GAS CHROMATOGRAPHIC ANALYSIS WITH CHIRAL CYCLODEXTRIN PHASES REVEALS THE ENANTIOSELECTIVE FORMATION OF HYDROXYLATED POLYCHLORINATED BIPHENYLS BY RAT LIVER MICROSOMES

SUPPORTING INFORMATION

Izabela Kania-Korwel[§], *Michael W. Duffel*[‡], *Hans-Joachim Lehmler*[§]*

[§] Department of Occupational and Environmental Health, College of Public Health, University of Iowa, Iowa City, Iowa 52242

[‡] Department of Pharmaceutical Sciences and Experimental Therapeutics, College of Pharmacy, University of Iowa, Iowa City, Iowa 52242

RECEIVED DATE

Corresponding Author: Dr. Hans-Joachim Lehmler, The University of Iowa,

Department of Occupational and Environmental Health,

University of Iowa Research Park, #221 IREH, Iowa City, IA 52242-5000

Phone: (319) 335-4211, Fax: (319) 335-4290, e-mail: hans-joachim-lehmler@uiowa.edu

INDEX:

Animal treatment and preparation of microsomes

Experiments involving animals were approved by the Institutional Animal Care and Use Committee at the University of Iowa. Six Sprague-Dawley rats were purchased from Harlan, Inc. (Indianapolis, IN, USA) and allowed to acclimatize for one week. The animals received intraperitoneal injections of phenobarbital (3 doses on consecutive days, 102 mg/kg b.w./day in saline). Rats were euthanized by CO_2 asphyxiation followed by cervical dislocation 24 h after the last phenobarbital treatment. Livers were excised *en bloc* and immediately placed in ice-cold 0.05 M Tris-HCl buffer (pH 7.5) containing 0.15 M KCl. The liver microsomes were isolated by differential centrifugation as described previously (1, 2). In short, liver homogenates were prepared from individual livers from each treatment groups and centrifuged at 9,000 g for 20 min at 5˚C. The supernatant was centrifuged at 100,000 g for 60 min at 5˚C to separate cytosol from the pellet. The pellet was subsequently re-suspended in EDTA-KCl buffer and centrifuged again at 100,000 g for 60 min at 5˚C. The pooled microsomal pallet from this separation was re-suspended in 0.25 M sucrose and ~1 mL aliquots were stored at -80°C.

Cytochrome P450 enzyme activities

The protein levels in microsomes were measured using Lowry method (3), using bovine serum albumin as standard. The activity of cytochrome P450 isoforms in the liver microsomes were obtained using 7-ethoxyresorufin-O-deethylase (EROD), 7-benzyloxyresorufin-O-debenzylase (BROD) and 7-benzyloxyquinoline debenzylation (BQ) activity using established methods (4, 5). The activities were 1300, 13100 and 15800 pmol/min/mg protein for EROD, BROD and BQ respectively.

Table S1.A. Structure, IUPAC name, abbreviation and resolution of methoxylated polychlorinated biphenyls on different enantioselective columns and using different temperature programs[1,2].

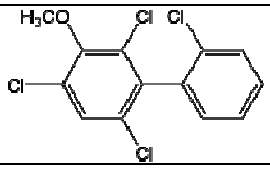
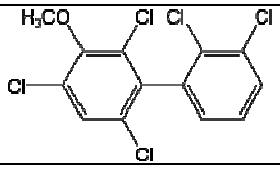
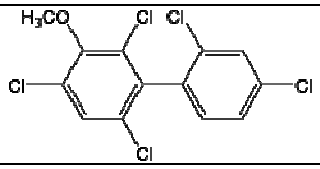
A.		3-Methoxy-2,2',4,6-tetrachlorobiphenyl **(3-50)**[3]				
Column	Programmed temperature	Isothermal				
		140°C	150°C	160°C	170°C	180°C
20B	nr (95.5)					
BGB	nr (89.3)					
BDM	nr (75.4)					
BPM	nr (70.3)					
CB	nr (86.0)					
CD	nr (69.9)					
GTA	nr (77.1)					
B.		3-Methoxy-2,2',3',4,6-pentachlorobiphenyl **(3-98)**[3]				
Column	Programmed temperature	Isothermal				
		140°C	150°C	160°C	170°C	180°C
20B	nr (112.5)					
BGB	nr (106.3)					
BDM	0.55 (92.0)	0.81 (224.1)	0.68 (129.9)	0.60 (80.4)	0.49 (53.6)	0.25 (38.8)
BPM	nr (113.5)					
CB	0.58 (102.9)	0.85 (388.4)	0.73 (220.4)	0.71 (132.1)	0.57 (84.0)	0.46 (57.2)
CD	0.36 (84.6)	0.64 (169.3)	0.49 (98.1)	0.29 (61.5)	0.10 (42.0)	nr (31.3)
GTA	0.70 (109.9)	0.97 (244.4)	0.75 (142.5)	0.73 (88.3)	0.67 (58.6)	0.41 (42.0)
C.		3-Methoxy-2,2',4,4',6-pentachlorobiphenyl **(3-100)**[3]				
Column	Programmed temperature	Isothermal				
		140°C	150°C	160°C	170°C	180°C
20B	nr (108.9)					
BGB	nr (105.0)					
BDM	nr (90.1)					
BPM	nr (83.8)					
CB	nr (100.4)					
CD	nr (82.3)					
GTA	0.60 (103.0)	0.77 (215.0)	0.60 (124.5)	0.52 (79.0)	0.29 (53.1)	nr (38.7)

Table S1.B. Structure, IUPAC name, abbreviation and resolution of methoxylated polychlorinated biphenyls on different enantioselective columns and using different temperature programs[1,2].

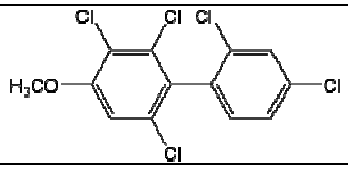
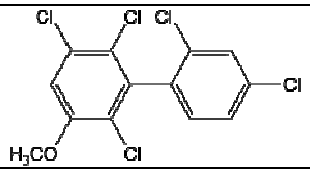
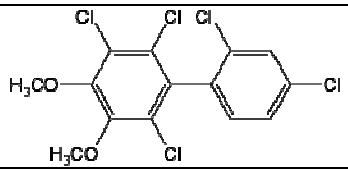
D.		4-Methoxy-2,2',3,4',6-pentachlorobiphenyl **(4-91)**[3]				
Column	Programmed temperature	Isothermal				
		140°C	150°C	160°C	170°C	180°C
20B	nr (125.8)	na	na	nr	nr	nr
BGB	nr (116.6)					
BDM	nr (106.2)	0.79 (404.7)	0.71 (213.8)	0.62 (131.0)	nr (81.6)	nr (54.8)
BPM	nr (100.4)					
CB	nr (114.0)					
CD	nr (94.2)					
GTA	nr (155.3)					
E.		3-Methoxy-2,2',4',5,6-pentachlorobiphenyl **(5-91)**[3]				
Column	Programmed temperature	Isothermal				
		140°C	150°C	160°C	170°C	180°C
20B	nr (124.8)	na	na	0.94 (347.0)	0.78 (205.2)	0.59 (127.6)
BGB	0.75 (116.8)	na	0.83 (416.1)	0.90 (252.1)	0.76 (152.9)	0.68 (97.8)
BDM	2.1 (105.5)	3.7 (426.9)	3.3 (232.5)	2.1 (134.3)	1.5 (82.9)	1.4 (55.3)
BPM	0.50 (99.3)	1.3 (331.4)	0.84 (184.5)	0.77 (109.2)	0.54 (69.2)	0.46 (47.5)
CB	1.3 (113.3)	na	3.3 (398.8)	2.8 (230.3)	1.8 (137.4)	1.4 (87.1)
CD	0.97 (93.9)	3.0 (309.8)	1.9 (167.5)	1.4 (97.1)	0.97 (61.0)	0.75 (41.9)
GTA	nr (146.5)	na	na	nr (131.6)	nr (82.2)	nr (55.3)
F.		3,4-Dimethoxy-2,2',4',5,6-pentachlorobiphenyl **(4,5-91)**[3]				
Column	Programmed temperature	Isothermal				
		140°C	150°C	160°C	170°C	180°C
20B	nr (126.5)					
BGB	nr (119.0)					
BDM	nr (106.5)					
BPM	nr (101.0)					
CB	nr (114.6)					
CD	nr (95.5)					
GTA	nr (151.9)					

Table S1.C. Structure, IUPAC name, abbreviation and resolution of methoxylated polychlorinated biphenyls on different enantioselective columns and using different temperature programs[1,2].

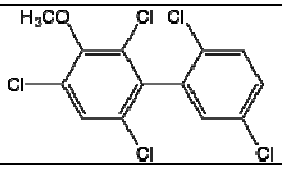
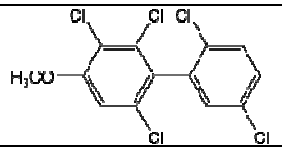
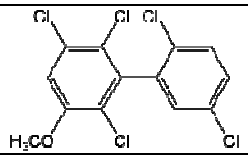
G.		3-Methoxy-2,2',4,5',6- pentachlorobiphenyl **(3-103)**[3]				
Column	Programmed temperature	Isothermal				
		140°C	150°C	160°C	170°C	180°C
20B	nr (107.9)					
BGB	0.65 (102.1)	0.85 (323.0)	0.79 (191.9)	0.73 (119.3)	0.63 (78.4)	0.54 (54.8)
BDM	nr (88.7)					
BPM	nr (83.0)					
CB	nr (99.6)					
CD	nr (81.4)					
GTA	nr (97.8)					
H.		4-Methoxy-2,2',3,5',6-pentachlorobiphenyl **(4-95)**[3]				
Column	Programmed temperature	Isothermal				
		140°C	150°C	160°C	170°C	180°C
20B	nr (124.8)					
BGB	0.39 (114.5)	na (479.5)	0.78 (370.3)	0.69 (217.7)	0.61 (134.4)	0.53 (87.6)
BDM	0.41 (104.1)	0.76 (380.0)	0.69 (210.9)	0.58 (124.0)	0.39 (77.7)	nr (52.6)
BPM	nr (99.6)					
CB	nr (113.0)					
CD	nr (93.6)					
GTA	nr (145.3)					
I.		3-Methoxy-2,2',5,5',6-pentachlorobiphenyl **(5-95)**[3]				
Column	Programmed temperature	Isothermal				
		140°C	150°C	160°C	170°C	180°C
20B	nr (123.3)	na	na	0.50 (331.8)	nr (196.9)	nr (122.7)
BGB	nr (113.4)					
BDM	0.88 (102.9)	1.3 (389.1)	1.1 (213.5)	0.90 (124.3)	0.70 (77.5)	0.62 (52.3)
BPM	nr (98.1)					
CB	0.54 (111.9)	na	0.99 (369.8)	0.95 (210.7)	0.89 (126.9)	0.76 (0.69)
CD	0.49 (92.9)	1.0 (284.7)	0.86 (155.5)	0.69 (91.2)	0.52 (57.9)	nr (40.3)
GTA	nr (137.4)	0.61 (400.3)	0.50 (222.4)	0.32 (130.9)	nr (82.1)	nr (55.3)

Table S1.D. Structure, IUPAC name, abbreviation and resolution of methoxylated polychlorinated biphenyls on different enantioselective columns and using different temperature programs[1,2].

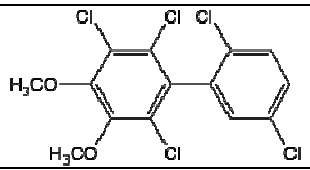
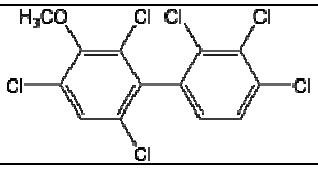
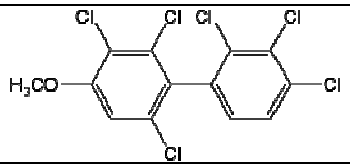
J.		3,4-Dimethoxy-2,2',5,5',6-pentachlorobiphenyl **(4,5-95)**[3]				
Column	Programmed temperature	Isothermal				
		140°C	150°C	160°C	170°C	180°C
20B	nr (124.6)					
BGB	nr (115.4)					
BDM	0.49 (105.6)	0.86 (404.4)	0.72 (228.6)	0.55 (129.8)	0.43 (80.8)	0.29 (54.2)
BPM	nr (99.4)					
CB	nr (113.1)	na	0.60 (377.0)	0.53 (242.0)	0.58 (130.7)	0.41 (85.4)
CD	nr (94.3)					
GTA	nr (141.9)					
K.		3-Methoxy-2,2',3',4,4',6-hexachlorobiphenyl **(3'-140)**[3]				
Column	Programmed temperature	Isothermal				
		140°C	150°C	160°C	170°C	180°C
20B	nr (103.0)					
BGB	0.54 (121.5)	na	na	0.72 (295.8)	0.72 (180.2)	0.69 (115.2)
BDM	nr (112.9)					
BPM	nr (104.4)					
CB	nr (117.8)					
CD	nr (98.5)					
GTA	0.76 (171.3)	na	1.0 (314.9)	0.90 (180.8)	0.73 (110.2)	0.60 (71.9)
L.		4-Methoxy-2,2',3,3',4',6-hexachlorobiphenyl **(4'-132)**[3]				
Column	Programmed temperature	Isothermal				
		140°C	150°C	160°C	170°C	180°C
20B	nr (161.0)	na	na	na	nr	nr
BGB	nr (133.2)					
BDM	nr (144.2)					
BPM	nr (134.8)					
CB	nr (130.8)					
CD	nr (110.0)					
GTA	nr (293.1)					

Table S1.E. Structure, IUPAC name, abbreviation and resolution of methoxylated polychlorinated biphenyls on different enantioselective columns and using different temperature programs[1,2].

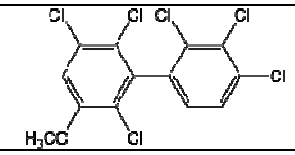
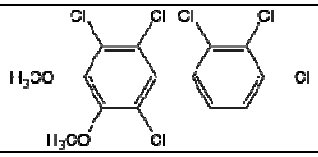
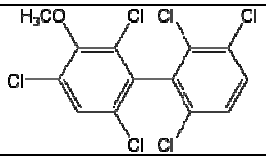
M.		3-Methoxy-2,2',3',4',5,6-hexachlorobiphenyl **(5'-132)**[3]				
Column	Programmed temperature	Isothermal				
		140°C	150°C	160°C	170°C	180°C
20B	nr (158.4)	na	na	na	na	na
BGB	0.99 (132.6)	na	na	na	0.85 (260.7)	1.0 (193.6)
BDM	1.2 (143.3)	na	na	2.5 (319.8)	1.4 (181.5)	1.5 (109.9)
BPM	0.49 (131.9)	na	na	0.75 (256.9)	0.72 (149.5)	0.54 (92.8)
CB	0.91 (130.1)	na	na	na	1.4 (183.6)	1.5 (314.2)
CD	0.74 (109.7)	na	2.3 (420.2)	1.8 (224.3)	1.2 (128.1)	0.92 (78.8)
GTA	nr (269.9)	na	na	nr (344.5)	nr (198.0)	nr (120.5)
N.		3,4-Dimethoxy-2,2',3',4',5,6-hexachlorobiphenyl **(4',5'-132)**[3]				
Column	Programmed temperature	Isothermal				
		140°C	150°C	160°C	170°C	180°C
20B	nr (161.4)	na	na	na	nr	nr
BGB	nr (134.8)					
BDM	nr (147.4)					
BPM	nr (134.9)					
CB	nr (131.1)					
CD	nr (111.0)					
GTA	nr (280.3)					
O.		3-Methoxy-2,2',3',4,6,6'-hexachlorobiphenyl **(3'-150)**[3]				
Column	Programmed temperature	Isothermal				
		140°C	150°C	160°C	170°C	180°C
20B	nr (117.7)					
BGB	nr (111.6)	nr (477.0)	0.12 (306.3)	0.08 (184.2)	nr (116.1)	nr (77.4)
BDM	0.31 (97.6)	0.60 (287.3)	0.52 (163.5)	0.42 (98.8)	0.25 (64.0)	nr (44.9)
BPM	nr (91.6)					
CB	nr (107.5)					
CD	0.39 (89.2)	1.2 (290.8)	0.91 (158.3)	0.63 (92.5)	0.49 (58.5)	0.20 (40.5)
GTA	nr (122.9)					

Table S1.F. Structure, IUPAC name, abbreviation and resolution of methoxylated polychlorinated biphenyls on different enantioselective columns and using different temperature programs[1,2].

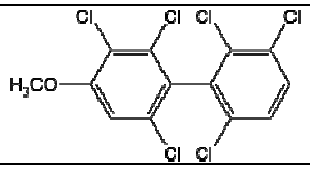
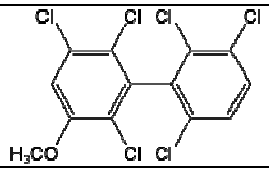
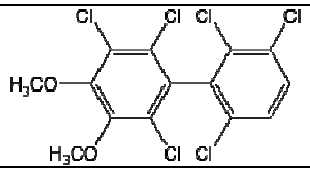
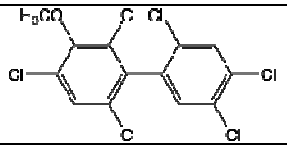
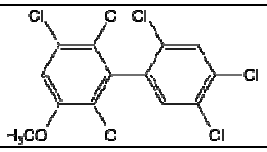
P.		4-Methoxy-2,2',3,3',6,6'-hexachlorobiphenyl **(4-136)**[3]				
Column	Programmed temperature	Isothermal				
		140°C	150°C	160°C	170°C	180°C
20B	nr (141.0)					
BGB	nr (123.9)					
BDM	0.34 (119.1)	na	0.75 (337.1)	0.66 (191.7)	0.51 (115.5)	0.43 (74.5)
BPM	nr (113.5)					
CB	0.18 (121.5)	na	0.83 (420.4)	0.64 (316.7)	0.58 (195.3)	0.53 (120.9)
CD	nr (101.5)	0.64 (445.5)	0.47 (239.0)	0.30 (136.5)	nr (83.5)	nr (55.1)
GTA	nr (195.5)					
Q.		3-Methoxy-2,2',3',5,6,6'-hexachlorobiphenyl **(5-136)**[3]				
Column	Programmed temperature	Isothermal				
		140°C	150°C	160°C	170°C	180°C
20B	nr (137.5)					
BGB	0.54 (114.5)	na	na	0.67 (316.2)	0.67 (194.8)	0.63 (122.2)
BDM	nr (115.2)					
BPM	nr (109.7)					
CB	nr (119.6)					
CD	nr (99.9)	0.64 (427.4)	nr (228.8)	nr (129.8)	nr (79.1)	nr (52.3)
GTA	nr (178.5)					
R.		3,4-Dimethoxy-2,2',3',5,6,6'-hexachlorobiphenyl **(4,5-136)**[3]				
Column	Programmed temperature	Isothermal				
		140°C	150°C	160°C	170°C	180°C
20B	nr (138.0)					
BGB	nr (123.2)					
BDM	nr (118.1)					
BPM	nr (110.2)					
CB	nr (120.1)					
CD	nr (101.2)					
GTA	nr (179.1)					

Table S1.G. Structure, IUPAC name, abbreviation and resolution of methoxylated polychlorinated biphenyls on different enantioselective columns and using different temperature programs[1,2].

S.		3-Methoxy-2,2',4,4',5',6-hexachlorobiphenyl **(3'-154)**[3]				
Column	Programmed temperature	Isothermal				
		140°C	150°C	160°C	170°C	180°C
20B	nr (120.0)					
BGB	0.96 (116.2)	na (480.2)	1.8 (403.2)	1.4 (236.5)	1.3 (145.4)	1.1 (94.1)
BDM	0.52 (104.4)	1.1 (398.1)	0.87 (218.3)	0.69 (126.9)	0.47 (79.0)	0.39 (53.1)
BPM	nr (96.0)					
CB	0.48 (112.1)	na	0.90 (373.3)	0.85 (212.7)	0.68 (128.2)	0.49 (82.3)
CD	0.46 (93.1)	0.91 (281.8)	0.76 (155.3)	0.57 (91.8)	0.43 (58.7)	nr (41.0)
GTA	nr (141.5)					
T.		3-Methoxy-2,2',4',5,5',6-hexachlorobiphenyl **(5-149)**[3]				
Column	Programmed temperature	Isothermal				
		140°C	150°C	160°C	170°C	180°C
20B	nr (142.8)	na	na	nr	nr	nr
BGB	nr (126.8)					
BDM	0.29 (127.5)	na	1.0 (426.9)	0.90 (234.4)	0.68 (136.5)	0.48 (85.0)
BPM	nr (117.5)					
CB	nr (124.3)					
CD	nr (104.3)	na	0.77 (302.0)	0.77 (166.2)	0.61 (98.2)	nr (62.8)
GTA	0.50 (217.3)	na	0.67 (462.6)	0.57 (257.2)	0.35 (151.1)	nr (94.5)

[1] The resolution R_s of atropisomers was calculated as $R_s=(t_{R2}-t_{R1})/0.5(BW_1+BW_2)$, where T_{R1} and T_{R2} are the retention time and BW_1 and BW_2 are the baseline peak width of the first and the second eluting atropisomer, respectively (6); the retention time of first eluting peak is given in parentheses. [2] Abbreviations of columns: 20B - HP-Chiral-20B; BGB - BGB-172; BDM - Chiral-Dex B-DM, BPM - Chiral-Dex B-PM; CB - Cyclosil-B; CD - Chirasil-Dex (CD); GTA - Chiral-Dex G-TA. See Table S3 for additional information. [3] Abbreviated name of the metabolite. nr – no peak resolution was observed. na – the compound did not elute from the column within 220 min at 180°C or 500 min at 140°C.

Table S2. Summary of the best resolution achieved in the isothermal analysis for the atropisomers of the MeO-PCBs.[a]

Compound	Column						
	20B	BGB	BDM	BPM	CB	CD	GTA
3-98	nr	nr	0.81	nr	0.85	0.64	**0.97**
3-100	nr	nr	nr	nr	nr	nr	**0.77**
4-91	nr	nr	**0.79**	nr	nr	nr	nr
5-91	0.94 #	0.90 #	**3.7**	1.3	3.3*	3.0	nr
3-103	nr	**0.85**	nr	nr	nr	nr	nr
4-95	nr	**0.78** *	0.76	nr	nr	nr	nr
5-95	0.50*****	nr	**1.3**	nr	0.99 *	1.0	0.61
4,5-95	nr	nr	**0.86**	nr	0.60 *	nr	nr
3'-140	nr	0.72 #	nr	nr	nr	nr	**1.0** *
5'-132	nr	1.0 ‡	**2.5** #	0.75 #	1.5 ‡	2.3 *	nr
3'-150	nr	nr	0.60	nr	nr	**1.2**	nr
4-136	nr	nr	0.75 *	nr	**0.83** *	0.64	nr
5-136	nr	**0.67** #	nr	nr	nr	0.64	nr
3'-154	nr	**1.8** *	1.1	nr	0.90*****	0.91	nr
5-149	nr	nr	**1.0** *	nr	nr	0.77 *	0.67 *

[a] This table contains only MeO-PCBs for which atropisomers resolved on at least one column. For a full list of the MeO-PCBs studied, see Table S1. Unless otherwise stated, the isothermal analysis was performed at 140°C using the following temperature program: 50°C for 1 min, 10°C/min to 140°C, hold for 500 min, 10°C to 225°C, hold for 10 min. The best separation for each compound in highlighted in **bold**; ***** - isothermal analysis performed at 150°C; # - isothermal analysis performed at 160°C; ‡ - isothermal analysis performed at 180°C. Abbreviations of columns: 20B - HP-Chiral-20B; BGB - BGB-172; BDM - Chiral-Dex B-DM, BPM - Chiral-Dex B-PM; CB - Cyclosil-B; CD - Chirasil-Dex (CD); GTA - Chiral-Dex G-TA. See Table S3 for additional information regarding the columns.

Table S3. List of the characteristics of the enantioselective columns used for the separation of methoxylated PCB derivatives.

Designation	Abbreviation	Chiral stationary phase	Cyclodextrin type	Max temp.	Parameters	Supplier
HP-Chiral-20B	20B	20% in (35%-phenyl)-methylpolysiloxane	β-cyclodextrin	250°C	30 m x 250 µm x 0.25 µm	Agilent Santa Clara, CA
BGB-172	BGB	20% tert-butyldimethyl-silyl-	β-cyclodextrin	250°C	30 m x 250 µm x 0.25 µm	BGB Analytic Boeckten, Switzerland
ChiralDex B-DM	BDM	2,3-di-O-methyl-6-tert-butyl-silyl-	β-cyclodextrin	200°C	30 m x 250 µm x 0.12 µm	Supelco Analytical St. Louis, MO
ChiralDex B-PM	BPM	2,3,6-tri-O-methyl-silyl-	β-cyclodextrin	200°C	30 m x 250 µm x 0.12 µm	Supelco Analytical St. Louis, MO
Cyclosil-B	CB	30% hepatkis (2,3-di-O-methyl-6-O-t-butyl dimethyl-silyl)-	β-cyclodextrin	250°C	30 m x 250 µm x 0.25 µm	Agilent Santa Clara, CA
Chirasil-Dex	CD	2,3,6-tri-O-methyl-	β-cyclodextrin	225°C	30 m x 250 µm x 0.39 µm	Varian (Agilent) Santa Clara, CA
ChiralDex G-TA	GTA	2,6-di-O-pentyl-3-trifluoroacetyl-	γ-cyclodextrin	180°C	30 m x 250 µm x 0.12 µm	Supelco Analytical St. Louis, MO

Table S4. Amounts and enantiomeric fractions of hydroxylated PCBs (analyzed as methoxylated derivatives)s from microsomal incubations of PCBs 91, 95, 132 and 149 determined on six enantioselective columns[1] during isothermal analysis at 160˚C. The resolutions of the atropisomers in the racemic standards are given in parentheses.

Enantioselective column			BGB*	BDM	BPM	CB	CD	GTA*
Parent	Metabolite	Amount formed [nmol]						
PCB 91	3-100	0.4	nr	nr	nr	nr	nr	E1 (0.40)
	5-91	160	0.46 (0.81)	0.54 $^{\$}$	0.54 $^{\$}$ (0.94)	0.54 $^{\#}$ (3.0)	0.54 $^{\$}$ (1.2)	nr
	4-91	1.2	nr	nd	nr	nr	nd	nr
	4,5-91	0.4	nr	nd	nr	nr	nd	nr
PCB 95	3-103		E2 (0.58)	nr	nd	nr	nr	nr
	4-95		nr	0.51 (0.69)	nd	nr	nd	nr
	5-95	70	nr	0.36 $^{\$}$	nr	0.32 $^{\$}$	0.33 $^{\$}$ (0.70)	E1 $^{\wedge}$ (0.26)
	4,5-95		nr	nd	nd	nd	nd	nr
	X-95		0.68	nr	nr	0.33	0.33	nr
PCB 132	3'-140	1	E1 (0.40)	nd	nr	nd	nd	nd
	4'-132	120	nr	nr	nr	nr	nr	nr
	5'-132	8	0.71 $^{\$,\&}$	0.30 $^{\$}$	0.28 $^{\$}$	0.31 $^{\$,\&}$	0.31 (1.8)	nr
	4',5'-132	0.5	nr	nr	nr	nr	nr	nr
PCB149	3-154	3	E2 (1.0)	nd	nr	E1 (0.64)	nd	nr
	5-149	20	nr	0.66 (0.83)	nr	0.65 (0.68)	0.65 (0.75)	0.29 (0.45)

[1] The 20B column was not used because it only resolved 5-91. * Reverse elution order of atropisomers compared to the other columns investigated; $^{\$}$ the major metabolite co-elutes with minor metabolites and the EF was not determined in the racemic standard and/or the incubation sample; $^{\#}$ EF of standard = 0.55; $^{\wedge}$ the EF value was not determined due to the poor resolution; however, a small shoulder corresponding to the second eluting peak indicated an enrichment of the first eluting atropisomer; $^{\&}$ analysis at 170˚C. Abbreviations of columns: BGB - BGB-172; BDM - Chiral-Dex B-DM, BPM - Chiral-Dex B-PM; CB - Cyclosil-B; CD - Chirasil-Dex (CD); GTA - Chiral-Dex G-TA. See Table S3 for additional information; nr – metabolite does not resolve on the column; E1 and E2 – only the first or second atropisomer was observed for these minor metabolites; nd – metabolite not detected in the incubation.

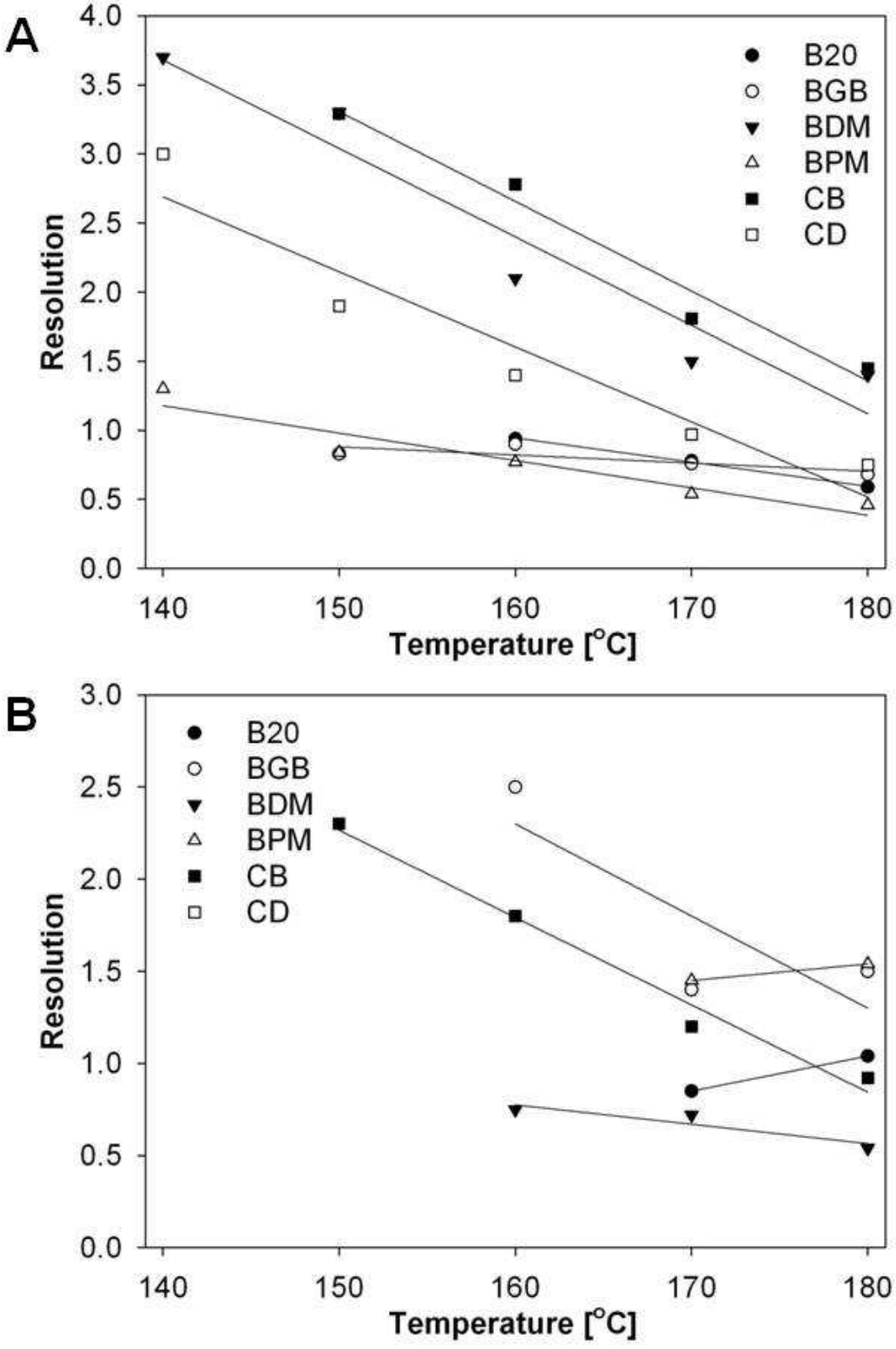
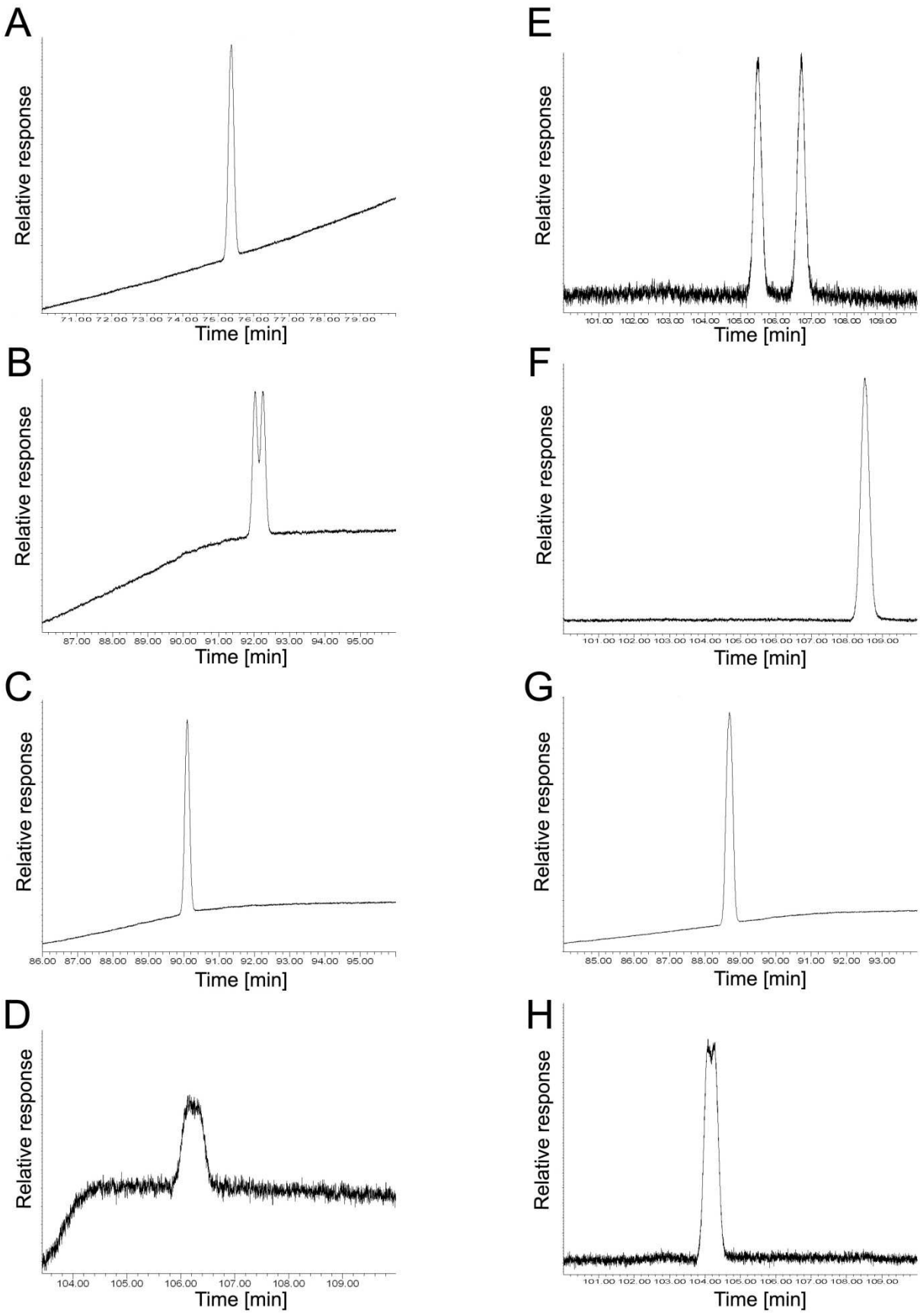
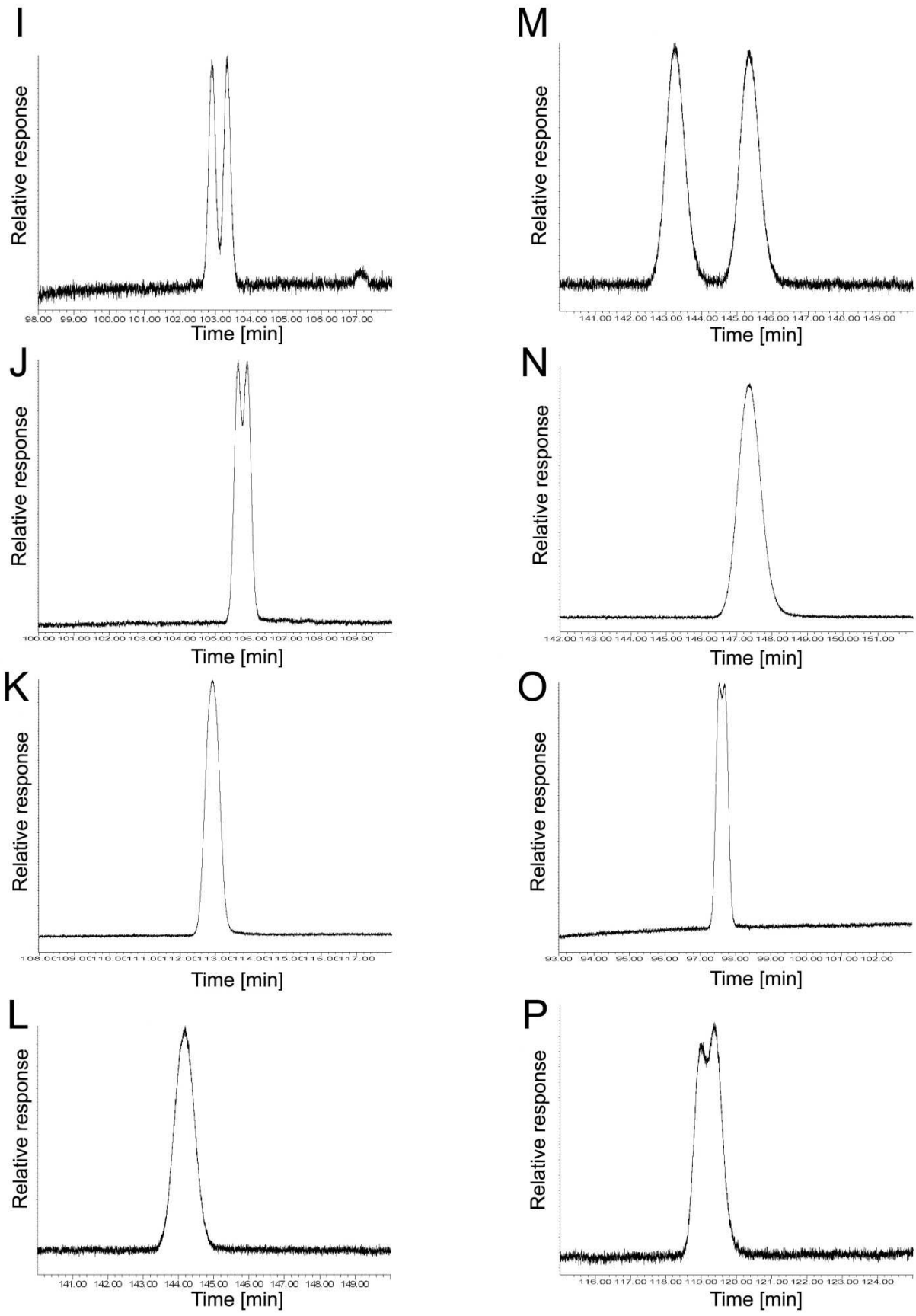


Figure S1. The resolution of 5-methoxylated PCBs decreases with increasing temperature. The resolution on different enantioselective columns is plotted against temperature of isothermal analysis in the range of 140°C to 180°C for (A) 5-91 and (B) 5'-132. Abbreviations of columns: 20B - HP-Chiral-

20B; BGB - BGB-172; BDM - Chiral-Dex B-DM, BPM - Chiral-Dex B-PM; CB - Cyclosil-B; CD - Chirasil-Dex (CD); GTA - Chiral-Dex G-TA. See Table S3 for additional information. The resolution R_s of atropisomers was calculated as $R_s=(t_{R2}-t_{R1})/0.5(BW_1+BW_2)$, where T_{R1} and T_{R2} are the retention time and BW_1 and BW_2 are the baseline peak width of the first and the second eluting atropisomer, respectively (6).


A
Relative response
71.00 72.00 73.00 74.00 75.00 76.00 77.00 78.00 79.00
Time [min]
B
Relative response
87.00 88.00 89.00 90.00 91.00 92.00 93.00 94.00 95.00
Time [min]
C
Relative response
86.00 87.00 88.00 89.00 90.00 91.00 92.00 93.00 94.00 95.00
Time [min]
D
Relative response
104.00 105.00 106.00 107.00 108.00 109.00
Time [min]
E
Relative response
101.00 102.00 103.00 104.00 105.00 106.00 107.00 108.00 109.00
Time [min]
F
Relative response
101.00 102.00 103.00 104.00 105.00 106.00 107.00 108.00 109.00
Time [min]
G
Relative response
85.00 86.00 87.00 88.00 89.00 90.00 91.00 92.00 93.00
Time [min]
H
Relative response
101.00 102.00 103.00 104.00 105.00 106.00 107.00 108.00 109.00
Time [min]


I
Relative response
98.00 99.00 100.00 101.00 102.00 103.00 104.00 105.00 106.00 107.00
Time [min]
M
Relative response
141.00 142.00 143.00 144.00 145.00 146.00 147.00 148.00 149.00
Time [min]
J
Relative response
100.00 101.00 102.00 103.00 104.00 105.00 106.00 107.00 108.00 109.00
Time [min]
N
Relative response
142.00 143.00 144.00 145.00 146.00 147.00 148.00 149.00 150.00 151.00
Time [min]
K
Relative response
108.0C109.0C110.0C111.0C112.0C113.0C114.0C115.0C116.0C117.00
Time [min]
O
Relative response
93.00 94.00 95.00 96.00 97.00 98.00 99.00 100.00 101.00 102.00
Time [min]
L
Relative response
141.00 142.00 143.00 144.00 145.00 146.00 147.00 148.00 149.00
Time [min]
P
Relative response
116.00 117.00 118.00 119.00 120.00 121.00 122.00 123.00 124.00
Time [min]

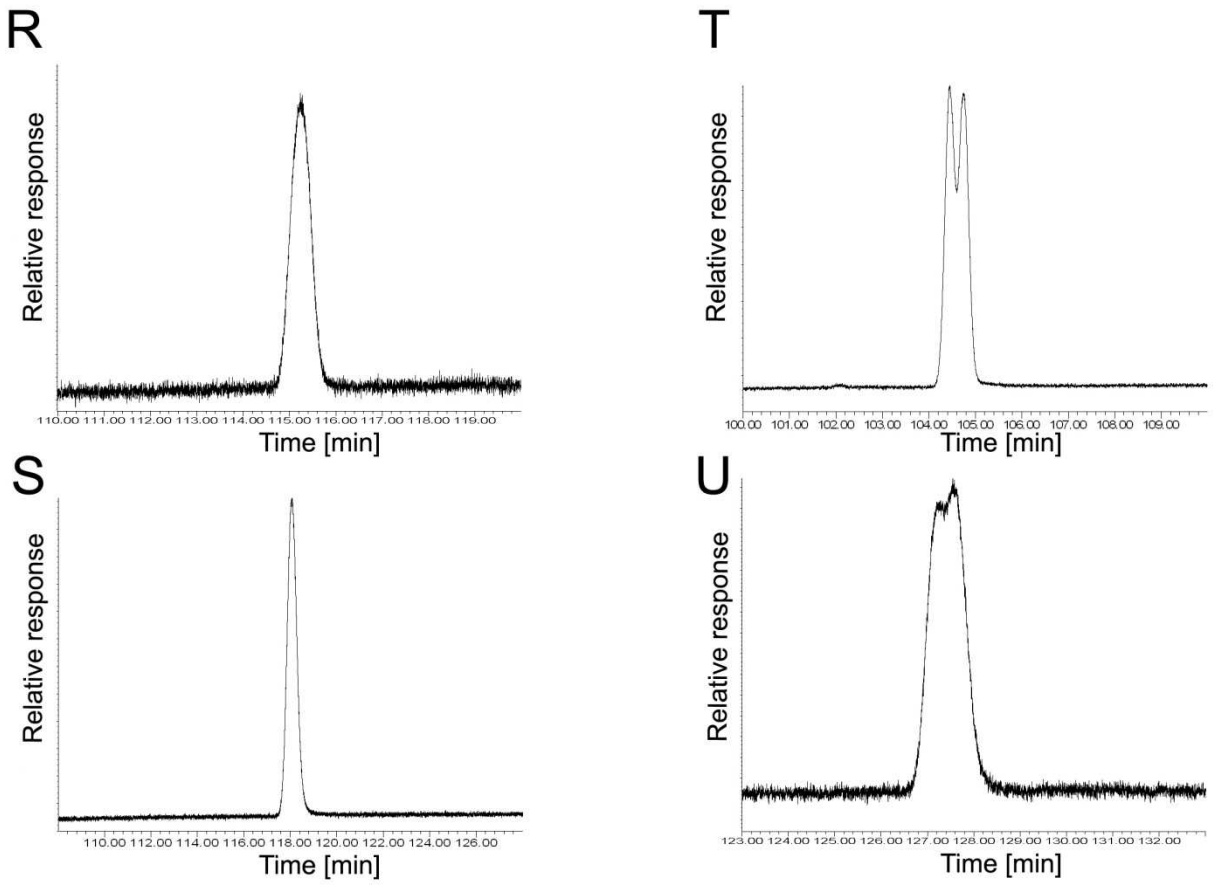


Figure S2A. Resolution of all studied methoxylated compounds on the ChiralDex B-DM column using programmed temperature analysis. This column resolved 11 out of 20 studied metabolites. A. 3-50; B. 3-98 (R_s=0.55); C. 3-100; D. 4-91; E. 5-91 (R_s=2.1); F. 4,5-91; G. 3-103; H. 4-95 (R_s=0.41); I. 5-95 (R_s=0.88); J. 4,5-95 (R_s=0.49); K. 3'-140; L. 4'-132; M. 5'-132 (R_s=1.2); N. 4',5'-132; O. 3-150 (R_s=0.31); P. 4-136 (R_s=0.34); R. 5-136; S. 4,5-136; T. 3-154 (R_s=0.52); U. 5-149 (R_s=0.29). The resolution R_s of atropisomers was calculated as $R_s=(t_{R2}-t_{R1})/0.5(BW_1+BW_2)$, where T_{R1} and T_{R2} are the retention time and BW_1 and BW_2 are the baseline peak width of the first and the second eluting atropisomer, respectively (6).

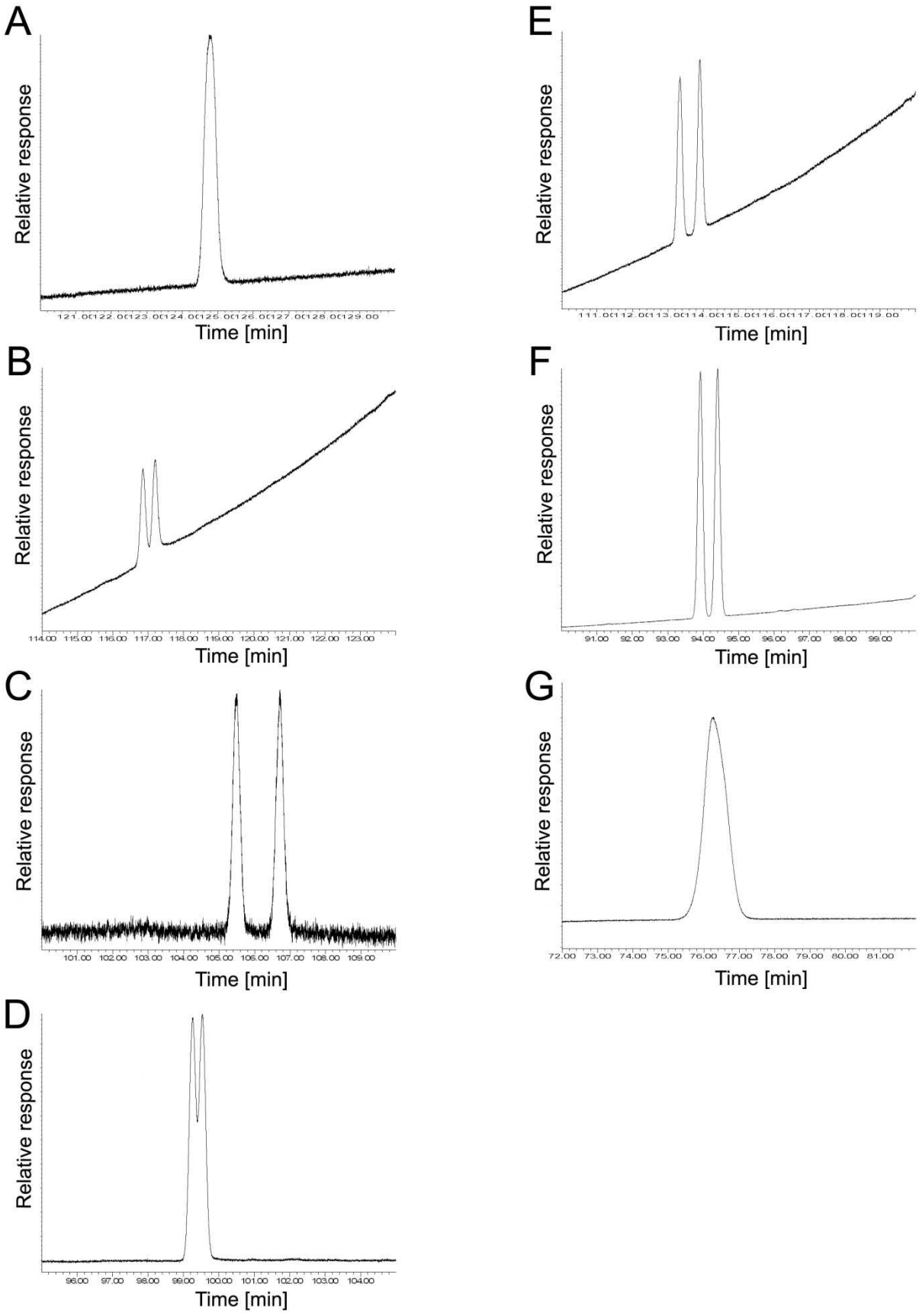

A
Relative response
121.00 122.00 123.00 124.00 125.00 126.00 127.00 128.00 129.00
Time [min]
B
Relative response
114.00 115.00 116.00 117.00 118.00 119.00 120.00 121.00 122.00 123.00
Time [min]
C
Relative response
101.00 102.00 103.00 104.00 105.00 106.00 107.00 108.00 109.00
Time [min]
D
Relative response
96.00 97.00 98.00 99.00 100.00 101.00 102.00 103.00 104.00
Time [min]
E
Relative response
111.00 112.00 113.00 114.00 115.00 116.00 117.00 118.00 119.00
Time [min]
F
Relative response
91.00 92.00 93.00 94.00 95.00 96.00 97.00 98.00 99.00
Time [min]
G
Relative response
72.00 73.00 74.00 75.00 76.00 77.00 78.00 79.00 80.00 81.00
Time [min]

Figure S2B. Resolution of 5-91 on all enantioselective columns using programmed temperature analysis. A. HP Chiral 20B; B. BGB-172 (R_s=0.75); C. Chiral-Dex B-DM (R_s=2.1); D. Chiral-Dex B-PM (R_s=0.50); E. Cyclosil-B (R_s=1.3); F. Chirasil-Dex (R_s=0.97); G. Chiral-Dex G-TA. The resolution R_s of atropisomers was calculated as $R_s=(t_{R2}-t_{R1})/0.5(BW_1+BW_2)$, where T_{R1} and T_{R2} are the retention time and BW_1 and BW_2 are the baseline peak width of the first and the second eluting atropisomer, respectively (6).

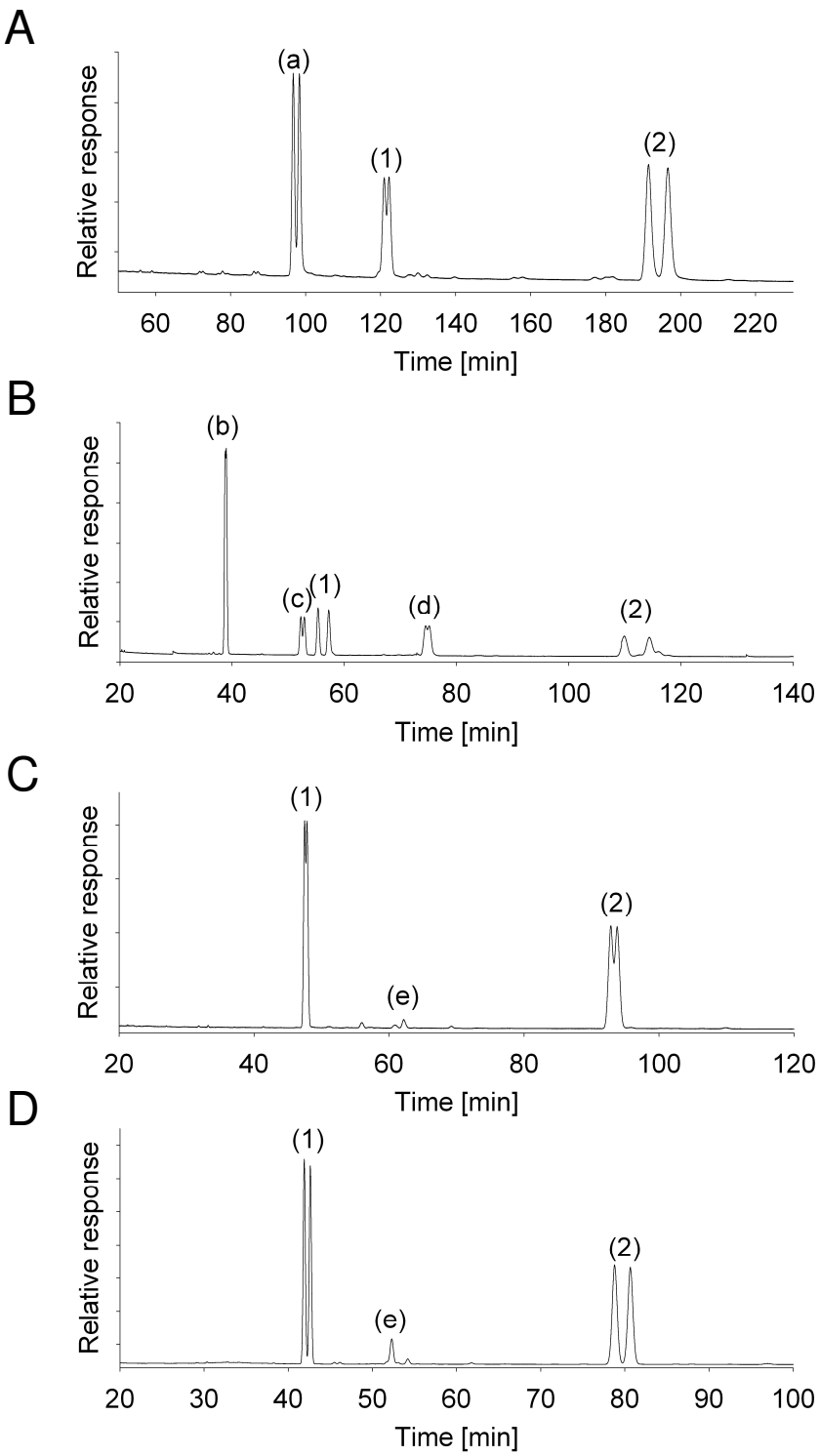
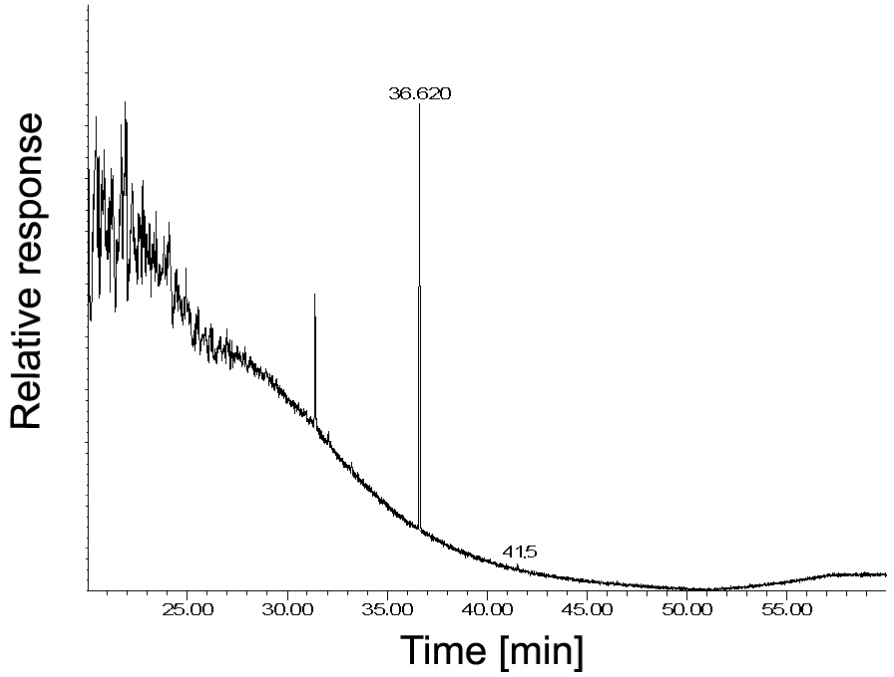
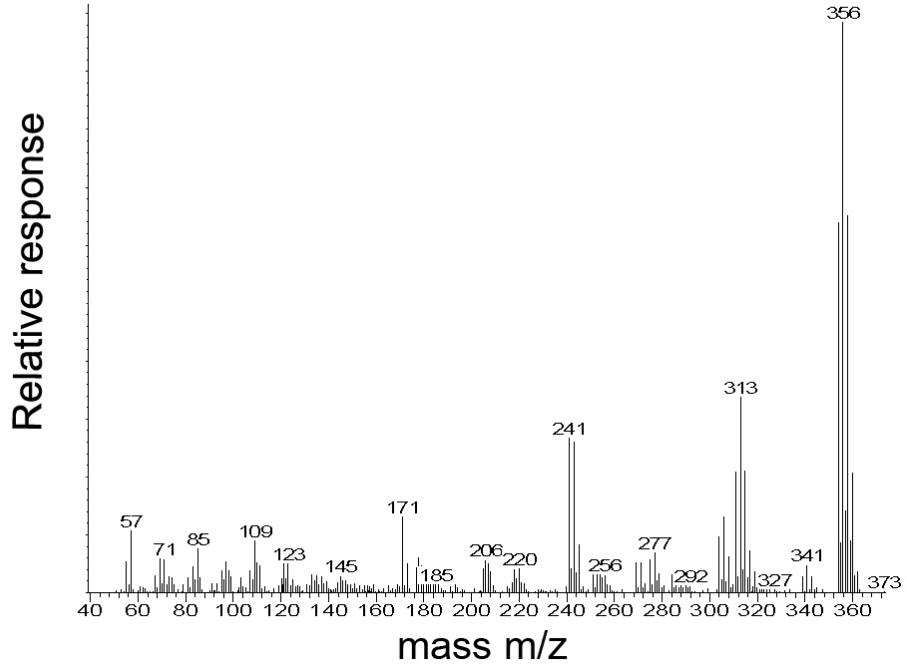


Figure S3. The isothermal resolution of 5-91 (1) and 5'-132 (2) on different columns: A. 180°C on BGB column (R_{s1} = 0.68; R_{s2}=1.0); B. 180°C on BDM column (R_{s1} = 1.4; R_{s2} = 1.5); C. 180°C on BPM column (R_{s1} = 0.46; R_{s2} = 0.54); D. 180°C on CD column (R_{s1} = 0.75; R_{s2} = 0.92). In all cases the flow is 3 mL/min. (a) 5'-132; (b) 3-98; (c) 5-92; (d) 4-136; (e) unknown contamination.

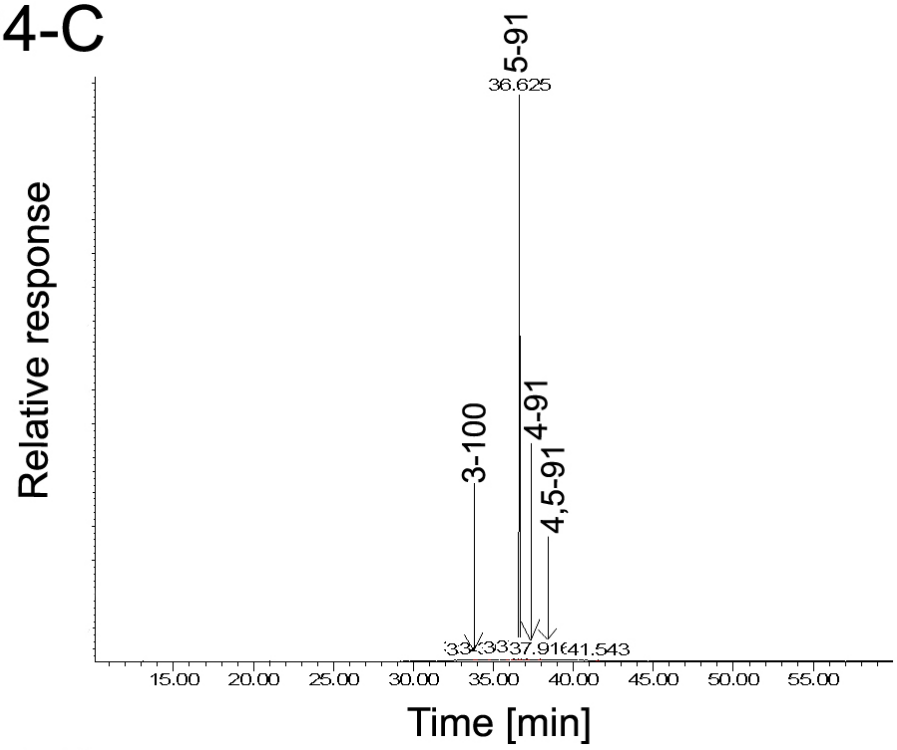
S4-A


36.620
41.5
Relative response
25.00
30.00
35.00
40.00
45.00
50.00
55.00
Time [min]

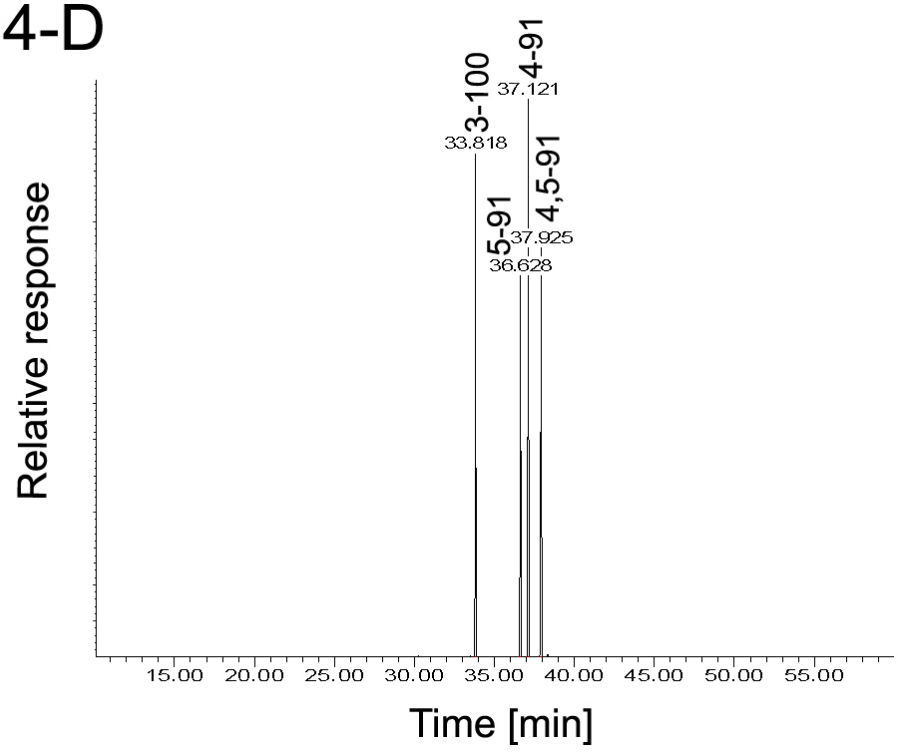
S4-B


356
313
241
171
57
71
85
109
123
145
185
206
220
256
277
292
327
341
373
Relative response
40 60 80 100 120 140 160 180 200 220 240 260 280 300 320 340 360
mass m/z

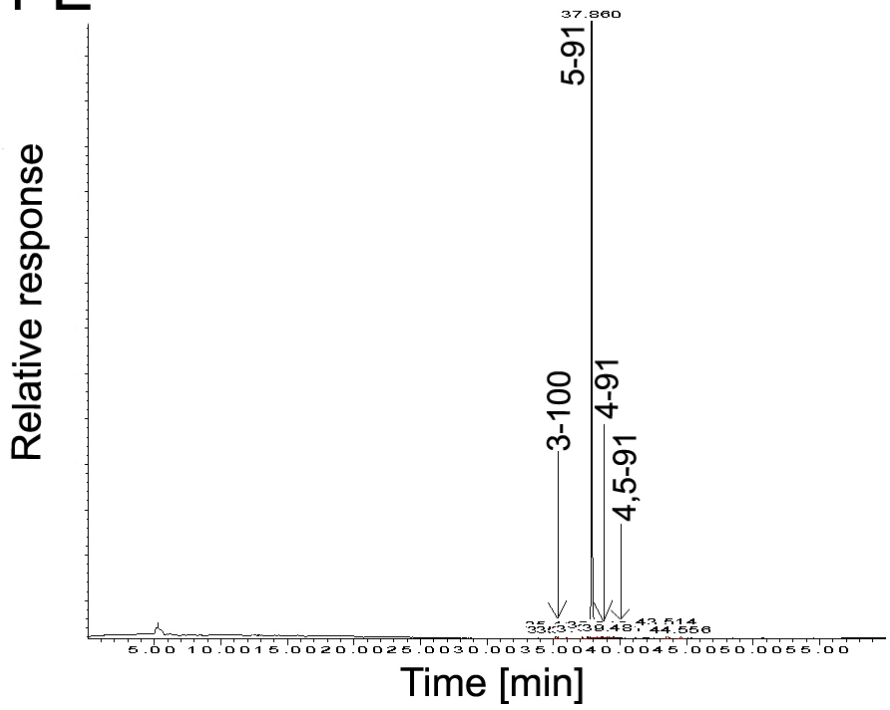
S4-C


5-91
36.625
3-100
4-91
4,5-91
37.916
41.543
Relative response
15.00
20.00
25.00
30.00
35.00
40.00
45.00
50.00
55.00
Time [min]

S4-D


3-100
33.818
4-91
37.121
5-91
36.628
4,5-91
37.925
Relative response
15.00
20.00
25.00
30.00
35.00
40.00
45.00
50.00
55.00
Time [min]

S4-E


37.860
5-91
3-100
4-91
4,5-91
Relative response
5.00 10.00 15.00 20.00 25.00 30.00 35.00 40.00 45.00 50.00 55.00
Time [min]

S4-F

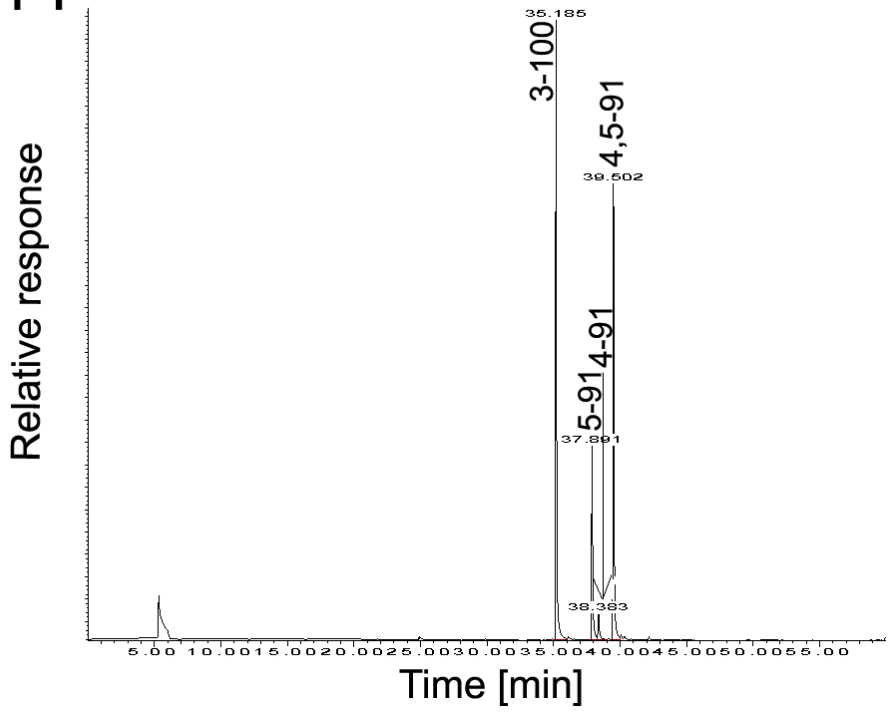
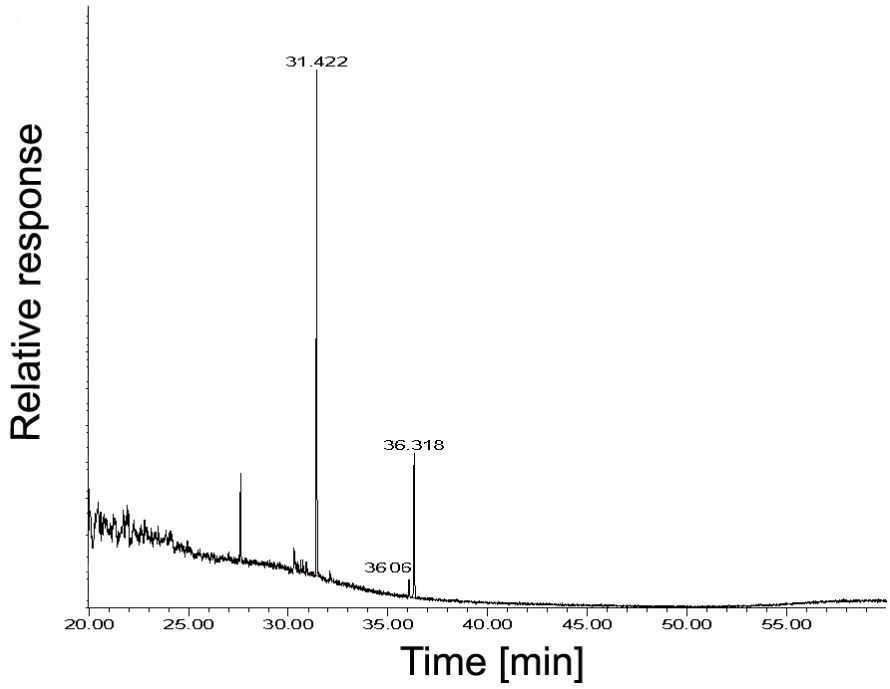
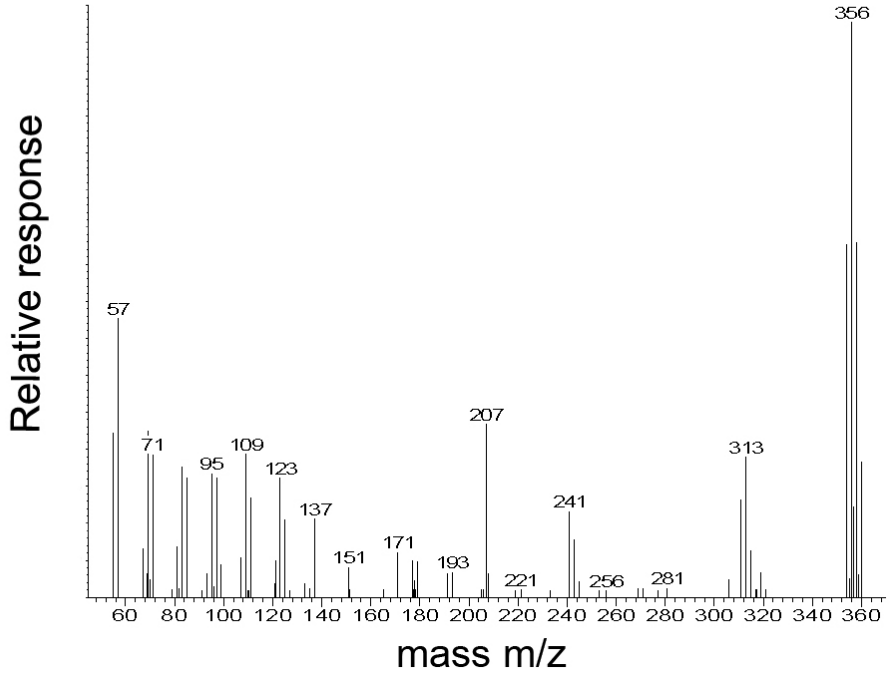

35.185
3-100
4,5-91
39.502
4-91
5-91
37.891
38.383
Relative response
5.00 10.00 15.00 20.00 25.00 30.00 35.00 40.00 45.00 50.00 55.00
Time [min]

Figure S4. Microsomal metabolism of PCB 91 by liver microsomes. One major mono-methoxylated metabolite of PCB 91, corresponding to 5-91, is formed in the microsomal incubation according to GC-MS and GC-ECD analysis. **A-B.** Chromatogram (A) and mass spectrum of major metabolite (B) recorded in the TIC mode. The chromatogram shows a major peak at 36.6 min with a molecular ion corresponding to a mono-methoxylated pentachlorobiphenyl (*m/z* = 356) and a small peak at 41.5 min with a molecular ion of *m/z* = 383. **C-D.** Chromatograms of the incubation sample (C) and a standard containing authentic metabolites of PCB 91 (D) recorded in the SIM mode. The chromatogram of the incubation sample shows a single major peak at 36.6 min, corresponding to 5-91. Several minor peaks corresponding to 3-100 (33.8 min), 4-91 (37.1 min) and 4,5-91 (37.9 min) were also detected. The metabolite ratio is 5-91 : 3-100 : 4-91 : 4,5-91 = 1 : 0.004 : 0.003 : 0.002. **E-F.** Chromatograms of the incubation sample (E) and the authentic standard (F) analyzed with GC-ECD. The chromatogram of the incubation sample shows a single major peak at 37.9 min, corresponding to 5-91. Several minor peaks corresponding to 3-100 (35.2 min), 4-91 (38.4 min) and 4,5-91 (39.5 min) were also observed. The metabolite ratio is 5-91 : 3-100 : 4-91 : 4,5-91 = 1 : 0.003 : 0.010 : 0.002.

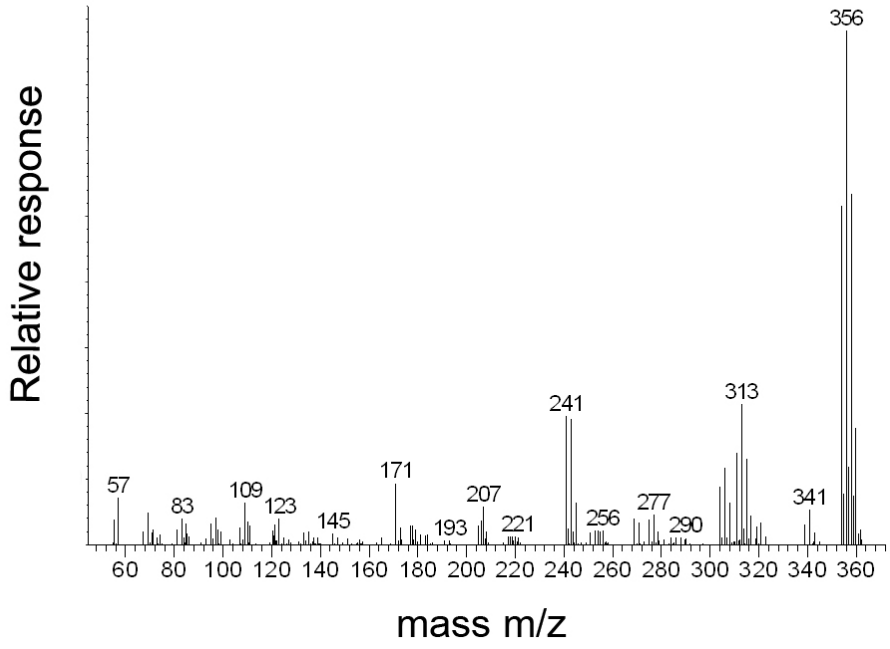
S5-A


31.422
36.318
36.06
Relative response
20.00
25.00
30.00
35.00
40.00
45.00
50.00
55.00
Time [min]

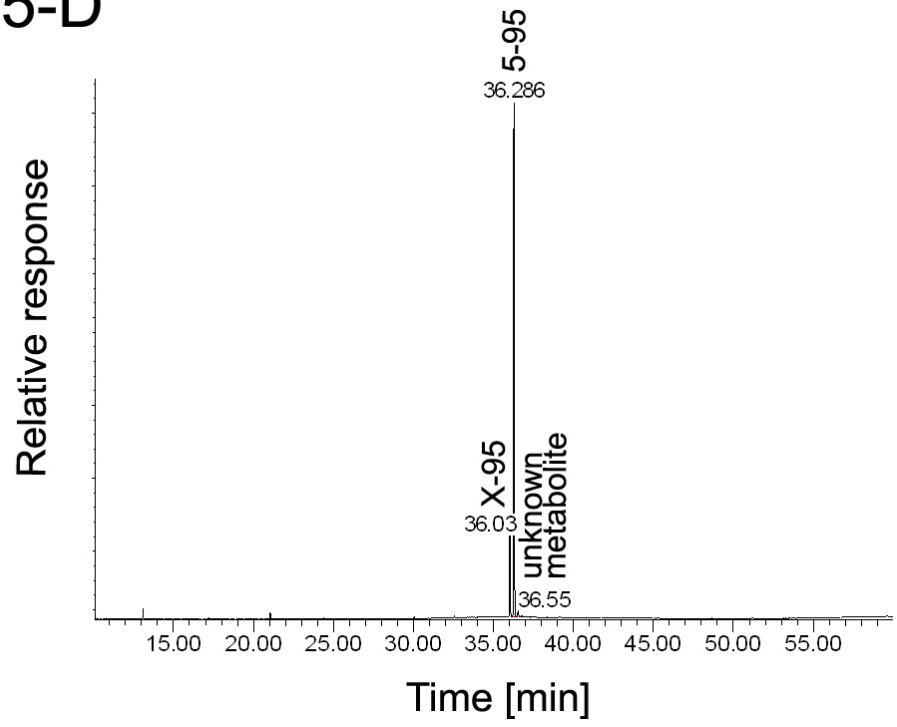
S5-B


356
57
71
95
109
123
137
151
171
193
207
221
241
256
281
313
Relative response
60
80
100
120
140
160
180
200
220
240
260
280
300
320
340
360
mass m/z

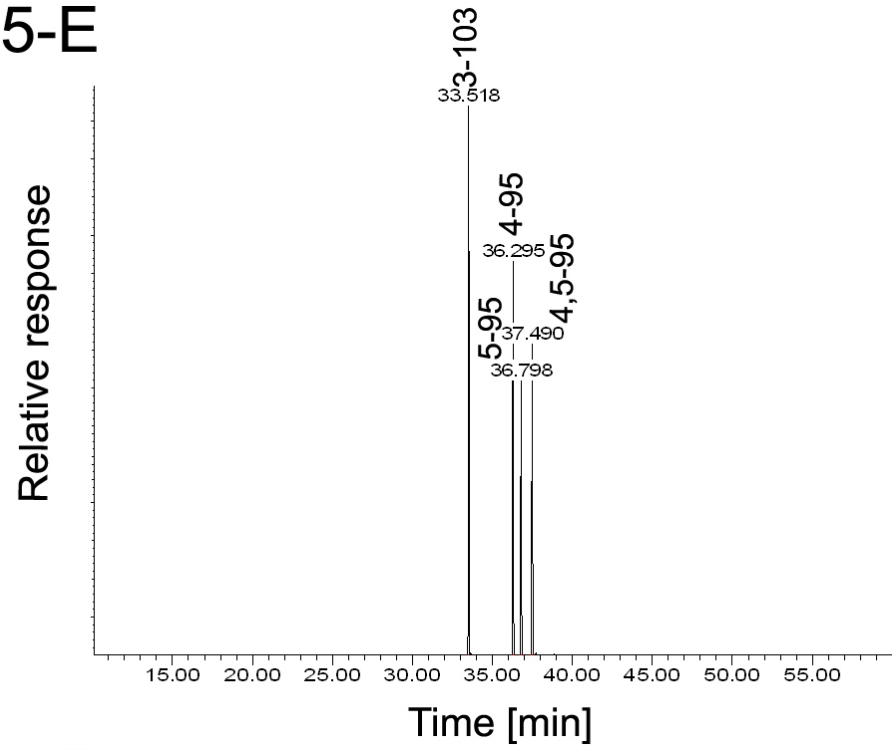
S5-C


Relative response
356
313
241
171
207
57
83
109
123
145
193
221
256
277
290
341
60 80 100 120 140 160 180 200 220 240 260 280 300 320 340 360
mass m/z

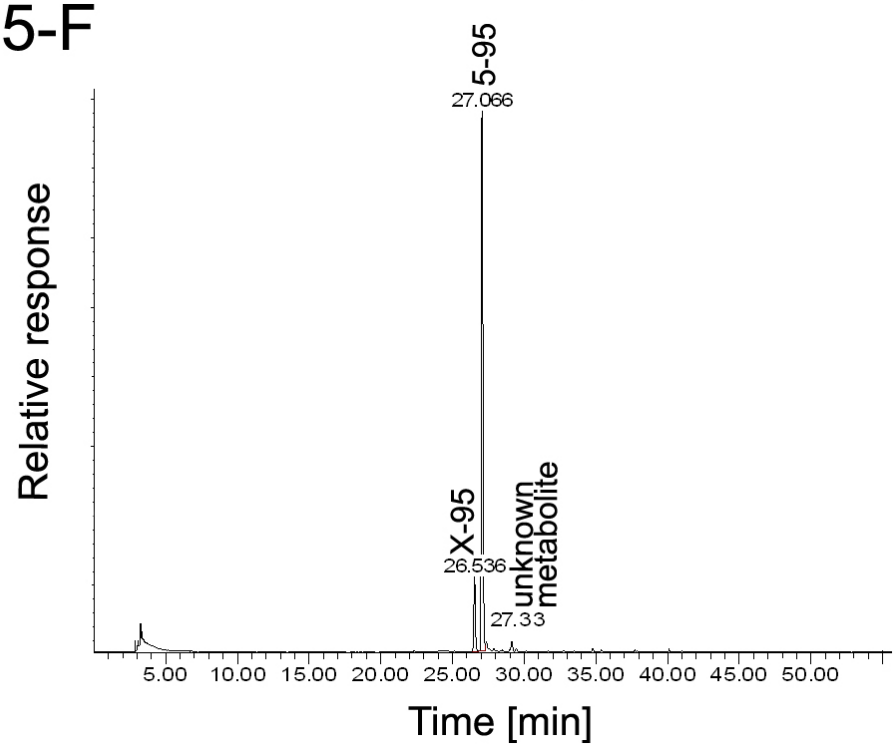
S5-D


Relative response
5-95
36.286
X-95
36.03
unknown metabolite
36.55
15.00 20.00 25.00 30.00 35.00 40.00 45.00 50.00 55.00
Time [min]

S5-E


3-103
33.518
4-95
36.295
4,5-95
5-95
37.490
36.798
Relative response
15.00 20.00 25.00 30.00 35.00 40.00 45.00 50.00 55.00
Time [min]

S5-F


5-95
27.066
X-95
26.536
unknown metabolite
27.33
Relative response
5.00 10.00 15.00 20.00 25.00 30.00 35.00 40.00 45.00 50.00
Time [min]

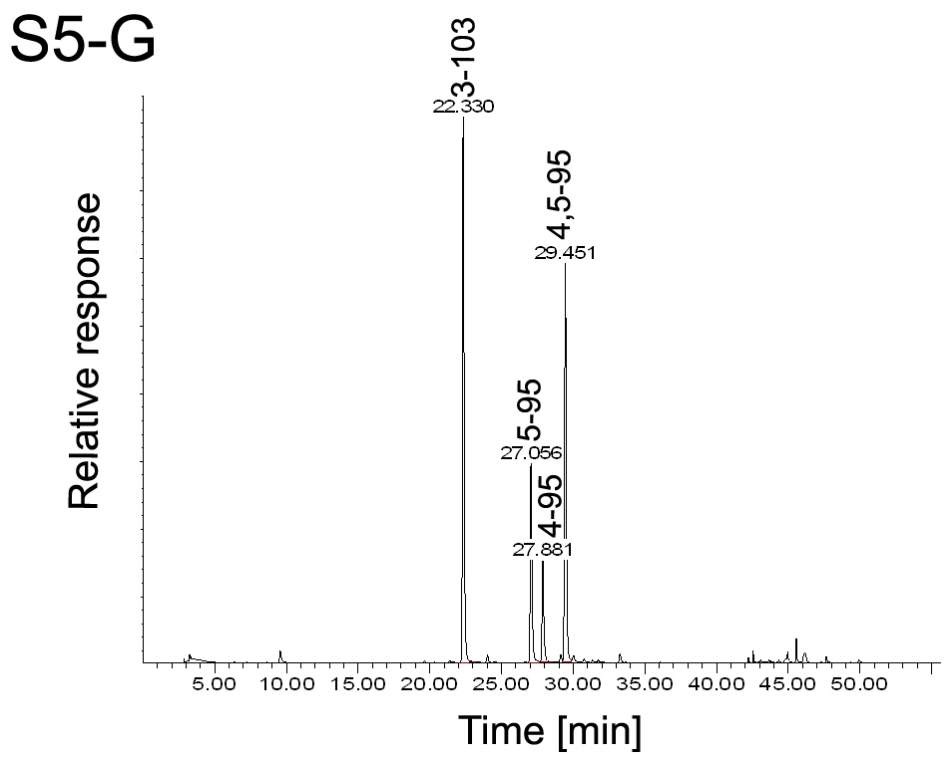
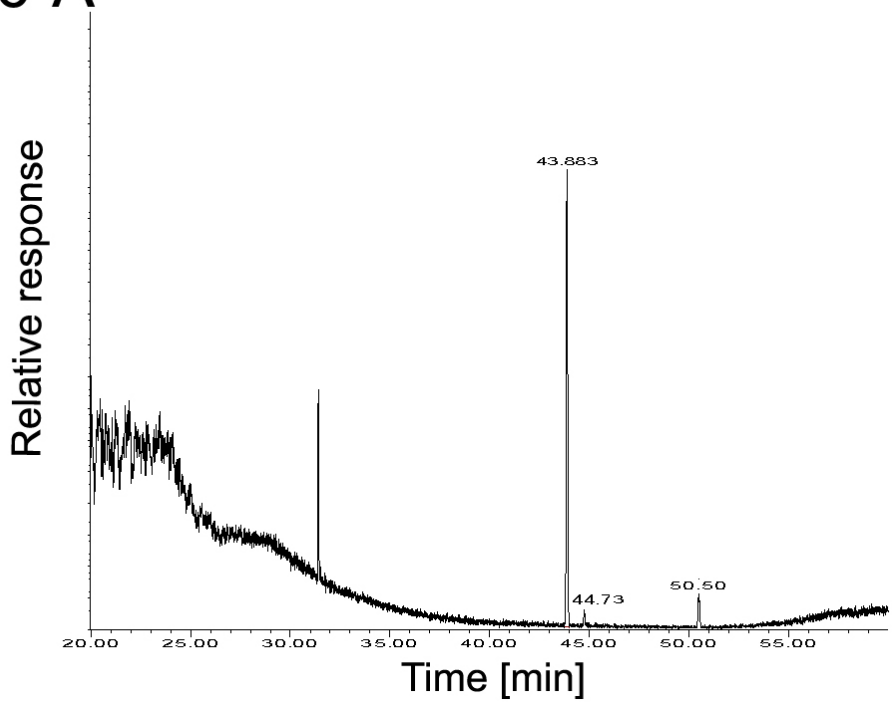
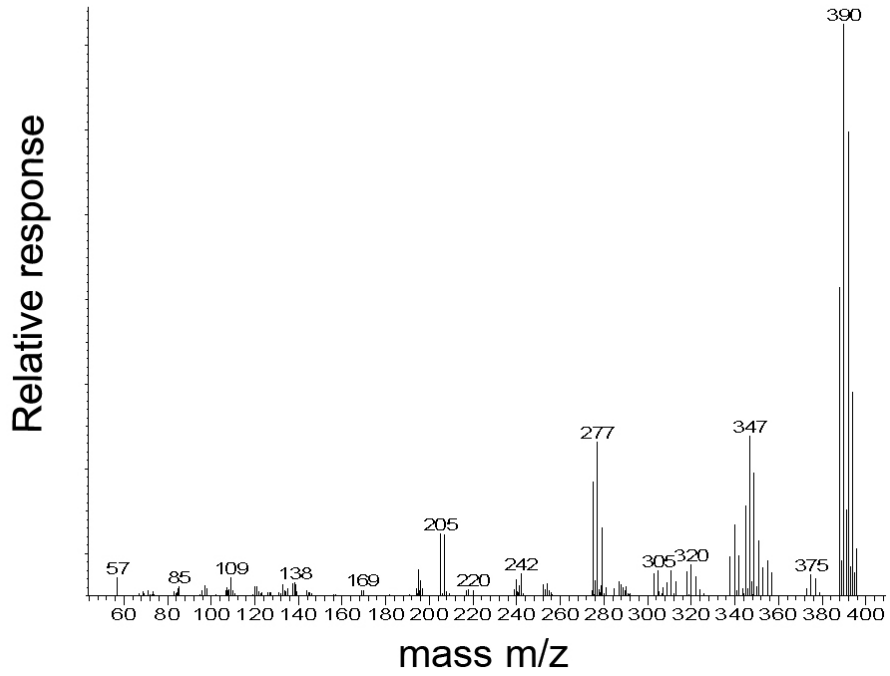


Figure S5. Microsomal metabolism of PCB 95 by liver microsomes. Two mono-methoxylated metabolites of PCB 95 are formed according to GC-MS and GC-ECD analysis. One metabolite corresponds to 5-95. The structure of the other mono-methoxylated metabolite, X-95, is unknown. **A-C.** Chromatogram (A) and mass spectra of metabolites (B and C) recorded in the TIC mode. The chromatogram shows a major peak at 36.32 min and minor peak at 36.1 min, with molecular ion at *m/z* 356, corresponding to mono-methoxylated pentachlorobiphenyls. **D-E.** Chromatograms of the incubation sample (D) and a standard containing authentic metabolites of PCB 95 (E) recorded in the SIM mode. The chromatogram of the incubation sample shows a major peak at 36.3 min, corresponding to 5-95, and a peak at 36.0 min, corresponding to an unknown mono-methoxylated metabolite X-95. **F-G.** Chromatograms of the incubation sample (F) and the authentic standard (G) analyzed with GC-ECD. The chromatogram of the incubation sample shows a major peak at 27.1 min, corresponding to 5-95, and a peak at 26.5 min, corresponding to the unknown mono-methoxylated metabolite X-95.

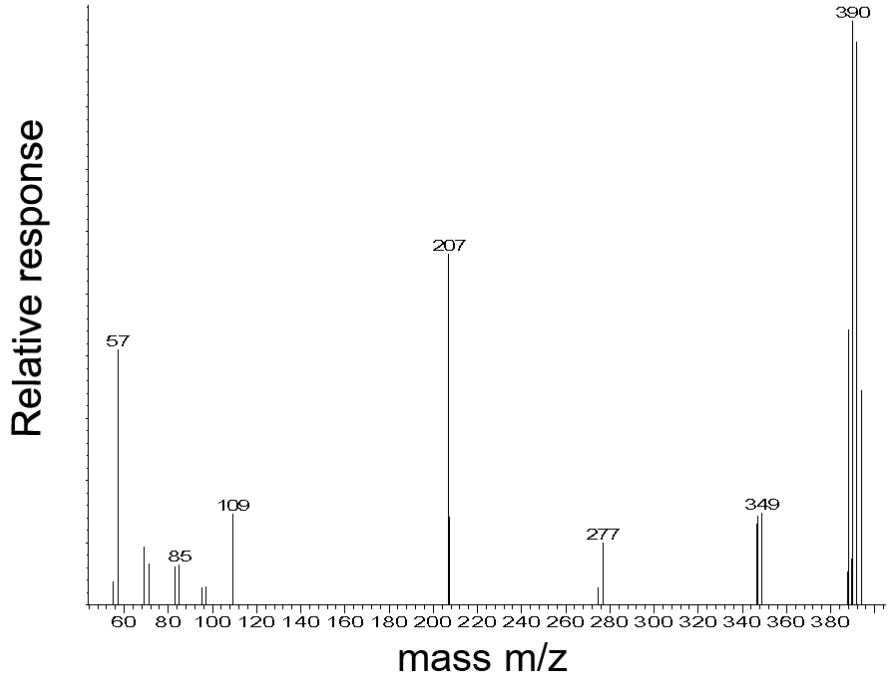
S6-A


Relative response
43.883
44.73
50.50
20.00
25.00
30.00
35.00
40.00
45.00
50.00
55.00
Time [min]

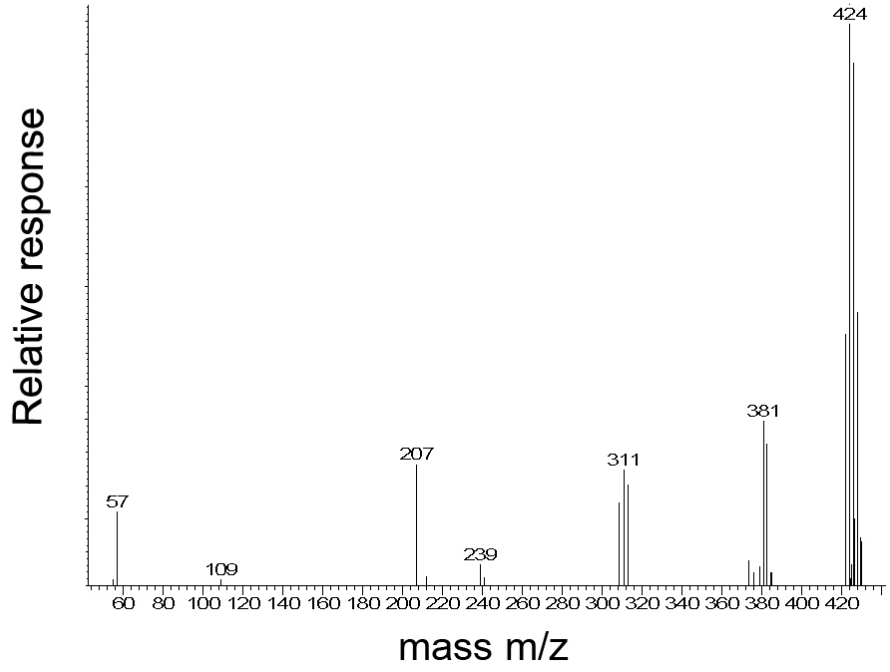
S6-B


Relative response
390
347
277
205
57
85
109
138
169
220
242
305
320
375
60 80 100 120 140 160 180 200 220 240 260 280 300 320 340 360 380 400
mass m/z

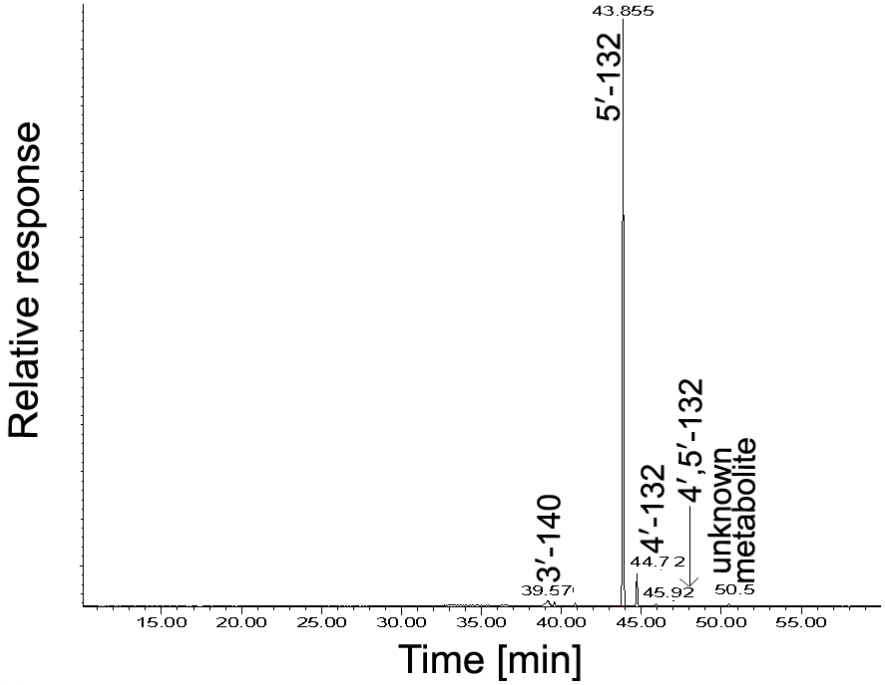
S6-C


Relative response
390
207
57
109
349
277
85
60 80 100 120 140 160 180 200 220 240 260 280 300 320 340 360 380
mass m/z

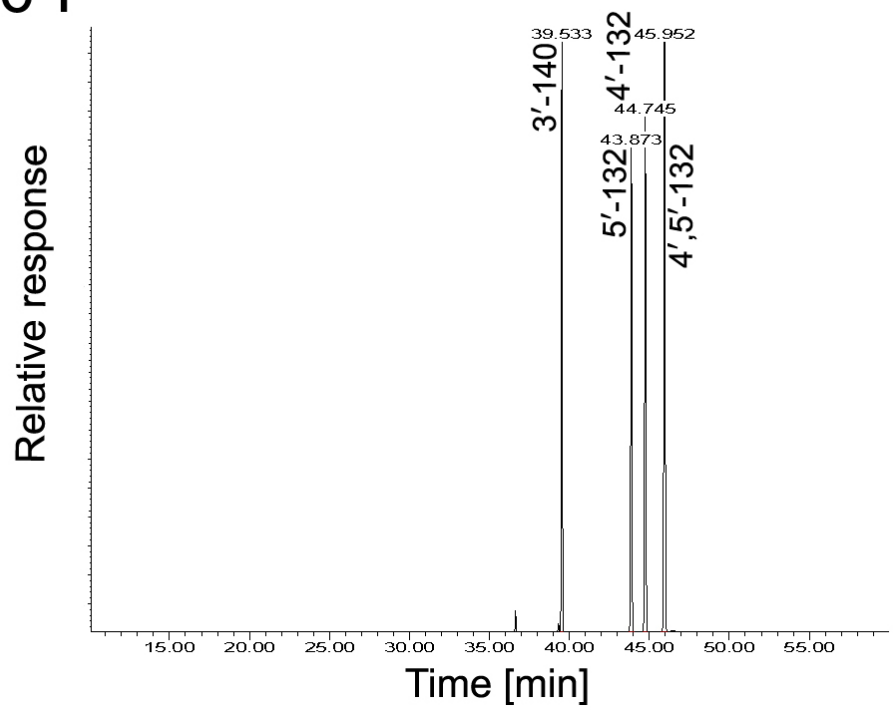
S6-D


Relative response
424
381
207
311
57
239
109
60 80 100 120 140 160 180 200 220 240 260 280 300 320 340 360 380 400 420
mass m/z

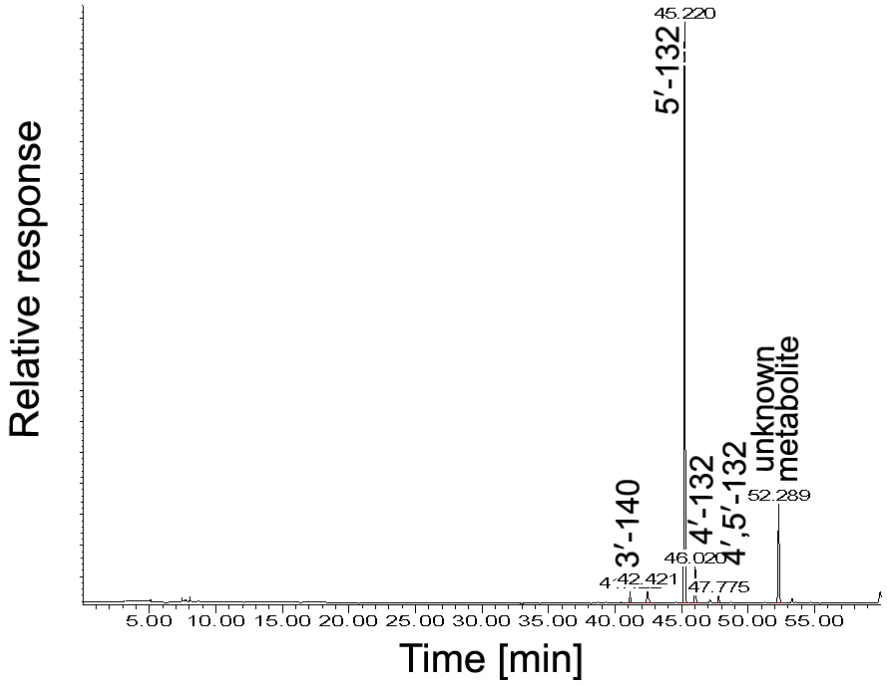
S6-E


43.855
5′-132
3′-140
39.57
44.72
4′-132
45.92
4′,5′-132
unknown
metabolite
50.5
Relative response
15.00
20.00
25.00
30.00
35.00
40.00
45.00
50.00
55.00
Time [min]

S6-F


39.533
3′-140
4′-132
45.952
44.745
43.873
5′-132
4′,5′-132
Relative response
15.00
20.00
25.00
30.00
35.00
40.00
45.00
50.00
55.00
Time [min]

S6-G


Relative response
45.220
5′-132
3′-140
42.421
46.020
4′-132
4′,5′-132
47.775
unknown metabolite
52.289
5.00 10.00 15.00 20.00 25.00 30.00 35.00 40.00 45.00 50.00 55.00
Time [min]

S6-H

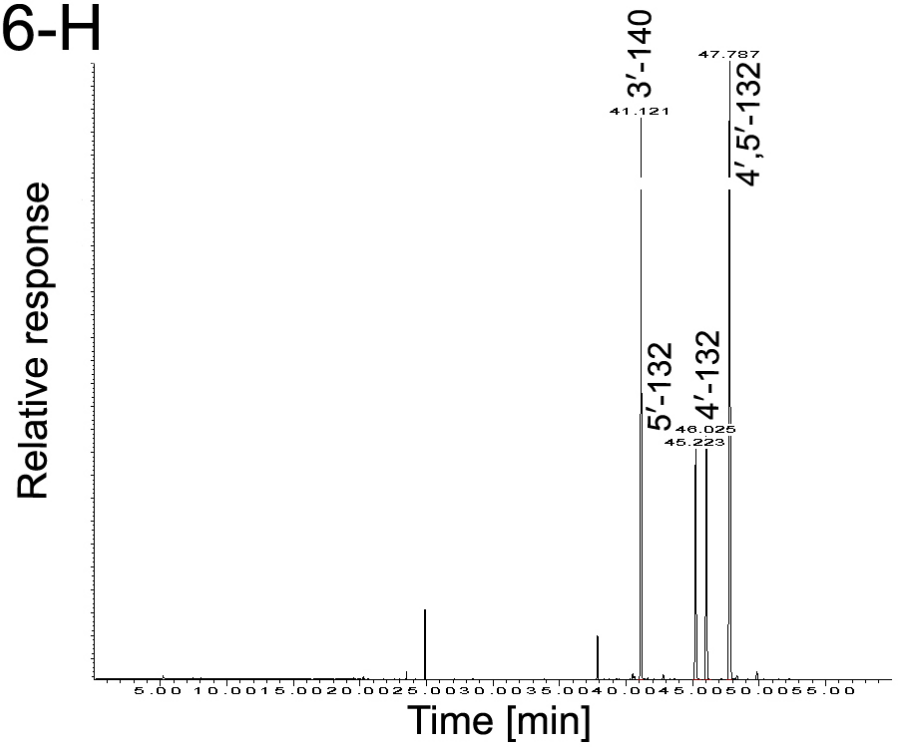
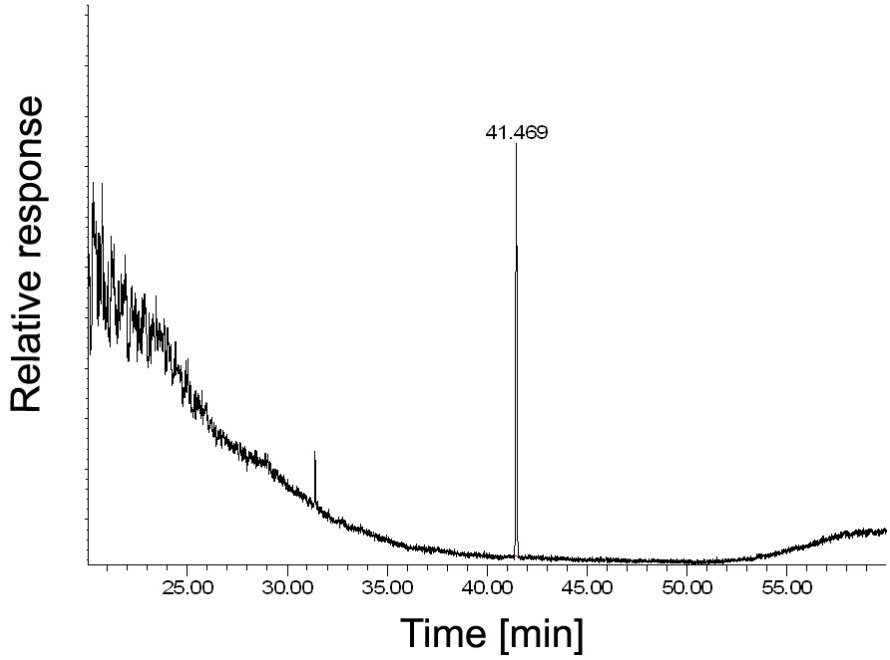
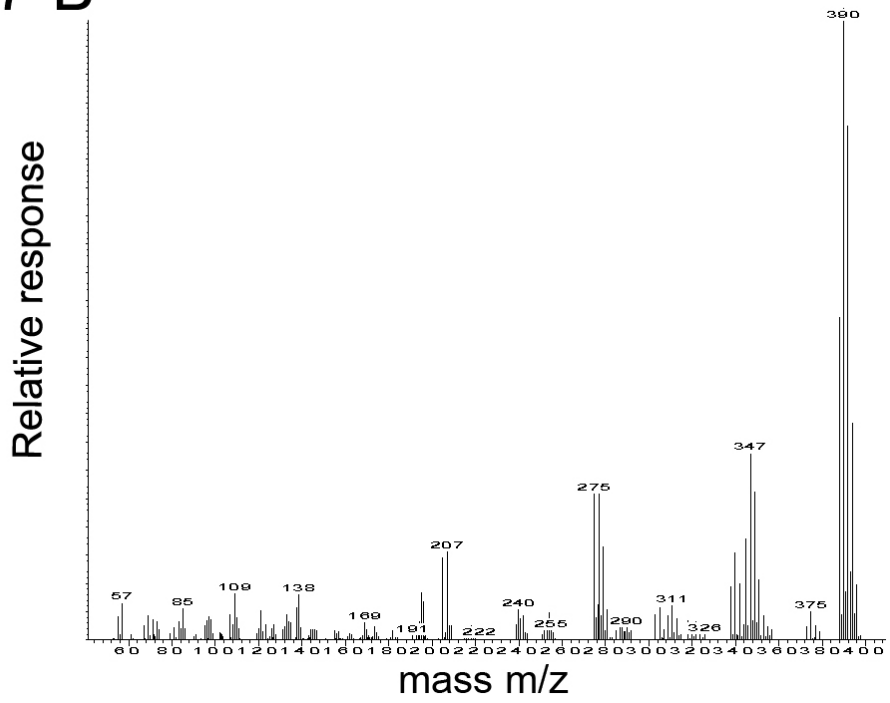

Relative response
3′-140
41.121
47.787
4′,5′-132
5′-132
46.025
4′-132
45.223
5.00 10.00 15.00 20.00 25.00 30.00 35.00 40.00 45.00 50.00 55.00
Time [min]

Figure S6. Microsomal metabolism of PCB 132 by liver microsomes. Four metabolites of PCB 132, including 5'-132, 3'-140, 4'-132 and 4',5'-132, are formed in the microsomal incubation according to GC-MS and GC-ECD analysis. **A-D.** Chromatogram (A) and mass spectra of metabolites (B-D) recorded in the TIC mode. The chromatogram shows a major peak at 43.9 min corresponding to a mono-methoxylated hexachlorobiphenyl (*m/z* 390), a minor peak of a mono-methoxylated hexachlorobiphenyl (*m/z* 390) at 44.7 min, and a peak at 50.5 min with a molecular ion at *m/z* 424 corresponding to a di-methoxylated hexachlorobiphenyl. **E-F.** Chromatograms of the incubation sample (E) and a standard containing authentic metabolites of PCB 132 (F) recorded in the SIM mode. The chromatogram of the incubation sample shows a major peak at 43.9 min, corresponding to 5'-132. Several minor peaks corresponding to 3'-140 (39.6 min), 4'-132 (44.7 min) and 4',5'-132 (45.9 min) were also detected. The metabolite ratio is 5'-132 : 3'-140 : 4'-132 : 4',5'-132 = 1 : 0.1 : 0.06 : 0.005. **G-H.** Chromatograms of the incubation sample (G) and the authentic standard (H) analyzed with GC-ECD. The chromatogram of the incubation shows a major peak at 45.2 min, corresponding to 5'-132. Several minor peaks corresponding to 3'-140 (41.1 min), 4'-132 (46.0 min) and 4',5'-132 (47.8 min) were also detected. The metabolite ratio is 5'-132 : 3'-140 : 4'-132 : 4',5'-132 = 1: 0.01 : 0.06 : 0.002.

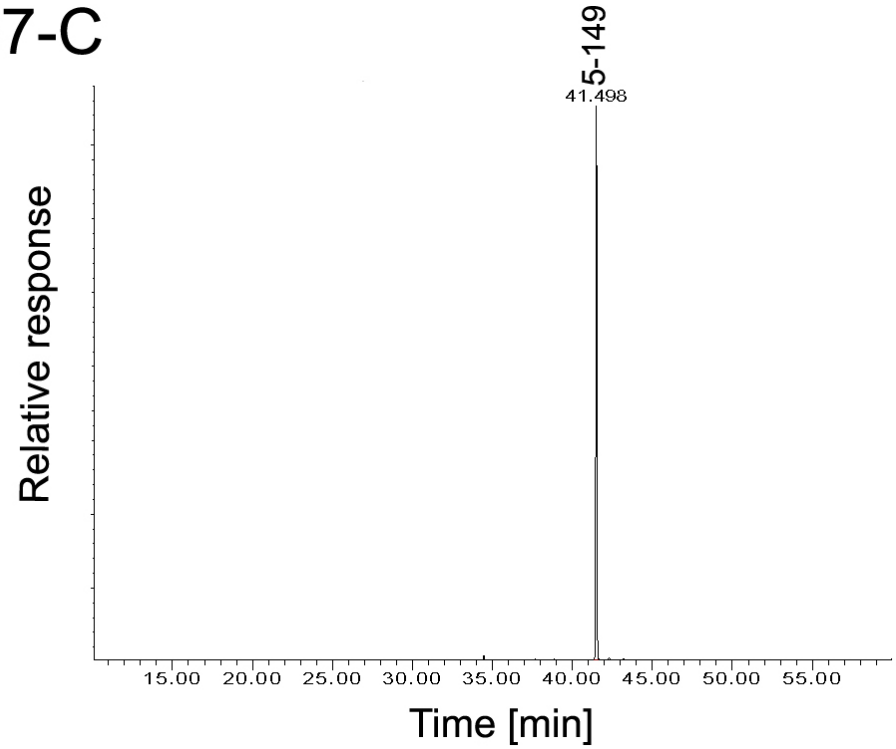
S7-A


Relative response
41.469
25.00
30.00
35.00
40.00
45.00
50.00
55.00
Time [min]

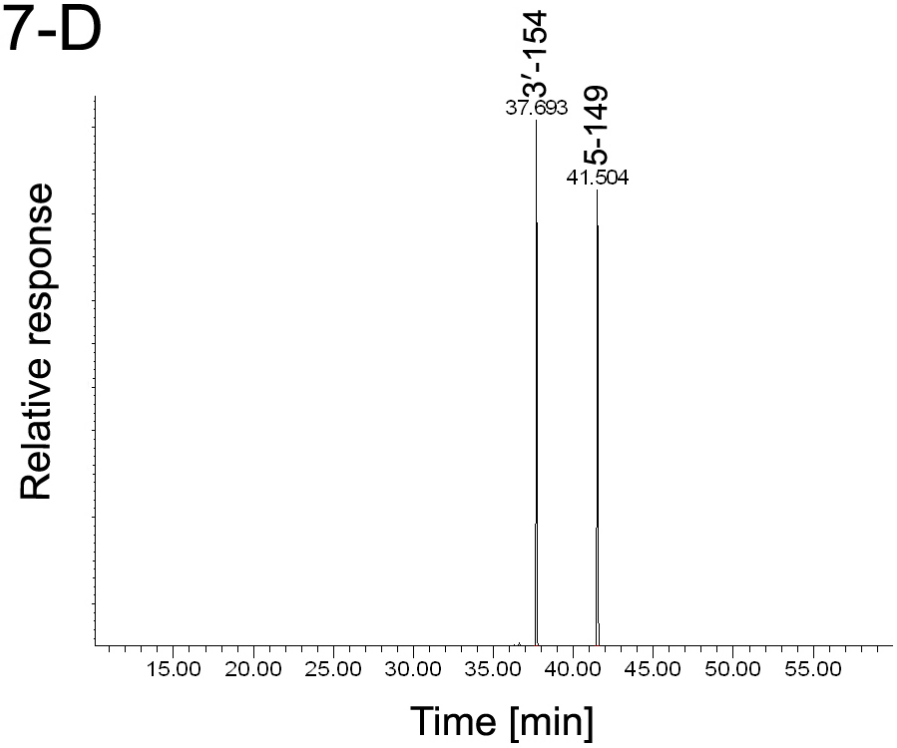
S7-B


Relative response
390
347
275
207
57
85
109
138
169
191
222
240
255
280
311
326
375
mass m/z

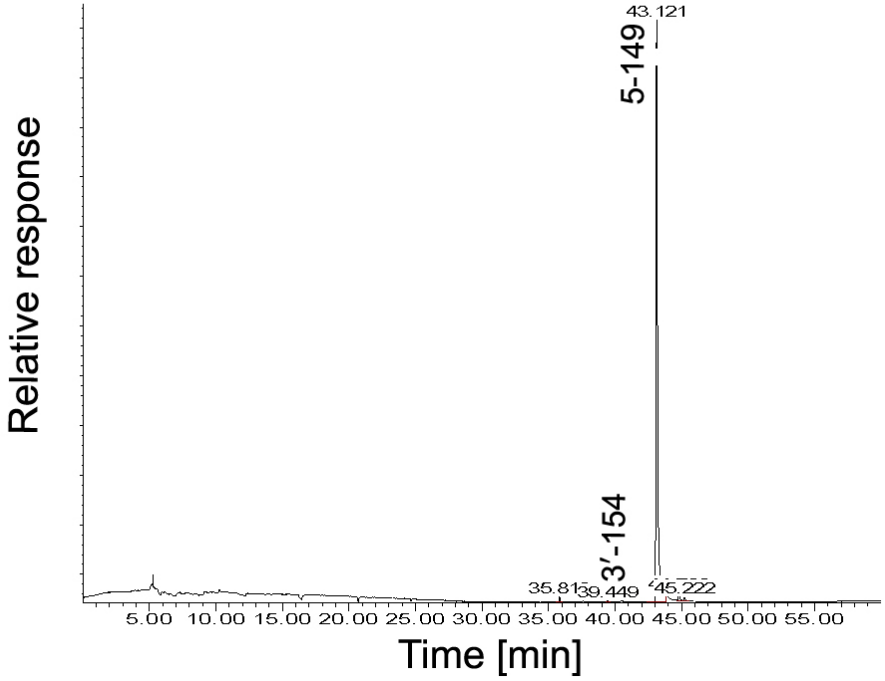
S7-C


5-149
41.498
Relative response
15.00 20.00 25.00 30.00 35.00 40.00 45.00 50.00 55.00
Time [min]

S7-D


3′-154
37.693
5-149
41.504
Relative response
15.00 20.00 25.00 30.00 35.00 40.00 45.00 50.00 55.00
Time [min]

S7-E


43.121
5-149
3′-154
35.813
39.449
45.222
Relative response
5.00 10.00 15.00 20.00 25.00 30.00 35.00 40.00 45.00 50.00 55.00
Time [min]

S7-F

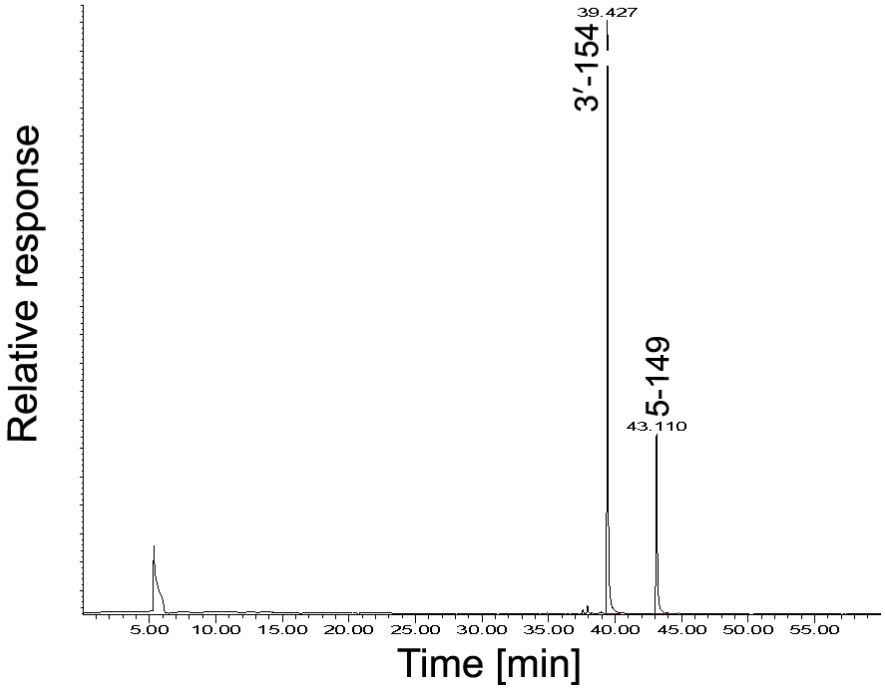
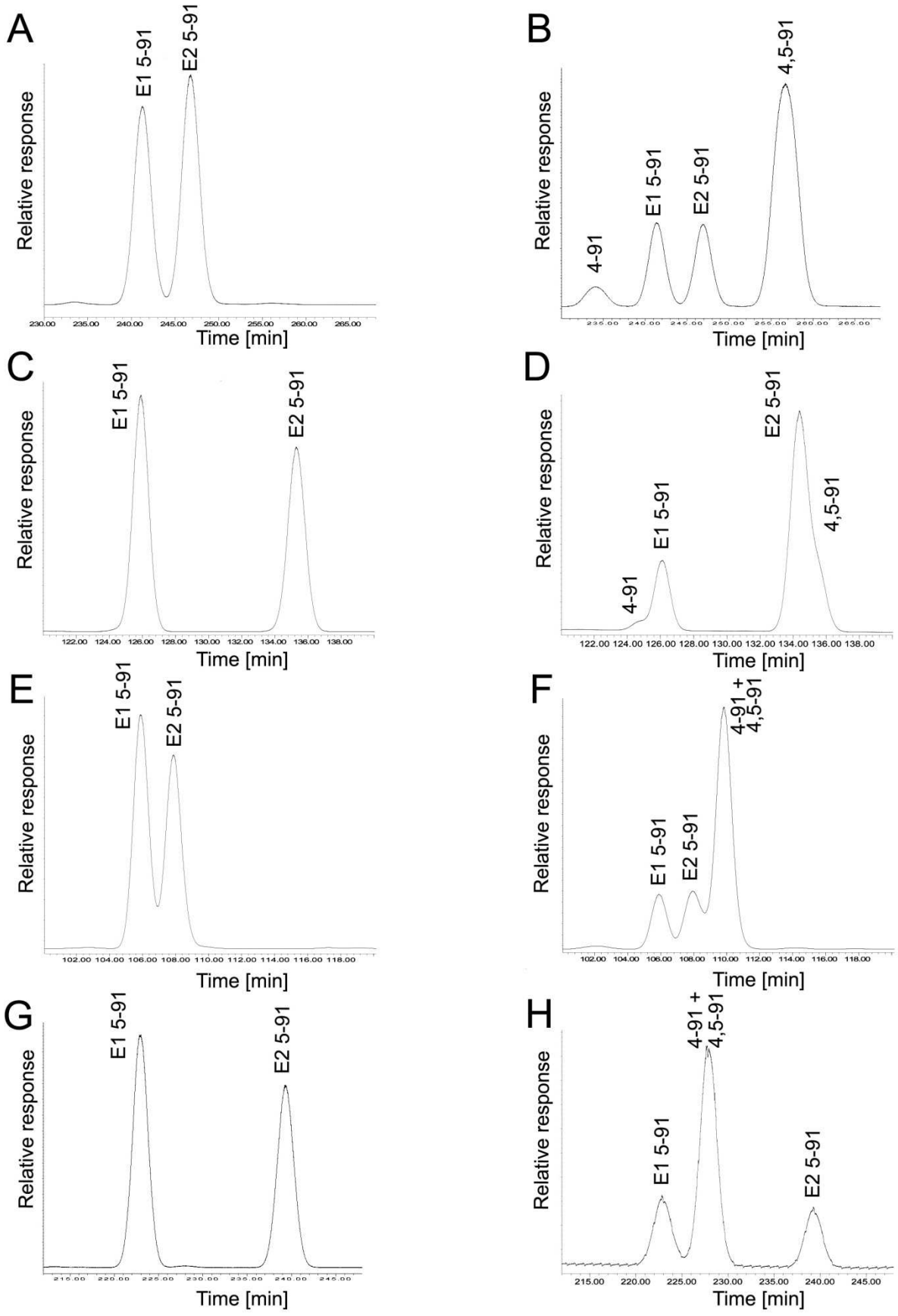

39.427
3′-154
5-149
43.110
Relative response
5.00 10.00 15.00 20.00 25.00 30.00 35.00 40.00 45.00 50.00 55.00
Time [min]

Figure S7. Microsomal metabolism of PCB 149 by liver microsomes. One major mono-methoxylated metabolite of PCB 149, corresponding to 5-149, is formed in microsomal incubation according to GC-MS and GC-ECD analysis. **A-B.** Chromatogram (A) and mass spectrum of the major metabolite (B) recorded in the TIC mode. The chromatogram shows a major peak at 41.5 min with a molecular ion corresponding to a mono-methoxylated hexachlorobiphenyl (*m/z* = 390). **C-D.** Chromatograms of the incubation sample (C) and a standard containing authentic metabolites of PCB 149 (D) recorded in the SIM mode. The chromatogram of the incubation sample shows a single major peak at 41.5 min, corresponding to 5-149. **E-F.** Chromatograms of the incubation sample (E) and a standard containing authentic metabolites of PCB 149 (F) analyzed with GC-ECD. Chromatogram of the incubation sample shows a single major peak at 43.1 min, corresponding to 5-149, and a minor peak at 39.4 min, corresponding to 3-154. The metabolite ratio is 5-149 : 3-154 = 1: 0.001.


A
E1 5-91
E2 5-91
Relative response
Time [min]
B
4-91
E1 5-91
E2 5-91
4,5-91
Relative response
Time [min]
C
E1 5-91
E2 5-91
Relative response
Time [min]
D
4-91
E1 5-91
E2 5-91
4,5-91
Relative response
Time [min]
E
E1 5-91
E2 5-91
Relative response
Time [min]
F
E1 5-91
E2 5-91
4-91 +
4,5-91
Relative response
Time [min]
G
E1 5-91
E2 5-91
Relative response
Time [min]
H
E1 5-91
4-91 +
4,5-91
E2 5-91
Relative response
Time [min]

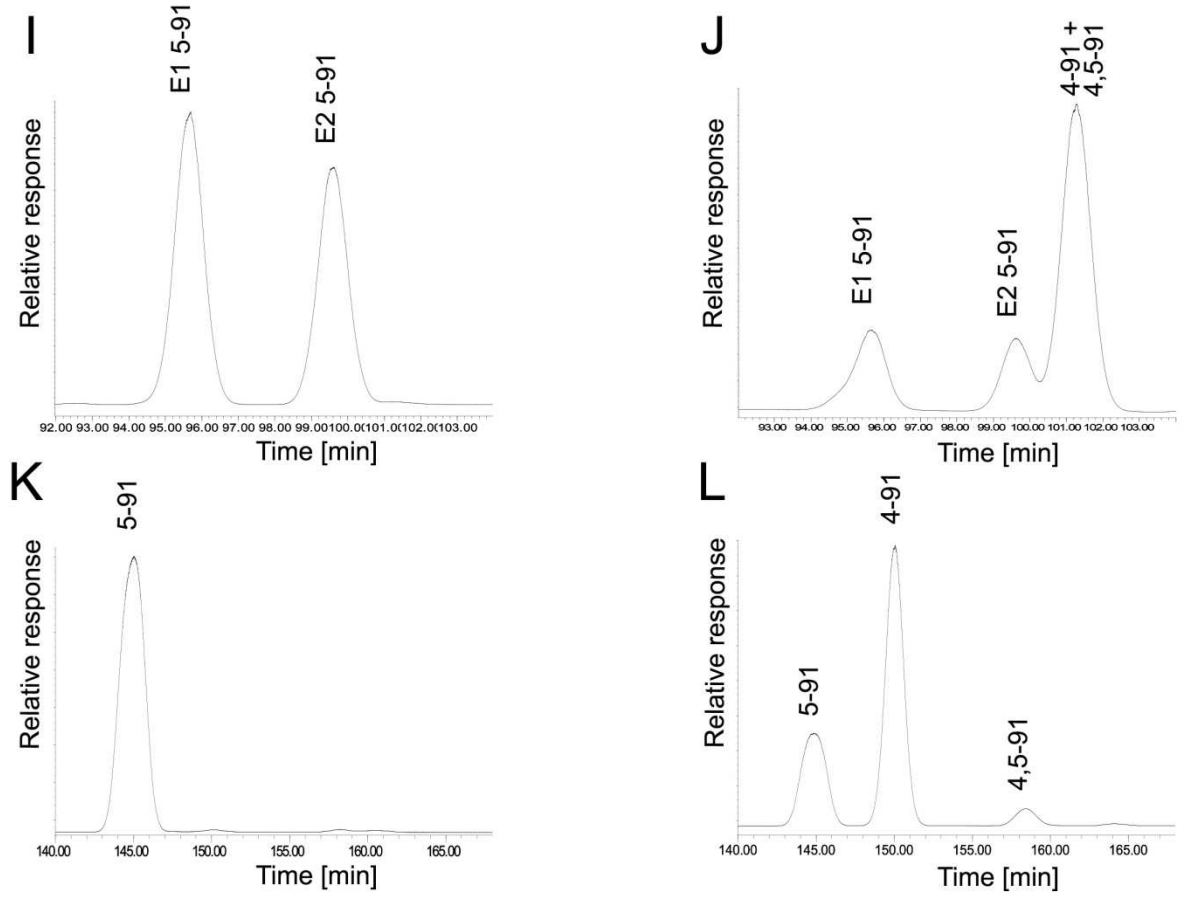
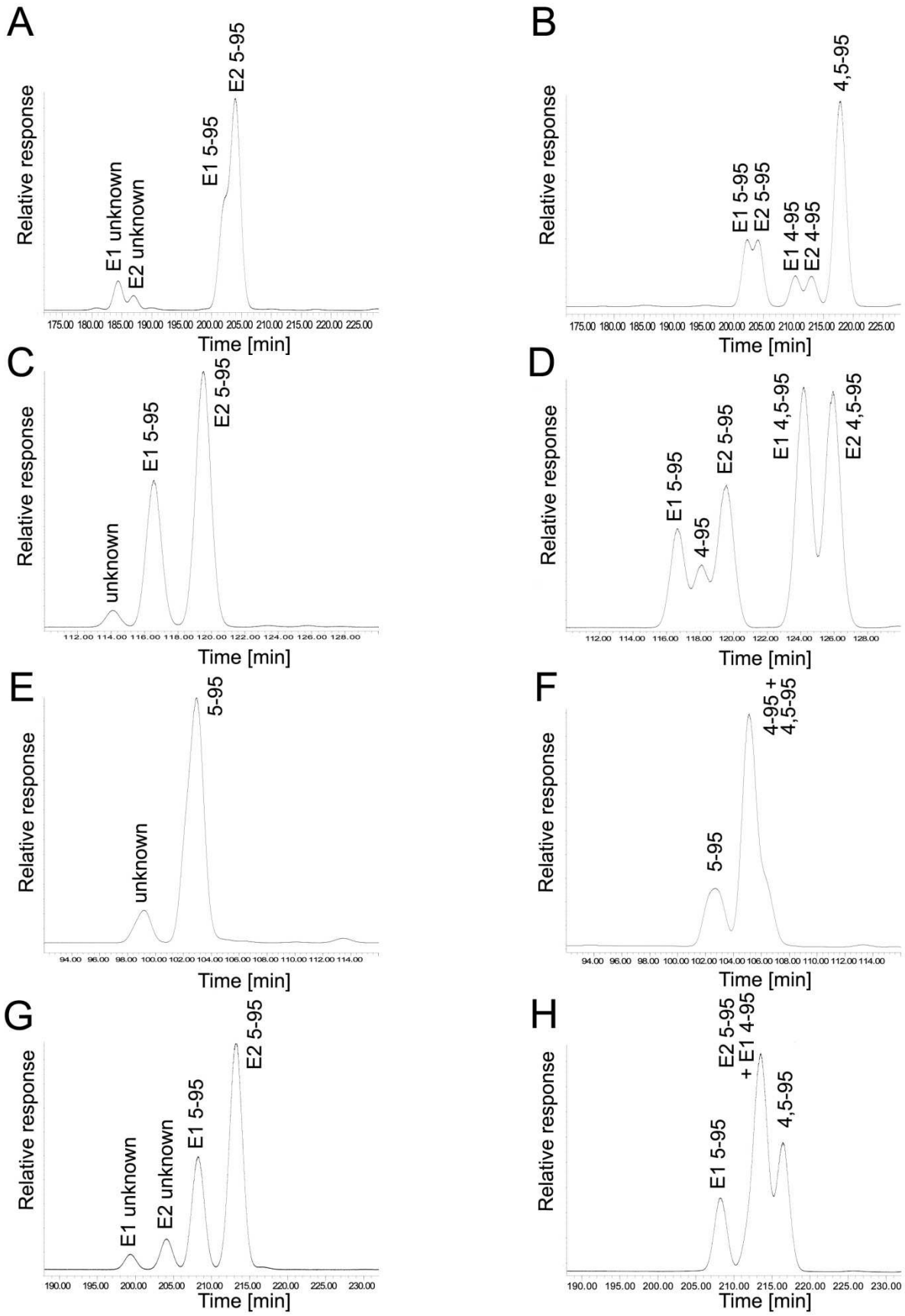


Figure S8. Enantiomeric enrichment of 5-91 isolated from an incubation of PCB 91 with liver microsomes. The diazomethane derivatized metabolite extract was analyzed on different enantioselective columns (left column) and compared to the corresponding metabolite standards (right column). **A.** incubation, BGB column, EF = 0.46. **B.** racemic standard, BGB column, EF = 0.50. **C.** incubation, BDM column, EF = 0.54. **D.** racemic standard, BDM column, no EF was determined because of co-elution of the second peak of 5-91 with 4,5-91. **E.** incubation, BPM column, EF = 0.54. **F.** racemic standard, BPM column, no EF was determined because the second eluting peak of 5-91 overlaps with 4- and 4,5-91. **G.** incubation, CB column, EF = 0.54. **H.** racemic standard, CB column, EF = 0.54. **I.** incubation, CD column, EF = 0.54. **J.** racemic standard, CD column, no EF was determined because first eluting peak of 5-91 co-elutes with 4-91 and the second eluting peak co-elutes with 4,5-91. **K.** incubation, GTA column, atropisomers of 5-91 are not resolved. **L.** racemic standard,

GTA column, metabolites of PCB 91 are not resolved. Note the reverse of the elution order of the atropisomers on the BGB column (A) compared to BDM (C), BPM (E), CB (G) and CD columns (I). Abbreviations of columns: BGB - BGB-172; BDM - Chiral-Dex B-DM, BPM - Chiral-Dex B-PM; CB - Cyclosil-B; CD - Chirasil-Dex (CD); GTA - Chiral-Dex G-TA. See Table S3 for additional information.


A
Relative response
E1 unknown
E2 unknown
E1 5-95
E2 5-95
175.00 180.00 185.00 190.00 195.00 200.00 205.00 210.00 215.00 220.00 225.00
Time [min]
B
Relative response
E1 5-95
E2 5-95
E1 4-95
E2 4-95
4,5-95
175.00 180.00 185.00 190.00 195.00 200.00 205.00 210.00 215.00 220.00 225.00
Time [min]
C
Relative response
unknown
E1 5-95
E2 5-95
112.00 114.00 116.00 118.00 120.00 122.00 124.00 126.00 128.00
Time [min]
D
Relative response
E1 5-95
4-95
E2 5-95
E1 4,5-95
E2 4,5-95
112.00 114.00 116.00 118.00 120.00 122.00 124.00 126.00 128.00
Time [min]
E
Relative response
unknown
5-95
94.00 96.00 98.00 100.00 102.00 104.00 106.00 108.00 110.00 112.00 114.00
Time [min]
F
Relative response
5-95
4-95 +
4,5-95
94.00 96.00 98.00 100.00 102.00 104.00 106.00 108.00 110.00 112.00 114.00
Time [min]
G
Relative response
E1 unknown
E2 unknown
E1 5-95
E2 5-95
190.00 195.00 200.00 205.00 210.00 215.00 220.00 225.00 230.00
Time [min]
H
Relative response
E1 5-95
E2 5-95
+ E1 4-95
4,5-95
190.00 195.00 200.00 205.00 210.00 215.00 220.00 225.00 230.00
Time [min]

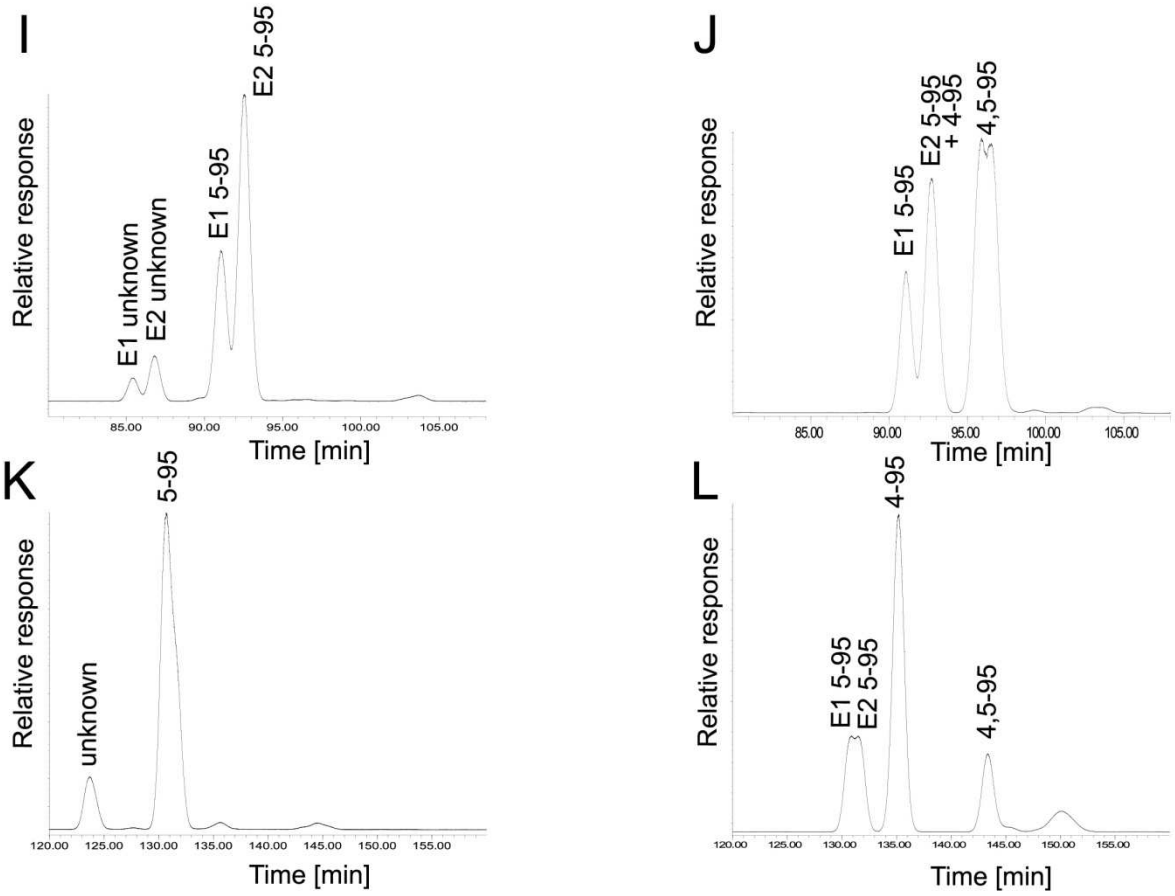
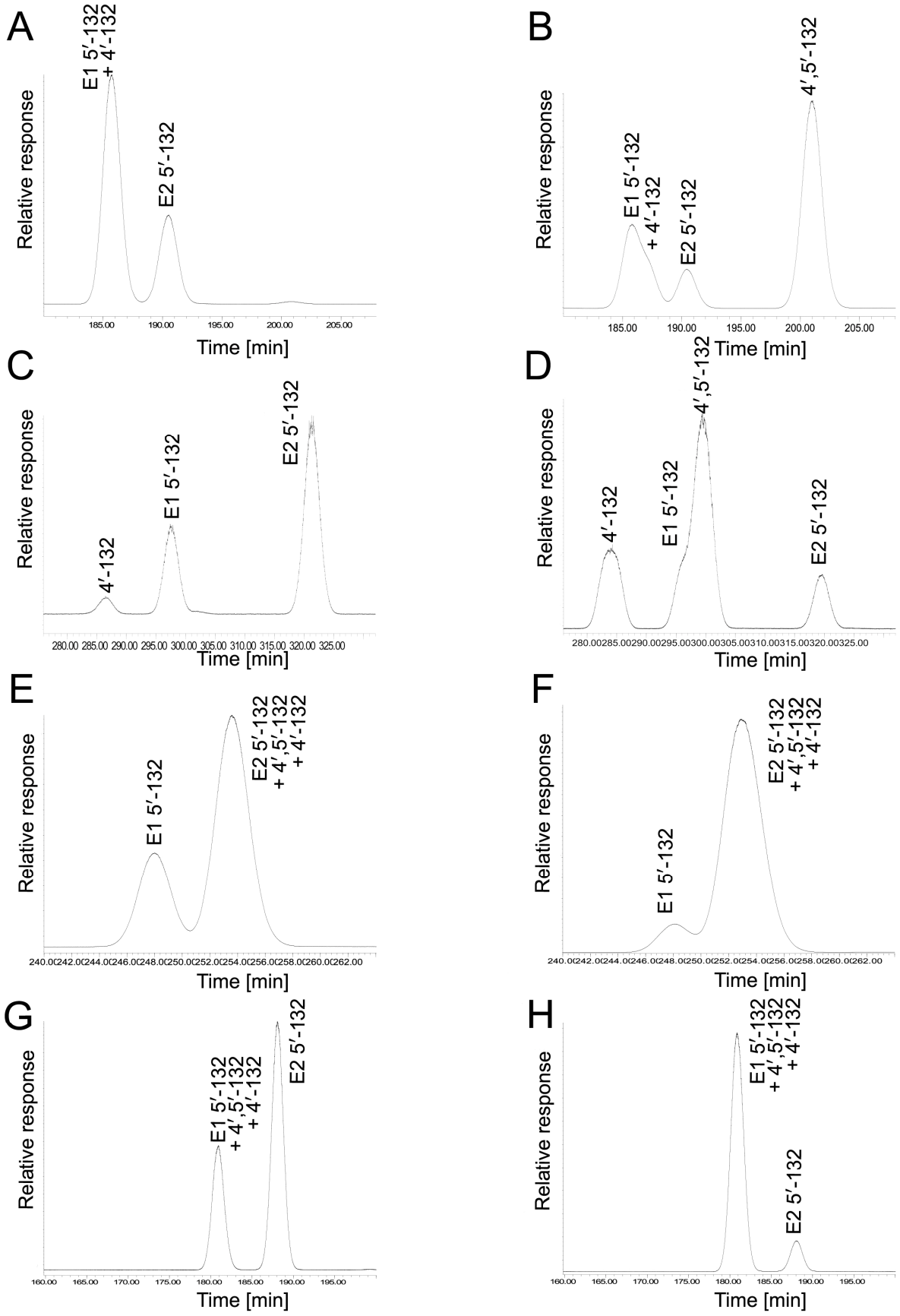


Figure S9. Enantiomeric enrichment of 5-95 and X-95 isolated from an incubation of PCB 95 with liver microsomes. The diazomethane derivatized metabolite extract was analyzed on different enantioselective columns (left column) and compared to the corresponding metabolite standards (right column). **A.** incubation, BGB column, resolution is not sufficient to determine EF of 5-95. X-95 EF = 0.68. **B.** racemic standard, BGB column, resolution is not sufficient to determine EF of 5-95. **C.** incubation, BDM column, 5-95 EF = 0.36. **D.** racemic standard, BDM column, no EF was determined because of co-elution of both peaks of 5-95 with 4-95. **E**. incubation, BPM column, atropisomers of 5-95 are not resolved. **F.** racemic standard, BPM column, metabolites of PCB 95 are not resolved. **G.** incubation, CB column, 5-95 EF = 0.32 and X-95 EF = 0.33. **H.** racemic standard, CB column, no EF was determined because of co-elution of second atropisomer of 5-95 with 4-95. **I.** incubation, CD column, 5-95 EF = 0.33. X-95 EF = 0.33. **J.** racemic standard, CD column, no EF was determined

because the second eluting peak of 5-95 co-elutes with 4-95. **K.** incubation, GTA column, resolution is not sufficient to determine EF; however, the first eluting atropisomer appears to be enriched. **L.** racemic standard, GTA column, resolution is not sufficient to determine EF. Abbreviations of columns: BGB - BGB-172; BDM - Chiral-Dex B-DM, BPM - Chiral-Dex B-PM; CB - Cyclosil-B; CD - Chirasil-Dex (CD); GTA - Chiral-Dex G-TA. See Table S3 for additional information.


A
E1 5′-132
+ 4′-132
E2 5′-132
Relative response
185.00 190.00 195.00 200.00 205.00
Time [min]
B
4′,5′-132
E1 5′-132
+ 4′-132
E2 5′-132
Relative response
185.00 190.00 195.00 200.00 205.00
Time [min]
C
E2 5′-132
E1 5′-132
4′-132
Relative response
280.00 285.00 290.00 295.00 300.00 305.00 310.00 315.00 320.00 325.00
Time [min]
D
4′,5′-132
4′-132
E1 5′-132
E2 5′-132
Relative response
280.00285.00290.00295.00300.00305.00310.00315.00320.00325.00
Time [min]
E
E2 5′-132
+ 4′,5′-132
+ 4′-132
E1 5′-132
Relative response
240.0242.0244.0246.0248.0250.0252.0254.0256.0258.0260.0262.00
Time [min]
F
E2 5′-132
+ 4′,5′-132
+ 4′-132
E1 5′-132
Relative response
240.0242.0244.0246.0248.0250.0252.0254.0256.0258.0260.0262.00
Time [min]
G
E2 5′-132
E1 5′-132
+ 4′,5′-132
+ 4′-132
Relative response
160.00 165.00 170.00 175.00 180.00 185.00 190.00 195.00
Time [min]
H
E1 5′-132
+ 4′,5′-132
+ 4′-132
E2 5′-132
Relative response
160.00 165.00 170.00 175.00 180.00 185.00 190.00 195.00
Time [min]

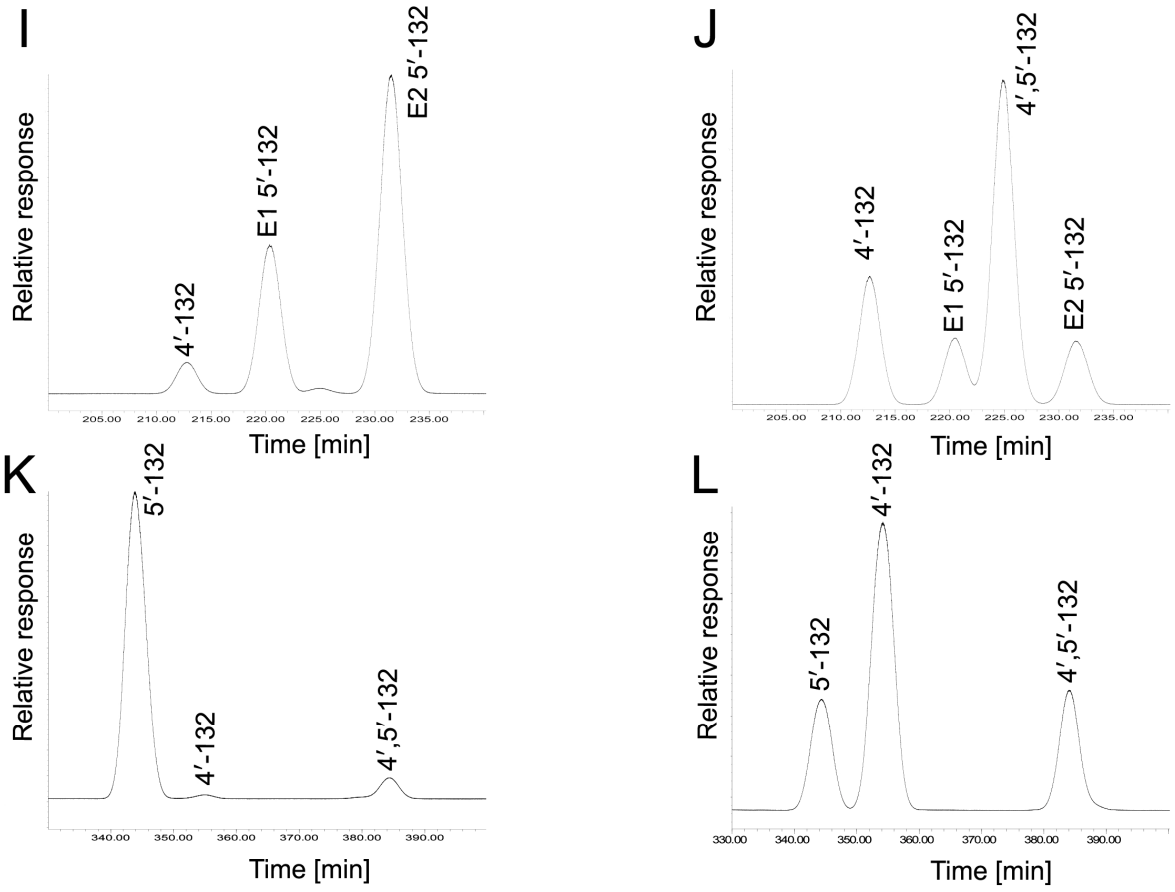
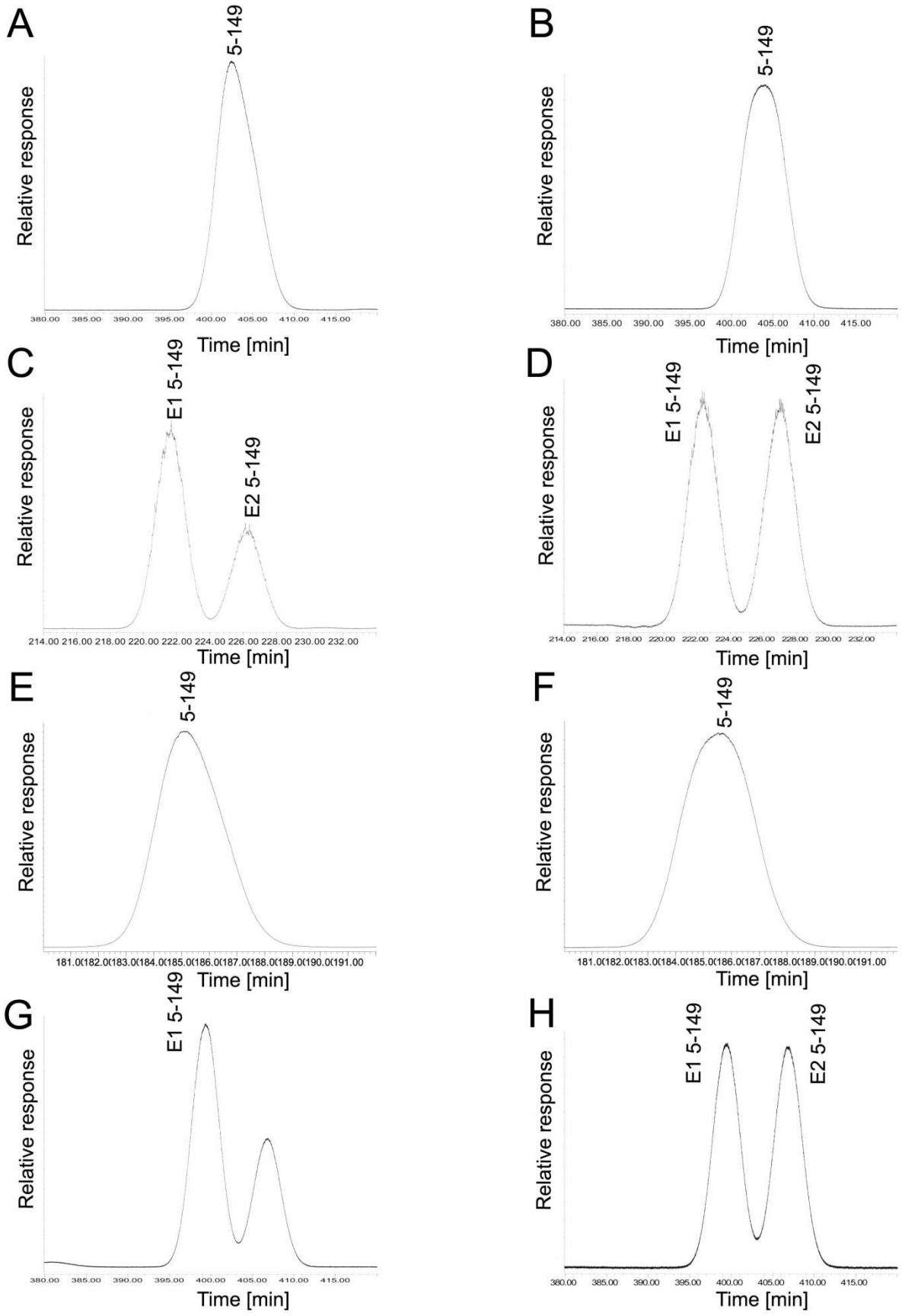


Figure S10. Enantiomeric enrichment of 5'-132 isolated from an incubation of PCB 132 with liver microsomes. The diazomethane derivatized metabolite extract was analyzed on different enantioselective columns (left column) and compared to the corresponding metabolite standards (right column). **A.** incubation, BGB column, EF = 0.71. First eluting peak of 5'-132 co-elutes with 4'-132. **B.** racemic standard, BGB column, no EF was determined because of co-elution of the first eluting peak of 5'-132 with 4'-132. **C.** incubation, BDM column, EF = 0.30. **D.** racemic standard, BDM column, no EF was determined because of co-elution of first eluting peak of 5'-132 with 4',5'-132. **E.** incubation, BPM column, EF = 0.28. Second eluting atropisomer of 5'-132 co-elutes with 4'- and 4',5'-132. **F.** racemic standard, BPM column, no EF was determined because the second eluting atropisomer of 5'-132 co-elutes with 4'- and 4',5'-132. **G.** incubation, CB column, EF = 0.31. First eluting peak of 5'-132 co-elutes with 4'- and 4',5'-132. **H.** racemic standard, CB column, no EF was determined because the first eluting peak of 5'-132 co-elutes with 4'- and 4',5'-132. **I.** incubation, CD column, EF = 0.31. **J.** racemic

standard, CD column, EF = 0.50. **K.** incubation, GTA column, the atropisomers of 5'-132 are not resolved. **L.** racemic standard, GTA column, methoxylated metabolites of PCB 132 are not resolved. Note the reverse of the elution order of the atropisomers on the BGB column (A) compared to BDM (C), BPM (E), CB (G) and CD columns (I). Abbreviations of columns: BGB - BGB-172; BDM - Chiral-Dex B-DM, BPM - Chiral-Dex B-PM; CB - Cyclosil-B; CD - Chirasil-Dex (CD); GTA - Chiral-Dex G-TA. See Table S3 for additional information.


A
5-149
Relative response
380.00 385.00 390.00 395.00 400.00 405.00 410.00 415.00
Time [min]
B
5-149
Relative response
380.00 385.00 390.00 395.00 400.00 405.00 410.00 415.00
Time [min]
C
E1 5-149
E2 5-149
Relative response
214.00 216.00 218.00 220.00 222.00 224.00 226.00 228.00 230.00 232.00
Time [min]
D
E1 5-149
E2 5-149
Relative response
214.00 216.00 218.00 220.00 222.00 224.00 226.00 228.00 230.00 232.00
Time [min]
E
5-149
Relative response
181.0(182.0(183.0(184.0(185.0(186.0(187.0(188.0(189.0(190.0(191.00
Time [min]
F
5-149
Relative response
181.0(182.0(183.0(184.0(185.0(186.0(187.0(188.0(189.0(190.0(191.00
Time [min]
G
E1 5-149
Relative response
380.00 385.00 390.00 395.00 400.00 405.00 410.00 415.00
Time [min]
H
E1 5-149
E2 5-149
Relative response
380.00 385.00 390.00 395.00 400.00 405.00 410.00 415.00
Time [min]

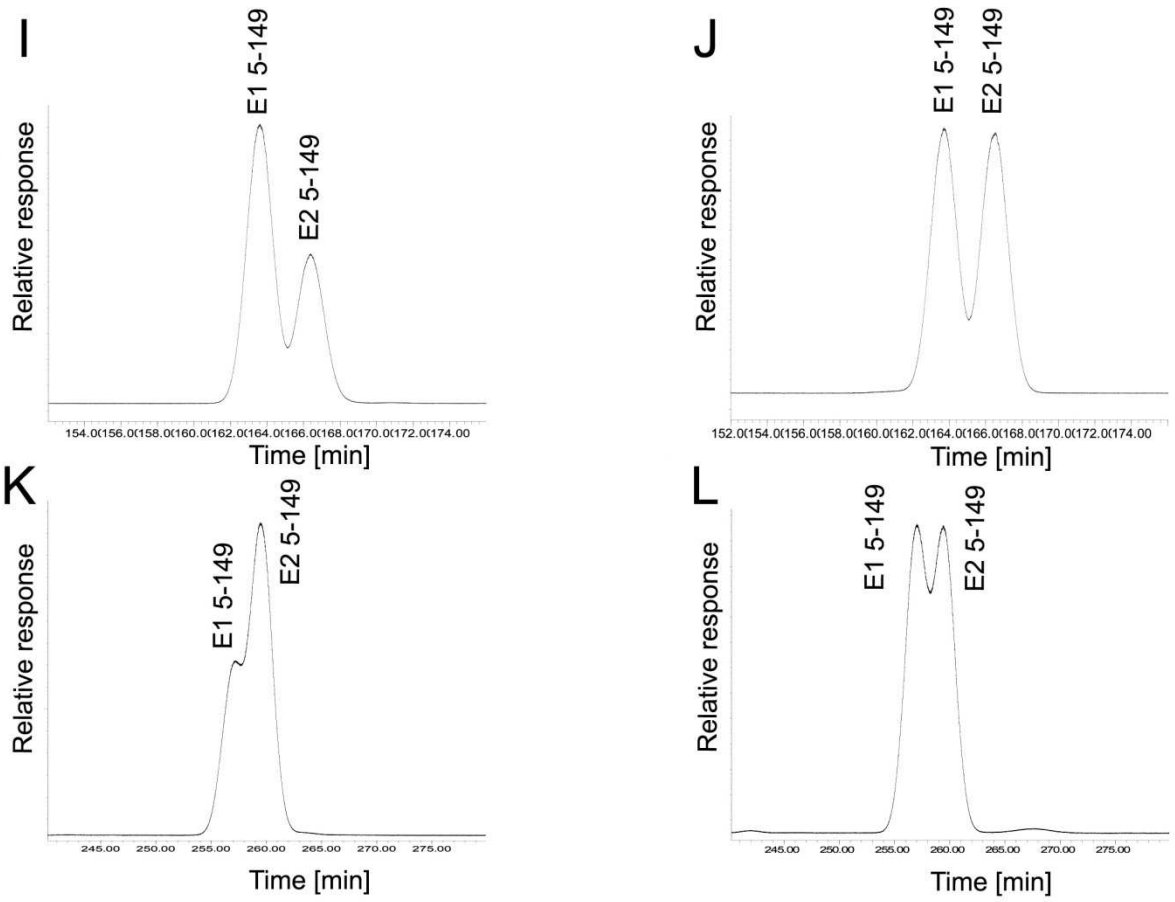


Figure S11. Enantiomeric enrichment of 5-149 isolated from an incubation of PCB 149 with liver microsomes. The diazomethane derivatized metabolite extract was analyzed on different enantioselective columns (left column) and compared to the corresponding metabolite standards (right column). **A.** incubation, BGB column, the atropisomers of 5'-132 are not resolved. **B.** racemic standard, BGB column, the atropisomers of 5'-132 are not resolved. **C.** incubation, BDM column, EF = 0.66. **D.** racemic standard, BDM column, EF = 0.50. **E.** incubation, BPM column, the atropisomers of 5'-132 are not resolved. **F.** racemic standard, BPM column, the atropisomers of 5'-132 are not resolved. **G.** incubation, CB column, EF = 0.65. **H.** racemic standard, CB column, EF = 0.50. **I.** incubation, CD column, EF = 0.65. **J.** racemic standard, CD column, EF = 0.50. **K.** incubation, GTA column, EF = 0.29. **L.** racemic standard, GTA column, EF = 0.50. Note the reverse of elution order of the atropisomers on the GTA column (K) compared to BDM (C), CB (G) and CD columns (I). Abbreviations of columns: BGB - BGB-172; BDM - Chiral-Dex B-DM, BPM - Chiral-Dex B-PM; CB -

Cyclosil-B; CD - Chirasil-Dex (CD); GTA - Chiral-Dex G-TA. See Table S3 for additional information.

References

1. Thomas, P. E.; Reik, L. M.; Ryan, D. E.; Levin, W., Induction of two immunochemically related rat liver cytochrome P-450 isozymes, cytochromes P-450c and P-450d, by structurally diverse xenobiotics. J. Biol. Chem. 1983, 258, (7), 4590-4598.

2. Kania-Korwel, I.; Hrycay, E. G.; Bandiera, S.; Lehmler, H.-J., 2,2',3,3',6,6'-hexachlorobiphenyl (PCB 136) atropisomers interact enantioselectively with hepatic microsomal cytochrome P450 enzymes. Chem. Res. Toxicol. 2008, 21, (6), 1295-1303.

3. Lowry, O. H.; Rosenbrough, N. J.; Rarr, A. L.; Randall, R. J., Protein measurement with Folin Phenol reagent. J. Biol. Chem. 1951, 193, 265-275.

4. Renwick, A. B.; Lavignette, G.; Worboy, P. D.; Williams, B.; Surry, D.; Lewis, D. F.; Price, R. J.; Lake, B. G.; Evans, D. C., Evaluation of 7-benzyloxy-4-trifluoromethylcoumarin, some other 7-hydroxy-4-trifluoromethylcoumarin derivatives and 7-benzyloxyquinoline as fluorescent substrates for rat hepatic cytochrome P450 enzymes. Xenobiotica 2001, 31, (12), 861-78.

5. Burke, D. M. T., S.; Elcombe, C. R.; Halpert, J.; Haaparanta, T.; Mayer, R. T.;, Ethoxy-, pentoxy- and benzyloxyphenoxazones and homologues: A series of substrates to distinguish between different induced cytochromes P-450. Biochem. Pharmacol. 1985, 34, (18), 3337-3345.

6. McNaught, A. D.; Wilkinson, A., IUPAC Compendium of Chemical Terminology. 2nd Edition ed.; Blackwell Science Ltd: Oxford, UK, 1997.