2	57,2	50,0	87%	5,12	14,34	10,2%	13,88	38,86	27,8%	50,2%

Tables 3 and 4: GC/ECD determination of toxaphene indicator compounds 3 and 4 - Interlaboratory method performance (*concentrations in µg/kg*)

Toxaphene indicator compound 3	No. of labs with valid results	No. of valid results	'True' conc. at 100% level	'True' conc. at 90% level	Calc. 'true' conc. (mean of both levels)	Conc. found (mean of both levels)	Mean recovery	Repeat-ability standard deviation	Repeat-ability limit	Repeat-ability relative standard deviation	Repro-ducibility standard deviation	Repro-ducibility limit	Repro-ducibility relative standard deviation	Highest acceptable reproducibility relative standard deviation
			x_1	x_2	$(x_1+x_2)/2$	$\bar{x}$		S_r	$r = 2{,}8 \cdot S_r$	$S_r / \bar{x}$	S_R	$R = 2{,}8 \cdot S_R$	$S_R / \bar{x}$	$2 \cdot 2^{(1-0{,}5 \log c)}$
Corn oil, not spiked	6	12	0,0	0,0	0,0	n.d.								
Corn oil, low spiked	8	16	12,2	11,0	11,6	10,4	90%	0,56	1,57	5,4%	5,25	14,70	50,5%	63,6%
Corn oil, high spiked	6	12	36,6	32,9	34,8	29,6	85%	1,75	4,90	5,9%	2,85	7,98	9,6%	54,4%
Cod liver oil, not spiked	6	12	15,6	14,0	14,8	14,8		2,3	6,44	15,5%	5,12	14,34	34,6%	60,3%
Cod liver oil, low spiked	4	8	27,8	25,0	26,4	20,3	77%	0,96	2,69	4,7%	1,86	5,21	9,2%	57,5%
Cod liver oil, high spiked	9	18	52,2	47,0	49,6	39,2	79%	6,08	17,02	15,5%	15,94	44,63	40,6%	52,1%

Toxaphene indicator compound 4	No. of labs with valid results	No. of valid results	'True' conc. at 100% level	'True' conc. at 90% level	Calc. 'true' conc. (mean of both levels)	Conc. found (mean of both levels)	Mean recovery	Repeat-ability standard deviation	Repeat-ability limit	Repeat-ability relative standard deviation	Repro-ducibility standard deviation	Repro-ducibility limit	Repro-ducibility relative standard deviation	Highest acceptable reproducibility relative standard deviation
			x_1	x_2	$(x_1+x_2)/2$	$\bar{x}$		S_r	$r = 2{,}8 \cdot S_r$	$S_r / \bar{x}$	S_R	$R = 2{,}8 \cdot S_R$	$S_R / \bar{x}$	$2 \cdot 2^{(1-0{,}5 \log c)}$
Corn oil, not spiked	4	8	0,0	0,0	0,0	n.d.								
Corn oil, low spiked	7	14	14,4	13,0	13,7	13,1	96%	1,3	3,64	9,9%	2,32	6,50	17,7%	61,4%
Corn oil, high spiked	7	14	43,2	38,9	41,0	39,1	95%	2,29	6,41	5,9%	10,87	30,44	27,8%	52,1%
Cod liver oil, not spiked	6	12	0,0	0,0	0,0	2,5								78,8%
Cod liver oil, low spiked	5	10	14,4	13,0	13,7	12,6	92%	0,59	1,65	4,7%	2,16	6,05	17,2%	61,8%
Cod liver oil, high spiked	7	14	43,2	38,9	41,0	41,1	100%	3,12	8,74	7,6%	8,97	25,12	21,8%	51,7%

5. Results

The proposed cleanup resulted in sufficiently resolved chromatograms. The PCBs included in the extracts of cod liver samples did not complicate the determination of indicator CHBs. Some participants would have preferred a cleanup, separating PCBs from the slightly more polar CHBs. Only in some laboratories, coelution of CHBs with other compounds has been observed (critical pairs: indicator compound **3** and diethylhexylphthalate or mirex; compound **4** and cis-nonachlor or pp'-DDD or PCB 153). With a second capillary the resolution of interesting peaks could always be achieved. The influence of the injector temperature on the response of the indicator compounds **2** and **3** has been confirmed by the participants. Injector temperatures higher than 240 °C should be avoided. Sometimes, such capillaries working well in most laboratories have shown drastically reduced intensities of particular indicator compounds. Decomposition on active sites has been discussed as possible reason for this phenomenon.
A graphic representation of concentrations found versus true concentrations is shown in Figures 1-4. No significant systematic bias was found in the data submitted. The summary statistics calculated after removal of outliers are presented in Tables 1 - 4. Reproducibility standard deviations were found comparable to the Horwitz equation ($RSD_R = 2^{(1-0,5 \log c)}$), which is dependent from analyte concentration (c). This equation had been derived empirically from an examination of more than 3000 method performance studies. It has been proposed that reproducibility standard deviation values found within a range of 0.5 - 2 times the RSD_R may be considered as an acceptable precision of method performance between laboratories[3]. Horwitz' RSD_R values multiplied by a factor of 2 are used in Tables 1 - 4 as "highest acceptable relative standard deviation". Higher reproducibility standard deviations were never calculated from the data of this collaborative study.

6. Conclusions

The analytical method for the determination of the proposed toxaphene indicator compounds in a fatty matrix was shown to be quantitative even at concentrations of 10µg/kg fat. Recovery from a vegetable and a fish oil was uniform and reasonably precise. Based on the range of fat content in commercially significant fish species (1 - 20%) and the assumption of quantitative extraction of toxaphene residues together with the fish fat, a limit of determination of about 1-2µg/kg can be achieved on a wet weight basis. These results demonstrate the validity of the analytical method tested and the applicability of the toxaphene indicator compound concept.

7. References

1) Alder L., B. Vieth (1995): A Congener Specific Method for the Determination of Camphechlor (Toxaphene) Residues in Fish and Other Foodstuffs. Fresenius' J. Anal. Chem., in press
2) AOAC (1989): Guidelines for collaborative study procedure to validate characteristics of a method of analysis. J. Assoc. Off. Anal. Chem. **72**, 694-704
3) IUPAC, Analytical, applied and clinical chemistry divisions; Interdivisional working party for harmonization of quality assurance schemes for analytical laboratories (1990): Harmonized protocol for the adoption of standardized analytical methods and for the presentation of their performance characteristics. Pure Appl. Chem. **62**, 149-162