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Triclosan, a commonly used bactericide found in human milk and in the aquatic environment in Sweden

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Abstract

High levels of the commonly used, effective bactericide Triclosan was found in three out of five randomly selected human milk samples. It was also found in the bile of fish exposed to municipal wastewater and in wild living fish from the receiving waters of the three wastewater treatment plants. © 2002 Elsevier Science Ltd. All rights reserved.

Keywords: Municipal wastewater; Antibacterial; Fish bile; Exposure; Bio available

1. Introduction

Triclosan (Fig. 1) is a very common antibacterial agent used in a number of products as diverse as toothpaste, detergents, household sponges, plastic cutting boards, socks and underwear. The main manufacturer is Ciba-Geigy that uses the names Irgasan® DP 300 and Irgacare® MP. Impregnated fibres and polymers have names like Ultra-Fresh®, Amicor®, Microban®, Monolith®, Bactonix® and Sanitized®. Triclosan is a stable, liphophilic compound ($\log K_{ow} = 4.8$) able to form low chlorinated dioxins on incineration and under the influence of sunlight, under certain circumstances even more highly chlorinated isomers, as demonstrated by Kanetoshi et al. (1988a,b).

Triclosan is active against both gram-positive and gram-negative bacteria and its mechanism of action and structural binding has recently been elucidated by a number of researchers (McMurry et al., 1998; Levy et al., 1999; Heath and Rock, 2000). The enzyme ENR (enoyl–acyl carrier protein reductase) involved in the bacterial lipid biosynthesis is effectively inhibited by triclosan. It has not yet been shown toxic to mammals, but it is toxic to water living organisms such as fish (LC_{50}, rainbow trout = 0.35 mg/l) and Daphnia magna ($EC_{50} = 0.39$ mg/l) and especially to algae ($EC_{50} = 1.5$ μg/l) (Ciba, 1998). Very little is known about sublethal effects in fish living in the recipient. The use of this type of phenolic antibacterial substances was abandoned in most hospitals in Sweden several years ago, since they were considered unnecessary in practical use (Myrbäck, 1999). In the late 1970s it was shown that one of these substances, hexachlorophene used in soaps, was absorbed through the skin and caused damage to the nervous system of infants (de Groot et al., 1994).

In order to investigate human exposure for triclosan, five randomly selected samples of human milk were analysed using GC/MS. Triclosan occurrence in the aquatic environment was examined by exposing rainbow trout in cages in the receiving water outside a small wastewater treatment plant and in tanks to treated wastewater from two large plants. Wild living fish was also caught in the receiving waters of the three plants. Bile fluid from the exposed fish was analysed for triclosan. As it was found impossible to get information on what active ingredient an antibacterial product contains, other that toothpaste and cosmetics, a number of products on the Swedish market was collected in

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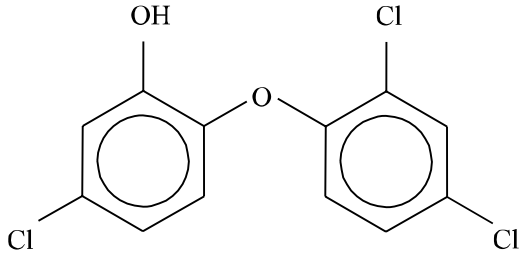

Fig. 1. Triclosan (5-chloro-2-(2,4-dichlorophenoxy)-phenol).

co-operation with the Swedish Consumer Agency and analysed by GC/MS.

2. Materials and methods

2.1. Chemicals and reagents

Ethyl acetate SupraSolv and hexane LiChrosolv, were obtained from Merck, methyl-*t*-butyl-ether HPLC-grade came from Rathburn, β-glucuronidase (101200 β-glucuronidase units/ml and 290 sulfatase units/ml) from Sigma, acetic anhydride pro analysi from Prolab. Formic acid, Na_2CO_3, H_2SO_4, HCl and KOH were all pro analysi from Merck. Fluoranthene and β,β-binaphthyl with purity >99% were purchased from Larodan. Triclosan (Irgasan DP300) was a gift from Ciba-Geigy.

2.2. Consumer products

Bicycle shorts, socks, cutting boards, shoe insoles, kitchen sponges and WC-cleaners with antibacterial treatment were collected from the Swedish market. The products were cut in small pieces and extracted with 10 ml ethyl acetate in test tubes with ultrasonication for a few minutes. Extracts from socks, cutting boards, bicycle shorts and shoe insoles were filtered through glass wool and analysed directly by GC/MS. Extracts from household sponges and WC-cleaners were washed with 2 ml distilled water, pH 2. The organic phase was evaporated to dryness and phenolic compounds were distributed to 2 ml 0.5 M KOH/50% EtOH. The alkaline phase was washed twice with 2 ml hexane and the compounds reextracted into 2×3 ml hexane/methyl-*t*-butyl-ether (90:10) after addition of 1 ml 1 M HCl. The organic phase was evaporated to dryness in small test tubes with teflon lined screw-caps, 2 ml hexane and 4 ml 0.1 M Na_2CO_3 was added together with 100 μl acetic anhydride and immediately shaken for two minutes. The organic phase was analysed by GC/MS.

2.3. Human milk

Five individual samples of human milk were randomly collected from the Mothers' Milk Center in Stockholm. To 10 ml milk sample, 10 ml formic acid was added and extracted twice with 25 ml hexane. The hexane extract was evaporated to dryness and the hexane extractable fraction was considered as "fat". The fat extract was redissolved in 2 ml hexane and the phenolic compounds in the extracts were distributed to 2 ml 0.5 M KOH/50% EtOH. The alkaline phase was washed twice with 2 ml hexane and reextracted into 2×3 ml hexane/methyl-*t*-butyl-ether (90:10) after addition of 1 ml 1 M HCl. The organic phase was evaporated to dryness, redissolved in 2 ml hexane and treated with 2 ml $H_2SO_4 \cdot H_2O$. After evaporation of the organic phase, 2 ml hexane containing fluorantene as internal standard and 4 ml 0.1 M Na_2CO_3 was added together with 100 μl acetic anhydride and immediately shaken for 2 min. The organic phase was analysed by GC/MS.

3. Fish exposed to treated wastewater

3.1. Wastewater treatment plants

Three municipal wastewater treatment plants were investigated. Gråbo is a small conventional plant situated in the south west of Sweden. It uses no anaerobic digestion, receives mainly domestic wastewater from approximately 3500 persons and is not connected to any large industries. Ryaverken is a big modern plant on the Swedish west coast treating sewage water from the second largest town in Sweden, Göteborg. The water is subjected to both anaerobic and aerobic digestion. Henriksdal is a big modern plant with similar cleaning steps as Ryaverken, but uses slow sand filtration as an additional cleaning step and is one out of three plants in Stockholm, situated on the east coast of Sweden. Table 1 summarises some data on the investigated plants.

3.2. Tank experiments

At Henriksdal, rainbow trout (*Oncorhynchus mykiss*) was exposed for three weeks in 70 l tanks with treated wastewater "on line" before and after sand filtration at a flow rate of 1 l/min. About 15 fish weighing 60 g each was held in each tank. At Ryaverken, rainbow trout was exposed "on line" four weeks, the tank size was 17 l, the flow rate 0.5 l/min, 15 fish were in each tank and they weighed 45 g each.

3.3. Cage experiments

Rainbow trout was caged for three weeks upstream, just outside the plant and 1 and 2 km downstream Gråbo plant. The contribution of wastewater was ≈50%

Table 1
Some data on the investigated plants

Sewage treatment plant	Connected persons	Wastewater treated daily 1000 m^3	Major cleaning steps	Approx. industrial sewage (%)
Gråbo	3500	0.88	Chemical precipitation aerobic treatment	0
Ryaverken	584 500	365	Chemical precipitation aerobic and anaerobic treatment	11
Henriksdal	607 000	227	Chemical precipitation aerobic and anaerobic treatment sand filtration	9

in the creek. About 20 fish weighing 60 g each was held in each cage.

3.4. Wild living fish

Roach (*Rutilus rutilus*) was caught 2.5 km downstream Gråbo plant, eelpout (*Zoarces viviparus*) 1 and 2 km downstream Ryaverken and perch (*perca fluviatilis*) 2 km from the Henriksdal outlet.

3.5. Fish bile analysis

Bile fluid from the fish was collected, each sample was a pool of five fish. Approximately 200 mg of bile was enzymatically hydrolysed by adding 20 μl β-glucuronidase in 1 ml 0.2 M acetic buffer pH 5 and the mixture was incubated for two hours. After addition of 2 ml water, free compounds were extracted with 2 × 3 ml hexane/methyl-*t*-butyl-ether (2:1). The combined organic phases were evaporated to dryness, 100 μl

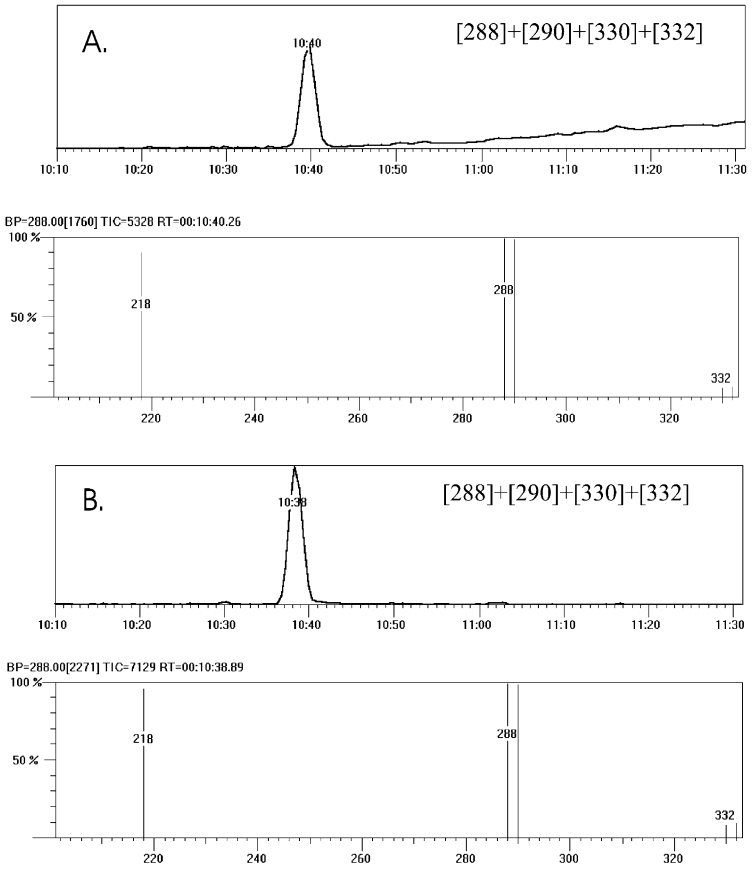

Fig. 2. Ion chromatogram and SIM-spectra from (A) Human milk and (B) Irgasan DP300.

β,β-binaphthyl in ethyl acetate as internal standard was added and the extract was analysed by GC/MS.

3.6. GC/MS determination

The extracts were analysed in splitless mode on a HP 5890 series II GC coupled with a JEOL LR automass with EI ionisation, 70 eV. The ion source was 200 °C and the interface 250 °C. The column (30 m × 0.25 mm J&W 0.25 μm MSDB5) was 90 °C for 1 min, then quickly raised to 200 °C followed by an increase of 10 °C/min up to 300 °C. The injector temperature was 275 °C. Bile samples and consumer product extracts were analysed in full scan mode and human milk samples in SIM-mode (Fig. 2) using the ions 288, 290, 330 and 332. For identification and quantification purposes, ion distribution and retention time was compared to Irgasan DP300 and in the case of human milk and consumer products, to acetylated Irgasan DP300. The identity of triclosan in milk samples was further confirmed by analysing one sample by GC/HRMS at a resolution of 5000. Determination of triclosan in consumer products was only made qualitatively.

4. Results and discussion

4.1. Consumer products

Most of the investigated consumer products with antibacterial treatment contained triclosan. One household sponge was treated with dichlorophene, three WC-blocks with chlorophene and two shoe insoles had an ingredient which still remains to be identified.

4.2. Human milk

High levels of triclosan were determined in three out of five individual samples of human milk, in one sample as high as 300 μg/kg lipid weight (Table 2).

Table 2
Triclosan concentrations in fish and human milk

Sewage treatment plant	Exposure	Fish (species)	Distance from discharge (km)	Concentration in bile (mg/kg fresh weight)
Fish bile exposed to treated wastewater				
Gråbo	Cages	Rainbow trout	Upstream	0.71
			0	47
			1	25
			2	17
	Wild living	Roach	Reference site	<0.01
			2.5	4.4
Ryaverken	Control	Rainbow trout	–	<0.08
	Sewage water		–	34–53
	Wild living	Eelpout	Reference site	<0.01
			1	0.63–0.90
			2.5	0.24–0.37
Henriksdal	Control	Rainbow trout	–	<0.08
	Sewage water		–	83–120
	Sand filtrated sewage water		–	59–94
	Wild living	Perch	Reference site	<0.01
			2.5	0.44

Human milk Sample	Concentration in milk (μg/kg lipid weight)
Cow's milk (control)	<20
Human milk 1	<20
Human milk 2	<20
Human milk 3	60
Human milk 4	130
Human milk 5	300

4.3. Fish exposure to treated wastewater

In all fish bile samples, high levels of triclosan was found: in the bile fluid of rainbow trout caged in the receiving water of the small plant, in wild living fish caught further downstream and in rainbow trout exposed to treated water in tanks (Table 2). Triclosan was also identified in the wastewater and in the sludge.

These findings indicate that substantial amounts of triclosan are released into the environment, which can create unwanted effects. Due to its specific action, it can foster resistant bacteria and it may also be connected with the increase of allergies. Dioxins may be formed when manufacturing triclosan and when incinerating products containing triclosan. It is difficult to estimate the flux of triclosan in the Swedish environment, since unknown quantities are imported in products. Its main use is probably in household products and thus it eventually turns up in the sewage water. About 25% of the total amount of toothpaste sold in Sweden contains triclosan, corresponding to 2 ton of active substance in 1998. Soaps, deodorants etc. comprises about 300 kg (Holmer, 1999). The fact that triclosan contaminates human milk and turns up in unexpected high amount in the aquatic environment urgently calls for further studies.

5. Conclusion

The commonly used, effective bactericide triclosan contaminate human milk, survives several microbial degradation steps in the wastewater treatment process, enters the aquatic environment where it is bio available to fish.

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