Comprehensive Subchronic Inhalation Toxicity Assessment of an Indoor School Air Mixture of PCBs

*Hui Wang[1], Andrea Adamcakova-Dodd[1], Susanne Flor[1], Laura Gosse[2], Violet E. Klenov[3], Jeffrey Stolwijk[2], Hans-Joachim Lehmler[1,2], Keri C. Hornbuckle[2,4], Gabriele Ludewig[1,2], Larry W. Robertson[1,2], Peter S. Thorne[1,2]**

[1]Department of Occupational and Environmental Health, [2]Interdisciplinary Graduate Program in Human Toxicology, [3]Department of Obstetrics and Gynecology, [4]Department of Civil and Environmental Engineering, the University of Iowa, Iowa City, Iowa 52242, United States

*Corresponding Author:
Peter S. Thorne, Ph.D.
University of Iowa College of Public Health
100 CPHB S341A, 145 N Riverside Dr.
Iowa City, IA 52246
TEL: (319) 335-4216
peter-thorne@uiowa.edu

Data generated in this research are available at https://doi.org/10.25820/data.006120.

Table of Contents

Table S3.8 Average PCB congeners concentration in adipose tissue of SAM-exposure group. Values are expressed in ng/g lipid weight.

Table S3.9 Average PCB congeners concentration in serum of SAM-exposure group. Values are expressed in ng/g wet weight.

Table S3.10 Average PCB congeners concentration in serum of SAM-exposure group. Values are expressed in ng/g lipid weight.

Table S4. Redox parameters and redox potentials (E_h) in plasma.

Figures:

Figure S1. Schematic of PCB generation and nose-only exposure system.

Figure S2. PCB congener profile (in mass percentage, %) in sequential three-day collections of PCB vapor using XAD.

Figure S3. PCB homologue composition (in mass percentage, %) in sequential three-day collections of PCB vapor using XAD.

Figure S4. PCB homolog composition (in mass percentage, %) in tissues of SAM-exposed rats and in generated SAM vapor.

Figure S5. Body weight (g) and relative tissue weight (g/g body weight) of rats in SAM- and sham-exposed groups.

Figure S6. Cytokines concentration (pg/ml) in plasma from rats in sham- and SAM-exposure groups.

Figure S7. Thyroid hormones (total T4 and total T3) concentration (nmol/L) in serum after exposed to SAM vapor.

Figure S8. Antioxidant enzyme activities in liver. A: GPx-1, B: GPx-4, and C: catalase activity.

Figure S9. Trace elements content (Cu, Mn, Se, and Zn) in dried liver from rats exposed to SAM and sham.

Figure S10. Micronuclei (MN) number expressed as per 1000 reticulocytes (immature erythrocytes) from acridine orange coated slides in SAM-exposed and sham-exposed group.

Figure S11. Ovarian follicle number in a single section from SAM- and sham-exposed rats.

Abbreviations

AMH	anti-mullerian hormone
ASE	accelerated solvent extractor
BP	bromopropane
CAT	catalase
CFC	colony-forming cell
CFU-G	colony forming unit-granulocytes
CFU-GM	colony forming unit-granulocytes, macrophage
CFU-M	colony forming unit-macrophages
CYPs	cytochrome P450
Cys	cysteine
CySS	cystine
CySSG	cysteine glutathione disulfide
DE	diatomaceous earth
EPA	Environmental Protection Agency
FSH	follicle stimulating hormone
GC	gas chromatography
GPx	glutathione peroxidase
GSH	glutathione
GSSG	glutathione disulfide
H&E	haematoxylin and eosin
HNE	hydroxynonenal
HPLC	high performance liquid chromatography
HSC	hematopoietic stem cells
IL	interleukin
LH	luteinizing hormone
MDA	malondialdehyde
MDL	method detection limit
MN	micronuclei
MS	mass spectrometry
MWM	Morris Water Maze
PCBs	polychlorinated biphenyls
PLSDA	partial least squares discriminant analysis
RNS	reactive nitrogen species
ROS	reactive oxygen species
SAM	school air mixture
TERT	telomerase reverse transcriptase
T3	triiodothyronine
T4	thyroxine
TNF	tumor necrosis factor
TRAP	telomeric repeat amplification protocol
TRF	telomeric repeat binding factors

PCB Extraction and Quantification from Tissue and XAD. About 1 g of tissue was homogenized with diatomaceous earth (DE) and split into two equal fractions: fraction A (for PCB extraction) and fraction B (for lipid content analysis). Fraction A or XAD were spiked with a surrogate standard mixture (PCB 14, deuterium (^{2}H)-labeled PCB 65, and PCB 166) before accelerated solvent extraction (ASE 350, Dionex, Sunnyvale, CA) with acetone-hexane (1:1, v/v) at 100°C and 10,340 kPa (1500 psi) with preheat for 6 min, 60% cell flush volume, and 1 static cycle of 5 min. Collected extracts were concentrated to 1 ml using a TurboVap (Caliper Life Science Inc., Hopkinton, MA) followed by mixing with 2 ml sulfuric acid for cleanup. The organic phase (top) was eluted through another sulfuric acid-acidified silica gel column for additional cleanup. Ultimately, concentrated extracts were transferred to vials and spiked with internal standard (^{2}H-labeled PCB 30 and PCB 204) for PCB quantification using gas chromatography with tandem mass spectrometry (GC-MS/MS) modified from EPA method 1668A[1] as previously described.[2] Fraction B was extracted with chloroform-methanol (2:1, v/v) using an ASE 200 as above. Lipid content in tissue was determined gravimetrically after evaporating all solvents in a vacuum concentrator (SpeedVac, Fisher Scientific, Pittsburgh, PA). Serum lipid content was calculated using the following formula:

$$\text{Serum total lipid} = (2.27 \times \text{total cholesterol}) + \text{total triglycerides} + 62.3\ \text{mg/dL}.$$[3]

Total cholesterol and triglycerides were measured at the University of Iowa Diagnostic Lab.

Morris Water Maze Test. The method was adapted from Vorhees and Williams [4]. A black circular tank (170 cm diameter, 60 cm high), surrounded with a black curtain, was filled with water (23 to 25℃). Four different cues (circle, square, triangle, and plus sign) were affixed on the tank wall above the water in four positions: north (N), east (E), south (S), and west (W). The tank was divided into four equal-sized quadrants (NE, SE, SW and NW). A circular transparent platform (11 cm diameter, 30 cm high), submerged 1 to 2 cm below the surface of water, was placed in the center of SE quadrant and was unmoved during spatial acquisition test. In spatial acquisition test**,** all rats performed four trials each day for five consecutive days. During the four trials, rats were released into water facing the wall randomly from four positions (N, E, S and W). For each trial, rats were allowed to swim until they found the platform and remained on the platform for 20 s. If the rat didn't find the platform within 120 s, it was gently guided onto the platform to stay for 20 s, then was removed from the tank and dried with towels. The swimming paths were recorded by an overhead video tracking system. Several parameters reflecting the spatial learning abilities were analyzed, including the time latency to reach the platform, the length of the swimming path and the swimming speed. On the sixth day of probe trial, the hidden platform was removed, and the rats were allowed to swim for 60 s. The number of crossing platform and the time spent in the target quadrant where the previous platform was located were compared among groups to evaluate the reference memory.

Toxicity assessment

GPx1 activity assay. Tissue samples were flash frozen in liquid nitrogen after harvesting and stored at -80 °C until analyses. On the day of the assay, samples were homogenized in 1× RIPA lysate buffer (containing protease and phosphatase inhibitors) and centrifuged at 10,000 g for 10 min, 4 °C. All activities were normalized to protein content, measured using the Lowry protein assay (Bio-Rad, Hercules, CA).[5] GPx1 activity was determined using UV-Vis spectroscopy, by following the oxidation of NADPH as earlier described.[6, 7] First, a 55.6 mM (pH 7.4) potassium phosphate buffer (PB) was made containing: 1.1 mM NaN_3 and 1.1 mM EDTA. Secondly, the working buffer was made containing: 1.33 mM glutathione and 1.33 U/mL glutathione disulfide reductase (GR) in stock buffer. A 2.5 mM H2O2 solution and a 4 mM NADPH solution were prepared in PB and kept on ice to prevent oxidation. All components, except H_2O_2 were combined, homogenized and incubated at 25 °C for 5 min. Then H_2O_2 solution was added to start the reaction. The rate of NADPH oxidation was then monitored at 340 nm ($\varepsilon = 6{,}220\ M^{-1}\ cm^{-1}$).

GPx4 activity assay. The activity assay is based on a version described by Stolwijk, et al. [8]. A 100 mM (pH 8.0) Tris/HCl buffer was made containing: 1.5 mM NaN_3 to inhibit catalase, and 2.0 mM EDTA (chelator). Additionally, Triton X-100, peroxide free (Sigma-Aldrich: X100PC), was added as a surfactant to achieve a 0.1% volume percentage of Triton X-100. Glutathione disulfide reductase, from backer's yeast (Sigma-Aldrich: G3664), solution of 15 U/mL was made. A 60 mM glutathione (Sigma-Aldrich: G4251) was made in assay buffer, as well as, 4 mM NADPH in 0.1% $NaHCO_3$. The buffer, GR solution, GSH solution, NADPH solution, and sample were combined in a cuvette and incubated at 37 °C for 5 min. To monitor the kinetics, the oxidation of NADPH was monitored for 480 s (8 min). Background oxidation of NADPH was monitored for 4 min, then, 15 µL of ~2 mM PCOOH solution was added into the cuvette to start the reaction. The rate of NADPH oxidation was then monitored at 340 nm ($\varepsilon = 6{,}220\ M^{-1}\ cm^{-1}$).

Catalase activity assay. Catalase activity was determined using UV-Vis spectroscopy by following the rate of removal (oxidation) of hydrogen peroxide due to catalase.[9, 10] Hydrogen peroxide has an absorbance at wavelength of 240 nm, $\varepsilon_{240} = 39.4\ M^{-1}\ cm^{-1}$ [11] that was monitored. The protein content (Lowry protein assay) in each assay was determined and used to normalize the activity of catalase per mg protein (m*k* U/mg protein). The assay was conducted in a 55.6 mM (pH 7.0) potassium phosphate buffer. Then a 30 mM H_2O_2 solution was added and the analysis was started. The absorbance of hydrogen peroxide was monitored over 120 s at 10 s intervals.

Micronuclei test [12]. Acridine orange (AO) was dissolved in distilled water (1 mg/ml) and spread 10 μl using a glass rod onto pre-heated (70°C) clean glass slides and left to air dry. Five ul of whole blood was pipetted onto the center of the AO-coated slide and coverslipped. Scoring was done with the combination of a blue excitation (e.g. 490 nm) and a yellow to orange barrier filter (e.g. 525 nm) and coded slides were analyzed by counting micronuclei per 1000 reticulocytes (immature erythrocytes).

Bone marrow cells isolation. Bone marrow nucleated cells were isolated from femurs of Sprague-Dawley rats as described by Zhang, et al. [13]. The cells were washed with Dulbecco's Modified Eagle Medium DMEM supplemented with 20% heat inactivated fetal bovine serum (FBS) and 1% penicillin/streptomycin (complete DMEM) and passed through a 70 μm nylon strainer to remove cell clumps and debris. After initial centrifugation, red blood cells were lysed and clearing of turbidity was achieved by incubating the cells with Flow Cytometry Mouse Lyse Buffer (R&D Systems, Minneapolis, MN) for 10 min at room temperature. After washing with Iscove's Modified Dulbecco's Medium IMDM/2% FBS, cell numbers were counted by an Accuri flow cytometer and 3 different cell suspensions were made in IMDM/2% FBS.

Colony forming cell (CFC) assay. We performed a CFC assay to measure the absolute number of viable and functional progenitor cells in the bone marrow. A total of 2×10^6 cells in 4 ml IMDM were counted again and a new cell suspension of 6×10^5 cells in 2 ml IMDM/2% FBS was made and 300 μl of this was added into 3 ml rat Methycellulose Complete Media without Epo (R&D Systems, Minneapolis, MN) to set the final cell number at 3×10^4 cells/ml. Then 1.1 ml of the cell suspension was plated in duplicate in 35-mm untreated culture dishes. Dishes were incubated in a humid incubator at 37°C, 5% CO_2 for 12 days prior to enumeration. Three different colonies: colony forming unit-granulocytes (CFU-G), colony forming unit-macrophages (CFU-M) and colony forming unit-granulocytes, macrophage (CFU-GM) were counted via light microscopy and gridded scoring dish.

Telomerase activity. 3×10^6 bone marrow cells were washed once with Phosphate buffered saline (PBS) and were resuspended in cell lysis buffer and incubated on ice for 20 min. After centrifugation at 16,000×g for 20 min at 4°C, the supernatant was transferred into new tubes and stored at -80°C. The protein concentration of the supernatant was determined by Bradford Protein Assay (Bio-Rad, Hercules, CA). The SYBR Green based quantitative PCR reaction was performed by adding 100 ng of TS primer (5'-AATCCGTCGGAGCAGGTT-3'), 50 ng of CX primer (5'-CCCTTACCCTTACCCTTACCCTAA-3')[14], 2.5 µl cell lysate supernatant, 12.5 µl Power SYBR Green PCR master mix from Applied Biosystems (Foster City, CA) and 9.7 µl of DNase- and RNase-free water into the well of a 96-well microplate. Samples were incubated for 20 min at room temperature and PCR was performed by incubating the sample at 95°C for 10 min followed by 35 cycles of 95°C for 30 s and 60°C for 90 s using an Eppendorf RealPlex Thermal Cycler (Eppendorf, Hamburg, Germany). The threshold cycle (Ct) value was established from the semi-log amplification plots (log increase in fluorescence versus cycle number) and compared with the standard curves generated from serial dilutions of cell extracts. Each sample was analyzed in duplicate.

Quantitative real-time PCR. 4×10^6 bone marrow cells were washed once with PBS and total RNA was extracted from each sample using RNeasy Plus mini kit from Qiagen (Valencia, CA) following the manufacturer's protocol and quantified using a NanoDrop2000 (Thermo Fisher

Scientific, Wilmington, DE). Total RNA was reverse transcribed using the High Capacity cDNA Reverse Transcription Kit from Applied Biosystems (Foster City, CA). The cDNA was subsequently used for quantitative real-time RT-PCR (qRT-PCR) using an Eppendorf RealPlex thermal cycler. The PCR program used started with 95°C for 10 min followed by 35 cycles at 95°C for 30 s, 60°C for 30 s, and 72°C for 1 min. The primers for the quantification of selected genes were purchased from Integrated DNA Technologies (IDT) (Coralville, IA) and were as follows: *Tert*: Forward, 5'-AGCTGCTGAGGTCATTCTTC-3', and reverse, 5'-GCTTGCTCCACACACTCTTA-3'; *Trf-1*: Forward, 5'-CAGCTCACTACAGGACAGTTAAA-3', and reverse, 5'-CTCTCTCACACACACACACAA-3'; *Trf-2*: Forward, 5'-GTGTTTCCCTTCAGTGGTTTG-3', and reverse, 5'-GAGAGCCTATGTGCTCCTAATG-3' (all designed by PrimerQuest); *Gadph*: forward, 5'-TGGAGAAACCTGCCAAGTATG-3', and reverse, 5'-GGGTGGTCCAGGGTTTCTTA-3' [15]. An amplification threshold in the linear range of each sample was selected to calculate the C_T for each sample. The relative mRNA levels were calculated with the relative standard curve method.

Gut Microbiota analysis. The colon fecal samples were sent to Argonne National Laboratory (Argonne, IL) where DNA was extracted from all rat feces samples stored at -20℃. Following the methods outlined by Denner, et al. [16], primers specific for the V4 region of the created 16S rRNA encoding gene was used for PCR amplification. These amplicons were then quantified using the Illumina MiSeq (Illumina, San Diego, CA) at the Argonne National Laboratory. Once quantified, amplicons were sequenced using customized sequencing primers to process for operational taxonomic unity (OTU) picking.[16] Subsequent analysis was then completed by using QiiME2 [17] which was able to produce taxonomic profiles and metadata files. These files were then analyzed through METAGENassist to produce relative abundance tables of genera, heatmaps of total taxonomic ranks, and Partial Least Squares Discriminant Analysis (PLSDA) plots.[18]

Table S1. Method Detection Limit (MDL) for each PCB congener or co-eluted congeners. MDL was determined from method blanks analyzed in parallel with samples and expressed as the upper limit of 99% confidence interval of method blanks (average + t_{n-1}* standard deviation, t_{n-1} is Student's t-value of 99% confidence level with n-1 degree of freedom). Values are expressed in ng.

PCB	MDL	PCB	MDL	PCB	MDL	PCB	MDL
1	0.024	**51**	0.007	**106**	0.002	**161**	0.002
2	0.031	**52**	0.177	**107**	0.004	**162**	0.017
3	0.051	**54**	0.002	**108/124**	0.003	**164**	0.002
4	0.062	**55**	0.007	**110**	0.129	**165**	0.002
5	0.008	**56**	0.032	**111**	0.002	**167**	0.018
6	0.024	**57**	0.003	**112**	0.001	**169**	0.003
7	0.010	**58**	0.001	**114**	0.068	**170**	0.008
8	0.047	**59/62/75**	0.022	**115**	0.106	**171/173**	0.008
9	0.007	**60**	0.021	**117**	0.269	**172**	0.012
10	0.014	**61/70/74/76**	0.241	**118**	0.089	**174**	0.008
11	0.109	**63**	0.006	**120**	0.002	**175**	0.008
12/13	0.061	**64**	0.045	**121**	0.001	**176**	0.002
15	0.044	**66**	0.116	**122**	0.003	**177**	0.007
16	0.044	**67**	0.004	**123**	0.002	**178**	0.005
17	0.033	**68**	0.086	**126**	0.002	**179**	0.005
18/30	0.057	**72**	0.008	**127**	0.002	**180/193**	0.012
19	0.018	**73**	0.023	**129/138/163**	0.062	**181**	0.028
20/28	0.081	**77**	0.006	**130**	0.003	**182**	0.058
21/33	0.041	**78**	0.006	**131**	0.003	**183**	0.006
22	0.024	**79**	0.001	**132**	0.016	**184**	0.069
23	0.011	**80**	0.001	**133**	0.004	**185**	0.007
24	0.008	**81**	0.002	**134**	0.004	**186**	0.003
25	0.017	**82**	0.017	**135/151**	0.021	**187**	0.018
26/29	0.043	**83**	0.007	**136**	0.015	**188**	0.034
27	0.030	**84**	0.052	**137**	0.003	**189**	0.009
31	0.057	**85/116**	0.081	**139/140**	0.002	**190**	0.018
32	0.026	**86/97/109/119**	0.051	**141**	0.008	**191**	0.007
34	0.017	**87/125**	0.060	**142**	0.003	**192**	0.007
35	0.004	**88**	0.004	**143**	0.003	**194**	0.010
36	0.004	**89**	0.002	**144**	0.002	**195**	0.006
37	0.020	**90/101/113**	0.137	**145**	0.002	**196**	0.004
38	0.007	**91**	0.015	**146**	0.004	**197**	0.028
39	0.003	**92**	0.032	**147/149**	0.060	**198/199**	0.007
40/71	0.053	**93/100**	0.003	**148**	0.003	**200**	0.017
41	0.006	**94**	0.002	**150**	0.002	**201**	0.003
42	0.022	**95**	0.123	**152**	0.002	**202**	0.028
43	0.004	**96**	0.002	**153/168**	0.047	**203**	0.002
44/47/65	0.290	**98**	0.002	**154**	0.002	**205**	0.004
45	0.005	**99**	0.058	**155**	0.002	**206**	0.078
46	0.056	**102**	0.003	**156/157**	0.008	**207**	0.013
48	0.016	**103**	0.002	**158**	0.002	**208**	0.008
49/69	0.077	**104**	0.002	**159**	0.013	**209**	0.086
50/53	0.063	**105**	0.035	**160**	0.002		

Table S2. Recovery rate (%) of surrogate standards (PCB 14, d-PCB 65, and PCB 166) in tissues and SAM vapor extraction. Values are expressed as mean ± standard deviation.

	Liver (n = 15)	Lung (n = 20)	Brain (n = 14)	Adipose (n = 19)	Serum (n = 19)	SAM vapor (n = 13)
PCB 14	87 ± 9	85 ± 7	86 ± 8	91 ± 13	82 ± 11	81 ± 10
d-PCB 65	93 ± 11	93 ± 7	91 ± 9	75 ± 23	101 ± 13	62 ± 10
PCB 166	105 ± 11	101 ± 7	101 ± 6	83 ± 20	108 ± 15	63 ± 14

Table S3.1 Average PCB congener concentrations in **liver** of SAM-exposure group. Values are expressed in ng/g wet tissue weight.

PCB	Conc.	PCB	Conc.	PCB	Conc.	PCB	Conc.
1	0.03	**51**	0.01	**107**	0.25	**162**	0.02
2	0.04	**52**	1.86	**108/124**	0.01	**164**	0.02
3	0.06	**54**	0.00	**110**	1.86	**165**	0.00
4	0.07	**55**	0.11	**111**	0.00	**167**	0.02
5	0.01	**56**	0.06	**112**	0.05	**169**	0.01
6	0.03	**57**	0.00	**114**	0.22	**170**	0.02
7	0.01	**58**	0.00	**115**	0.76	**171/173**	0.02
8	0.06	**59/62/75**	0.12	**117**	0.62	**172**	0.02
9	0.01	**60**	2.40	**118**	4.84	**174**	0.03
10	0.02	**61/70/74/76**	9.10	**120**	0.00	**175**	0.02
11	0.13	**63**	0.14	**121**	0.00	**176**	0.00
12/13	0.07	**64**	0.21	**122**	0.01	**177**	0.02
14	0.00	**66**	12.1	**123**	0.14	**178**	0.01
15	0.06	**67**	0.01	**126**	0.00	**179**	0.01
16	0.15	**68**	0.10	**127**	0.00	**180/193**	0.06
17	0.04	**72**	0.01	**128/166**	0.00	**181**	0.03
18/30	0.22	**73**	0.04	**129/138/163**	1.26	**182**	0.07
19	0.15	**77**	0.03	**130**	0.08	**183**	0.05
20/28	0.68	**78**	0.01	**131**	0.00	**184**	0.08
21/33	0.22	**79**	0.01	**132**	0.08	**185**	0.01
22	0.15	**80**	0.02	**133**	0.01	**186**	0.00
23	0.03	**81**	0.01	**134**	0.00	**187**	0.08
24	0.11	**82**	0.10	**135/151**	0.05	**188**	0.04
25	0.05	**83**	0.02	**136**	0.02	**189**	0.01
26/29	0.10	**84**	0.06	**137**	0.12	**190**	0.03
27	0.04	**85/116**	3.81	**139/140**	0.14	**191**	0.02
31	0.23	**86/97/109/119**	1.09	**141**	0.05	**192**	0.02
32	0.26	**87/125**	1.08	**142**	0.00	**194**	0.02
34	0.09	**88**	0.02	**143**	0.00	**195**	0.01
35	0.04	**89**	0.00	**144**	0.01	**196**	0.01
36	0.01	**90/101/113**	6.51	**145**	0.00	**197**	0.04
37	0.02	**91**	0.12	**146**	0.16	**198/199**	0.02
38	0.06	**92**	0.85	**147/149**	0.40	**200**	0.02
39	0.01	**93/100**	0.04	**148**	0.00	**201**	0.00
40/71	0.06	**94**	0.00	**150**	0.00	**202**	0.03
41	0.01	**95**	0.51	**152**	0.00	**203**	0.01
42	0.07	**96**	0.00	**153/168**	1.08	**204**	0.00
43	0.01	**98**	0.00	**154**	0.04	**205**	0.01
44/47/65	1.94	**99**	15.0	**155**	0.00	**206**	0.09
45	0.01	**102**	0.01	**156/157**	0.01	**207**	0.02
46	0.07	**103**	0.00	**158**	0.15	**208**	0.01
48	0.05	**104**	0.00	**159**	0.02	**209**	0.10
49/69	2.08	**105**	1.81	**160**	0.00	**SUM**	78.6
50/53	0.08	**106**	0.01	**161**	0.00		

Table S3.2 Average lipid-adjusted PCB congener concentrations in **liver** of SAM-exposure group. Values are expressed in ng/g lipid weight.

PCB	Conc.	PCB	Conc.	PCB	Conc.	PCB	Conc.
1	0.47	**51**	0.14	**107**	4.15	**162**	0.33
2	0.61	**52**	30.6	**108/124**	0.10	**164**	0.26
3	1.01	**54**	0.05	**110**	30.2	**165**	0.03
4	1.22	**55**	1.78	**111**	0.04	**167**	0.35
5	0.15	**56**	1.02	**112**	0.80	**169**	0.16
6	0.48	**57**	0.05	**114**	3.57	**170**	0.35
7	0.20	**58**	0.03	**115**	12.5	**171/173**	0.36
8	0.94	**59/62/75**	1.89	**117**	10.1	**172**	0.38
9	0.14	**60**	39.4	**118**	79.2	**174**	0.44
10	0.28	**61/70/74/76**	149	**120**	0.06	**175**	0.36
11	2.15	**63**	2.24	**121**	0.03	**176**	0.05
12/13	1.20	**64**	3.49	**122**	0.19	**177**	0.37
14	0.00	**66**	198	**123**	2.26	**178**	0.10
15	0.92	**67**	0.09	**126**	0.05	**179**	0.13
16	2.49	**68**	1.71	**127**	0.05	**180/193**	1.05
17	0.65	**72**	0.15	**128/166**	0.00	**181**	0.56
18/30	3.74	**73**	0.72	**129/138/163**	20.5	**182**	1.14
19	2.33	**77**	0.51	**130**	1.23	**183**	0.83
20/28	11.4	**78**	0.17	**131**	0.06	**184**	1.36
21/33	3.45	**79**	0.19	**132**	1.40	**185**	0.21
22	2.53	**80**	0.33	**133**	0.14	**186**	0.06
23	0.47	**81**	0.20	**134**	0.07	**187**	1.30
24	1.93	**82**	1.70	**135/151**	0.88	**188**	0.67
25	0.77	**83**	0.29	**136**	0.34	**189**	0.20
26/29	1.67	**84**	1.02	**137**	1.97	**190**	0.48
27	0.69	**85/116**	62.2	**139/140**	2.33	**191**	0.25
31	3.73	**86/97/109/119**	17.8	**141**	0.86	**192**	0.28
32	4.25	**87/125**	17.7	**142**	0.06	**194**	0.29
34	1.43	**88**	0.34	**143**	0.05	**195**	0.14
35	0.60	**89**	0.05	**144**	0.17	**196**	0.12
36	0.20	**90/101/113**	107	**145**	0.03	**197**	0.68
37	0.39	**91**	1.99	**146**	2.64	**198/199**	0.30
38	1.05	**92**	14.0	**147/149**	6.63	**200**	0.37
39	0.13	**93/100**	0.63	**148**	0.05	**201**	0.07
40/71	1.06	**94**	0.05	**150**	0.03	**202**	0.55
41	0.13	**95**	8.37	**152**	0.03	**203**	0.18
42	1.09	**96**	0.05	**153/168**	17.6	**204**	0.00
43	0.17	**98**	0.04	**154**	0.60	**205**	0.16
44/47/65	31.8	**99**	245	**155**	0.03	**206**	1.55
45	0.12	**102**	0.10	**156/157**	0.17	**207**	0.26
46	1.10	**103**	0.05	**158**	2.45	**208**	0.17
48	0.84	**104**	0.03	**159**	0.26	**209**	1.69
49/69	34.1	**105**	29.7	**160**	0.06	**SUM**	1290
50/53	1.25	**106**	0.12	**161**	0.03		

Table S3.3 Average PCB congener concentrations in **lung** of SAM-exposure group. Values are expressed in ng/g wet tissue weight.

PCB	Conc.	PCB	Conc.	PCB	Conc.	PCB	Conc.
1	0.08	**51**	0.02	**107**	0.42	**162**	0.05
2	0.10	**52**	3.15	**108/124**	0.02	**164**	0.01
3	0.16	**54**	0.01	**110**	2.29	**165**	0.01
4	0.20	**55**	0.08	**111**	0.01	**167**	0.06
5	0.02	**56**	0.24	**112**	0.07	**169**	0.01
6	0.08	**57**	0.01	**114**	0.28	**170**	0.03
7	0.03	**58**	0.00	**115**	1.14	**171/173**	0.03
8	0.15	**59/62/75**	0.16	**117**	1.11	**172**	0.04
9	0.02	**60**	7.91	**118**	8.64	**174**	0.03
10	0.05	**61/70/74/76**	14.5	**120**	0.01	**175**	0.02
11	0.35	**63**	0.18	**121**	0.00	**176**	0.01
12/13	0.20	**64**	0.26	**122**	0.02	**177**	0.03
14	0.00	**66**	22.2	**123**	0.18	**178**	0.01
15	0.14	**67**	0.01	**126**	0.01	**179**	0.02
16	0.14	**68**	0.28	**127**	0.01	**180/193**	0.10
17	0.11	**72**	0.02	**128/166**	0.00	**181**	0.09
18/30	0.19	**73**	0.07	**129/138/163**	3.65	**182**	0.19
19	0.06	**77**	0.02	**130**	0.12	**183**	0.09
20/28	2.07	**78**	0.02	**131**	0.01	**184**	0.22
21/33	0.13	**79**	0.00	**132**	0.09	**185**	0.02
22	0.08	**80**	0.00	**133**	0.01	**186**	0.01
23	0.04	**81**	0.01	**134**	0.01	**187**	0.14
24	0.03	**82**	0.25	**135/151**	0.10	**188**	0.11
25	0.05	**83**	0.02	**136**	0.05	**189**	0.03
26/29	0.14	**84**	0.17	**137**	0.19	**190**	0.06
27	0.10	**85/116**	6.74	**139/140**	0.19	**191**	0.02
31	0.18	**86/97/109/119**	1.97	**141**	0.07	**192**	0.02
32	0.08	**87/125**	2.04	**142**	0.01	**194**	0.03
34	0.05	**88**	0.01	**143**	0.01	**195**	0.02
35	0.01	**89**	0.01	**144**	0.01	**196**	0.01
36	0.01	**90/101/113**	11.9	**145**	0.01	**197**	0.10
37	0.06	**91**	0.11	**146**	0.37	**198/199**	0.02
38	0.02	**92**	1.54	**147/149**	0.62	**200**	0.05
39	0.01	**93/100**	0.02	**148**	0.01	**201**	0.01
40/71	0.17	**94**	0.01	**150**	0.01	**202**	0.09
41	0.02	**95**	1.18	**152**	0.01	**203**	0.01
42	0.08	**96**	0.01	**153/168**	2.90	**204**	0.00
43	0.02	**98**	0.01	**154**	0.07	**205**	0.01
44/47/65	3.76	**99**	32.0	**155**	0.01	**206**	0.25
45	0.02	**102**	0.01	**156/157**	0.03	**207**	0.04
46	0.18	**103**	0.01	**158**	0.29	**208**	0.03
48	0.07	**104**	0.01	**159**	0.04	**209**	0.28
49/69	1.31	**105**	5.30	**160**	0.01	**SUM**	149
50/53	0.20	**106**	0.01	**161**	0.01		

Table S3.4 Average lipid-adjusted PCB congener concentrations in **lung** of SAM-exposure group. Values are expressed in ng/g lipid weight.

PCB	Conc.	PCB	Conc.	PCB	Conc.	PCB	Conc.
1	1.76	**51**	0.51	**107**	9.66	**162**	1.25
2	2.32	**52**	72.1	**108/124**	0.48	**164**	0.24
3	3.80	**54**	0.17	**110**	52.5	**165**	0.13
4	4.61	**55**	1.96	**111**	0.15	**167**	1.33
5	0.56	**56**	5.69	**112**	1.73	**169**	0.26
6	1.80	**57**	0.19	**114**	6.32	**170**	0.60
7	0.77	**58**	0.09	**115**	25.9	**171/173**	0.70
8	3.53	**59/62/75**	3.58	**117**	25.4	**172**	0.86
9	0.54	**60**	183	**118**	198	**174**	0.65
10	1.06	**61/70/74/76**	332	**120**	0.16	**175**	0.56
11	8.12	**63**	4.22	**121**	0.10	**176**	0.18
12/13	4.53	**64**	5.93	**122**	0.43	**177**	0.77
14	0.00	**66**	509	**123**	4.11	**178**	0.34
15	3.25	**67**	0.30	**126**	0.18	**179**	0.38
16	3.32	**68**	6.44	**127**	0.14	**180/193**	2.19
17	2.45	**72**	0.58	**128/166**	0.00	**181**	2.13
18/30	4.29	**73**	1.72	**129/138/163**	84.1	**182**	4.30
19	1.33	**77**	0.47	**130**	2.79	**183**	2.01
20/28	47.7	**78**	0.46	**131**	0.22	**184**	5.12
21/33	3.04	**79**	0.11	**132**	2.05	**185**	0.54
22	1.79	**80**	0.09	**133**	0.30	**186**	0.20
23	0.83	**81**	0.13	**134**	0.27	**187**	3.30
24	0.58	**82**	5.72	**135/151**	2.36	**188**	2.52
25	1.24	**83**	0.52	**136**	1.15	**189**	0.68
26/29	3.18	**84**	3.85	**137**	4.43	**190**	1.31
27	2.23	**85/116**	155	**139/140**	4.38	**191**	0.56
31	4.25	**86/97/109/119**	45.3	**141**	1.47	**192**	0.49
32	1.97	**87/125**	46.8	**142**	0.22	**194**	0.77
34	1.24	**88**	0.26	**143**	0.19	**195**	0.47
35	0.31	**89**	0.18	**144**	0.30	**196**	0.33
36	0.26	**90/101/113**	272	**145**	0.12	**197**	2.37
37	1.48	**91**	2.53	**146**	8.54	**198/199**	0.53
38	0.51	**92**	35.39	**147/149**	14.1	**200**	1.26
39	0.24	**93/100**	0.50	**148**	0.19	**201**	0.22
40/71	3.99	**94**	0.17	**150**	0.12	**202**	2.08
41	0.43	**95**	27.3	**152**	0.13	**203**	0.21
42	1.90	**96**	0.14	**153/168**	66.6	**204**	0.00
43	0.35	**98**	0.18	**154**	1.64	**205**	0.31
44/47/65	86.2	**99**	734	**155**	0.12	**206**	5.84
45	0.37	**102**	0.24	**156/157**	0.63	**207**	0.97
46	4.17	**103**	0.20	**158**	6.59	**208**	0.61
48	1.51	**104**	0.14	**159**	0.99	**209**	6.39
49/69	30.0	**105**	122	**160**	0.12	**SUM**	3420
50/53	4.72	**106**	0.15	**161**	0.13		

Table S3.5 Average PCB congener concentrations in **brain** of SAM-exposure group. Values are expressed in ng/g wet tissue weight.

PCB	Conc.	PCB	Conc.	PCB	Conc.	PCB	Conc.
1	0.03	**51**	0.01	**107**	0.25	**162**	0.03
2	0.04	**52**	1.37	**108/124**	0.04	**164**	0.02
3	0.07	**54**	0.00	**110**	1.59	**165**	0.00
4	0.13	**55**	0.08	**111**	0.00	**167**	0.03
5	0.01	**56**	0.05	**112**	0.12	**169**	0.01
6	0.04	**57**	0.00	**114**	0.25	**170**	0.02
7	0.02	**58**	0.00	**115**	0.55	**171/173**	0.02
8	0.06	**59/62/75**	0.12	**117**	0.62	**172**	0.03
9	0.01	**60**	2.05	**118**	4.28	**174**	0.01
10	0.06	**61/70/74/76**	9.00	**120**	0.00	**175**	0.12
11	0.19	**63**	0.13	**121**	0.00	**176**	0.00
12/13	0.08	**64**	0.19	**122**	0.01	**177**	0.01
14	0.00	**66**	10.5	**123**	0.13	**178**	0.02
15	0.06	**67**	0.01	**126**	0.00	**179**	0.01
16	0.14	**68**	0.12	**127**	0.00	**180/193**	0.06
17	0.04	**72**	0.02	**128/166**	0.00	**181**	0.04
18/30	0.21	**73**	0.04	**129/138/163**	1.05	**182**	0.13
19	0.75	**77**	0.01	**130**	0.08	**183**	0.06
20/28	0.87	**78**	0.01	**131**	0.01	**184**	0.09
21/33	0.11	**79**	0.00	**132**	0.07	**185**	0.02
22	0.03	**80**	0.00	**133**	0.02	**186**	0.00
23	0.02	**81**	0.00	**134**	0.01	**187**	0.11
24	0.71	**82**	0.09	**135/151**	0.06	**188**	0.05
25	0.02	**83**	0.08	**136**	0.02	**189**	0.08
26/29	0.06	**84**	0.08	**137**	0.11	**190**	0.04
27	0.16	**85/116**	3.09	**139/140**	0.09	**191**	0.01
31	1.16	**86/97/109/119**	1.03	**141**	0.05	**192**	0.01
32	0.04	**87/125**	0.84	**142**	0.00	**194**	0.01
34	0.20	**88**	0.00	**143**	0.00	**195**	0.01
35	0.04	**89**	0.00	**144**	0.01	**196**	0.01
36	0.00	**90/101/113**	5.20	**145**	0.00	**197**	0.04
37	0.03	**91**	0.10	**146**	0.17	**198/199**	0.02
38	0.01	**92**	0.76	**147/149**	0.30	**200**	0.03
39	0.00	**93/100**	0.03	**148**	0.00	**201**	0.01
40/71	0.10	**94**	0.00	**150**	0.00	**202**	0.04
41	0.01	**95**	0.44	**152**	0.00	**203**	0.01
42	0.06	**96**	0.00	**153/168**	0.97	**204**	0.00
43	0.01	**98**	0.00	**154**	0.03	**205**	0.01
44/47/65	1.68	**99**	12.0	**155**	0.00	**206**	0.11
45	0.01	**102**	0.00	**156/157**	0.01	**207**	0.02
46	0.08	**103**	0.00	**158**	0.12	**208**	0.01
48	0.05	**104**	0.00	**159**	0.02	**209**	0.12
49/69	0.59	**105**	1.60	**160**	0.00	**SUM**	69.7
50/53	0.09	**106**	0.01	**161**	0.00		

Table S3.6 Average lipid-adjusted PCB congener concentrations in **brain** of SAM-exposure group. Values are expressed in ng/g lipid weight.

PCB	Conc.	PCB	Conc.	PCB	Conc.	PCB	Conc.
1	0.35	**51**	0.10	**107**	2.69	**162**	0.29
2	0.46	**52**	15.0	**108/124**	0.50	**164**	0.18
3	0.76	**54**	0.03	**110**	17.4	**165**	0.03
4	1.47	**55**	0.94	**111**	0.03	**167**	0.30
5	0.15	**56**	0.53	**112**	1.28	**169**	0.07
6	0.47	**57**	0.04	**114**	2.70	**170**	0.25
7	0.18	**58**	0.02	**115**	5.93	**171/173**	0.19
8	0.71	**59/62/75**	1.27	**117**	6.70	**172**	0.37
9	0.14	**60**	22.3	**118**	46.7	**174**	0.16
10	0.65	**61/70/74/76**	98.3	**120**	0.03	**175**	1.25
11	2.07	**63**	1.43	**121**	0.02	**176**	0.04
12/13	0.91	**64**	2.09	**122**	0.16	**177**	0.15
14	0.00	**66**	115	**123**	1.37	**178**	0.19
15	0.65	**67**	0.06	**126**	0.04	**179**	0.08
16	1.54	**68**	1.29	**127**	0.04	**180/193**	0.70
17	0.49	**72**	0.20	**128/166**	0.00	**181**	0.42
18/30	2.25	**73**	0.38	**129/138/163**	11.5	**182**	1.43
19	8.15	**77**	0.09	**130**	0.86	**183**	0.60
20/28	9.53	**78**	0.09	**131**	0.06	**184**	1.02
21/33	1.15	**79**	0.02	**132**	0.80	**185**	0.17
22	0.36	**80**	0.04	**133**	0.19	**186**	0.04
23	0.17	**81**	0.03	**134**	0.09	**187**	1.22
24	7.35	**82**	1.02	**135/151**	0.70	**188**	0.50
25	0.25	**83**	0.92	**136**	0.23	**189**	0.92
26/29	0.64	**84**	0.86	**137**	1.25	**190**	0.42
27	1.81	**85/116**	33.7	**139/140**	1.02	**191**	0.16
31	12.0	**86/97/109/119**	11.2	**141**	0.59	**192**	0.14
32	0.39	**87/125**	9.11	**142**	0.05	**194**	0.15
34	2.27	**88**	0.05	**143**	0.04	**195**	0.14
35	0.38	**89**	0.04	**144**	0.15	**196**	0.09
36	0.05	**90/101/113**	56.7	**145**	0.03	**197**	0.47
37	0.30	**91**	1.05	**146**	1.84	**198/199**	0.24
38	0.12	**92**	8.31	**147/149**	3.30	**200**	0.31
39	0.05	**93/100**	0.37	**148**	0.04	**201**	0.10
40/71	1.09	**94**	0.03	**150**	0.02	**202**	0.42
41	0.09	**95**	4.85	**152**	0.03	**203**	0.07
42	0.65	**96**	0.03	**153/168**	10.6	**204**	0.00
43	0.10	**98**	0.03	**154**	0.34	**205**	0.06
44/47/65	18.3	**99**	130	**155**	0.02	**206**	1.17
45	0.09	**102**	0.04	**156/157**	0.13	**207**	0.23
46	0.83	**103**	0.03	**158**	1.35	**208**	0.15
48	0.54	**104**	0.02	**159**	0.20	**209**	1.28
49/69	6.42	**105**	17.5	**160**	0.04	**SUM**	760
50/53	0.94	**106**	0.08	**161**	0.03		

Table S3.7 Average PCB congener concentrations in **adipose tissue** of SAM-exposure group. Values are expressed in ng/g wet tissue weight.

PCB	Conc.	PCB	Conc.	PCB	Conc.	PCB	Conc.
1	0.06	**51**	0.05	**107**	12.5	**162**	0.04
2	0.07	**52**	147	**108/124**	0.77	**164**	0.76
3	0.12	**54**	0.00	**110**	130	**165**	0.01
4	0.15	**55**	0.02	**111**	0.01	**167**	0.64
5	0.02	**56**	4.18	**112**	0.00	**169**	0.01
6	0.13	**57**	0.01	**114**	7.79	**170**	0.78
7	0.06	**58**	0.00	**115**	24.9	**171/173**	0.66
8	0.46	**59/62/75**	6.13	**117**	6.67	**172**	0.21
9	0.05	**60**	129	**118**	226	**174**	0.40
10	0.03	**61/70/74/76**	585	**120**	0.01	**175**	0.06
11	0.26	**63**	9.46	**121**	0.00	**176**	0.03
12/13	0.14	**64**	16.1	**122**	0.01	**177**	0.99
14	0.00	**66**	738	**123**	6.02	**178**	0.27
15	0.10	**67**	0.08	**126**	0.01	**179**	0.04
16	0.11	**68**	0.21	**127**	0.01	**180/193**	1.76
17	0.16	**72**	0.16	**128/166**	0.00	**181**	0.13
18/30	1.14	**73**	0.05	**129/138/163**	93.3	**182**	0.09
19	0.04	**77**	0.01	**130**	4.72	**183**	1.38
20/28	56.2	**78**	0.01	**131**	0.37	**184**	0.09
21/33	0.10	**79**	0.01	**132**	6.78	**185**	0.02
22	0.06	**80**	0.00	**133**	1.23	**186**	0.01
23	0.03	**81**	0.00	**134**	0.52	**187**	1.60
24	0.02	**82**	9.14	**135/151**	5.76	**188**	0.08
25	0.13	**83**	0.02	**136**	0.55	**189**	0.02
26/29	0.24	**84**	3.34	**137**	6.77	**190**	0.14
27	0.07	**85/116**	187	**139/140**	7.35	**191**	0.05
31	4.76	**86/97/109/119**	61.3	**141**	3.95	**192**	0.02
32	0.35	**87/125**	52.5	**142**	0.01	**194**	0.19
34	0.04	**88**	0.01	**143**	0.02	**195**	0.09
35	0.01	**89**	0.09	**144**	1.38	**196**	0.09
36	0.01	**90/101/113**	355	**145**	0.01	**197**	0.08
37	0.62	**91**	6.59	**146**	11.0	**198/199**	0.29
38	0.02	**92**	57.9	**147/149**	28.5	**200**	0.04
39	0.01	**93/100**	1.88	**148**	0.07	**201**	0.05
40/71	3.58	**94**	0.04	**150**	0.01	**202**	0.22
41	0.12	**95**	28.3	**152**	0.01	**203**	0.15
42	3.96	**96**	0.02	**153/168**	65.4	**204**	0.00
43	0.24	**98**	0.03	**154**	2.62	**205**	0.01
44/47/65	126	**99**	633	**155**	0.01	**206**	0.19
45	0.23	**102**	0.33	**156/157**	1.87	**207**	0.04
46	0.13	**103**	0.20	**158**	10.5	**208**	0.07
48	2.50	**104**	0.00	**159**	0.03	**209**	0.25
49/69	49.6	**105**	86.8	**160**	0.09	**SUM**	4050
50/53	0.45	**106**	0.01	**161**	0.02		

Table S3.8 Average lipid-adjusted PCB congener concentrations in **adipose tissue** of SAM-exposure group. Values are expressed in ng/g lipid weight.

PCB	Conc.	PCB	Conc.	PCB	Conc.	PCB	Conc.
1	0.07	**51**	0.06	**107**	15.8	**162**	0.05
2	0.09	**52**	184	**108/124**	0.96	**164**	0.95
3	0.15	**54**	0.00	**110**	164	**165**	0.01
4	0.18	**55**	0.02	**111**	0.01	**167**	0.80
5	0.03	**56**	5.22	**112**	0.01	**169**	0.01
6	0.16	**57**	0.01	**114**	9.83	**170**	0.97
7	0.07	**58**	0.00	**115**	32.1	**171/173**	0.82
8	0.57	**59/62/75**	7.68	**117**	8.85	**172**	0.25
9	0.07	**60**	161	**118**	285	**174**	0.50
10	0.04	**61/70/74/76**	732	**120**	0.01	**175**	0.07
11	0.32	**63**	11.8	**121**	0.00	**176**	0.03
12/13	0.18	**64**	20.1	**122**	0.01	**177**	1.22
14	0.00	**66**	923	**123**	7.61	**178**	0.34
15	0.13	**67**	0.10	**126**	0.01	**179**	0.04
16	0.13	**68**	0.25	**127**	0.01	**180/193**	2.18
17	0.20	**72**	0.20	**128/166**	0.00	**181**	0.17
18/30	1.44	**73**	0.07	**129/138/163**	117	**182**	0.11
19	0.05	**77**	0.02	**130**	5.90	**183**	1.72
20/28	70.8	**78**	0.02	**131**	0.48	**184**	0.12
21/33	0.12	**79**	0.01	**132**	8.56	**185**	0.02
22	0.07	**80**	0.00	**133**	1.55	**186**	0.01
23	0.03	**81**	0.01	**134**	0.67	**187**	1.98
24	0.02	**82**	11.4	**135/151**	7.22	**188**	0.10
25	0.17	**83**	0.02	**136**	0.70	**189**	0.03
26/29	0.30	**84**	4.23	**137**	8.46	**190**	0.17
27	0.09	**85/116**	235	**139/140**	9.29	**191**	0.06
31	5.98	**86/97/109/119**	77.0	**141**	4.94	**192**	0.02
32	0.43	**87/125**	66.5	**142**	0.01	**194**	0.23
34	0.05	**88**	0.01	**143**	0.02	**195**	0.11
35	0.01	**89**	0.11	**144**	1.75	**196**	0.11
36	0.01	**90/101/113**	446	**145**	0.01	**197**	0.10
37	0.78	**91**	8.30	**146**	13.7	**198/199**	0.36
38	0.02	**92**	73.2	**147/149**	36.1	**200**	0.05
39	0.01	**93/100**	2.36	**148**	0.09	**201**	0.06
40/71	4.48	**94**	0.05	**150**	0.01	**202**	0.26
41	0.17	**95**	35.7	**152**	0.01	**203**	0.19
42	4.94	**96**	0.02	**153/168**	82.0	**204**	0.00
43	0.28	**98**	0.03	**154**	3.29	**205**	0.01
44/47/65	158	**99**	801	**155**	0.01	**206**	0.23
45	0.29	**102**	0.42	**156/157**	2.33	**207**	0.05
46	0.16	**103**	0.25	**158**	13.2	**208**	0.08
48	3.13	**104**	0.01	**159**	0.04	**209**	0.30
49/69	62.3	**105**	109	**160**	0.12	**SUM**	5090
50/53	0.57	**106**	0.01	**161**	0.02		

Table S3.9 Average PCB congener concentrations in **serum** of SAM-exposure group. Values are expressed in ng/g wet weight.

PCB	Conc.	PCB	Conc.	PCB	Conc.	PCB	Conc.
1	0.03	**51**	0.01	**107**	0.01	**162**	0.02
2	0.04	**52**	0.49	**108/124**	0.00	**164**	0.00
3	0.07	**54**	0.00	**110**	0.25	**165**	0.00
4	0.09	**55**	0.02	**111**	0.00	**167**	0.03
5	0.01	**56**	0.05	**112**	0.00	**169**	0.00
6	0.03	**57**	0.00	**114**	0.00	**170**	0.01
7	0.01	**58**	0.00	**115**	0.00	**171/173**	0.01
8	0.07	**59/62/75**	0.03	**117**	0.00	**172**	0.02
9	0.01	**60**	0.29	**118**	0.59	**174**	0.01
10	0.02	**61/70/74/76**	0.93	**120**	0.00	**175**	0.01
11	0.15	**63**	0.02	**121**	0.00	**176**	0.00
12/13	0.09	**64**	0.06	**122**	0.00	**177**	0.01
14	0.00	**66**	1.16	**123**	0.01	**178**	0.01
15	0.06	**67**	0.01	**126**	0.00	**179**	0.01
16	0.06	**68**	0.12	**127**	0.00	**180/193**	0.02
17	0.05	**72**	0.01	**128/166**	0.00	**181**	0.01
18/30	0.08	**73**	0.03	**129/138/163**	0.10	**182**	0.01
19	0.03	**77**	0.01	**130**	0.00	**183**	0.01
20/28	0.11	**78**	0.01	**131**	0.00	**184**	0.01
21/33	0.06	**79**	0.00	**132**	0.02	**185**	0.01
22	0.03	**80**	0.00	**133**	0.01	**186**	0.04
23	0.02	**81**	0.00	**134**	0.01	**187**	0.02
24	0.01	**82**	0.02	**135/151**	0.03	**188**	0.02
25	0.02	**83**	0.01	**136**	0.02	**189**	0.01
26/29	0.06	**84**	0.07	**137**	0.01	**190**	0.02
27	0.04	**85/116**	0.46	**139/140**	0.01	**191**	0.01
31	0.08	**86/97/109/119**	0.10	**141**	0.01	**192**	0.01
32	0.04	**87/125**	0.20	**142**	0.00	**194**	0.01
34	0.02	**88**	0.01	**143**	0.00	**195**	0.01
35	0.01	**89**	0.00	**144**	0.00	**196**	0.01
36	0.01	**90/101/113**	1.15	**145**	0.00	**197**	0.04
37	0.03	**91**	0.02	**146**	0.01	**198/199**	0.01
38	0.01	**92**	0.17	**147/149**	0.09	**200**	0.02
39	0.00	**93/100**	0.00	**148**	0.00	**201**	0.00
40/71	0.08	**94**	0.00	**150**	0.00	**202**	0.04
41	0.01	**95**	0.17	**152**	0.00	**203**	0.00
42	0.03	**96**	0.00	**153/168**	0.14	**204**	0.00
43	0.01	**98**	0.00	**154**	0.01	**205**	0.01
44/47/65	0.29	**99**	1.49	**155**	0.00	**206**	0.11
45	0.01	**102**	0.00	**156/157**	0.01	**207**	0.02
46	0.08	**103**	0.00	**158**	0.00	**208**	0.01
48	0.02	**104**	0.00	**159**	0.02	**209**	0.12
49/69	0.29	**105**	0.28	**160**	0.00	**SUM**	12.0
50/53	0.09	**106**	0.00	**161**	0.00		

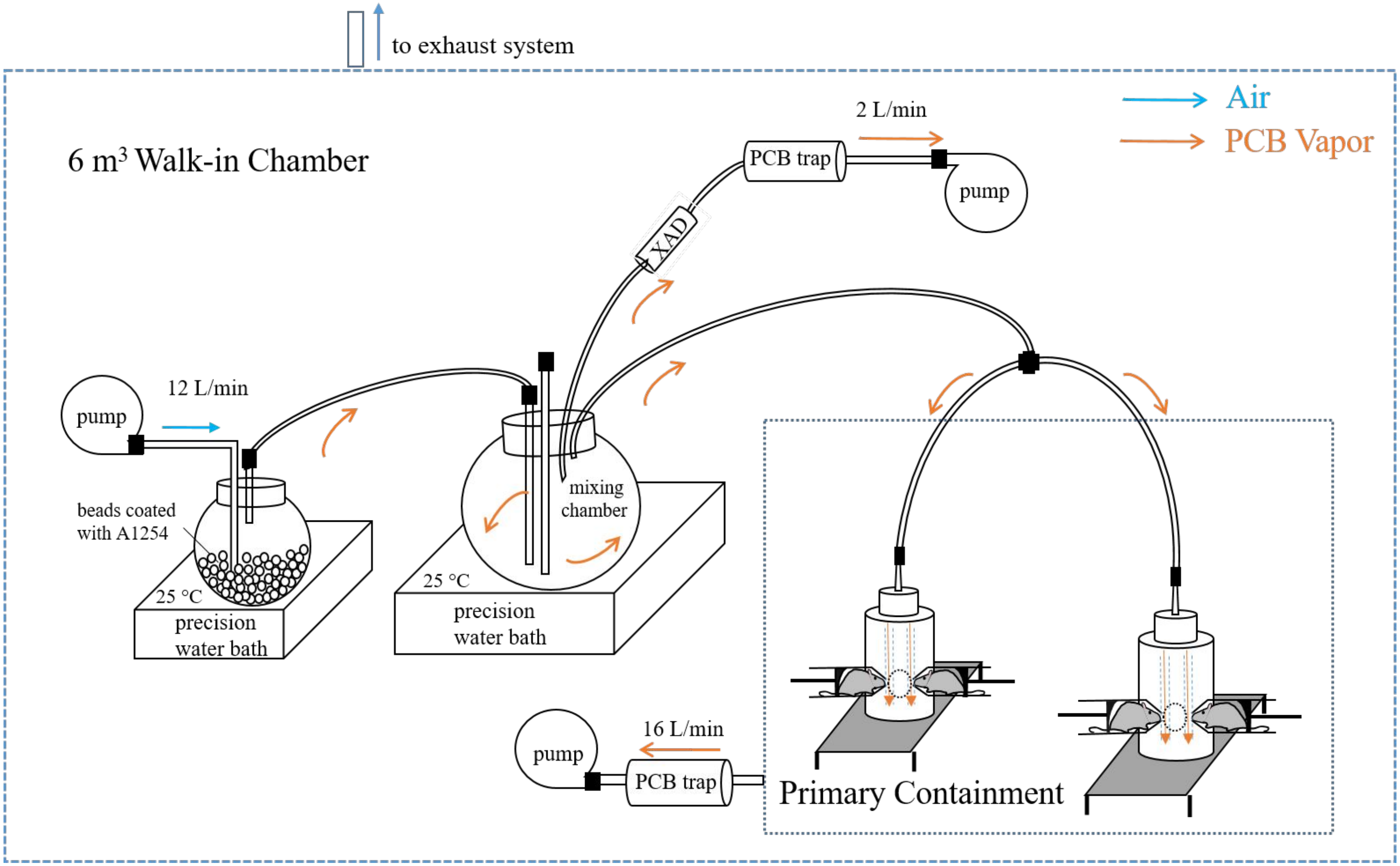
Table S3.10 Average lipid-adjusted PCB congener concentrations in **serum** of SAM-exposure group. Values are expressed in ng/g lipid weight.

PCB	Conc.	PCB	Conc.	PCB	Conc.	PCB	Conc.
1	9.38	**51**	2.73	**107**	2.77	**162**	6.66
2	12.4	**52**	137	**108/124**	1.06	**164**	0.69
3	20.2	**54**	0.61	**110**	71.3	**165**	0.69
4	24.5	**55**	5.81	**111**	0.79	**167**	7.10
5	2.99	**56**	12.9	**112**	0.58	**169**	1.37
6	9.56	**57**	1.03	**114**	0.35	**170**	3.20
7	4.10	**58**	0.50	**115**	0.74	**171/173**	3.18
8	18.8	**59/62/75**	8.77	**117**	0.67	**172**	4.60
9	2.89	**60**	81.1	**118**	165	**174**	3.29
10	5.66	**61/70/74/76**	260	**120**	0.87	**175**	3.01
11	43.2	**63**	4.46	**121**	0.56	**176**	0.96
12/13	24.1	**64**	17.7	**122**	1.34	**177**	2.65
14	0.00	**66**	322	**123**	1.80	**178**	1.80
15	17.3	**67**	1.60	**126**	0.00	**179**	2.05
16	17.7	**68**	34.31	**127**	0.74	**180/193**	4.72
17	13.1	**72**	3.07	**128/166**	0.00	**181**	2.50
18/30	22.8	**73**	9.13	**129/138/163**	29.3	**182**	2.60
19	7.08	**77**	2.50	**130**	1.22	**183**	2.54
20/28	32.2	**78**	2.45	**131**	1.15	**184**	2.58
21/33	16.2	**79**	0.58	**132**	6.41	**185**	2.86
22	9.53	**80**	0.50	**133**	1.59	**186**	9.72
23	4.43	**81**	1.05	**134**	1.43	**187**	7.02
24	3.11	**82**	6.65	**135/151**	8.36	**188**	4.54
25	6.58	**83**	3.23	**136**	6.10	**189**	3.60
26/29	17.0	**84**	20.5	**137**	3.72	**190**	6.96
27	11.9	**85/116**	127	**139/140**	3.80	**191**	2.68
31	22.7	**86/97/109/119**	28.4	**141**	3.28	**192**	2.59
32	10.5	**87/125**	56.7	**142**	1.17	**194**	4.08
34	6.62	**88**	1.41	**143**	0.99	**195**	2.52
35	1.63	**89**	0.96	**144**	0.97	**196**	1.77
36	1.41	**90/101/113**	322	**145**	0.65	**197**	10.9
37	7.88	**91**	6.04	**146**	2.70	**198/199**	2.83
38	2.73	**92**	47.8	**147/149**	23.9	**200**	6.69
39	1.27	**93/100**	1.31	**148**	1.21	**201**	1.19
40/71	21.2	**94**	0.93	**150**	0.94	**202**	11.1
41	2.27	**95**	48.9	**152**	0.67	**203**	0.99
42	8.85	**96**	0.76	**153/168**	39.4	**204**	0.00
43	1.47	**98**	0.88	**154**	1.62	**205**	1.68
44/47/65	81.9	**99**	416	**155**	0.64	**206**	31.1
45	1.95	**102**	1.17	**156/157**	3.37	**207**	5.18
46	22.2	**103**	0.78	**158**	1.11	**208**	3.25
48	6.23	**104**	0.64	**159**	5.27	**209**	34.0
49/69	82.2	**105**	78.2	**160**	0.63	**SUM**	3350
50/53	25.1	**106**	0.78	**161**	0.68		

Table S4. Redox parameters and redox potentials (E_h) in plasma.

	CySS (μM)	Cys (μM)	CySSG (μM)	GSH (μM)	GSSG (μM)	E_h (GSSG/GSH) (mV)	E_h (CySS/Cys) (mV)	Total GSH (μM)	Total Cys (μM)
SAM	62.6 ± 9.5	29.4 ± 7.3	25.1 ± 6.9	28.0 ± 8.0	4.8 ± 2.2	-152.1 ± 13.5	-103.7 ± 5.7	62.6 ± 16.3	179.6 ± 25.1
Sham	64.4 ± 12.6	28.3 ± 3.5	25.3 ± 9.9	25.1 ± 4.0	5.2 ± 3.8	-148.1 ± 6.4	-102.9 ± 4.5	60.9 ± 19.5	182.4 ± 28.4

Values are expressed as mean ± standard deviation (n = 8). CySS: cystine; Cys: cysteine; CySSG: cysteine-glutathione disulfide; GSH: glutathione; GSSG: glutathione disulfide. E_h were calculated with the Nernst equation for pH 7.4: E_h (GSSG/GSH) = -264 + 30 * log (GSSG / GSH^2); Eh (CySS / Cys) = -250 + 30 * log (CySS / Cys^2).[19]



248

249 **Figure S1.** Schematic of PCB generation and nose-only exposure system.

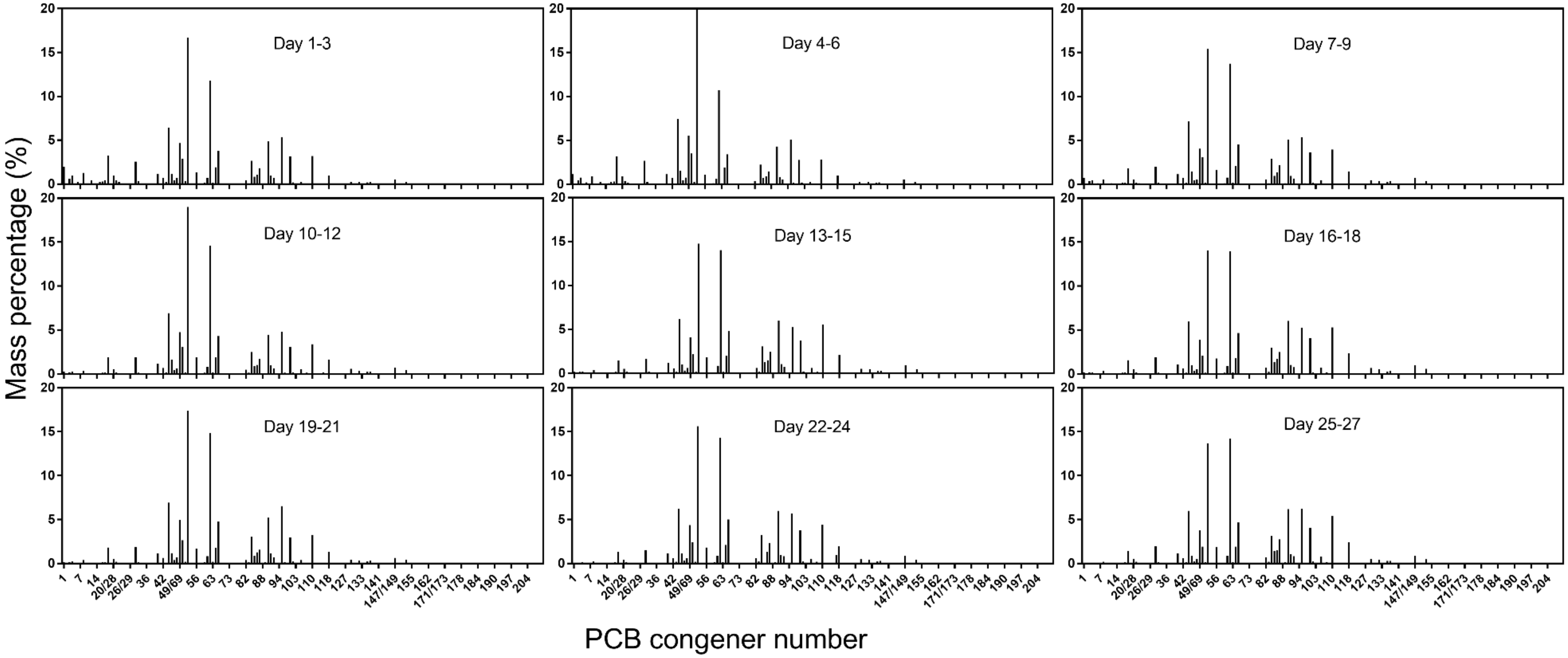


Figure S2. PCB congener profile (in mass percentage, %) in sequential three-day collections of PCB vapor using XAD.

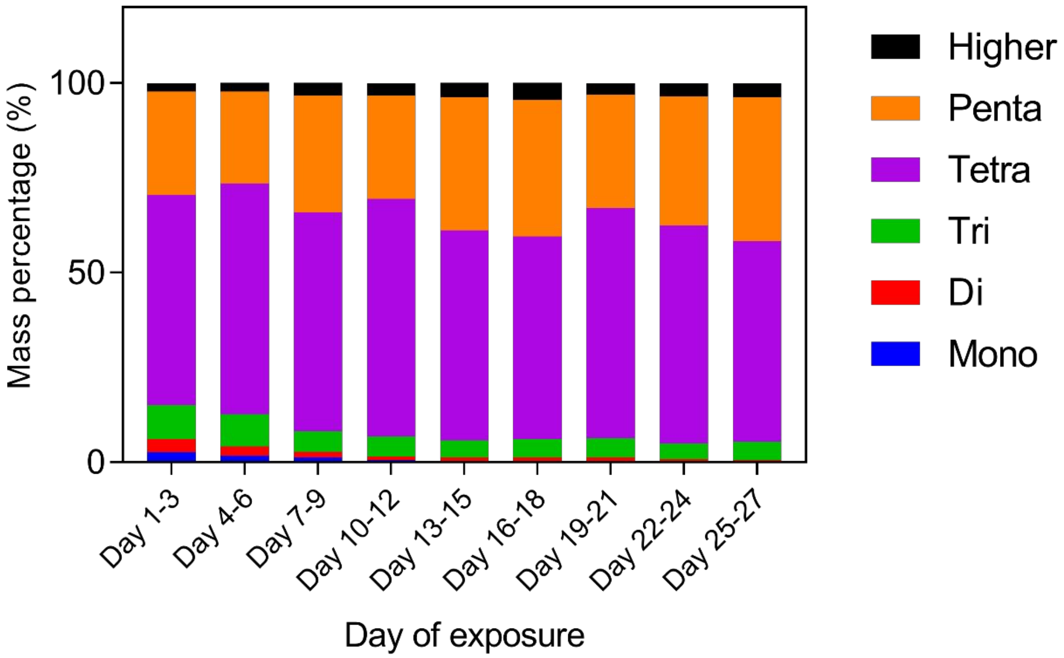


Figure S3. PCB homologue composition (in mass percentage, %) in sequential three-day collections of PCB vapor using XAD.

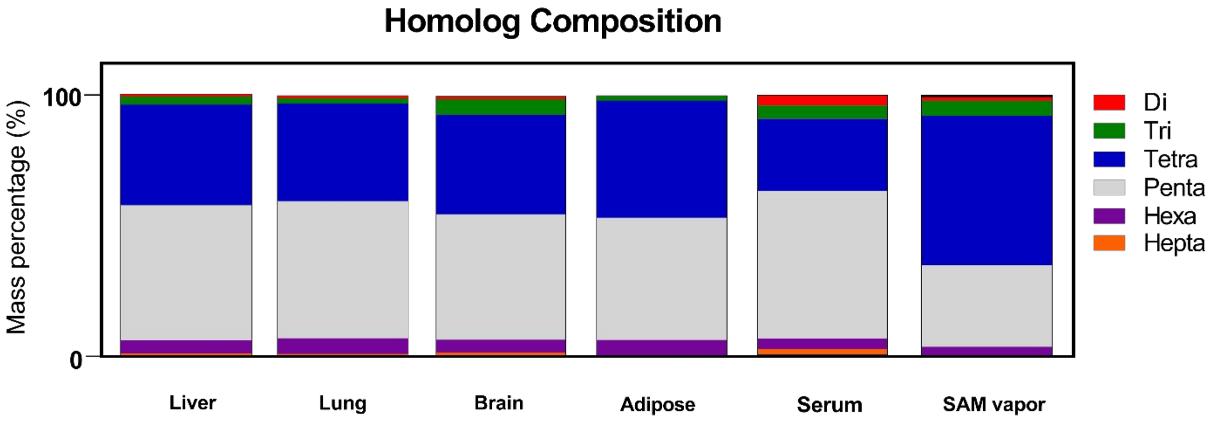


Figure S4. PCB homolog composition (in mass percentage, %) in tissues of SAM-exposed rats and in generated SAM vapor.

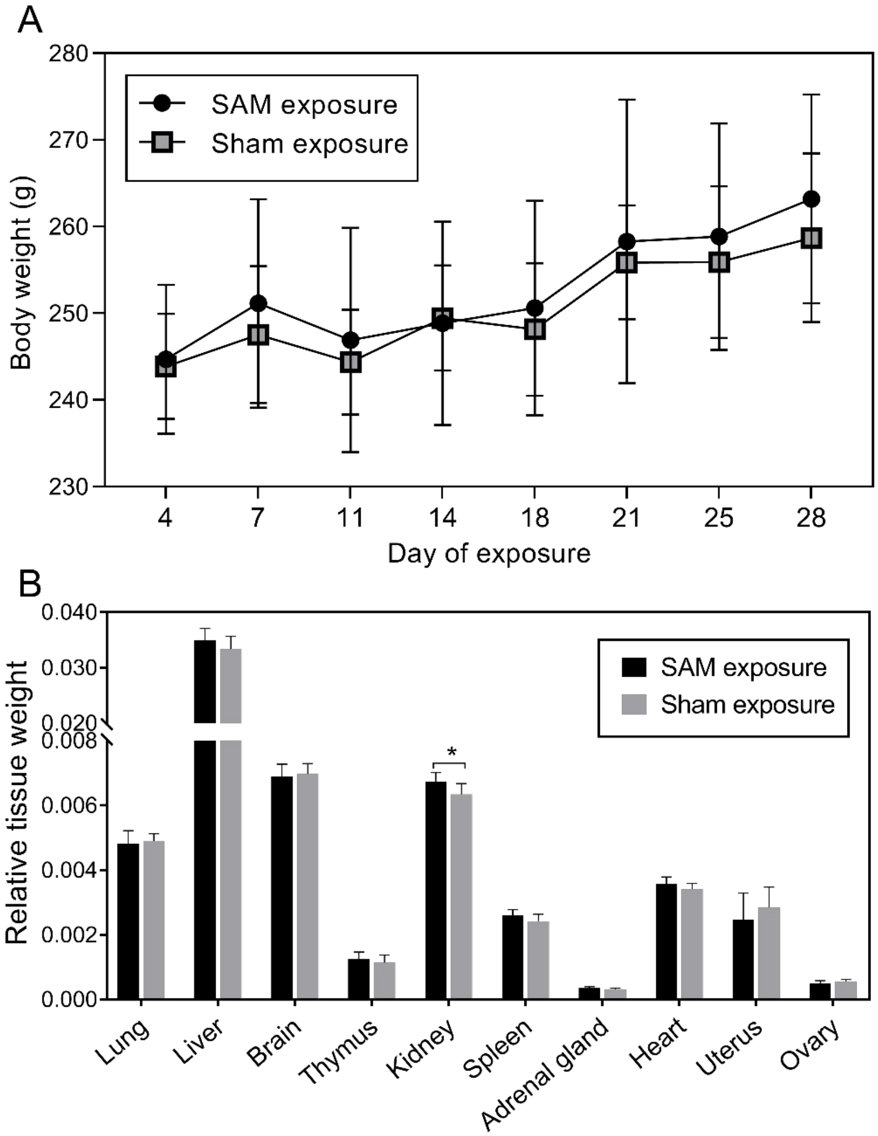


Figure S5. Body weight (S5A, g) and relative tissue weight (S5B, g/g body weight) of rats in SAM- and sham-exposed groups. No significant differences were found between the groups in body weight. Relative kidney weight in SAM was significantly higher than sham (*$p < 0.05$, Student's t-test). Values were expressed as mean ± standard deviation (n = 8).

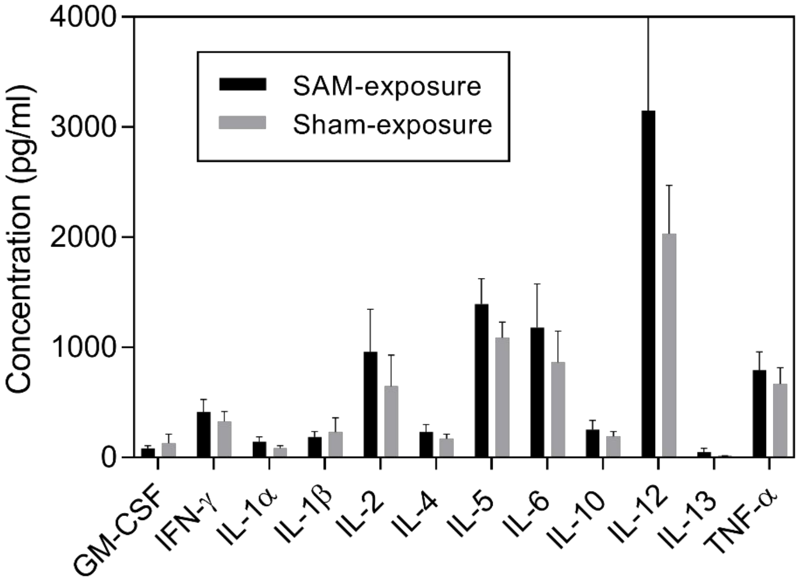


Figure S6. Cytokine concentrations (pg/ml) in plasma from rats in sham- and SAM-exposure groups. Values are expressed as mean ± standard error (n = 5). No significant differences were found between sham and SAM rats.

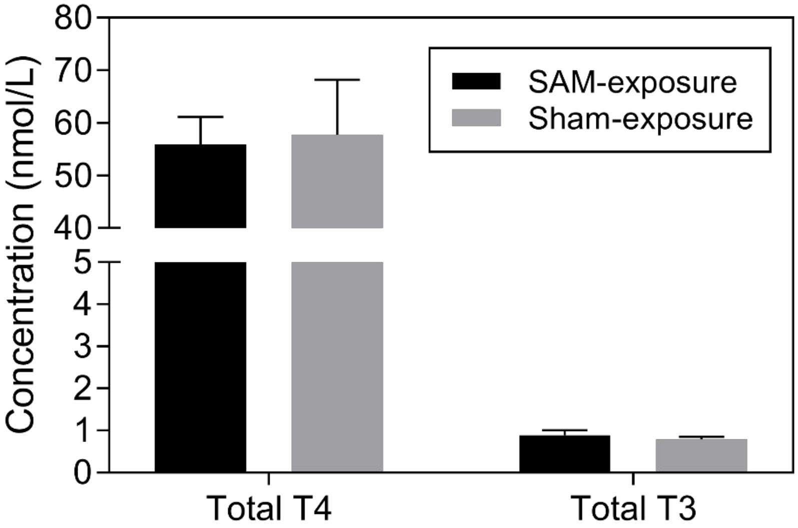


Figure S7. Thyroid hormone (total T4 and total T3) concentrations (nmol/L) in serum after exposed to SAM vapor. Values are graphed as mean ± standard deviation (n = 8). There were no significant differences between sham and SAM rats.

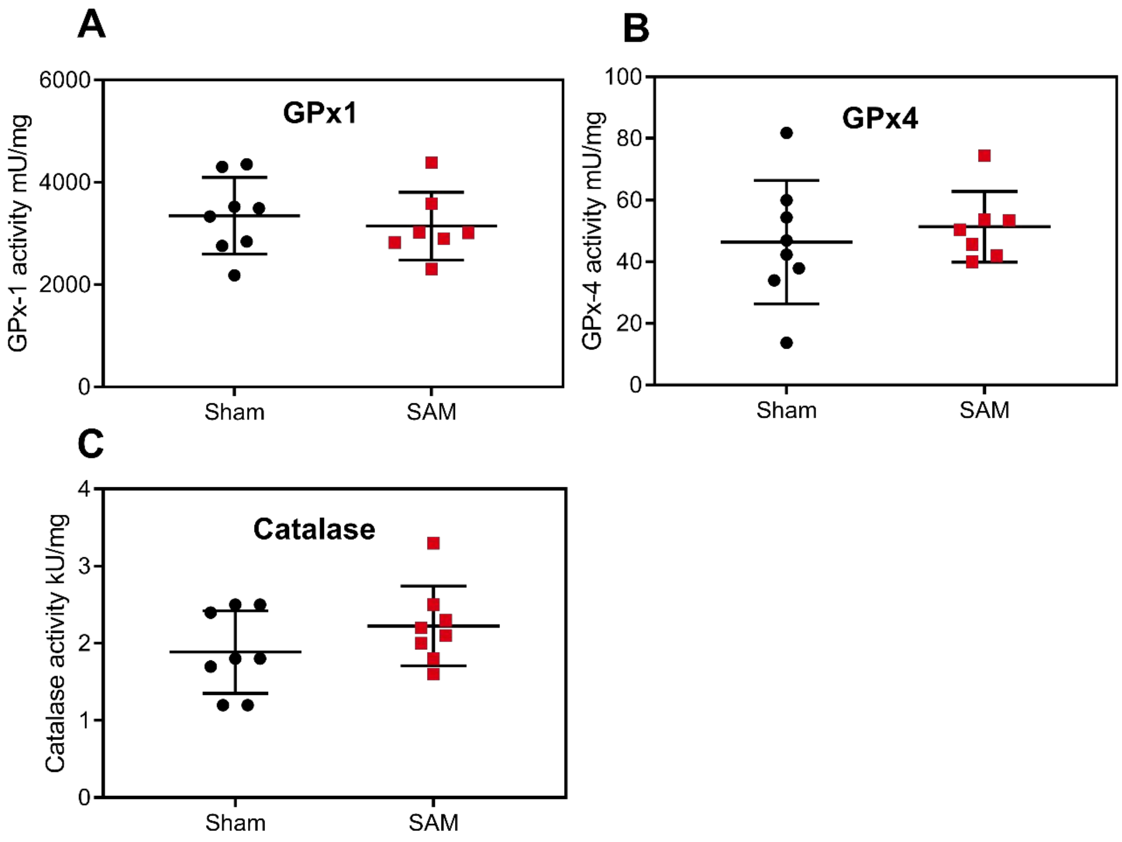


Figure S8. Antioxidant enzyme activities in liver. A: GPx1, B: GPx4, and C: catalase activity. Activities were normalized by protein content. Each data point represents the mean of three replicates of one sample of liver tissue. Values are expressed as mean ± standard deviation (n = 8 for sham, n = 7 for SAM). No significant differences were found between sham and SAM rats (*p* >0.05).

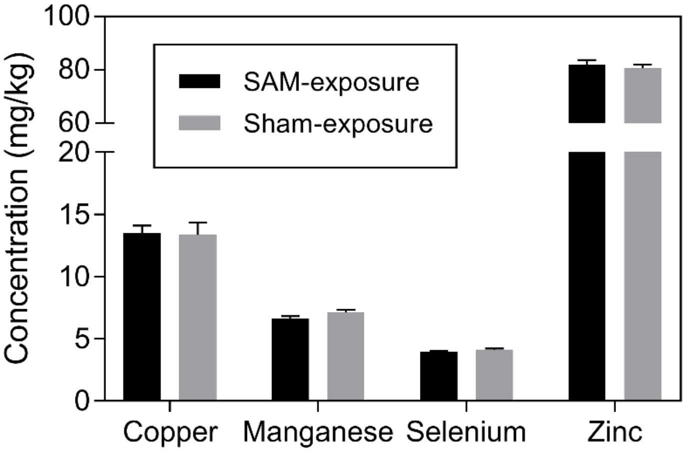


Figure S9. Trace elements content (Cu, Mn, Se, and Zn) in dried liver from rats exposed to SAM and sham. Values are expressed as mean ± standard error (n = 6). None of the trace elements were significantly different between SAM and sham exposed rats.

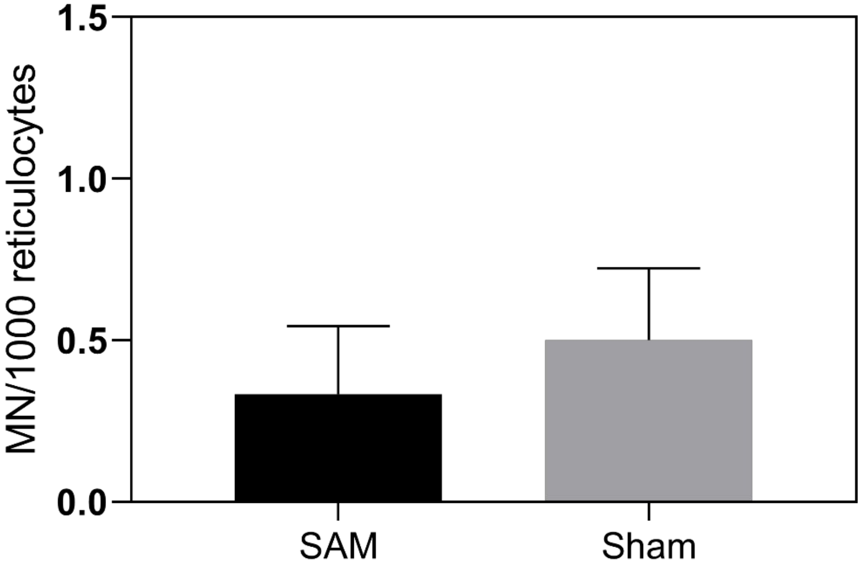


Figure S10. Micronuclei (MN) number expressed as per 1000 reticulocytes (immature erythrocytes) from acridine orange-coated slides in SAM-exposed and sham-exposed group. Values are graphed as mean ± standard error (n = 6). No significant differences were found between sham and SAM rats.

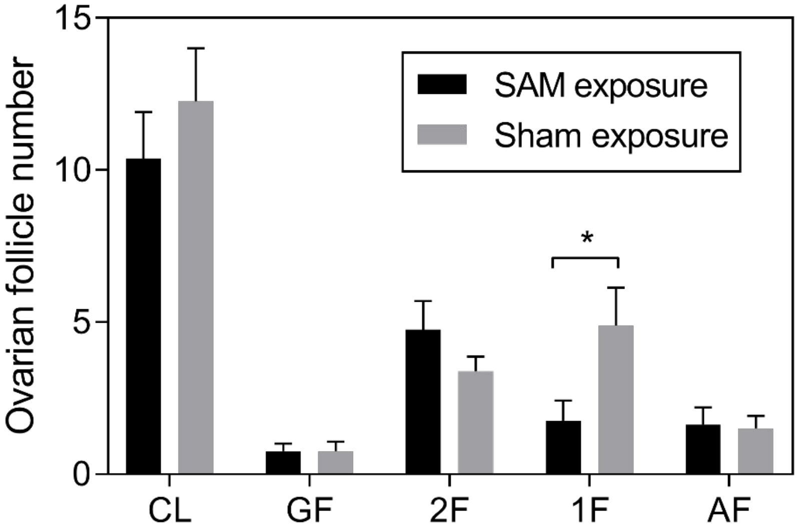


Figure S11. Ovarian follicle number in a single section from SAM- and sham-exposed rats. Values are expressed as mean ± standard error (n = 7). *$p < 0.05$, compared to sham. CL: corpus luteum; GF: graafian follicle; 2F: secondary follicle; 1F: primary follicle; AF: atretic follicle.

REFERENCES

1. USEPA, Method 1668, revision A: Chlorinated biphenyl congeners in waters, soil, sediment, and tissue by HRGC/HRMS. In United States Environmental Protection Agency Office of Water: Washington, DC: 1999.

2. Marek, R. F.; Thorne, P. S.; Herkert, N. J.; Awad, A. M.; Hornbuckle, K. C., Airborne PCBs and OH-PCBs Inside and Outside Urban and Rural U.S. Schools. *Environ Sci Technol* **2017,** *51*, (14), 7853-7860.

3. Phillips, D. L.; Pirkle, J. L.; Burse, V. W.; Bernert, J. T.; Henderson, L. O.; Needham, L. L., Chlorinated hydrocarbon levels in human serum: effects of fasting and feeding. *Archives of environmental contamination toxicology* **1989,** *18*, (4), 495-500.

4. Vorhees, C. V.; Williams, M. T., Morris water maze: procedures for assessing spatial and related forms of learning and memory. *Nat Protoc* **2006,** *1*, (2), 848-58.

5. Lowry, O. H.; Rosebrough, N. J.; Farr, A. L.; Randall, R. J., Protein measurement with the Folin phenol reagent. *The Journal of biological chemistry* **1951,** *193*, (1), 265-75.

6. Lawrence, R. A.; Burk, R. F., Glutathione peroxidase activity in selenium-deficient rat liver. *Biochem Biophys Res Commun* **1976,** *71*, (4), 952-8.

7. Greenwald, R. A., *CRC handbook of methods for oxygen radical research*. CRC Press: Boca Raton, FL, 1985; p 447.

8. Stolwijk, J. M.; Falls-Hubert, K. C.; Searby, C. C.; Wagner, B. A.; Buettner, G. R., Simultaneous detection of the enzyme activities of GPx1 and GPx4 guide optimization of selenium in cell biological experiments. *Redox Biol* **2020,** *32*, 101518.

9. Beers, R. F., Jr.; Sizer, I. W., A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. *The Journal of biological chemistry* **1952,** *195*, (1), 133-40.

10. Aebi, H., Catalase in vitro. *Methods Enzymol* **1984,** *105*, 121-6.

11. Nelson, D. P.; Kiesow, L. A., Enthalpy of decomposition of hydrogen peroxide by catalase at 25C (with molar extinction coefficients of H2O2 solutions in the UV). *Analytical biochemistry* **1972,** *49*, (2), 474-8.

12. Hayashi, M.; Morita, T.; Kodama, Y.; Sofuni, T.; Ishidate, M., Jr., The micronucleus assay with mouse peripheral blood reticulocytes using acridine orange-coated slides. *Mutat Res* **1990,** *245*, (4), 245-9.

13. Zhang, X.; Goncalves, R.; Mosser, D. M., The isolation and characterization of murine macrophages. *Curr Protoc Immunol* **2008,** *83*, (1), 14.1.1-14.1.14.

14. Banerjee, P. P.; Banerjee, S.; Zirkin, B. R.; Brown, T. R., Telomerase activity in normal adult Brown Norway rat seminal vesicle: regional distribution and age-dependent changes. *Endocrinology* **1998,** *139*, (3), 1075-81.

15. Li, X.; Li, Y.; Kang, X.; Guo, K.; Li, H.; Gao, D.; Sun, L.; Liu, Y., Dynamic alteration of protein expression profiles during neoplastic transformation of rat hepatic oval-like cells. *Cancer science* **2010,** *101*, (5), 1099-107.

16. Denner, D. R.; Sangwan, N.; Becker, J. B.; Hogarth, D. K.; Oldham, J.; Castillo, J.; Sperling, A. I.; Solway, J.; Naureckas, E. T.; Gilbert, J. A.; White, S. R., Corticosteroid therapy and airflow obstruction influence the bronchial microbiome, which is distinct from that of bronchoalveolar lavage in asthmatic airways. *J Allergy Clin Immunol* **2016,** *137*, (5), 1398-1405.

17. Caporaso, J. G.; Kuczynski, J.; Stombaugh, J.; Bittinger, K.; Bushman, F. D.; Costello, E. K.; Fierer, N.; Pena, A. G.; Goodrich, J. K.; Gordon, J. I.; Huttley, G. A.; Kelley, S. T.; Knights, D.; Koenig, J. E.; Ley, R. E.; Lozupone, C. A.; McDonald, D.; Muegge, B. D.; Pirrung, M.; Reeder, J.; Sevinsky, J. R.; Turnbaugh, P. J.; Walters, W. A.; Widmann, J.; Yatsunenko, T.;

Zaneveld, J.; Knight, R., QIIME allows analysis of high-throughput community sequencing data. *Nat Methods* **2010,** *7*, (5), 335-6.

18. Arndt, D.; Xia, J.; Liu, Y.; Zhou, Y.; Guo, A. C.; Cruz, J. A.; Sinelnikov, I.; Budwill, K.; Nesbo, C. L.; Wishart, D. S., METAGENassist: a comprehensive web server for comparative metagenomics. *Nucleic Acids Res* **2012,** *40*, (W1), W88-95.

19. Jones, D. P.; Carlson, J. L.; Mody, V. C.; Cai, J.; Lynn, M. J.; Sternberg, P., Redox state of glutathione in human plasma. *Free Radic Biol Med* **2000,** *28*, (4), 625-35.