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## Biomarkers of exposure to triclocarban in urine and serum

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### ABSTRACT

3,4,4'-Trichlorocarbanilide (triclocarban, TCC) is widely used as an antimicrobial agent in a variety of consumer and personal care products. TCC is considered a potential endocrine disruptor, but its potential toxic effects in humans are still largely unknown. Because of its widespread uses, the potential for human exposure to TCC is high. In order to identify adequate exposure biomarkers of TCC, we investigated the metabolic profile of TCC in adult female Sprague Dawley rats after administering TCC once (500 mg/kg body weight) by oral gavage. Urine was collected 0–24 h before dosing, and 0–24 h and 24–48 h after dosing. Serum was collected at necropsy 48 h after dosing. We identified several metabolites of TCC in urine and serum by on-line solid phase extraction-high performance liquid chromatography–mass spectrometry. We unambiguously identified two major oxidative metabolites of TCC, 3'-hydroxy-TCC and 2'-hydroxy-TCC, by comparing their chromatographic behavior and mass spectral fragmentation patterns with those of authentic standards. By contrast, compared to these oxidative metabolites, we detected very low levels of TCC in the urine or serum. Taken together these data suggest that in rats, oxidation of TCC is a major metabolic pathway. We also measured TCC and its oxidative metabolites in 50 urine and 16 serum samples collected from adults in the United States. The results suggest differences in the metabolic profile of TCC in rats and in humans; oxidation appears to be a minor metabolic pathway in humans. Total (free plus conjugated) TCC could serve as a potential biomarker for human exposure to TCC.

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### 1. Introduction

3,4,4'-Trichlorocarbanilide (TCC) is widely employed as an antimicrobial agent in a variety of consumer and personal care products including bar soap, detergents, toothpaste, deodorant, and cleansing lotions (The Personal Care Products Council, 2011). With an annual production and/or import volume in the United States of at least half a million pounds, TCC is classified as a high production volume chemical requiring environmental risk assessment by the U.S. Environmental Protection Agency (EPA, 2002). TCC may persist in water even after wastewater treatment (Heidler et al., 2006). As a result, TCC has been detected in surface waters, municipal waste water treatment effluents, estuarine sediments, and in streams nationwide (Halden and Paul, 2005; Heidler and Halden, 2009; Miller et al., 2008; Sapkota et al., 2007; Young et al., 2008).

Some toxicologic studies suggested that TCC could impair mammalian reproduction by reducing birth weight and survival rate in rats (Nolen and Dierckman, 1979). In humans, exposure to TCC has been associated with methemoglobinemia (Johnson et al., 1963). TCC is considered an endocrine disruptor even though TCC does not compete with some endogenous hormones for receptor binding, but at high concentrations could enhance estradiol (E<sub>2</sub>)-dependent or testosterone (T)-dependent activation of ER- and AR-responsive gene expression in cell-based assays and in rats (Ahn et al., 2008; Chen et al., 2008). Although the potential adverse health effects of TCC in humans are still largely unknown, human exposure to TCC is known to occur and ongoing in the general population because of the widespread use of this compound. Therefore, a need exists to identify adequate exposure biomarkers of TCC. For this purpose, understanding the metabolic fate of TCC is necessary.

Dermal exposure is believed to be the main route of human exposure to TCC because TCC is largely used in some personal care products (EPA 2002). Of interest, early publications on the metabolism of TCC in rats suggested that after oral, intravenous, or dermal administration of <sup>14</sup>C-TCC, TCC was mainly excreted in bile and feces (Hiles, 1977; Howes and Black, 1976; Warren et al., 1978),

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regardless of the route of administration (Warren et al., 1978). Less than 6% of the administered dose was excreted in urine (Warren et al., 1978). The major biliary and fecal metabolites were identified as nonconjugated and conjugated TCC and 2'-hydroxy-TCC (2'-OH-TCC) (Jeffcoat et al., 1977; Warren et al., 1978). Other research in monkeys and humans following oral or intravenous administration of TCC also suggested that TCC and its metabolites were mainly excreted in bile (Birch et al., 1978; Hiles et al., 1978; Hiles and Birch, 1978; Scharpf et al., 1975). Unfortunately, bile and feces are not the most adequate matrices for biomonitoring purposes primarily because of collection-related issues. By contrast, validated protocols for the collection of blood and urine for biomonitoring purposes do exist.

The main objective of the present study was to identify metabolites of TCC in the urine and serum of adult female Sprague Dawley rats administered with TCC. We also evaluated the usefulness of these TCC urinary and serum metabolites as biomarkers of TCC exposure in humans by measuring these compounds in 50 urine and 16 serum specimens collected from U.S. adults without known occupational exposure to TCC.

## 2. Materials and methods

### 2.1. Animal experiments

The animal treatment protocol was reviewed and approved by the EPA Internal Animal Care and Use Committee. Twelve female Sprague-Dawley rats (Harlan Laboratories, Indianapolis, IN) about 60 days of age were housed individually in clear polycarbonate cages (20 cm × 25 cm × 47 cm) with laboratory grade pine shavings as bedding at the Reproductive Toxicology animal facility of the U.S. Environmental Protection Agency (EPA) with a 12:12 light/dark cycle (lights off at 7:00 pm), temperature of 20–22 °C and 45–55% relative humidity, and provided with filtered (5 micron) water and NTP 2000 rat chow ad libitum. After a 12-day acclimation period, rats were placed for four days in Fisher Nalgene Rodent metabolism cages (Fisher catalogue # 01-287-6A) and given powdered feed and deionized/filtered water via water bottles; three of the rats were used as controls. The first urine samples ( $U_0$ ) were collected from all twelve rats on day 2, 0–24 h after they were placed in the metabolism cage. Then, we administered to nine of the rats 500 mg/kg body weight of TCC with purity at 99% (Sigma Aldrich Laboratories, Inc., St. Louis, MO, USA) in corn oil by gavage. The second and third urine samples for both control (3 rats) and dosed (9 rats) groups were collected on days 3 and 4, 0–24 h ( $U_1$ ) and 24–48 h ( $U_2$ ), respectively, after TCC administration. After the third urine collection, the rats were euthanized by exposure to excess  $CO_2$ , and serum samples (S) were collected. The urine and serum samples were placed in polypropylene vials and frozen at  $-70$  °C until shipped on dry ice to the Centers for Disease Control and Prevention's National Center for Environmental Health laboratory. On arrival, the samples were stored at  $-70$  °C until analyzed.

### 2.2. Materials and standards

HPLC-grade methanol was obtained from Tedia (Fairfield, OH) and analytical-grade formic acid (98%) was purchased from EM Science (Gibbstown, NJ). 4-methylumbelliferyl glucuronide, 4-methylumbelliferyl sulfate,  $\beta$ -glucuronidase/sulfatase (*Helix pomatia*, H1), and ammonium acetate (>98%) were purchased from Sigma Aldrich Laboratories, Inc.  $D_7$ -TCC and  $^{13}C_4$ -4-methylumbelliferone were obtained from Cambridge Isotope Laboratories, Inc (Andover, MA). 2'-OH-TCC and 3'-hydroxy-TCC (3'-OH-TCC) were synthesized by coupling the appropriate isocyanate and amine compounds as described previously (Ahn et al., 2008; Warren et al., 1978).

### 2.3. Human urine and serum specimens collection

Fifty urine samples were collected anonymously in 2010 from a diverse group of male and female adult volunteers in Atlanta, GA. A waiver of informed consent was requested under 45 CFR 46.116(d) and the Centers for Disease Control and Prevention Human Subjects Institutional Review Board reviewed and approved the study protocol. No personal or demographic data were available. Sixteen commercially available serum samples collected between 1998 and 2003 from 5 male and 11 female donors were purchased from Tennessee Blood Services (Memphis, TN). The potential exposure to TCC from any of the blood or urine donors was unknown.

### 2.4. Analytical method

For metabolite identification, 100  $\mu$ L of rat urine were spiked with 50  $\mu$ L of  $\beta$ -glucuronidase/sulfatase (*H. pomatia*, 463 000 U/g solid) in buffer solution at pH-5.0 (100 U) and incubated at 37 °C for 4 h to hydrolyze the conjugates. The incubated

samples were then diluted to 1 mL with 0.1 M formic acid. TCC and its metabolites were extracted by the on-line solid phase extraction (on-line SPE), separated using high performance liquid chromatography (HPLC) and detected with mass spectrometry (MS). The on-line SPE-HPLC system used was similar to one described previously (Ye et al., 2008). Briefly, it consisted of two binary pumps with degassers, an autosampler with a 900- $\mu$ L injection loop, and one column compartment with a 10-port switching valve (Agilent Technologies, Wilmington, DE). The SPE column was a LiChrosphere RP-18 ADS (25 mm × 4 mm, 25  $\mu$ m particle size, 60 Å pore size, Merck KGaA, Germany), and the HPLC columns were two Chromolith™ Performance RP-18 (100 mm × 4.6 mm; Merck KGaA). The mobile phases A and B were water and 100% MeOH, respectively, for both SPE and HPLC. TCC and its metabolites were detected by negative electrospray ionization using an API 4000 Q Trap™ mass spectrometer (Applied Biosystems, Foster City, CA).

For the quantitative detection of total and free TCC and its metabolites in rat and human urine (or serum), we used a modified on-line SPE-HPLC-MS/MS method (Ye et al., 2008) similar to the one used for metabolite identification described above. We spiked urine or serum (100  $\mu$ L) with 50  $\mu$ L of internal standard solution ( $D_7$ -TCC), 50  $\mu$ L of  $\beta$ -glucuronidase/sulfatase (*H. pomatia*, 463 000 U/g solid) in buffer (pH 5.0) (or 50  $\mu$ L of pH 5 buffer without enzyme for free concentration measurements), and 50  $\mu$ L of 0.5 ppm 4-methylumbelliferyl glucuronide/4-methylumbelliferyl sulfate/ $^{13}C_4$ -4-methylumbelliferone mixed standard. The 4-methylumbelliferone/ $^{13}C_4$ -4-methylumbelliferone peak area ratio was monitored to evaluate the extent of the deconjugation reaction. Samples were incubated for 4 h at 37 °C, then acidified with 0.1 M formic acid (750  $\mu$ L) before analysis by on-line SPE-HPLC-MS/MS. We prepared analytical standards, quality control (QC) materials, and blanks using the same procedure as described above, except that we replaced the urine/serum by the same volume of standard stock solution, QCs, or HPLC grade  $H_2O$  (for blanks). The limits of detection, calculated as  $3S_0$  where  $S_0$  is the standard deviation as the concentration approaches zero (Taylor, 1987), were 0.1 ng/mL in both urine and serum for all three analytes. The spiked recovery in urine or serum ranged from 83% to 120% for the three analytes at four spiking levels: 1, 2.5, 5, and 10 ng/mL.

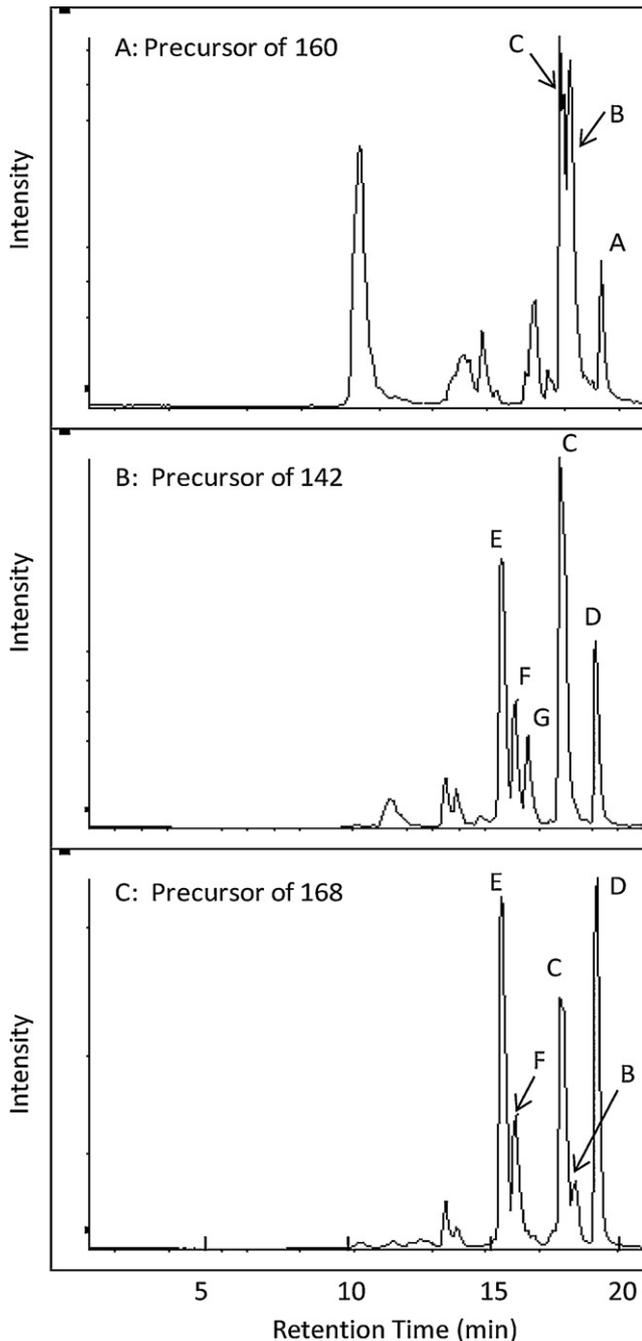
## 3. Results and discussion

### 3.1. In vivo metabolism of TCC in rats

First, we obtained the full scan mass spectra of a TCC standard. The molecular ion and the most abundant fragment ions were 313 ( $[M-H]^-$ ), 160 ( $[C_6H_4NCl]^-$ ), and 126 ( $[C_6H_3NCl_2]^-$ ). Under the experimental conditions described above, TCC eluted at 19.4 min. Because the metabolites of TCC could potentially fragment like the parent compound, we obtained a precursor ion mass spectrum of  $m/z = 160$ , the most abundant fragment ion of TCC, from the rat urine samples ( $U_1$  and  $U_2$ ) after deconjugation and on-line SPE-HPLC separation. We observed three major precursors: A ( $m/z = 313$ , retention time [RT] = 19.4 min), B ( $m/z = 345$ , RT = 18.3 min), and C ( $m/z = 329$ , RT = 17.9 min), but only in the urine ( $U_1$  and  $U_2$ ) of the dosed rats (Fig. 1A). We did not observe these three compounds in any of the three control rats urine samples or in the urine collected before dosing ( $U_0$ ) from the 9 dosed rats (data not shown).

Oxidized metabolites of TCC, such as 2'-OH-TCC, had been reported to be the major biliary metabolites of TCC (Jeffcoat et al., 1977). Therefore, we also obtained the precursor ion scans of the most abundant fragment ions for 2'-OH-TCC ( $m/z = 168$ ) and 3'-OH-TCC ( $m/z = 142$ ) from urine collected from both dosed and control rats. We found a total of six precursors of  $m/z = 142$  and  $m/z = 168$  only in the urine collected from dosed rats after administering TCC (Fig. 1B and C). These precursors were B, C, D ( $m/z = 329$ , RT = 19.2 min), E ( $m/z = 345$ , RT = 15.6 min), F ( $m/z = 295$ , RT = 16.1 min), and G ( $m/z = 295$ , RT = 16.6 min) (Fig. 1B and C).

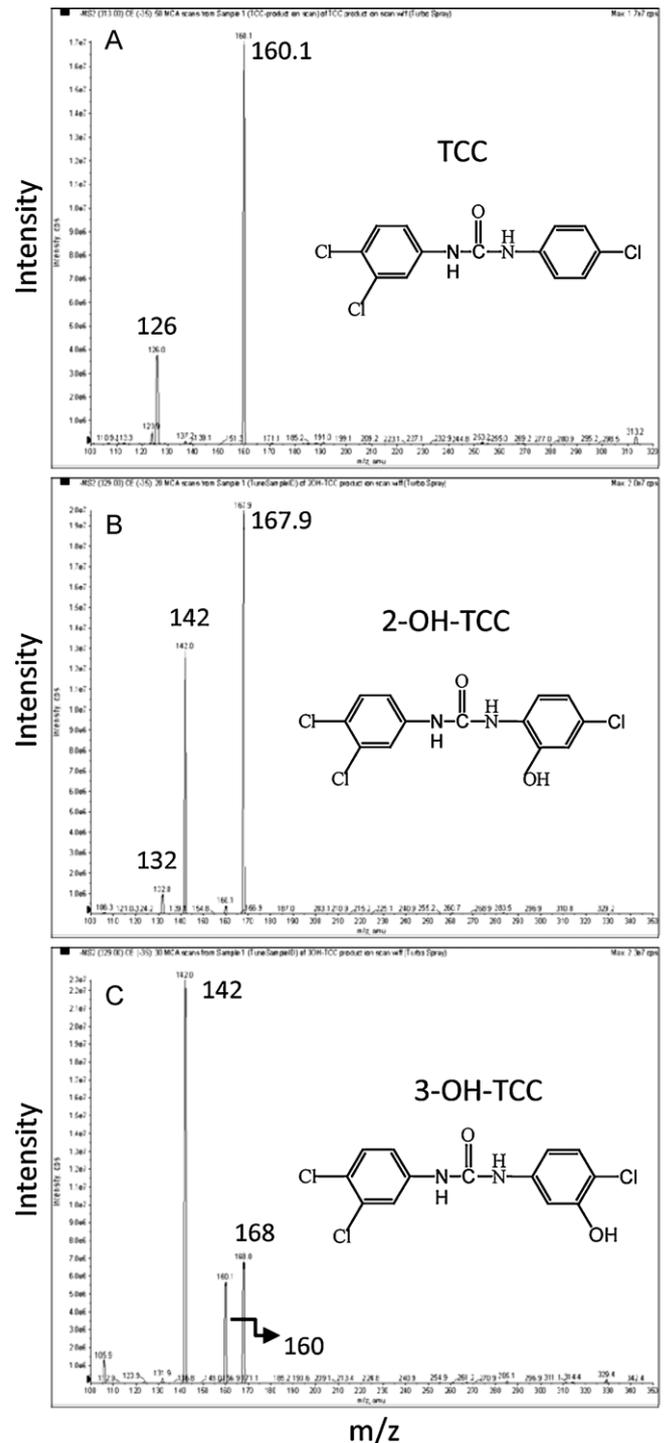
To identify these metabolites, we obtained the enhanced product ion (EPI) mass spectra of the after-dose urine ( $U_1$ ) after on-line SPE-HPLC separation. By comparing with the EPI data and retention times of the authentic standards, we unequivocally identified A as the parent compound TCC (Fig. 2A), D as the oxidized metabolite 2'-OH-TCC (Fig. 2B), and C as 3'-OH-TCC, another oxidized metabolite (Fig. 2C). Metabolites B and E shared the same molecular ion at  $m/z = 345$ , but metabolite E eluted earlier (15.6 min) than metabolite B (18.3 min). EPI scans of these two metabolites showed fragments at  $m/z = 329$ , 160, 168 for metabolite B, and  $m/z = 329$ , 142, 168 for



**Fig. 1.** Total ion chromatograms of precursor ion scans ( $m/z$  160, 142, and 168) from urine samples collected from nine rats after the administration of TCC. Note: A- $m/z$ -313 (RT=19.4 min); B- $m/z$ -345 (RT=18.3 min); C- $m/z$ -329 (RT=17.9 min); D- $m/z$ -329 (RT=19.2 min); E- $m/z$ -345 (RT=15.6 min); F- $m/z$ -295 (RT=16.1 min) and G- $m/z$ -295 (16.6 min).

metabolite E (data not shown). These data suggested that metabolites B and E were di-hydroxylated-TCC isomers, with the second hydroxyl group on different sites of the ring. Similarly, we tentatively identified the two other minor metabolites F and G (both with the same molecular ion  $m/z$  295) as a pair of dechlorinated hydroxy-TCC isomers. However, because of the lack of the authentic standards, the structures of these two pairs of isomers could not be unequivocally determined, and additional NMR experiments are needed to fully characterize the chemical structures of these compounds.

We measured the total (free plus conjugated) and free concentrations of TCC and its two metabolites, 2'-OH-TCC and 3'-OH-TCC, in rats urine and serum with multiple-reaction-monitoring (MRM) after the target analytes were extracted and separated by the on-line SPE-HPLC system. To increase the accuracy of the measurements, we added D<sub>7</sub>-TCC to the samples as the internal standard for all of the three analytes. The ion transitions used for quantitation (or confirmation) and the retention time of each target analyte are listed in Table 1. Fig. 3 shows the total and free median concen-

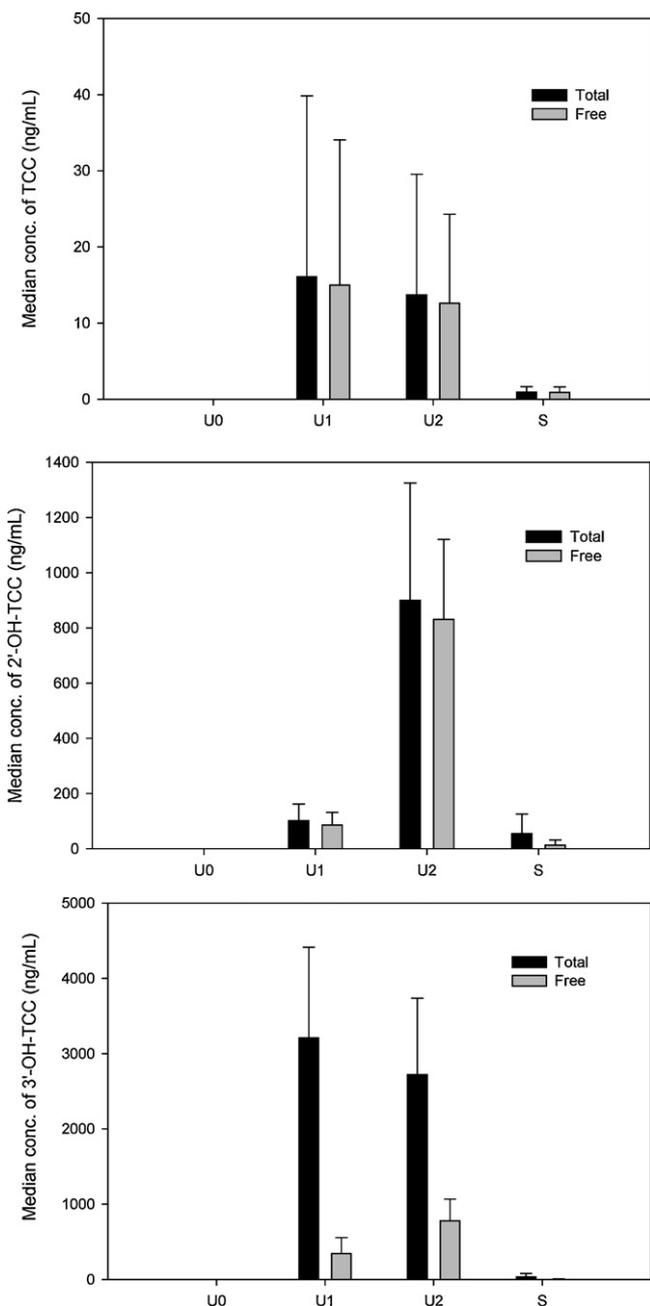


**Fig. 2.** Enhanced product ion (EPI) mass spectra of different precursors in urine collected from rats after the administration of TCC: (A) precursor A, (B) precursor D, and (C) precursor C.

**Table 1**  
Analyte retention time (RT), and precursor ion → product ion transitions monitored for quantitation (and confirmation).<sup>a</sup>

Analyte	RT (min)	Precursor ion → product ion (m/z)
TCC	19.4	313 → 160 (126)
2'-OH-TCC	19.2	329 → 168 (142)
3'-OH-TCC	17.9	329 → 168 (142)

<sup>a</sup> For all of the analytes, we used D<sub>7</sub>-TCC as the internal standard and monitored the m/z ion transition 320 → 163.



**Fig. 3.** Median total and free concentrations of TCC (A), 2'-OH-TCC (B), and 3'-OH-TCC (C) from urine (U<sub>0</sub>, U<sub>1</sub>, and U<sub>2</sub>) and serum samples (S) collected from 9 rats dosed with 500 mg/kg body weight TCC. The error bars indicate the standard deviation of the measurements.

trations of TCC, 2'-OH-TCC, and 3'-OH-TCC from urine and serum samples collected from the 9 rats dosed with TCC on day 2 (pre-dose, U<sub>0</sub>), day 3 (U<sub>1</sub>), and day 4 (U<sub>2</sub>, S). We did not detect TCC or its metabolites in the pre-dose urine (U<sub>0</sub>) samples (Fig. 3). Similarly, we did not detect TCC or TCC metabolites in any of the urine (U<sub>0</sub>, U<sub>1</sub>, and U<sub>2</sub>) and serum samples collected from three control rats (data not shown). Within 24 h after dosing, TCC was rapidly metabolized to the oxidative metabolites. 3'-OH-TCC appeared to be the main metabolite, with median total urinary concentrations of 3210 ng/mL versus 102 ng/mL (2'-OH-TCC) and 16 ng/mL (TCC) (Fig. 3). The median urinary concentrations of 3'-OH-TCC after dosing dropped about 15% from day 3 to day 4 to 2720 ng/mL, and urinary median concentration of 2'-OH-TCC increased from 102 ng/mL to 900 ng/mL (Fig. 3). We also detected 2'-OH-TCC and 3'-OH-TCC in serum, but at much lower levels than in the urine collected in the same after administration; median serum concentrations were 55.1 ng/mL (2'-OH-TCC) and 33.4 ng/mL (3'-OH-TCC). All of these findings suggest that TCC metabolized rapidly in rats through phase I metabolism, and the major urinary metabolites of TCC were 3'-OH-TCC and 2'-OH-TCC.

Furthermore, we detected 3'-OH-TCC mainly in its conjugated form; mean conjugate percent (%), defined as the ratio of concentrations of conjugated and total species, was 88% for urine collected in day 3 and 71% for urine collected in day 4. However, we detected TCC and 2'-OH-TCC in urine primarily in their free form, and the mean conjugate ranged from 14% to 25%. In serum, the main 3'-OH-TCC species was also its conjugate, with the mean conjugate of 3'-OH-TCC at 95%, while the mean conjugate of TCC and 2'-OH-TCC in serum were 1% and 65%, respectively.

Taken together, our data suggest that in the rat, after oral ingestion, TCC undergoes phase I metabolism to form 3'- and 2'-OH-TCC, similar to di(2-ethylhexyl)phthalate in humans (Koch et al., 2004, 2005). We speculate that most of 3'-OH-TCC was further metabolized through phase II metabolism to its conjugate before urinary excretion. By contrast, the formation and elimination of 2'-OH-TCC was slower than that of 3'-OH-TCC and did not appear to involve extensive phase II metabolism. Unfortunately, we collected urine samples for only 48 h after dosing the animals. Therefore, we could not determine whether additional amounts of 2'-OH-TCC conjugate would have excreted in urine more than 2 days after dosing. Nonetheless, the study design was adequate to demonstrate that TCC was rapidly metabolized through phase I and phase II metabolism, and that the oxidative metabolites 3'-OH-TCC and 2'-OH-TCC were the major urinary metabolites of TCC. Previous studies in rats (Hiles, 1977; Howes and Black, 1976; Warren et al., 1978), monkeys, and humans (Birch et al., 1978; Hiles et al., 1978; Hiles and Birch, 1978; Scharpf et al., 1975) suggest similar metabolic pathways of TCC regardless of administration route (e.g., oral, intravenous, dermal). Therefore, our results can be extrapolated to exposure routes other than oral ingestion. However, additional research is needed to identify unequivocally the metabolic profile of TCC by these other routes of exposure.

Of interest, our findings were also in agreement with a previous study which suggested that 2'-OH-TCC and 3'-OH-TCC were the main urinary oxidative metabolites based on the percentage of the extracted radioactivity after repeated oral administration of TCC to three rats (Warren et al., 1978). However, in that particular study, the majority of the urinary radioactivity was recovered from the parent TCC, which did not happen in our study. We speculate that these differences might be related to the much higher dose used in that study (2000 ppm TCC in the diet for 10 days), which could potentially saturate the regular metabolic pathway. Furthermore, our results are also in agreement with those from a recent *in vitro* study suggesting that di-hydroxylated TCC and dechlorinated hydroxyl-TCC could be formed by the oxidative metabolism of TCC (Baumann et al., 2010). However, we could not confirm

**Table 2**

Mean and median concentrations (ng/mL) of total and free TCC, 2'-OH-TCC and 3'-OH-TCC from 50 human urine.<sup>a</sup>

Urine	Total TCC (free TCC)	Total 2'-OH-TCC (free 2'-OH-TCC)	Total 3'-OH-TCC (free 3'-OH-TCC)
Mean conc.	3.85 (<LOD)	0.15 (<LOD)	<LOD (<LOD)
Median conc.	<LOD (<LOD)	<LOD (<LOD)	<LOD (<LOD)
Conc. range	<LOD–84.10 (<LOD–0.1)	<LOD–3.17 (<LOD)	<LOD–0.55 (<LOD)
Detection frequency (%)	28 (2)	10.7 (0)	5.4 (0)

<sup>a</sup> The limits of detections (LOD) of TCC, 2'-OH-TCC and 3'-OH-TCC were 0.1 ng/mL in urine.

the formation of quinone imines in vivo, even though these compounds were also suggested as potential oxidative metabolites of TCC in vitro (Baumann et al., 2010).

### 3.2. Measurements of TCC, 2'-OH-TCC and 3'-OH-TCC in human urine and serum

To check the usefulness of 2'-OH-TCC and 3'-OH-TCC, the major urinary metabolites of TCC in rats, as biomarkers of human exposure to TCC for biomonitoring purposes, we measured the total and free concentrations of TCC, 2'-OH-TCC and 3'-OH-TCC in 50 urine samples collected anonymously in 2010 from adult volunteers in Atlanta, GA with no documented occupational exposure to TCC (Table 2). We also measured the same compounds in 16 commercial available serum samples collected between 1998 and 2003 from persons with no documented occupational exposure to TCC. In urine, we detected the total species of TCC, 2'-OH-TCC and 3'-OH-TCC in up to a third of the samples; the frequency of detection of TCC, 2'-OH-TCC and 3'-OH-TCC in urine ranged from 5.4% to 28% (Table 2). However, we detected TCC, not 3'-OH-TCC, at the highest frequency and highest concentration ranges in urine. The administered dose (500 mg/kg body weight) was relatively high and may have resulted in induction or inhibition of phase I or phase II metabolic pathways. Nonetheless, our findings are consistent with a biotransformation study conducted using rats, monkeys, and men which also suggested possible species differences (human versus rat) in TCC metabolism (Birch et al., 1978). Furthermore, we found that the conjugated forms of TCC, 3'-OH-TCC, 2'-OH-TCC were the main species in urine. Our findings, albeit limited to a relatively small number of samples, are in agreement with early research data in which people were orally dosed with TCC (Hiles and Birch, 1978).

We did not detect 3'-OH-TCC and 2'-OH-TCC in any of the human serum samples, but we detected TCC in about 50% of the serum examined, both in total and free forms. However, TCC was detected in serum at a much lower level than in urine, with a mean concentration at 0.45 ng/mL versus 3.85 ng/mL in urine. Like for other non-persistent compounds (e.g., phthalates, bisphenol A) to which humans are likely exposed through episodic – rather than chronic – events, the concentrations in serum are much lower than the urinary concentrations. This is the main reason why urine is the preferred biomonitoring matrix for assessing exposure to non-persistent compounds (Needham and Sexton, 2000). It is also important to note that the urine and serum results presented here are not from the same individuals and collected years apart. Furthermore, the TCC serum results from these 16 samples must be interpreted with caution because TCC can be used in a variety of consumer and personal care products, and we had no information on the procedures for collection, processing, and storage of the samples analyzed. Therefore, we could not rule out the possibility of contamination with the parent compound during the collection and processing of the samples.

## 4. Conclusions

We investigated the metabolism of TCC in rats administered with a single oral dose of TCC. We unambiguously identified two oxidative metabolites, 3'-OH-TCC and 2'-OH-TCC, as the main urinary metabolites; we also detected the same metabolites in serum, but at much lower concentrations than in urine. Interestingly, we detected TCC at higher frequency and concentrations than those of its oxidative metabolites in 50 human urine samples, suggesting that species differences in TCC's metabolic profiles may exist. The total concentration of TCC in urine was the most suitable biomarker for TCC exposure in humans. 2'-OH-TCC and 3'-OH-TCC could be used to confirm TCC exposure in specific populations where exposures may be higher than background levels or in situations of suspected contamination of the urine specimen with TCC.

### Conflict of interest statement

There are no conflicts of interest.

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### References

- Ahn, K.C., Zhao, B., Chen, J., Cherednichenko, G., Sanmarti, E., Denison, M.S., Lasley, B., Pessah, I.N., Kultz, D., Chang, D.P.Y., Gee, S.J., Hammock, B.D., 2008. In vitro biologic activities of the antimicrobials triclocarban, its analogs, and triclosan in bioassay screens: receptor-based bioassay screens. *Environ. Health Perspect.* 116, 1203–1210.
- Baumann, A., Lohmann, W., Rose, T., Ahn, K.C., Hammock, B.D., Karst, U., Schebb, N.H., 2010. Electrochemistry-mass spectrometry unveils the formation of reactive triclocarban metabolites. *Drug Metab. Dispos.* 38, 2130–2138.
- Birch, C.G., Hiles, R.A., Eichhold, T.H., Jeffcoat, A.R., Handy, R.W., Hill, J.M., Willis, S.L., Hess, T.R., Wall, M.E., 1978. Biotransformation products of 3,4,4'-trichlorocarbanilide in rat, monkey, and man. *Drug Metab. Dispos.* 6, 169–176.
- Chen, J.G., Ahn, K.C., Gee, N.A., Ahmed, M.I., Duleba, A.J., Zhao, L., Gee, S.J., Hammock, B.D., Lasley, B.L., 2008. Triclocarban enhances testosterone action: a new type of endocrine disruptor? *Endocrinology* 149, 1173–1179.
- EPA, 12-30-2002. TCC HPVC Submission. U.S. Environmental Protection Agency. 31 January 2011.
- Halden, R.U., Paull, D.H., 2005. Response to comment on “co-occurrence of triclocarban and triclosan in US water resources”. *Environ. Sci. Technol.* 39, 6335–6336.
- Heidler, J., Halden, R.U., 2009. Fate of organohalogen in US wastewater treatment plants and estimated chemical releases to soils nationwide from biosolids recycling. *J. Environ. Monit.* 11, 2207–2215.
- Heidler, J., Sapkota, A., Halden, R.U., 2006. Partitioning, persistence, and accumulation in digested sludge of the topical antiseptic triclocarban during wastewater treatment. *Environ. Sci. Technol.* 40, 3634–3639.
- Hiles, R.A., 1977. Metabolism and toxicity of halogenated carbanilides: absorption, distribution and excretion of radioactivity from 3,4,4'-trichloro[14C]carbanilide (TCC) and 3-trifluoromethyl-4,4'-dichloro[14C]carbanilide (TFC) in rats. *Food Cosmet. Toxicol.* 15.
- Hiles, R.A., Birch, C.G., 1978. The absorption, excretion, and biotransformation of 3,4,4'-trichlorocarbanilide in humans. *Drug Metab. Dispos.* 6, 177–183.
- Hiles, R.A., Caudill, D., Birch, C.G., Eichhold, T., 1978. The metabolism and disposition of 3,4,4'-trichlorocarbanilide in the intact and bile duct-cannulated adult and in the newborn rhesus monkey (*M. mulatta*). *Toxicol. Appl. Pharmacol.* 46, 593–608.
- Howes, D., Black, J.G., 1976. Percutaneous absorption of triclocarban in rat and man. *Toxicology* 6, 67–76.
- Jeffcoat, A.R., Handy, R.W., Francis, M.T., Willis, S., Wall, M.E., Birch, C.G., Hiles, R.A., 1977. The metabolism and toxicity of halogenated carbanilides. Biliary metabolites of 3,4,4'-trichlorocarbanilide and 3-trifluoromethyl-4,4'-dichlorocarbanilide in the rat. *Drug Metab. Dispos.* 5, 157–166.
- Johnson, R.R., Navone, R., Larson, E.L., 1963. An unusual epidemic of methemoglobinemia. *Pediatrics* 31, 222–225.
- Koch, H.M., Bolt, H.M., Angerer, J., 2004. Di(2-ethylhexyl)phthalate (DEHP) metabolites in human urine and serum after a single oral dose of deuterium-labelled DEHP. *Arch. Toxicol.* 78, 123–130.

- Koch, H.M., Bolt, H.M., Preuss, R., Angerer, J., 2005. New metabolites of di(2-ethylhexyl)phthalate (DEHP) in human urine and serum after single oral doses of deuterium-labelled DEHP. *Arch. Toxicol.* 79, 367–376.
- Miller, T.R., Heidler, J., Chillrud, S.N., Delaquil, A., Ritchie, J.C., Mihalic, J.N., Bopp, R., Halden, R.U., 2008. Fate of triclosan and evidence for reductive dechlorination of triclocarban in estuarine sediments. *Environ. Sci. Technol.* 42, 4570–4576.
- Needham, L.L., Sexton, K., 2000. Assessing children's exposure to hazardous environmental chemicals: an overview of selected research challenges and complexities. *J. Expo. Anal. Environ. Epidemiol.* 10, 611–629.
- Nolen, G.A., Dierckman, T.A., 1979. Reproduction and teratogenic studies of a 2:1 mixture of 3,4,4'-trichlorocarbanilide and 3-trifluoromethyl-4,4'-dichlorocarbanilide in rats and rabbits. *Toxicol. Appl. Pharmacol.*, 51.
- Sapkota, A., Heldler, J., Halden, R.U., 2007. Detection of triclocarban and two co-contaminating chlorocarbanilides in US aquatic environments using isotope dilution liquid chromatography tandem mass spectrometry. *Environ. Res.* 103, 21–29.
- Scharpf, L.G.J., Hill, I.D., Maibach, H.I., 1975. Percutaneous penetration and disposition of triclocarban in man: body showering. *Arch. Environ. Health*, 30.
- Taylor, J.K., 1987. *Quality Assurance of Chemical Measurements*. Lewis Publishers, Chelsea, MI.
- The Personal Care Products Council, 2011. Available: <http://www.cosmeticsinfo.org/HBI/11/>.
- Warren, J.T., Allen, R., Carter, D.E., 1978. Identification of the metabolites of trichlorocarbanilide in the rat. *Drug Metab. Dispos.* 6, 38–44.
- Ye, X., Tao, L.J., Needham, L.L., Calafat, A.M., 2008. Automated on-line column-switching HPLC-MS/MS method for measuring environmental phenols and parabens in serum. *Talanta* 76, 865–871.
- Young, T.A., Heidler, J., Matos-Perez, C.R., Sapkota, A., Toler, T., Gibson, K.E., Schwab, K.J., Halden, R.U., 2008. Ab initio and in situ comparison of caffeine, triclosan, and triclocarban as indicators of sewage-derived microbes in surface waters. *Environ. Sci. Technol.* 42, 3335–3340.