Title

Microbiota-mediated reactivation of triclosan oxidative metabolites in colon tissues.

Authors

Zhang, Hongna

Sanidad, Katherine

Zhang, Jianan

et al.

Publication Date

2023-03-05

DOI

10.1016/j.jhazmat.2022.130509

Peer reviewed

HHS Public Access

Author manuscript

J Hazard Mater. Author manuscript; available in PMC 2024 March 05.

Published in final edited form as:

J Hazard Mater. 2023 March 05; 445: 130509. doi:10.1016/j.jhazmat.2022.130509.

Microbiota-mediated reactivation of triclosan oxidative metabolites in colon tissues

Hongna Zhang[a,b,1], **Katherine Z. Sanidad**[c,1], **Jianan Zhang**[c], **Guangqiang Wang**[c,d], **Rong Zhang**[b], **Chengchen Hu**[a], **Yongfeng Lin**[a], **Thomas D. Haggerty**[e], **Julie Parsonnet**[e], **Yuxin Zheng**[a], **Guodong Zhang**[c,f,*], **Zongwei Cai**[b,*]

[a]Department of Occupational and Environmental Health, School of Public Health, Qingdao University, Qingdao 266071, China

[b]State Key Laboratory of Environmental and Biological Analysis, Department of Chemistry, Hong Kong Baptist University, Hong Kong, China

[c]Department of Food Science, University of Massachusetts, Amherst, MA 01003, USA

[d]School of Medical Instrument and Food Engineering, University of Shanghai for Science and Technology, Shanghai 200093, China

[e]Department of Medicine and Department of Health Research and Policy, Stanford University, Stanford, CA 94305, USA

[f]Department of Food Science and Technology, National University of Singapore, Singapore

Abstract

[*]Corresponding author: Zongwei Cai, Tel: +852-34117070, zwcai@hkbu.edu.hk; and Guodong Zhang, Tel: +65-66017652, zhanggd@nus.edu.sg.

[1]Hongna Zhang and Katherine Z. Sanidad contributed equally to this work.

Credit Author Statement

Hongna Zhang: Methodology, Investigation, Data curation, Formal analysis, Writing-original draft. **Katherine Z. Sanidad:** Methodology, Investigation, Writing-original draft. **Jianan Zhang:** Methodology, Investigation, Formal analysis. **Guangqiang Wang:** Methodology, Investigation. **Rong Zhang:** Formal analysis, Data curation. **Chengchen Hu:** Investigation, Data curation. **Yongfeng Lin:** Software, Data curation. **Thomas D. Haggerty:** Methodology, Investigation. **Julie Parsonnet:** Resources, Funding acquisition. **Yuxin Zheng:** Validation, Writing-reviewing & editing. **Guodong Zhang:** Conceptualization, Supervision, Writing-reviewing & editing, Funding acquisition. **Zongwei Cai:** Supervision, Project administration, Funding acquisition.

Declaration of Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Environmental implication

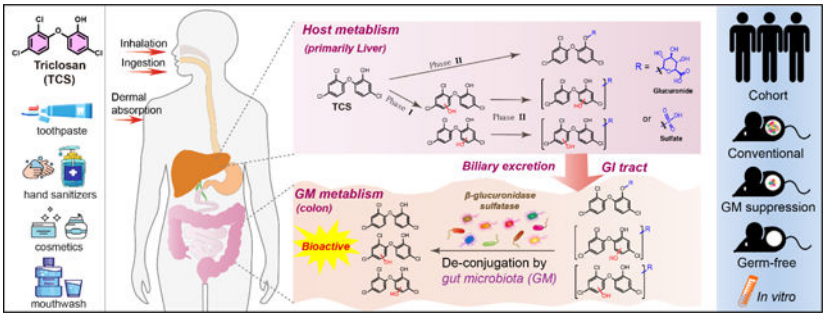
TCS is an environmental toxicant associated with many adverse health risks. Its toxicological effects have been linked to the generation of bioactive metabolites. Using strategies including human cohort study, antibiotic-treated and germ-free mice, as well as *in vitro* bacterial culture, this study demonstrated that gut microbiota efficiently converts glucuronide/sulfate-conjugated OH-TCS, which are generated from host metabolism, back to their bioactive free-forms in colon tissues. Through elucidating host-gut microbiota metabolic interactions, our findings shed light on the crucial roles of microbial metabolism in TCS toxicity, and highlight the importance of incorporating gut microbial transformations in the safety evaluation of environmental chemicals.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org…

Triclosan (TCS) is a widespread antimicrobial agent that is associated with many adverse health outcomes. Its gut toxicity has been attributed to the molecular modifications mediated by commensal microbes, but microbial transformations of TCS derivatives in the gut lumen are still largely unknown. Aromatic hydroxylation is the predominant oxidative metabolism of TCS that linked to its toxicological effects in host tissues. Here, we aimed to reveal the biological fates of hydroxyl-TCS (OH-TCS) in the colon, where intestinal microbes mainly reside. Unlike the profiles generated via host metabolism, OH-TCS species remain unconjugated in human stools from a cohort study. Through tracking molecular compositions in mouse intestinal tract, elevated abundance of free-form OH-TCS while reduced abundance of conjugated forms was observed in the colon digesta and mucosa. Using antibiotic-treated and germ-free mice, as well as *in vitro* approaches, we demonstrate that gut microbiota-encoded enzymes efficiently convert glucuronide/sulfate-conjugated OH-TCS, which are generated from host metabolism, back to their bioactive free-forms in colon tissues. Thus, host-gut microbiota metabolic interactions of TCS derivatives were proposed. These results shed light on the crucial roles of microbial metabolism in TCS toxicity, and highlight the importance of incorporating gut microbial transformations in health risk assessment of environmental chemicals.

Graphical Abstract



Keywords

triclosan; hydroxylation; gut microbiota; metabolism; colon

1. Introduction

Metabolic transformations are essential for the biological activities of xenobiotics, either increasing or decreasing their toxicities in humans (Croom, 2012). When evaluating the safety of xenobiotic chemicals, it is thus crucial to elucidate their metabolic fates and identify the bioactive metabolites. While most previous studies have focused on host tissue metabolism (Xu et al., 2005), it is now increasingly clear that gut microbes co-metabolize xenobiotic molecules, catalyze unique metabolic routes that are different from those mediated by host enzymes (Koppel et al., 2017; Lindell et al., 2022). Environmental chemicals are typical xenobiotics that usually deposit in the gut lumen via the biliary excretion or during passage through the gastrointestinal (GI) tract. Mounting evidence indicates that the interactions between gut microbial ecology and environmental chemicals are associated with elevated health risks of many diseases, such as obesity, diabetes,

inflammatory bowel disorders, and cancer (Piovani et al., 2019; Snedeker and Hay, 2012; Song and Chan, 2019), but how microbial transformations of xenobiotic contaminants affect human health remains largely unknown.

Triclosan (TCS) is an antibacterial agent that is formulated into over 2,000 consumer and personal-care products including toothpastes, hand sanitizers, mouthwash, cosmetics, clothing and food packaging (Halden et al., 2017; Wang et al., 2022). Humans could be continuously exposed to TCS at each life stage due to its widespread incorporation in commercial products and ubiquitous presence in the environment (Halden, 2014; Tohidi and Cai, 2017; Wu et al., 2019). Emerging research has shown that exposure to TCS is associated with health risks such as liver tumor promotion, endocrine disruption, and disturbance of immune function (Yueh and Tukey, 2016). Specific to the GI tract, growing evidence demonstrates that TCS has detrimental impacts on the gut health (Sanidad et al., 2019). It is becoming a major concern because at human relevant levels, TCS exacerbated the development of colitis and colitis-associated colon cancer in mouse models, supporting that TCS could be a risk factor for intestinal diseases (Sanidad et al., 2022). Of particular note, the presence of gut microbiota is crucial for these adverse outcomes because the gut toxicity of TCS diminished in germ-free mice (Yang et al., 2018). The mechanisms of TCS-induced gut pathology have recently been attributed to metabolic transformations mediated by the commensal microbes (Zhang et al., 2022).

After human exposure to TCS, it is generally recognized that TCS is dominantly transformed to the non-active derivative, TCS-glucuronide (TCS-G), in host tissues, which facilitates its elimination from the body (Arbuckle et al., 2015). The biliary-fecal excretion is a key deposition route of TCS metabolites (Fang et al., 2016), whereas our study revealed that specific gut microbial β-glucuronidase (GUS) enzymes catalyze the de-conjugation of TCS-G to the biologically active TCS, thereby driving the colitis-promoting effects (Zhang et al., 2022). It should be pointed out that TCS derivatives that enter the intestine also include a cluster of its oxidative metabolites. Indeed, metabolic activation of TCS frequently occurs via aromatic hydroxylation in the liver (Ashrap et al., 2017; Zhang et al., 2021). The generation of hydroxyl-TCS (OH-TCS) has received increasing attention because compared with TCS, these molecules could elicit stronger binding activity of the constitutive androstane receptor (CAR) (Ashrap et al., 2017), which contributes to the susceptibility of liver tumorigenesis (Dong et al., 2015; Wang et al., 2020b; Yamamoto et al., 2004; Yueh et al., 2014). Moreover, the OH-TCS species are chemically reactive and could covalently bind to cellular proteins that are involved in reproductive and development functions, endocrine and immune effects, and carcinogenesis, giving rise to the risk for adverse health outcomes (Liu et al., 2020). However, OH-TCS generation may be easily underestimated due to the subsequent conjugation with glucuronic acid and sulfate in host tissues (Zhang et al., 2019; Zhang et al., 2021). These results led us to pinpoint the host-gut microbiota metabolic interactions of TCS hydroxylated metabolites.

We hypothesize that intestinal microbiota could convert the conjugated OH-TCS back to bioactive free forms in the gut and contributes to its gut toxicity. To prove this hypothesis, we used ultra-high-performance liquid chromatography high-resolution mass spectrometry (UHPLC–HRMS) to analyze OH-TCS profiles in human stools, and track their metabolic

progress across the mouse GI tract. Through a series of *in vivo* and *in vitro* approaches, we elucidated the functional roles of gut microbial flora in the metabolic reactivation of OH-TCS. The obtained results were expected to improve our understanding of the metabolic fates of TCS and the underlying mechanisms for its health risks.

2. Materials and methods

2.1. Chemicals

TCS with a purity of 99% was purchased from Alfa Aesar (Haverhill, MA, USA). HPLC-grade methanol was procured from Duksan Chemicals Co. (Seoul, Korea). Other chemicals were obtained from Sigma-Aldrich (Milwaukee, WI, USA), unless otherwise indicated.

2.2. Sampling of human stools

Human stools from a previous cohort study were used for the UHPLC–HRMS determination (Poole et al., 2016). Briefly, after a washout period, healthy adult volunteers were given household and personal-care products (HPCPs) with/without TCS for the 4-months duration. These products include toothpaste, hard and liquid hand soap, and dishwashing liquid. Stool samples from volunteers in TCS group (6 subjects) and control group (7 subjects) were collected once a month. This study was conducted with the approval of the Institutional Review Board of Stanford University (ClinicalTrials.gov identifier NCT01509976).

2.3. Animal experiments

Animal studies were carried out in accordance with protocols approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Massachusetts Amherst and Massachusetts Host-Microbiome Center at the Brigham and Women's Hospital (Boston, MA, USA). C57BL/6 mice were procured from Charles River Laboratories (Wilmington, MA, USA) and maintained under specific pathogen-free conditions. The mice were fed with modified AIN-93G diets according to previous studies (Xie et al., 2020; Yang et al., 2018), diet ingredients are provided in Table S1. Polyethylene glycol 400 (PEG 400) was used as the solvent vehicle.

2.3.1 Animal study 1: OH-TCS profiles in the intestine of TCS-exposed mice

—Male C57BL/6 mice (6-week-old) were randomly divided into two groups (n = 10 in each group). The mice were fed a diet containing either 80 ppm TCS or no TCS. After 28 days, the mice were sacrificed, the tissues of heart, liver and bile were collected, and the distal small intestine and colon (digesta and mucosa) were harvested for the analysis of TCS concentrations and OH-TCS profiles.

2.3.2. Animal study 2: Effects of gut microbiota depletion on OH-TCS profiles in the mouse colon—

Male C57BL/6 mice (6-week-old) received drinking water with/without a broad-spectrum antibiotic cocktail ABX throughout the experiment (n = 10 in each group). The composition of ABX (1 g/L ampicillin and 0.5 g/L neomycin) was used based on previous studies (Martin et al., 2019; Wang et al., 2020a; Yang et al., 2020). After 5 days, the mice were fed a diet containing 80 ppm TCS for the 28-days duration. The mice

were then sacrificed, and the colon digesta and mucosa were harvested for UHPLC–HRMS analysis.

To validate the bacteria-depleting effect of the antibiotic cocktail ABX, we analyzed total fecal microbial biomass using the 16S rRNA gene as a marker (Lynn et al., 2018). Briefly, mouse fecal samples were collected before and at the end of TCS exposure. Fecal DNA was extracted using QIAamp DNA Stool Mini Kit (Qiagen, Valencia, CA, USA) following the manufacturer's instruction. The quantity of DNA was measured using a NanoDrop Spectrophotometer (Thermo Fisher Scientific). The extracted DNA was subjected to real-time quantitative PCR (*q*PCR) analysis using a DNA Engine Opticon System (Bio-Rad Laboratories, Hercules, CA, USA), and DNA was normalized to 5 ng/μL per reaction. Details of the 16S rRNA primers are listed in Table S2.

2.3.3. Animal study 3: Effects of gut microbiota depletion on OH-TCS kinetic changes in the mouse colon—

Male C57BL/6 mice (6-week-old) received drinking water with/without the antibiotic cocktail ABX for a week, then the mice were orally gavaged with TCS at 8 mg/kg body weight (bw). At the time points of 4, 8, 12 and 24 h post-gavage (n = 5 for each time point), the mice were sacrificed to obtain the colon digesta and mucosa for UHPLC–HRMS analysis.

2.3.4. Animal study 4: OH-TCS profiles in the colon of germ-free mice—

Germ-free male C57BL/6 mice or conventional controls received a single oral gavage administration of TCS at 8 mg/kg bw (n = 5 in each group). At 8 h post-gavage, the mice were sacrificed to obtain the colon digesta and mucosa for UHPLC–HRMS analysis.

2.4. Sample preparations

Human stools and mouse samples were homogenized in 1 mL methanol using a bead-disruptor, then centrifuged at 7,000 ×*g* for 3 min to collect the supernatant. After centrifugation again at 13,000 ×*g* for 5 min, an aliquot (500 μL) of the supernatant was removed and vacuum centrifuged to dryness. Before the UHPLC–HRMS determination, the extracts were re-dissolved with the volume of methanol proportional to the sample weights, then centrifuged (15,000 ×*g*, 10 min, 4°C) to harvest the supernatants for analysis.

2.5. UHPLC–HRMS determination of OH-TCS species

Mono-hydroxyl TCS metabolites, including free-form OH-TCS and glucuronide/sulfate conjugates (OH-TCSG and OH-TCSS) were analyzed by an Ultimate 3000 UHPLC coupled with an Orbitrap Fusion Tribrid Mass Spectrometer (Thermo Fisher Scientific, Waltham, MA, USA). Chromatographic separation was performed on an ACQUITY UPLC HSS T3 column (2.1 mm × 100 mm, 1.7 μm particle size, Waters Corp., Milford, MA, USA). Column temperature was set at 30°C. The injection volume was 10 μL with a mobile phase flow rate of 0.3 mL/min. The gradient program consisted of mobile phase A (5 mM ammonium acetate in water) and mobile phase B (methanol). Parallel reaction monitoring (PRM) acquisition was conducted in negative ionization mode. Detailed instrumental methods are in accordance with the protocol in previous study (Zhang et al., 2021), which are summarized in Table S3.

All UHPLC–HRMS data were acquired and initially processed by Xcalibur v.4.1 (Thermo Fisher Scientific). OH-TCS species were identified based on their retention times and accurate mass-to-charge ratios of the parent and product ions in PRM mode (mass tolerance 10 ppm). The chromatograms and mass spectra of OH-TCS species determined by UHPLC–HRMS are provided in Figure S1. They were semi-quantified with arbitrary units based on the MS/MS peak area (PA) per unit mass of sample due to the lack of standard chemicals. The MS detection parameters for quantitative analysis are supplied in Table S4.

2.6. In vitro experiments

2.6.1. In vitro metabolism of TCS by gut microbiota—Human stool or mouse samples (feces, colon and small intestine) were collected and dispersed in sterile phosphate buffered saline (PBS) with 0.05% L-cysteine, followed by centrifugation at 900 ×*g* for 5 min. The supernatants were anaerobically fermented in de Man, Rogosa and Sharpe (MRS) broth at 37°C. When the OD600 value reached 0.5, bacterial cultures were inoculated into MRS broth at a 1/10 ratio (*v*/*v*) with 20 μM TCS or DMSO vehicle. After 48 h, the samples were collected to analyze TCS and OH-TCS species using the methods in Tables S3–S5.

2.6.2. Effects of fecal bacteria on biotransformation of OH-TCS species—Human stool or mouse feces were dispersed in sterile PBS containing 0.05% L-cysteine, followed by centrifugation at 900 ×*g* for 5 min. The supernatants that contained fecal bacteria were fermented in MRS broth at 37°C. When the OD600 value reached 0.5, bacterial cultures were inoculated into MRS broth at a 1/10 ratio (*v*/*v*) with the extract containing OH-TCS species (from small intestine digesta of TCS-treated mice). After 24 h, the samples were collected to analyze OH-TCS species by UHPLC–HRMS.

2.6.3. Effects of microbe-derived enzymes on biotransformation of OH-TCS species—To decode the gut microbial enzymes involved in the de-conjugation of OH-TCS species, we determined the *in vitro* catalysis of microbe-derived GUS (from *Escherichia coli*, Sigma-Aldrich) and sulfatase (from *Aerobacter aerogenes*, Sigma-Aldrich). Reaction mixtures contained 100 U/mL GUS, 1 U/mL sulfatase, and 20 μL extract of OH-TCS species in 1 mL assay buffer (50 mM sodium acetate, 50 mM NaCl). The extract was from small intestine digesta of TCS-treated mice. Control group replaced enzymes with buffer. Reactions were incubated at 37 °C for 24 h, then quenched with methanol. After certification (15,000 ×*g*, 10 min, 4°C), the supernatants were subjected to analyze OH-TCS species by UHPLC–HRMS.

2.7. Statistical analysis

All data are presented as the mean ± standard error of the mean (SEM). Shapiro–Wilk test was used to verify the normality of data and Levene's test was applied to assess equal variance of data. Statistical comparison of two groups was determined using two-side *t*-test (normally distributed data) or Wilcoxon-Mann-Whitney test (non-normally distributed data), and comparison of three groups was analyzed by one-way ANOVA followed by Bonferroni post hoc test. The statistical analysis was conducted using SPSS v.26.0 (SPSS Inc., Chicago, IL, USA) and GraphPad Prism v.8.0 (GraphPad Software Inc., San Diego, CA, USA). The value of $P < 0.05$ was considered as statistically significant.

3. Results

3.1. Profiles of OH-TCS species in human stools

Hydroxylation is one of the major oxidative metabolic reactions of TCS in host tissues (Ashrap et al., 2017; Zhang et al., 2021). Our previous study showed that free-form TCS was dominant in the gut tract due to the metabolic functions of commensal microbes, resulting in different metabolic profiles in the gut compared with other tissues (Zhang et al., 2022). This leads to our hypothesis that OH-TCS exhibited similar profiles in the intestinal tract. That is, the gut microbes would convert conjugated OH-TCS, mainly OH-TCSG and OH-TCSS generated via host metabolism (Zhang et al., 2021), to their free forms (Figure 1A). To test our hypothesis, we first analyzed the abundances of OH-TCS species, including the free-form and conjugated forms in human stools. The samples of a previous cohort study were utilized (Poole et al., 2016), in which the subjects used HPCPs with or without TCS for 4 months (Figure 1B). UHPLC–HRMS analysis showed that only free-form OH-TCS was detected in the stools, while the conjugated OH-TCSG and OH-TCSS were not observed. Compared with those in control group, the subjects used TCS-containing HPCPs had higher abundance of free-form OH-TCS in their stools over a 4-month period (Figure 1C). Notably, OH-TCS detection in control samples indicates the ubiquitous presence of TCS in our surroundings. Indeed, except for the direct usage of TCS-containing HPCPs, humans could also be daily exposed to TCS via other products and routes such as food digestion and air inhalation. We would like to point out that the absence of UHPLC–HRMS signals of OH-TCSG and OH-TCSS doesn’t mean these conjugates are absent in human stools due to the lack of standard compounds, animal studies were thus conducted for further investigation.

3.2. Profiles of OH-TCS species in the mouse intestine

We fed mice for 28 days with a diet containing 80 ppm TCS (Figure 2A), and used UHPLC–HRMS to analyze the metabolic profiles of OH-TCS in a range of mouse samples. This exposure scheme was employed according to previous study (Yang et al., 2018), in which the plasma levels of TCS were comparable to those in TCS-exposed human subjects. TCS concentrations in host tissues and their profiles in the GI tract of mice are shown in Figure S2. As the central organ for host metabolism, the liver could catalyze TCS hydroxylation and excrete a series of metabolites into the bile, with the conjugates as dominant forms (Zhang et al., 2021). In agreement with previous studies, we showed a similar pattern of the metabolic profile of TCS in host tissues (Figure 2B). Notably, the detection of OH-TCS species in mouse heart suggested the blood circulation of these compounds. Host-derived metabolites could flow through the bile duct into the small intestine, eventually enter the colon via the intestine tract. We further analyzed the profiles of free-form OH-TCS, OH-TCSG and OH-TCSS in distal small intestine and the colon, and compared their abundances in the digesta and mucosa. Our findings showed that compared with those in distal small intestine, free-form OH-TCS was significantly increased (~8.5 folds based on HRMS signals), while OH-TCSG and OH-TCSS were obviously decreased in both colon digesta and mucosa (Figure 2C). These results demonstrate that the composition of OH-TCS species altered while passing through the intestine tract, with elevated abundance of the free-form metabolites. This is consistent with the observations in human samples, in

which only the free-form OH-TCS detected in the stools. Of note, within the GI tract of human body, the vast majority of commensal microbes reside in the colon with estimates of about 10^{14} bacteria, while the small intestine makes negligible contributions (less than 10^{12} bacteria) (de Vos et al., 2022; Sender et al., 2016). We thus focused on the commensal microbial metabolism in colon tissues.

3.3. Effects of gut microbiome depletion on TCS hydroxylation in the mouse colon

We used the approach of ABX-mediated suppression of gut microbiota to determine the functional roles of the microbiota in the formation of OH-TCS (Figure 3A). In agreement with previous studies (Wang et al., 2020a; Yang et al., 2020), this antibiotic strategy efficiently depleted most gut bacteria in the mouse intestine, as assessed by *q*PCR assays targeting the 16S rRNA genes (Figure S3). At the end of the experiment, the colon digesta and mucosa were collected to compare the abundances of free-form OH-TCS, OH-TCSG and OH-TCSS with or without the antibiotic treatment. UHPLC–HRMS analysis showed that antibiotic suppression of gut microbiome dramatically reduced the levels of free-form OH-TCS (~86% reduction based on HRMS signals), while increased the abundances of conjugated species, OH-TCSG and OH-TCSS, in colon digesta (Figure 3B). Consistent tendencies were also observed in the corresponding mucosa (Figure S4).

Next, we performed a time-course study to further validate this observation in the gut lumen. Specifically, the mice were pre-treated with the antibiotics for a week to suppress the microbial biomass, then received a single oral gavage of TCS (Figure 3C). The profiles of OH-TCS metabolites in colon tissues at time points of 4, 8, 12 and 24 h were analyzed. During the period of 24 h post-gavage, gut microbiome depletion by antibiotic pre-treatment significantly decreased the abundance of free-form OH-TCS, while elevated the levels of OH-TCSG and OH-TCSS in mouse colon digesta, as assessed using both the time-dependent curves and area under curve (AUC) analyses (Figure 3D–F). Similar tendencies were observed in colon mucosa (Figure S5). These results are well consistent with the findings of Figure 3B, supporting that the microbiota was required for the regeneration of the active free-form OH-TCS in the colon.

3.4. Profiles of OH-TCS species in germ-free mice

Furthermore, germ-free mice were used to confirm the roles of gut microbiota in the metabolic fates of OH-TCS in the colon. We treated germ-free or conventional mice with a single oral administration of TCS, then analyzed the profiles of OH-TCS metabolites in colon tissues at 8 h post-gavage (Figure 4A). Compared with conventional mice, the abundance of free-form OH-TCS was reduced, while the abundances of OH-TCSG and OH-TCSS were dramatically increased, in the colon digesta and mucosa of germ-free mice (Figure 4B–C). These findings are in good accordance with the results using the antibiotic approaches (Figure 3), supporting our hypothesis that the microbiota converts the conjugated OH-TCS metabolites (e.g., OH-TCSG and OH-TCSS) back to the unconjugated species, resulting in the accumulation of free-form OH-TCS in colon tissues.

3.5. Effects of gut bacteria on TCS metabolism in vitro

To further explore the roles of gut microbiota in TCS metabolism, we cultured gut bacteria under anaerobic conditions and tested whether the bacteria can catalyze the conversion of TCS to OH-TCS species. We incubated TCS with gut bacteria from human stools and mouse feces, colon and small intestine, for 48 h (Figure 5A). UHPLC–HRMS failed to detect the formation of free-form OH-TCS, OH-TCSG or OH-TCSS (Figure 5B, Table S6). This result suggests that at least the culturable gut bacteria can't directly convert TCS to OH-TCS, and the OH-TCS species in the gut lumen are from the host metabolism. As shown in Figure 2C, small intestine digesta of TCS-exposed mice contain high abundance of conjugated OH-TCS species. Next, we analyzed whether *in vitro* incubation with fecal bacteria could alter the metabolic profile of OH-TCS species in the extract of small intestine digesta. The results showed that compared with the control group, incubation of fecal bacteria from human or mice increased free-form OH-TCS, while reduced conjugated OH-TCSG and OH-TCSS, demonstrating the de-conjugation of OH-TCS species (Figure 5C–D). We further conducted *in vitro* experiment to decode the gut microbial enzymes involved. Our findings clearly showed that microbe-derived GUS and sulfatase efficiently convert conjugated OH-TCSG and OH-TCSS to free-form OH-TCS (Figure 5E–F).

4. Discussion

First introduced to commerce in 1964, TCS has been widely utilized as an antimicrobial agent over five decades (Halden et al., 2017). This highly lipophilic compound is readily absorbed from the GI tract and skin, causing that a very large proportion of populations around the world have detectable TCS concentrations in human fluids and tissues (Weatherly and Gosse, 2017). Previous research indicates that after TCS enters the human body, it is rapidly metabolized in host tissues, predominantly in the liver, to generate metabolic products mainly via phase Ⅱ reactions of glucuronidation and sulfonation. These conjugated metabolites are less active than the parent compound and are thought to be quickly excreted from the body through the urinary and fecal elimination. The dominant metabolite found in humans was TCS-G, and rapid turnover occurred with the terminal plasma half-life shorted than 24 hours (Sandborgh-Englund et al., 2006). However, over the past decade, TCS has aroused great public concern due to its cancer-promoting potential to the liver and colon, the tissues that TCS metabolites are generated and stored for fecal removal (Yang et al., 2018; Yueh et al., 2014). Further studies support that the biotransformation process of TCS contributes to the mechanisms of its toxicity. In the liver, CYP-mediated oxidative metabolism also generates reactive metabolites that could induce the hepatic toxicity, although these metabolites underwent subsequent inactivation by mammalian phase Ⅱ metabolism via glucuronidation and sulfonation (Ashrap et al., 2017; Zhang et al., 2019). In the colon, our recent study first proved that after the elimination of TCS-G into the gut, intestinal commensal microbes mediate the regeneration of parent compound TCS from TCS-G and drive its gut toxicology (Zhang et al., 2022). Indeed, previous studies regarding the biological fates of TCS have primarily focused on the metabolism in mammalian host tissues, leaving gut microbiota-mediated transformation being considerably understudied. Here, we elucidate that this de-conjugation process extensively occurred in the gut lumen,

also resulting in the regeneration of TCS reactive metabolites, the host tissue-toxic products that are easily underestimated.

In this study, we provide a series of evidences that strongly supporting the above hypothesis. Previous studies about TCS host metabolism have demonstrated that TCS hydroxylation frequently occurred in the liver, accompanied by subsequent conjugation with glucuronic acid and sulfate (Wu et al., 2010; Zhang et al., 2021). To reveal the gut microbial metabolism involved, we first used a cohort study to determine OH-TCS profiles in human stools, the results showed that for volunteers used TCS-containing products, hydroxylated metabolites were present as free-form OH-TCS in stool samples (Figure 1C). We next sought to tract the generation and elimination of OH-TCS via animal experiments. In TCS-exposed mice, the profiles of OH-TCS species substantially changed during these metabolites transit through the intestinal tract. Notably, free-form OH-TCS increased while the conjugated forms decreased in colon tissues (Figure 2). The strategies of antibiotic suppression of gut microbiota were then applied to evaluate the metabolic roles of gut microbes. Our data showed that depletion of gut microbiota dramatically depleted free-form OH-TCS, while enhanced abundances of OH-TCSG and OH-TCSS in the colon digesta and mucosa (Figure 3). This notion is further supported by the results in germ-free mice (Figure 4). Moreover, results of *in vitro* experiments suggested that gut bacteria mainly mediate the de-conjugation of OH-TCS species via microbial-encoded GUS and sulfatase enzymes (Figure 5). Collectively, these findings clearly demonstrate that gut microbiota efficiently de-conjugates glucuronide/sulfate-conjugated OH-TCS, which are derived from the host oxidative metabolism, to re-generate the bioactive free-form OH-TCS in colon tissues.

Therefore, we proposed the host-gut microbiota metabolic interactions of TCS derivatives based on this and previous studies (Zhang et al., 2021; Zhang et al., 2022). As depicted in Figure 6, TCS in the products and environment generally enter the human body through ingestion, dermal absorption or inhalation. It is rapidly absorbed into the blood circulation and enter the liver-gut axis for metabolic disposition. For the host tissue-specific metabolism, TCS undergoes phase Ⅰ hydroxylation and/or phase Ⅱ conjugation to form biologically inactive conjugates with glucuronic acid and sulfate, which are subsequently excreted in the bile and enter the intestinal tract for elimination. However, the gut microbiota mediates the de-conjugation of these compounds via the encoded enzymes, resulting in the accumulation of free-form TCS and hydroxylated species in colon tissues. Of note, intestinal mucosae are the primary sites at which the colon tissue interacts with the microbial products. We proved that the abundances of TCS-derived components in colon mucosa are positively related with those in the lumen, thus posing potential risks to the host health. To the best of our knowledge, this is the most complete metabolic networks of TCS by combing gut microbial transformations. Also note that renal-urinary and biliary-fecal excretions are two elimination routes after conjugation reactions. Molecular weight (MW) has been suggested as an important factor in biliary excretion levels of compounds. Generally, most compounds with low molecular weights are quickly cleared through the kidneys and are poorly excreted in the bile, whereas those with higher MW (> 325 kDa) can significantly excreted via biliary route (Claus et al., 2016). Specific to TCS, the glucuronide/sulfate conjugates of TCS and OH-TCS (all > 325 kDa) could be secreted into bile and enter the small intestine, then move down to the colon where microbial metabolism mainly takes place. Indeed, gut microbes

collectively encode 150-fold more genes than the human genome, including a rich repository of enzymes that can mediate diverse biochemical reactions (Zimmermann et al., 2019).

The sequencing data from the Human Microbiome Project (HMP) suggest that gut microbiota contains hundreds of unique microbial GUS enzymes, which clustered into six categories based on active-site features (Creekmore et al., 2019; Pollet et al., 2017). Distinct microbiome-encoded sulfatase proteins are also extensively expressed in the colon (Ervin et al. 2020). Consistent with the *in vitro* results as shown in Figure 5E–F, the actions of these microbial enzymes can explain hydrolytic transformations of glucuronide and sulfate conjugates in the gut tract. Of note, the reactivation of TCS and its oxidative metabolites gives rise to several health concerns. The first one is their direct GI toxicity. When microbial enzymes hydrolyze the conjugates, they release the active free-form compounds into the intestinal lumen, which could be the underlying mechanisms that exacerbate inflammatory bowel diseases (IBDs) (Yang et al., 2018; Zhang et al., 2022). Indeed, IBD is characterized by a chronic inflammation of the intestinal mucosa (Chang, 2020), our results clearly showed that these free-form reactive metabolites could accumulate in the mucosal tissue. Another key concern is the enterohepatic recirculation of these bioactive compounds. This process controls the recycling of many endogenous and xenobiotic chemicals, leading to their increased residence time in the body (Claus et al., 2016; Collins and Patterson, 2020). Therefore, free-form TCS and OH-TCS generated via bacteria-dependent metabolism may be re-absorbed into the bloodstream and modulate the toxicity for the host tissues. Moreover, long-term activation of TCS-related compounds in the GI tract could shape an individual's microbiotype or induce antibiotic resistance. It is well known that the composition of gut microbiome is predominantly shaped by environmental factors (Rothschild et al., 2018). Previous study showed that enrichment of phylum *Proteobacteria* was observed in stool samples from mothers using TCS-containing toothpaste, and infants with higher urinary TCS levels also showed a significant enrichment of *Proteobacteria* species in their stools (Ribado et al., 2017). Notably, an expansion of *Proteobacteria* may serve as a potential diagnostic marker of dysbiosis, risk of disease and increased antibiotic resistance genes (Bengtsson-Palme et al., 2015; Shin et al., 2015). Animal study also revealed that TCS treatment caused a ~75% reduction in the gut abundance of genus *Bifidobacterium*, which has been linked to anti-inflammatory effects (Yang et al., 2018).

Although de-conjugation of TCS metabolites occur universally under the catalysis of gut microbial enzymes, inter-individual variations of reaction efficiency exist in the gut tract. Different human subjects have their own specific gut microbial composition that expresses a unique set of microbial GUS and sulfatase enzymes, causing varied capacities to convert conjugates of environmental compounds (Ervin et al., 2020; Pollet et al., 2017). Our previous study revealed that specific Loop 1 and flavin mononucleotide (FMN)-binding GUS were the most effective enzyme orthologs at converting TCS-G to TCS *in vitro*, whereas Loop 1 GUS exhibited a wide range of abundance levels and ~40% of the sampled 139 human subjects did not have this category in the fecal microbiota (Zhang et al., 2022). We admit that the key limitation of this study is the lack of quantitative OH-TCS concentrations due to the absence of chemical standards. However, previous study showed that in the liver of mice exposed to TCS, the host generation of OH-TCS cannot be ignored (Ashrap et al., 2017). Additionally, the gut toxicity of free-form OH-TCS

needs further confirmation. Despite we demonstrated that OH-TCS can be readily absorbed by the intestinal mucosa, the direct effects of OH-TCS on intestinal health still remain unclear. In spite of these limitations, the data presented here provide sufficient evidences for the reactivation of TCS metabolites in colon tissues, and elaborate the metabolic pathways of TCS in combination with microbial-mammalian co-metabolism. It is of great significance for the safety evaluation of TCS application because it is very likely that humans would receive a lifetime exposure to this antimicrobial ingredient. This work also provides a methodology to explore the metabolic routes and the related health risks of other environmental compounds. Future research will include exploration of TCS-related gut toxicity using the standard chemicals of OH-TCS and its conjugates.

5. Conclusion

This study first described metabolic networks of TCS oxidative metabolites mediated by the gut microbiota. Aromatic hydroxylation is the predominant oxidative reaction of TCS in host tissues, followed by phase II inactivation through the addition of either a glucuronic acid or sulfate moiety prior to the biliary-fecal excretion. However, we found that OH-TCS metabolites are present unconjugated in human stools of TCS-exposed subjects. Further animal studies revealed elevated abundance of free-form OH-TCS in the colon compared with the small intestine. Using approaches including gut microbiome depletion by antibiotic treatment, germ-free mice, and *in vitro* bacterial culture, we demonstrated our hypothesis that gut microbes de-conjugate glucuronide/sulfate OH-TCS, which are derived from the host metabolism, to re-generate the bioactive free-forms via GUS and sulfatase enzymes in colon tissues. These results facilitate a deeper understanding of the metabolic fates of TCS, and highlight the importance of elucidating host-gut microbiota co-metabolism in the safety evaluation of environmental contaminants.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This research is supported by interdisciplinary faculty research award from the University of Massachusetts Amherst, USDA NIFA 2019-67017-29248 and 2020-67017-30844, and USDA/Hatch MAS00556 (to G. Zhang); General Research Fund (12303319) of Hong Kong Research Grants Council (to Z. Cai); NIH/NIEHS R21 ES023371 (to J. Parsonnet); National Natural Science Foundation of China (82273591), and State Key Laboratory of Environmental Chemistry and Ecotoxicology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences (KF2021-21) (to H. Zhang).

References

Arbuckle TE, Marro L, Davis K, Fisher M, Ayotte P, Bélanger P, Dumas P, Leblanc A, Bérubé R, Gaudreau É, 2015. Exposure to free and conjugated forms of bisphenol A and triclosan among pregnant women in the MIREC cohort. Environ. Health Perspect 123, 277–284. 10.1289/ehp.1408187. [PubMed: 25494523]

Ashrap P, Zheng G, Wan Y, Li T; Hu W, Li W, Zhang H, Zhang Z, Hu J, 2017. Discovery of a widespread metabolic pathway within and among phenolic xenobiotics. Proc. Natl Acad. Sci. USA 114, 6062–6067. 10.1073/pnas.1700558114. [PubMed: 28536195]

Bengtsson-Palme J, Angelin M, Huss M, Kjellqvist S, Kristiansson E, Palmgren H, Larsson DG, Johansson A, 2015. The human gut microbiome as a transporter of antibiotic resistance genes between continents. Antimicrob. Agents Chemother 59, 6551–6560. 10.1128/AAC.00933-15. [PubMed: 26259788]

Chang JT, 2020. Pathophysiology of inflammatory bowel diseases. N. Engl. J. Med 383, 2652–2664. 10.1056/NEJMra2002697. [PubMed: 33382932]

Claus SP, Guillou H, Ellero-Simatos S, 2016. The gut microbiota: A major player in the toxicity of environmental pollutants? npj Biofilms Microbiol. 2, 16003. 10.1038/npjbiofilms.2016.3.

Collins SL, Patterson AD, 2020. The gut microbiome: An orchestrator of xenobiotic metabolism. Acta Pharm. Sin. B 10, 19–32. 10.1016/j.apsb.2019.12.001.\ [PubMed: 31998605]

Creekmore BC, Gray JH, Walton WG, Biernat KA, Little MS, Xu Y, Liu J, Gharaibeh RZ, Redinbo MR, 2019. Mouse gut microbiome-encoded beta-glucuronidases identified using metagenome analysis guided by protein structure. mSystems 4, e00452–19. 10.1128/mSystems.00452-19.

Croom E, 2012. Metabolism of xenobiotics of human environments. Prog. Mol. Biol. Transl. Sci 112, 31–88. 10.1016/B978-0-12-415813-9.00003-9. [PubMed: 22974737]

de Vos WM, Tilg H, Van Hul M, Cani PD, 2022. Gut microbiome and health: Mechanistic insights. Gut 71, 1020–1032. 10.1136/gutjnl-2021-326789. [PubMed: 35105664]

Dong B, Lee JS, Park YY, Yang F, Xu G, Huang W, Finegold MJ, Moore DD, 2015. Activating CAR and beta-catenin induces uncontrolled liver growth and tumorigenesis. Nat. Commun 6, 5944. 10.1038/ncomms6944. [PubMed: 25661872]

Ervin SM, Simpson JB, Gibbs ME, Creekmore BC, Lim L, Walton WG, Gharaibeh RZ, Redinbo MR, 2020. Structural insights into endobiotic reactivation by human gut microbiome-encoded sulfatases. Biochemistry 59, 3939–3950. 10.1021/acs.biochem.0c00711. [PubMed: 32993284]

Fang J-L, Vanlandingham M, da Costa GG, Beland FA, 2016. Absorption and metabolism of triclosan after application to the skin of B6C3F1 mice. Environ. Toxicol 31, 609–623. 10.1002/tox.22074. [PubMed: 25410937]

Halden RU, 2014. On the need and speed of regulating triclosan and triclocarban in the United States. Environ. Sci. Technol 48, 3603–3611. 10.1021/es500495p. [PubMed: 24588513]

Halden RU, Lindeman AE, Aiello AE, Andrews D, Arnold WA, Fair P, Fuoco RE, Geer LA, Johnson PI, Lohmann R, McNeill K, Sacks VP, Schettler T, Weber R, Zoeller RT, Blum A, 2017. The Florence statement on triclosan and triclocarban. Environ. Health Perspect 125, 064501. 10.1289/EHP1788.

Koppel N, Maini Rekdal V, Balskus EP, 2017. Chemical transformation of xenobiotics by the human gut microbiota. Science 356, eaag2770. 10.1126/science.aag2770.

Lindell AE, Zimmermann-Kogadeeva M, Patil KR, 2022. Multimodal interactions of drugs, natural compounds and pollutants with the gut microbiota. Nat. Rev. Microbiol 10.1038/s41579-022-00681-5.

Liu M, Li N, Zhang Y, Zheng Z, Zhuo Y, Sun B, Bai L-P, Zhang M, Guo M-Q, Wu J-L, 2020. Characterization of covalent protein modification by triclosan *in vivo* and *in vitro* via three-dimensional liquid chromatography-mass spectrometry: New insight into its adverse effects. Environ. Int 136, 105423. 10.1016/j.envint.2019.105423.

Lynn MA, Tumes DJ, Choo JM, Sribnaia A, Blake SJ, Leong LEX, Young GP, Marshall HS, Wesselingh SL, Rogers GB, Lynn DJ, 2018. Early-life antibiotic-driven dysbiosis leads to dysregulated vaccine immune responses in mice. Cell Host Microbe 23, 653–660.e5. 10.1016/j.chom.2018.04.009. [PubMed: 29746836]

Martin AM, Yabut JM, Choo JM, Page AJ, Sun EW, Jessup CF, Wesselingh SL, Khan WI, Rogers GB, Steinberg GR, Keating DJ, 2019. The gut microbiome regulates host glucose homeostasis via peripheral serotonin. Proc. Natl Acad. Sci. USA 116, 19802–19804. 10.1073/pnas.1909311116. [PubMed: 31527237]

Piovani D, Danese S, Peyrin-Biroulet L, Nikolopoulos GK, Lytras T, Bonovas S, 2019. Environmental risk factors for inflammatory bowel diseases: An umbrella review of meta-analyses. Gastroenterology 157, 647–659.e644. 10.1053/j.gastro.2019.04.016. [PubMed: 31014995]

Pollet RM, D'Agostino EH, Walton WG, Xu Y, Little MS, Biernat KA, Pellock SJ, Patterson LM, Creekmore BC, Isenberg HN, Bahethi RR, Bhatt AP, Liu J, Gharaibeh RZ, Redinbo MR, 2017.

An atlas of β-glucuronidases in the human intestinal microbiome. Structure 25, 967–977.e965. 10.1016/j.str.2017.05.003. [PubMed: 28578872]

Poole AC, Pischel L, Ley C, Suh G, Goodrich JK, Haggerty TD, Ley RE, Parsonnet J, 2016. Crossover control study of the effect of personal care products containing triclosan on the microbiome. mSphere 1, e00056–00015. 10.1128/mSphere.00056-15.

Ribado JV, Ley C, Haggerty TD, Tkachenko E, Bhatt AS, Parsonnet J, 2017. Household triclosan and triclocarban effects on the infant and maternal microbiome. EMBO Mol. Med 9, 1732–1741. 10.15252/emmm.201707882. [PubMed: 29030459]

Rothschild D, Weissbrod O, Barkan E, Kurilshikov A, Korem T, Zeevi D, Costea PI, Godneva A, Kalka IN, Bar N, Shilo S, Lador D, Vila AV, Zmora N, Pevsner-Fischer M, Israeli D, Kosower N, Malka G, Wolf BC, Avnit-Sagi T, Lotan-Pompan M, Weinberger A, Halpern Z, Carmi S, Fu J, Wijmenga C, Zhernakova A, Elinav E, Segal E, 2018. Environment dominates over host genetics in shaping human gut microbiota. Nature 555, 210–215. 10.1038/nature25973. [PubMed: 29489753]

Sandborgh-Englund G, Adolfsson-Erici M, Odham G, Ekstrand J, 2006. Pharmacokinetics of triclosan following oral ingestion in humans. J. Toxicol. Environ. Health A 69, 1861–1873. 10.1080/15287390600631706. [PubMed: 16952905]

Sanidad KZ, Wang G, Panigrahy A, Zhang G, 2022. Triclosan and triclocarban as potential risk factors of colitis and colon cancer: Roles of gut microbiota involved. Sci. Total Environ 842, 156776. 10.1016/j.scitotenv.2022.156776. [PubMed: 35724794]

Sanidad KZ, Xiao H, Zhang G, 2019. Triclosan, a common antimicrobial ingredient, on gut microbiota and gut health. Gut Microbes 10, 434–437. 10.1080/19490976.2018.1546521. [PubMed: 30453815]

Sender R, Fuchs S, Milo R, 2016. Are we really vastly outnumbered? Revisiting the ratio of bacterial to host cells in humans. Cell 164, 337–340. 10.1016/j.cell.2016.01.013. [PubMed: 26824647]

Shin N-R, Whon TW, Bae J-W, 2015. *Proteobacteria*: microbial signature of dysbiosis in gut microbiota. Trends Biotechnol. 33, 496–503. 10.1016/j.tibtech.2015.06.011. [PubMed: 26210164]

Snedeker SM, Hay AG, 2012. Do interactions between gut ecology and environmental chemicals contribute to obesity and diabetes? Environ. Health Perspect 120, 332–339. 10.1289/ehp.1104204. [PubMed: 22042266]

Song M, Chan AT, 2019. Environmental factors, gut microbiota, and colorectal cancer prevention. Clin. Gastroenterol. H 17, 275–289. 10.1016/j.cgh.2018.07.012.

Tohidi F, Cai Z, 2017. Fate and mass balance of triclosan and its degradation products: Comparison of three different types of wastewater treatments and aerobic/anaerobic sludge digestion. J. Hazard. Mater 323, 329–340. 10.1016/j.jhazmat.2016.04.034. [PubMed: 27166780]

Wang M, Hu B, Zhou W, Huang K, Fu J, Zhang A, Jiang G, 2022. Enhanced hand-to-mouth exposure from hand sanitizers during the COVID-19 pandemic: A case study of triclosan. Sci. Bull 67, 995–998. 10.1016/j.scib.2022.03.016.

Wang Y, Yang J, Wang W, Sanidad KZ, Cinelli MA, Wan D, Hwang SH, Kim D, Lee KSS, Xiao H, Hammock BD, Zhang G, 2020a. Soluble epoxide hydrolase is an endogenous regulator of obesity-induced intestinal barrier dysfunction and bacterial translocation. Proc. Natl Acad. Sci. USA 117, 8431–8436. 10.1073/pnas.1916189117. [PubMed: 32220957]

Wang Z, Wu Q, Li X, Klaunig JE, 2020b. Constitutive androstane receptor (CAR) mediates dieldrin-induced liver tumorigenesis in mouse. Arch. Toxicol 94, 2873–2884. 10.1007/s00204-020-02781-8. [PubMed: 32435917]

Weatherly LM, Gosse JA, 2017. Triclosan exposure, transformation, and human health effects. J. Toxicol. Environ. Health. B 20, 447–469. 10.1080/10937404.2017.1399306.

Wu J-L, Ji F, Zhang H, Hu C, Wong MH, Hu D, Cai Z, 2019. Formation of dioxins from triclosan with active chlorine: A potential risk assessment. J. Hazard. Mater 367, 128–136. 10.1016/j.jhazmat.2018.12.088. [PubMed: 30594711]

Wu J-L, Liu J, Cai Z, 2010. Determination of triclosan metabolites by using in-source fragmentation from high-performance liquid chromatography/negative atmospheric pressure chemical ionization ion trap mass spectrometry. Rapid Commun. Mass Spectrom 24, 1828–1834. 10.1002/rcm.4558. [PubMed: 20533312]

Xie M, Zhang H, Wang W, Sherman HL, Minter LM, Cai Z, Zhang G, 2020. Triclocarban exposure exaggerates spontaneous colonic inflammation in Il-10−/− Mice. Toxicol. Sci 174, 92–99. 10.1093/toxsci/kfz248. [PubMed: 31868902]

Xu C, Li CY-T, Kong A-NT, 2005. Induction of phase I, II and III drug metabolism/transport by xenobiotics. Arch. Pharm. Res 28, 249–268. 10.1007/bf02977789. [PubMed: 15832810]

Yamamoto Y, Moore R, Goldsworthy TL, Negishi M, Maronpot RR, 2004. The orphan nuclear receptor constitutive active/androstane receptor is essential for liver tumor promotion by phenobarbital in mice. Cancer Res. 64, 7197–7200. 10.1158/0008-5472.CAN-04-1459. [PubMed: 15492232]

Yang H, Sanidad KZ, Wang W, Xie M, Gu M, Cao X, Xiao H, Zhang G, 2020. Triclocarban exposure exaggerates colitis and colon tumorigenesis: Roles of gut microbiota involved. Gut Microbes 12, 1690364. 10.1080/19490976.2019.1690364.

Yang H, Wang W, Romano KA, Gu M, Sanidad KZ, Kim D, Yang J, Schmidt B, Panigrahy D, Pei R, Martin DA, Ozay EI, Wang Y, Song M, Bolling BW, Xiao H, Minter LM, Yang G-Y, Liu Z, Rey FE, Zhang G, 2018. A common antimicrobial additive increases colonic inflammation and colitis-associated colon tumorigenesis in mice. Sci. Transl. Med 10, eaan4116. 10.1126/scitranslmed.aan4116.

Yueh M-F, Taniguchi K, Chen S, Evans RM, Hammock BD, Karin M, Tukey RH, 2014. The commonly used antimicrobial additive triclosan is a liver tumor promoter. Proc. Natl Acad. Sci. USA 111, 17200–17205. 10.1073/pnas.1419119111. [PubMed: 25404284]

Yueh MF, Tukey RH, 2016. Triclosan: A widespread environmental toxicant with many biological effects. Ann. Rev. Pharmacol. Toxicol 56, 251–272. 10.1146/annurev-pharmtox-010715-103417. [PubMed: 26738475]

Zhang H, Sanidad KZ, Zhu L, Parsonnet J, Haggerty TD, Zhang G, Cai Z, 2021. Frequent occurrence of triclosan hydroxylation in mammals: A combined theoretical and experimental investigation. J. Hazard. Mater 407, 124803. 10.1016/j.jhazmat.2020.124803.

Zhang H, Shao X, Zhao H, Li X, Wei J, Yang C, Cai Z, 2019. Integration of metabolomics and lipidomics reveals metabolic mechanisms of triclosan-induced toxicity in human hepatocytes. Environ. Sci. Technol 53, 5406–5415. 10.1021/acs.est.8b07281. [PubMed: 30964272]

Zhang J, Walker ME, Sanidad KZ, Zhang H, Liang Y, Zhao E, Chacon-Vargas K, Yeliseyev V, Parsonnet J, Haggerty TD, Wang G, Simpson JB, Jariwala PB, Beaty VV, Yang J, Yang H, Panigrahy A, Minter LM, Kim D, Gibbons JG, Liu L, Li Z, Xiao H, Borlandelli V, Overkleeft HS, Cloer EW, Major MB, Goldfarb D, Cai Z, Redinbo MR, Zhang G, 2022. Microbial enzymes induce colitis by reactivating triclosan in the mouse gastrointestinal tract. Nat. Commun 13, 136. 10.1038/s41467-021-27762-y. [PubMed: 35013263]

Zimmermann M, Zimmermann-Kogadeeva M, Wegmann R, Goodman AL, 2019. Separating host and microbiome contributions to drug pharmacokinetics and toxicity. Science 363, eaat9931. 10.1126/science.aat9931.

Highlights

- Hydroxyl-TCS (OH-TCS) metabolites remain unconjugated in human stools.
- Abundance of free-form OH-TCS increased while passing through the intestine tract.
- Free-from OH-TCS reduced in colon tissues of antibiotic-treated and germ-free mice.
- Gut microbes de-conjugate glucuronide/sulfate OH-TCS to bioactive free-forms.
- Host-gut microbiota metabolic interactions of TCS derivatives were proposed.

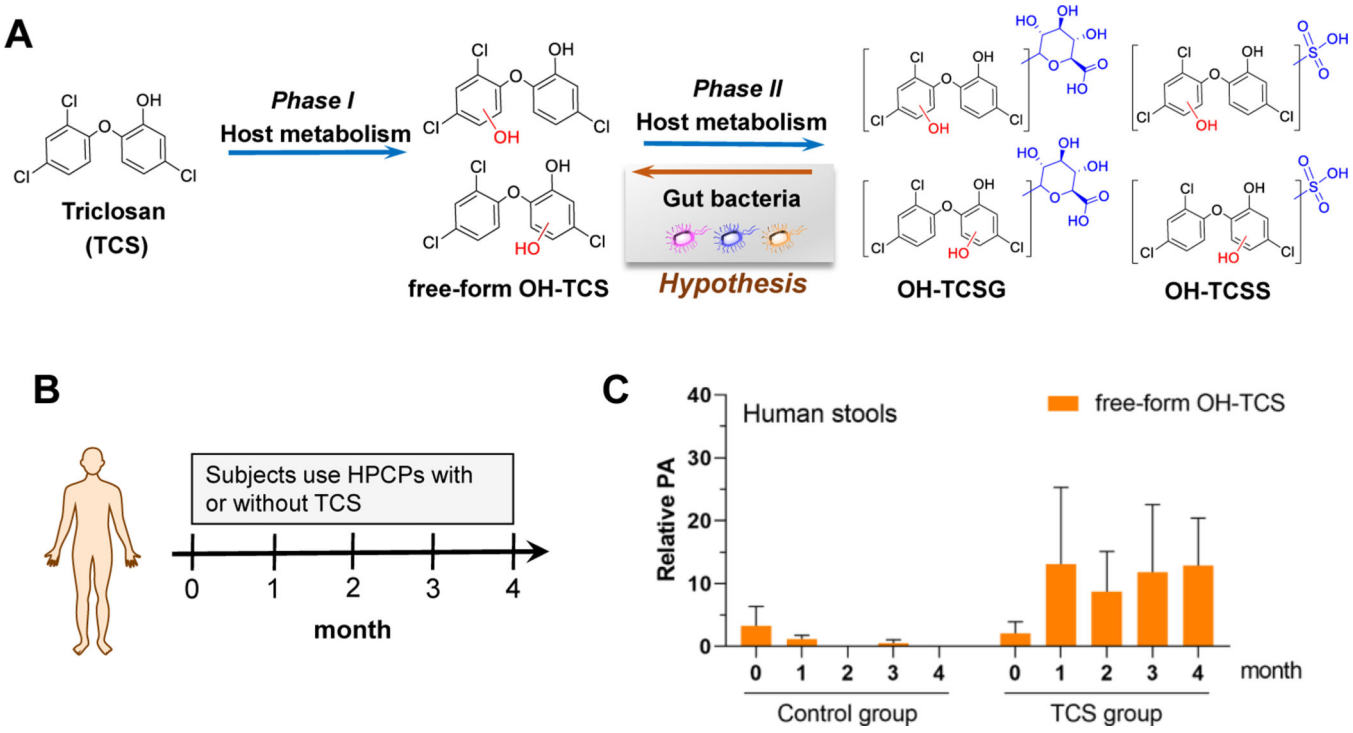


Figure 1. Profiles of OH-TCS metabolites in human stools.

(A) Hypothesis of the metabolic fates of TCS oxidative metabolites. **(B)** Scheme of experiment. Human subjects used HPCPs without TCS (control group, n = 7) or with TCC (TCS group, n = 6) for up to 4 months. The stool samples were collected once a month for analysis. **(C)** Semi-quantification of OH-TCS in human stools. PA, peak area detected by UHPLC–HRMS, relative to the mean value in control group.

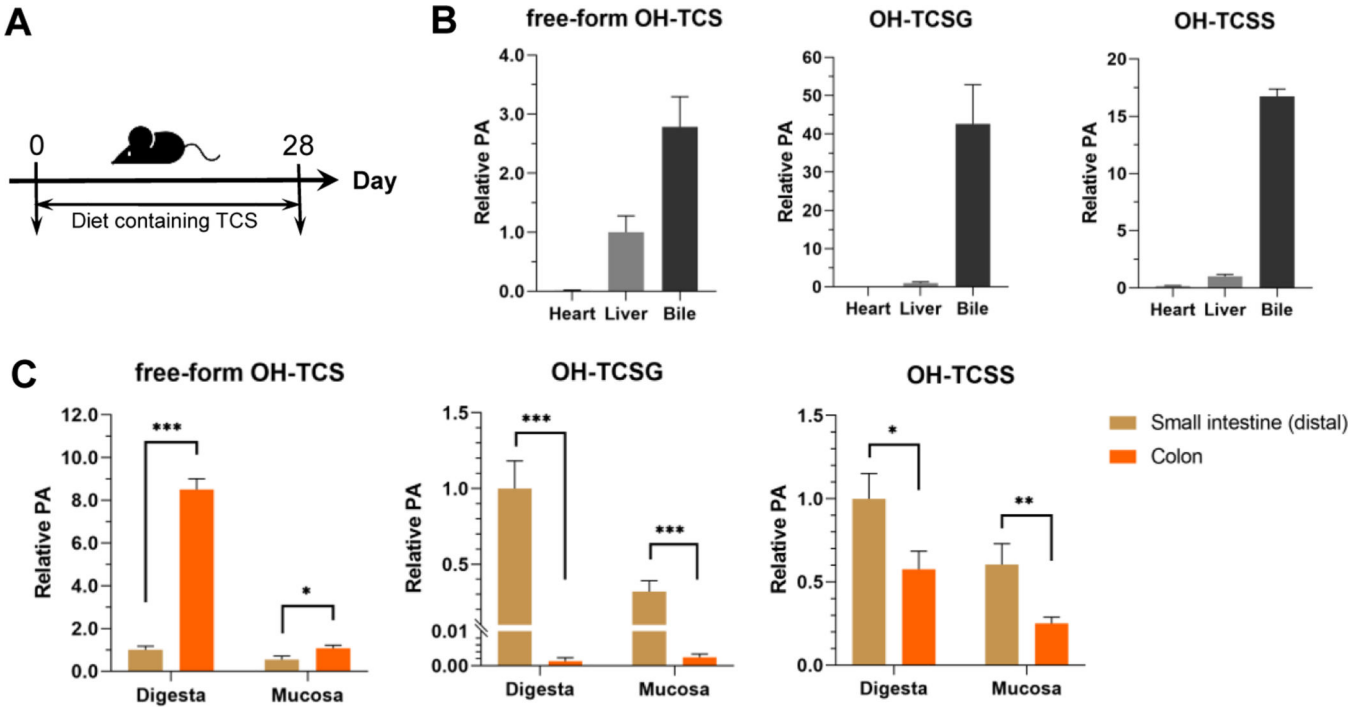


Figure 2. The generation of OH-TCS species in host tissues and their profiles in the GI tract of mice.

(A) Scheme of experiment. Mice were fed a diet containing 80 ppm TCS for 28 days (n = 10 in each group). **(B)** Semi-quantification of OH-TCS metabolites in mouse liver, bile and heart. PA is relative to that in the liver. **(C)** Semi-quantification of OH-TCS species in the small intestine and colon (both the digesta and mucosa), PA is relative to that in the digesta of small intestine. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

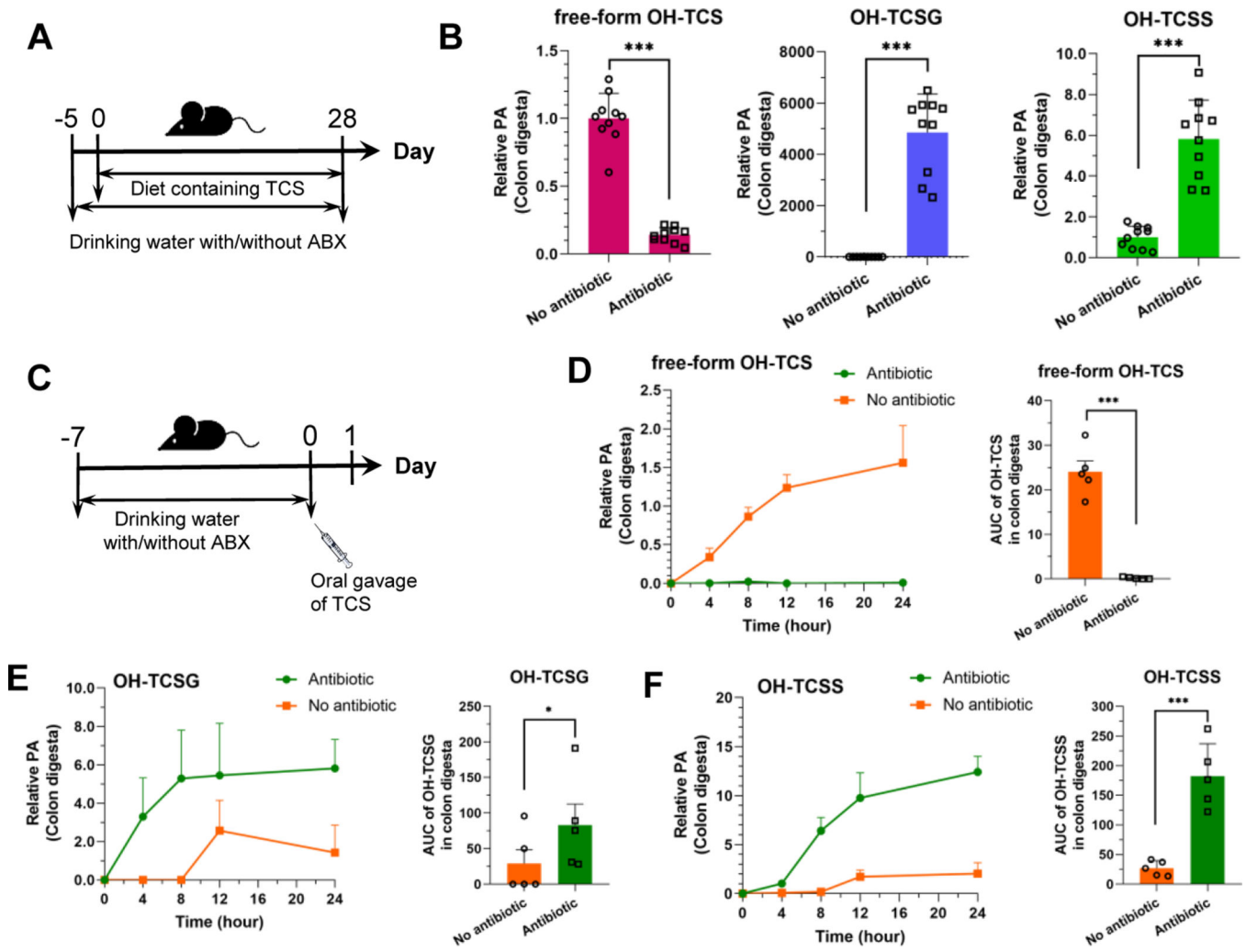


Figure 3. Effects of antibiotic-mediated suppression of gut microbiota on TCS hydroxylation in the colon.

(A) Scheme of experiment. Mice received drinking water with or without an antibiotic cocktail, and treated with a diet containing TCS (n = 10 in each group). **(B)** Antibiotic treatment reduced free-form OH-TCS and increased OH-TCSG and OH-TCSS in colon digesta. **(C)** Scheme of experiment. Mice were pre-treated with or without antibiotics for a week and then orally gavaged with TCS (n = 5 for each time point). **(D–F)** Antibiotic treatment reduced free-form OH-TCS and increased OH-TCSG and OH-TCSS in the colon digesta in a time-dependent manner. AUC, area under curve. * $P < 0.05$, *** $P < 0.001$.

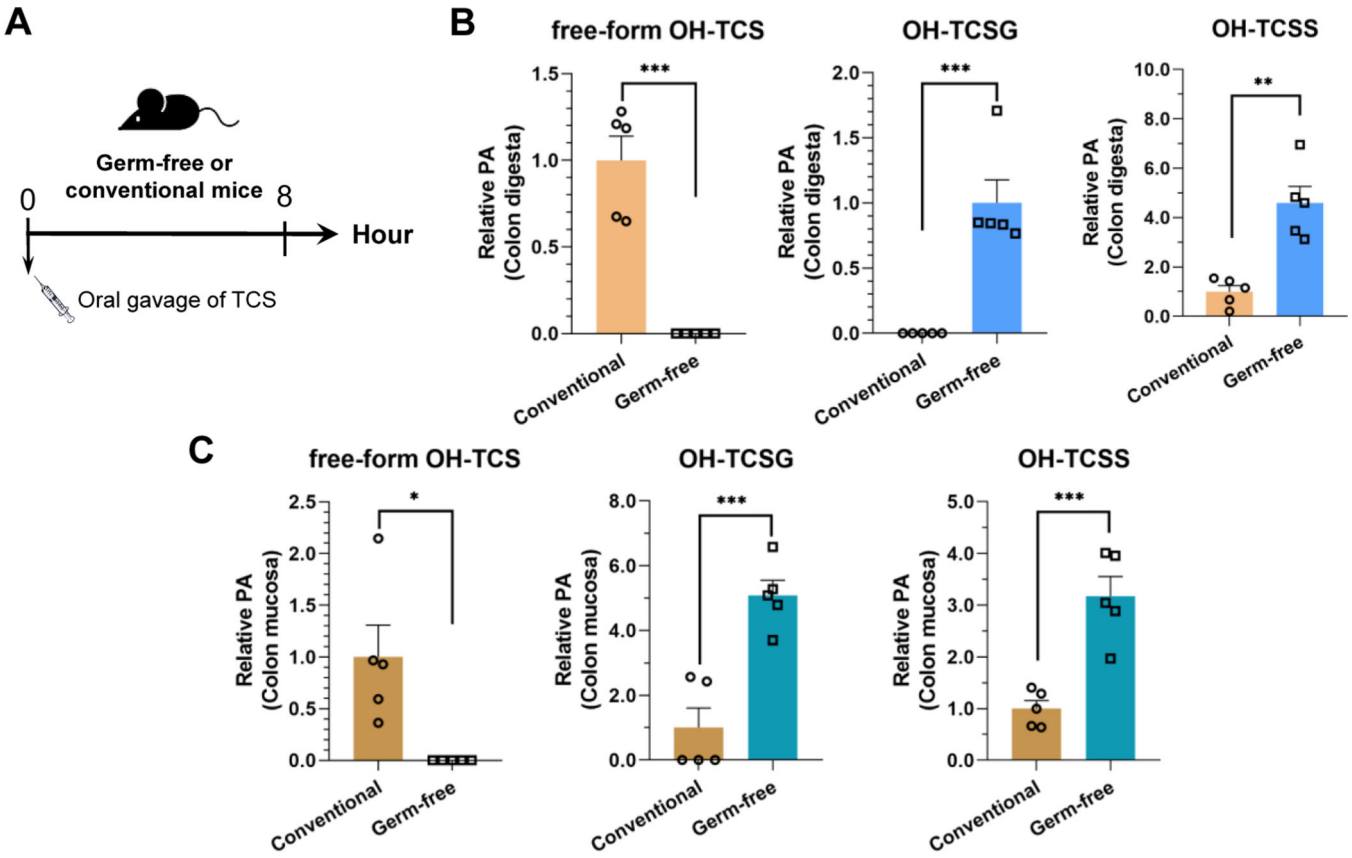


Figure 4. Profiles of OH-TCS metabolites in the colon of germ-free mice.

(A) Scheme of experiment. Germ-free or conventional mice received a single oral gavage administration of TCS (n = 5 in each group). **(B–C)** Compared with conventional mice, germ-free mice had reduced free-form OH-TCS and increased OH-TCSG and OH-TCSS in colon digesta and mucosa. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

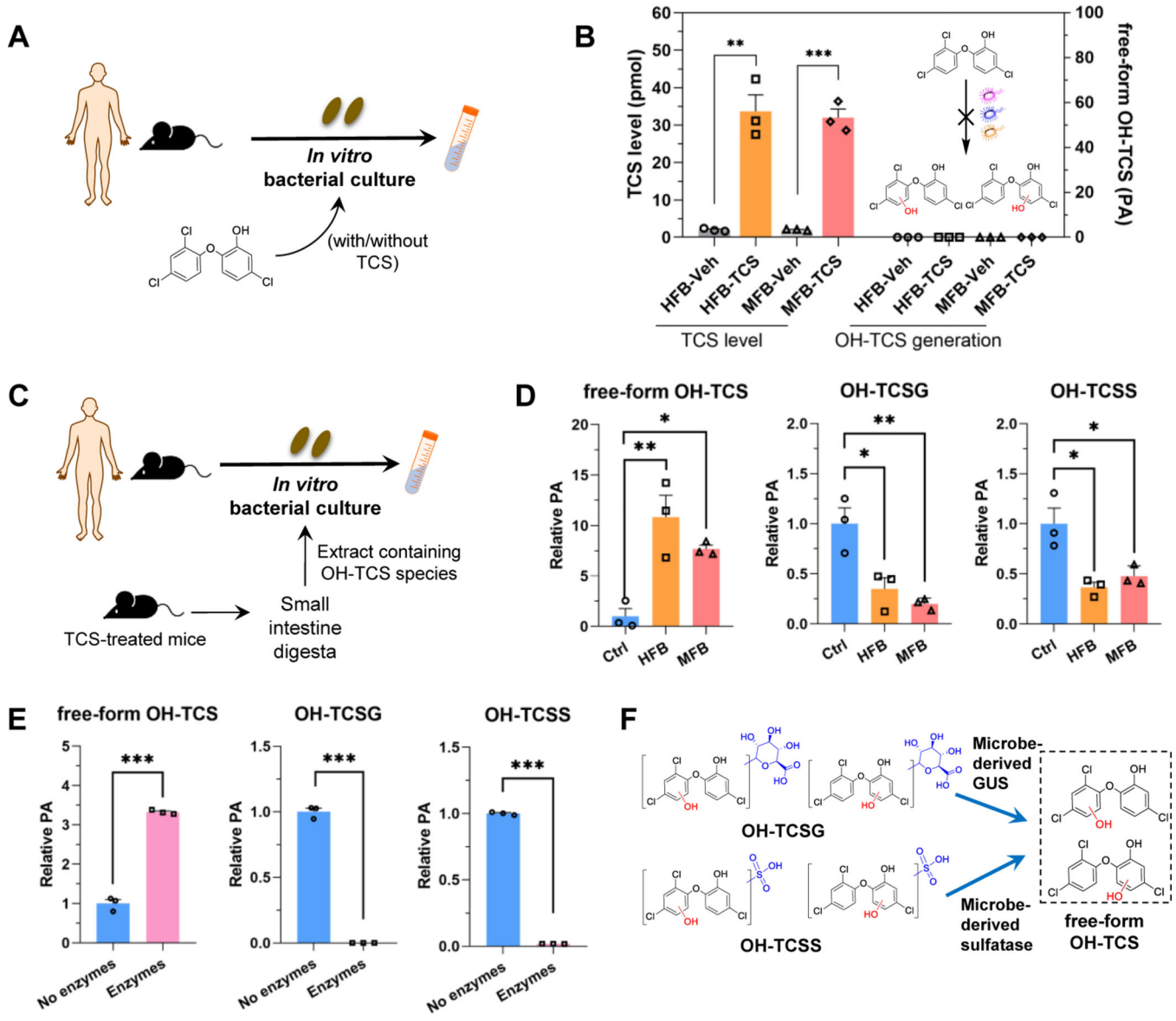


Figure 5. ***In vitro*** **experiments.**

(A) Scheme of experiment. TCS was incubated with gut bacteria for 48 h, then the formation of OH-TCS species was analyzed (n = 3 in each group). DMSO was used as the solvent vehicle (Veh). **(B)** Gut microbiota could not directly catalyze TCS hydroxylation *in vitro*. HFB, human fecal bacteria. MFB, mouse fecal bacteria. **(C)** Scheme of experiment. We incubated gut bacteria with the extract of small intestine digesta from TCS-treated mice, then analyzed OH-TCS species by UHPLC–HRMS (n = 3 in each group). **(D)** Relative levels of OH-TCS species. HFB, human fecal bacteria. MFB, mouse fecal bacteria. **(E)** Relative levels of OH-TCS species treated with/without microbe-derived GUS and sulfatase. **(F)** *In vitro* evidences showed that microbe-derived GUS and sulfatase are involved in the de-conjugation of OH-TCS species. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

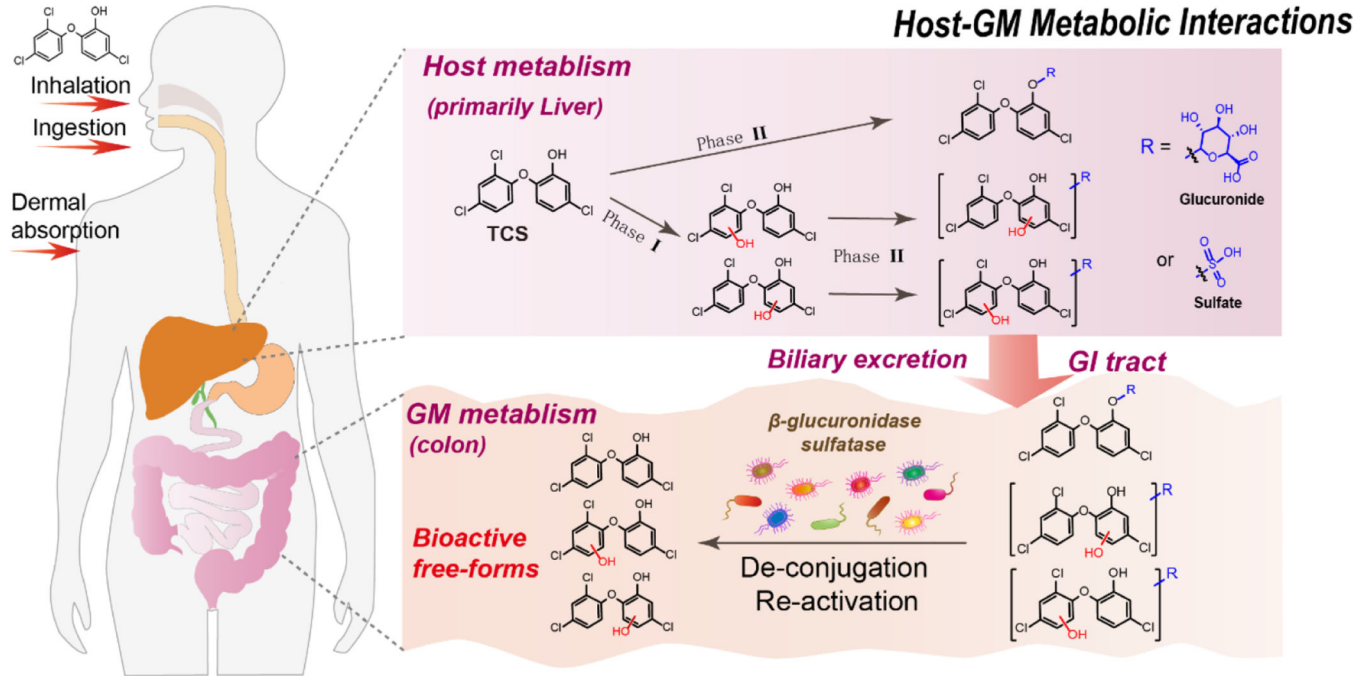


Figure 6.

Primary metabolic fates of TCS proposed in this study through combing the interactions between the host tissues and gut microbiota. GM, gut microbiota; GI tract, gastrointestinal tract.