Tris(4-chlorophenyl)methane and -methanol in Biota from the Polish Coastal Waters in the Baltic Sea

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Abstract
Tris(4-chlorophenyl)methane (TCPM-H) and *tris*(4-chlorophenyl)methanol (TCPM-OH) were detected (3.7-120000 ng/g lipid) in Baltic fish, marine mammals, fish-eating birds and white-tailed sea eagle but were undetected (<0.3 ng/g lipid) in plankton, mollusc and crustacean. Fish, mostly edible species, contained TCPM-H and TCPM-OH in concentration from 1.4 to 30 (11±8) and from <0.3 to 11 (4.4±3.0) ng/g on a fresh weight basis, respectively. TCPM-H/OH seems to be much more persistent under environmental conditions than DDT and its metabolites - possibly an exclusive or a main source of TCPM-H in the environment. A positive correlation ($p < 0.05$ - < 0.001)) was found between TCPM-H/OH and DDTs (*p,p'*-DDT + *p,p'*-DDD + *p,p'*-DDE + *p,p'*-DDMU) in fish and white-tailed sea eagles.

Key words: *Tris*(4-chlorophenyl)methane; TCPM-H; *tris*(4-chlorophenyl)methanol; TCPM-OH; DDT; plankton; mollusc; crustacean; fish; fish-eating birds; marine mammals; eagle

Introduction

Tris(4-chlorophenyl)methanol (TCPM-OH) was first identified as an environmental contaminant by Walker *et al.* (1) in biota in the North America in 1989 and its occurrence in the global scale was further indicated by other scientists (2, 3). Apart from TCPM-OH, recently also *tris*(4-chlorophenylo)methane (TCPM-H) was quantified (3, 4) in wildlife and

human milk, while both those xenobiotics were undetected (> 0.5 ng/g lipid wt) in fish such as tuna *Katsuwonus pelamis*, sward-fish *Xiphias gladius*, perch *Perca fluviatilis*, eel *Anguilla anguilla*, angler *Lophius piscatorius*, mullet *Mullus surmuletus* and octopus *Octopus vulgaris* caught in the Mediterranean Sea and sold in Italy (4).
TCPM-H (4,4′,4′′-TCPM-H) and its isomers (2,2′,4′′- and 2,4′,4′′- TCPM-H) were identified recently in technical DDT including a formulation produced more than 40 years ago (5). Additionally, TCPM-H and its isomers are formed in the reaction of chloral, chlorobenzene and fumic sulphuric acid under the same conditions as in the manufacture of the insecticide DDT, while all ten theoretically possible isomers are produced in the reaction of DDT with chlorobenzene in the presence of anhydrous $AlCl_3$ (5). It was suggested that DDT but not the synthetic (optically active) high polymers and compounds used in the production of synthetic lightfast dyes for acrylic fibres are a possible source of the environmental pollution with TCPM-H and TCPM-OH. TCPM-OH was detected in archived sample of beluga whale fat collected at the time (1952 year), when possible sources like the optically active polymers and synthetic dyes containing precursors of TCMP-OH were of less importance or absent.

This communication reports the results of the study on occurrence and relationship of TCPM-H/OH and DDTs in biota from the southern part of the Baltic Sea.

Materials and Methods
Fish (perch, lamprey, three-spined stickleback, flounder, sand eel, lesser sand-eel, round goby, eelpout, pikeperch, herring and cod) were caught in south-western part of the Gulf of Gdańsk in 1992, harbour porpoises were collected in the southern part of the Baltic Sea in 1991-1993, black cormorants were collected in the Gulf of Gdańsk in 1992, white-tailed sea eagles were collected from the coastal and inland breeding sites in Poland in 1991-1995 and an egg of white-tailed sea eagle was collected from the pair breeding at the Baltic coast in north-western Poland in 1995. The analytical method used for determination of TCPM-H/OH is a part of a multi-residue procedure allowing to determine simultaneously many organochlorines and polynuclear aromatic hydrocarbons (PAH) (6). After homogenisation of the sample with anhydrous sodium sulphate, the powdered mixture was spiked with an internal standard 1 ($^{13}C_{12}$ - *p,p′*-DDT). After gravimetric extraction in an open tube with 500 ml of a mixture of acetone and *n*-hexane followed by 500 ml of *n*-hexane and diethyl ether the solvents were carefully evaporated on a water bath under vacuum pressure, using a rotary evaporator. Bulk lipid removal was performed by means of the polyethylene film dialysis method (7). The extract was split into two parts, of which 10% was used for the analysis of TCPM-H, TCPM-OH, some organochlorine pesticides and PCBs, while 90% was used for analysis of some planar compounds such as PCNs, PCDDs, PCDFs, R-PCDFs and non-*ortho* PCBs. The remaining fat was removed on *n*-hexane wetted a Florisil gel column, and TCPM-H (fraction 2) and TCPM-OH (fraction 3) were eluted with 38 ml (15:85, v/v) methylene chloride in *n*-hexane (F2) and 30 ml (50:50, v/v) methylene chloride in *n*-hexane (F 3) (2). Before concentration of each fraction down to a final volume tetradecane (30 μl) was added to the vials as a keeper. An extract was than spiked with an internal standard 2 - recovery standard ($^{13}C_{12}$ - 2,2′,4,5,5′-pentachlorobiphenyl), and the

results were corrected for recoveries. Analysis and detection was accomplished by means of 60 m long capillary column (Rtx-5) gas chromatography and low resolution mass spectrometry (HRGC/LRMS). Additional procedural blanks were also performed and no interference were found. The mass spectrometer used was a Fisons MD 800 coupled to a Fison GC 8000 working in the Electron Impact (EI) ionisation mode using selected ion recording (SIR). Authentic reference material was used in identification and quantification both of TCPM-H and TCPM-OH.

Results and Discussion

TCPM-H/OH were detected in all fish, harbour porpoises, black cormorants and white-tailed sea eagles examined and in an egg of white-tailed sea eagle, while were below detection limit of the method (< 0..3 ng/g on a lipid weight basis) in plankton, blue mussel and crab (Table 1).

Surprisingly, both TCPM-H and TCPM-OH were not detected in organisms lower in their position in marine food web such as mixed fito- and zooplankton, mollusc and crustacean, while were quantified in collected from the same area, a plankton feeding herring, molluscivorous flounder and also in nearly exclusively feeding on herring harbour porpoise. All fish in this study contained detectable concentrations of TCPM-H, while TCPM-OH was not detected (<0.3 ng/g lipid) except of herring.

Fish examined contained TCPM-H, TCPM-OH and TCPM-H/OH in concentration 0.56±0.46 (0.08-1.6), 0.21±0.13 (ND-0.4) and 0.75±0.55 (0.30-2.0) ng/g when expressed on a wet (fresh) weight basis. The fish TCPM-H/OH data (Table 1) can explain an origin and route of exposure of both those xenobiotics to human.

TCPM-H/OH were quantified also in cod-liver oil and mackerel oil of the North Sea origin and TCPM-H was a minor component (0.6-2.9 ng/g) when compared to TCPM-OH (35-51 ng/g) (4). In fish in our study concentration of TCPM-H was more than twice that of TCPM-OH, on the average (Table 1). TCPM-H is a possible substrate to TCPM-OH in the environment. A fish-eating animals such as harbour porpoise and black cormorant contained TCPM-OH in higher percentage that TCPM-H, what implies a metabolism of the later compound. Nevertheless, TCPM-OH seems to be highly retained in liver and the breast muscles of black cormorant. In the tissues and an egg of white-tailed sea eagle the concentrations of TCPM-OH and TCPM-H were roughly similar (Table 1).

TCPM-H content of two technical DDT formulations examined by Buser was ~0.0038 % (~0.0015 - ~0.006) (5). The contribution of TCPM-H/OH when added to DDTs content of fish, harbour porpoise, black cormorant and white-tailed sea eagle (DDTs data are not shown in Table 1) is 1.0, 0.27, 2.4-7.0 and 0.26-2.0 %, respectively. A much higher contribution of TCPM-H/OH to DDTs (*p,p'*-DDT + *o,p'*-DDT + *p,p'*-DDD + *o,p'*-DDD + *p,p'*-DDE + *o,p'*-DDE + *p,p'*-DDMU) in biota examined in this study than of TCPM-H to DDTs content in technical DDT formulation implies, assuming that insecticide DDT is a sole or a main source of TCPM-H/OH in the environment, a very high environmental

Table 1. TCPM-H and TCPM-H concentrations (ng/g lipid) in fish, porpoise, black cormorant and white-tailed sea eagle from the southern part of the Baltic Sea

Sample	No.	TCPM-H	TCPM-OH	TCPM-H/OH
Plankton	4	<0.3	<0.3	<0.3
Blue mussel	2 (700)*	<0.3	<0.3	<0.3
Crab	1 (3)	<0.3	<0.3	<0.3
Fish				
- whole body	18**	11±8 (1.4-30)	4.4±3.0 (<0.3-11)	16±10 (3.7-37)
Harbour porpoise				
- blubber	4	6.8±1.7 (2.9-12)	15±2 (10-19)	21±7 (13-30)
Black cormorant				
- breast muscle	3	56±16 (25-77)	170±14 (93-190)	230±77 (120-300)
- liver	3	75±16 (60-82)	550±58 (530-580)	620±5 (620-630)
White-tailed sea eagle				
- breast muscle	13	5300±9800 (9.4-33000)	6700±15000 (<1-54000)	12000±24000 (13-73000)
- liver	6	20000±44000 (<1-110000)	5300± 8100 (<1-22000)	25000±48000 (<1-120000)
- adipose fat	2	4.5 (2.0-7.0)	10 (9.0-10)	15 (12-16)
- egg	1	1200	1300	2500

*number of pooled samples and number of animals (in parentheses)
**11 species and 18 pooled samples (total 207 specimens)

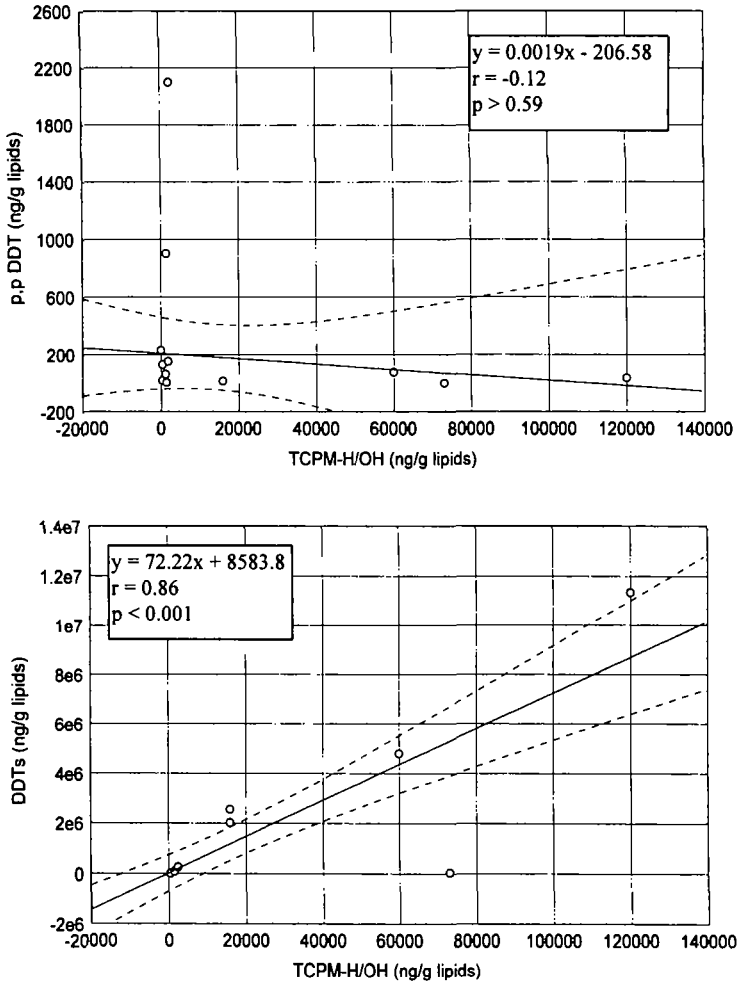

Figure 1. Regression between TCPM-H/OH and *p,p'*-DDT/ DDTs in white-tailed sea eagles.

persistency of TCPM-H/OH. The concentrations of TCPM-H/OH are positively correlated to *p,p'*-DDD, *p,p'*-DDE, *p,p'*-DDMU and DDTs (*p,p'*-DDT, *p,p'*-DDD, *p,p'*-DDE, *p,p'*-DDMU) (<0.001 $p < 0.01$) but not to *p,p'*-DDT in sea eagles (Figure 1). There is a positive correlation also between *p,p'*-DDD and TCPM-H/OH ($p < 0.05$), and *p,p'*-DDMU and TCPM-H/OH ($p < 0.01$) in fish but not for *p,p'*-DDT, *p,p'*-DDE and DDTs ($p < 0.05$) (Figure 2). In the case of harbour porpoise only few animals were examined and DDTs to TCPM-H/OH relationship seem to be apparent. The data obtained suggest a connection of TCPM-H/OH concentrations with the application rates of the insecticide DDT.

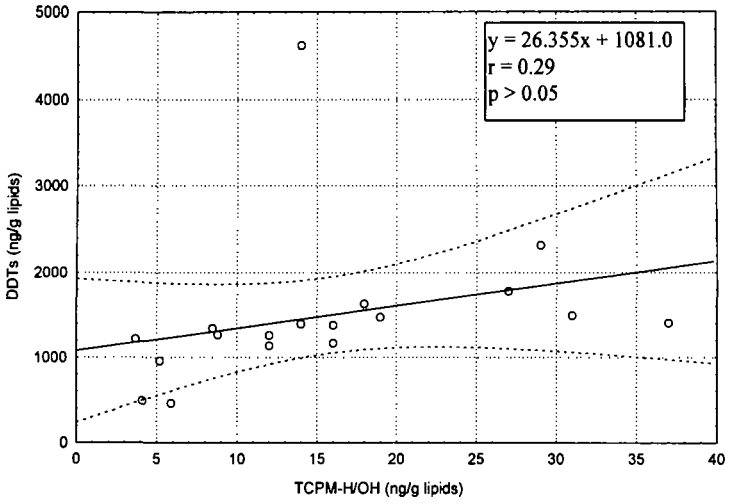

Figure 2. Regression between TCPM-H/OH and DDTs in fish.

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