Bull. Environ. Contam. Toxicol. (1998) 61:411-418


Bulletin of Environmental Contamination and Toxicology

# Spatial Distribution of TCPM-H and TCPM-OH in Blue Mussel and Fish from the Gulf of Gdansk, Baltic Sea

J. Falandysz[1] B. Strandberg,[2] L. Strandberg,[1] P.-A. Bergqvist,[2] C. Rappe[2]

[1] Department of Environmental Chemistry and Ecotoxicology, University of Gdansk, ul. Sobieskiego 18, PL. 80-952 Gdansk, Poland
[2] Institute of Environmental Chemistry, Umea University, S-901 80 Umea, Sweden

Received: 15 April 1998/Accepted: 30 June 1998

Tris(4-chlorophenyl)methanol (TCPM-OH) was for the first time quantified as an environmental pollutant in harbor seal *Phoca vitulina* from Puget Sound in the North America by Walker *et al.* (1989). Further a global scale distribution of TCPM-OH in the animals higher in their position in a trophic web such as herring gull *Larus argentatus,* peregrine falcon *Falcon peregrinus anatum,* great blue heron *Ardea heroidas,* beluga whale *Delphinapterus leucas,* harp seal *Phoca groenlandica,* polar bear *Ursus maritimus,* northern fur seal *Callorhinus ursinus,* Antarctic fur seal *Arctocephalus gazela,* Australian sea lion, *Neophoca cinerea* and Californian sea lion *Zalophus californianus* was indicated by Jarman *et al.* (1992). TCMP-OH was also quantified in marine mammal such as ringed seal *Phoca hispida* from the Baltic Sea (Zook *et al.* 1992).

Tris(4-chlorophenyl)methane (TCPM-H) is a possible substrate to TCPM-OH. TCPM-H was identified in parallel to TCPM-OH in peregrine falcon eggs and in blubber and liver of ringed seal (Jarman *et al.* 1992, Zook *et al.* 1992). Both TCPM-H and TCPM-OH were recently quantified in Italian and Swedish human milk, cod *Gadus morhua* liver oil and mackerel *Scomber scombrus* oil of the North Sea origin, while were undetected (< 0.5 ng/g lipid) in tuna *Katsuwonus pelamis,* sword-fish *Xiphias gladius,* perch *Perca fluviatilis,* eel *Anguilla anguilla,* angler *Lophius piscatorius,* mullet *Mullus surmuletus* and octopus *Octopus vulgaris* caught from the Mediterranean Sea and sold at the Italian market (Rahman *et al.* 1993).

The synthetic (optically active) high polymers and compounds used in the production of synthetic lightfast dyes for acrylic fibres were suggested to be a possible sources of the environmental pollution with TCPM-H and TCPM-OH, nevertheless, no such links could be confirmed. 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 (Buser, 1995). The agrochemicals such as the insecticides DDT and dicofol, due to their structural analogy to TCPM-H and TCPM-OH, respectively, were suggested also as a

*Correspondence to:* J. Falandysz

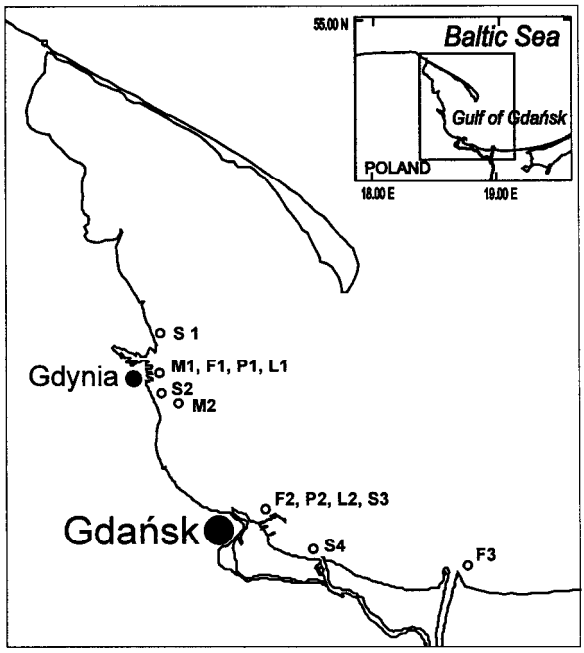


**Figure 1.** The sampling sites of blue mussel (M1 and M2), three-spined stickleback (S1-S4), flounder (F1-F3), perch (P1 and P2) and lamprey (L1 and L2) in the Gulf of Gdansk.

plausible source of environmental pollution with TCPM-H/OH. Both the technical DDT and dicofol (Kelthane®) formulations examined by Walker *et al.* (1989) did not contain impurities of TCPM-OH in concentration above the detection limit of the method (0.1%), while TCPM-H was not searched for.

This study reports the data about spatial distribution of TCPM-H/OH and their relationships with DDTs in blue mussel and fish from the Gulf of Gdansk.

## MATERIALS AND METHODS

Two samples, each with 350 specimens of blue mussel *Mytilus trossulus* of different body size (around 0.5-4.5 cm in shell length), were collected at the Gdynia (M1) and Orlowo (M2) sites in the western part of the Gulf of Gdansk in November 1992 (Figure 1). 30 male and female adults of the three-spined sticklebacks *Gasterosteus aculeatus* were collected at four sites (Oksywie, Redlowo, Orlowo and Gorki Zachodnie) in the beach zone in the south-western part of the Gulf of Gdansk from June 2 to July 1, 1992. Perch *Perca fluviatilis* and lamprey *Lampetra fluviatilis* were collected at the Gdynia and Gdansk sites, while flounder *Platychthis flesus* were collected at the Gdynia, Gdansk and Mikoszewo sites in November 1992 (Figure 1). The mussels were kept for 24 h in fresh water (in ± 6-8°C) taken at the sampling site, and were then dissected and the soft

tissues, pooled in clean polyethylene bags and kept deep frozen (-20°C) until analysis. In the case of fish, pooled samples of whole three-spinned stickleback (8-10 cm), adult lamprey, perch (10-17 cm) and small flounder (13-17 cm in body length) were subjected to chemical analysis.

The analytical method used for determination of TCPM-H/OH is a part of a multi-residue procedure allowing simultaneously determination of many organochlorines and polynuclear aromatic hydrocarbons (PAH) (Bergqvist *et al.*, 1992). After homogenisation of the sample containing the soft tissue of mussel (69-94 g) and from 3 to 30 a whole individuals of fish (69-380 g) ground with anhydrous sodium sulphate (1:7; baked at 550°C for 2 days), the powdered mixture was packed into a wide bore open glass column, spiked with an internal standard 1 ($^{13}C_{12}$ - *p,p'*-DDT), extracted with 500 ml of a mixture of acetone and n-hexane (2.5:1) followed by 500 ml of n-hexane and diethyl ether (9:1), to obtain a fat extract. The solvents were carefully evaporated on a water bath under vacuum pressure, using a rotary evaporator. Then, pure ethanol (99.5%) was added, to remove azeotropically co-extracted water, also under vacuum and using rotary evaporator. Bulk lipid removal was performed by means of the polyethylene film dialysis method (Strandberg *et al.* 1998). After dissolving the extracted lipids in cyclopentane, dialysis through the polymeric membrane was accomplished by changing the dialysate after 24 and 48 hours. The three dialysate fractions, containing normally between 1-10% of the original lipids, depending on sample size and matrix type, were combined and concentrated to a few millilitres using a rotary evaporator. 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 polychlorinated naphthalenes (PCNs) and some other planar compounds not described here. The remaining fat was removed on n-hexane wetted Florisil gel (8 g) 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 (F3) (Zook *et al.* 1992). 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 long non-polar capillary column DB-5 (60 m x 0.32 mm ID and 0.25 µm film thickness) gas chromatography and low resolution mass spectrometxy (HRGC/LRMS). Additional procedural blanks were also performed and no interference was 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

Both TCPM-H and TCPM-OH were absent (> 0.3 ng/g lipid) in blue mussel but were identified in all fishes examined in this study (Table 1, Figure 2).

**Table 1.** TCPM-H, TCPM-OH and DDTs content of blue mussel and fish in the Gulf of Gdansk (ng/g lipid wt)

| Species | Site | No. | Lipids (%) | TCPM-H | TCPM-OH | DDTs |
|---|---|---|---|---|---|---|
| Blue mussel | Gdynia | 1 (350)* | 1.3 | ND | ND | 840 |
| | Orłowo | 1 (350) | 1.7 | ND | ND | 980 |
| Three-spined stickleback | Oksywie | 1 (30) | 2.4 | 12 | 6.4 | 1800 |
| | Redłowo | 1 (30) | 2.4 | 6.4 | 2.4 | 1400 |
| | Port Północny | 1 (30) | 2.7 | 7.3 | 4.4 | 1300 |
| | Górki Zachodnie | 1 (30) | 2.4 | 18 | 11 | 2600 |
| Perch | Gdynia | 1 (4) | 5.9 | 9.2 | 2.7 | 1300 |
| | Gdańsk | 1 (4) | 5.2 | 30 | 7.1 | 1500 |
| Lamprey | Gdynia | 1 (3) | 6.3 | 4.0 | 1.2 | 980 |
| | Gdańsk | 1 (3) | 22.6 | 6.8 | 1.7 | 1400 |
| Flounder | Gdynia | 1 (5) | 4.8 | 11 | 2.8 | 1500 |
| | Gdańsk | 1 (5) | 4.2 | 16 | 3.4 | 1600 |
| | Mikoszewo | 1 (5) | 4.8 | 24 | 7.3 | 1700 |

DDTs = *p,p* '-DDT + *o,p* '-DDT + *p,p* '-DDD + *o,p* '-DDD + *p,p* '-DDE + *o,p* '-DDE + *p,p* '-DDMU; *Number of samples and number of animals (in parentheses); ND (< 0.3 ng/g lipid wt)

A link between an environmental occurrence of DDTs and TCPM-OH was suggested after a regression analysis of the concentrations found in some environmental matrices. TCPM-OH highly correlated with *p,p'* -DDT and *p,p'*-DDE ($p<0.001$; n = 17) and *p,p'* -DDD ($p<0.01$) in harbor seal from Puget Sound (Walker *et* al. 1989). TCPM-OH was significantly correlated ($p<0.01$; r = 0.68; n = 39) also to DDTs *(p,p'*- DDT + *p,p'*- DDE + *p,p'*- DDD) in all samples examined by Jarman *et* al. (1992) but it was not significantly correlated to TCPM-H in the eggs of peregrine falcon (TCPM-H was twice the concentration of TCPM-OH). In study by Rahman *et al.* (1993) the concentration of TCPM-OH in human milk samples did not correlate to DDTs.

Very recently TCPM-H (4,4',4"-TCPM-H) and its isomers (2,2',4"- and 2,4',4"-TCPM-H) were identified in technical DDT and included as impurities in the formulations produced more than 40 years ago (Buser, 1995). Additionally, TCPM-H and its isomers are formed in the reaction of chloral, chlorobenzene and fuming 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 $AlCl_3$ (Buser, 1995).

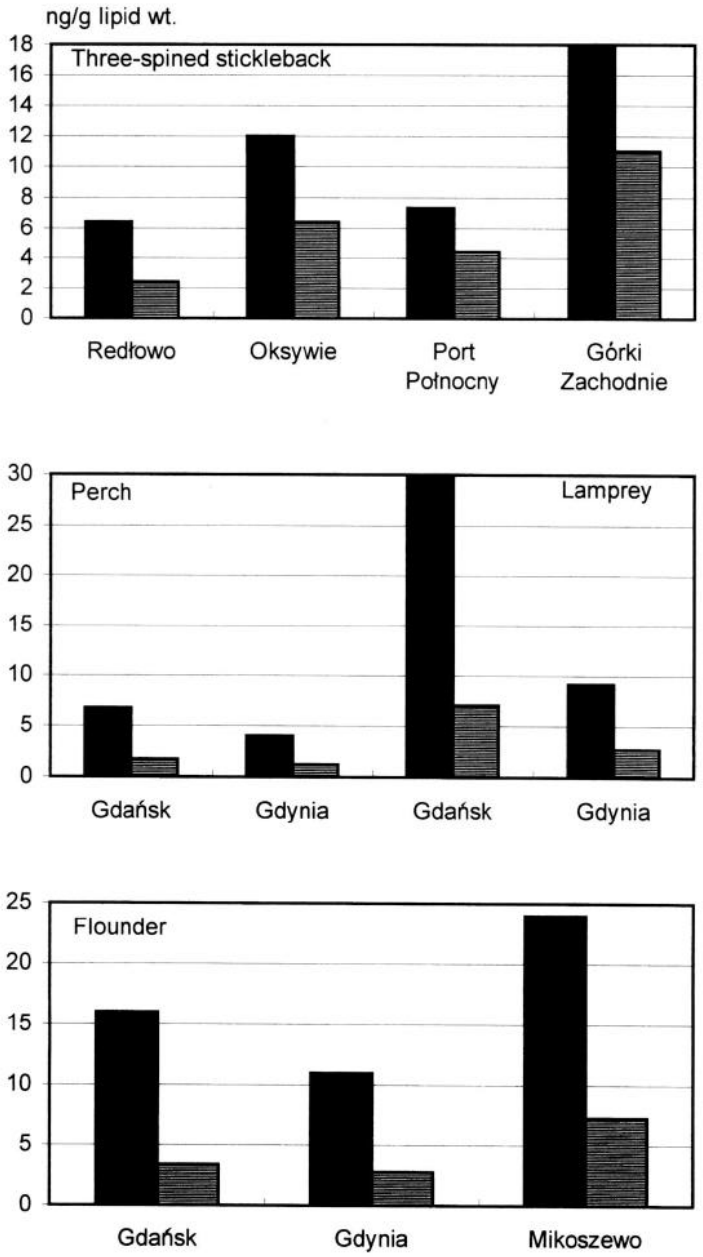


**Figure 2.** Spatial distribution and species related differences of TCPM-H (black bars) and TCPM-OH (striated bars) content of three-spined stickleback, perch, lamprey and flounder from the Gulf of Gdansk.

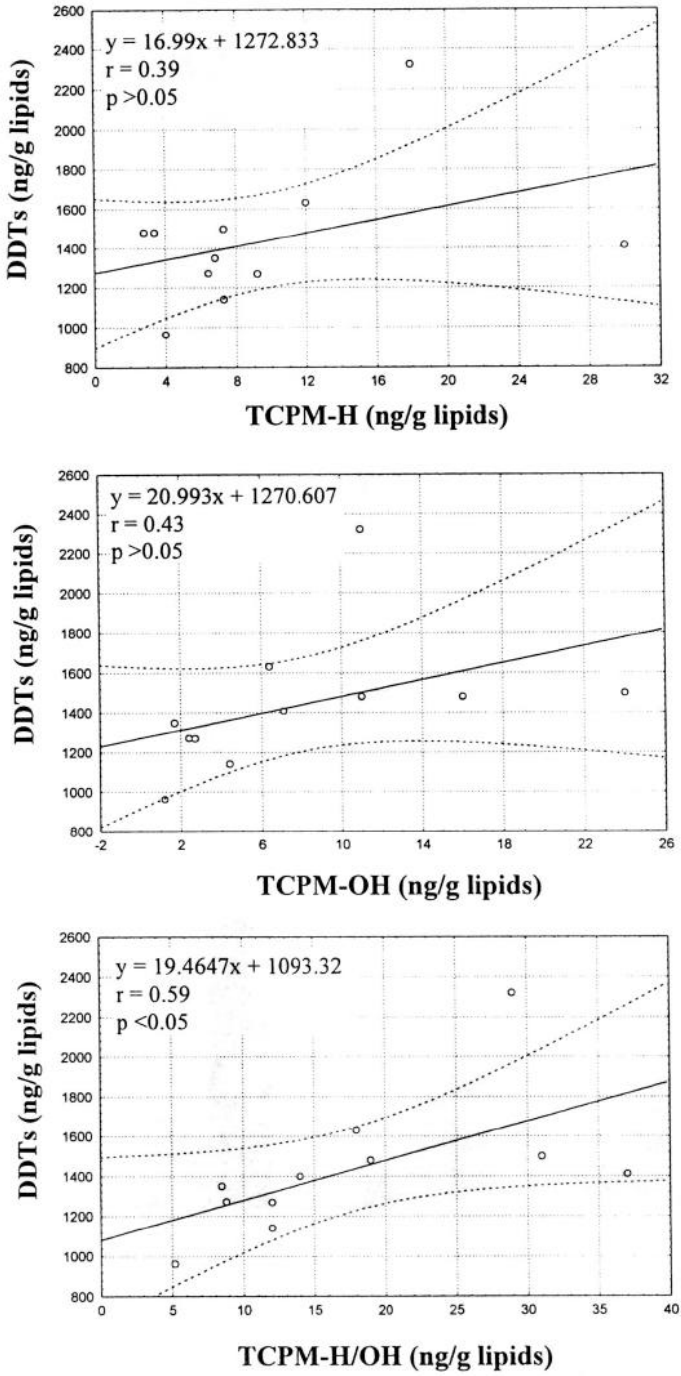


**Figure 3.** Regression between TCPM-H, TCPM-OH, TCPM-H/OH and DDTs (*p,p'* -DDT, *p,p'* -DDD, *p,p'* -DDE and *p,p'* -DDMU) in fish examined.

There is a positive correlation ($p<0.05$) for TCPM-H/OH and DDTs, but not for TCPM-H and TCPM-OH alone and DDTs in fish examined (Figure 3).

Since DDTs could be quantified in blue mussel at the concentrations nearly half of that what was found in all fish in this study (Table 1), the reason for the absence of TCPM-H and TCPM-OH in molluscs is unknown. Even flounder collected at the same site as blue mussel (the Gdynia site F1/M1; Figure 1) did contain TCPM-H and TCPM-OH while its most abundant prey organism at the area did not contain any detectable amounts (<0.3 ng/g lipid). TCPM-H and TCPM-OH are compounds of moderate polarity and their detailed physicochemical properties as well as half-life time in various environmental matrices and especially when related to the persistency of DDT and its analogues are unknown. Blue mussel is an effective filter type organism and one of the reasons of the absence of detectable amounts of TCPM-H and TCPM-OH in their body can be a much lower uptake rates from the water column by such gill filtering organisms when compared to the uptake rates of DDT and its analogues.

Food but not water is considered as a main source of highly hydrophobic (log $K_{O/W}$ > 5) and persistent organochlorine compounds for fish under environmental conditions. The bioaccumulation factors (BAF) of the persistent substances such as *p,p'* -DDE and DDTs in flounder at the Gdynia site when related to blue mussel are 1.8 and 1.7, respectively, while ~70 is for TCPM-H and ~20 for TCPM-OH.

The TCPM-H (4,4',4''-TCPM-H) content of two technical DDT formulations examined by Buser (1995) was ~0.006% (Promochem) and ~0.0015% (Maag). The contribution of TCPM-H/OH when added to DDTs content of fish in this study is between 0.5 and 2.5%, which is two or three orders of magnitude higher than in technical DDT.

Both relatively high BAF values for TCPM-H/OH compared to DDTs in flounder and a much higher contribution of TCPM-H/OH when added to DDTs content of fish than of TCPM-H in technical DDT suggest a very high enrichment rates of TCPM-H/OH in aquatic food web.

*Acknowledgments*. This study was partly supported by Statens Naturvardsverk (Stockholm), Umea University (Umea), and Polish Committee on Scientific Research (KBN) (Warsaw) under grant DS. TCPM-H and TCPM-OH standards were a generous gift from Dr. Jacob de Boer (the Netherlands).

## REFERENCES

Bergqvist P-A, Bandh C, Broman D, Ishaq R, Lundgren K, Naf C, Pettersen H, Rappe C, Rolff C, Strandberg B, Zebuhr Y, Zook D (1992) Multi-residue analytical method including planar PCB, dioxins and other organic contaminants for marine samples. Organohalogen Compd 9: 17-20

Buser H-R (1995) DDT, a potential source of environmental *tris*(4-chlorophenyl)methane and *tris*(4-chlorophenyl)methanol. Environ Sci Technol 29: 2133-2139

Jarman WM, Slmon M, Norstrom RJ, Burns SA, Bacon CA, Simonetit BRT, Risebrough RW (1992) Global distribution of *tris*(4-chlorophenyl)methanol in high trophic level birds and mammals. Environ Sci Technol 26: 1770-1774

Lukovits I, Tóth B, Varjas L, Matolcsy G (1978) Quantitative relationship between structure and anti-ecdysone activity of triarimol analogues. Acta Phytopathol Acad Scien Hung 13: 227-234

Poon R, Lecavalier P, Bergman A, Yagminas A, Chu I, Valli VE (1997) Effects of *tris*(4-chlorophenyl)methanol on the rat following short-term oral exposure. Chemosphere 34: 1-12.

Rahman MS, Montanarella L, Johansson B, Larsen BR (1993) Trace levels of *tris*(4-chlorophenyl)-methanol and -methane in human milk. Chemosphere 27: 1487-1497

Strandberg B, Bergqvist P-A, Rappe C (1998) Dialysis with semipermeable membranes as an efficient lipid removal method in the analysis of bioaccumulative chemicals. Anal Chem 70: 526-533

Walker W, Risebrough RW, Jarman WM, de Lappe BW, Tefft JA, De Long RL (1989) Identification of *tris*(chlorophenyl)methanol in blubber of harbor seals from Puget Sound. Chemosphere 18: 1799-1804

Zook DR, Buser H-R, Bergqvist P-A, Rappe C, Olsson M (1992) Detection of *tris*(chlorophenyl) methane and *tris*(4-chloropnenyl) methanol in ringed seal (*Phoca hispida*) from the Baltic Sea. Ambio 21: 557-560