Supporting Information

for

Discovery of a Widespread Metabolic Pathway within and among Phenolic Xenobiotics

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Chemicals and Reagents.

Triclosan (TCS) and ^{13}C-TCS were purchased from Wellington Laboratories Inc. (Guelph, ON, Canada). Chlorophenol (ClPhOH), phenol (PhOH) and benzene (Ph) were purchased from Dr. Ehrenstorfer GmbH (Germany). Benzo(a)pyrene (B[a]P) and pyrene (Py) was obtained from AccuStandard (New Haven, CT). 2-phenoxyphenol (2-Ph-O-PhOH), 3-phenoxyphenol (3-Ph-O-PhOH), and 4-phenoxyphenol (4-Ph-O-PhOH) were obtained from Ark Pharma Inc. (IL, USA), Alfa Aesar (Ward Hill, MA) and CNW Technologies GmbH (Germany), respectively. BPA was obtained from Kanto Chemical Co. (Tokyo, Japan), and ClBPA was synthesized in our previous study (1). Dichloromethane (DCM), n-hexane, water, acetone, acetonitrile, and methanol of pesticide residue grade were purchased from Fisher Chemicals (NJ, USA). N,O-bis(trimethylsilyl)trifluoroacetamide with 1% trimethylchlorosilane (BSTFA+1% TMCS) was obtained from Supelco (Bellefonte, PA, USA). Dansyl chloride (DNS), 4-(dimethylamino)-pyridine (DMAP), uridine 5'-diphosphate-glucuronic acid trisodium salt (UDPGA), adenosine 3'-phosphate 5'-phosphosulphate (PAPs), dithiothreitol (DTT), 2,6-dichloro-4-nitrophenol (DCNP), and dimethyl sulfoxide (DMSO) were obtained from Sigma-Aldrich (St. Louis, MO, USA). 4-methylumbelliferone (4-MU) was purchased from J&K Technology Limited (Beijing, China). Alamethicin was purchased from TRC (Toronto, ON). Pure water was prepared by a Milli-Q Synthesis water purification system (Millipore, Bedford, MA, USA). The NADPH regenerating system was purchased from Promega (Madison, WI, USA). High performance liquid chromatography (HPLC) grade ammonium acetate was purchased from Dima-Tech Inc. (Richmond Hill, ON, Canada). Sodium sulfate (analytical reagent grade, Beijing Chemicals

(Beijing, China) was baked at 450°C for 4 hours and stored in a drying oven before use. Monopotassium phosphate, dipotassium phosphate, ethylene diamine tetraacetic acid (EDTA), magnesium chloride ($MgCl_2$) and glycerol were purchased from Beijing Chemicals. Human liver microsomes (HLM), human liver cytosols (HLCYT) and human S9 which were pooled (n= 20) from mixed genders, were obtained from BD Biosciences (USA), and stored at -80°C prior to *in vitro* studies. 1,2,4-trichloro-5-nitrobenzene was purchased from TCI organic chemicals. 1,3,5-trimethylbenzene, potassium hydroxide, 4-chloro-2-methoxy-pheno, iron, 1,4-dioxane, hydrochloric acid, sodium nitrite, and boron tribromide were purchased from Adamas Reagent. Cuprous chloride was obtained from Acros chemicals.

Microsome Preparations.

Liver microsome of weever (*Lateolabras japonicus*) and common quails (*Coturnix coturnix*) were prepared according to the method of Dyer et al. (2). HLM, S9, and HLCYT were purchased from the BD biological chemical company (USA) and stored at –80°C before incubation. The weever used in this study was purchased from an aquaculture farm in Qingdao, Shangdong Province, China, and their body weight at the time of use was about 2 kg. Briefly, approximately 5 g of tissue was homogenized in 25 mM phosphate buffer (pH = 7.4, 1.25 mM EDTA, 1mM DTT, 10% (v/v) glycerol), and samples were centrifuged for 15 min at 10,000 × *g*. Following centrifugation, the supernatant (S9 fraction) was removed and then centrifuged at 100,000 × *g* for 60 min. The resulting pellet was dissolved in 10 mL of phosphate buffer (50 mM phosphate buffer, pH = 7.4, 1 mM EDTA, 20% (v/v) glycerol) and stored in liquid nitrogen. Quails were purchased from a farm in Rizhao, Shangdong Province, China and their body weight was about 0.35 kg. Liver microsomes of quail were extracted

according to the method of Diaz et al. (3). Approximately 5 g liver tissue was homogenized in 20 mM phosphate buffer (pH = 7.4, 1 mM EDTA, 250 mM sucrose) and then centrifuged for 30 min at 10,000 × *g*. Following centrifugation, the supernatant (S9 fraction) was removed and then centrifuged at 100,000 × *g* for 90 min. The resulting pellet was dissolved in 10 mL of phosphate buffer (20 mM phosphate buffer, pH = 7.4, 1 mM EDTA, 250 mM sucrose, and 20% (v/v) glycerol) and stored in liquid nitrogen before use. All centrifugation steps were carried out at 4°C, and samples were kept on ice throughout the procedure. The protein concentrations were determined by the Bradford method using bovine serum albumin as a standard according to the manufacturer's protocol (Sigma-Aldrich Corp.) (4).

***In Vitro* Microsomal Incubations.**

For *in vitro* incubations of weever microsomes, reactions were performed in 50 mM phosphate buffer (pH = 7.4) containing 1 mM tetraacetic acid (EDTA), 1 mM dithiothreitol (DTT), and 20% (v/v) glycerol. For *in vitro* incubations of quail microsomes, 50 mM phosphate buffer (pH = 7.4) containing 5 mM $MgCl_2$ and 0.5 mM EDTA were added to the incubation vials. For *in vitro* incubations of human microsomes, the assay was performed in 50 mM phosphate buffer (pH = 7.4) containing 3 mM $MgCl_2$. The reaction mixtures (200 µL), consisting of 100 µL liver microsomes (10 mg/mL protein), 60 µL NADPH regenerating system and 1 uL substrate (TCS dissolved in dimethyl sulfoxide [DMSO]), were incubated in 1.5-mL amber glass vials. After 5 min pre-incubation in a shaking incubator at 25°C, 39°C and 37°C for weever, quail, and human microsomes, respectively, reactions were initiated by the addition of a NADPH-generating system (6.5 mM NADP, 16.5 mM glucose 6-phosphate, 16.5 mM magnesium chloride, and 2 U/mL glucose 6-phosphate dehydrogenase). The protein

concentrations in the incubation mixtures were adjusted to 1 mg/mL. The incubation time was 1 h for kinetic studies, and 12 h for metabolic tests within and among phenolic compounds. After incubations, the reactions were quenched by adding 200 µL of ice-cold acetone. The vials were stored at –20°C until chemical analysis.

***In Vitro* S9 and Cytosol Incubations.**

To investigate TCS metabolites produced by phase I and phase II enzymes, TCS was separately incubated with human S9, HLM, and HLCYT. Incubations of TCS with human S9 were prepared in the microsomes experiments described above using human S9 (2 mg/mL protein) instead of HLM. To investigate TCS metabolites produced by UGT enzymes, reaction mixtures (200 µL) consisting of pooled HLM (1 mg/mL protein), alamethicin (10 µg/mL, 1% DMSO v/v), and TCS were incubated in 1.5-mL amber glass vials. The reaction was initiated by the addition of 1mM trisodium salt (UDPGA). A new aliquot of UDPGA was added after 9 h incubation to ensure a sufficient cofactor amount throughout the experiment. To investigate TCS metabolites produced by SULT enzymes, samples were prepared as described above for the UGT experiments without the addition of alamethicin, using 1 mg/mL HLCYT instead of HLM and starting the reaction by the addition of 0.1 mM adenosine 3’-phosphate 5’-phosphosulphate (PAPs) instead of UDPGA. Phase II enzyme inhibition experiments were conducted by adding enzyme inhibitors to the incubation mixtures described above. 2,6-dichloro-4-nitrophenol (DCNP) and 4-methylumbelliferone (4-MU) were inhibitors of UGT and SULT enzymes in HLM, respectively, and the inhibitors were added using the corresponding reaction buffer as the solvent.

All reactions were performed in triplicate at 37°C and quenched after 18 h by adding 200 μL acetone. The vials were stored at −20°C until chemical analysis. Incubations with deactivated enzymes and standards were used as negative controls to assess potential background interference and the possibility of non-enzymatic changes.

Analysis of Incubation Mixtures.

For analysis of TCS incubation mixtures, the mixtures were spiked with 100 μg internal standards (^{13}C-TCS) and extracted with 1 mL hexane three times. The aquatic fraction was then passed through a Pasteur pipe filled with sodium sulfate to remove moisture and then eluted by 2 mL hexane and 2 mL DCM, which were also used to rinse the glass vial before elution. All of the extracts were combined and concentrated to 200 μL in hexane for instrumental analysis. An aliquot of extract (100 μL) was derivatized with BSTFA+1% TMCS for analysis of TCS. Briefly, 100 μL BSTFA+1% TMCS was added into the vial containing the extracts, which was dried under a gentle stream of nitrogen gas before the derivatization. The mixture was allowed to react for 1 h at 70°C. After cooling for 5 min, the final sample volume was adjusted to 100 μL using hexane for gas chromatography–mass spectrometry (GC-MS) analysis. The remaining 100 μL of extract was reconstituted with 100 μL of methanol for the untargeted screening of biotransformation products by ultrahigh-pressure liquid chromatography (UPLC) coupled to a quadrupole time-of-flight mass spectrometer (QTOF-MS). For analysis of incubation mixtures of ClBPA, ClPhOH, BPA, PhOH, B[a]P, and Py, the extraction procedures were same to those of TCS and the extracts were directly reconstituted with 100 μL of methanol for UPLC-QTOF-MS analysis. For analysis of incubation mixtures of Ph, the extracts were derivatized with BSTFA+1%

TMCS for GC-MS analysis.

Analysis of Rat Liver and Urine Samples.

Approximately 1 g of tissue was homogenized in 1 mL ultrapure water, spiked with 100 μg internal standard (^{13}C-TCS), and then extracted with 2 mL hexane three times. The aquatic fraction was then passed through sodium sulfate to remove any moisture and eluted by hexane and DCM. All of the extracts were combined and concentrated to 50 μL for dansylation before UPLC-MS/MS analysis. Human urine samples (25 mL) were acidified with 0.1 M formic acid, ^{13}C-TCS was added to obtain a final concentration of 5 ng/L, and then the samples were extracted with 25 mL hexane two times and 25 mL (hexane: DCM = 3: 1). The extract was dried and dissolved in 1 mL acetonitrile for dansylation. Dansylation was applied to improve the analytical sensitivities of TCS and its biotransformation products. Briefly, 200 μL of 30 mg/mL dansyl chloride and DMAP in DCM was added to the extract solution. The mixture solution was shaken vigorously for 1 min. The resulting mixture was kept at 65°C for 60 min and then extracted with 2 mL hexane three times. The extract was evaporated to dryness and reconstituted with 25 μL of methanol prior to UPLC-MS/MS analysis.

GC-MS Analysis.

Sample analyses were performed with an Agilent 6890 equipped with a 5975C mass spectrometer (Agilent Technologies) in EI (electron impact) mode. Analytes were separated on a HP-5ms column (30 cm × 0.25 mm × 0.25 μm film thickness; J&W Scientific) with helium as the carrier gas (1.0 mL/min). The oven temperature program was 80°C for 1 min, 30°C/min to 180°C, 5°C/min to 260°C, 20°C/min to 300°C, and then 300°C for 10 min. The ion source temperature was maintained at 250°C and the electron energy was 70 eV. Sample

(1 μL) was injected into the GC-MS system in splitless mode, and the injector was held at 280°C. The following masses were used for confirmation and quantification of TCS: m/z = 345, 347, and 200 for the derivative of TCS, m/z = 357, 359, and 200 for internal standard ^{13}C-TCS, m/z =258, and 243 for the derivatives of Ph-O-PhOH, m/z =526, and 453 for the derivatives of for Py-O-PyOH, and m/z=626, and 285 for the derivatives of for B[a]P-O-B[a]POH.

UPLC-QTOF-MS Analysis.

Analysis was performed on an ACQUITY^{TM} UPLC system (Waters, Milford, MA, USA) coupled to a Xevo QTOF-MS (G2, Waters) equipped with an electrospray ionization source. Instrument control was performed using MassLynx Software (Waters, software version V4.1). UPLC separation was achieved using a Waters ACQUITY^{TM} UPLC BEH C8 column (50 × 2.1 mm, 1.7-μm particle size). The column was maintained at 60°C at a flow rate of 0.6 mL min^{-1} and the injection volume was 3 μL. The mobile phases consisting of ultrapure water containing 5 mM ammonium acetate and 0.05% acetic acid (A) and acetonitrile (B) were used with gradient elution. The gradient applied was as follows: 0 min, 5% B; 7 min, 95% B; 8 min, 95% B; 8.1 min, 5% B; and 10 min, 5% B. The mass spectra were acquired in the negative ion mode. The analysis was performed in full scan mode in the mass range of 80-700 Da with a 1-s scan time. The optimized parameters were as follows: source capillary voltage, -0.7 kV; sampling cone voltage, 35 V; extraction cone voltage, 4.0V; source temperature, 100°C; cone gas flow rate, 50 L/h; and desolvation gas flow rate, 950 L/h. Nitrogen and argon were used as cone and collision gases, respectively. The [M-H]$^-$ ion of leucine-enkephalin (200 pg/μL infused at 5 μL/min) was used as a reference lock mass (m/z = 554.2615). The QTOF detector

was calibrated with a sodium formate solution to achieve a mass accuracy below 3 ppm by using leucine-enkephalin as the lock mass in negative mode. The accuracy of mass measurement in combination with the retention times in UPLC was used to calculate empirical formulae for TCS. For MS/MS analysis, the experimental conditions were the same as above except for the collision energy. Three levels of collision energy were applied: 5~15V, 15~25V, and 25~35V.

UPLC-MS/MS analysis.

The LC apparatus was an ACQUITY^{TM} Ultra Performance LC system (Waters). Separations were conducted using a Waters ACQUITY^{TM} UPLC BEH C8 column (50 × 2.1 mm, 1.7-μm particle size). The column was maintained at 40°C, and the flow rate and injection volume were 0.6 mL/min and 5 μL, respectively. Ultrapure water containing 0.1% formic acid (A) and acetonitrile (B) were chosen as the mobile phases. The gradient applied was as follows: 0 min, 5%B; 7 min, 95% B; 8 min, 95% B; 8.1 min, 5% B; and 10 min, 5% B. The optimized parameters were as follows: source temperature, 110°C; desolvation temperature, 350°C; capillary voltage, 3.50 kV; desolvation gasflow, 800 L/h; cone gas flow, 50 L/h; and multiplier, 650 V. The mass spectra were acquired in the positive ion mode. Data acquisition was performed by multiple reaction monitoring (MRM). The optimized MRM transitions, cone voltages, and collision energies were summarized in the Table S3.

Separate Isolation of Metabolites.

Preparative HPLC was performed to isolate the individual metabolites (e.g. individual TCS-O-TCS isomers) with a Shimadzu high performance liquid chromatography system (LC-20AT) equipped with a UV detector (SPD-20A), using an YMC C18 preparative column

(5 μm particle size, 250 mm x 10 mm i.d.). The processing conditions for the gradient elution were as follows: 0-2 min, acetonitrile-water (5:95, v/v); 2-52 min, acetonitrile-water (100:0, v/v); 52-60.5 min, acetonitrile-water (5:95, v/v); 60.5-66 min, acetonitrile-water (5:95, v/v). The flow rate was 4.5 mL/min. The target fractions were collected, confirmed by UPLC-QTOF-MS analysis.

Statistical analysis of Nontargeted Screening.

Spectral peaks were deconvoluted and aligned using Waters MarkerLynx with the following parameters: initial retention time of 1 min, final retention time of 8 min, and mass in the range 50–1200 Da, with a mass tolerance of 0.02 Da, mass window of 0.02 Da, retention time window of 0.2 min, and noise elimination level 6. For peak integration, the peak width at 5% of the height was 1 s, the peak-to-peak baseline noise was automatically calculated, and the peak intensity threshold was 100. No specific mass or adduct was excluded. Isotopic peaks were excluded for analysis. The data sets were exported to SIMCA-P+ (ver. 12.0, Umetrics, Umea, Sweden) for multivariate statistical analysis. Principal component analysis (PCA) and orthogonal partial least-squares-discriminant analysis (OPLS-DA) were conducted to identify the discriminatory chemicals based on the comparison of exposed and control samples. The accurate mass composition and fragmentation pattern of each marker feature of interest (as determined by multivariate techniques) were further determined by UPLC-QTOF-MS/MS analysis.

Quality Assurance and Quality Control (QA/QC).

All equipment was rinsed with acetone and hexane to avoid sample contamination. Procedural blanks were prepared with Milli-Q water to determine background contaminations.

Concentrations of TCS and TCS-O-TCS in sample extracts were quantified relative to ^{13}C-TCS. Efficiencies of the sample preparation procedures for urine samples were assessed by analyzing samples spiked with standard solutions of TCS and purified standards of TCS-O-TCS and OH-TCS. The absolute recoveries of target compounds in triplicate spiked samples were 72 ± 11%. Surrogate standards were spiked to samples prior to extraction to compensate for the loss of target compounds during the extraction process and correct the variation of instrument response and matrix effect. The recoveries of ^{13}C-TCS were 85 ± 13% in all of the sample analyses. Instrumental method detection limits for TCS on GC-MS were both below 0.3 μg/mL and were 0.03, 0.02, and 0.01 ng/mL for TCS, OH-TCS, and TCS-O-TCS, respectively, on UPLC-MS/MS.

Chemical Synthesis of a TCS-O-TCS isomer.

As shown in SI Appendix, Fig. S3, a solution of compound B (1.0 eq) and KOH (1.0 eq) in mesitylene was heated to 150°C for 30 min, then a solution of compound A (1.0 eq) was added into the solution, and stirred at this temperature for 16 hours, then the mixture was concentrated and purified by Prep-TLC to give compound C (400 mg, 50%) as a yellow treacly appearance. A solution of compound D (1.0 eq) and KOH (1.0 eq) in mesitylene was heated to 150°C for 30 min, then a solution of compound C (1.0 eq) was added into the solution, and stirred at this temperature for 16 hours, then the mixture was concentrated and purified by Prep-TLC to give compound E (20 mg, 20%) as a white solid. A solution of compound E (1.0 eq) in H_2O/dioxane=1/1 was added with Fe (10 eq) and 3 drops of conc.HC. This mixture was heated at 100°C for 3 hours, and then filtered, concentrated, and purified by Prep-TLC to give compound F (10 mg, 45%) as a yellow oil. A solution of compound F (1.0

eq) in H_2O/conc.HCl=1/1 was added with $NaNO_2$ (1.5 eq) at 0°C and stirred for 30 mins, then $CuCl_2$ (2.0 eq) was added into the solution, warmed to room temperature, and stirred for 3 hours. Then the solution was extracted by DCM, dried over Na_2SO_4, filtered and concentered to give compound G (8.0 mg) as a crude material. A solution of compound G (1.0 eq) in DCM was cooled to -78°C and then BBr_3 (3.0 eq) was added into the solution, warmed to room temperature for 3 hours, then 2 mL MeOH was added into the solution, concentrated and purified by Prep-TLC to give product (1.2 mg) as a light red solid.

The synthesized substance was dissolved in 600 μL chloroform-d, and used for ^1H nuclear magnetic resonance (NMR) analysis. ^1H NMR spectra were recorded at 500 MHz on a Bruker AVANCE III 500 spectrometer. The substance was also redissolved in methanol for UPLC-QTOF-MS analysis.

CAR Activation Assay.

The yeast two-hybrid assay was used to test the binding activity of TCS and its metabolites with the CAR. This system is based on the ligand-dependent interaction of two proteins, a human CAR and a coactivator transcriptional intermediary factor 2. The extracts of the incubation mixtures or isolated metabolites were treated with yeast cells to determine their CAR activities. The microsomes and NADPH system were re-added to the incubation mixtures every 12 h to supplement the protein. The yeast cells were pre-incubated overnight at 30°C (about 14-16 h) with vigorous shaking in 5 mL SD medium (6.7 g/L Difco yeast nitrogen base without amino acids, 2% glucose, 300 mg/L L-isoleucine, 1,500 mg/L L-valine, 200 mg/L L-adenine hemisulfate salt, 200 mg/L L-arginine HCl, 200 mg/L L-histidine HCl monohydrate, 300 mg/L L-lysine HCl, 200 mg/L L-methionine, 500 mg/L L-phenylalanine,

200 mg/L L-threonine, 300 mg/L L-tyrosine, and 200 mg/L L-uracil [Sigma-Aldrich]) until the cell density reached an optical density (OD) level of 0.4. The OD levels were measured by a microplate reader (Bio RAD 550, Richmond, CA, USA) operating at a wavelength of 595 nm. Cell cultures (50 μL) and 2.5 μL TCS standards or extracts of incubations dissolved in DMSO solution were then added to 200 μL fresh medium, respectively. After incubation at 30°C for 4 h, 150-μL aliquots of the culture mixtures were removed to determine the absorbance at 595 nm. The residual culture was centrifuged (12,000 rpm) for 5 min at 4°C, and the collected cells were digested enzymatically by resuspension in 200 μL Z buffer (0.1 M sodium phosphate, 10 mM KCl, and 1 mM $MgSO_4$) containing 1 mg/mL Zymolyase 20T (Seikagaku, Tokyo, Japan) at 30°C for 20 min. The lysate was mixed with 40 μL 4 mg/mL 2-nitrophenyl-b-D-galactoside (ONPG, Tokyo Kasei, Tokyo, Japan) at 30°C and reacted until the development of a yellow color (usually 20 min). Then, 100 μL 1 M Na_2CO_3 was added to finalize the enzymatic reaction and 150-μL aliquots were added into each well in a 96-well microplate. Absorbances at 415 and 570 nm were read on the microplate reader, and based on the absorbances at 595, 415, and 570 nm, CAR activity was calculated by the following equation:

$$U = 1{,}000 \times ([OD_{415}] - [1.75 \times OD_{570}]) / ([v] \times t \times [OD_{595}]),$$

where U = CAR activity, t = time of reaction (min), v = volume of culture used in the assay (ml), observed density $(OD)_{595}$ = cell density at the start of the assay, OD_{415} = absorbance by o-nitrophenol at the end of the reaction, and OD_{570} = light scattering at the end of the reaction. CITCO, a human CAR selective agonist, was used as a positive control. Stock solutions of test chemicals were subjected to a 2-fold serial dilution with DMSO to prepare 11

concentrations in the range of 9.77×10^{-2} to 100 μM. All results are given as the mean ± standard deviation (SD) of at least triplicate assays. The REC50 is the concentration of the test chemical corresponding to 50% of the maximum activity of TCS. Sigmoidal dose-effect curves for human CAR binding activities were analyzed using GraphPad Prism 5 Software (GraphPad Software, Inc., San Diego, CA).

Data Analysis.

After the Pareto scaling (mean centering and scaling to the square root of variance), the data sets were exported to SIMCA-P 13.0 (Umetrics) for multivariate statistical analysis including PCA and OPLS-DA analysis. Each OPLS-DA model was evaluated by both the internal permutation test and external validation test. Unpaired Student's *t*-tests, with a Bonferronni correction for multiple comparisons, were used to ensure that the markers extracted with holistic OPLS-DA analysis were significantly differentially expressed between the exposed and control groups. A *p*-value threshold of 0.05 was used to define significance. Enzyme kinetic data were fit to the Michaelis-Menten model using GraphPad Prism 5 Software (GraphPad Software, Inc.).

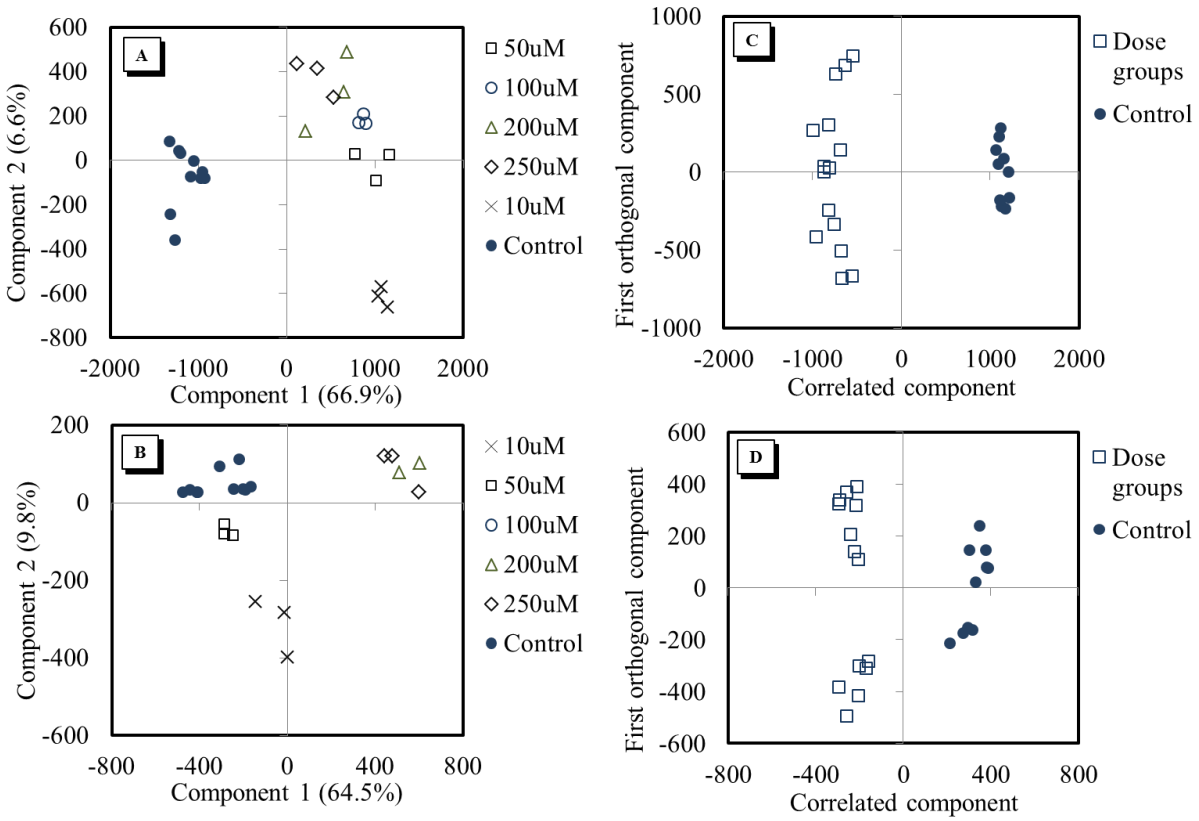

Fig. S1. A) PCA score plots of samples from control and dosed groups incubated with quail microsomes; B) OPLS-DA score plots of samples from control and dosed groups incubated with quail microsomes; C) PCA score plots of samples from control and dosed groups incubated with weever microsomes; D) OPLS-DA score plots of samples from control and dosed groups incubated with weever microsomes

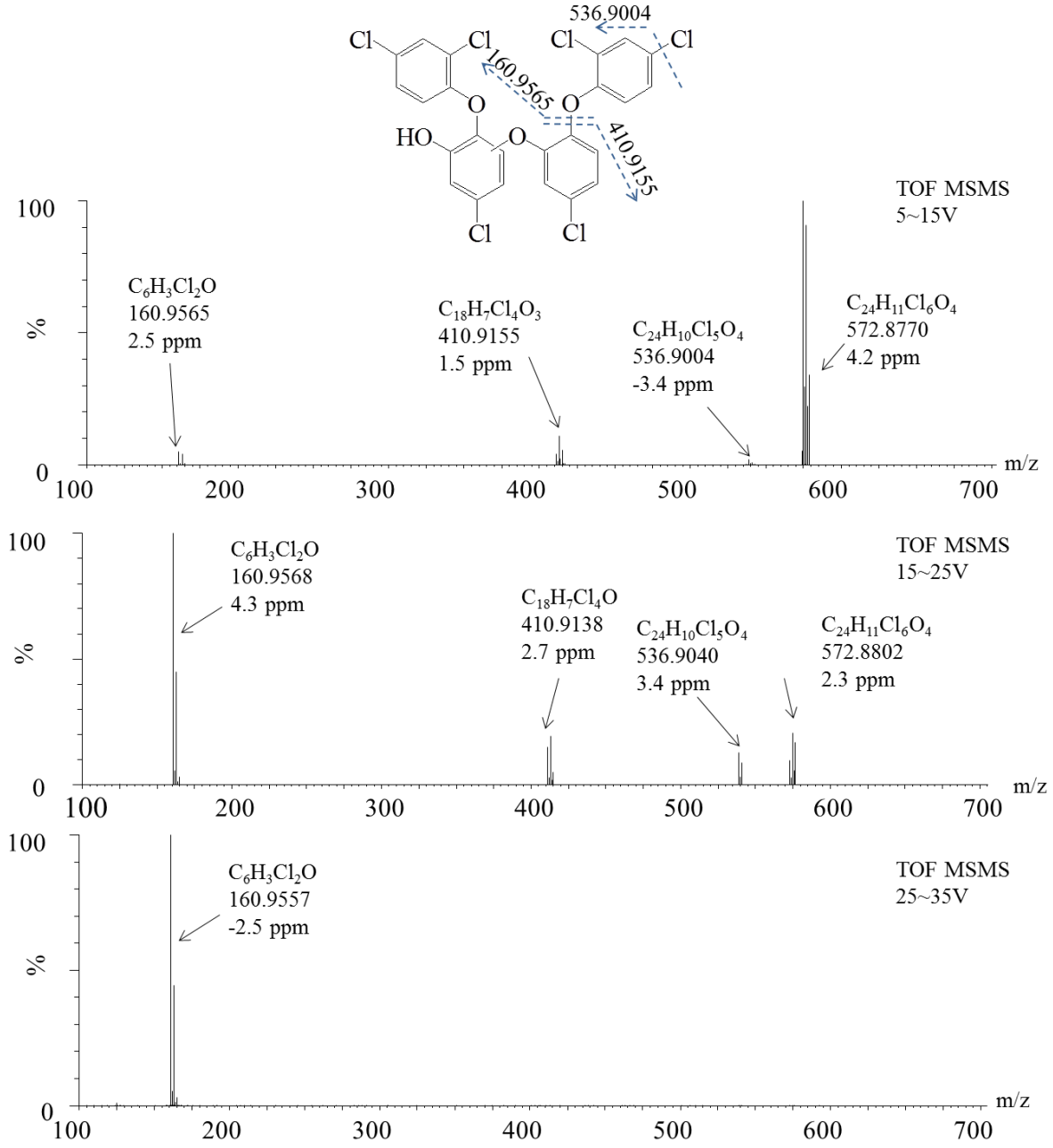

Fig. S2. MS/MS spectra under different collision energies and proposed structures of TCS-O-TCS.

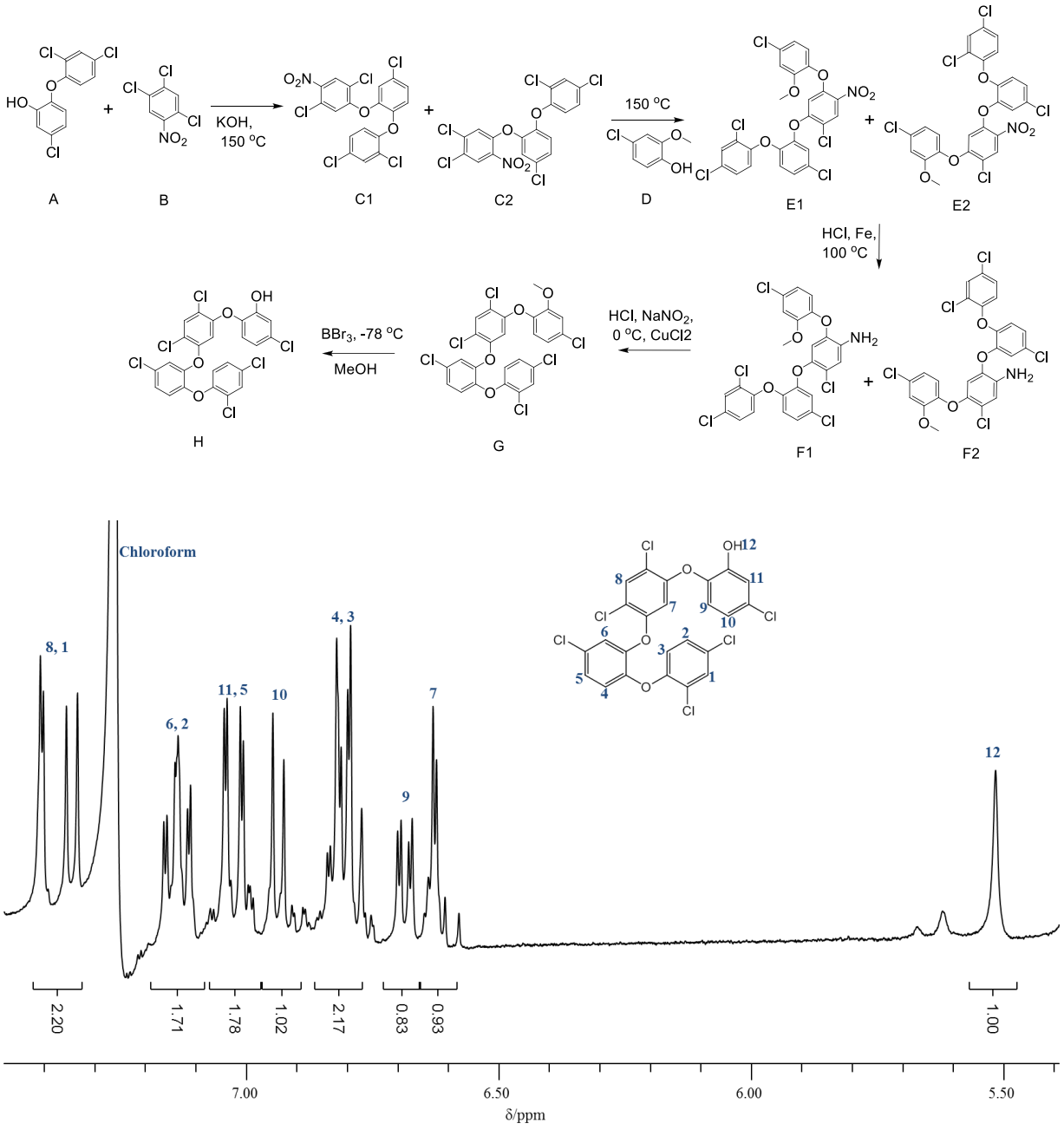

Fig. S3. Synthesis route of TCS-O-TCS (M6), and ^{1}H NMR spectrum (500 MHz, chloroform-d, p.p.m.) of the metabolite.

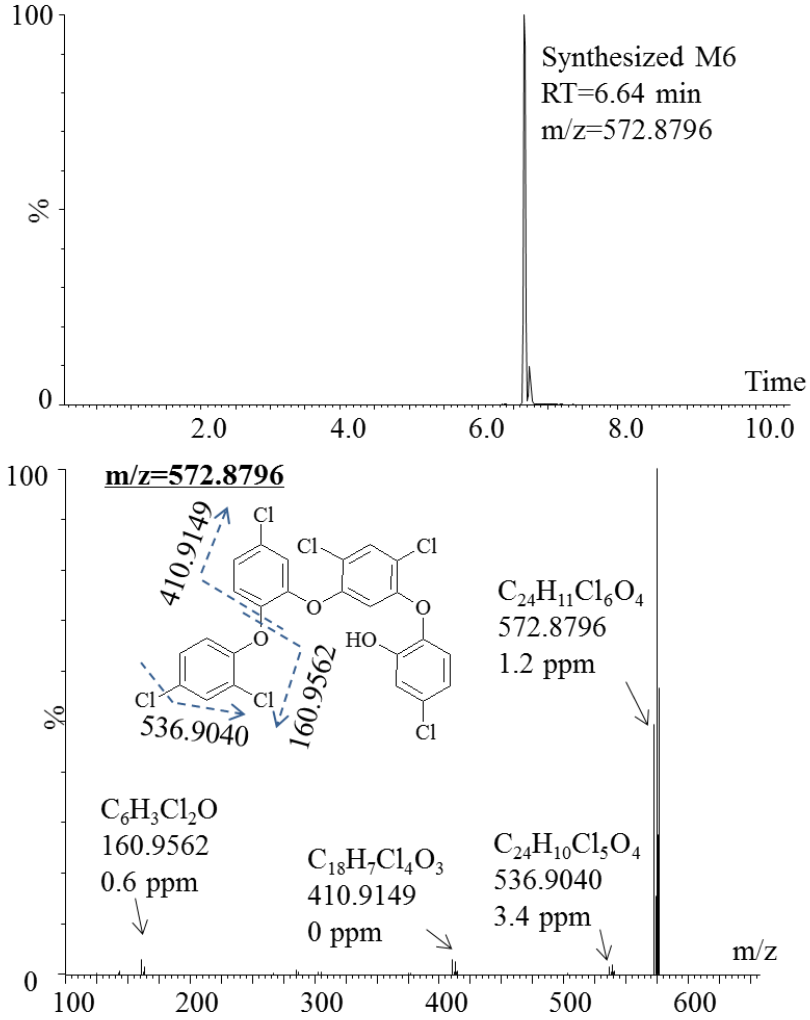

Fig. S4. Chromatogram and mass spectrum of synthesized TCS-O-TCS (M6).

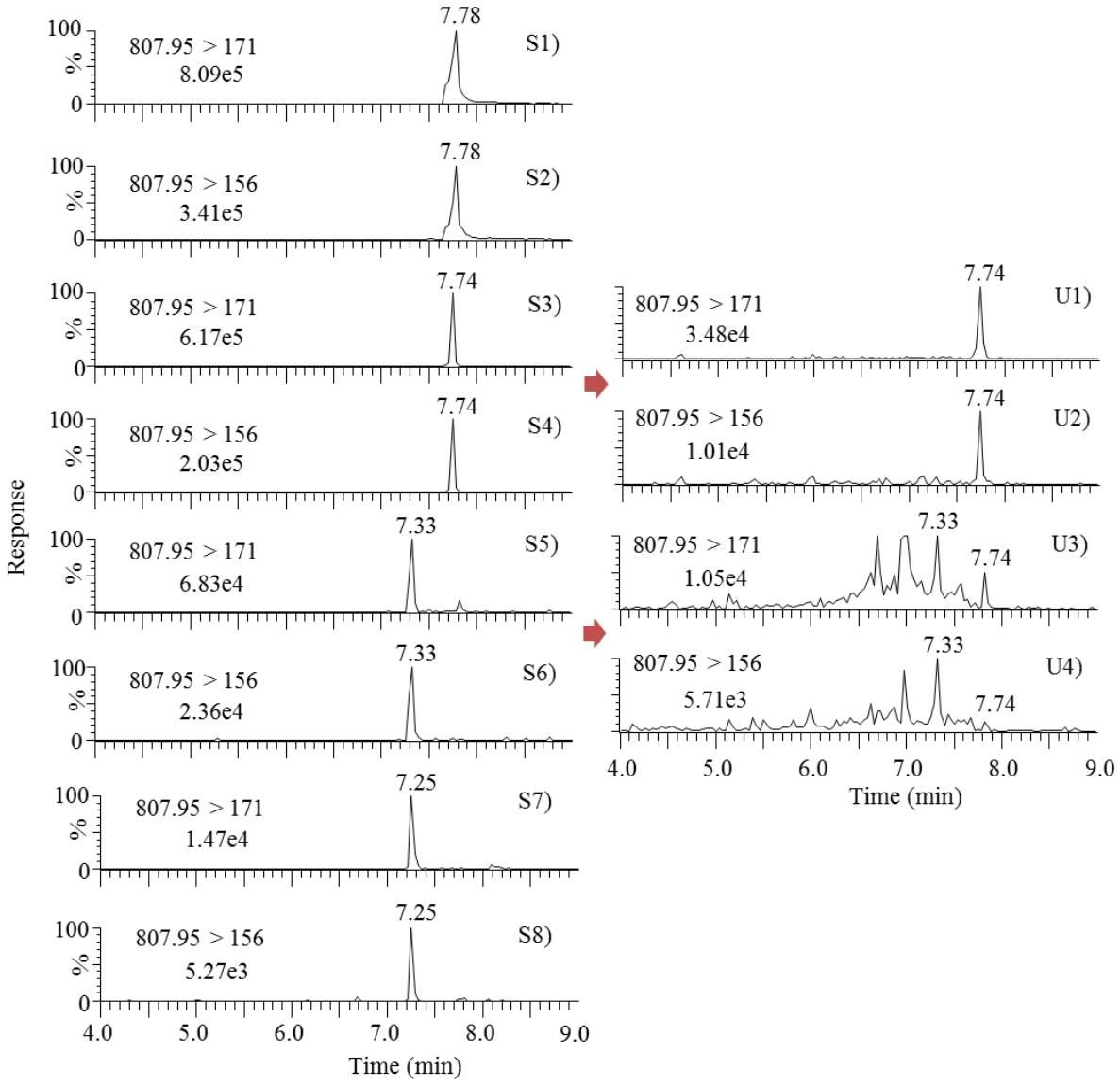

Fig. S5. Chromatogram of TCS-O-TCS derivertized with dansyl chloride (TCS-O-TCS-DNS) in separately purified TCS-O-TCS standards (S1-8) and extracts of urine samples (U1-4). Peaks showed in S1/2, S3/4, S5/6, and S7/8 were M7, M6, M3, and M2, respectively, and M4 and M5 cannot react with dansyl chloride. M6 (U1/2) and M3 (U3/4) were detected in the urine samples. Each TCS-O-TCS isomers were purified through preHPLC. The structure of the predominant metabolite (M6) was confirmed by chemically synthesized compound as shown in SI Appendix, Fig. S3.

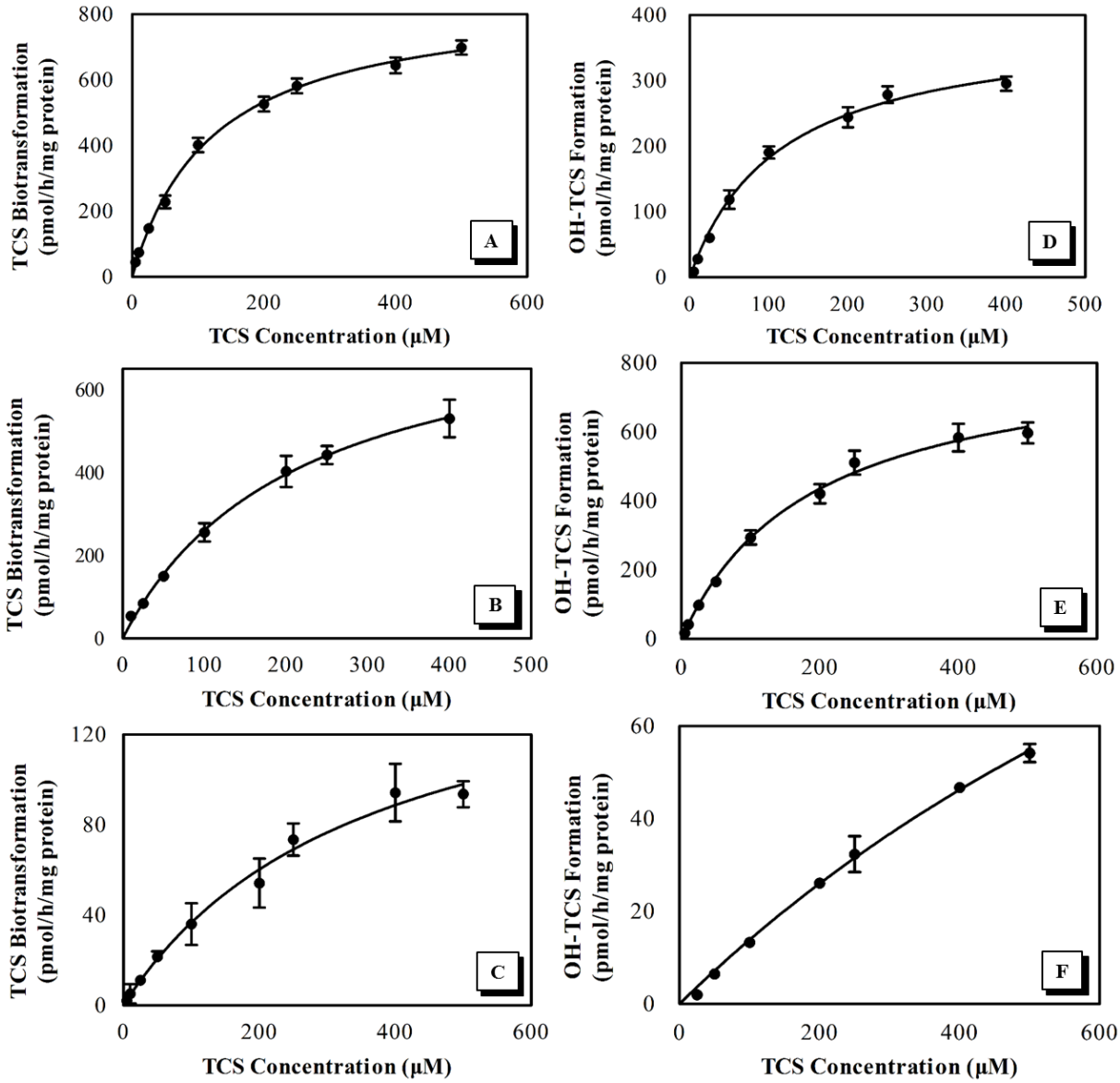

Fig. S6. Biotransformation kinetics of TCS in microsomes of human (A), quail (B) and weever (C), and formation kinetics of OH-TCS from TCS in microsomes of human (D), quail (E) and weever (F).

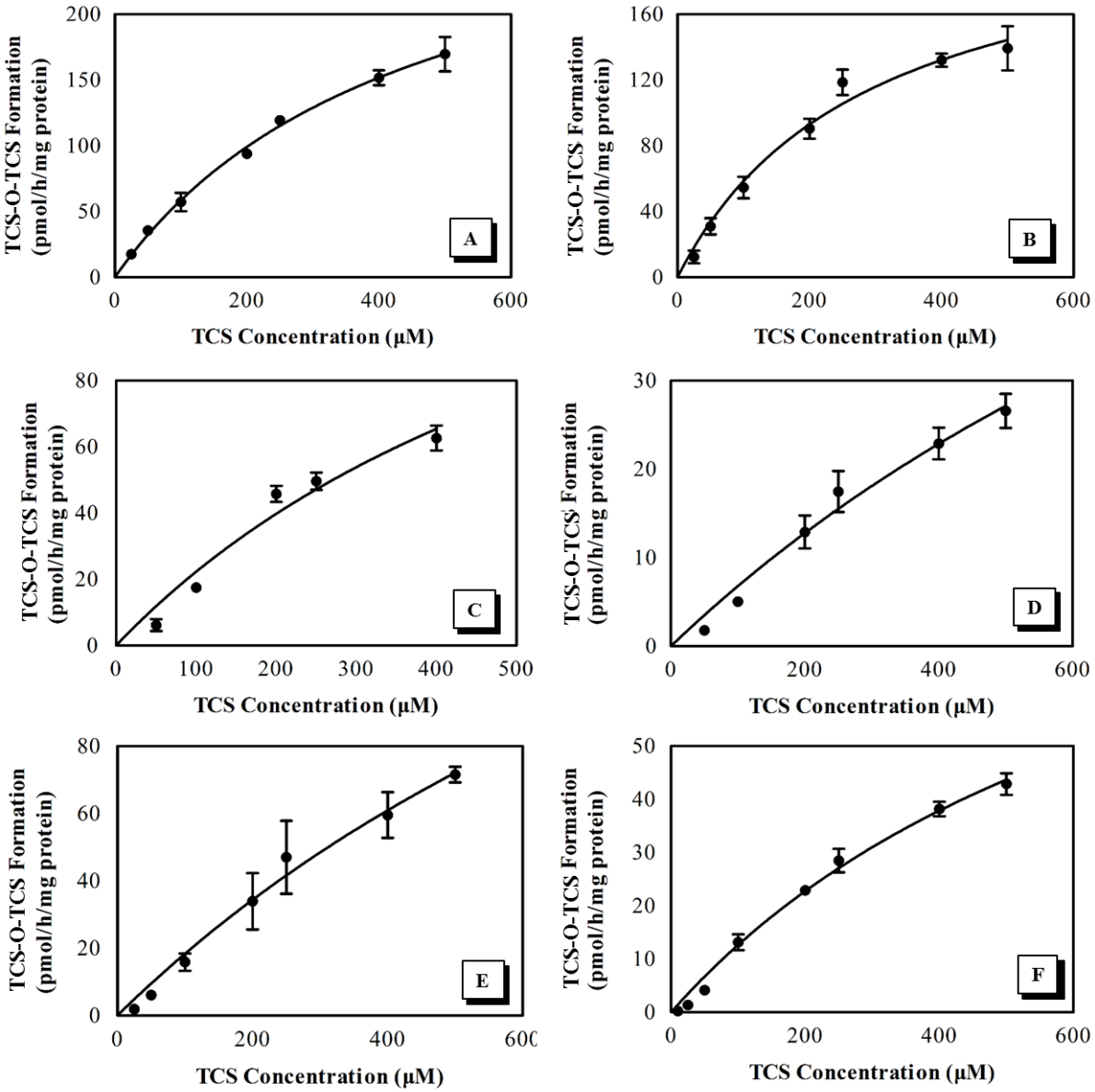

Fig. S7. Formation kinetics of two predominant TCS-O-TCS from TCS in microsomes of human (A, B), quail (C, D) and weever (E, F).

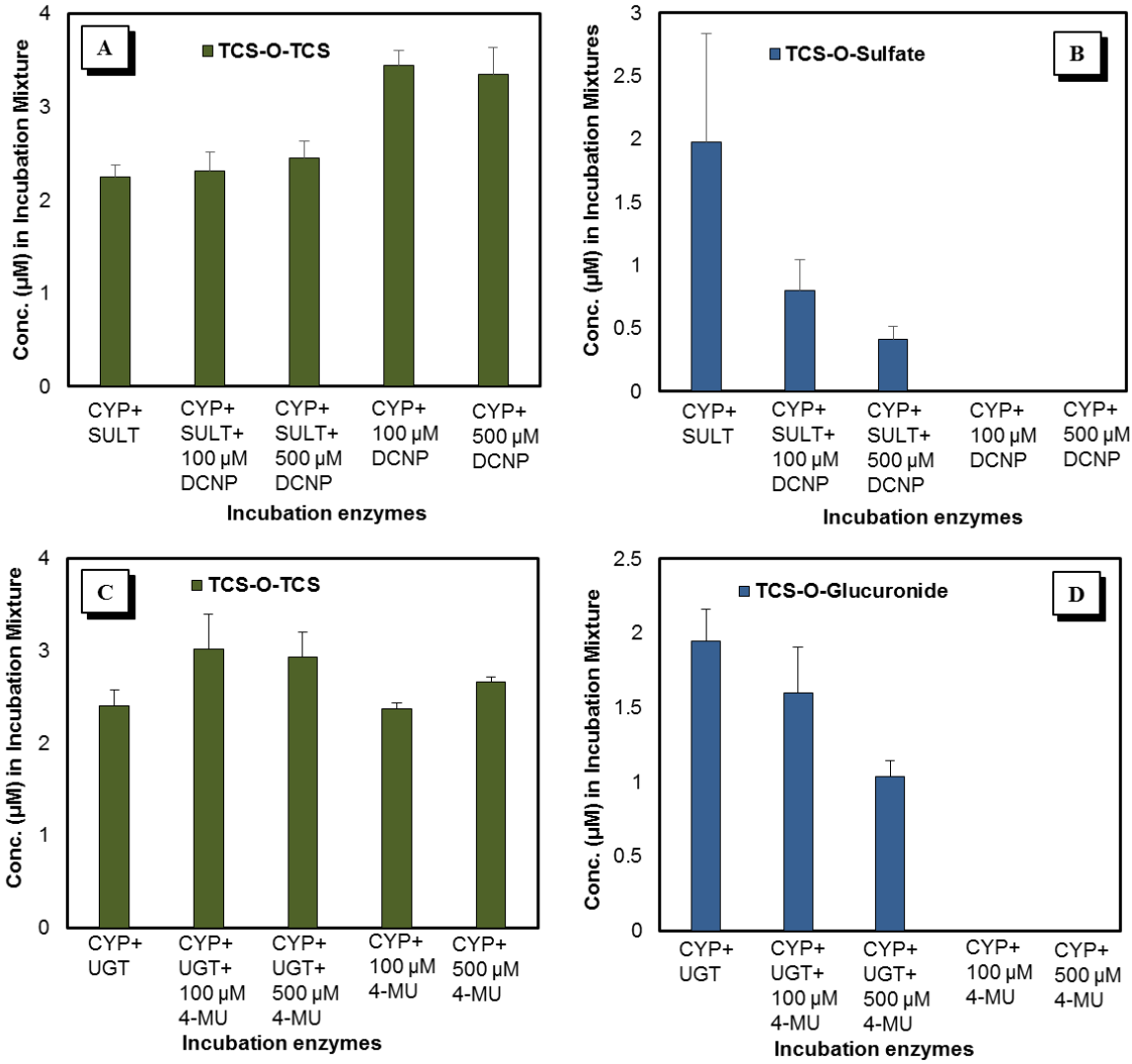

Fig. S8. Effects of SULT and UGT inhibitors (DCNP and 4-MU) on the concentrations of TCS-O-TCS, TCSO-Sulfate and TCS-O-Glucuronide after incubations in human microsomes. A) concentrations of TCS-O-TCS after incubation with phase I+II enzymes, phase I+II enzymes+DCNP, and phase I enzymes+DCNP; B) concentrations of TCSO-Sulfate after incubation with phase I+II enzymes, phase I+II enzymes+DCNP, and phase I enzymes+DCNP; C) concentrations of TCS-O-TCS after incubation with phase I+II enzymes, phase I+II enzymes+4-MU, and phase I enzymes+4-MU; D) concentrations of TCS-O-Glucuronide after incubation with phase I+II enzymes, phase I+II enzymes+4-MU, and phase I enzymes+4-MU.

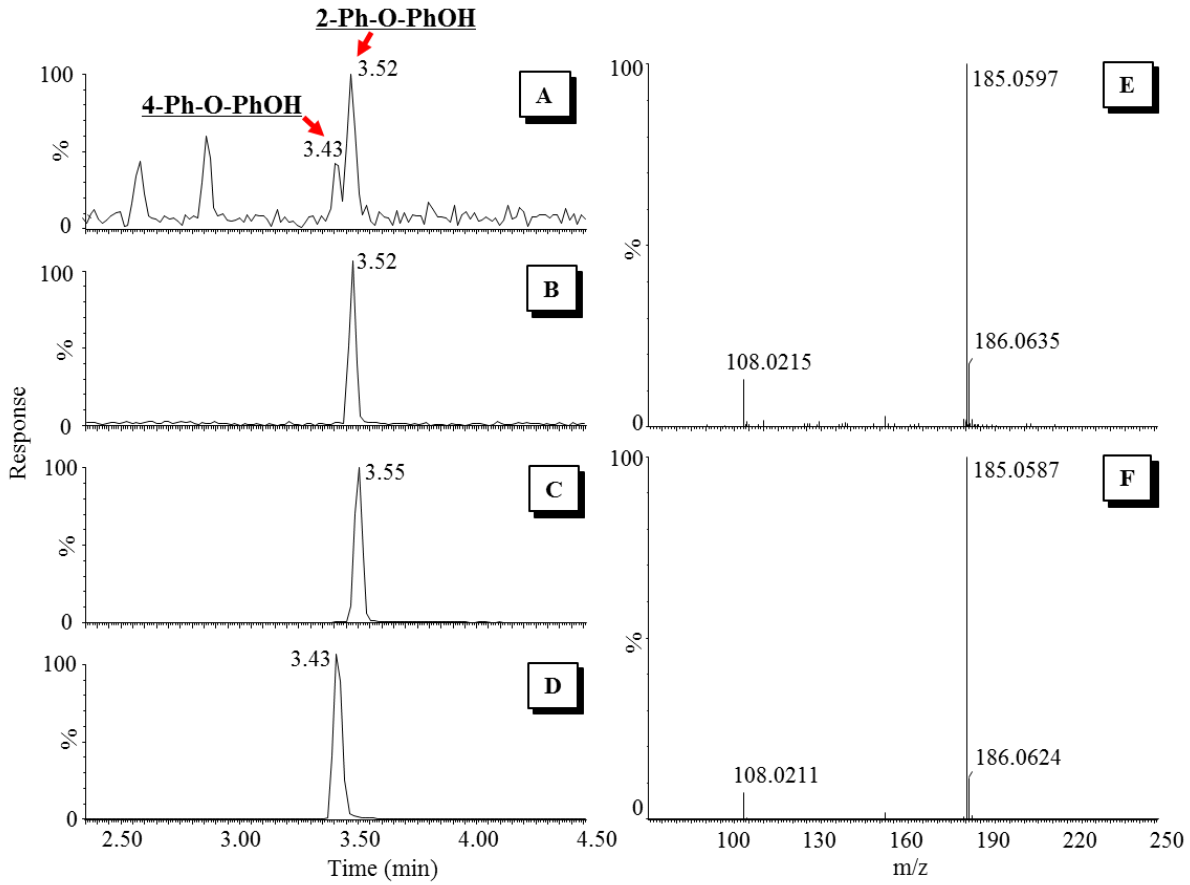

Fig. S9. Liquid chromatogram and QTOF mass spectra of the metabolic products of PhOH and standards of 2-Ph-O-PhOH, 3-Ph-O-PhOH, and 4-Ph-O-PhOH. A) chromatogram of the metabolic products of PhOH; B) chromatogram of 2-Ph-O-PhOH standard; C) chromatogram of 3-Ph-O-PhOH standard; D) chromatogram of 4-Ph-O-PhOH standard; E) mass spectrum of the peak at 3.52 min in incubation mixtures; F) mass spectrum of 2-Ph-O-PhOH.

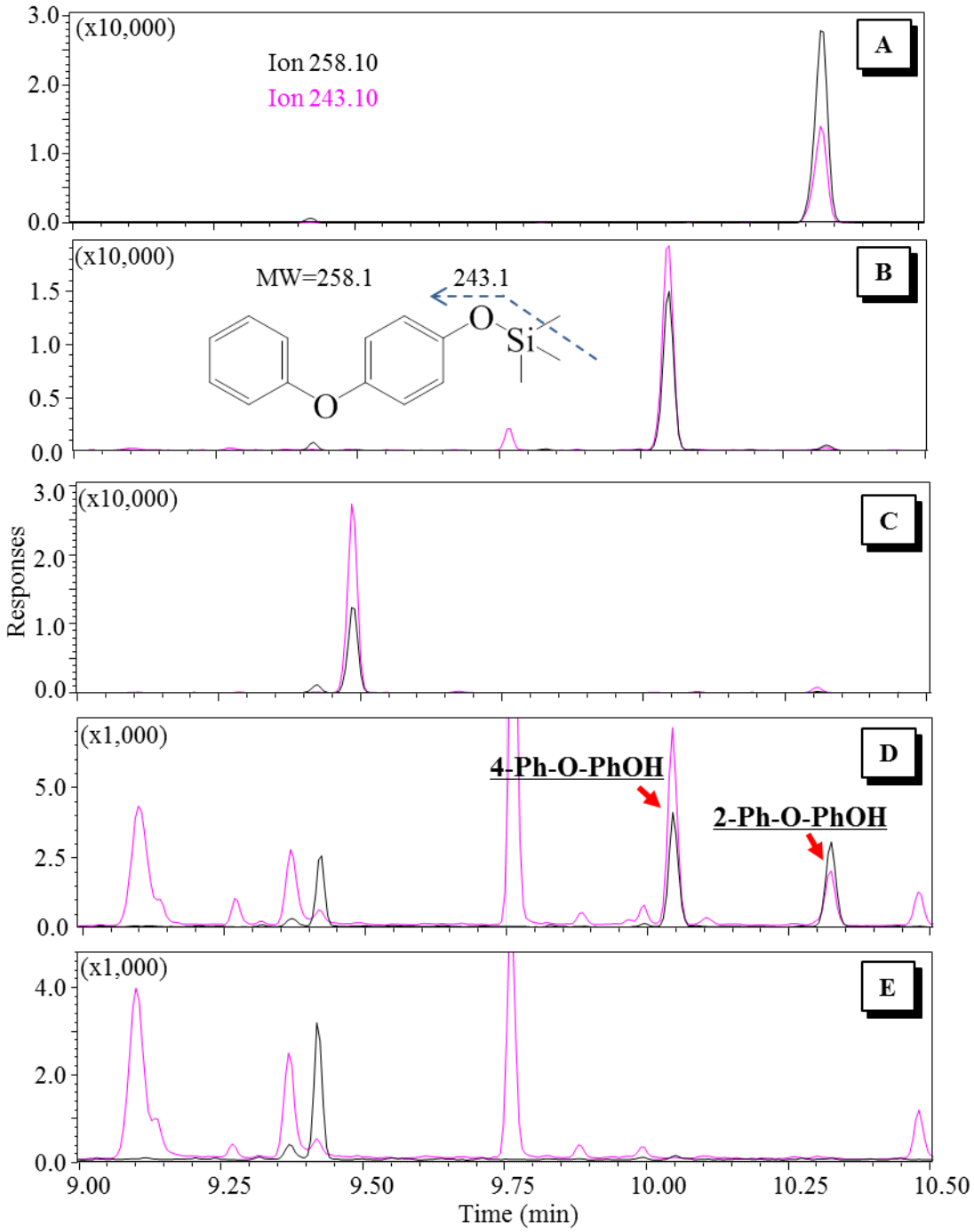

Fig. S10. GC-MS chromatogram of derivertized Ph-O-PhOH standards and the metabolic products of Ph. A) chromatogram of derivertized 2-Ph-O-PhOH standard; A) chromatogram of derivertized 4-Ph-O-PhOH standard; B) chromatogram of derivertized 3-Ph-O-PhOH standard; D) chromatogram of the metabolic products of Ph after incubation with liver microsomes; E) chromatogram of control samples after incubation with liver microsomes.

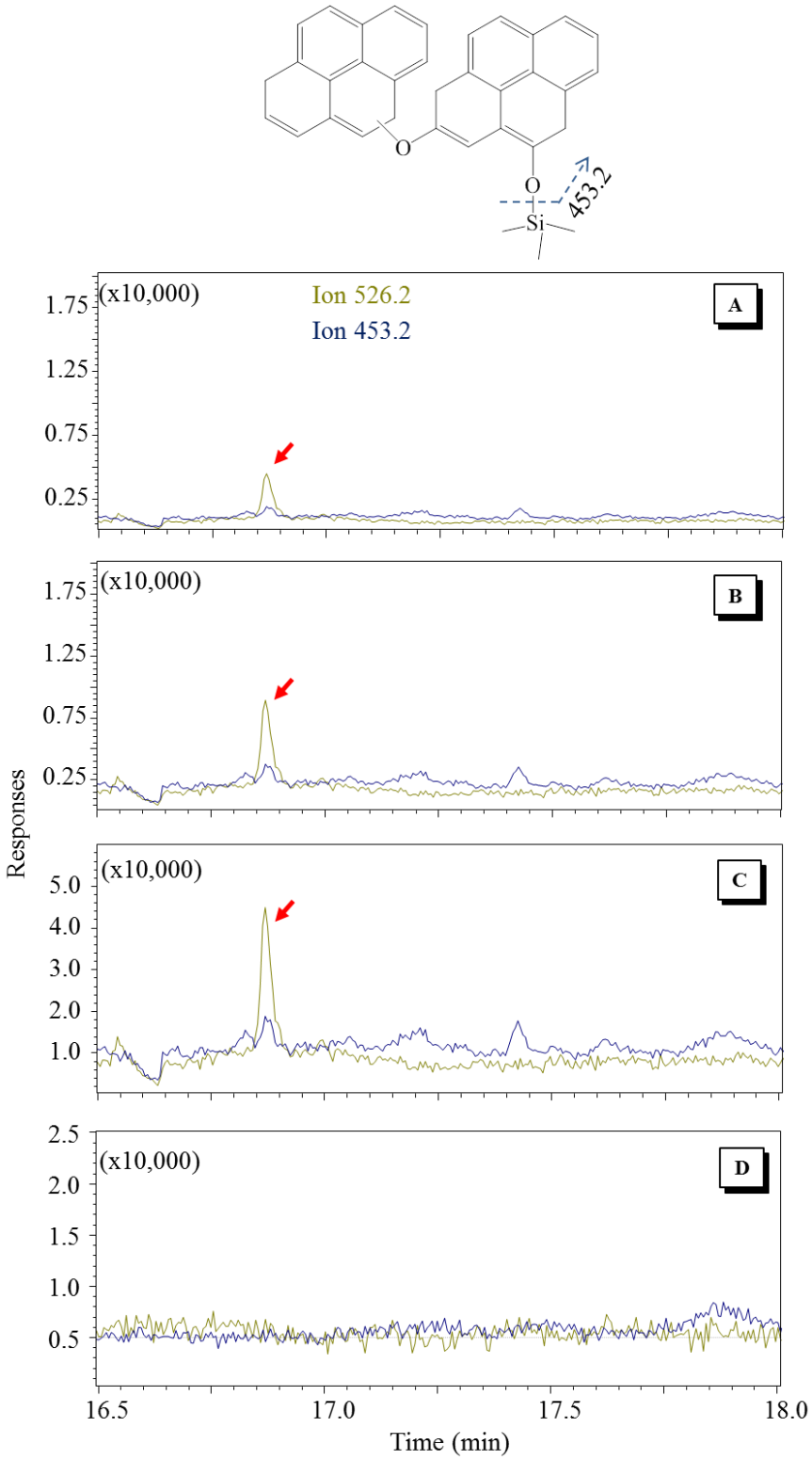

Fig. S11. GC-MS chromatograms of derivertized Py-O-PyOH when incubation concentrations of Py were 5 μM (A), 10 μM (B), 50 μM (C), and control samples (D) after incubation with liver microsomes.

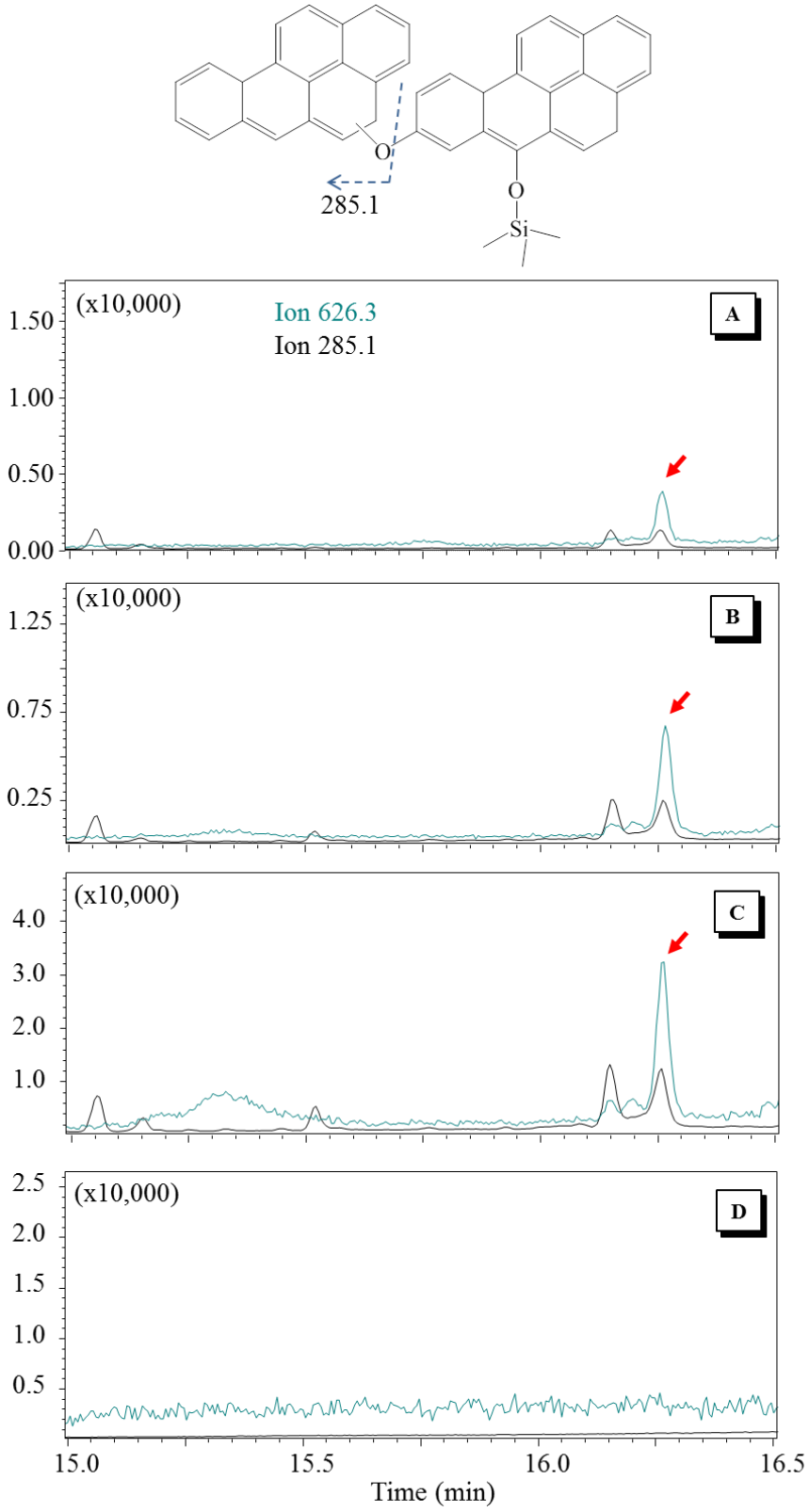

Fig. S12. GC-MS chromatograms of derivertized B[a]P-O-B[a]POH when incubation concentrations of B[a]P were 5 μM (A), 10 μM (B), 50 μM (C), and control samples (D) after incubation with liver microsomes.

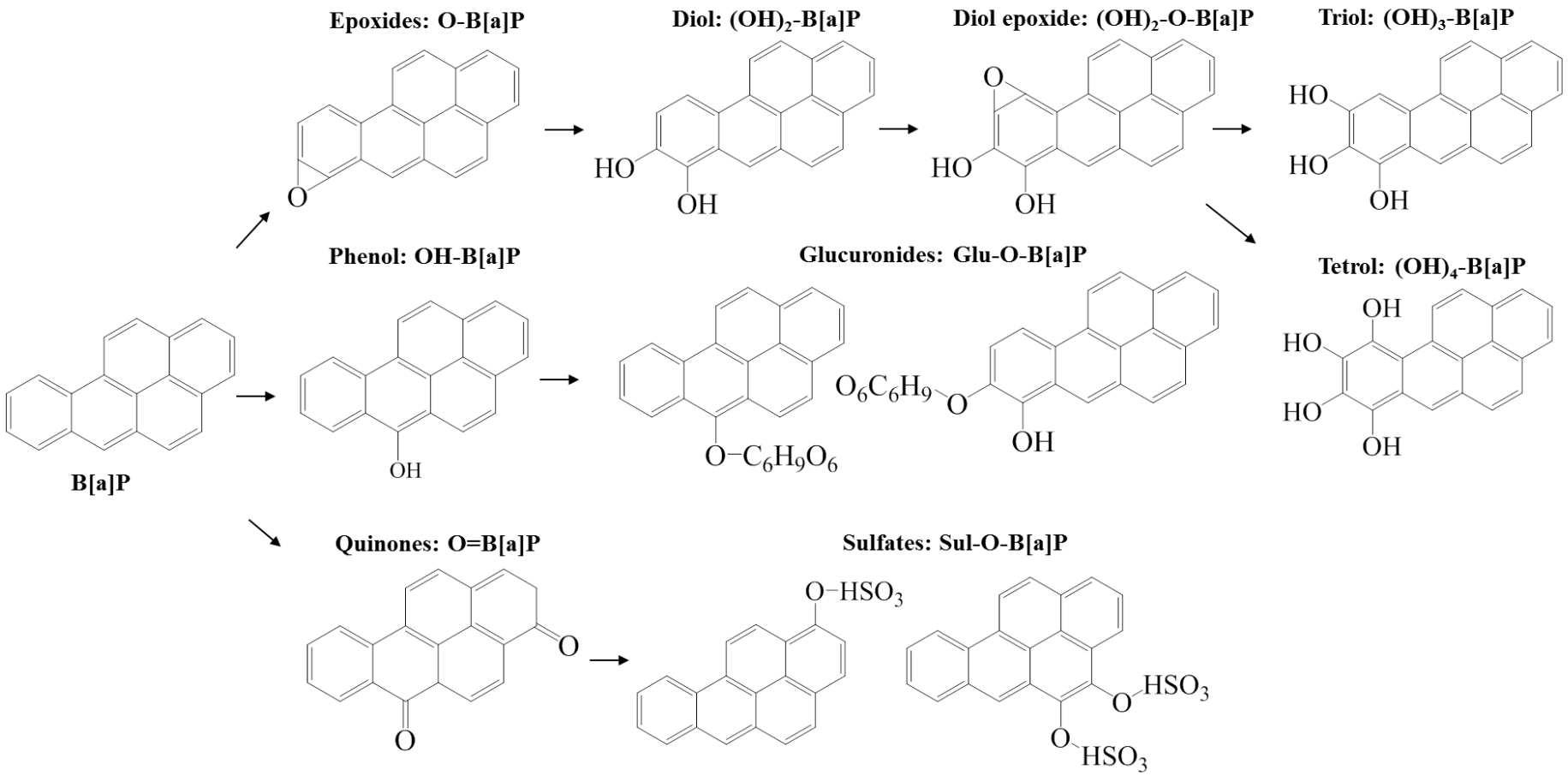

Fig. S13. Reported metabolic pathways of B[a]P.

Table S1. Characteristics of the population and concentrations of TCS and its biotransformation products in urine samples of the population.

Characteristic	Sample number
Enrolled	83
Age, year	31.2±4.9
BMI	22.1±3.3
Resident history, year	5±2.5
Sex, n (%)	
Male	48 (57.8)
Female	35 (42.2)
Smoking, n (%)	
Current smokers	16 (19.5)
Former smokers	7 (8.5)
Non-smokers	59 (72.0)
missing	1
Education, n (%)	
Primary school or below	1 (1.2)
Secondary school	13 (15.7)
High school	40 (48.2)
Bachelor degree	28 (33.7)
Master degree or above	1 (1.2)
Concentrations of TCS (ng/L)	
Geometric mean	11.9
Median	10.8
Maximum	190
Concentrations of TCS-O-TCS (ng/L)	
Geometric mean	0.19
Median	0.17
Maximum	47.9

Table S2. Kinetics of TCS and their biotransformation products in microsomes of various species.

Conditions	K_m (μM)	V_{max} (pmol/h/mg)	V_{max}/K_m (μL/h/mg)
TCS biotransformation			
Human microsomes	122.5±8.5	858.7±20.8	7
Quail microsomes	173.8±14.2	736.3±22.2	4.2
Fish microsomes	376.2±43.5	173.0±9.2	0.5
OH-TCS formation			
Human microsomes	114.2±12.7	390.1±16.5	3.4
Quail microsomes	189.3±18.7	847.3±34.6	4.5
Fish microsomes	1413±290.9	209.5±34.0	0.1
TCS-O-TCS formation			
Human microsomes	290.8~1154	103~1009	0.2~1.2
Quail microsomes	434.2~1931	35.3~837.9	0.1~0.4
Fish microsomes	795.4~1372	113.1~269.9	0.1~0.3

Table S3 Analytes and reaction monitoring conditions of derivertized TCS and TCS-O-TCS in UPLC-MS/MS analysis.

Analyte	MRM transition	Cone voltage (V)	Collision energy (eV)
TCS-DNS	523.74>171.0	50	31
	523.74>156.0	50	57
	523.74>114.6	50	70
TCS-O-TCS-DNS	807.95>171.0	50	31
	807.95>156.0	50	57
	809.94>171.0	50	31

Supporting Information references

1. Fan, Z. L., Hu, J. Y., An, W., Yang, M. (2013) Detection and occurrence of chlorinated byproducts of bisphenol A, nonylphenol, and estrogens in drinking water of China: comparison to the parent compounds. *Environ. Sci. Technol.* 47: 10841-10850.
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