Supporting Information

for

Physiologically Based Pharmacokinetic Modeling for Chlorinated Paraffins in Rat and Human: Importance of Biliary Excretion

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Chemicals and Reagents.

The standard mixtures of SCCPs contained C10-C13 CP congeners, and three SCCP standard mixtures can be purchased from Dr. Ehrenstorfer (Augsburg, Germany) with chlorine percentages about 51.0%, 55.5%, and 63.0%. The standard mixtures of MCCPs contained C14-C17 CP congeners, and three LCCP standard mixtures can also be purchased form Dr. Ehrenstorfer (Augsburg, Germany) with chlorine percentages about 42.0%, 52.0%, and 57.0%. The standard mixtures of LCCPs contained C18-C30 CP congeners, and two LCCP standard mixtures can be purchased form Dr. Ehrenstorfer (Augsburg, Germany) with chlorine percentages about 36.0% and 49%. $^{13}C_{10}$-anti-Dechlorane Plus (^{13}C10-anti-DP) was obtained from Cambridge Isotope Laboratories (Andover, MA). Pesticide residue-grad methanol, dichloromethane (DCM), *n*-hexane, and acetonitrile were purchased from Fisher Chemicals (Bridgewater, NJ). The NADPH regenerating system was purchased from Promega (Madison, WI, USA). Rat and human liver microsomes were obtained from iPhaseBiosciences (Beijing, China) and stored at -80°C prior to *in vitro* studies. Distilled water was prepared by a Milli-Q Synthesis water purification system (Millipore, Bedford, MA). Granular anhydrous sodium sulfate and aluminum oxide (200–300 mesh) were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China).

Sample Collection.

Samples of major exposure matrices including air, food and soil were collected in a residential area in Shenzhen, in the South of China. About 1200–1500 m^3 of air was collected in each sampling location at a speed of 500 L/min using a high-volume air sampler (SIBATA Scientific Technologies, Japan). The air samples were passed through a glass fiber filter

(Whatman GF/F, 70 mm diameter; pre-baked at 450°C for 4 h) and two polyurethane foam filters (PUF: 75 × 85 mm; pre-cleaned by 24 h Soxhlet extractions in acetone and dichloromethane before sampling). Blank field samples were also collected by loading the filter and PUF plugs in the air sampler for 10 s. A total of 72 samples and 12 blank samples were collected.

During the one-year air-sampling period, 46 soil samples and 88 food samples were also collected in this area. Surface soil samples (0–5 cm depth) were collected, wrapped in aluminum foil and stored in sealed polyethylene bags. The collected food samples were selected according to the local population's dietary composition. The food samples included 7 types of fruit, 27 types of vegetables, 5 types of cereals, 14 types of fish and shrimp, 4 types of meat and 4 kinds of eggs. Detailed information on the food samples is shown in Table S1.

Animal Experiments.

Sprague-Dawley rats (6 weeks old) were obtained from the Beijing Vital River Laboratory Animal Technology Company (Beijing, China). Standards of SCCPs, MCCPs and LCCPs were dissolved in corn oil and administered by gavage at a dose of 13.9, 9.3, and 3.2 mg/kg, respectively. The rats were housed at the Beijing Vital River Laboratory Animal Technology Company at a temperature of 22 ± 2°C, a relative humidity of 40–60%, and a 12-h light/dark cycle. After CP administrations, three rats were killed at each sampling time (0.5 h, 2 h, 8 h, 24h, 3 d, 7 d, 14 d and 28 d), and samples of blood, liver, fat, blood, kidney, lung, heart, muscle, and stomach/intestine tissues were collected. Feces and urine were collected for exposure periods of 0–0.5 h, 0.5–2 h, 4–8 h, 20–24 h, 68–72 h, 164–168 h, 332–336 h, 668–672 h. All the samples were freeze-dried and kept at -20°C before analysis.

106 ***In vitro* Microsomal Incubations.**

107 Standards of SCCPs, MCCPs and LCCPs were incubated with rat (Sprague-Dawley) and
108 human microsomes to assess their metabolic rates in organisms. All incubations were
109 performed in triplicate at 37°C. The final reaction volume was 200 μL, containing 39 μL 50
110 mM phosphate buffer (pH 7.4) containing 1 mM EDTA, 1 mM DTT, and 20% (v/v) glycerol,
111 60 μL NADPH regenerating system (NADP 6.5 mM, glucose 6-phosphate 16.5 mM, $MgCl_2$
112 16.5 mM, and glucose 6-phosphate dehydrogenase 2 U/mL), 100 μL of microsomes and 1 uL
113 of CP standards, with final incubated concentrations of 0.1, 0.5, 1, 5, 10, 25, 50 μmol/L.
114 Incubations without chemicals and without microsomes were used as negative controls to
115 assess background contaminants. After the incubation, the samples were diluted with cold
116 acetone and analyzed immediately to determine metabolic rates.

117 The metabolic rates of CPs were determined by a Michaelis-Menten-type model, which is
118 commonly used to describe chemical metabolism in liver microsomes. The metabolism of
119 CPs per hour (A_M) in rat and human microsomes can be estimated by equation (1):

120 $$\frac{dA_M}{dt} = \frac{V_{\max} C_{li}}{K_s + C_{li}} \qquad (1)$$

121 where C_{li} is the concentration of CPs in liver (ng/g ww), V_{max} is the apparent maximum
122 reaction rate (ng/g/h) and K_s is the apparent half-saturation constant (ng/g). The resulting
123 calculated values for V_{max} and K_s can then be applied to determine the metabolic rates of CPs
124 in the PBPK models.

125 **Sample Preparation.**

126 Approximately 5 g food, 10 g soil, 200 μL microsome incubation mixtures and 0.1 g
127 tissue samples of exposed rat were transferred to a Teflon vessel added with surrogate

(^{13}C10-anti-DP) and 20 mL hexane/DCM (1:1, v/v) for microwave digestion extraction. The microwave digestion unit (CEM Mars-6, USA) was set to 600 W and programmed with the following conditions: ramp to 100°C over 5 min, maintain this temperature for 20 min, then cool to room temperature over 30 min. The extraction was repeated for three times and the extract solutions were combined. For air samples, the filter and PUF were spiked with ^{13}C10-anti-DP and then Soxhlet-extracted with 250 mL toluene for 24 h. For blood, urine and microsomal incubation mixtures, the samples were mixed with 25 mL of a 1:3 mixture of ethanol and *n*-hexane after spiked with surrogate (^{13}C10-anti-DP). The solutions were shaken for 30 min and the organic layer was then collected. This extraction procedure was repeated with 20 mL *n*-hexane, and the organic layers were combined and washed with 20 mL of water.

The sample extracts from each matrix were concentrated to a volume of approximately 1 mL, and passed through a glass column containing 8 g of 5% H_2O-deactivated active Al_2O_3, which had been pre-baked by heating at 600°C for 4 hours. The column was pre-cleaned with 30 mL DCM and 30 mL hexane. After loading the sample extracts, the column was eluted with 30 mL hexane and a 30 mL mixture of hexane and DCM (3:1). The eluent was concentrated to about 1 mL using a rotary evaporator, and then evaporated until dry under a stream of nitrogen. The samples were finally redissolved in 100 μL acetonitrile for UPLC-QTOFMS analysis. The details of instrument analysis and quality assurance and quality control were provided in the Supplemental Material.

UPLC-QTOFMS Analysis.

CPs were analyzed by an ACQUITY UPLC system (Waters, Milford, MA) coupled with

a Xevo QTOF-MS (G2, Waters). Instrument control was performed using MassLynx Software (version V4.1, Waters). All standards and samples were separated on a Waters ACQUITY UPLC BEH C18 column (1.7 μm, 2.1 × 50 mm). The flow rate was set as 0.1 mL min^{-1}, the column temperature was 40°C, and 3 μL of samples was injected. Ultrapure water (A) and methanol (B) were used as the mobile phases for gradient elution. The initial conditions were 10% B for 1 minute, ramped to 30% by 1.5 minute, ramped to 60% by 2 minute, ramped to 80% by 3 minute, ramped to 90% by 3.5 minute, ramped to 100% by 4 minute, held from 4.5 to 8.5 minute, ramped to 30% by 9 minute, and held for 1 minute before returning to the initial conditions, which were equilibrated for 1 minute before the next injection. DCM was added to the sample, separated by the column between the UPLC and the ion source, with a syringe pump at a flow of 10 μL min^{-1} using a T-connection in the period of 5.5 to 8.5 minute.[1]

The atmospheric pressure ionization-electrospray ionization (API-ESI) source was operated in negative ion mode. The optimized analytical parameters were as follows. Source capillary voltage: 2.5 kV; sampling cone voltage: 40 V; extraction cone voltage: 4.0 V; source temperature: 100°C; desolvation temperature: 250°C; cone gas flow rate: 50 L/h; desolvation gas flow rate: 600 L/h. Full-scan mode in the mass range of 250 to 1600 Da with a 1-second scan time was performed. Leucine-enkephalin was used as a reference lock mass (200 pg/μL infused at 5 μL/min, m/z 554.2615). The detector of QTOFMS was calibrated with a sodium formate solution. The achieved mass accuracy is lower than 3 ppm.

Toxicokinetics of CPs in the Developed of PBPK Models.

Absorption and excretion in GI and kidney. The uptake of CPs from the GI tract was assumed

to follow first-order kinetics:

$$dA_{GI} / dt = -K_a \times A_{GI} - K_f \times A_{GI} \quad (2)$$

where A_{GI} represents the amounts of CPs in the GI tract (mg), and t and K_a are the time and absorption rate, respectively. The initial condition is $A(0)$ = administration dose. $K_f \times A_{GI}$ is used to describe the fraction of CPs that enters the feces (non-absorption), and thus K_f here was termed as GI feces elimination.

For the kidney, the urinary excretion was also considered to follow these kinetics:

$$dA_{KI} / dt = Q_{KI} \times (C_B - A_{KI}/P_{KI}/W_{KI}) - K_u \times A_{KI} \quad (3)$$

where A_{KI} represents the amounts of CPs in the kidney (mg), Q_{KI}, P_{KI} and W_{KI} are blood flow, partition coefficient and weight of kidney, respectively. C_B is defined as the blood concentration of CPs, and K_u is the urinary elimination rate. In this study, the partition coefficients for each tissue were determined to be the average CP concentration ratios between tissue and blood in rat after 24 h exposure.

Metabolism and excretion in liver. The metabolic rates of CPs in rat livers were obtained by the microsomal incubation and analysis method described above. The mass balance model for CPs in liver was expressed as:

$$dA_{LI} / dt = K_a \times A_{GI} + Q_{LI} \times (C_B - A_{LI}/P_{LI}/W_{LI}) - K_m \times A_{LI} - K_b \times A_{LI} \quad (4)$$

where the A_{LI} is the amount of CP in the liver, and the Q_{LI}, W_{LI}, P_{LI} are the liver blood flow, liver weight and partition coefficient between liver and blood of CPs, and K_m and K_b are metabolism rate and biliary elimination rate, respectively.

Distribution. Distribution of CPs in various tissues is determined by the blood flow to the target compartments and partition coefficients between various tissues and blood. The

distribution in tissue *i* can be calculated by the following equation:

$$dA_i / dt = Q_i \times (C_b - A_i/P_i/W_i) \quad (5)$$

where A_i is the amount of CPs in tissue *i* (include fat and the rest of body), Q_i is the blood flow to tissue *i*, W_i is the weight of tissue *i* and P_i is the partition coefficient between tissue *i* and blood.

The lung is the respiratory system enabling the exchange of oxygen and carbon dioxide. Because the amount of CPs in lung tissue was negligible, the blood levels of CPs entering and leaving the lung were treated as equivalent. Therefore, the blood concentration was estimated using the following equations:

$$C_B = \frac{Q_{KI} \times A_{KI}/P_{KI}/W_{KI} + Q_{LI} \times A_{LI}/P_{LI}/W_{LI} + Q_F \times A_F/P_F/W_F + Q_{RB} \times A_{RB}/P_{RB}/W_{RB}}{Q_{LI} + Q_{KI} + Q_{RB} + Q_F} \quad (6)$$

Quality Assurance and Quality Control.

SCCPs, MCCPs, and LCCPs were quantified using the $[M+Cl]^-$ ions of each CP congener group, and the details of the quantifications were reported in our previous study.[1] Strict quality assurance and quality control (QA/QC) was applied to ensure the quantification of chemical concentration. All equipment was thoroughly rinsed with DCM and hexane before the experiment, and the samples were prepared in a clean lab to reduce background contamination. The procedure described above was validated for the recovery experiment by analyzing the spiked samples. The recoveries of the six spiked samples (SCCPs 0.2 μg; MCCPs 0.2 μg; LCCPs 0.2 μg) in air samples were in the range of 88.9-94.8%, 100.3-112.4%, and 105.6-110.8%, respectively. The recoveries of SCCPs, MCCPs and LCCPs in the six spiked soil samples were in the range of 93.2-115.0%, 99.8-109.0%, and 105.6-120.8%, respectively, and those in the six spiked food samples (meat was chosen due to

the complicate sample matrix) were in the range of 92.7-112.7%, 102.1-113.5%, and 98.6-120.8%, respectively. To automatically correct for losses of analytes during extraction or sample preparation and to compensate for variations in the instrumental response from injection to injection, analyte quantification was achieved using a surrogate standard method with calibration against standard solutions. $^{13}C_{10}$-anti-dechlorane plus ($^{13}C_{10}$-anti-DP) was used as the surrogate standard, and the recovery of $^{13}C_{10}$-anti-DP in the prepared samples was in the range of 85-120%. A procedural blank was analyzed in each batch of seven samples to check for interfering peaks and to correct the sample values. For chemicals with detectable blank contamination, the method detection limits (MDLs) were set at three times the standard deviation of the procedural blanks, and the final concentrations of these compounds were blank-corrected. The MDLs for the other congeners (those not detected in the blank samples) were set to the instrumental minimum detectable amounts. The MDLs of ∑SCCPs, ∑MCCPs, and ∑LCCPs were estimated to be 37, 40, and 27 pg/m^3 wet weight in air, 1.9, 2.0, and 0.4 ng/g dry weight in soil, and 4.0, 4.0, and 0.9 ng/g dry weight in food, respectively,

Parameter Estimation for the Rat PBPK Model.

A PBPK model includes physical parameters, partition coefficients, metabolic rates and excretion and absorption parameters. In this study, the physical parameters of a rat were obtained from a previous study,[2] and partition coefficients and metabolic rates were determined in the exposure experiment described above. The urinary rates (k_u) were optimized based on the urinary excretion of CPs compared with the levels of CPs that remained in the kidney of rats. The rates of absorption, fecal excretion, biliary excretion and urinary elimination were considered as unknown parameters. A log-transform algorithm-based error

function was used as the likelihood function to determine the minimum error and unknown parameters:[3]

$$E=\sum_{i=1}^{6}\sum_{j=1}^{8}(\mathrm{Ln}(A_{ij\text{-obs}})-Ln(A_{ij\text{-}sim}))^2+\sum_{j=1}^{8}(\mathrm{Ln}(C_{bj-\mathrm{obs}})-Ln(C_{bj\text{-}sim}))^2 \quad (7)$$

where the *i* represents the six types of biomarker used in this study: liver, fat, kidney, the rest of body, urine and feces, *j* represents eight time-points, *A* is the amount of CPs in tissues, and C_b is the concentrations of CPs in blood. *obs-* and *sim-* denote the observed (experimental) and simulated values, respectively.

Average Daily Dose Assessment.

Three main heavy metal exposure pathways in local residents were considered: ingestion, inhalation and dermal contact. The risk estimates were determined based on the US Environmental Protection Agency (EPA) health risk handbook.[4] The risk of exposure was expressed in terms of the average daily dose (ADD) (ng kg^{-1} day^{-1}), which was calculated using Eqs. (1), (2) and (3).

The dose through the ingestion of food and soil was calculated using Eq (8)

$$\mathrm{ADDingest}=\frac{\mathrm{C}\times\mathrm{lngR}\times\mathrm{EF}\times\mathrm{ED}}{\mathrm{BW}\times\mathrm{AT}} \quad (8)$$

The dose through the inhalation of air and soil was calculated using Eq (9)

$$\mathrm{ADDinhale}=\frac{\mathrm{C}\times\mathrm{lnhR}\times\mathrm{EF}\times\mathrm{ED}}{\mathrm{BW}\times\mathrm{AT}\times\mathrm{PEF}} \quad (9)$$

The dose absorbed through dermal contact with soil was calculated using Eq (10)

$$\mathrm{ADDdermal}=\frac{\mathrm{C}\times\mathrm{SA}\times\mathrm{SL}\times\mathrm{ABS}\times\mathrm{EF}\times\mathrm{ED}}{\mathrm{BW}\times\mathrm{AT}} \quad (10)$$

where C is the concentration of metals in the matrix (ng/g or ng/m^3), IngR is the ingestion rate in mg/day, InhR is the inhalation rate in m^3/day, SA is the surface area of the skin exposed to pollutants in cm^2, SL is the skin adherence factor in mg/cm^2, EF is the exposure frequency in

260 days/year, ED is the exposure duration in years, AT is the averaging time, BW is the
261 residents' body weights obtained through the questionnaire-based the survey, ABS is the
262 dermal absorption factor, and PEF is the particle emission factor representing an estimate of
263 the relationship between soil contaminant concentrations and the concentration of these
264 contaminants in air as a consequence of particle suspension. To calculate the ADD of each
265 pathway, the exposure parameters were obtained from the literatures and listed in Table S3.[4-5]
266

Table S1. Sampling details of collected food samples.

Food category	Items
Meat	duck, chicken, pork, beef
Fish & shrimp	bighead carp, crucian, mullet, bass, grass carp, tilapia, tuna, spadefish, shellfish, shrimp
Eggs	quail eggs, duck eggs, chicken eggs
Cereals	rice, rice flour, wheat flour
Fruits	litchi, peach, strawberry, orange, banana, pear, apple
Vegetables	tomato, lettuce, romaine lettuce, eggplant, potato, spinach, green bean, baby cabbage, red amaranth, scallion, green pepper, chilli, kidney bean, leaf mustard, balsam pears, sweet potato leaves, cucumber, leaf of lettuce, Chinese cabbage, garlic bolt, water spinach, green soya bean, greengrocery

Table S2. Percentages of ∑SCCPs, ∑MCCPs and ∑LCCPs accumulated in liver, blood, fat, kidney, lung, muscle, heart and stomach/intestine of exposed rat.

Time	24 h	72 h	168 h	336 h	672 h
Liver	57.0±9.6%	72.7±10.2%	76.5±10.1%	73.5±7.8%	65.6±9.1%
Blood	0.5±0.2%	0.6±0.2%	0.3±0.1%	0.7±0.3%	1.4±0.4%
Fat	42.4±31.4%	26.7±5.0%	23.1±5.9%	25.7±9.2%	32.8±9.2%
Kidney	0.01±0.002%	0.01±0.02%	0.01±0.001%	0.02±0.005%	0.04±0.009%
Lung	0.003±0.001 %	0.003±0.001%	0.002±0.001 %	0.014±0.002 %	0.022±0.007%
Muscle	0.01±0.002%	0.01±0.002%	0.02±0.003%	0.05±0.008%	0.09±0.014%
Heart	<0.001%	<0.001%	<0.001%	0.001±0.0003%	0.003±0.0005%
Stomach/intestine	<0.001%	<0.001%	<0.001%	0.002±0.0004%	0.004±0.0006%

Table S3. Estimated mass of three classed of CPs in each tissue (mg).

Time	Excretions via feces [a]	Excretions via urine [a]	Lung	Liver	Fat	Kidney	Other tissues
				∑SCCPs			
336	2.50E+00	2.93E-03	1.21E-05	1.35E-01	5.77E-02	4.39E-05	1.92E-03
672	3.04E+00	3.57E-03	1.16E-05	5.80E-02	3.84E-02	4E-05	1.83E-03
				∑MCCPs			
336	1.77E+00	1.79E-03	1.67E-05	8.98E-02	3.86E-02	2.92E-05	1.28E-03
672	2.08E+00	2.10E-03	7.81E-06	2.64E-02	1.43E-02	1.48E-05	6.77E-04
				∑LCCPs			
336	6.49E-01	6.96E-04	1.06E-05	8.98E-02	3.86E-02	2.92E-05	1.28E-03
672	7.28E-01	7.82E-04	6.04E-06	2.64E-02	1.43E-02	1.48E-05	6.77E-04

[a] Accumulated excretion amounts.

[b] The dose amounts of ∑SCCPs, ∑MCCPs, and ∑LCCPs were 3.2, 2.1, and 0.74 mg, respectively.

Table S4. Exposure factors for the exposure assessments.

Parameters for exposure assessment		Value
Body weight/kg		59
Averaging time /days		ED×365
Exposure duration/year		25
Exposure frequency/(days/year)		250
Particulate emission factor /(m^3/kg)		1.316×10^9
Dermal absorption factor		0.001
Surface area/cm^2		3300
Skin adherence factor /(mg/cm^2)		0.2
Ingestion Rate/(g/day)	cereals	239.6
	fish & shrimp	59.9
	eggs	31.3
	poultry	46.6
	meat	10.1
	pork	94
	fruits	97.9
	vegetables	336.7
	water	1500
	soil	100
Inhalation Rate/(m^3/day)	air	20
	soil	20

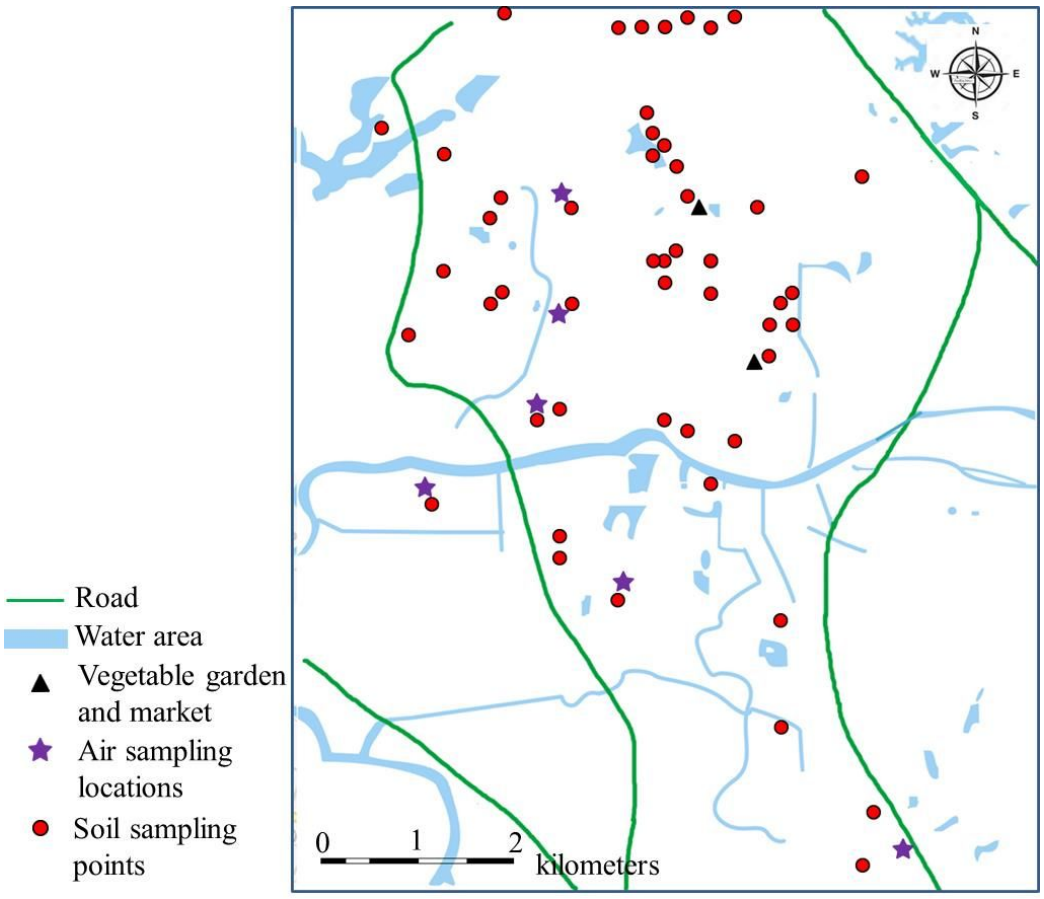
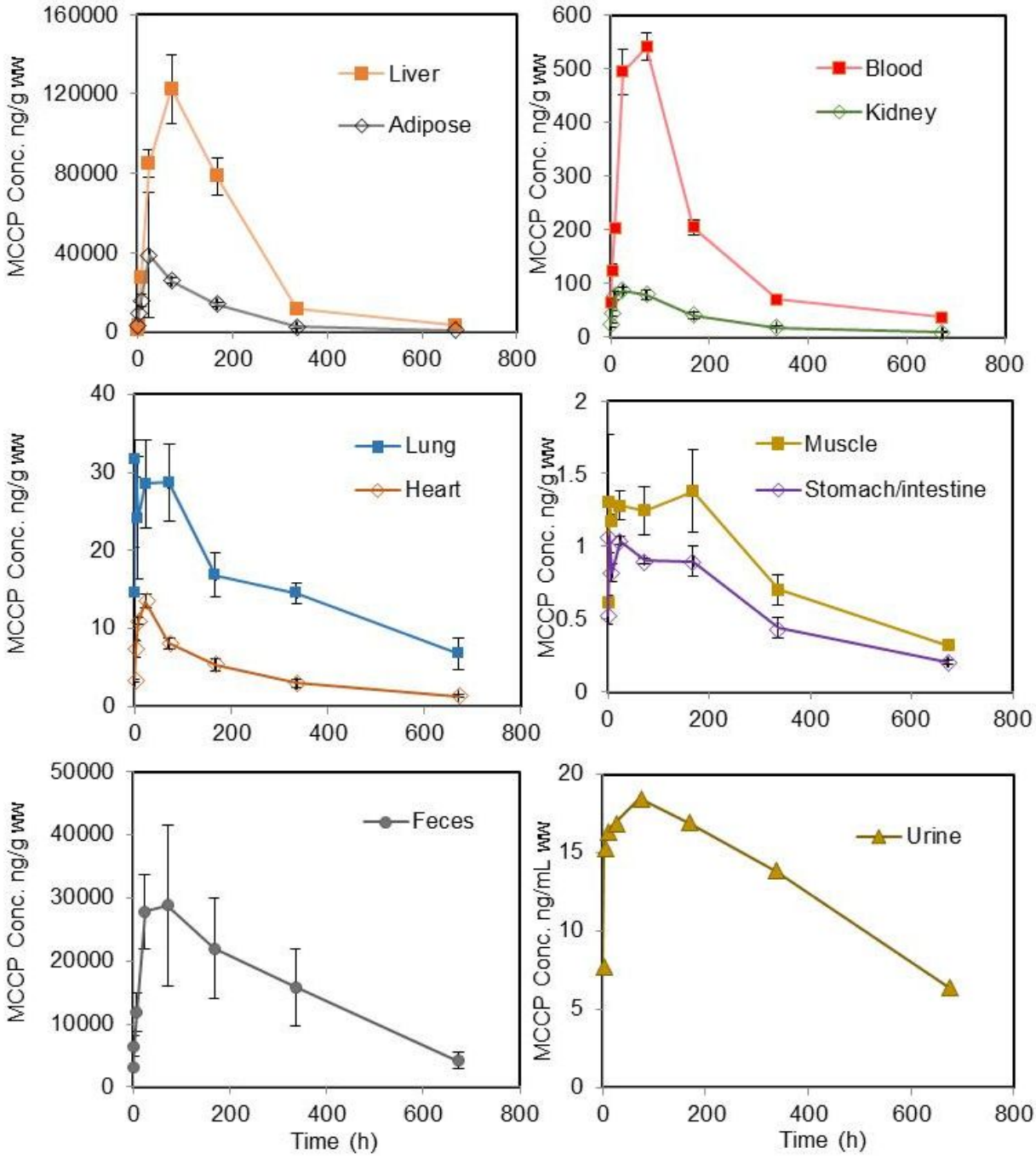


Figure S1. Sampling locations of soil, air and food samples in an area in Shenzhen, South China.

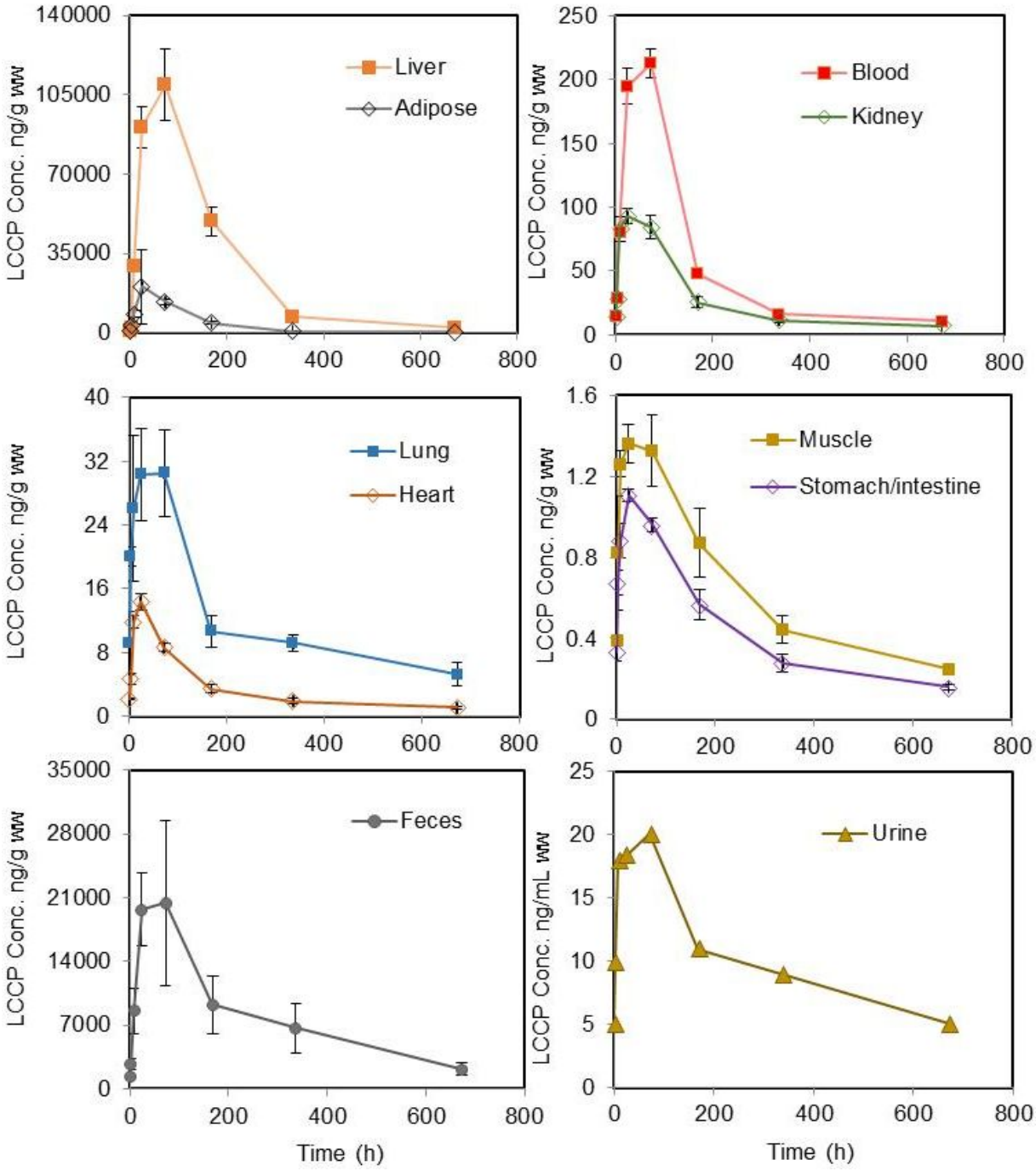


289

290 **Figure S2**. Concentration-time curves of ∑MCCPs in liver, fat, blood, kidney, lung, heart,
291 muscle, stomach/intestine, feces and urine in rats after administered of the chemicals.

292

293



294

295 **Figure S3**. Concentration-time curves of ∑LCCPs in liver, fat, blood, kidney, lung, heart,
296 muscle, stomach/intestine, feces and urine in rats after administered of the chemicals.

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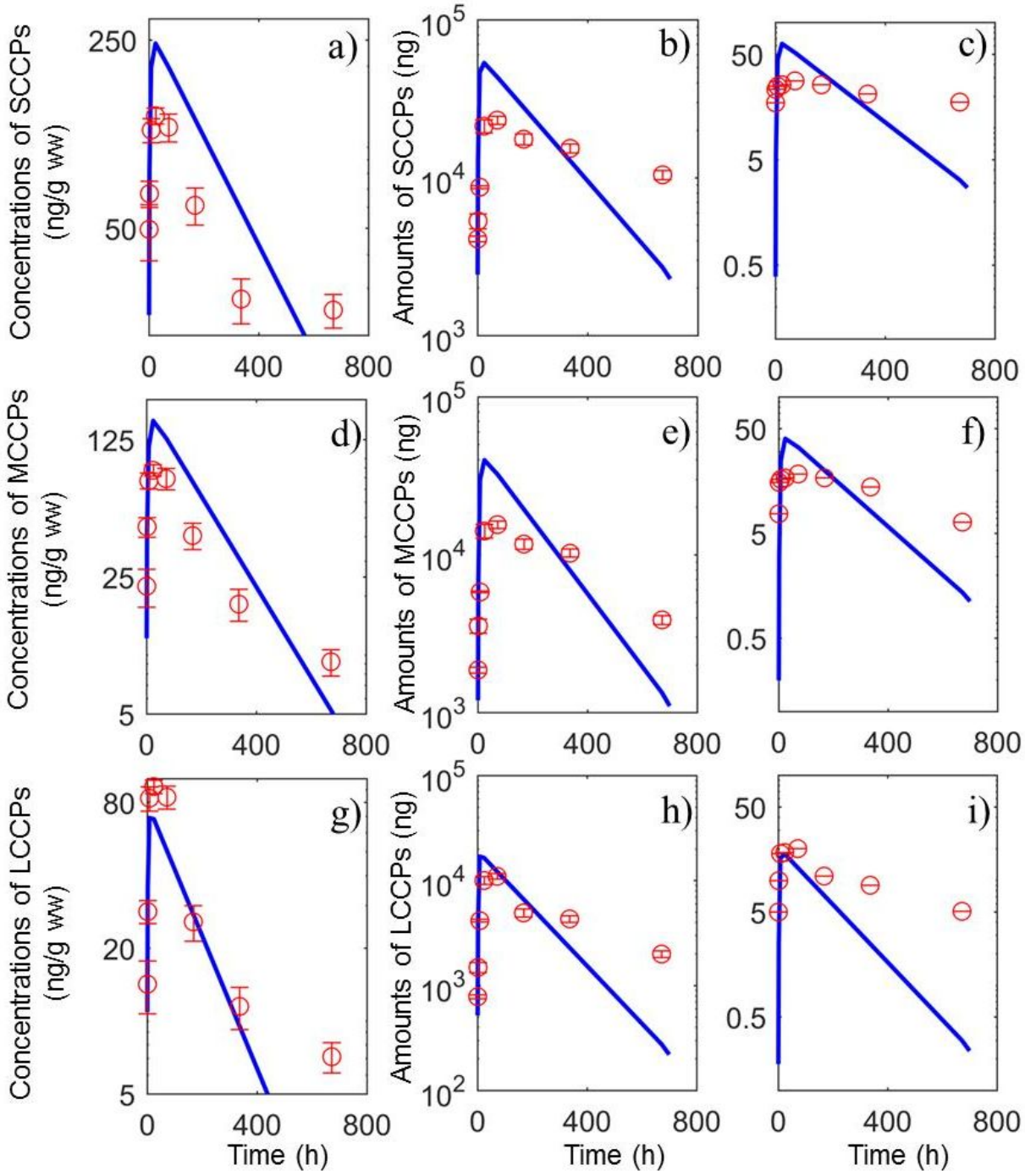


Figure S4. Simulated concentrations (blue line, ng/g ww) of ∑SCCPs, ∑MCCPs, and ∑LCCPs in tissues of kidney (a, d, g), and simulated amounts (blue line, ng) of ∑SCCPs, ∑MCCPs, and ∑LCCPs in feces (b, e, h) and urine (c, f, i) in exposed rats.

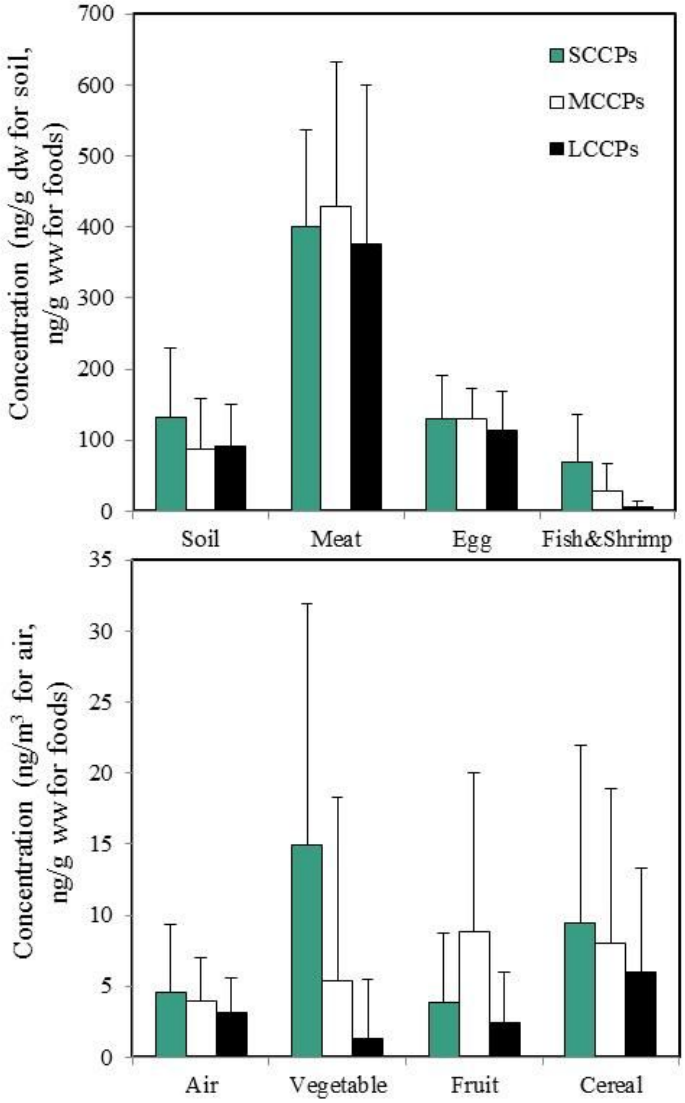


Figure S5. Concentrations of ∑SCCPs, ∑MCCPs, and ∑LCCPs in collected soil, air and food samples.

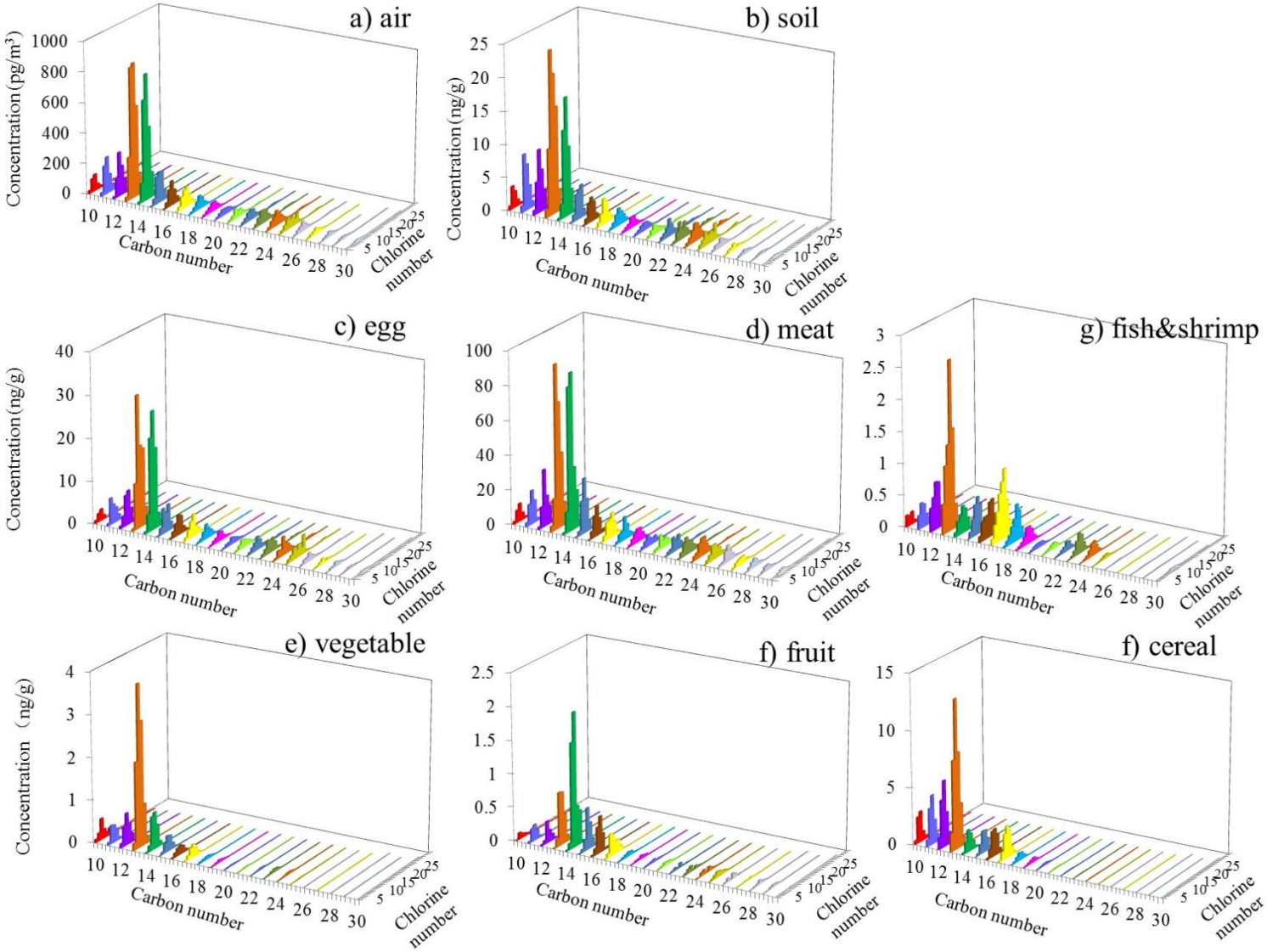
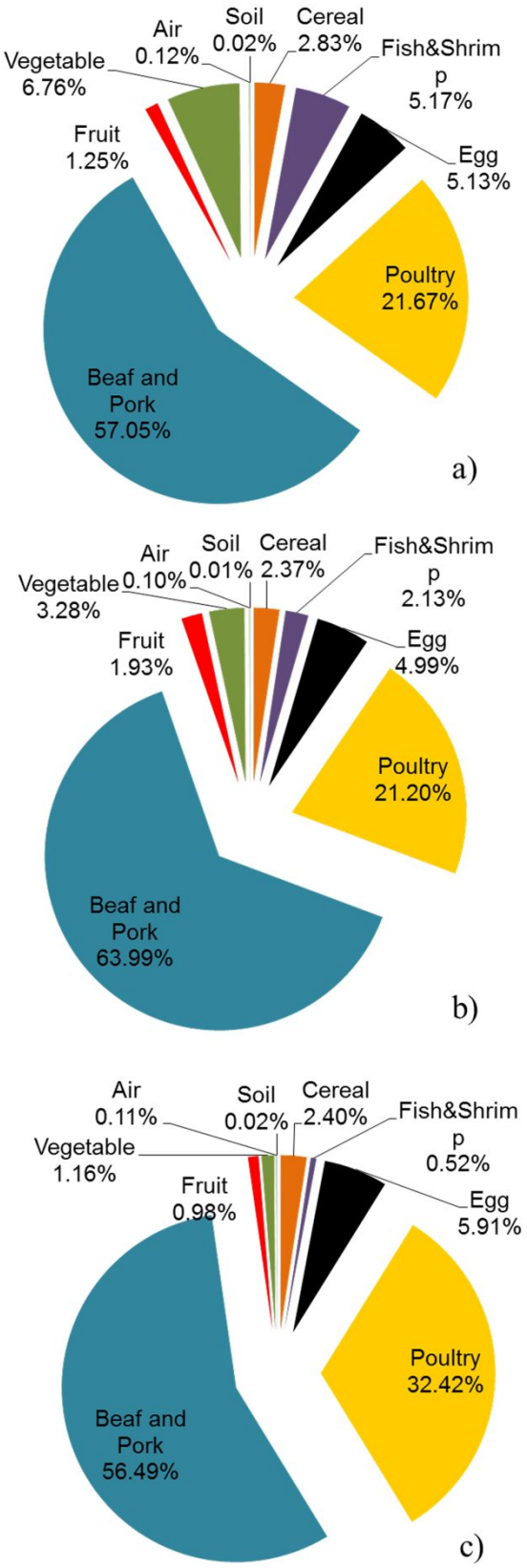


Figure S6. Profiles of CPs in collected air, soil and food samples.



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313 **Figure S7**. Percentages of contributions to the average daily doses of ∑SCCPs, ∑MCCPs and
314 ∑LCCPs through multiple exposure routes.

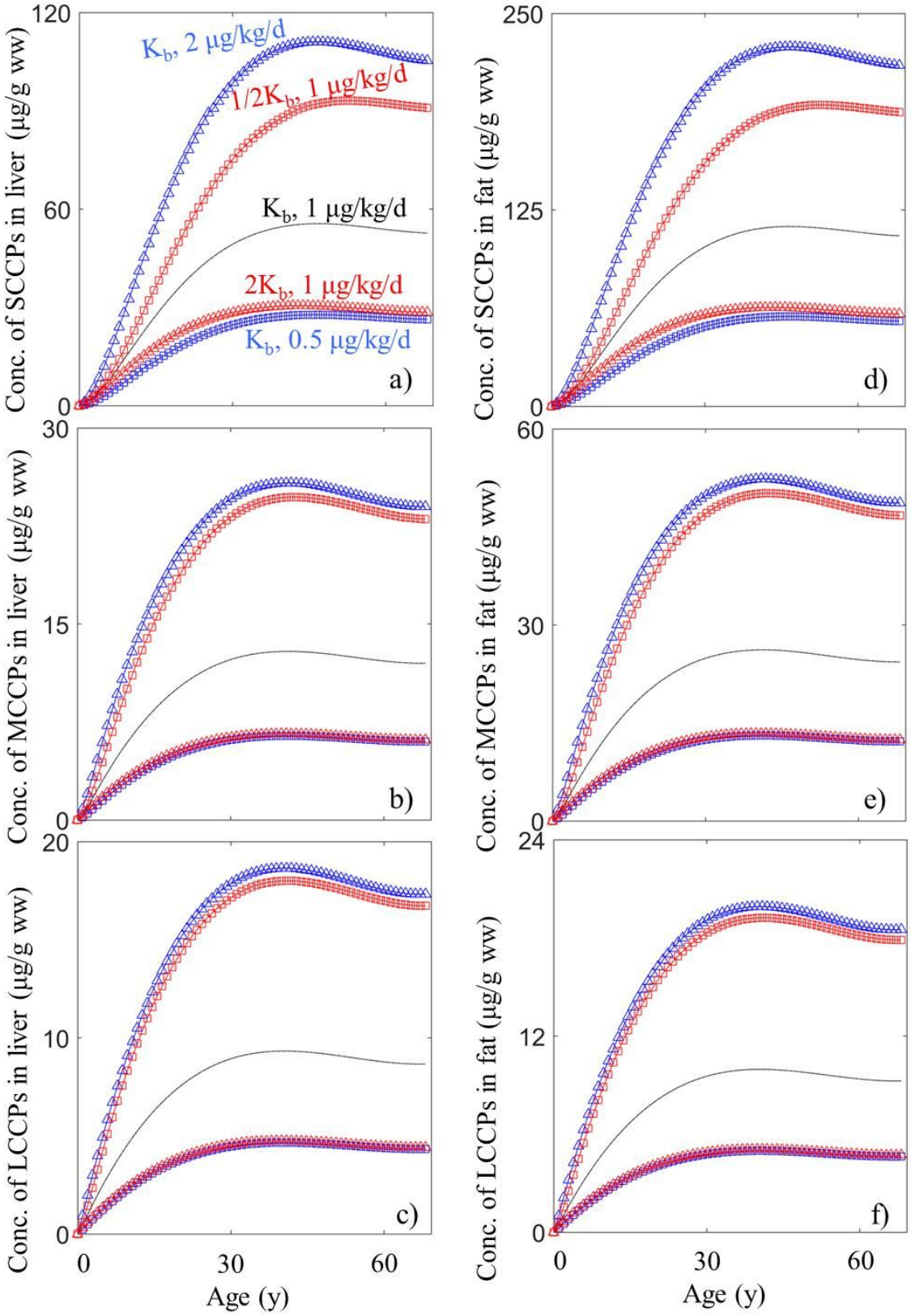

Figure S8. Modeled concentrations of ∑SCCPs (a, d), ∑MCCPs (b, e) and ∑LCCPs (c, f) in human liver (a, b, c) and fat (d, e, f) through the human PBPK models with different doses (0.5, 1, and 2 μg/kg/d, blue line) and biliary excretion rates ($1/2K_b$, K_b, $2K_b$, red line). K_b is

the biliary excretion rate.

Code of Rat PBPK Model.

```
function [tp xamount xcon]=pbpk_CP_rat(dose,index);

%unit for dose: mg
%unit for con: mg/kg;

% index:1 for SCCPs; 2 for MCCPs; 3 for LCCPs
BW=0.23; %bod weight, Unit:kg

%% the fraction of tissue weight
WFO=0.07; %fat
WLIO=0.034; %liver
WKIO=0.007; %kidney
WRBO = 1-WFO-WLIO-WKIO;% the rest of body

WF=WFO*BW;
WLI=WLIO*BW;
WKI=WKIO*BW;
WRB=WRBO*BW;

%% tissue blood flow fraction

QC=6.62; %L per hour
QLIF=0.183; %liver
QFF=0.07; %fat
QKIF=0.141; %kidney
QRBF=1-(QFF+QLIF+QKIF); % the rest of body

QF=QFF*QC;
QRB=QRBF*QC;
QLI=QLIF*QC;
QKI=QKIF*QC;



%% partition

if index==1
PKI=0.21; %kidney
PF=50.3; %fat
PRB=0.0073; % the rest of body
PLI=203.7; %liver

```

```
%% other factors
Ka=0.17; %absorption
Kf=0.0013; %fecal excretion
Kb=0.007;% biliary excretion

Km=1.31*1E-06; %metabolism
Ku=0.04; %urinary excretion

elseif index==2
    PKI=0.21;
PF=50.4;
PRB=0.0073;
PLI=207;

%% other factors
Ka=0.13; %absorption
Kf=0.0009; %fecal excretion
Kb=0.008;% biliary excretion

Km=2.54*1E-06; %metabolism
Ku=0.04; %urinary excretion

elseif index==3
     PKI=0.55;
PF=67.9;
PRB=0.002;
PLI=529.5;

%% other factors
Ka=0.33; %absorption
Kf=0.00085; %fecal excretion
Kb=0.008;% biliary excretion

Km=3.55*1E-06; %metabolism
Ku=0.04; %urinary excretion

end
%% initial condition
options=odeset('NonNegative',[1:7]);
x00=[dose 0 0 0 0 0 0];
timestep=0:1000; % from start to 1000 hours;
  [tp xt]=ode15s(@sub_CPs,timestep,x00,options);
  xcon(:,4)=xt(:,4)./WKI; %kidney
  xcon(:,3)=xt(:,3)./WF; % fat
```

```
411       xcon(:,2)=xt(:,2)./WLI; % liver
412       xcon(:,5)=xt(:,5)./WRB; % the rest
413     xcon(:,1)=tp;
414     CB=(QF'.*xt(:,3)./PF/WF+QRB'.*xt(:,5)./PRB/WRB+QKI'*xt(:,4)/PKI/WKI+QLI'.*xt(:,2)./PLI/WLI)./(Q
415     C);
416     xamount(:,2)=xt(:,2);    % liver
417     xamount(:,3)=xt(:,3);    %fat
418     xamount(:,4)=xt(:,4);      %kidney
419     xamount(:,5)=xt(:,5);      %others
420     xcon(:,6)=CB; %blood
421     xamount(:,1)=tp;
422     xamount(:,7)=xt(:,6);
423     xamount(:,8)=xt(:,7);
424        function dx=sub_CPs (t,x)
425     dx=zeros(7,1);
426     dx(1)=-Ka*x(1)-Kf*x(1); %GI tract
427     CB=(QF*x(3)/PF/WF+QRB*x(5)/PRB/WRB+QLI*x(2)/PLI/WLI+QKI*x(4)/PKI/WKI)/(QC);       %blood
428     concentration
429     dx(2)=QLI*(CB-x(2)/PLI/WLI)-Km*x(2)-x(2)*Kb+Ka*x(1); % liver
430     dx(3)=QF*(CB-x(3)/PF/WF);%Fat
431     dx(4)=QKI*(CB-x(4)/PKI/WKI)-x(4)*Ku;% kidney
432     dx(5)=QRB*(CB-x(5)/PRB/WRB); % the rest of
433     dx(6)=x(2)*Kb+Kf*x(1); %feces
434     dx(7)=x(4)*Ku; %urine
435     end
436     end
437
```

Code of Human PBPK Model.

```
function [tp xamount xcon]=pbpk_CP_human(dose,index);

%unit for dose: mg/day;
% repeated daily exposure
%unit for con: mg/kg;

% index:1 for SCCPs; 2 for MCCPs; 3 for LCCPs

timestep=[0:365*24:70*365*24]; % all life exposure
tt=timestep./365./24;
BW=(0.00058*tt.^3-0.0948*tt.^2+4.8434*tt+2.2785)'; %body weight; human

%% the fraction of tissue weight
WFO=0.2142; %fat
WLIO=0.0257; %liver
WKIO=0.0044; %kidney
WRBO = 1-WFO-WLIO-WKIO;% the rest of body

WF=WFO*BW;
WLI=WLIO*BW;
WKI=WKIO*BW;
WRB=WRBO*BW;

%% tissue blood flow fraction


QC=312; %L per hour
QLIF=0.2270; %liver
QFF=0.052; %fat
QKIF=0.1750; %kidney
QRBF=1-(QFF+QLIF+QKIF); % the rest of body

QF=QFF*QC;
QRB=QRBF*QC;
QLI=QLIF*QC;
QKI=QKIF*QC;

%% partition
if index==1
PKI=0.21; %kidney
PF=50.3; %fat
PRB=0.0073; % the rest of body
```

```
PLI=203.7; %liver

%% other factors
Ka=0.17; %absorption
Kf=0.0013; %fecal excretion
Kb=0.00005;% biliary excretion

Km=3.0*1E-06; %metabolism
Ku=0.04; %urinary excretion

elseif index==2
     PKI=0.21;
PF=50.4;
PRB=0.0073;
PLI=207;

%% other factors
Ka=0.13; %absorption
Kf=0.0009; %fecal excretion
Kb=0.00025;% biliary excretion

Km=4.47*1E-06; %metabolism
Ku=0.04; %urinary excretion

elseif index==3
      PKI=0.55;
PF=67.9;
PRB=0.002;
PLI=529.5;

%% other factors
Ka=0.33; %absorption
Kf=0.00085; %fecal excretion
Kb=0.00035;% biliary excretion

Km=5.56*1E-06; %metabolism
Ku=0.04; %urinary excretion

end
%% initial condition
options=odeset('NonNegative',[1:7]);
x00=[0 0 0 0 0 0 0];
  [tp xt]=ode15s(@sub_CPs,timestep,x00,options);
  xcon(:,4)=xt(:,4)./WKI; %kidney
```

```
526       xcon(:,3)=xt(:,3)./WF; % fat
527       xcon(:,2)=xt(:,2)./WLI; % liver
528       xcon(:,5)=xt(:,5)./WRB; % the rest
529     xcon(:,1)=tp./365/24;
530     CB=(QF'.*xt(:,3)./PF/WF+QRB'.*xt(:,5)./PRB/WRB+QKI'*xt(:,4)/PKI/WKI+QLI'.*xt(:,2)./PLI/WLI)./(Q
531     C);
532     xamount(:,2)=xt(:,2);    % liver
533     xamount(:,3)=xt(:,3);    %fat
534     xamount(:,4)=xt(:,4);      %kidney
535     xamount(:,5)=xt(:,5);      %others
536     xcon(:,6)=CB; %blood
537     xamount(:,1)=tp./365/24;
538
539        function dx=sub_CPs(t,x)
540     t1=t./365./24;
541               BW=(0.00058*t1.^3-0.0948*t1.^2+4.8434*t1+2.2785);
542
543               WF=WFO*BW;
544     WLI=WLIO*BW;
545     WKI=WKIO*BW;
546     WRB=WRBO*BW;
547
548     dx=zeros(7,1);
549
550     dx(1)=-Ka*x(1)-Kf*x(1)+dose*BW./24; %GI tract
551     CB=(QF*x(3)/PF/WF+QRB*x(5)/PRB/WRB+QLI*x(2)/PLI/WLI+QKI*x(4)/PKI/WKI)/(QC);       %blood
552     concentration
553     dx(2)=QLI*(CB-x(2)/PLI/WLI)-Km*x(2)-x(2)*Kb+Ka*x(1); % liver
554     dx(3)=QF*(CB-x(3)/PF/WF);%Fat
555     dx(4)=QKI*(CB-x(4)/PKI/WKI)-x(4)*Ku;% kidney
556     dx(5)=QRB*(CB-x(5)/PRB/WRB); % the rest of
557     dx(6)=x(2)*Kb+Kf*x(1); %feces
558     dx(7)=x(4)*Ku; %urine
559     end
560     end
561
562
563
564
565
```

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