

Assessment of the acute toxicity of triclosan and methyl triclosan in wastewater based on the bioluminescence inhibition of *Vibrio fischeri*

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Abstract In this work, the contributions of triclosan and its metabolite methyl triclosan to the overall acute toxicity of wastewater were studied using *Vibrio fischeri*. The protocol used in this paper involved various steps. First, the aquatic toxicities of triclosan and methyl triclosan were determined for standard substances, and the 50% effective concentrations (EC₅₀) were determined for these compounds. Second, the toxic responses to different mixtures of triclosan, methyl triclosan, and surfactants were studied in different water matrices, i.e., Milli-Q water, groundwater and wastewater, in order to evaluate (i) the antagonistic or synergistic effects, and (ii) the influence of the water matrices. Finally, chemical analysis was used in conjunction with the toxicity results in order to assess the aquatic toxicities of triclosan and its derivative in wastewaters. In this study, the toxicities of 45 real samples corresponding to the influents and effluents from eight wastewater treatment works (WWTW) were analyzed. Thirty-one samples were from a wastewater treatment plant (WWTP) equipped with

two pilot-scale membrane bioreactors (MBR), and the influent and the effluent samples after various treatments were characterized via different chromatographic approaches, including solid-phase extraction (SPE), liquid chromatography coupled to tandem mass spectrometry (LC–MS/MS), and SPE coupled to gas chromatography–mass spectrometry (GC–MS). The toxicity was determined by measuring the bioluminescence inhibition of *Vibrio fischeri*. In order to complete the study and to extrapolate the results to different WWTPs, the toxicity to *V. fischeri* of samples from seven more plants was analyzed, as were their triclosan and methyl triclosan concentrations. Good agreement was established between the overall toxicity values and concentrations of the biocides, indicating that triclosan is one of the major toxic organic pollutants currently found in domestic wastewaters.

Keywords Triclosan · Methyl triclosan · Surfactants · Toxicity · *Vibrio fischeri* · Bioluminescence · Wastewater

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Introduction

Triclosan (2,4,4'-trichloro-2'-hydroxydiphenyl ether; synonym: Irgasan DP 300), is a synthetic, broad-spectrum antimicrobial agent that in recent years has exploded onto the consumer market in a wide variety of household and personal care products (PCP).

Triclosan was first introduced into the health care industry in 1972 and into toothpaste in Europe in 1985 [1]. In the United States, triclosan has been used for over 40 years and is recognized by the Food and Drug Administration as being an over-the-counter or a prescription drug [1]; however, in recent years its usage has increased. Triclosan is stable and relatively soluble in water

(10 mg/l at 20 °C). It is a lipophilic compound (with $\log K_{ow}=4.8$) which can be bioaccumulated. Triclosan and some of its derivatives, such as methyl triclosan, have been detected in the environment and in some food sources [2, 3]. Triclosan has also been found in human breast milk [4, 5], and plasma [6].

Most antiseptic products are disposed of down residential drains, where they undergo treatment by local wastewater treatment plants (WWTPs). Triclosan has been detected in WWTP influents at concentrations ranging from 62 to 21,900 ng/L [7–10]. During wastewater treatment, many of the chemicals, including biocides, are removed, but some chemicals still reach surface waters. The efficiency with which WWTPs remove contaminants depends upon the particular wastewater treatment. However, municipal sewage treatment plants are not designed to remove pharmaceutical and personal care products (PPCPs) [11], and it has been demonstrated that triclosan readily survives conventional wastewater treatment methods.

Despite the continual introduction of triclosan into water sources, a number of mechanisms have been developed for its removal. Some of the triclosan leaves surface waters via sedimentation. In addition, several investigators have shown that triclosan in surface waters can degrade in the presence of sunlight. However, this photodegradation can result in harmful products, and triclosan could be a source of highly chlorinated and toxic dioxins in the environment [12].

In addition, the chemical structure of triclosan closely resembles certain estrogens, and one study suggests that triclosan is weakly androgenic, causing changes in fin length and sex ratios in fish [13, 14].

There is also concern that, rather than being a general biocide (a chemical substance that disrupts so many cellular functions at once that bacteria encountering it simply cannot survive), triclosan may instead be a specific biocide, killing bacteria by targeting very specific cellular functions. Triclosan blocks essential enzymes during fatty acid synthesis. This allows the bacteria to mutate, thus building up resistance and developing into “superbugs” [15].

Finally, triclosan has adverse effects on aquatic organisms, and it is highly toxic to algae [16] and to some species of fish, particularly in their early stages of development [13].

Most of the research carried out to evaluate the toxicity of triclosan has been carried out using long-term exposure assays, such as its effects on *Daphnia magna* survival, growth, and reproduction [17], fish reproduction [13], or algae [16]. Only a few studies have been based on the use of rapid microorganism tests in order to quickly establish toxicity for wastewater applications.

The objectives of the present work were:

- To evaluate the toxicity of triclosan and methyl triclosan to the standard organism *Vibrio fischeri*.

- To evaluate possible synergistic or antagonist effects by other organic pollutants commonly detected at high concentrations in wastewater [e.g., linear alkyl benzene sulfonates (LAS)] on triclosan toxicity, as well as matrix effects of different types of waters.
- To explore the further use of the inhibition of bioluminescence by triclosan as a rapid tool for assessing the activity of this biocide in wastewater.

In this work, the toxicities to *V. fischeri* of 45 samples, corresponding to the influents and the effluents from eight domestic WWTPs located along the Llobregat and Ebro rivers (Spain), were evaluated, and the concentrations of triclosan and methyl triclosan were analyzed by gas chromatography–mass spectrometry (GC–MS). The chemical characterization of the organic pollutants most commonly found in domestic wastewater, such as surfactants and their degradation products, was carried out by liquid chromatography followed by tandem mass spectrometry (LC–MS/MS).

Experimental section

Chemical and reagents

Liquid- or freeze-dried luminescent bacterial reagent *V. fischeri* NRRL B-111 77 was used. The bacterial reagents as well as the reconstitution reagents were purchased from AZUR Environmental (Carlsbad, CA, USA), while the test was carried out with Microtox™.

All solvents (water, acetonitrile and methanol) were of high-performance liquid chromatography (HPLC) grade and were purchased from Merck (Darmstadt, Germany).

The standards used in this study were of the highest purity available. Triclosan and methyl triclosan standards were obtained from Sigma-Aldrich (St. Louis, MO, USA). High-purity (98%) 4-*tert*-OP and 4-NP were obtained from Aldrich (Milwaukee, WI, USA). NP₁EO, NP₂EO, OP₁EC, OP₂EC, NP₁EC and NP₂EC were synthesized according to a method described elsewhere (Diaz et al. 2002). Additionally, the technical mixture of NPEOs containing chain isomers and oligomers with an average of ten ethoxy units (Findet 9Q/22) was from Kao Corporation (Barcelona, Spain). Commercial linear alkylbenzene sulfonates (LAS) with low dialkyltetralinsulfonate contents (<0.5%) were supplied by Petroquímica Española S.A. in a single standard mixture with a proportional composition of the four homologs of: C10: 3.9%, C11: 37.4%, C12: 35.4%, C13: 23.1%. The mixture of coconut diethanol amide (CDEA) was kindly supplied by H. Fr. Schröder. The proportional composition of the five homologs is: C7: 7%, C9: 7.5%, C11: 60.9%, C13: 18%, C15: 6.6%. 4-NP₁EO-d₂ and 4-*n*-NP-d₈, which

were used as the internal standard, were obtained from Dr. S. Ehrenstorfer (Augsburg, Germany).

Stock solutions (1 mg/ml) of individual standards and standard mixtures were prepared by dissolving accurate amounts of pure standards in methanol. Working standard solutions were obtained by further dilution of stock solutions with methanol.

Toxicity procedure

The experimental procedure for the bacterial bioluminescence assay was based on the ISO 11348 standard protocol [18]. The bacterial assay used the commercially available system Microtox™ (Carlsbad, CA, USA).

The analysis was carried out with all dilution and reagents tempered at 15 °C. To enhance test performance, the osmolality was adjusted in order to obtain 2% saline in each solution or sample.

Bacterial reagents were reconstituted just prior to analysis and the pre-incubation times used were those given in the device protocols. In all measurements, the percent inhibition (%I) was determined by comparing the response to a saline control solution to the response to the diluted sample.

The concentration of toxicant in the test that caused a 50% reduction in light (inhibition=50%) after exposure for 15 or 30 min was designated the 15 or 30 min EC₅₀ (effective concentration) value. Tests were performed at 15 °C. Light measurements were made using a luminometer.

Sampling and sample preparation

Thirty-one samples were collected during March 2007 from the influents and the effluents of a WWTP-1 located near Barcelona (NE of Spain), which discharged into the River Llobregat. This WWTP-1 was equipped with two different pilot-scale membrane bioreactors (MBR), the first using Kubota membranes (Osaka, Japan), and the second using the Koch system (Wilmington, MA, USA). These MBRs were operating in parallel with a conventional biological treatment, and the influents and the effluents undergoing the different treatments were characterized. Triclosan, methyl triclosan, and surfactants were quantified by SPE–LC–MS/MS and GC–MS, and the acute toxicities of the samples were assessed by the Microtox™ test. Detailed information on the MBRs has been provided by Gonzalez et. al. [19, 20]. Influent water samples (samples after primary treatment—from now on we will refer to these as “influent”), CAS effluent and MBR effluent water samples were taken as composite samples (24 h).

The second set of samples came from seven domestic WWTPs located along the Ebro River, and they were designated as WWTP-2 to WWTP-8. The triclosan and

methyl triclosan were quantified in these samples and they were related to the acute toxicity.

All of the samples included in this study were collected in Pyrex borosilicate amber glass containers previously rinsed with tap water and high-purity water and kept in the dark at 4 °C. Before toxicity evaluations, the water samples were adjusted to a neutral pH and 2% NaCl content.

Sample preparation

All samples were filtered through 0.45- μ m membrane filter and preconcentrated on ISOLUTE C₁₈ solid-phase extraction (SPE) cartridges (IST, Hengoed, UK) within 48 h in order to avoid any degradation of target compounds and loss of sample integrity.

Different volumes were taken depending on the type of sample: 200 ml of CAS effluent and MBR effluent, and 100 ml of the influent.

The complete SPE procedure has been described elsewhere by Petrovic [21]. After preconcentration, SPE cartridges were wrapped in aluminum foil and kept at 20 °C until analysis. Cartridges were eluted with 2 \times 4 ml of methanol. The eluents were evaporated to dryness with a gentle stream of nitrogen and reconstituted with methanol to a final volume of 1 ml for LC–MS/MS analysis. For GC–MS analysis, the samples were reconstituted with 500 μ l of methylene chloride.

Chemical analysis

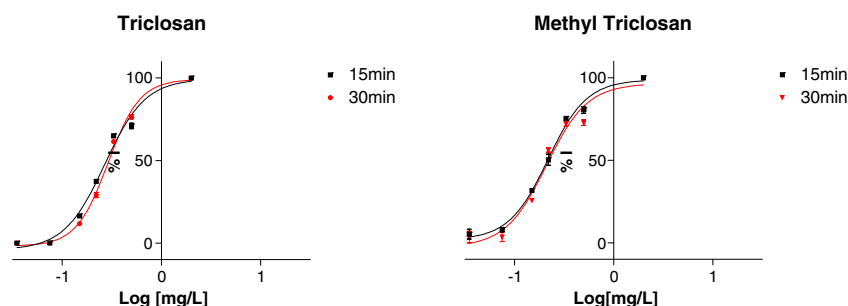
Gas chromatography–mass spectrometry GC–MS analyses were run on a Trace 2000 gas chromatograph from Thermo Electron (San Jose, CA, USA) coupled to a mass spectrometer from Thermo Electron with an electron ionization (EI) mode at 70 eV.

Compound separation was achieved using an HP-5MS capillary column of length 30 m \times 0.25 mm i.d. and with a film thickness of 0.25 μ m from J&W Scientific (Folsom, CA, USA) with the following temperature program: from 100 °C (holding time 1 min) to 130 °C at 30 °C/min, from 130 °C to 220 °C at 8 °C/min, then to 280 °C at 30 °C/min, and finally to 310 °C at 6 °C/min (holding time 10 min). Injection was achieved in the splitless mode, with the split valve closed for 0.8 min.

Helium was used as carrier gas at a flow rate of 1 mL/min.

The injector, transfer line, and ion source temperatures were set at 280 °C, 250 °C, and 200 °C, respectively, and the detector voltage at 400 V. The injection volume was 2 μ L. Acquisition was achieved in time-scheduled selected ion monitoring (SIM) mode to increase sensitivity and selectivity. Quantification was carried out using an external

Fig. 1 *Vibrio fischeri* inhibition curves for standard substances after 30 min of exposure. The curves show the percent inhibition vs. the logarithm of the concentration expressed in $\mu\text{g/ml}$



standard curve and the molecular weight; the diagnostic ions used for the GC–SIM–MS analysis were 290, 288, and 218 for triclosan, and 304, 302, 252 for methyl triclosan, and the quantification masses were 288 and 302 for triclosan and methyl triclosan, respectively.

Identification and quantification were carried out automatically by the Xcalibur software.

Liquid chromatography–tandem mass spectrometry Alkylphenol ethoxylates (APEOs) and their metabolites [alkylphenols (APs) and alkylphenoxy carboxylates (APECs)], as well as other surfactants (LAS and CDEA) were analyzed using a liquid chromatography–mass spectrometry (LC–MS) method as described by Gonzalez et al. [22]. The HPLC system consisted of an HP 1100 autosampler with a 100- μl loop and an HP 1090 A LC binary pump, both from Helwett-Packard (Palo Alto, CA, USA). HPLC separation was achieved on a 5 μm , 125 \times 2 mm i.d., C18 reversed-phase column (Purospher_STAR RP-18), preceded by a guard column (4 \times 4, 5 μm) of the same packing material, both from Merck (Darmstadt, Germany).

The injection volume was set at 15 μl . A binary mobile phase gradient with methanol (A) and water (B) was used for analyte separation at a flow rate of 400 $\mu\text{l}/\text{min}$. The elution gradient was linearly increased from 30% A to 80% A in 10 min, then increased to 90% A in 5 min, then to 95%, and kept isocratic for 5 min. Detection was carried out using an LC–MSD HP 1100 mass-selective detector, equipped with an atmospheric-pressure ionization source and electrospray interface.

The detection was performed under negative ionization (NI) for LAS ($n=10$ –13), NPECs, OPECs, NP and OP, and under positive ionization (PI) for CDEA ($n=7, 9, 11, 13, 15$) and NPEOs ($n=1$ –15).

Quantitative analysis was performed in selected ion monitoring (SIM) mode using external calibration (the internal standards 4-NP1EO-d2, in the PI mode, and 4-*n*-NP-d8, in the NI mode, were used to check the extent of ion suppression in MS detection). A series of injections of target compounds in the concentration range from 50 ng/ml

to 50 $\mu\text{g}/\text{ml}$ were used to determine the linear concentration range.

Results and discussion

Validation studies

Quantitative information on the toxicity was obtained by calculating the toxicity quantifying capacity in terms of EC_{50} , toxicity units (TU), and the toxicity detection capacity (in terms of the lowest observed effect concentration, LOEC).

A wide range of concentrations of triclosan and methyl triclosan were measured (2, 0.5, 0.33, 0.22, 0.150, 0.075, 0.0375 mg/L), inhibition curves were fitted based on a four-parameter equation, and the corresponding 50% effective concentrations (EC_{50}) were calculated.

The acute toxicities (to *V. fischeri*) and the potential hazards to aquatic systems of triclosan and methyl triclosan were evaluated. Figure 1 shows the bioluminescence inhibition curves obtained for the biocide and its degradation product after 15 and 30 min of exposure.

Table 1 Toxicity parameters obtained using *V. fischeri*, expressed as 50% bioluminescence inhibition (EC_{50} ; $\mu\text{g}/\text{mL}$), toxicity units (TU), and lowest observed effect concentration (LOEC; $\mu\text{g}/\text{mL}$) for standard substances

Compound	EC_{50} ($\mu\text{g}/\text{mL}$)	TU	LOEC ($\mu\text{g}/\text{mL}$)
Triclosan	0.28	357	0.10
Methyl triclosan	0.21	476	0.075
NP	0.36	278	0.12
OP	0.30	333	0.12
NP ₂ EO	2.04	49	0.90
NP _{9–5} EO	2.70	37	1.25
OP ₁ EC	2.38	42	1.00
NP ₁ EC	2.64	38	1.22
NP ₂ EC	3.05	33	1.00
LAS	144	0,7	55
CDEA	5.46	18	6.0

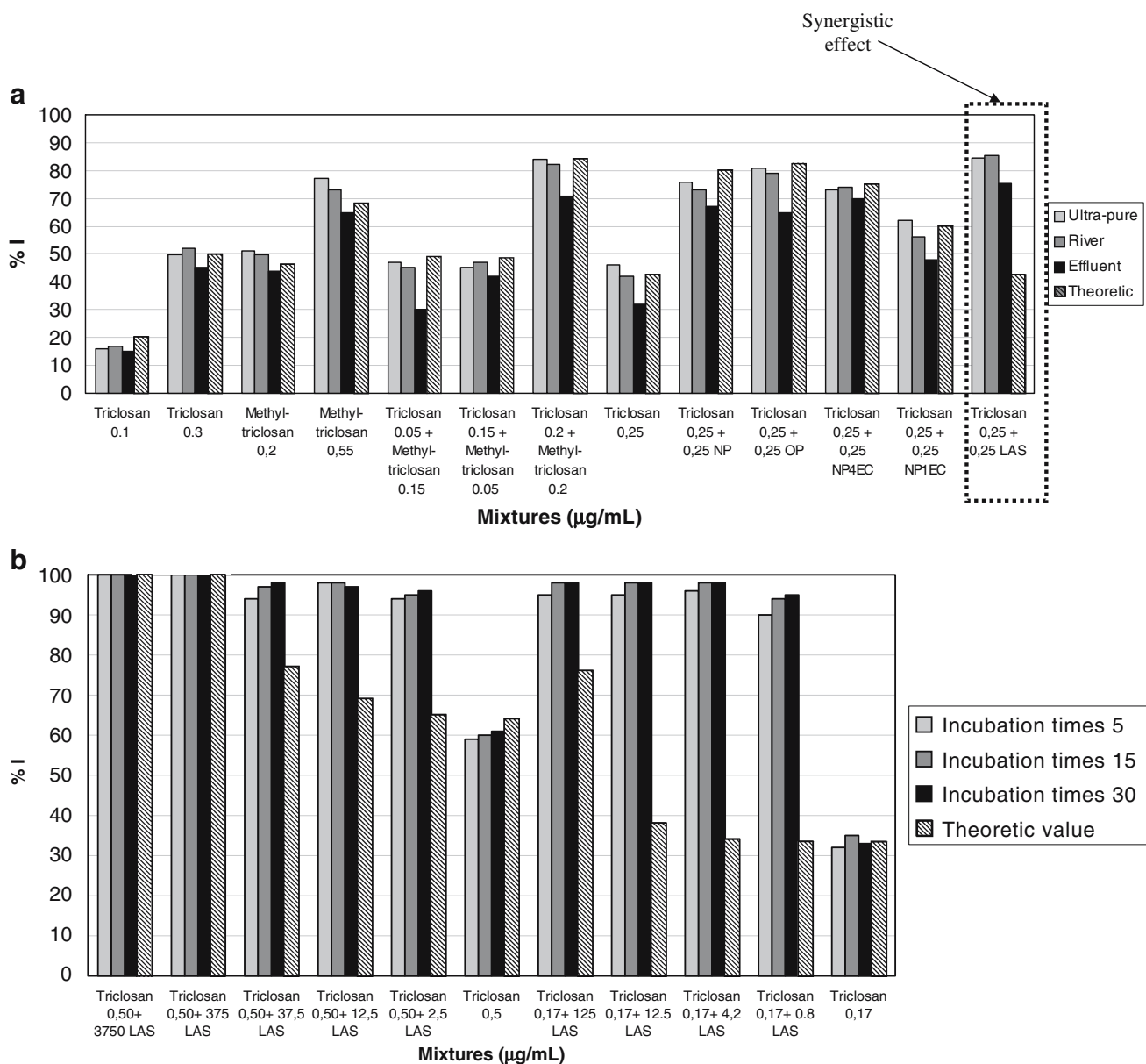


Fig. 2 a *Vibrio fischeri* bioluminescence inhibition percentages of different mixtures of triclosan and surfactants in different types of wastewater in comparison with the inhibition percentages calculated according to a simple additive model. **b** *Vibrio fischeri* biolumines-

cence inhibition percentages of different mixtures of triclosan and LAS after 5, 15 and 30 min of exposure in comparison with the inhibition percentages calculated according to a simple additive model

Together with the EC_{50} values, to make graphical expression and interpretation of the toxicity data more convenient, the toxicity values of the standard substances were converted into toxicity units according to the formula:

$$TU = 100(EC_{50})^{-1}$$

Table 1 summarizes the toxicity parameters for triclosan and methyl triclosan, as well as those for a series of surfactants commonly found in domestic wastewater, whose possible synergistic or antagonistic effects were evaluated in this work.

The toxicity of the samples was expressed as %I of bioluminescence. The percentage of samples producing 20% inhibition (EC_{20}) was calculated, and for the most toxic samples the EC_{50} values were also calculated. According to the formula for TU, the toxicity impact index TII_{50} is defined for real samples as:

$$TII_{50} = 100(EC_{50})^{-1}$$

This change in nomenclature is due to the fact that they are different parameters; TU is related to the concentration of a known substance, whereas TII_{50} is related to a

percentage of a sample, and therefore is used for a mixture of unknown composition. TII_{50} is directly proportional to the toxicity and it is expressed as a percentage.

Matrix effects and synergic/antagonistic studies

Prepared water samples with different organic carbon contents, namely surface water from the River Llobregat (TOC 29.7 mg/L), groundwater from a well in Barcelona (TOC 2.0 mg/L), and wastewater effluent (TOC 230.3 mg/L), were spiked with different proportions of triclosan, methyl triclosan and different mixtures of these with surfactants, in order to evaluate the influence of matrix effects on the Microtox™ toxicity values in different water matrices and possible synergistic or antagonistic effects between these organic pollutants. In order to ensure the initial absence of triclosan, methyl triclosan and surfactants in the different water matrices used in this study, a solid-phase extraction procedure was applied (based on that described before) to the different types of water, and the percolated waters were collected. Later these percolated waters were fortified with the different mixtures studied.

As can be seen in Fig. 2a, in general no significant differences were obtained between the theoretical value calculated according to an additive model and the results for ultrapure water and river water. However, for wastewater samples, the percentage of bioluminescence inhibition was in general slightly lower than those obtained for other types of water, and also lower than the calculated values. This toxicity diminution can be linked to the adsorption of

some of the toxicants in colloidal organic material, causing a drop in bioavailability. However, mixtures containing linear alkyl benzene sulfonates (LAS) showed greater inhibitions than those calculated by the additive approach. In these cases synergistic effects should be considered.

Figure 2b presents different mixtures of triclosan and LAS, and the synergistic effect between these compounds is confirmed. In all cases the toxicities exhibited by the mixtures were much higher than those calculated using the simple additive approach.

On the other hand, the rapid toxicity of triclosan to *V. fischeri* should be noted. In all cases the inhibition of bioluminescence after 5 min of exposure was not significantly different from the value obtained after 30 min of exposure. The rapid response of *V. fischeri* to triclosan could be further exploited as a tool for assessing biocide efficacy instead of conventional time-consuming methods.

The target site of triclosan is believed to be FabI, an enoyl-acyl carrier protein reductase. Triclosan subsequently prevents lipid biosynthesis [23, 24]. However, the immediate effect on *V. fischeri* bioluminescence observed is probably due to increased cell permeability, causing cell leakage of essential metabolites, and this effect is also increased by LAS.

In order to evaluate the increase of toxicity across the range of wastewater concentrations, a double checkerboard set of concentrations was prepared. Figure 3 presents the inhibition curves obtained for a range of concentrations of triclosan with different concentrations of LAS, ranging from 0 to 800 $\mu\text{g/L}$ (Table 2). The curve fitted for triclosan

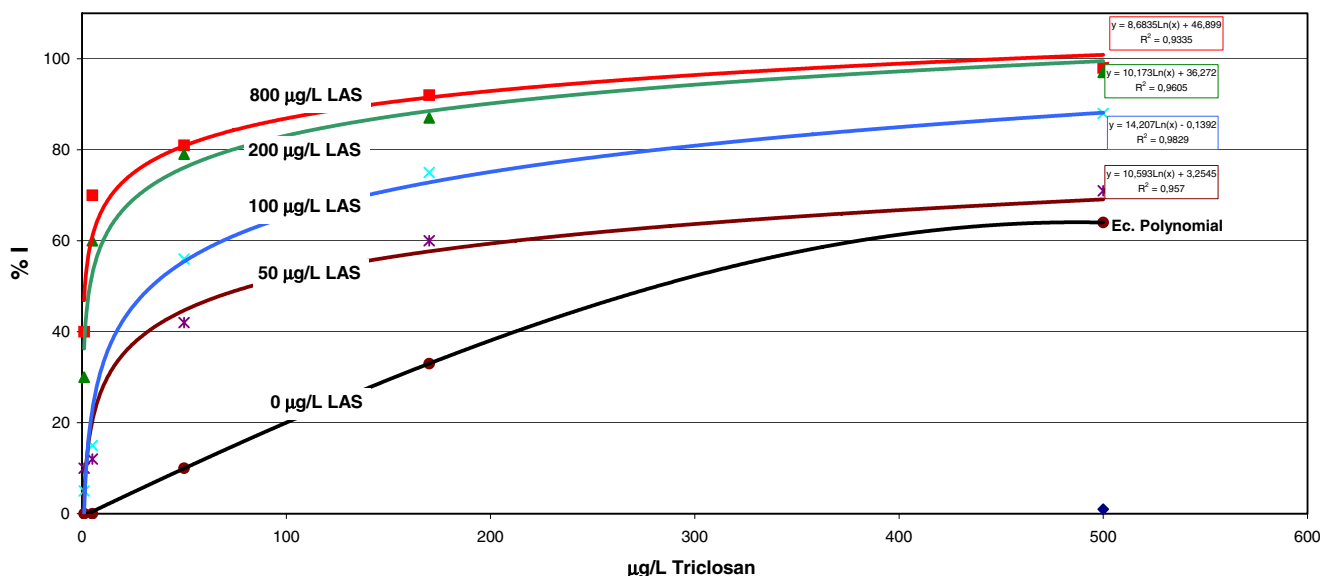


Fig. 3 Exponential curves for *Vibrio fischeri* bioluminescence inhibition percentages due to different concentrations of triclosan ($\mu\text{g/L}$) along with different concentrations of LAS ($\mu\text{g/L}$), and a cubic

polynomial equation fitted for the bioluminescence inhibition percentages obtained for different concentrations of triclosan ($\mu\text{g/L}$) without LAS

Table 2 Range of concentrations of the major classes of compounds in the different types of water from WWTP-1, analyzed by solid-phase extraction followed by liquid chromatography coupled to tandem mass

spectrometry (SPE–LC–MS/MS), or in the case of triclosan and methyl triclosan, by gas chromatography–mass spectrometry (GC–MS)

	Compound	Type of sample				
		Influent, range of 8 samples	CAS, range of 7 samples	MBR (Koch), range of 8 samples	MBR (Kubota), range of 8 samples	
Range of concentrations of major organic pollutants ($\mu\text{g/L}$)	NP ₂ EO	0.0–2.2	0.0	0.0	0.0	
	NP _{9–5} EO	8.5–21.0	0.0–4.1	0.1–1.0	0.2–0.7	
	OP ₁ EC	0.0–1.0	2.4–5.6	0.7–4.5	1.4–2.5	
	OP ₂ EC	0.0–5.5	0.6–1.1	0.9–4.4	0.3–1.0	
	NP ₁ EC	0.4–1.0	1.2–1.8	0.5–1.5	0.5–1.4	
	NP ₂ EC	0.0–1.4	1.0–2.29	2.2–4.2	0.7–3.8	
	OP	0.0	0.9–2.3	0.0	0.0	
	NP	3.3–6.5	0.9–1.2	0.0–1.7	0.0–1.34	
	LAS	1100–1700	4.4–45.8	2.3–73.2	1.3–45.2	
	CDEA	56.6–285	0.0–12.2	0.0–5.3	0.0–24.2	
	Triclosan	0.4–9.4	0.2–0.7	0.1–0.2	0.1–0.6	
	Methyl triclosan	0.1–1.0	0.0–0.2	0.0	0.0	
	Theoretical values	Additive %I	1.0–5.0	0.3–1	0.1–1	0.1–1
		Synergic %I	20–26	19–20	14–20	14–20
Measured toxicity parameters	%I	54–95	19–37	6–18	5–22 (44)	
	TII ₂₀	2.7–4.8	0.8–2.5	0.5–1	1.1–2.2	
	TII ₅₀	1.0–2.2	0.5–1.0	NT	0.4–1.0	

Theoretical values are the theoretical range of inhibitions calculated using a simple additive approach and the theoretical range of inhibitions according to a model that includes the synergism between LAS and triclosan.

Measured values are the ranges obtained for the different types of wastewater using the MICROTOX™ test.

without LAS is derived from a cubic polynomial equation, whereas in the presence of LAS the values of inhibition followed an exponential equation. The figure shows an exponential fit with good regression values (R^2) for the different LAS concentrations.

For mixtures including LAS and triclosan the additive approach cannot be used and so an exponential approach should be included.

The cubic model for the rate of light loss (without LAS) probably reflects the cascade of intracellular events that occurs due to the inhibition of cellular metabolism and membrane disruption. The exponential fit in the presence of LAS confirms this theory, and suggests that the double polarity of the LAS molecules increases the processes of membrane disruption.

Wastewater studies

A comparison of the %I with the triclosan concentrations measured by SPE–GC–MS for the samples from WWTP-1 is shown in Fig. 4. As can be seen in this figure, the toxicity and the concentration values of triclosan in the samples followed the same trend. Figure 5 shows the TII₅₀ values for more toxic samples, and the TII₂₀ for less toxic ones, plotted against the triclosan + methyl triclosan concen-

trations. Good correlations between the toxicity and the concentrations were obtained in both cases. On the other hand, chemical characterization of the samples showed that triclosan and methyl triclosan were two of the most important organic toxicants present in these wastewaters, especially when LAS was present to enhance biocide toxicity.

Even though a large proportion of the organic pollution remains unidentified, and in this study a selection of organic substances were considered, using a mixed model including the additive approach and the exponential increase in triclosan toxicity in the presence of LAS, approximately 25% of the total toxicity of the influent samples can be identified, which indicates the important contribution of triclosan to the total organic toxicity of the wastewater.

From an environmental point of view, it should be pointed out that triclosan was present in all of the samples, and that methyl triclosan was present in most of them, which is a clear indication of the inability of wastewater treatments to completely remove these biocides. However, a general reduction in toxicity, and better triclosan and methyl triclosan removal rates, were obtained after the MBR treatments; the concentration of triclosan was high (554 ng/L) and was toxic after MBR treatment in just one of the analyzed samples.

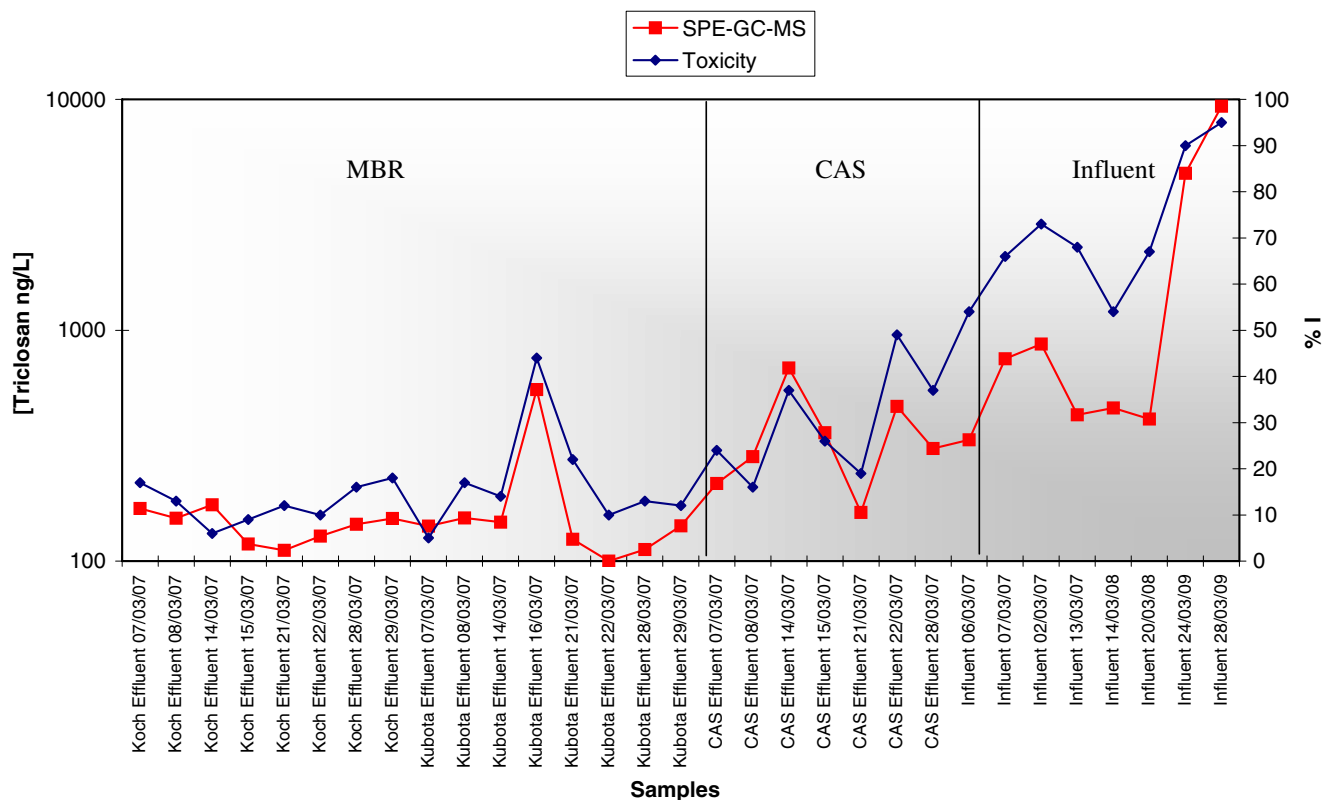


Fig. 4 Toxicity as the percent bioluminescence inhibition, and concentrations of triclosan in ng/L (logarithmic scale), for wastewaters from WWTP-1 after treatment in the Koch and Kubota membrane

bioreactors (MBR), after a conventional secondary treatment (CAS), and for the influents. R^2 indicates the goodness of the fit

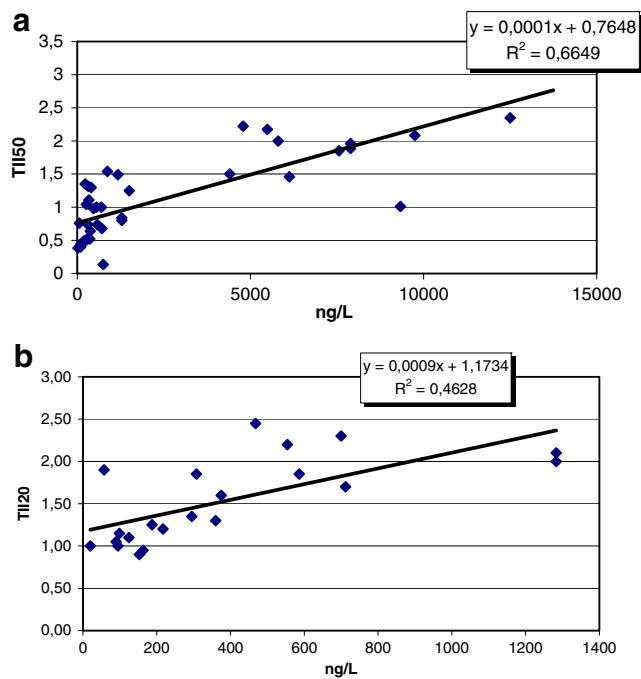


Fig. 5 Linear regressions between concentrations of triclosan+methyl triclosan (ng/L) and toxicity impact index (TII_{20}) for less toxic samples, and between concentrations of triclosan+methyl triclosan (ng/L) and toxicity impact index (TII_{50}) for more toxic samples. R^2 indicates the goodness of the linear regression

Conclusions

Using a standard organism (*Vibrio fischeri*), triclosan and methyl triclosan have been identified as two of the major organic pollutants that currently contribute to the acute toxicity of domestic wastewater.

The response to triclosan is similar to the response to nonylphenol, which is included in the blacklist, and triclosan has been related to endocrine-disruptor effects, which is also the case for nonylphenol. Moreover, triclosan is a chronic toxicant and is a precursor of dioxins.

Good agreement was found between the concentrations of triclosan and methyl triclosan and the range of acute toxicity. In addition, a strong synergic effect between triclosan and LAS was observed and quantified. However, no synergistic or antagonistic effects with other surfactants or their degradation products were observed.

In terms of the influence of matrix effects on toxicity, in general only wastewater samples with high amounts of organic material can influence the acute toxicity. Samples with high TOCs generally showed decreased responses because some of the triclosan and other organic toxicants are adsorbed and therefore the bioavailability decreases.

Triclosan and methyl triclosan were present in the 45 samples analyzed in this study, which indicates that these

compounds are not totally removed during the water treatment process. However, significant decreases in their concentrations and the toxicity were seen after MBR treatments, in comparison with the values obtained after conventional treatments with activated sludge.

Finally, the rapid response of *V. fischeri* to triclosan could be further exploited as a tool for assessing biocide efficacy, in contrast to conventional time-consuming methods involving plate counts.

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