MATT: MONITORING, ANALYSIS AND TOXICITY OF TOXAPHENE - IMPROVEMENT OF ANALYTICAL METHODS

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Introduction

The European Research Project MATT (Investigation into the Monitoring, Analysis and Toxicity of Toxaphene) started in 1997 and has the following objective: to provide information on toxicological risks to the consumer of toxaphene residues in fish from European waters. The way to realise this objective is sub-divided in the following way:

- a study on the improvement of analytical methods for the determination of toxaphene
- a baseline survey on toxaphene concentrations in fish from European waters
- studies on the carcinogenicity of toxaphene extracts from fish.

This report includes information on the analytical block of this project. Progress in the toxicity studies is reported elsewhere in this volume (1). The baseline survey is currently being carried out.

The analytical block consists of three main studies:

- a series of stepwise-designed interlaboratory studies
- a study on gas chromatographic (GC) separation
- a study on GC injection techniques.

The results of these three studies are presented here. The studies were initially focused on the three congeners (CHBs) Parlar 26 [B12012-202] (2), 50 [B12012-212] and 62 [B30030-122], but at a later stage the nrs 40 [B12012-112], 41 [B21020-122] and 44 [B20030-122] were added, because they were reported to be the six most persistent toxpahene congeners (3). Total-toxaphene analyses were not carried out. Two of the partners were relatively experienced in the toxaphene analysis, two others had no experience at all at the start of the project.

Methods

A series of four interlaboratory studies was set-up between four partners of the project to gain insight in the possible sources of error in the analysis of a congener-specific toxaphene analysis. The partners were asked to analyse the following solutions and samples:

1) standard solutions of the CHBs 26, 50 and 62
2) cleaned-up extracts of cod liver and herring, herring oil and cod liver oil
3) cod liver, mackerel and plaice
4) herring oil.

The analytical methods used by the partners are given in Table 1. The internal standards used were chlorobiphenyl (CB) 112 (syringe standard), CHB 32 and ε-HCH. The GC columns used generally had lengths of 50-60 m and internal diameters of 0.15-0.25 mm.
Table 1. Analytical methods used in the interlaboratory studies

Method	Partner 1	Partner 2	Partner 3	Partner 4
Extraction				
Soxhlet extraction	x			
Pentane/dichloromethane1:1	x			
Ultraturrax		x	x	x
acetone + acetone/hexane1:3		x	x	
water/acetone/petroleum ether				x
Clean-up				
Gel permeation chromato-graphy (GPC, Biobeads®)				x
HP-GPC				x
Alumina column	x			
Silicagel column	x	x	x	x
Sulphuric acid	x	x	x	
GC separation and detection				
GC/ECD		x	x	x
GC/NCI-MS	x			
CP Sil8 type column	x	x	x	x
CP Sil19 column	x		x	x
HT 8 column	x			

The fish oils and wet sterilised fish samples were all tested on homogeneity which was found to be sufficient for the purpose of this exercise.

A qualitative multi-dimensional GC (MDGC) study was carried out in which a series of samples was analysed by MDGC and also by single column GC. The MDGC conditions were the following:
GC: Siemens Sichromat 2-8
Monitor column: CP Sil 8, 50 m, 0.15 mm, film thickness 0.25 mm
Main column: DX 4, 15 m, 0.25 mm, 0.25 µm
Carrier gas: nitrogen, monitor column: 3.5 bar, main column: 0.9 bar
Make-up gas: nitrogen
Injector: splitless (3 min), 235 °C, injection volume: 2 µl
Detector temperature: 310 °C
Temperature programmes: Monitor column: 1 min 80 °C, 30 °C/min to 180 °C, 2.5 °C/min to 250 °C, 40 min 250 °C; Main column: 1 min 80 °C, 30 °C/min to 180 °C, 28 min 180 °C, 2.5 °C/min to 240 °C, 40 min 240 °C.

A study on possible degradation of toxaphene congeners in the GC injector was carried out. Splitless injection was compared with on-column injection. The following parameters were studied: 1) optimisation of GC parameters by studying combined effects of pressure at injection, detector make-up gas flow and injection temperature, 2) different injection temperatures in pulsed splitless injection (after optimisation) and automatic on-column

injection. The experiments were carried out at an HP 6890 GC. Each time a CP Sil 8 column, 50m, 0.25 mm with a 0.25 µm film was used, with a ^{63}Ni ECD at 320 °C and hydrogen as a carrier gas. Specific parameters for the two studies:
1) Injector and detector parameters were tested in two full factorial designs with 3 variables in each test. The variables were tested in 8 experiments at two levels (Table 2).
2) Injection temperatures: 240-300 °C, 1 µl pulsed injection at 30 psi, tapered liner; on-column: 90 °C; optimised system.

Table 2. Optimisation of GC parameters

Test 1a			Test 1b		
Parameter	1	-1	Parameter	1	-1
Liner	Open	Tapered	Liner	Open	Tapered
Pressure	15 psi	30 psi	Pressure	15 psi	30 psi
Make-up gas	30 ml/min	60 ml/min	Temperature	240 ˚C	300 ˚C

Results and discussion
The coefficients of variation (CV) obtained in the four interlaboratory studies are given in Table 3. The results reflect on one hand the difficulties that particularly the inexperienced laboratories had in setting up a reliable toxaphene analysis, which shows the degree of difficulty of this analysis. On the other hand the results show that a CV value of ca. 20% is about the best of what can be obtained at the moment, even for more experienced laboratories. Although statistics based on n=4 do not tell very much, this value is in agreement with results obtained in other interlaboratory studies, such as a recent study organised by QUASIMEME in which CV values of 16-39% were obtained for the CHBs 26, 50 and 62 in cleaned marine mammal and fish extracts (4). The best results until now were obtained in a German study in which CV values of 9-50% with a mean of 23% were obtained for the CHBs 26, 50 and 62 in cod liver oil and corn oil (5). However, it should be mentioned that in this study the clean-up method was prescribed for all participants. CHB 62 shows higher CV values in most interlaboratory studies carried out until now, indicating the degree of difficulty of the determination of this congener.

Table 3. Coefficients of variation obtained in the MATT interlaboratory studies* (n=4).

Round	CHB 26	CHB 50	CHB 62	CHB 40	CHB 41	CHB 44
1. Standard Solution	2.4	20	15			
2a. Cleaned extract	31-46	34-35	32-42			
2b. Fish oil	12-13	21-25	30-36			
3. Fish matrices	20-37	19-29	34-74	21-37	19-38	19-38
4. Herring oil	26	22	23			

* CHB concentrations were: 1. ca. 200 µg/kg, 2a. 2-45 µg/kg, 2b. 30-70 µg/kg, 3. 0.04-45 µg/kg, 4. 12-24 µg/kg

The MDGC study showed that it is very difficult to separate CHB 26 properly from other CHBs. By using a 50m x 0.15 mm CP Sil 8 column as a first column, it was possible to obtain complete separation for the CHBs 50 and 62. This result was actually better than those of earlier MDGC experiments in which 25m columns were used as a first column (6). However, for CHB 26 no real improvement was obtained. It is expected that for the CHBs 40, 41 and 44

further separation problems will be encountered when using single-column capillary GC. Other columns are not of much help. At a CP Sil 19 column the CHBs 41 and 44 co-elute, as can the CHBs 40 and 50, dependent of the temperature programme used. The use of coupled columns may offer a solution, provided they are optimised for certain target CHBs (7). The determination of CHB 26 in particular may remain very difficult, as also a good fractionation of this congener at a silica column is difficult to achieve.
The first injection study showed that the use of a tapered liner is a great advantage in this type of analysis. The positive effect on the peak areas is greater for lower boiling compounds, thus showing more improvement for CB 112 compared to the CHBs. A higher inlet pressure in combination with the use of a tapered liner had particularly a positive effect on the determination of CHB 62. In an open liner higher pressures had a negative effect on the peak areas. In the second injection study higher temperatures resulted in slightly smaller peak areas for the CHBs but the peak areas of CB 112 increased almost linearly (Table 4). This indicates that CB 112 is not an ideal internal standard for CHB analyses. The repeatability under optimised conditions (tapered liner, pulsed injection, injector temperature 240 °C) was better with ca. 50% lower RSDs than under non-optimised conditions. In on-column injection the CHB peak areas were considerably larger, particularly for CHB 62, but lower for CB 112. The RSDs were smaller with on-column injection. However, these experiments were carried out with standard solutions. The injection of sample extracts may of course result in different results with on-column injection due to contamination of the first part of the column.

Table 4. Results of injection study 2. Shown are Area/RSD% values, n=5.

	CHB 26	CHB 50	CHB 62	CB 112
Splitless, 230 ˚C	11/3	14/4	6/6	131/2
Splitless, 250 ˚C	11/3	13/4	6/4	138/1
Splitless, 270 ˚C	10/3	13/2	6/4	142/1
On-column, 90 ˚C	15/1	25/1	15/1	85/1

Conclusions

- According to the current state-of-the-art between-laboratory CV values for a congener-specific toxaphene analysis cannot expected to be much lower than 20%.
- MDGC experiments show that particularly for CHB 26 and presumably also for a number of other toxaphene congeners co-elution occurs on CP Sil 8 type of columns.
- Injector temperatures above 240 °C cause thermal degradation of some toxaphene congeners. The use of on-column injection avoids thermal degradation, but may result in contamination of the column. Currently, pulsed splitless injection seems to be the most attractive technique.

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References

1. Besselink H, Brouwer A*; Organohalogen Compounds* **1999**, this volume
2. Wester PG, de Geus H-J, de Boer J, Brinkman UATh; *Chemosphere* **1997**, 35, 1187
3. Parlar H, Schulz-Jander D, Fingerling G, Koske G, Angerhöfer D, Burhenne J; *Organohalogen Compounds* **1998**, 35, 221
4. Anon.; QUASIMEME Laboratory Performance Studies Round 14, Exercise 379 DE-2,

1998, Aberdeen, UK
5. Alder L, Bache K, Beck H, Parlar H; *Chemosphere* **1997**, 35, 1391
6. de Boer J, de Geus H-J, Brinkman UATh, *Environ Sci Technol* **1997**, 31 , 873
7. Baycan-Keller R, Oehme M, Galliker B, *Organohalogen Compounds* **1998**, 35, 229