Retrospective study of triclosan and methyl-triclosan residues in fish and suspended particulate matter: results from the German Environmental Specimen Bank

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Abstract

A retrospective monitoring of triclosan (TCS; period 1994-2003 and 2008) and its potential transformation product methyl-triclosan (MTCS; period 1994-2008) was performed using archived fish samples from German rivers (16 sites, including Elbe and Rhine). At four of these sites suspended particulate matter (SPM) was also investigated covering the period 2005-2007. Samples were analyzed by GC/MS, either directly (MTCS) or after derivatization (TCS). TCS burdens of fish muscle tissue ranged from <0.2-3.4 ng g^{-1} ww (wet weight; corresponding to <2-69 ng g^{-1} lw, lipid weight) without apparent concentration trends over time. MTCS was detected at considerably higher concentrations in fish ranging from 1.0-33 ng g^{-1} ww (47-1010 ng g^{-1} lw) and increased until about 2003-2005. Thereafter, concentrations generally were lower, although at some sites single higher values were observed in recent years. In SPM, decreasing MTCS concentrations in the range 1-4 ng g^{-1} dry weight were detected while TCS was always below the limit of quantification. Assuming that MTCS concentrations are correlated to TCS consumption, the observed decrease in MTCS levels may be partly a result of the voluntary renunciation of TCS use in detergents for, e.g., laundry or dishwashing declared by a manufacturers' association in 2001. Because of a lack of ecotoxicity studies for MTCS, a QSAR-derived predicted no effect concentration (PNEC) was compared to averaged ambient water concentrations of fish which were calculated from maximum tissue residues by applying an appropriate bioconcentration factor from literature. Since these calculated water concentrations were below the PNEC it is assumed that MTCS alone poses no immediate risk to aquatic organism. The conversion to a PNEC for SPM organisms and comparison with detected SPM levels of MTCS also revealed no risk.

Keywords

biocides; biota; environmental specimen bank; retrospective monitoring; time series; transformation product

1. Introduction

Triclosan (5-chloro-2-(2,4-dichlorphenoxy)phenol, CAS no. 3380-34-5, short TCS) is a widely used antimicrobial agent. In the European Union (EU) TCS is regulated by the Biocidal Product Directive 98/8/EC and currently in the review program for 'existing substances' for authorization for several biocidal product types (e.g., as disinfectant or as preservative for materials). TCS is constituent of many personal care products (PCPs; also referred to as cosmetic products or cosmetics in the EU) like liquid soaps, deodorants, toothpastes and creams, and is also used for the impregnation of textiles to reduce microbial growth on these materials. During production and use (e.g., of TCS containing disinfectants or washing of impregnated textiles) TCS is emitted into wastewaters. In wastewater treatment plants (WWTP) the vast majority of TCS is eliminated by biodegradation and adsorption to sewage sludge (Singer et al., 2002; McAvoy et al., 2002; Bester, 2003; Heidler and Halden, 2008). A minor amount, however, can still be detected in WWTP effluents. Furthermore, the TCS metabolite methyl-triclosan (2,4-dichloro-1-(4-chloro-2-methoxyphenoxy)benzene, CAS no. 4640-01-1, short MTCS) can be found in the effluents. MTCS is suspected to be a result of microbial methylation, e.g., during the water treatment process (Lindström et al., 2002) and may be applied as chemical marker for lipophilic WWTP-derived contaminants (Buser et al., 2006). The application of sewage sludge on land and subsequent run-off into aquatic systems is a further potential source of TCS and MTCS to the environment, although the run-off potential for TCS seems to be low (Sabourin et al., 2009).

TCS is highly toxic to bacteria and phytoplankton and toxic to many other aquatic organisms (Schweizer, 2001; Orvos et al. 2002; DeLorenzo et al. 2008). Its environmental half-life, however, is assumed to be relatively low as it is susceptible to photo- and biodegradation (Lindström et al., 2002; Reiss et al. 2002). The transformation product MTCS is more persistent under environmental conditions (Balmer et al., 2004; Lindström et al., 2002), but little is known about its ecotoxicity (Balmer et al., 2004; Stevens et al. 2009; Lyndall et al.

2010; Brausch and Rand, 2011). Both compounds have a logarithmic octanol/water partition coefficient (log Kow) of greater than 3 (TCS: 4.7, MTCS: 5.2; estimated with EPI Suite, EPA 2008) and are thus potentially bioaccumulative (e.g., according to the criteria discussed in Gobas et al. 2009).

Triclosan is suggested as one of the substances of possible concern on the new EU watch list for monitoring of surface waters in the context of the Water Framework Directive (WFD). By gathering monitoring data on these compounds the European Commission intends to break the vicious circle of the necessity of monitoring substances in order to regulate them, and of regulating substances in order to monitor them (European Parliament, 2012).

An environmental risk assessment by the international Association for Soaps, Detergents and Maintenance Products came to the result that the total input of TCS into the environment should not be increased. Based on this outcome, the German Cosmetic, Toiletry, Perfumery and Detergent Association (IKW) recommended to their member companies in 2001 to voluntarily refrain from using TCS in new formulations of detergents and to substitute TCS in formulations of existing products (IKW, 2001).

In order to investigate the effectiveness of the renunciation a retrospective monitoring of TCS and MTCS in bream of six representative German rivers was performed using samples of the German Environmental Specimen Bank. The preliminary study, covering the years 1994 - 2003, revealed low TCS levels as well as strong regional differences in MTCS concentrations until 2003 (Boehmer et al. 2004; only wet weight data). The present study expands the MTCS time series to 2008, provides lipid-normalized concentration data, and thus allows a comprehensive overview of MTCS levels in fish from German rivers in the time period after the voluntary reduction measure (for the follow up TCS was only measured in 2008). In addition, suspended particulate matter (SPM) samples from the German ESB were investigated for TCS and MTCS loads. SPM may be regarded as equivalent to freshly

deposited sediment. Thus, SPM monitoring data are an alternative to assess the potential risk of contaminants for sediment organisms (European Commission, 2010; Schubert et al. 2012).

2. **Materials and methods**

2.1. Environmental Specimen Bank sampling and sample treatment

Sampling and storage were performed according to the concept of the ESB (UBA 2008) following detailed standard operating procedures. Bream (*Abramis brama*) is a non-predatory freshwater fish that mainly feeds on benthos organisms, particularly on chironomid larvae and molluscs (Löffler 1984, Persson and Brönmark 2002, Persson and Stenberg 2006, Specziar and Rezsu 2009). Briefly, at every sampling site routinely a minimum of twenty 8 - 12 year-old bream is sampled annually after spawning (Klein et al., 2010). Tissues are immediately shock-frozen in liquid nitrogen and kept at a temperature below -150°C during all treatment steps and storage. Muscle tissue of the left body side of bream from one sampling site and year are pooled, grinded and stored as homogenized powder in sub-samples of approx. 10 g at temperatures below -150 °C in an inert atmosphere of evaporating liquid nitrogen (details are given in previous reports by Wenzel et al., 2004, and Rüdel et al., 2006). In addition to the pooled samples the muscle tissue of the right body side were taken as individual samples at some sites in 2007 and prepared by cryo-milling. Bream are collected at the following rivers and sites (Fig. 1): Saar: sampling sites Güdingen (km 91) and Rehlingen (km 54); Rhine: Weil (km 174), Iffezheim (km 334), Koblenz (km 590), and Bimmen (km 865); Elbe: Prossen (km 13), Zehren (km 93), Barby (km 296), Cumlosen (km 470), and Blankenese (km 632); Mulde: Dessau; Saale: Wettin; Danube: Ulm (km 2.593), Kelheim (km 2.404), and Jochenstein (km 2.210). Lake Belau in northern Germany serves as reference area because anthropogenic impact is low in this area.

Since 2005, SPM is also sampled within the framework of the German ESB. Monthly SPM samples are collected passively in traps made of stainless steel (Schulze et al. 2007).

Immediately after sampling the material is frozen in the gas phase above liquid nitrogen. Routinely, equal amounts of monthly samples from one site are combined to an annual composite sample which is freeze-dried and homogenized. Then, sub-samples of about 10 g are prepared and kept in the ESB archive at a temperature below -150°C. For this study the sites Rehlingen, Wettin, Dessau, and Prossen are covered.

2.2. Analytical method for fish

This procedure has also been applied in an earlier study (Boehmer et al. 2004). Briefly, aliquots of 1 - 2.5 g frozen and grinded bream muscle samples were mixed with sodium sulfate (1:6 weight/weight) and extracted by pressurized liquid extraction (ASE system from Dionex GmbH, Idstein, Germany). After clean-up by gel permeation chromatography on Bio-Beads S-X3 with a dichlormethane/cyclohexane mixture (1:1; volume/volume, v/v) the analyte containing fraction was concentrated by evaporation and transferred to an activated silica gel column. MTCS was eluted with n-hexane, concentrated and analyzed directly by gas chromatography with tandem ion trap mass spectrometric detection (Magnum GC/MS/MS; Finnigan MAT, Bremen, Germany). The m/z values for detection by selective ion monitoring (SIM) were 232 for MTCS and 235+254 for ^{13}C-MTCS (selected precursor ions 302 and 307, respectively). A polar fraction containing TCS was eluted from the silica gel column with n-hexane/acetone (9:1; v/v). After evaporation to dryness and re-dissolving in 2 mL acetone, 250 µL of a 10 % aqueous potassium carbonate solution and 10 µL 2,3,4,5,6-pentafluoro-benzylbromide were added for derivatization. After 1 h at 60°C the solution was transferred into a vessel containing a mixture of purified water and cyclohexane (5:1; v/v). After mixing, the cyclohexane phase was analyzed by GC/NCI-MS (NCI - negative chemical ionization; with an Agilent quadrupol 5973 Mass Selective Detector from Agilent, Waldbronn, Germany). For quantification, TCS (from Ehrenstorfer GmbH, Augsburg, Germany) and MTCS (from VeZerf Laborsynthesen GmbH, Idar-Oberstein, Germany) were used as well as $^{13}C_6$-TCS (kindly

provided by CIBA, Basel, Switzerland) and MTCS-D3 (from Syntheselabor Dr. Weiss, Stahnsdorf, Germany) as internal standards.

2.3. Analytical method for SPM

SPM samples of 3 g were extracted with 50 ml hexane/acetone (1:1; v/v) by means of ultrasonically treatment (first 2 x 5 min, then 2 x 30 min), filtered through 0.2 µm filters and subsequently cleaned-up on silica gel according to Schwarzbauer et al. (2001) into 6 fractions. The TCS-containing fraction was derivatized with N-methyl-N-(tert-butyldimethylsilyl) trifluoroacetamide (MTBSTFA; 1:12 v/v) by treatment in an ultrasonic bath for 5 min. The final solutions were analyzed on a Thermo GCQ-ITD (Thermo Quest, Austin, USA) equipped with a 30 m VFx-5ms column (0.25 mm i.d., 0.25 µm film) (Varian, Palo Alto, USA). The samples were injected splitless at 100°C (2 µl) into an OPTIC2 PTV (ramped at 10°C/s to 290°C) and the oven program was ramped at 5°C/min from 80°C (1 min) to 300°C (16 min hold). Helium was used as carrier gas at a pressure of 1.7 bar in constant flow mode. The MS parameters were: transfer line temperature 275°C, source temperature 180°C, EI mode at 70 eV, EMV 1125, AGC 50, 10 microscans. The m/z values for detection by selective ion monitoring (SIM) were 302/304 for MTCS, 347/349/73 for TCS-D3, and 344/345/73 for TCS. Quantification was realized via external standards (50 pg μl^{-1}, 100 pg μl^{-1}, 500 pg μl^{-1}, 1000 pg μl^{-1}, 2000 pg μl^{-1}) as 5-point linear calibration. All SPM samples were analyzed in duplicate. TCS-D3 was obtained from Dr. Ehrenstorfer (Augsburg, Germany).

2.4. Quality control

Fraunhofer IME holds an ISO/IEC 17025 accreditation from DAkkS (Berlin) for the described protocol for fish tissue analysis. Recovery experiments with fish were performed for six concentration levels in the range of sample levels (spike range 2.4 - 20 ng g^{-1} plus blank). The average recoveries $\pm$ relative method standard deviations for spiked MTCS and TCS derived

from the recovery functions were 100.6 ± 1.9 % and 101.0 ± 1.3 %, respectively. The limit of quantification (LOQ) for MTCS was 0.25 ng g^{-1} ww (corresponding to 13 - 63 ng g^{-1} lw MTCS, depending on lipid content) as determined according to the German standard method DIN 32645 (2008). Blank values were < LOQ for MTCS. Since the determined LOQ for TCS was in the range of the blank values (0.20 ng g^{-1} ww), this value was applied as effective LOQ (corresponding to 3.0 - 50 ng g^{-1} lw TCS).

In time series, usually only one pooled fish sub-sample per year was analyzed. However, sufficient repeatability of the method was verified for a number of samples by analyzing 3 - 4 sub-samples each. For TCS and MTCS in fish no reference material is available. Therefore one bream muscle tissue sample which was available in sufficient quantity was used as laboratory reference material and analyzed along with each batch of ESB samples. The results from these analyses also revealed a sufficient repeatability: 0.41 ± 0.1 ng g^{-1} (± 13 %) for TCS and 6.9 ± 0.4 ng g^{-1} (± 5.8 %) for MTCS (n = 12).

The SPM extraction procedure was also checked by recovery experiments since no reference material for the analytes was available. Concentrations in the range of sample levels of MTCS (4 ng μl^{-1}) and TCS-D3 (2 ng μl^{-1}) were spiked into typical raw extracts for quality assurance. The recovery of spiked MTCS was 88.5 ± 4.9 % (n = 11) and the recovery of the spiked TCS-D3 throughout the analytical scheme was 85.0 ± 5.1 % (n = 11). The limits of detection (LOD) were 0.1 ng g^{-1} dw for TCS and MTCS, respectively (for a signal/noise-ratio of 3:1). The LOQ was determined as 3 * LOD.

2.5. Calculations and statistical evaluations

No corrections for analytical recovery were performed. Gravimetrically determined lipid contents of bream muscle tissue for calculation of lipid-related concentrations of analytes in fish were retrieved from the German ESB database. For arithmetic mean and median value calculations concentrations below the LOQ were substituted by values of 50 % of the

respective LOQ. Statistical evaluations of possible trends for all time series (or relevant parts of time series with at least four datasets) were performed with the two-sided non-parametric Mann-Kendall test using a dedicated Microsoft Excel application (Salmi et al., 2002). As output the significance level of the respective trend is received. All time series were also analyzed with a statistical program retrieved from the Arctic Monitoring and Assessment Programme homepage (Bignert, 2007). The basic statistical methodology applied is a robust regression-based analysis to detect trends in time-series datasets (Nicholson et al., 1998). The concentration change is derived from the slope of a log-linear regression line (transformed to yield annual % change data).

For Quantitative Structure-Activity Relationship (QSAR) estimation of MTCS ecotoxicity models for non-polar, non-specific toxic chemicals were applied according to requirements of the selected models. Input data was the n-octanol/water-partition coefficient K_{ow} (as log K_{ow}).

3. **Results and discussion**

3.1. Fish data

3.1.1. Overview

Concentration trends of TCS and MTCS in bream from different German rivers and sampling sites are shown in Figs. 2 - 3 and S3 (Supplementary Information). Additional information, i.e., mean values ± standard deviations, concentration ranges for the whole study period and concentrations in samples from 2008 (or 2007 for Lake Belau) are summarized in Tables S1 - S4 (Supplementary Information). TCS was not analyzed in samples from 2004 to 2007 because levels during the first study period (until 2003; Boehmer et al., 2004) had been very low. Figures are based on lipid weight data for better comparison of MTCS and TCS although TCS, other than MTCS, is easily ionized at pH > 7 which is accompanied by a much lower bioaccumulation that is not solely associated with body lipids (Schettgen, 2000). Wet weight-based data for TCS are provided in Table S2 (Supplementary Information).

3.1.2. Comparison of TCS and MTCS levels

The present results confirm that MTCS accumulation in fish muscle tissue is considerably higher compared to TCS. The MTCS:TCS ratio was in the range of about 3 to 300 (TCS concentrations below the LOQ were substituted by a value corresponding to 50 % of the LOQ). Lower MTCS:TCS ratios were found in fish sampled in the 1990s (e.g., 1995, Saale and Mulde sites) while higher ratios were detected after 2000 (e.g., 2008 at all sites > 10). During the whole study period, TCS ranged from < 2 to 69 ng g^{-1} lw (corresponding to < 0.2 to 3.4 ng g^{-1} ww) compared to MTCS levels of 47 - 1010 ng g^{-1} lw (corresponding to 1.0 - 33 ng g^{-1} ww).

Highest levels of TCS (based on ww) were detected in the rivers Saar and Saale as well as in the upper part of the river Rhine. At the Saar sampling sites Güdingen and Rehlingen mean TCS concentrations were 0.65 and 0.77 ng g^{-1} ww, respectively (period 1994-2003 and 2008). Slightly lower TCS residues were observed in bream from the Saale (mean: 0.32 ng g^{-1} ww) and the Rhine site Weil (mean: 0.41 ng g^{-1} ww) (Table S2, Supplementary Information). TCS was lowest in fish from the rivers Danube and Elbe, and from the reference site Lake Belau (< 0.2 ng g^{-1} ww). Mann-Kendall tests revealed that no significant time trends were apparent for TCS at any site for the whole investigated period. However, at Güdingen an increase of TCS concentrations until 1999 was observed followed by a steady decline until 2003 (Fig. 2). An especially high TCS concentration was found in fish from the Saar site Rehlingen in 1998 (Fig. 2). Generally, at most sites highest TCS values were observed in the 1990s.

Like TCS, MTCS concentrations in bream were also highest in the rivers Saar and Saale with mean values of 378 and 361 ng g^{-1} lw at the Saar sites Güdingen and Rehlingen and 417 ng g^{-1} lw in the Saale (Figs. 2 and 3 and Table S3, Supplementary Information). High levels (mean values about 170 - 220 ng g^{-1} lw) were also detected in fish collected from the middle and lower part of the Rhine, the upper and middle part of the Elbe, and the Elbe tributary

Mulde. Danube bream had clearly lower MTCS levels (mean values 71 - 75 ng g^{-1} lw; Fig. S3, Supplementary Information). Low concentrations (mean values about 80 - 100 ng g^{-1} lw) were also observed in fish from the lower Elbe (Cumlosen, Blankenese) and the upper Rhine (Weil) (Figs. 2 and 3). At the reference site Lake Belau MTCS contents in bream were below the LOQ.

Analysis of individual fish sampled from two sites in 2007 indicate a relative heterogeneous exposure with MTCS concentrations ranges of 215 - 719 ng g^{-1} lw at Güdingen and 59 - 102 ng g^{-1} lw at Weil (n = 10, respectively; see Supplementary Information Fig. S4).

3.1.3. Temporal and spatial comparison

Temporal trends of MTCS differed between rivers and sampling sites (Figs. 2 - 3 and Fig. S3, Supplementary Information). At all sites, however, clear decreases of MTCS residues were not observed before 2003 or 2005. In the Saar, MTCS in bream muscle tissue increased about 3-fold between 1994 and 2004/2005 and decreased thereafter. In 2008, however, a new slight increase was noticeable.

All time series were applied to log-linear regression analysis to identify possible trends (Bignert, 2007). Periods from the start of the monitoring until the maximum MTCS level and from the maximum until the end were evaluated separately. For the sites Rehlingen and Güdingen (both Saar), Koblenz and Bimmen (both Rhine), Blankenese (Elbe), Wettin (Saale), and Dessau (Mulde) significant increasing trends were detected ($p < 0.035$ - < 0.001; Table 1) between the mid-1990s and the early 2000s. The highest increase of MTCS bream levels was observed in the Saale (average + 32 % per year for the period 1995 - 2003). Annual increases at the other sites with positive trends were in the range of 6 - 15 %. Significant decreases after peak concentrations in the early 2000s were observed at Weil and Iffezheim (Rhine) and Cumlosen (Elbe) where MTCS levels in bream decreased by -10 %, -18 %, and -16 % per

year for the period 2003 - 2008, respectively (see Table 1 for significance levels and confidence intervals).

The observed temporal variation of MTCS levels seems not to be correlated to changes of the performance of WWTPs and respective discharges to the rivers. One indication is that the observed trends for MTCS in fish samples are different from those found for compounds with similar consumption pattern in ESB samples (i.e., ingredients of personal care products or detergents which are also emitted via wastewater to WWTPs). In further sub-samples of the pooled annual fish samples alkylphenolic compounds and polycyclic musk fragrances were investigated previously. In the period 1992 - 2001 peak concentrations of 4-nonylphenol and 4-*tert.*-octylphenol and the respective monoethoxylates were detected in samples from the mid-1990s (with highest levels in fish from the Saar river site Güdingen in 1994; Wenzel et al., 2004). Peak concentrations for the synthetic fragrances AHTN (1-(5,6,7,8-Tetrahydro-3,5,5,6,8,8-hexamethyl-2-naphthalenyl)-ethanone; trade name Tonalide) and HHCB (1,3,4,6,7,8-Hexahydro-4,6,6,7,8,8-hexamethyl-cyclopenta[g]-2-benzopyrane; Galaxolide) have also decreased from maximum values in the mid-1990s (covered period 1995 - 2003; Rüdel et al., 2006).

Spatial trends for MTCS were observable in the rivers Rhine (four sites) and Elbe (five sites). In the Rhine, MTCS levels increased downstream with on average clearly higher levels in bream at Koblenz and Bimmen as compared to Weil and Iffezheim (Fig. 2). Spatial trends in Elbe fish revealed mostly lower levels at downstream sites (Fig. 3). Especially fish from the sampling sites Zehren, downstream from the city of Dresden, and Barby, downstream of the tributaries Saale and Mulde, had higher MTCS levels as compared to fish from Cumlosen and Blankenese (downstream of Hamburg harbor, tidal influence).

For fish from the Elbe site Barby and from the Saale river similar concentration trends were detected (Fig. 3; e.g., peak in 2007). The Saale flows into the Elbe shortly upstream of Barby and apparently influences the MTCS levels of bream sampled downstream the confluence.

3.1.4. Literature data for TCS/MTCS in fish

The finding that mainly the more persistent MTCS is detected in fish has also been reported by others. The concentration levels, however, vary widely depending on sampling location and fish species. In Europe, similar MTCS levels were reported by Balmer et al. (2004) for fish from different Swiss lakes, i.e. < 2 - 365 ng g^{-1} lw (corresponding to about < 0.01 - 35 ng g^{-1} ww) whereas much higher MTCS contents were detected in carp from German aquaculture (140 ng g^{-1} ww resp. 1100 ng g^{-1} lipid; Hiebl, 2000). Reported TCS levels in fish are mostly low, although occasionally also relatively high levels were detected in fish sampled near WWTPs, e.g., 0.24 to 4.4 µg g^{-1} ww in bile of fish caught downstream of WWTPs in Sweden (Adolfsson-Erici et al., 2002). Subedi et al. (2012) investigated also German ESB bream samples from 2008. TCS was not detected in any sample. The different results for these parallel investigations may be explained by the fact that these authors applied a multi-method for the analysis of a larger set of substances with a TCS method detection limit of 3.4 ng g^{-1} ww (the TCS LOQ in this study was 0.2 ng g^{-1} ww and detected TCS levels for the samples from 2008 were < 1 ng g^{-1} ww). MTCS was not analyzed in these studies.

The reasons for the - in comparison to TCS - higher MTCS levels in whole fish and fish muscle tissue are not fully understood yet. They are possibly the result of higher persistence and a higher accumulation potential of MTCS in combination with an internal methylation of TCS in fish (Lyndall et al., 2010).

3.1.5. Ecotoxicological relevance of the observed tissue concentrations

By means of a bioconcentration factor (BCF) the fish tissue concentrations can be used to calculate ambient water concentrations to which the fish had been exposed. For MTCS and TCS lipid-based fish BCF values of up to 260,000 and up to 165,000, respectively, have been reported (Balmer et al., 2004; Schettgen, 2000). By comparing the estimated water

concentrations to effect concentrations it can be assessed whether the TCS/MTCS levels are harmful for aquatic organisms.

For TCS ecotoxicological data are available (for a recent review, see Bedoux et al., 2012) and several authors derived predicted no effect concentrations (PNECs). A PNEC of 50 ng L^{-1} for TCS in surface waters was proposed by Samsøe-Petersen et al. (2003) while Capdevielle et al. (2008) used a log-logistic species sensitivity distribution to estimate a PNEC of 1550 ng L^{-1} TCS.

In contrast to TCS, ecotoxicity data for MTCS are limited to two proprietary studies (cited in Lyndall et al., 2010) in which no acute toxicity to *Daphnia magna* was observed at MTCS concentrations up to 180 µg L^{-1} and algal growth was unaffected at 40 µg L^{-1} (72-h-NOEC). Stevens et al. (2009) even observed that methylation reduces TCS toxicity to wetland plants. These findings indicate a lower toxic potential of MTCS compared to TCS. Brausch and Rand (2011) concluded that the current data do not allow a risk assessment of MTCS in the aquatic environment due to a lack of information on potential effects.

In view of the scarcity of ecotoxicity data for MTCS, assessments by QSAR were performed to estimate the effect concentrations of MTCS and evaluate potential risks for the organisms in the rivers under investigation. The calculations were performed with different QSAR-models which are all based on the partition coefficient log K_{ow} and are appropriate for nonpolar, unspecific acting compounds (see Supplementary Information, Table S5, for models and complete results). The QSAR-derived toxic effect concentrations for MTCS were highest for algae with an EC50 (72 h growth inhibition) of 1450 µg L^{-1} for *Selenastrum capricornutum* (model from Clements, 1996). Lowest effect concentrations were derived for daphnids (NOEC, 16 d reproduction: 15 µg L^{-1}; model from Verhaar et al. 1995). Due to the uncertainty of this approach a safety factor of 1,000 on this value is applied resulting in a Q-PNEC (QSAR-derived PNEC) of 15 ng L^{-1} for MTCS.

For risk assessment MTCS water concentrations were calculated using the maximum MTCS concentrations detected in bream and a BCF of 180,000 (mean, whole fish) which has been determined for white fish from Swiss lakes (Balmer et al. 2004). The resulting water concentrations are within the range of 1 - 6 ng L^{-1} MTCS. Lindström et al. (2002) reported slightly lower maximum MTCS water concentrations of 0.5 - 2 ng L^{-1} for Swiss rivers and lakes.

Since the calculated MTCS water concentrations are clearly below the here derived Q-PNEC of 15 ng L^{-1} it is unlikely that MTCS exerted any harm to freshwater organisms in the German rivers investigated in this study.

3.2. SPM data

3.2.1. TCS/MTCS levels in SPM

Between 2005 and 2007 SPM was sampled at four sampling sites (Prossen/Elbe, Wettin/Saale, Dessau/Mulde and Rehlingen/Saar) and analyzed for TCS and MTCS. TCS could not be detected in any sample (< 0.1 ng g^{-1} dw). Table 2 summarizes the MTCS concentrations for SPM. Highest MTCS values of about 4 ng g^{-1} dw were detected in 2005. At Prossen and Wettin MTCS levels were about 80 % lower in 2007 as compared to 2005. At Dessau and Rehlingen lowest levels were detected in the SPM sampled in 2006.

The ratios of the MTCS concentrations of bream (lw data): SPM (dw data) range between about 30 (Elbe/Prossen 2005) and 1000 (Saale Wettin 2007). A clear pattern of a relation between biota and SPM levels of MTCS is not apparent. For example, no relation is obvious at the Saar site Rehlingen where SPM levels are low while concentrations in bream are quite high.

A direct comparison of detected MTCS concentrations in SPM with ecotoxicological effect levels of organisms is not possible due to a lack of data. Therefore SPM effect concentrations were estimated from the derived aquatic Q-PNEC on the basis of partitioning of MTCS

between solids and water. This equilibrium partitioning approach is described in the guidance document of risk assessment of chemicals within the framework of the European REACH Directive (ECHA, 2010). Details of the calculation are presented as Supplementary Information. By this means SPM pore water concentrations between < 1 and 6 ng L^{-1} MTCS were derived from the measured MTCS concentrations in SPM. These levels are below the derived Q-PNEC (15 ng L^{-1}; see section 3.1.5.). Thus, a risk to SPM organisms by the detected MTCS levels is not likely.

3.2.2. Literature data for TCS/MTCS in SPM and sediments

Studies on TCS and MTCS in SPM from Europe are scarce. One study from Greece reported TCS levels in SPM in the range 16 - 31 ng g^{-1} (Gatidou et al., 2007). In the present study TCS concentrations for SPM were all < 0.1 ng g^{-1}. Since SPM can be regarded as equivalent to freshly deposited sediment, SPM levels were also compared to sediment data. Data are contradictory since in some previous studies TCS was detected but no MTCS, and *vice versa*. However, reported sediment levels of TCS and MTCS from previous studies were higher than the here determined SPM concentrations. For surface sediments in Spain, TCS concentrations in the range of 8 - 200 ng g^{-1} dw were reported while no MTCS was detected (González-Mariño et al., 2010). Dated sediment cores from Switzerland showed a preservation of TCS in sediment samples (Singer et al., 2002; due to the applied analytical method TCS concentrations also included MTCS residues which, however, were below the LOQ in typical samples tested). Residues dated to the mid-1970s were 75 ng g^{-1} dw TCS. In the following years concentrations were in the range of 35 - 60 ng g^{-1} dw (Singer et al., 2002). In a survey of surface sediments along the German Lippe River no TCS was detected (nine sites, four samplings in the period 1999-2001). MTCS concentrations were in the range < 0.5 - 450 ng g^{-1} dw (Kronimus et al., 2004). In a follow up study with a dated sediment core from

the Lippe only MTCS was detected with concentrations of a few ng g^{-1} in the mid-1960s, peak values of 30 ng g^{-1} dw in the mid-1980s and decreasing levels thereafter (Heim et al., 2004).

3.3. TCS usage pattern

Assuming that MTCS levels are correlated to TCS consumption, the observed concentration pattern may be partly related to the voluntary renunciation of the use of TCS in detergents for laundry, dishwashing, hard surfaces etc. as declared by IKW (2001; see Introduction). It is assumed that the renunciation was fully implemented in the production process until 2005 (personal communication, B. Glassl, IKW; August 2012). From a TCS production company it was also confirmed that the usage of TCS increased around the year 2000 due to new applications, while around 2005 the usage generally decreased. According to IKW, member companies also confirmed that the usage of TCS in PCPs was clearly decreasing after the year 2000 (personal communication, K. Rettinger, IKW, August 2012). Until October 2005, formulations of detergents had to be notified to the German Environment Agency (Umweltbundesamt). An evaluation of the products data base by the Umweltbundesamt for the period January 2002 to October 2005 revealed that the TCS usage in detergents was constant or slightly decreasing (estimated annual usage in detergents for laundry, dishwashing, hard surfaces etc. during this period was about 4 - 7 tons TCS). In October 2005, TCS was still used in 46 products. However, in the years 2004 and 2005 only three new detergent products with TCS were notified while the total number of notified products was as high as 7700 in 2004 (personal communication M. Gast / B. Rechenberg, Umweltbundesamt; August 2012).

Taken together, this information seems to confirm that the decrease of MTCS levels in fish mirrors the lower consumption of TCS in personal care products and detergents in Germany.

4. **Conclusions**

MTCS levels in bream seem to decrease in the investigated German rivers in recent years. Assuming that MTCS levels are correlated to TCS consumption, these decreases may be at least partly related to the voluntary renunciation of the use of TCS in detergents by the manufacturers' association IKW in 2001. However, at some locations, i.e., at both Saar sites, the Rhine site Bimmen and the Elbe sites Prossen and Zehren, a new increase was noticeable in 2008 which should be followed up in the coming years.

MTCS water concentrations presumably pose no risk for aquatic organism. The same is true for the detected MTCS concentrations in SPM because estimated MTCS pore water concentrations were below the QSAR derived PNEC (conversion of the aquatic PNEC to a SPM PNEC by applying the equilibrium portioning method; ECHA, 2010).

5. Supplementary Information

Supplementary information associated with this article is available online.

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FIGURE CAPTIONS

Figure 1: River sampling sites of the German ESB.

Figure 2: Methyl-Triclosan (left scale) and Triclosan (right scale) in muscle tissue of bream from the ESB sampling sites in the rivers Saar (a) and Rhine (b). Concentrations for pooled annual samples are given as ng g^{-1} lipid weight. Triclosan was not analyzed in the period 2004 - 2007. Values < LOQ were included as 0.5 of the respective LOQ and shown as open squares (relevant only for triclosan). For some samples replicates were analyzed (n = 2 - 3) and mean and standard deviations are shown.

Figure 3: Methyl-Triclosan (left scale) and Triclosan (right scale) in muscle tissue of bream from the ESB sampling sites in the river Elbe (a) and its tributaries Saale and Mulde (b). Concentrations for pooled annual samples are given as ng g^{-1} lipid weight. Triclosan was not analyzed in 2004 - 2007. Values < LOQ were included as 0.5 of the respective LOQ and shown as open squares (relevant only for triclosan). In Elbe bream triclosan concentrations were always < LOQ and are not shown. For one sample (Saale 2007) replicates were analyzed (n = 3) and mean and standard deviations are shown.

Fig 1

Fig 2

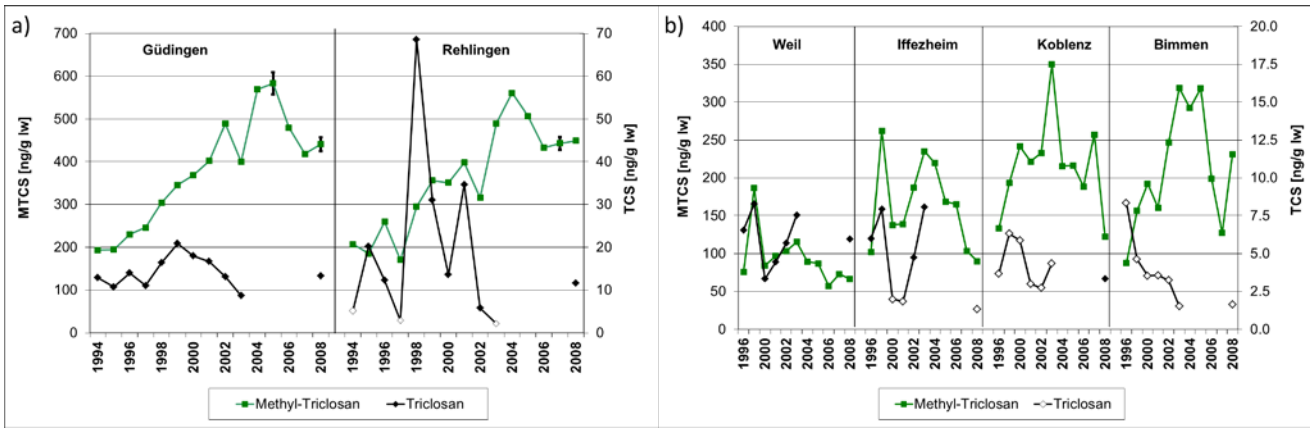

Fig 3

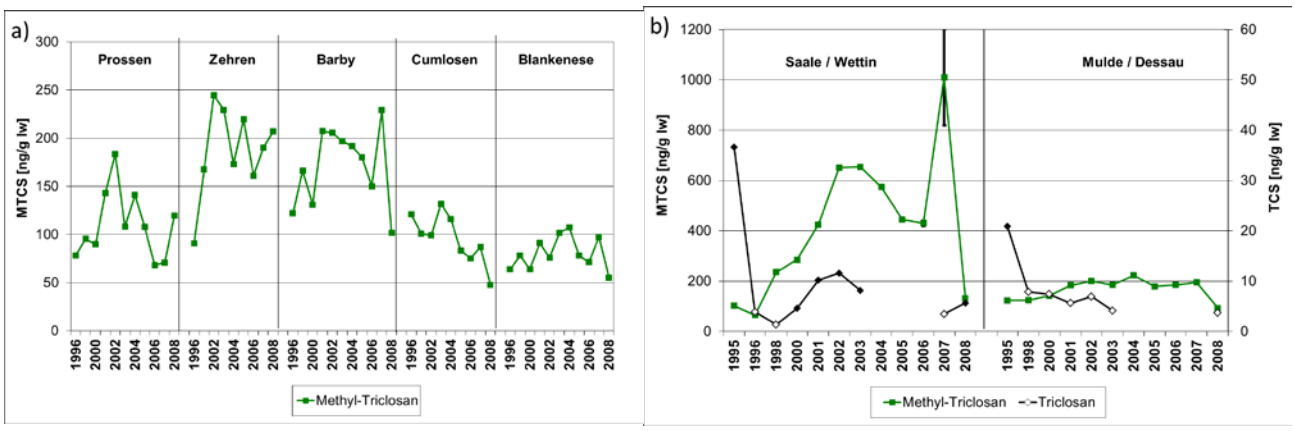

Table 1: Data from log-linear regression analysis (Bignert, 2007) of time trends of MTCS in muscle tissue of bream.

Site	evaluated period#		change in % per year	confidence interval in % per year		significance
Saar /	1994	2005	10.5	9.3	12.7	$p < 0.001$
Güdingen	2005	2008	no trend			
Saar /	1994	2005	10.4	6.8	13.9	$p < 0.001$
Rehlingen	2005	2008	no trend			
Rhine /	1996	2005	no trend			
Weil	2003	2008	-10.4	-19.7	0.0	$p < 0.05$
Rhine /	1996	2003	no trend			
Iffezheim	2003	2008	-18.1	-24.4	-12.2	$p < 0.003$
Rhine /	1996	2003	11.6	4.7	19.7	$p < 0.010$
Koblenz	2003	2008	no trend			
Rhine /	1996	2005	15.0	9.5	20.9	$p < 0.001$
Bimmen	2005	2008	no trend			
Elbe /	1996	2002	no trend			
Prossen	2002	2008	no trend			
Elbe /	1996	2002	no trend			
Zehren	2002	2008	no trend			
Elbe /	1996	2001	no trend			
Barby	2001	2008	no trend			
Elbe /	1996	2003	no trend			
Cumlosen	2003	2008	-15.6	-24.4	-5.7	$p < 0.014$
Elbe /	1996	2004	6.0	0.6	11.6	$p < 0.035$
Blankenese	2004	2008	no trend			
Saale /	1995	2003	32.3	19.7	46.2	$p < 0.001$
Wettin	2003	2008	no trend			
Mulde /	1995	2004	7.3	3.7	10.5	$p < 0.004$
Dessau	2004	2008	no trend			
Danube / Ulm	2003	2008	no trend			
Danube / Kelheim	2003	2008	no trend			
Danube / Jochenstein	2003	2008	no trend			

from beginning of the monitoring period until maximum or from maximum until end of the monitoring period; compare Fig. 2 - 3 and S3 (Supplementary Information) for monitored years.

Table 2: MTCS concentrations in SPM (ng g^{-1} dry weight, dw) and muscle tissue of bream (ng g^{-1} lipid weight, lw) from German rivers. TCS values were always < LOQ. Data are means of two or three samples ± standard deviation (SD).

year:	**2005**		**2006**		**2007**	
	mean	**SD**	**mean**	**SD**	**mean**	**SD**
SPM (MTCS in ng g^{-1} dw; n = 2)						
Saar / Rehlingen	-	-	0.6	< 0.1	1.2	< 0.1
Elbe / Prossen	3.8	0.1	1.4	< 0.1	0.6	< 0.1
Saale / Wettin	4.1	0.1	3.5	0.1	1.0	< 0.1
Mulde / Dessau	4.5	< 0.1	1.1	< 0.1	2.8	< 0.1
bream (MTCS in ng g^{-1} lw; n = 1 - 3)						
Saar / Rehlingen	507	-	433	-	443	15
Elbe / Prossen	108	2	68.1	-	70.9	-
Saale / Wettin	445	-	430	10	1010	192
Mulde / Dessau	179	-	185	-	196	-