Tris(4-chlorophenyl)methanol and tris(chlorophenyl)methane in marine mammals and fish from the North Sea and Dutch Wadden Sea

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Abstract

An analytical method for the determination of tris(4-chlorophenyl)methanol (TCP) in organisms was developed. With this method, based on soxhlet extraction, gel permeation chromatography, fractionation over silica and GC/NCI-MS analysis, TCP could be determined in marine organisms with a detection limit of 0.02 µg/kg and a recovery of 90%. TCP concentrations in marine mammals from the North Sea and the Dutch Wadden Sea varied between 0.2 and 2 mg/kg on a lipid weight basis. Tris(chlorophenyl methane) was also identified, but could not be quantified due to lack of a standard.

1. Introduction

Several scientists have reported the occurrence of tris(4-chlorophenyl)methanol (TCP) and tris(chlorophenyl)methane (TCPMe) (Fig. 1) in environmental samples from various parts of the world. Walker et al.[1] reported TCP concentrations in seals, found dead between 1972 and 1982 in Puget Sound, Northwest USA, of 23-750 µg/kg on a lipid weight basis. Jarman et al.[2] reported the presence of TCP in various birds and marine mammals from the Arctic and Antarctica, Australia and the US. They reported TCP levels up to 4 mg/kg lipid weight in bird eggs from British Columbia and a TCP concentration of 6.8 mg/kg lipid weight in a polar bear from the St. Lawrence river, Canada[2]. Zook et al.[3] reported TCP concentrations up to 3 mg/kg lipid weight in seal livers from the Baltic. In most samples TCPMe was also identified, but no quantitative data on that compound are

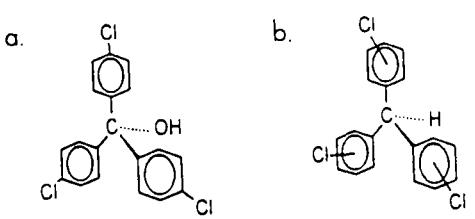

Figure 1. Structures of TCP (a.) and TCPMe (6).

available[1-3]. There is only few information available on sources of TCP. Possibly it is a derivate of TCPMe or TCP methylchloride, which are used in the production of dyes [2,4,5]. Until now data on TCP and TCPMe in samples from the North Sea were not available. We have, therefore, developed a method for the determination of TCP and TCPMe, and carried out a screening on these compounds in marine mammals and fish from the North Sea and the Dutch Wadden Sea.

2. Experimental

TCP was analysed by GC/ECD, GC/EI-MS and GC/NCI-MS. Relative retention times vs. 1,2,3,4-tetrachloronaphthalene (TCN) were measured. Retention times and detection limits are given in Table 1. CP-Sil 8 and CP-Sil 12 columns with lengths of 45-50 m, internal diameters of 0.15-0.25 mm and film thicknesses of 0.2-0.3 µm were used. The temperature of the GC oven was programmed from 90°C to 290°C. A splitless injection technique was used.

Table 1. Detection limits and retention times of TCP

Method: Column:	GC/ECD CP-Sil 8	GC/EI-MS CP-Sil 12	GC/NCI-MS CP-Sil 12
Detection limit (pg)	10	30	3
Detection limit (µg/kg)	0.07	0.2	0.02
Retention time (min)	85.88	33.46	33.46
Retention time relative to TCN	3.22	2.26	2.26

The ions used for identification were: GC/EI-MS: 111, 139 (quantification), 141, 251, 362 and 364 and for GC/NCI-MS: 362 (quantification), 364 and 366.
It was not possible to elute TCP from an alumina column without co-eluting the lipids present in the extract after soxhlet extraction. TCP was not stable when mixing with sulphuric acid. Therefore, gel permeation chromatography (GPC) was tested as a method for the separation of TCP and lipids. A column with a length of 33 cm and an internal diameter of 2 cm, filled with Bio beads S-X3 in dichloromethane/hexane (1:1 v/v) was used under atmospheric pressure. TCP eluted between 70 and 150 ml, whereas 99% of the lipids eluted before 70 ml. Under nitrogen pressure the elution was accelerated from 1-2 ml/min to 10 ml/min and an improved separation was obtained. In order to separate TCN, together with chlorinated pesticides, from PCBs, a silica fractionation was carried out over a 1.8 g SiO_2 2% H_2O column (i.d.: 0.6 cm). TCN eluted in the second fraction of 10 ml 15% diethylether in iso-octane, after a first fraction with PCBs of 11 ml iso-octane.
The recovery of a TCP-spiked seal blubber sample (306 ng TCP in 1 g seal blubber) after soxhlet extraction with 120 ml dichloromethane/pentane (1:1 v/v), GPC and fractionation over silica was 90%.

3. Results and discussion

Table 1 shows that there is not much difference between detection limits obtained with GC/ECD and GC/NCI-MS. Because of its higher selectivity GC/NCI-MS is to be preferred. Generally compounds with less than four Cl-atoms cannot be measured properly with GC/NCI-MS because of a too low sensitivity. In this case the presence of the electronegative oxygen apparently causes a sufficient response for TCP. Results of TCP

determinations in marine mammals and cod liver from the North Sea and the Dutch Wadden Sea are given in Table 2.

Table 2. Concentration of TCP in blubber of marine mammals and in cod liver from the North Sea and Dutch Wadden Sea

Species	Location	Year	Age (years)	Sex	TCP-conc. (µg/kg lipid w.)	Fat content (g/kg)
Common Seal (1)	Wadden Sea	1990	2	f	2,000	385
Common Seal (2)	Wadden Sea	1992	0,01	[c]	750	563
Common Dolphin	Wadden Sea	1992	5	f	220	824
Dolphin [a]	Central North Sea	1989	[d]	f	190	839
Whitebeaked dolphin	Wadden Sea	1992	[e]	m	570	614
Whitebeaked dolphin	Dutch coast	1990	10	m	1,400	719
Harbour porpoise	Dutch coast	1990	>10	m	1,000	666
Cod (liver) [b]	Dutch coast	1993			18	448

[a] Precise species unknown; [b] pooled sample of 25 livers; [c] sex unknown; [d] age unknown, length 220 cm; [e] age unknown, length 216 cm, weight ca. 85 kg.

The TCP concentrations found correspond with those reported by Zook et al. [3] in Baltic seal blubber (up to 3 mg/kg lipid weight). TCP concentrations in seals from Puget Sound, USA, were lower (23-750 µg/kg lipid weight)[1]. Apparently, there is not much difference in biomagnification of TCP between seals and other marine mammals. The much lower TCP concentration in cod liver, 25-35-fold lower than in harbour porpoise and whitebeaked dolphin from the same area, points to a strong biomagnification. This is confirmed by the value of the octanol-water partition coefficient, calculated by the C logP method[6]. The $\log K_{d,oct}$ value is relatively high, that is 6.0 for TCP and 6.5 for TCPMe. The TCP concentration in cod liver is comparable to concentrations of HCB and p,p'-DDT in the same matrix [7].
There are no data available on the toxicity of TCP. Dicofol, a pesticide with a structure comparable to TCP, has a relatively low acute toxicity compared to other organochlorine pesticides: LD_{50} for rats: 668-842 mg/kg[8].
In a screening with GC/EI-MS on masses 346, 348 and 350 the presence of TCPMe could also be confirmed. TCPMe was present in both fractions after the silica elution. Due to a lack of standard, TCPMe could not yet be quantified.

4. Conclusions

TCP and TCPMe were identified in marine mammals and cod liver from the North Sea and the Dutch Wadden Sea.
TCP can be determined in organisms by an analytical method consisting of soxhlet extraction, gel permeation chromatography, silica fractionation and GC/NCI-MS, with a detection limit of 0.02 µg/kg and a recovery of 90%. More information on the toxicity, production and/or application of TCP and TCPMe is requested.
TCP concentrations in marine mammals varied between 0.2 and 2 mg/kg on a lipid weight basis. These concentrations confirm the global presence of these compounds and indicate the need for a further research of the presence of these compounds in fish and sediment from the Dutch aquatic environment.

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