Investigation of Human Exposure to Triclocarban after Showering and Preliminary Evaluation of Its Biological Effects

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Supporting Information

INTRODUCTION

Triclocarban (TCC, 3,4,4'-trichlorocarbanilide) is widely used as an antibacterial agent in soaps.1 Many of the antimicrobial bar soaps sold in the United States contain up to 1.5% of TCC, amounting to a million pounds of this chemical used every year.2–3 Due to its environmental persistence and widespread use, TCC was found in surface water in concentrations up to microgram/liter levels, and recent studies show that TCC accumulates in snails and algae.2,4,5 Moreover, the Targeted National Sewage Sludge Survey published in 2009 (US EPA) reports the detection of TCC in all 84 sewage samples analyzed. TCC was found at concentrations up to 0.44 g/kg, the highest levels of all synthetic compounds included in the study.6 On the basis of the toxicological studies from the 1970s, the European Union regard the use of TCC in personal care products (PCP) as safe.7 However, recent findings from our group indicate that TCC has significant biological effects on mammalian targets. At high concentrations, it might act as an endocrine disruptor by enhancing the action of testosterone and other steroids.1,8 Moreover, it was recently shown that oxidative metabolism of TCC may lead to reactive intermediates that can covalently bind to proteins.9 A high-throughput in vitro screening study also showed that TCC potently inhibits the soluble epoxide hydrolase (sEH).10 Its inhibitory potency is similar to many sEH inhibitors, which in vivo alter the biological regulation of inflammation, pain, and blood pressure.11–13 Taken together, the high persistence of TCC in the environment, direct exposure by humans in PCP, and particularly the potent inhibition of sEH suggest that exposure to TCC could influence human health.

In order to assess the risk that TCC influences human health, human exposure levels have to be evaluated. Most human exposure studies on TCC were carried out in the 1970s using 14C-labeled compound.14–18 Studies demonstrated that TCC is extensively metabolized in rodents, monkeys, and humans.15,19–23 Cytochrome P-450 monoxygenases are thought to be largely responsible for the extensive oxidative metabolism. The main phase I metabolites in all species are monohydroxylated-TCC derivatives, bearing a hydroxyl function ortho to the aniline group.15,19–23 The sulfate conjugate of this 2'-OH-TCC is the major metabolite found in human and monkey plasma, though to a smaller extent 3'-OH-TCC has also been detected.15,20,24 A method able to quantify TCC and all these metabolites is necessary to accurately assess human exposure to TCC. Recently, several sensitive LC electrospray ionization (ESI) MS methods have been developed and validated to rapidly analyze TCC and its major metabolites in urine and other biological samples to assess human exposure. We measured human urine concentrations 0–72 h after showering with a commercial bar soap containing 0.6% TCC. The major route of renal elimination was excretion as N-glucuronides. The absorption was estimated at 0.6% of the 70 ± 15 mg of TCC in the soap used. The TCC-N-glucuronide urine concentration varied widely among the subjects, and continuous daily use of the soap led to steady state levels of excretion. In order to assess potential biological effects arising from this exposure, we screened TCC for the inhibition of human enzymes in vitro. We demonstrate that TCC is a potent inhibitor of the enzyme soluble epoxide hydrolase (sEH), whereas TCC’s major metabolites lack strong inhibitory activity. Topical administration of TCC at similar levels to rats in a preliminary in vivo study, however, failed to alter plasma biomarkers of sEH activity. Overall the analytical strategy described here revealed that use of TCC soap causes exposure levels that warrant further evaluation.

ABSTRACT: The antibacterial soap additive triclocarban (TCC) is widely used in personal care products. TCC has a high environmental persistence. We developed and validated a sensitive online solid-phase extraction–LC–MS/MS method to rapidly analyze TCC and its major metabolites in urine and other biological samples to assess human exposure. We measured human urine concentrations 0–72 h after showering with a commercial bar soap containing 0.6% TCC. The major route of renal elimination was excretion as N-glucuronides. The absorption was estimated at 0.6% of the 70 ± 15 mg of TCC in the soap used. The TCC-N-glucuronide urine concentration varied widely among the subjects, and continuous daily use of the soap led to steady state levels of excretion. In order to assess potential biological effects arising from this exposure, we screened TCC for the inhibition of human enzymes in vitro. We demonstrate that TCC is a potent inhibitor of the enzyme soluble epoxide hydrolase (sEH), whereas TCC’s major metabolites lack strong inhibitory activity. Topical administration of TCC at similar levels to rats in a preliminary in vivo study, however, failed to alter plasma biomarkers of sEH activity. Overall the analytical strategy described here revealed that use of TCC soap causes exposure levels that warrant further evaluation.
methods were developed for the detection and quantification of TCC, including, in some cases, the two byproducts of its synthesis, diclocarban (DCC, 4,4’-dichlorocarbanilide) and 3’-Cl-TCC. These methods were applied to environmental samples including water and sludge. However, these methods did not include the quantification of major metabolites. Moreover, they are not designed to investigate biological samples. Online solid-phase extraction (SPE) is a fast sample preparation approach for the analysis of biological samples that can be fully automated. This approach was successfully applied for the detection of TCC among other contaminants in breast milk. This multianalyte method allows the detection of TCC only and not TCC metabolites, and it employs a relatively long runtime of 24 min.

Therefore, in the present study, a fast online-SPE–LC–ESI-MS/MS method specifically designed for the monitoring of TCC and its metabolites (2’-OH-TCC, 3’-OH-TCC, and 2’-SO_2-O-TCC as well as its analogs DCC and 3’-Cl-TCC) in plasma and urine was developed and validated. This method was utilized to investigate the urine concentration of TCC in human volunteers. Exposure to TCC was accomplished following showering with a commercial soap containing TCC. In order to extrapolate the potential of eliciting biological effects, we tested a comparable amount of TCC administered topically in a cream to rats and monitored biomarkers of sEH activity in vivo and evaluated the inhibitory potency of the metabolites in vitro.

## MATERIALS AND METHODS

### Chemicals

3,4,4’-Trichlorocarbanilide (Triclocarban, TCC) was purchased from Aldrich (St. Louis, MO) and further purified (≥99.9%) by repeated recrystallization. β-Glucuronidase (GUS) from Helix pomatia Type I and Type II and GUS Type VA from Escherichia coli were obtained from Sigma Aldrich (St. Louis, MO). The internal standard (IS) (4-chlorophenyl-[13C_6]–TCC (99% 13C)) was obtained from Cambridge Isotope Laboratories Inc. (Andover, MA). All other chemicals were from Fisher Scientific (Pittsburgh, PA) and were of the highest purity available. The chemical structures of the analytes are displayed in Figure S1 (SI).

### Synthesis of Standards

The TCC congeners [4,4’-dichlorocarbanilide (DCC) and 3,3’,4,4’-tetrachlorocarbanilide (3’-Cl-TCC)] and metabolites (2’-OH-TCC, 3’-OH-TCC, and 2’-SO_2-O-TCC) were synthesized using the appropriate isocyanate and amine as described previously. The purity of the products was higher than 99% based on the peak areas following HPLC–UV analysis at 270 nm.

### Online-SPE–LC–MS/MS Analysis of TCC and Its Analogs

Online-SPE–LC analysis was performed in back-flush mode as described in the SI.

### Preparation of Standard Solutions and Spiked Samples

Analyte stock solutions (10 mM) were prepared in DMSO and stored at −20 °C. A solution of IS was prepared in ACN/HAc 98/2 to a final concentration of 20 nM for 1:1 and 12.5 nM for 1:4 dilutions with the sample. For calibration, a multistandard solution (100 μM in ACN) was prepared from stock solution and sequentially diluted and mixed with IS solution 1:1 (v/v), yielding concentrations of 0.015–1500 nM of each analyte. For determination of recovery rates, spiked sample solutions were prepared by mixing 90 μL of pooled blank human urine and pooled blank rat plasma with 10 μL of 1000, 300, and 100 nM of IS free standard solutions, respectively. Each spiked solution was prepared in triplicate and was treated like other samples.

### Human Exposure to TCC Following Showering with a TCC-Containing Soap

TCC exposure was investigated in a group of six healthy volunteers (information about the subjects is provided in the SI). This study was reviewed and approved by the UC Davis IRB committee and informed consent forms were obtained from the subjects prior to the study. The volunteers were questioned about their use of TCC-containing products, and instructed not to use them during the sampling period. Directly before the exposure a urine sample was collected from the volunteers (t = 0 h). The volunteers were then asked to take a shower with commercial 0.6% TCC containing soap (Dial Gold soap, Dial/Henkel, St. Louis, MO) obtained locally. The individuals took a shower, rubbing soap throughout the whole body except for head and genitals. In order to minimize interindividual differences in shower procedures, the individuals let the foam stand for 15 min prior to wash off. Aliquots from each urination over a time span of 24–48 h and a single sample after 72 h were collected and stored at −20 °C until analysis. To normalize the urine samples by renal fluid excretion rate, the creatinine concentrations of all urine samples were determined by a standard photometric method using 2,4,6-trinitrophenol.

### Sample Preparation

For analyses, 50–100 μL aliquots of samples were mixed with IS solution 1/1 (v/v), vortexed, and centrifuged at 16,000g at 4 °C for 5 min. The supernatant was directly injected into the online-SPE–LC–ESI-MS/MS system.

Acid TCC conjugate hydrolysis was carried out as described by Scharpf et al. with minor modifications. Hydrochloric acid (100 μL) was added to 500 μL of urine to a final acid concentration of 1 M in a 3 mL glass vial with a screw cap. The mixtures were vortexed and heated for 20 min to 100 °C, cooled down on ice, and neutralized with 90 μL of 6 M aqueous NaOH. An aliquot of the resulting solution was subsequently mixed with IS solution 1:4 (v/v) and analyzed in the same way as the other samples. For enzymatic hydrolysis, the samples were mixed (9:1, sample: enzyme) with GUS from E. coli type VA (10,000 units/mL in 100 mM potassium phosphate buffer) and incubated overnight at 37 °C. The optimization of the conjugate hydrolysis is described in the SI.

### Topical Administration of TCC to Rats

Four male rats (Charles River Inc. Boston, MA) with a body weight of 485 ± 20 g were used. This study was approved by the institutional UC Davis Animal Care and Use Committee. Animals were housed in UC Davis facilities with access to food and water ad libitum. In order to generate a human equivalent exposure to TCC in rats, we formulated TCC in a cream. The cream was prepared by dissolving TCC in PEG400 and mixing this 1:9 (v/v) with commercially available Vanicream as described to give a final TCC concentration of 1% (v/wt). This amount is similar to the concentrations found in commercially available soaps. Before the administration of TCC, a blood sample (500 μL) was taken from the tail vein of each animal and 2 h after treatment with TCC free Vanicream. These baseline samples from the first week served as controls. One week following the baseline sampling Vanicream formulated with TCC (a volume of 75 μL cream corresponding to a topical dose of 1.5 mg/kg body weight) was applied and blood (500 μL) was sampled 2 h following topical administration. In the following week Vanicream formulated with TCC (a volume of 200 μL cream corresponding to a topical dose of 4 mg/kg) was again administered and blood was sampled 2 h following administration. The cream was administered to one hind paw of...
the rat by gently rubbing for 1 min until the cream disappeared into the paw. The rats were monitored by the investigator between application and sample collection, and they did not lick the paw. Thus the possibility of oral absorption can be excluded.

Plasma was separated from heparinized blood samples and was stored at −70 °C until analysis. To monitor TCC, 20 μL of plasma and for its metabolites 18 μL of plasma were used. For the quantitative analysis of oxylipins, 200 μL of plasma was analyzed by LC−MS/MS, as described by Yang et al. with modifications (see the SI). Additionally, a single urine sample from the animals receiving the highest dose were collected 2−8 h after treatment and analyzed for the presence of TCC-N-glucuronides.

In Vitro Measurement of sEH Inhibition by TCC and Its Metabolites. The potency of TCC and its metabolites was determined for the recombinant human and rat sEH enzymes. Baculovirus expressed, purified enzymes were used in all assays. The inhibitory potencies were measured using two independent activity assays, one based on the generation of a fluorescent product and the other based on the hydrolysis of a putative endogenous substrate, 14(15)-epoxyeicosatrienoic acid [14,(15)-EET], using LC−MS detection of the resulting diol as described.

In vitro Screening for Inhibition of Human Enzymes. The effects of TCC and its metabolites on the activity of human esterases, microsomal amidase, cytochrome P-450 monoxygenases, glutathione-S-transferases, and mitochondrial epoxide hydrolase were investigated in a standard battery of in vitro assays described in the SI.

Data Analysis. All results are reported as mean of three determinations. For rat plasma the variation is reported as standard error (SE). Oxylipin data were analyzed by ANOVA followed by Dunnett’s two-sided t test for between group comparisons.

RESULTS

Development of an Automated Analytical Method for TCC Exposure Assessment. In order to rapidly analyze TCC and its metabolites in urine and plasma samples, an online-SPE−LC−ESI-MS/MS method allowing the direct injection of these crude biological samples (a detailed description is given in the SI) was developed and optimized. The method allows baseline separation, selective analysis, and quantification of TCC, its metabolites, and two analogs in less than 7 min including the online SPE step. The limit of detection (LOD, S/N = 3) for TCC and most of the analytes was 0.15 nM (50 pg/mL), equivalent to 6 fmol on the column (Table 1). The method provided a broad linear range of detection, over 3 orders of magnitude (r² ≥ 0.99, Table 1). Using plasma and urine samples spiked with 10, 30, and 100 nM of the analytes, we observed a near perfect accuracy for all compounds, with a mean recovery rate of 104 ± 8% for both urine and plasma (Table S2, SI). In addition to the high accuracy, the method precision was also excellent with an intersample variation of less than 5% and an intrasample variation of less than 10% for all analytes (Table S2, SI). Thus, the direct injection of crude samples after addition of IS and centrifugation in the fully automated ultrafast online-SPE−LC−MS/MS did not compromise the analytical performance and is ideally suited for the exposure measurement of TCC.

Investigation of Human Exposure to TCC following Showering with a TCC-Containing Soap. The findings from Hiles and Birch indicate that the renal excretion is suitable for the investigation of human exposure to TCC. Therefore, we chose urine analysis for this study. The dry weight of the soap bars was determined before and after shower by six human volunteers (A−F). The mean soap consumption was 11.7 ± 2.6 g (70 ± 15 mg of TCC), corresponding to an average maximal topical dose of 1 mg/kg body weight (40 mg/m² body surface area). This soap consumption is consistent with earlier findings from Howes and Black, who reported a soap consumption of 13.75 ± 4.40 g per use. Subjects B−F did not use other TCC containing PCPs prior to or after the exposure. Subject A however declared using 0.6% TCC containing soap on a daily basis. This subject was instructed to abstain from using TCC-containing soap for 3 weeks after the experiment to monitor the decline in TCC levels and obtain a baseline urine sample. This subject then restarted regular use and provided further urine samples.

Direct Urine Analysis. In all samples analyzed TCC was barely detectable and only a few samples exceeded the LOQ (0.3 nM in urine). The TCC concentration reached an apex between 10 and 18 h after exposure with a maximum concentration of 0.75 nM (Figure 1). The main oxidative metabolite, 2'-OH-TCC,
Figure 2. TCC concentration in the urine after conjugate hydrolysis. (A) Line graph of TCC levels in the urine of six healthy volunteers. (B) Volunteer A, a regular TCC-soap user restarted his/her normal use of soap containing 0.6% TCC after 18 days of abstinence. The concentration in urine (mean and SD of three injections) normalized by the creatinine content of the urine is demonstrated.

The highest concentrations of TCC-N-Gs measured as liberated TCC occurred 10–24 h after exposure (Figure 2). Despite using the same showering procedure and comparable soap consumption, a massive interindividual variation in the maximal TCC concentration was determined (119–1013 nM, Table S4, SI). Moreover, the urinary excretion of TCC-N-Gs profiles normalized by creatinine content also varied significantly (Figure 2). Volunteers A, B, and D displayed a steep increase in the TCC excretion 0–12 h after exposure, which decreased in a biphasic manner. The renal excretion of volunteers C, D, and E increased more slowly and the urinary concentration remained almost constant over a period of 12–36 h after exposure. Overall, the data suggest that complete clearance required more than 72 h (Figure 2).

Taking into account that 25% of TCC is excreted in the urine, 14,15 it can be roughly estimated that on average 0.5 ± 0.1 mg per shower per person was absorbed. This corresponds to an absorbed portion of 0.6 ± 0.2% of the TCC amount applied with the soap, consistent with the findings of Scharpf et al., who report 0.39% after a similar whole body shower experiment. 14

Bioactivity of TCC. In order to investigate if TCC exposure following showering can alter the activity of sEH, we administered a comparable topical dose of 1.5 mg/kg BW and a higher dose of 4 mg/kg BW topically to rats. In contrast to the human study, in which TCC was washed off after 15 min, in the rat study TCC was administered in a cream. Thus, we anticipated that this route of administration led to a higher or at least similar absorption to human exposure. The plasma levels of TCC reached a mean of 16 nM for the low dose and 94 nM for the high dose animal group (Figure S12, SI). Notably, glucuronidation of plasma samples with GUS isozymes did not change the TCC concentration. In urine samples collected from rats, no TCC-N-Gs were detected. This finding supports the earlier observations that in the rat TCC is not metabolized by N-glucuronidation. 19–21 No free 2'-OH-TCC and 3'-OH-TCC were detected in rat plasma. Following GUS treatment low amounts of 9 nM of 2'-OH-TCC and approximately 1 nM of 3'-OH-TCC were detected in the high dose group. In both high and low dose groups substantial concentrations of 2'-SO₃O-
Table 2. Inhibitory Potency of TCC on sEH

<table>
<thead>
<tr>
<th>compound</th>
<th>fluorescence assay</th>
<th>LC−MS/MS assay</th>
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<tbody>
<tr>
<td></td>
<td>human (nM)</td>
<td>rat (nM)</td>
</tr>
<tr>
<td>TCC</td>
<td>39 ± 3</td>
<td>27 ± 6</td>
</tr>
<tr>
<td>2’-OH-TCC</td>
<td>284 ± 19</td>
<td>926 ± 60</td>
</tr>
<tr>
<td>2’-SO₂O-TCC</td>
<td>1440 ± 130</td>
<td>1640 ± 53</td>
</tr>
<tr>
<td>3’-OH-TCC</td>
<td>133 ± 17</td>
<td>48 ± 8</td>
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</tbody>
</table>

*Potency (IC₅₀ values) for TCC and its metabolites was determined using the recombinant human and rat sEH with the fluorescence assay [substrate cyano(6-methoxynaphthalen-2-yl)methyl trans-[(3-phenyloxiran-2-yl)methyl] carbonate] and alternatively using the LC−MS/MS based assay with 14(15)-EET as the natural substrate.

TCC (>1600 nM), the main known TCC metabolite in rat and human plasma, were found (Figure S12, SI). These plasma levels indicate that a significant portion of the topically administered TCC was absorbed through the paw skin in both groups.

Next, we investigated if these plasma levels corresponded to changes in the biomarkers of sEH activity in the plasma. Plasma levels of epoxy fatty acids and their corresponding sEH-generated degradation products, the dihydroxy fatty acids, from arachidonic acid and docosahexaenoic acid, were quantified following topical administration of TCC. The ratio of epoxy to dihydroxy fatty acids is a reliable measure of in vivo sEH activity. ¹⁷,⁴¹ Significant changes in the ratio of epoxy to dihydroxy fatty acids were not observed in either the low dose or the high dose groups (Figure S13, SI). Since the ratio of epoxy to dihydroxy fatty acids reflects systemic inhibition of sEH, we conclude that topical TCC doses did not significantly alter systemic sEH activity.

In order to evaluate the in vitro potency of TCC and its metabolites as inhibitors of human and rat sEH, the IC₅₀ values were determined by utilizing two independent methods, one employing a fluorescent substrate and the other employing an endogenous substrate of sEH 14(15)-EET. ³⁸,³⁹ The inhibitory potency determined for TCC with the fluorescence assay are well in line with the natural substrate (Table 2) and previously reported values. ¹⁰ The human and the rat sEH are inhibited to a similar degree by TCC, regardless of the method used. All TCC metabolites show lower inhibition potency than the parent compound (Table 2). In particular, the main plasma metabolite 2’-SO₂O-TCC, bearing a bulky sulfate group ortho to the urea moiety, reduces the inhibitory activity toward sEH about 100-fold. Thus, the metabolism of TCC to these metabolites leads to a biological deactivation of TCC with respect to sEH inhibition.

In the in vitro screening for other inhibitory potencies, no effect of TCC and its metabolites was detected on the activity of human carboxylesterases, fatty acid amidase, microsomal epoxide hydrolase, cytochrome P450 activity, and glutathione S-transferases.

**DISCUSSION**

An analytical method suited for biological samples is indispensable for the determination of human exposure to TCC. The first aim of our study was to develop a rapid, accurate, and robust analytical approach for the parallel quantification of TCC, its analogs, and metabolites in biological fluids. Through the application of online-SPE sample preparation prior to LC−MS/MS analysis, sample preparation was minimized. Overall, only four steps required manual labor: (i) sample collection, (ii) mixing with IS solution, (iii) centrifugation, and (iv) transferring the supernatant to the vial. Considering that 30 samples are centrifuged at the same time, cumulative hands-on preparation time required per sample is less than 1 min per sample. The online SPE−LC−ESI-MS/MS approach developed herein is not only rapid (7 min/sample) but has excellent accuracy and precision for urine and plasma samples. Due to its sensitivity, it is possible to accurately monitor TCC and concurrently its major oxidative metabolites in these biological samples without the need to expose human subjects to radioactivity, as described earlier. ¹⁴,¹⁵

Following showering with a soap containing 0.6% TCC, the renal excretion profile obtained, including the presence of TCC-N-Gs as major metabolites and the lack of oxidative metabolites, is consistent with all previous studies on the human metabolism of TCC. ¹⁵,²⁰,⁴² The levels of TCC-N-Gs (Figure 2) are consistently higher than those reported from previous showering or bathing studies with TCC-containing soaps. Specifically, Gruenke et al. reported 30 ng/mL TCC-N-Gs (60 nM) in the urine of users of TCC-containing bar soaps, though the TCC content of these soaps and bathing procedures were not reported. ⁴² Interestingly, Howes and Black did not find TCC (<25 ng/mL) in the urine of human subjects after intensive 28-day bathing with 2% TCC containing soap. ¹⁶ However, this is most likely due to a lack of appropriate conjugate hydrolysis.

In contrast to earlier human exposure studies, here the amounts of excreted TCC in urine were quantified in each urination following exposure. Therefore, we demonstrated for the first time that renal excretion of TCC varies widely among individual subjects (Figure 2). The maximal TCC-N-Gs concentrations in the urine were detected 12–24 h after exposure with up to a 10-fold variation among individuals (Table S4, SI). These differences did not correlate with age, height or weight BMI, and body surface area of the volunteers (Table S3, SI) and are most likely due to individual differences in absorption, distribution, metabolism, and excretion. The total clearance of TCC in urine following a single exposure required approximately 72 h. In order to investigate if TCC accumulated due to its slow excretion, we quantified the urine concentrations of TCC and its metabolites over a time period of 2 weeks of daily showering with TCC in a single individual. No accumulation of TCC or its metabolites was detected (Figure 2), but urinary excretion reached a constant level of 79 ± 20 μg TCC g⁻¹ creatinine, indicating a steady state of TCC body burden.

The large amount of TCC extracted as TCC-N-Gs via the urine in the subjects demonstrates that a relevant portion of TCC (70 ± 15 mg, equivalent to a topical dose 1 mg/kg BW) was absorbed after showering. Considering a renal excretion rate of 25% ¹⁴,¹⁵ and a constant creatinine excretion rate of 1.5 g/24 h, we estimated that the mean absorption of TCC was 0.5 ± 0.1 mg, corresponding to 0.6 ± 0.2% of the dose applied (a detailed description of the model used is given in the SI). This result is consistent with previous findings of 0.4% absorption of the TCC applied in a similar showering study using ¹⁴C-labeled TCC. ¹⁴ This good correlation argues that the TCC-N-Gs levels in urine are highly predictive of the human TCC exposure and TCC-N-Gs levels are ideally suited for monitoring TCC exposure.

Our in vitro screening results indicate that TCC does not affect the activity of human carboxylesterases, fatty acid amidase, microsomal epoxide hydrolases, cytochrome P450, and glutathione S-transferases. However, we could show with two independent methods that TCC is a nanomolar inhibitor of sEH.
TCC exhibits a similar inhibitory potency (IC₅₀) when recombinant human and rat enzymes are compared (Table 2). Thus, the rat may be a suitable model for humans when investigating the biological effects of TCC mediated by sEH inhibition. Since the preliminary rat exposure study described here did not lead to systemic inhibition of sEH as measured by oxylipin profiles (Figure S13, SI), it seems possible that the systemic concentrations of free TCC (nonconjugated) found in humans through showering may not be sufficient to cause effects through sEH inhibition. However, at the site of topical application, the TCC concentration might reach levels that lead to local effects via sEH inhibition. Possible reasons for the lack of systemic effects of TCC could be nocovalent protein binding, causing a low uptake into the cells. A key finding of this study is the deactivation of the inhibitory potency of TCC on sEH by its metabolism. As demonstrated in this study, all investigated TCC metabolites show a lower potency than the parent compound (Table 2). The in vitro potency of TCC-N-Gs was not determined, although, on the basis of the extensive SAR of sEH inhibitors, it is highly unlikely that these major urinary metabolites would inhibit sEH. We predict that the glucuronic acid group would prevent hydrogen bonding of the urea moiety in the active site of the enzyme, and on the basis of our crystal structure data, TCC-N-Gs are too large to fit in the catalytic pocket.

Overall, our human exposure study in a small group of subjects showed that a portion of the TCC present in bar soaps is absorbed through the skin and is excreted in urine as N-glucuronides. These urinary glucuronides appear to be valuable biomarkers of TCC exposure. TCC is a nanomolar inhibitor of sEH, and inhibition of this enzyme has been shown to have profound though largely beneficial effects on mammalian physiology. Our studies of TCC exposure in human volunteers using a commercial soap when compared to preliminary exposure and efficacy data in the rat suggest that such TCC exposures are unlikely to elicit systemic changes based on sEH inhibition. However, local dermal effects cannot be ruled out. The TCC exposures found following showering also indicate that a careful risk benefit analysis of TCC in personal care products should be undertaken. In particular, long-term exposure studies in humans that include biomonitoring of TCC in blood should be carried out to evaluate if exposure after using PCPs containing up to 1.5% TCC provides a sufficient margin of safety. With the online-SPE—LC—MS/MS method described herein, we provide an excellent analytical tool to answer these questions.

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**NOMENCLATURE**

TCC-N-Gs triclocarban-glucuronides
TCC triclocarban
BW body weight
DCC diclocarban
EPA Environmental Protection Agency
ESI electrospray ionization
GUS glucuronidase
IS internal standard
LC liquid chromatography
MS mass spectrometry
PCP personal care product
SAR structure–activity relationship
sEH soluble epoxide hydrolase
SI Supporting Information
SPE solid phase extraction
SRM selected reaction monitoring
TCC triclocarban
TCC-N-Gs triclocarban-N-glucuronides

**ASSOCIATED CONTENT**

Supporting Information. Details of methods and results pertaining to the LC—MS analyses of TCC, its metabolites, oxylipins, and the enzyme inhibition assays are provided, and detailed results are presented in Tables S1—S6 and Figures S1—S13. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes
The authors declare that no competing financial interests exist.

**REFERENCES**


