

# Retrospective Monitoring of Triclosan and Methyl-triclosan in Fish: Results from the German Environmental Specimen Bank

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## Introduction

During the last years there were several reports on the appearance of the biocides triclosan (TCS; 5-chloro-2-(2,4-dichlorophenoxy)phenol; CAS No. 3380-34-5) and chlorophene (CP; 4-Chloro-2-(phenylmethyl)phenol; CAS No. 120-32-1) in the environment<sup>1,2,3</sup>. TCS is used in numerous personal care products like toothpaste and soaps, but also in textiles and shoes<sup>1</sup>. The current annual consumption in Germany is estimated to be 40 t (0.5 g per capita and year)<sup>4</sup>.

During use large amounts of TCS and CP are disposed into waste water. Modern waste water treatment plants (WWTP) eliminate approximately 95 % of TCS, mainly by biodegradation and adsorption to sludge<sup>2,3</sup>. It was observed that during waste water treatment methyl-triclosan (MTCS; 5-chloro-2-(2,4-dichlorophenoxy)anisole; CAS No. 4640-01-1) is formed, probably due to microbial methylation. In a WWTP effluent up to 1 % MTCS in relation to TCS was detected<sup>3</sup>.

The environmental half-life of TCS is assumed to be low, mainly because it is susceptible to photodegradation at environmental pH levels while MTCS is stable under these conditions<sup>5</sup>. The logPow values of 4.7 for TCS and 5.2 for MTCS (estimated with KowWin Vers. 1,67, respectively) indicate potential for bioaccumulation. In aquatic biota, the presence of MTCS was first reported for fish from the Tokyo Bay in Japan in 1984 where up to 38 ng/g MTCS were detected (whole body basis)<sup>6</sup>. In Swiss lakes influenced by wastewater effluents the occurrence of MTCS was observed<sup>5</sup> and levels of up to 35 ng/g MTCS (wet weight basis) were detected in fish from these lakes<sup>7</sup>. Another study revealed the presence of TCS and MTCS in plasma of fish from a North American river at levels of up to 10.4 ng/g and 0.0132 ng/g, respectively<sup>8</sup>. Based on these findings it is assumed that MTCS is more persistent than TCS<sup>8</sup>.

In order to investigate the exposure of aquatic organisms towards CP, TCS and MTCS in Germany a retrospective monitoring of breams (*Abramis brama*) from representative rivers was initiated. Samples from the period 1994 to 2003 were taken from the archive of the German Environmental Specimen Bank (ESB) to evaluate temporal changes and regional differences of the occurrence of the target compounds.

## Methods and Materials

**Sampling of ESB Material:** All procedures were performed according to ESB standard operating procedures<sup>9,10</sup>. Sampling and treatment of ESB samples prior to archiving was described previous-

ly<sup>10,11</sup>. In short, breams (*Abramis brama*) are caught annually after spawning between mid-July and mid-October. At least 20 fish aged between 8 and 12 years are taken at each sampling site. The muscles are pooled, grinded and stored as homogenized powder in sub-samples of approx. 10 g at temperatures below  $-150\text{ }^{\circ}\text{C}$  in an inert atmosphere resulting from evaporating liquid nitrogen. Sampling sites are in the river Elbe with Prossen (km 13), Zehren (km 93), Barby (km 296), Cumlosen (km 470), and Blankenese (km 632), the Elbe tributaries Mulde (Dessau, near the mouth) and Saale (Wettin), the river Rhine with Weil (km 174), Iffezheim (km 334), Koblenz (km 590), and Bimmen (km 865), the river Saar with GÜdingen (km 54) and Rehlingen (km 91), and the river Danube with Ulm (km 2.593), Kelheim (km 2.404), and Jochenstein (km 2.210). Further, samples from Lake Belau which represents a low polluted reference area were used.

**Analysis.** Aliquots of 1 – 2.5 g of frozen and grinded ESB samples were mixed with sodium sulfate in a ratio of 1:6 (w/w) and extracted by accelerated solvent extraction (ASE) with cyclohexane as solvent at  $100^{\circ}\text{C}$  and 14 MPa with an ASE system from Dionex. After clean-up by gel permeation chromatography on Bio-Beads S-X3 with a dichloromethane/cyclohexane 1:1 (v/v) mixture the analyte containing fraction was concentrated to 0.2 mL and filled up to 1.0 mL with n-hexane. This solution was transferred to an activated silica gel column which was rinsed with a mixture of n-hexane/acetone (9:1; v/v) and conditioned with n-hexane. A first non-polar fraction containing MTCS was eluted with n-hexane, concentrated and analyzed directly by gas chromatography with tandem ion trap mass spectrometer (Finnigan MAT Magnum GC/MS/MS). A second polar fraction containing the phenolic compounds TCS and CP was eluted with n-hexane/acetone (9:1; v/v) and evaporated to dryness. The residue was re-dissolved in 2 mL acetone. Then, 250  $\mu\text{L}$  of a 10 % aqueous potassium carbonate solution was added and 10  $\mu\text{L}$  PFBBR (2,3,4,5,6-pentafluorobenzylbromide) for derivatization. After 1 h at  $60^{\circ}\text{C}$  the mixture was transferred into a vessel with a mixture of purified water and cyclohexane (5:1; v/v). After mixing and phase separation the cyclohexane phase was analyzed by GC/NCI-MS (NCI – negative chemical ionization, with an Agilent quadrupol 5973 MSD). For quantification, TCS (from Ehrenstorfer GmbH, Augsburg), MTCS (from VeZerf Laborsynthesen GmbH, Idar-Oberstein) and CP (from ABCR, Karlsruhe) were used and  $^{13}\text{C}_6$ -TCS (provided by CIBA, Basel) and MTCS- $\text{D}_3$  (from Syntheselabor Dr. Weiss, Stahnsdorf) as internal standards. All data are reported on a wet weight basis.

**Quality control.** The limits of quantification (LOQ) were 0.25 ng/g for MTCS and CP, and 0.10 ng/g for TCS as estimated according to the German standard method DIN 32645<sup>12</sup>. Due to the scarcity of the ESB material usually only one sub-sample was analyzed. However, for a number of samples the repeatability of the method was tested by analyses of 3-4 samples (refer to results section). As further quality assurance measure one bream muscles sample which was available in larger quantities was used as laboratory reference material and analyzed along with each batch of ESB samples. The results from these analyses were:  $0.41 \pm 0.1$  ng/g ( $\pm 13\%$ ) for TCS;  $6.9 \pm 0.4$  ng/g ( $\pm 5.8\%$ ) for MTCS and  $< 0.25$  ng/g for CP (n = 12). Blank values were  $< \text{LOQ}$  for MTCS and CP and  $\leq 0.2$  ng/g for TCS (determining the effective LOQ to 0.2 ng/g).

### Results and Discussion

TCS and CP were only detected in a few samples, mainly from the river Saar while MTCS was detected in all analyzed fish muscle samples from rivers. However, in samples from the reference site Lake Belau concentrations of all compounds were below the respective LOQ (analyzed years: 1997, 2001, and 2003). For the rivers, lowest levels were found for the breams from the Danube.

Only MTCS was detected in samples from all three sampling sites at levels between 2 and 5 ng/g (analyzed years: 2002 and 2003).

Detailed data for the river Saar are presented in Table 1. At GÜdingen near the German-French border TCS levels in fish remained relatively constant in the whole period (0.4 – 0.9 ng/g). Highest CP levels were found in 1994 and 1996. Afterwards, CP concentrations declined and dropped finally below the LOQ in 2003. For MTCS, a clear increase of the concentrations in breams was detected from levels of approx. 10 ng/g in the mid 1990s to concentrations in the range 14 – 26 ng/g MTCS after 2000.

**Table 1:** Retrospective monitoring of TCS, MTCS and CP in breams from the river Saar. Data are referring to the wet weight. For replicate analyses mean values  $\pm$  standard deviation are reported. # n = 3; § n = 4.

Sampling site	Year	TCS [ng/g ww]	MTCS [ng/g ww]	CP [ng/g ww]
Saar, GÜdingen	1994	0.71 $\pm$ 0.03§	10.7 $\pm$ 1.0§	2.9 $\pm$ 0.2§
	1995	0.44	8.0	0.80
	1996	0.69	11.4	3.3
	1997	0.56	12.5	2.0
	1998	0.83 $\pm$ 0.12#	15.4 $\pm$ 1.1#	1.4 $\pm$ 0.1#
	1999	0.87	14.4	0.61
	2000	0.89	18.2	0.67
	2001	0.58	14.0	0.31
	2002	0.70 $\pm$ 0.04§	26.1 $\pm$ 0.8§	0.52 $\pm$ 0.01§
	2003	0.36	16.6	< LOQ
Saar, Rehlingen	1994	< LOQ	4.0	1.3
	1995	0.42	3.8	2.0
	1996	0.32	6.8	0.82
	1997	< LOQ	5.9	1.1
	1998	3.4	14.6	2.2
	1999	1.1	12.6	1.8
	2000	0.62	16.0	0.85
	2001	1.7	19.5	1.8
	2002	0.29	15.9	0.80
	2003	< LOQ	22.7	0.41

For breams from the second Saar site Rehlingen, TCS levels showed relatively high variations (Table 1). In some years TCS concentrations were higher as found for the breams from GÜdingen. Concentration of TCS were highest in 1998 and 2001. However, in other years (1994, 1997 and 2003) the levels were below the LOQ of 0.25 ng/g. Since the maximum concentration in 1998 a decline of the TCS levels is observed. MTCS levels were higher as those of TCS and CP. As for the GÜdingen site, also at Rehlingen a significant increase of the MTCS concentrations in breams was detected for the period 1994 – 2003 (by a factor of approx. 6).

For the Rhine, up to 0.7 ng/g TCS and up to 0.8 ng/g CP were detectable in breams from the upstream sampling sites Weil and Iffezheim. No CP and TCS was detected in breams from the downstream sites Koblenz and Bimmen. Data for MTCS in Rhine breams are listed in Table 2. For all sites an increase in MTCS levels in breams is obvious (if higher values for 1998 at Weil and Iffezheim are excluded). The relative increase was highest for the MTCS burden of breams sampled at Bimmen near the German-Dutch border (even if the high value in 2003 is excluded). In the years after 2000 MTCS levels of breams from all Rhine sites were between 5 and 12 ng/g (without the 2003 value for Bimmen). Currently there is no explanation for the partly very high values in single years for some sites (Bimmen 2003; Weil and Iffezheim 1998).

**Table 2:** Retrospective monitoring of MTCS in breams from the river Rhine. Data are referring to the wet weight.

Year	MTCS [ng/g ww]			
	Weil	Iffezheim	Koblenz	Bimmen
1996	4.3	3.6	3.6	1.0
1998	14.9	11.2	3.1	3.4
2000	6.0	7.0	4.1	5.4
2001	6.3	7.6	7.4	4.5
2002	7.1	10.6	8.5	7.6
2003	7.5	12.2	8.0	21.1

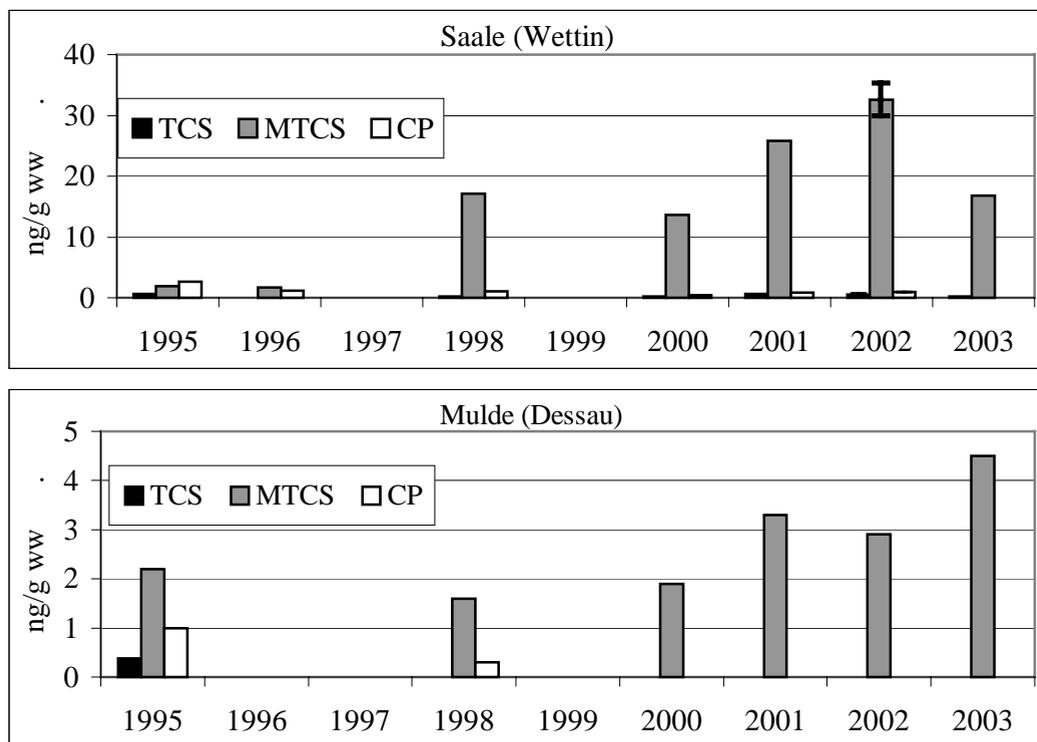
As for most samples from the Rhine, also in the river Elbe TCS levels of fish were below the LOQ of 0.2 ng/g. Only at the upstream sites Prossen, Zehren and Barby levels of up to 0.9 ng/g CP were found. MTCS levels in Elbe breams were all < 6 ng/g and therefore lower as compared to those of the Rhine breams (Table 3). However, as for Rhine and Saar a slight increase of MTCS levels is observed. At all sampling sites highest MTCS levels were found between 2001 and 2003.

**Table 3:** Retrospective monitoring of MTCS in breams from the river Elbe. Data are referring to the wet weight. n.a. – not analyzed.

Year	MTCS [ng/g ww]				
	Prossen	Zehren	Barby	Cumlosen	Blankenese
1996	1.1	1.1	3.4	2.7	1.8
1998	2.3	n.a.	3.4	n.a.	3.3
2000	2.2	n.a.	2.9	n.a.	2.1
2001	3.5	3.8	4.8	4.7	3.8
2002	4.2	3.4	2.5	3.6	3.9
2003	2.9	4.0	5.7	2.3	4.8

In Figure 1 the TCS, MTCS, and CP levels of the breams from the Elbe tributaries Mulde and Saale are shown. MTCS levels in the Saale breams were comparable to those from the Mulde in 1995 and 1996 (approx. 2 ng/g). However, afterwards the MTCS levels in the Saale increased significantly to levels of 14 – 33 ng/g while the levels for the Mulde breams increased only slightly up to approx. 5 ng/g in 2003. The reason for the sharp increase in the burden of the Saale bream after 1996 is not clear yet.

**Figure 1:** TCS, MTCS and CP levels in breams from the Elbe tributaries Mulde and Saale. For the sample 'Saale 2002' the mean value  $\pm$  standard deviation is shown (n = 4). No samples for the years 1996 (Mulde) and 1997 and 1999 (Mulde and Saale) were analyzed. No bar is shown where CP and TCS levels were < LOQ in the other years.



This retrospective monitoring revealed the presence of the biocides TCS and CP in biota from German rivers. While CP and TCS are of less concern due to low and partly decreasing concentrations the methylated transformation product of TCS, MTCS, is identified as persistent pollutant with increasing levels in fish muscles. MTCS levels detected here are in the range of those reported for fish from the Tokyo Bay and Swiss lakes<sup>6,7</sup>. There is an urgent need for studies of the ecotoxicological relevance of MTCS in order to allow the assessment of the potential risk of aquatic biota due to the exposure towards this compound.

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