Triclosan in plasma and milk from Swedish nursing mothers and their exposure via personal care products

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Abstract

The bactericide triclosan is commonly used in e.g. plastics, textiles and health care products. In vitro studies on rat and human biological systems indicate that triclosan might exert adverse effects in humans. Triclosan has previously been found in human plasma and milk, but neither the primary source of human exposure nor the efficiency of triclosan transfer to human milk is known. In this study, plasma and milk were sampled from 36 mothers and analyzed for triclosan. Scrutinization of the women’s personal care products revealed that nine of the mothers used toothpaste, deodorant or soap containing triclosan. Triclosan and/or its metabolites were omnipresent in the analyzed plasma and milk. The concentrations were higher in both plasma and milk from the mothers who used personal care products containing triclosan than in the mothers who did not. This demonstrated that personal care products containing triclosan were the dominant, but not the only, source of systemic exposure to triclosan. The concentrations were significantly higher in plasma than in milk, indicating that infant exposure to triclosan via breast milk is much less than the dose in the mother.

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1. Introduction

Triclosan (2,4,4′-trichloro-2′-hydroxydiphenyl ether, CAS 3380-34-5) (Fig. 1) is a lipophilic and phenolic compound (log KOW=4.76, pKₐ=7.9) (Syracuse Research Corporation, 2006; Merck Index 12:3, 2000). Due to its antibacterial properties, triclosan has found widespread use in a variety of consumer products including toothpastes, deodorants, soaps, polymers and fibers (Bhargava and Leonard, 1996; Adolfsson-Erici et al., 2002). An assessment of liquid soaps available on the market in the USA showed that 76% of 395 soaps of different brands contained triclosan (Perencevich et al., 2001). In Sweden, toothpaste is considered to be the main singular human source of exposure to triclosan; an estimated 20–25% of all toothpaste sold in Sweden contains triclosan, accounting for ~ 2 tons of use per year (Edwardsson et al., 2005). The European Commission (2002) stated that more than one third of the triclosan used within the EU in 2002 appeared to reach consumers in oral care products, and a similar amount in skin care products.
Triclosan is not acutely toxic to mammals but in vitro studies indicate that at low concentrations triclosan may disturb metabolic systems and hormone homeostasis (Hanioka et al., 1996; Schuur et al., 1998; Wang et al., 2004; Jacobs et al., 2005). Considering the potential adverse effects of triclosan, the magnitude of systemic exposure via the routine use of triclosan containing products in the general population should be clarified.

In humans, triclosan is absorbed through the mucosa in the mouth and intestinal tract when administered via dental care products (Lin, 2000; Sandborgh-Englund et al., 2006). In addition, in vitro studies have shown that triclosan is absorbed through human skin, although it is subject to substantial metabolism during absorption (Moss et al., 2000). Sandborgh-Englund et al. (2006) showed a rapid uptake and elimination of triclosan in human plasma after a single oral dose. The observed terminal half-life in plasma was 21 h and triclosan concentrations returned to baseline within 8 days after the experimental dose. Chronic accumulation of triclosan in humans has not been shown; however, Bagley and Lin (2000) showed that the continuous dose interval associated with normal use of toothpaste for 12 weeks resulted in a chronic systemic circulation of 14–21 ng/ml triclosan in the blood. In addition, Mustafa et al. (2003) demonstrated that triclosan was taken up by and distributed to the cytoplasm and nucleus of human gingival fibroblast cells in vitro, suggesting that a certain amount of triclosan may be retained in human cells. 

Triclosan has been identified in a pooled plasma sample from Swedish men (Hovander et al., 2002). Furthermore, Sandborgh-Englund et al. (2006) showed that triclosan was present in varying concentrations in plasma from 10 individuals, of which five were exposed and five not exposed to triclosan via personal care products. In that small study population, there was no apparent difference in triclosan concentrations in plasma between the exposed and control group, and other sources of exposure than personal care products were hypothesized.

Infants represent a sensitive subgroup of the population for which the exposure to contaminants is of central importance in risk assessment. Triclosan has previously been found in breast milk (Adolfsson-Erici et al., 2002), which indicates a route of exposure of the infant to triclosan via the food. Hence, there is a need to study to what extent triclosan is transferred from plasma to milk in the nursing mother.

In the present study, we investigated if the use of personal care products containing triclosan was the dominant source of systemic exposure to triclosan in a population of Swedish nursing mothers. We also studied the transfer of triclosan from plasma to breast milk in the mothers.

2. Materials and methods

The study was approved by the Regional Ethical Review Board, Huddinge University Hospital, Sweden (dnr: 395/03). Informed consent by the mothers was compulsory for their participation in the study.

2.1. Samples

Thirty-six mothers were recruited at a childcare center in the City of Stockholm between October 2003 and May 2004. Inclusion criteria were that the mother was a first time mother, healthy and that at least half the infant food intake was breast milk. Milk and plasma were sampled on two occasions, approximately 6 and 12 weeks after delivery. In order to avoid any direct contamination from personal care products, the mothers were given instructions to wash the breast with only water and dry it with a triclosan free paper tissue provided by us prior to sampling. The milk sample was expressed by the mother, preferably before the first breastfeeding of the day, collected in a polypropylene container (Sarstedt™, Nümbrecht, Germany), stored in a refrigerator and brought to the clinic the same day. There their blood was sampled in 10 ml sterile heparin tubes (Vacutainer™, Becton-Dickinson, Meylan Cedex, France). The whole blood was centrifuged at 900×g for 5 min and the plasma was transferred to polypropylene tubes (Sarstedt™, Nümbrecht, Germany). The samples were stored at −20 °C until analysis. Utensils used for sampling and storing the samples were extracted and analyzed for triclosan content before use. The mothers’ use of a breast milk pump was documented to control for potential contamination bias.

2.2. Monitoring of personal care products containing triclosan

The mothers were instructed to collect their personal care products (here defined as any chemical product used for personal care, e.g. toothpaste, mouth rinse, soap, shampoo, deodorant, cosmetic, moisturizer, etc.) and bring them to the clinic on the first sampling occasion. The product labels were scrutinized for triclosan
content. In Sweden, all ingredients in personal care products have to be labeled, as required in the Cosmetics Directive within the European Community. The mothers who used products containing triclosan were denoted as "exposed", while those who did not were denoted as "controls".

The mother’s were asked to judge their knowledge about triclosan on a scale ranging from “none” to “very good”.

2.3. Chemical analysis

The analytical method for the analyzed plasma and milk samples, including the sources and quantities of the reagents used, is described elsewhere (Allmyr et al., 2006). Briefly, 13C-labeled triclosan was added to 3 g milk or 5 g plasma as a surrogate standard. The sample was submitted to acid hydrolysis using sulphuric acid to release any triclosan bound in conjugates. The samples were then extracted using hexane/acetone and cleaned up using sulphuric acid. Triclosan was converted to its pentafluorobenzoyl ester, and the extract was analyzed using gas chromatography/mass spectrometry/electron capture negative ionization (GC/ECNI/MS).

2.4. Quantification

The triclosan concentration in the samples was quantified with an internal standard method. The limit of quantification (LOQ) was defined by the triclosan content in the blanks. The area ratio of triclosan to 13C-labeled triclosan (A_{triclosan}/A_{13C triclosan}) in the blanks was consistent, and hence 4 × (A_{triclosan}/A_{13C triclosan}) in the blank was defined as the LOQ. This corresponded to a triclosan amount of 0.06 ng, or 0.018 ng/g milk and 0.009 ng/g plasma. Concentrations below the LOQ were also quantified and these data were used in pair-wise statistics, as this was considered superior to excluding the data. All samples were corrected for the blanks. Triclosan concentrations were determined as the sum of unchanged and conjugated triclosan (ng/g). Since triclosan has been shown to be rapidly eliminated from the body (Sandborgh-Englund et al., 2006), the time elapsed since a given exposure was expected to be the major factor affecting the triclosan concentration, not properties of the body fluids such as lipid content. Therefore, the concentrations were calculated on a fresh weight basis.

2.5. Quality control

The method repeatability test was assessed by repeatedly analyzing triclosan in three different milk samples. The replicates were not analyzed together; rather they were analyzed on different occasions with freezing and thawing of the sample in between. Hence, the method repeatability test also included certain aspects of the sample handling procedures. The sample with higher concentration was among the highest concentrations in milk in this study and the lower concentration was near the LOQ for milk.

2.6. Statistics

The statistical analysis was performed using the SPSS statistical software program version 11.0.0 (SPSS Inc., Chicago, Illinois). Differences and correlations were tested with non-parametric tests: the Mann–Whitney U-test, the Wilcoxon signed rank test and the Spearman rank correlation. The impact of age, body mass index of the mother, days postpartum at sampling, the number of personal care products brought to the reception and the use of a breast milk pump on triclosan concentrations was evaluated with multiple linear regression. The level of significance was 0.05.

3. Results

3.1. Monitoring of products and grouping the study population

Nine mothers used personal care products labeled as containing triclosan and were denoted as exposed. Among these, seven used toothpaste, one used soap and one used deodorant containing triclosan. Twenty-six mothers were denoted as controls, based on the lack of labeling of triclosan on their personal care products. In addition, one mother used toothpaste purchased in Turkey, with no labeling of ingredients. However, when sold in Sweden, the same brand and sort contains triclosan. Since there was considerable uncertainty concerning her exposure, she was excluded from the statistical comparison.

None of the exposed mothers and 19 of the controls had any knowledge about triclosan; eight of the controls had some knowledge.

3.2. Quality control

The coefficient of variation was 6% for the high concentration (mean = 0.84 ng/g, n = 7), 1% for the intermediate concentration (mean = 0.31 ng/g, n = 3) and 5% for the low concentration (mean = 0.020 ng/g, n = 3). The instrumental precision was evaluated by analyzing two samples three times each in a sequence of 184
injections on the GC/ECNI/MS. In both cases, the instrumental coefficient of variation was 1%.

3.3. Triclosan in milk and plasma

In the following, triclosan concentrations refer to the sum of triclosan, in either unchanged or conjugated form, present in plasma or milk. The results from the analyzed plasma and milk samples are summarized in Table 1. Figs. 2 and 3 depict the results from the analyzed plasma and milk samples from the first sampling occasion. One individual could not sample milk at the second sampling occasion. She was not included in the statistical comparison of the two sampling occasions.

Triclosan was found in concentrations above the LOQ in all analyzed plasma samples. Triclosan was found in detectable amounts in all analyzed milk samples. However, on the first sampling occasion, 12 milk samples from the control group (n=26) had concentrations below the LOQ, while on the second sampling occasion the number of milk samples with concentrations below the LOQ was 17 (n=25).

Triclosan concentrations in plasma and milk were not influenced by the age or the body mass index of the mother, days postpartum at sampling or the number of personal care products brought to the reception. The use of a breast milk pump to express milk did not influence the triclosan concentration.

On both sampling occasions, the median triclosan concentration in plasma and milk was significantly higher in the exposed than in the control group (Mann–Whitney U, p<0.001) (Table 1) (Figs. 2 and 3). There was no significant difference in triclosan concentration between the first and second sampling occasion in either plasma or milk from the two groups (Wilcoxon signed rank test: control plasma, p=0.17; exposed plasma, p=0.59; control milk, p=0.70; exposed milk, p=0.14).

Table 1
Triclosan concentrations (ng/g fresh weight) in maternal blood plasma and milk from controls and exposed mothers at the first and second sampling occasion

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<td>9</td>
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Abbreviations: Cont., controls; Exp., exposed.

Fig. 2. Triclosan concentrations (ng/g fresh weight) in maternal blood plasma on the first sampling occasion. Open circle = control, filled circle = exposed, dashed line = LOQ.

Fig. 3. Triclosan concentrations (ng/g) in milk on the first sampling occasion. Open circle = control, filled circle = exposed, dashed line = LOQ.
The triclosan concentration ratios of paired milk and plasma samples (M/P) were calculated to obtain a measure of how much triclosan was transferred from plasma to milk. The M/P range was 0.01–2 (M/P > 1 in just two cases) and was, in both the exposed and control groups, negatively correlated with the triclosan concentration in plasma (Fig. 4) (Spearman rank correlation: first sampling occasion: exposed, \( r_s = -0.85, p = 0.004 \) and control, \( r_s = -0.58, p = 0.002 \); second sampling occasion: exposed, \( r_s = -0.77, p = 0.016 \) and control, \( r_s = -0.69, p < 0.001 \)).

4. Discussion

4.1. Triclosan in plasma and milk and the source of exposure

The triclosan concentration ratios of paired milk and plasma samples (M/P) were calculated to obtain a measure of how much triclosan was transferred from plasma to milk. The M/P range was 0.01–2 (M/P > 1 in just two cases) and was, in both the exposed and control groups, negatively correlated with the triclosan concentration in plasma (Fig. 4) (Spearman rank correlation: first sampling occasion: exposed, \( r_s = -0.85, p = 0.004 \) and control, \( r_s = -0.58, p = 0.002 \); second sampling occasion: exposed, \( r_s = -0.77, p = 0.016 \) and control, \( r_s = -0.69, p < 0.001 \)).

4.2. Milk-to-plasma ratio and infant exposure to triclosan via breast milk

On an individual basis, the triclosan concentration in milk was lower than in plasma (Fig. 4). It is well recognized that many acidic compounds have an M/P < 1, which may partly be explained by the fact that breast milk is slightly more acidic than plasma (Fleishaker, 2003). Triclosan is a phenol and a weak acid (\( pK_a = 7.9 \)), thus the differences in acid ionization equilibrium for triclosan in plasma (pH = 7.5) and milk (pH = 6.5) will favor the ionized form in plasma compared to milk. Assuming that the protonated triclosan equilibrates across the mammary membrane, the total concentration of triclosan in milk will be lower in milk than in plasma. Another factor may be the presence and behavior of triclosan conjugates in the plasma and milk.

Sandborgh-Englund et al. (2006) reported triclosan conjugates in plasma to be approximately 70% of the total triclosan concentration after a swallowed dose of 4 mg triclosan. The conjugated fraction remained relatively unchanged over a range of plasma concentrations. If the hydrophilic and highly acidic triclosan sulfate and glucuronide conjugates are not transported across the mammary membrane, the triclosan concentration as determined in this work (free triclosan + conjugates) would be sequentially placing mothers in the control group. Hence, no conclusions can be drawn from our data about the upper range of plasma and milk concentrations resulting from exposure to these other sources.
higher in plasma. Taken together with the influence of pH discussed above, this would suggest an M/P ratio of ~ 0.25.

Other factors, e.g. protein binding and partitioning to lipids in either matrix, may also affect the relative concentrations in plasma and milk. For example, Guvenius Meironyté et al. (2003) observed lower concentrations of polychlorobiphenyls (OH-PCBs) in milk than in plasma from nursing mothers, which they attributed to the specific and potent affinity of the identified OH-PCBs to the thyroid hormone (T₄) transport protein (transthyretin, TTR) in plasma.

The reason for the observed high variability and the negative correlation of triclosan M/P with plasma concentration is not known. The mothers in the present study were instructed to sample milk preferably before the first nursing of the day. Yet, the milk sample may have been expressed at any time before the time for plasma sampling and before, within or after a feeding, whenever it suited the mother. Perhaps more importantly, the individual dose and time point for exposure to triclosan is unknown; hence, the time between exposure and the sampling of milk and plasma respectively may have varied considerably. The above-mentioned inconsistencies are all likely to have affected the absolute and relative concentrations of triclosan in plasma and milk in the individual subjects, resulting in a variable M/P.

Two daily brushes with 1–2 g toothpaste containing 0.3% triclosan results in a theoretical maximal dose of 6–12 mg. Triclosan was found in breast milk at concentrations ranging from <0.018 to 0.95 ng/g, which are comparable to those determined by Adolfsson-Erici et al. (2002) (0.9, 1.6 and 2.0 ng/g milk, fresh weight basis by personal communication). The triclosan concentrations were significantly higher in the exposed group; thus, the infants’ exposure to triclosan was related to the mothers’ exposure. However, for an infant weighing 4 kg with an estimated milk intake of 150 ml/kg/day, extrapolating to the concentrations found in single milk samples in the present study, the daily intake of triclosan would be <11–570 ng/day. Conclusively, the infant is exposed to a considerably smaller dose of triclosan via the breast milk compared to the dose in the mother. The direct contact with products containing triclosan may be of more importance than breast milk for the exposure of the infant.

4.3. Health perspectives on triclosan exposure

The potential long-term negative effects should be weighed against the benefits of triclosan use. However, the long-term effects of a chronic systemic exposure to triclosan in humans are not fully understood. Triclosan is not acutely toxic to mammals as shown by traditional toxicity tests (Bhargava and Leonard, 1996). However, Schuur et al. (1998) demonstrated that triclosan inhibited the iodothyronine hormone sulfotransferase activity in rat liver cytosol in vitro. In addition, Wang et al. (2004) showed that triclosan, besides being a substrate for certain phase II metabolizing sulfotransferase and glucuronosyltransferase enzymes, was able to inhibit the sulfonation and glucuronidation of 3-hydroxy-benzo[a]pyrene in human liver preparations in vitro. Triclosan has also been shown to be a potent inhibitor of induced activity of the phase I metabolizing enzymes 7-pentoxyresorufin O-depentyldase and 7-pentoxyresorufin O-deethylase in liver microsomes obtained from rats treated with phenobarbital and 3-methylcholanthrene respectively (Hanioka et al., 1996). More recently, an in vitro study showed that triclosan was an activator of the human pregnane X receptor, which regulates the genetic transcription of, among other things, the broad specificity metabolic enzyme CYP3A4, an important mediator for biotransformation of steroids, pharmaceuticals and other xenobiotics (Jacobs et al., 2005). Whether the above-mentioned triclosan–enzyme interactions are of any clinical importance is unknown. Nevertheless, the findings suggest that triclosan may exert effects on biological systems in the sense that the biotransformation of other exogenous and endogenous compounds may be inhibited or induced. The possible long-term clinical adverse effects of a chronic systemic exposure to triclosan in humans need to be scrutinized. Additionally, since the conjugated triclosan might be biologically inactive, the fraction of unchanged triclosan in humans should be investigated with appropriate specific analytical methods and studied in controlled experiments with normal use of triclosan containing products.

The beneficial effects of triclosan to general hygiene and dental health are not clinically evident, and therefore the routine-like use of triclosan in toothpaste and soaps has been questioned (Edwardsson et al., 2005; Larson et al., 2003; Perencevich et al., 2001). In Sweden, all ingredients in personal care products, as defined here, have to be declared on the products. Moreover, in the case of toothpaste containing triclosan, there is an additional voluntary label stating that it should be used only on indication by a dentist. Apparently, this initiative to help the customer make an informed choice has not been very effective, since none of the exposed mothers in the present study were aware of their use of triclosan.

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