TRANSFER OF TOXAPHENE FROM ANIMAL FEED TO LAYING HENS AND THEIR EGGS - FIRST RESULTS OF A WITHDRAWAL STUDY

[1,3]M. Kaltenecker, [1]K.-H. Schwind, [2]K.-H. Ueberschär, [1]H. Hecht and [3]M. Petz

[1]Federal Centre for Meat Research, Institute for Chemistry and Physics, E.-C.-Baumann-Str. 20, D-95326 Kulmbach, Germany
[2]Federal Research Centre of Agriculture, Institute for Small Animals, Dörnbergstr. 25-27, D-29223 Celle, Germany
[3]University of Wuppertal, Department of Food Chemistry, Gauss Str. 20, D-42097 Wuppertal, Germany

Introduction

Because of its occurrence in sea fish toxaphene is coming more and more into the public interest. Since the year 1995 analytical standards are available for the congener specific determination of toxaphene residues [1-3]. Due to the lack of data concerning the carry-over of toxaphene in farmed animals and their food-products thereof, a feeding study with laying hens was started. An important aim of the study was to determine the toxaphene congeners with the highest accumulation potential in hens and eggs. From the withdrawal study biological half-life-times of these congeners should be calculated looking on their decrease in different tissues.

Material and Methods

The study was carried out with 39 hens (race LSL, in single cages) divided up into 2 groups. 21 hens were fed with 5.0 ppm technical toxaphene added to the diet, beginning from the 22nd week of life and continued up to the 60th week. A group of 18 hens (the control group) was fed with the same diet without toxaphene contamination. The withdrawal study started at the beginning of the 61th week of the hen's life and was continued for 15 weeks. Like the weeks before, always five eggs from five different animals were separated in yolk and eggwhite. After pooling and homogenizing, the samples were analysed for toxaphene residues. Over the whole time of the

feeding study faeces samples (pooled from three different animals) were taken. At seven defined dates always three hens were killed from the 5 ppm-toxaphene group and two, respectively three hens from the control group. Samples of kidney, liver, skin, blood, meat, fatty tissue and faeces from these animals each were pooled and then analysed for toxaphene.

Extraction and clean-up

Approximately 1 g fatty tissue, 3 g yolk, liver, kidney, meat, skin or 10 g faeces, meat, diet or eggwhite was mixed with 10 g seasand and up to 40 g dried Na_2SO_4 (depending on the water content of the sample). After adding $^{13}C_{12}$-PCB 77 as standard, these mixtures were extracted with hexane in a Soxhlet apparatus. After removing the solvent and weighing the residue, no more than 1 g of the lipid residue was diluted in 10 ml hexane and then chromatographed over a column (i.d. 20 mm) with 25g Florisil (deactivated with 3% water) to remove the lipids [4]. The toxaphene congeners were eluted with 300 ml hexane/dichloromethane (v,v 80+20). After rotary evaporation the samples were concentrated with nitrogen. Then $^{13}C_{12}$-PCB 81 as quantification standard was added and the samples carefully concentrated to 100 µl.

GC/HRMS analysis

The analytical determination of toxaphene in all samples was carried out on a GC/HRMS system. Twentytwo toxaphene congeners from a commercially available standard mixture were monitored.

GC: HP 5890 Series II; Carriergas: Helium; Injector: 240°C; Column: DB-5 (30 m x 0.25 mm x 0.25 µm); Temperature ramp: 70°C (2 min) - 225°C (22.5°C/min) - 230°C (1°C/min, 5 min) - 320°C (20°C/min, 1.5 min) [5]; Injection: 2 µl, 1.0 min splitless;

HRMS: VG Autospec; Interface temp.: 250°C; Ionsource temp.: 250°C; Ionisation: EI-Mode, 35 eV; Tuning: Manual tune; Mass resolution: 10.000; Detection mode: SIR;

For identification of the toxaphene congeners we monitored the dichlorotropylium-ion traces at 158.9769 and 160.9739 amu [6]. Quantification was done using the ion trace at 158.9769 amu.

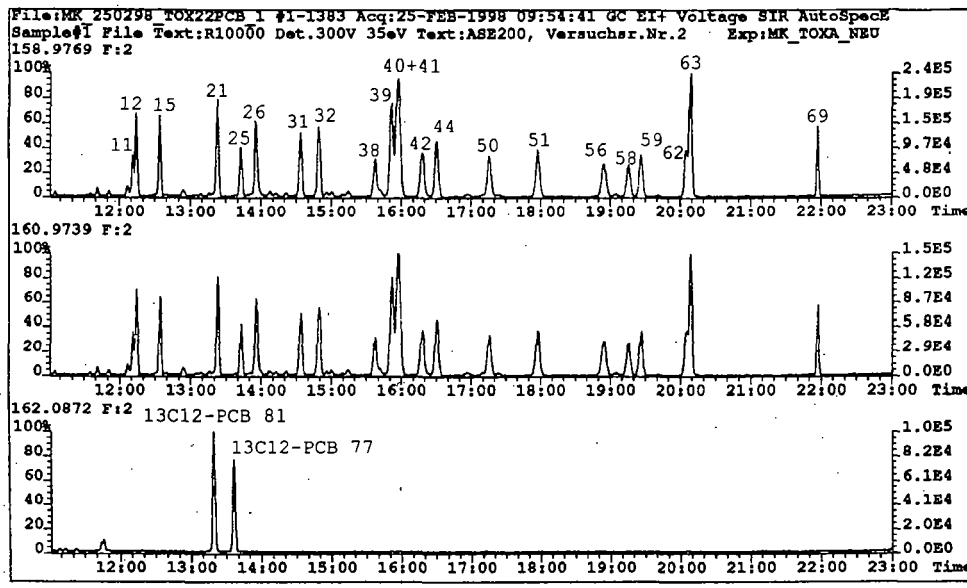

Fig.1: Chromatogram of the 22 congener standard mixture with the used $^{13}C_{12}$-PCB compounds

Standard compounds

For identification and quantification of toxaphene residues a 22 congener standard mixture (congener numbers according to Parlar [7]) obtained by Dr. Ehrenstorfer GmbH, Augsburg, was used. $^{13}C_{12}$-PCB standards were delivered from Promochem GmbH, Wesel. A chromatogram of all standard compounds used is given in [Fig. 1].

Results and Discussion

Measurement of toxaphene by a mass spectrometer working in EI-ionisation mode and monitoring the dichlorotropylium-fragment-ion may have some problems. There are some components eg. endosulfan [8], which produce the same dichlorotropylium-fragment-ion as toxaphene compounds. But in our investigations we were not confronted with one of these interfering compounds. The samples from hens of the control group, living under the same controlled conditions and fed with the same uncontaminated basic feed did not show interferences from such compounds. Another reason for the rare occurrence of interferences in the chromatograms is the fact that most of interferences resulting from non-toxaphene components can be eliminated efficiently by working with high resolution mass spectrometry. Although the mass spectrometer worked in the EI-ionisation mode routineously at a mass resolution of 10.000 detection limits for each of the 22 congeners in the tissue samples in the range of 1 to 10 µg/kg fat were reached.

In the eggwhite samples only traces of toxaphene could be found. They were about three orders of magnitude below the concentrations found in the yolk samples. This result is not surprising because eggwhite nearly is free of fat.

The congener pattern showed only small difference between yolk, fatty tissue [Fig. 2], meat, liver, blood, skin, meat and kidney [Fig. 3]. A noticeable difference resulted, however, in the case of congener 69. This component was found in increased concentrations in fatty tissue, skin and yolk, but in very low concentrations in liver, kidney and blood.

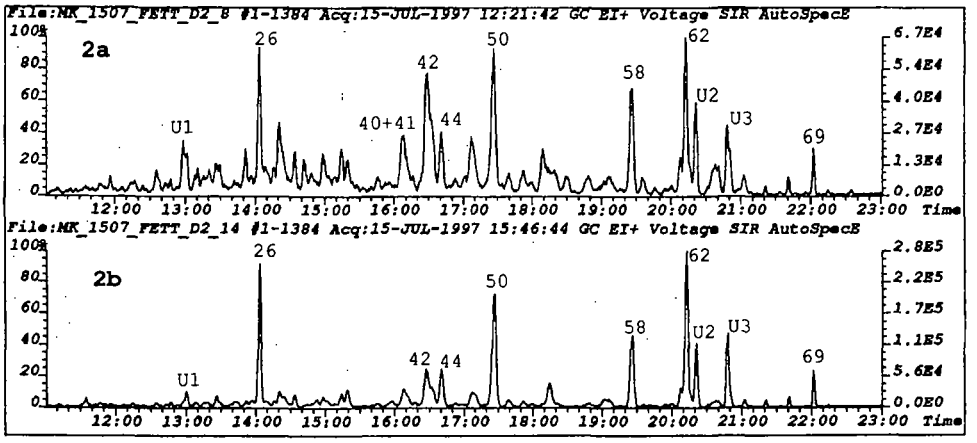

Fig. 2: Withdrawal of toxaphene in fatty tissue from hens
2a: Toxaphene pattern (m/z: 158.9769) at the beginning of the withdrawal study
2b: Toxaphene pattern (m/z: 158.9769) after 105 d

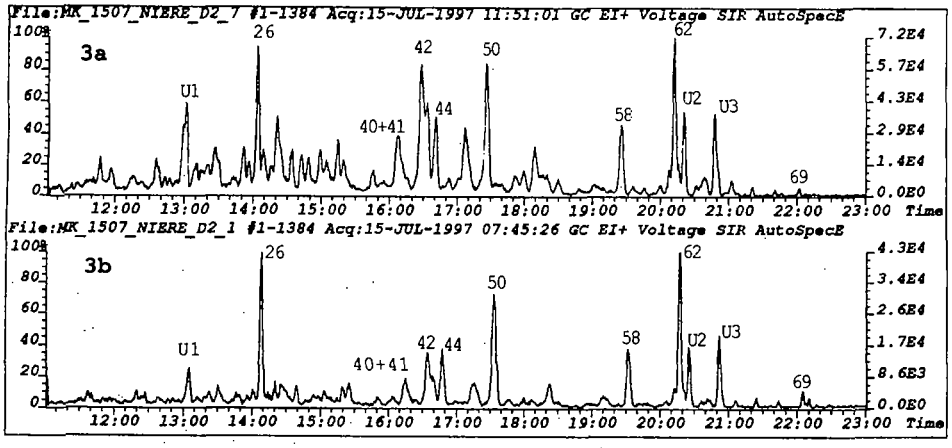

Fig. 3: Withdrawal of toxaphene in kidney of hens
3a: Toxaphene pattern (m/z: 158.9769) at the beginning of the withdrawal study
3b: Toxaphene pattern (m/z: 158.9769) after 105 d

Our investigations showed that there is a number of toxaphene congeners accumulating in all tissues and in yolk. We were able to identify 8 main congeners (Parlar 26, 40+41, 42, 44, 50, 58, 62, 69) in the samples by the aid of the 22 component standard. But there are some other unknown peaks which doubtlessly are toxaphene components, too. Three of the unknown peaks (U1, U2, U3) are shown in the chromatograms of Fig. 2 and Fig. 3. The peaks U2 and U3 seem to have an accumulation potential of the same order as the congeners 26, 50 and 62.

From the withdrawal study we could determine half-life-times for the identified toxaphene congeners in most of the investigated tissues. A selection of them (congeners with the highest accumulation potentials in kidney and fatty tissue) is given in Tab. 1.

Tab. 1: Half-life-times of toxaphen congeners in kidney and fatty tissue

	half-life-time in days						
	Parlar no. 26	Parlar no. 42	Parlar no.44	Parlar no. 50	Parlar no.58	Parlar no.62	Parlar no.69
kidney	44	29	36	40	51	49	---
fatty tissue	40	25	35	36	36	42	38

As can be seen there are surprisingly small differences in the congeners half-life-times between fatty tissue and kidney.
There was no possibility to determine biological half-life-times in liver, because the variation of the results was too high.

Fig. 4 shows the elimination of the congeners 26, 50 and 62 in fatty tissue. The shape of the curves seems to be approximately of the exponential type. But if the toxaphene tissue concentrations are drawn in logarithmic axis, it is possible to draw one straight line through the first four days of determination (up to 27 days) and another one through the last four points day (27 to day 105) but with different slopes. The first line reflects the biological half life for the reduction in the tissue itself, the second is determined by transportation effects. The biological half lifes calculated from the first line differ only very little from those given in tab.1. The biological half lifes ruled by the transportation effects are 53 days (congener 26), 56 days (congener 50) and 59 days (congener 62).
These effects are further investigated in our institutes.

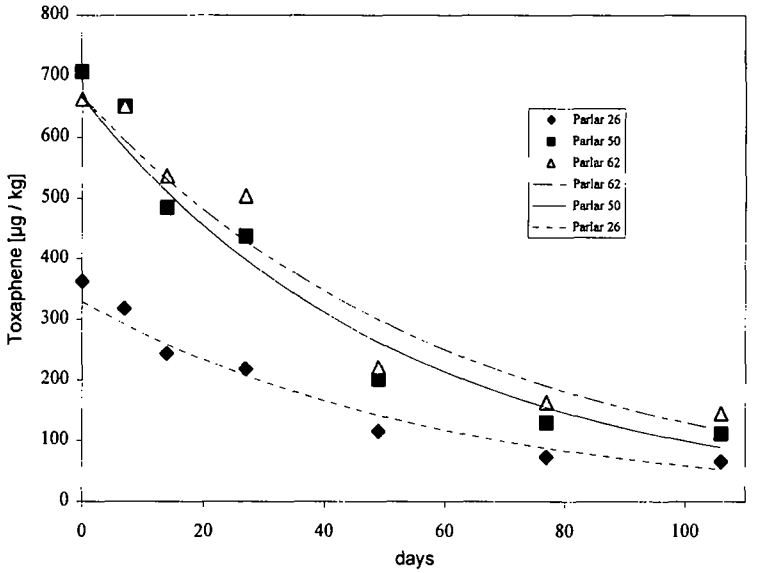

Fig.4: Progress of elimination of the congeners 26, 50, 62 in fatty tissue

To control the quality of the analytical results, a number of samples were measured on different GC-columns. One of these columns was coated with the phase Optima 17 from Machery - Nagel. This more polar column is able to separate the congener Parlar 42 into two isomeric components (42a and 42b). Moreover, a base line separation of the congener pair 40 and 41 is possible, in contrast to the DB-5 column. Nevertheless we used the DB-5 column because the results for the most important congeners obtained on this column showed only a very small difference then compared to the Optima 17 phase and furthermore chromatographic run time on a 30m DB-5 column is much shorter.
A chromatogram of the 22 congener standard mixture on an Optima 17 column is shown in Fig.5.

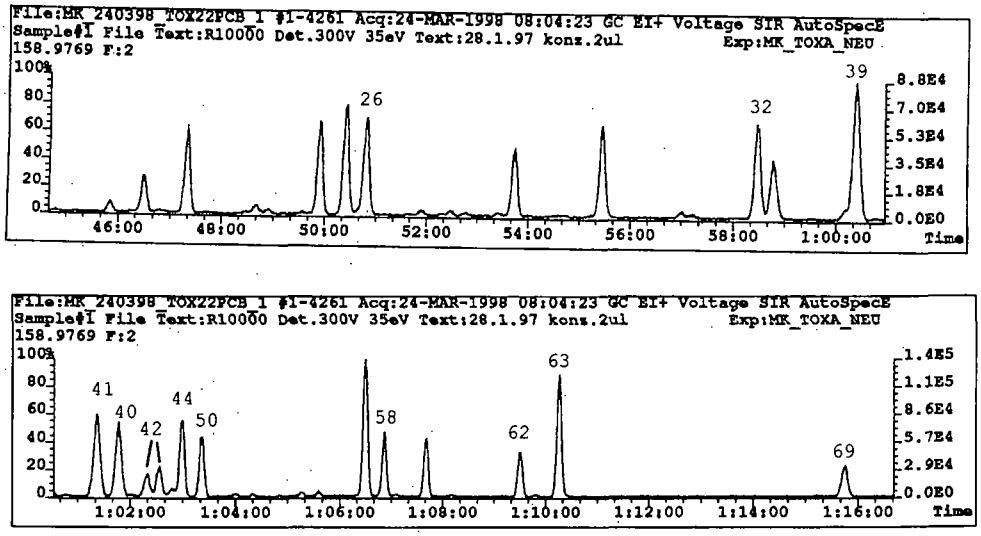

Fig.5: Separation of the 22 toxaphene congener standard mixture (m/z: 158.9769) on a MN Optima 17 column (50 m x 0.25 mm x 0.25 µm). Temperature ramp: 70°C (2 min) – 180°C (15°C/min) - 250°C (1,5°C/min, 5 min) - 290°C (10°C/min, 15 min) [9].

Acknowledgements

The authors would like to express their thanks to the Wilhelm-Schaumann-Stiftung Hamburg, Germany, for the grant for M. Kaltenecker. Furthermore they are very grateful to H. Parlar, University of Munich and W. Vetter University of Jena, for helpful discussions and their support.

References

[1] Hainzl D., Burhenne J., Barlas J., Parlar H.; *Fresenius Z. Anal. Chem.*, 351, 271-285 (1995)

[2] Burhenne J., Hainzl D., Xu L., Vieth B., Alder L., Parlar H.; *Fresenius Z. Anal. Chem.* 346, 779-785 (1993)

[3] Hainzl D., Burhenne J., Parlar H.; *GIT Fachz. Lab.* 4, 286-294 (1994)

[4] Beck H., Mathar W.; *Bundesgesundheitsblatt* 28, 1 - 12 (1985)

[5] Gill U.S., Schwartz H., Wheatly B., Parlar H., *Organohal. Compounds* 26, 383-387 (1995)

[6] Saleh, M.A.; *J.Agric.Food Chem.* 31, 748-751 (1983)

[7] Coelhan M., Parlar H.; *Chemosphere*, 32, 217-228 (1996)

[8] Lau B., Andrews P.; *Chemosphere*, 32, 1021-1041 (1996)

[9] Bartha R., Vetter W., Luckas B.; *Fresenius Z. Anal. Chem.* 358, 812-817 (1997)