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### **Supplemental Material**

#### **Cardiovascular Effects of Polychlorinated Biphenyls and Their Major Metabolites**

Fabian A. Grimm, William D. Klaren, Xueshu Li, Hans-Joachim Lehmler, Moumita Karmakar, Larry W. Robertson, Weihsueh A. Chiu, and Ivan Rusyn

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## References

**Additional File-** Excel Document

**Table S1.** Chemical names, abbreviations and references describing the synthesis and authentication of the PCB derivatives used in this study.

<b>Name</b>	<b>Abbreviation</b>	<b>References</b>
4-Chlorobiphenyl	PCB 3	(Espandiari et al. 2004)
3,3'-Dichlorobiphenyl	PCB 11	(Holland et al. 2017; Sethi et al. 2019)
2,2',5,5'-Tetrachlorobiphenyl	PCB 52	(Kania-Korwel et al. 2004)
2,2',3,5',6-Pentachlorobiphenyl	PCB 95	(Kania-Korwel et al. 2004)
3,3',4,4',5-Pentachlorobiphenyl	PCB 126	(Gadupudi et al. 2018)
2,2',4,4',5,5'-Hexachlorobiphenyl	PCB 153	(Kania-Korwel et al. 2004; Shaikh et al. 2006)
(-)-2,2',3, 3',6,6'-Hexachlorobiphenyl	(-)-PCB 136	(Kania-Korwel et al. 2008)
(+)-2,2',3,3',6,6'-Hexachlorobiphenyl	(+)-PCB 136	(Kania-Korwel et al. 2008)
4-Chlorobiphenyl-2'-ol	2'-OH-PCB3	(Dhakal et al. 2014)
4-Chlorobiphenyl-3'-ol	3'-OH-PCB3	(Dhakal et al. 2014)
4-Chlorobiphenyl-4'-ol	4'-OH-PCB3	(Dhakal et al. 2014)
3,3'-Dichlorobiphenyl-4-ol	4-OH-PCB11	(Alam et al. 2018)
3,3'-Dichlorobiphenyl-5-ol	5-OH PCB 11	(Joshi et al. 2011; Lehmler and Robertson 2001)
4-Chloro-2',5'-dihydroxybiphenyl	2',5'-diOH-PCB 3	(McLean et al. 1996)
2,2',5,5'-Tetrachlorobiphenyl-4-ol	4-OH-PCB 52	(Rodriguez et al. 2016)
4'-Methoxy-4-chlorobiphenyl	4'-MeO-PCB 3	(Joshi et al. 2011; Lehmler and Robertson 2001)
4-Methoxy-3,3'-dichlorobiphenyl	4-MeO-PCB 11	(Joshi et al. 2011; Lehmler and Robertson 2001)
4-Methoxy-2,2',5,5'-tetrachlorobiphenyl	4-MeO-PCB 52	(Rodriguez et al. 2016)
2-(4-Chlorophenyl)benzo-2,5-quinone	4-Cl-BQ	(Song et al. 2008)
Sulfuric acid mono-(4-chlorobiphenyl-2-yl) ester, ammonium salt	2-PCB 3 sulfate	(Dhakal et al. 2014)
Sulfuric acid mono-(4-chlorobiphenyl-3-yl) ester, ammonium salt	3-PCB 3 sulfate	(Dhakal et al. 2014)
Sulfuric acid mono-(4-chlorobiphenyl-2-yl) ester, ammonium salt	2'-PCB 3 sulfate	(Dhakal et al. 2014)
Sulfuric acid mono-(4-chlorobiphenyl-3'-yl) ester, ammonium salt	3'-PCB 3 sulfate	(Li et al. 2010)
Sulfuric acid mono-(4-chlorobiphenyl-4'-yl) ester, ammonium salt	4'-PCB 3 sulfate	(Li et al. 2010)
Sulfuric acid mono-(3,3'-dichlorobiphenyl-4-yl) ester, ammonium salt	4-PCB 11 sulfate	(Li et al. 2010; Rodriguez et al. 2016; Sethi et al. 2019)
Sulfuric acid mono-(2,2',5,5'-tetrachlorobiphenyl-4-yl) ester, ammonium salt	4-PCB 52 sulfate	(Li et al. 2010; Rodriguez et al. 2016)

**Table S2. Data files for all cell types and phenotypes used in this study (Supplemental Excel File)**

Sheet "Read\_me\_abbreviations" – all acronyms used in the data files

Sheet "iPSC-CM" – data for iCell cardiomyocytes

Sheet "iPSC-EC" – data for iCell endothelial cells

Sheet "HUVECs" – data for HUVECs

Sheet "POD\_summary" – POD data for all cell types and phenotypes

Sheet "ToxPi\_summary" – ToxPi data for all cell types and phenotypes

Sheet "ToxPi Slice Distribution" – Explanation for all slice abbreviations and which data were included in which slice in Figure 4

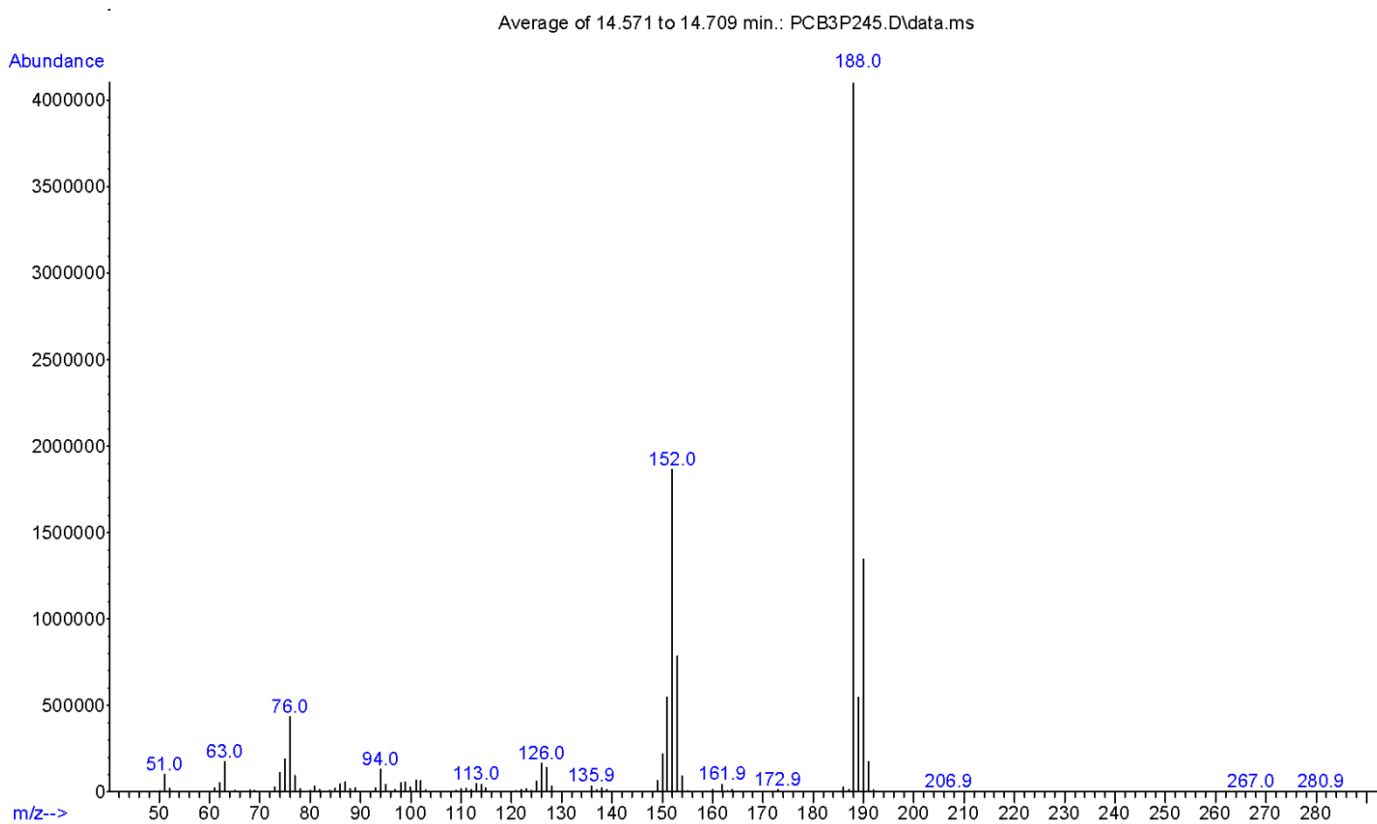
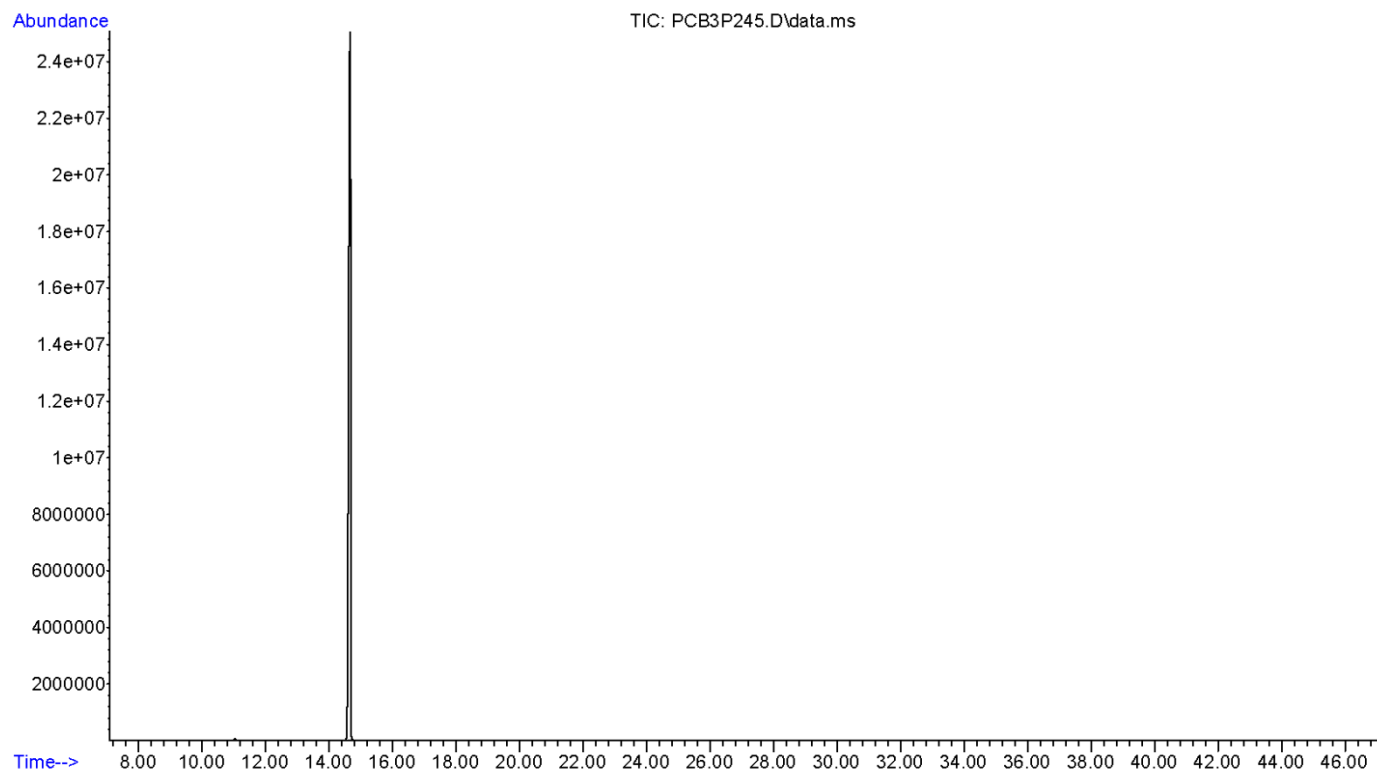
Sheet "ToxPi Scores" – ToxPi scores for each comparison shown in Figure 4

### **Supplemental Text 1. Purity determination by GC-MS**

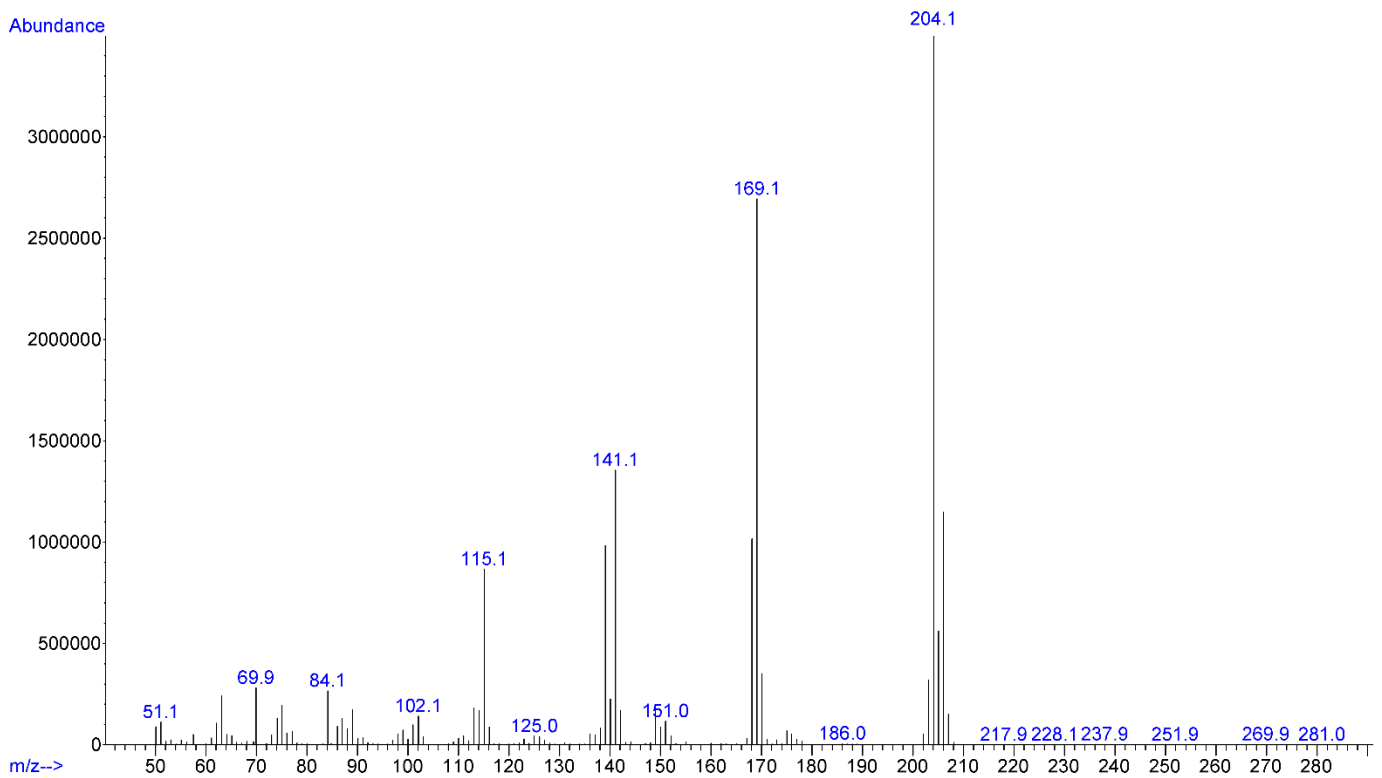
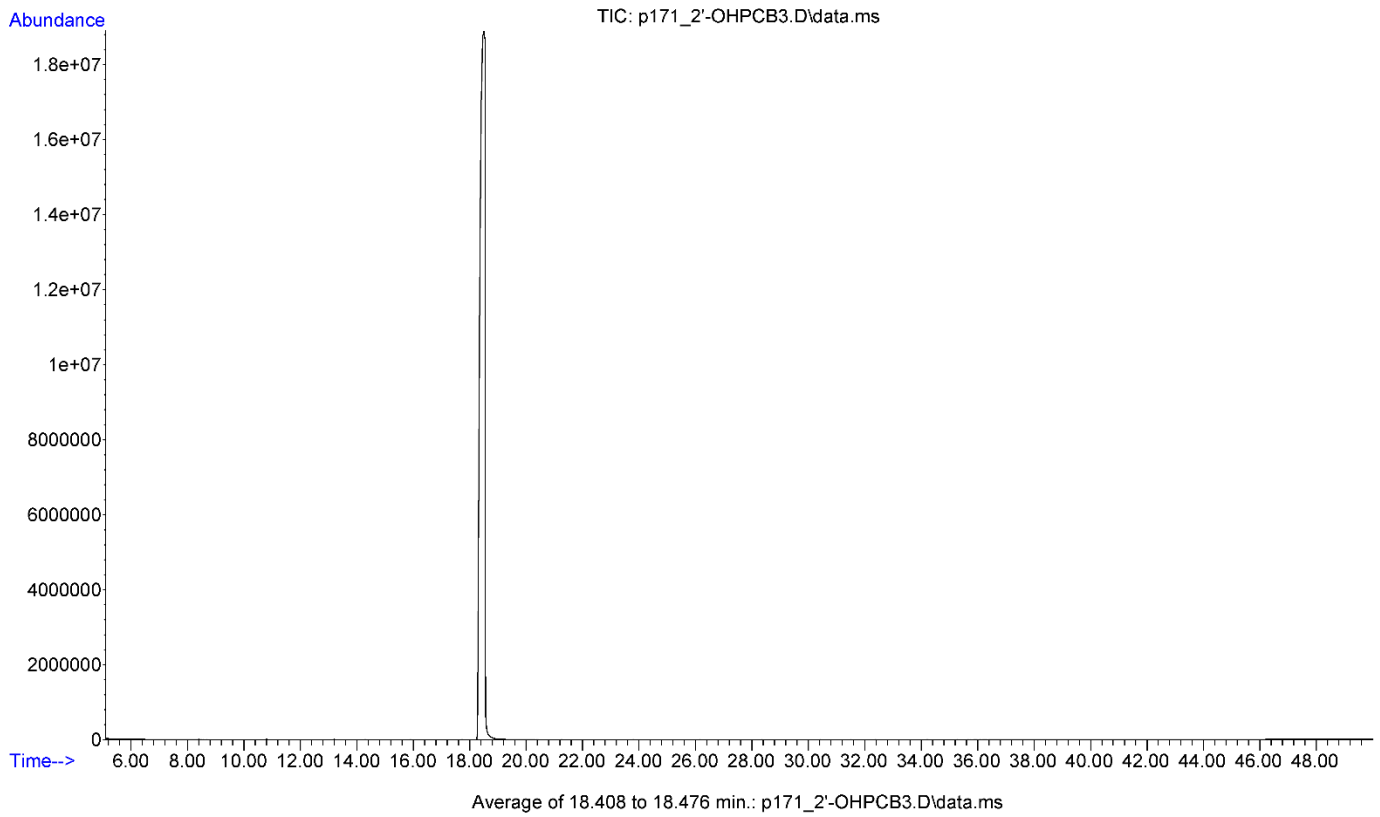
All PCBs, hydroxylated PCBs, methoxylated PCBs, and the PCB quinone were analyzed on an Agilent 7890A gas chromatography equipped with an Agilent 5975 Inert Mass Selective Detector (Agilent Technologies, CA, USA) operated in electronic ionization mode on a SLB-5MS capillary column (30 m length, 250  $\mu\text{m}$  inner diameter, 0.25  $\mu\text{m}$  film thickness; Supelco, Bellefonte, PA, USA). The temperature program was as described previously (Li et al. 2018). Briefly, the oven temperature started at 50  $^{\circ}\text{C}$ , 10  $^{\circ}\text{C}/\text{min}$  to 150  $^{\circ}\text{C}$ , then 5  $^{\circ}\text{C}/\text{min}$  to 280  $^{\circ}\text{C}$ , hold for 6 min, and then 10  $^{\circ}\text{C}/\text{min}$  to 300  $^{\circ}\text{C}$ , hold for 10 min. Helium was used as carrier gas with a constant flow rate of 1.5 mL/min. The injector temperature was 280  $^{\circ}\text{C}$ . The temperatures of transfer line, source and quadrupole were 280  $^{\circ}\text{C}$ , 230  $^{\circ}\text{C}$  and 150  $^{\circ}\text{C}$ , respectively.

### **Supplemental Text 2. Enantiomeric purity determination**

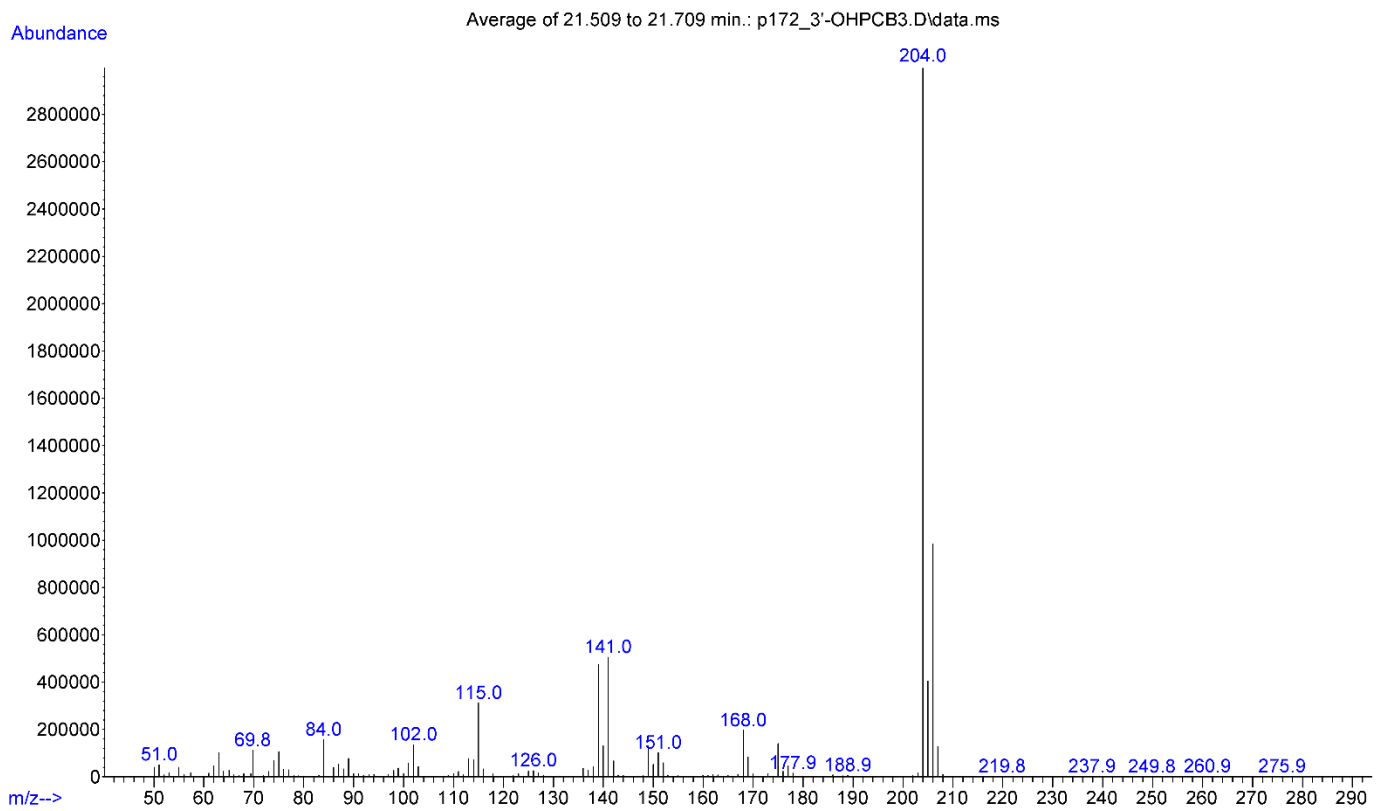
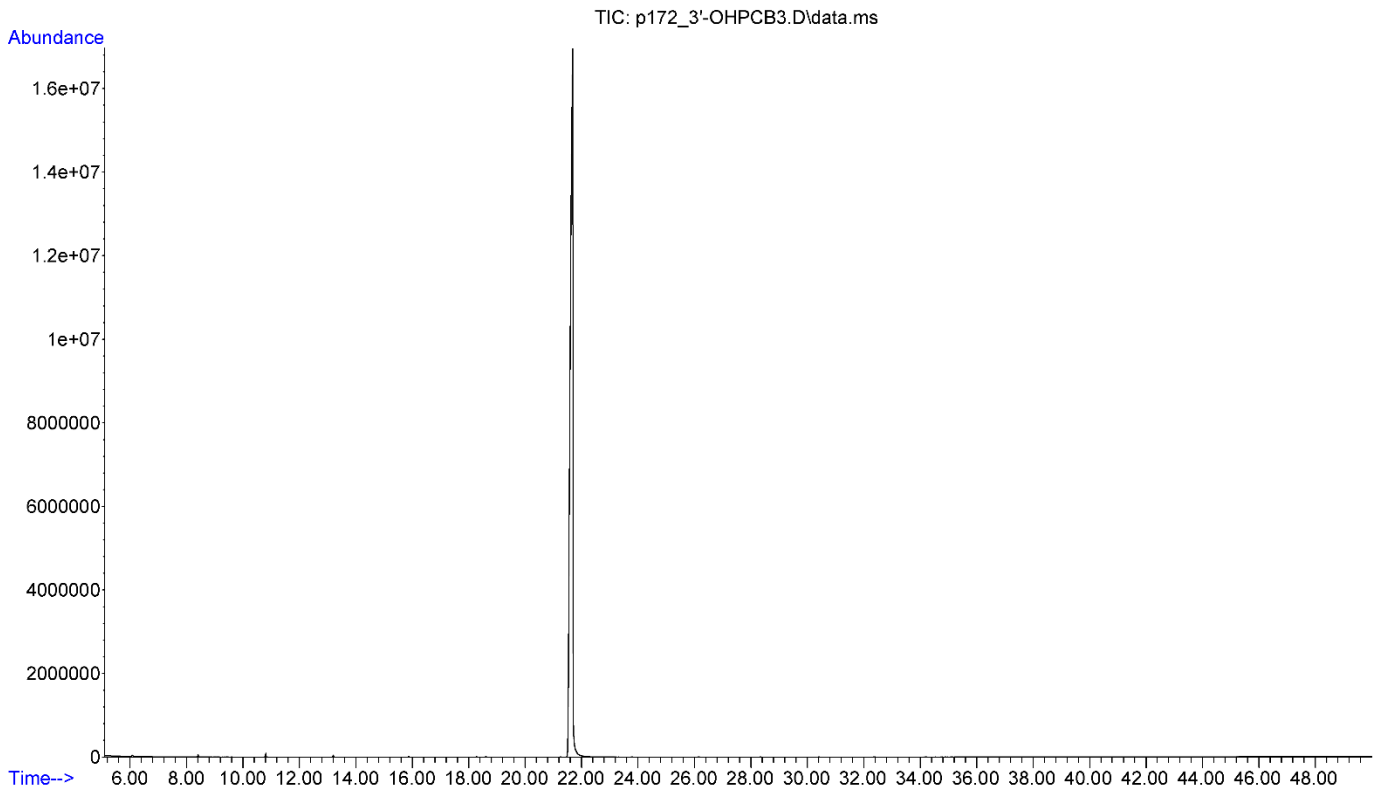
The purity of the enantiomeric pure PCB 136 was performed on an Agilent 7890A gas chromatography with a  $^{63}\text{Ni}$ - $\mu\text{ECD}$  detector and a Chiralsil-Dex (CD column, 25 m length, 250  $\mu\text{m}$  inner diameter, 0.25  $\mu\text{m}$  film thickness; Varian, Palo Alto, CA, USA). The temperature program was based on a previously published method (Wu et al. 2013) with minor modification: starting at 50  $^{\circ}\text{C}$ , then 10  $^{\circ}\text{C}/\text{min}$  to 155  $^{\circ}\text{C}$ , hold for 90 min, 20  $^{\circ}\text{C}/\text{min}$  to 200  $^{\circ}\text{C}$ , hold for 20 min. The temperatures of the injector and detector were 250  $^{\circ}\text{C}$  and 300  $^{\circ}\text{C}$ , respectively. Helium was used as carrier gas with a constant flow rate of 3 mL/min. Argon 95%-methane 5% was used as makeup gas with a constant flow rate of 60 mL/min.



**Fig. S1.** Gas chromatogram and mass spectrum of 4-chlorobiphenyl (PCB 3).



**Fig. S2.** Gas chromatogram and mass spectrum of 4-chlorobiphenyl-2'-ol (2'-OH PCB 3).



**Fig. S3.** Gas chromatogram and mass spectrum of 4-chlorobiphenyl-3'-ol (3'-OH PCB 3).



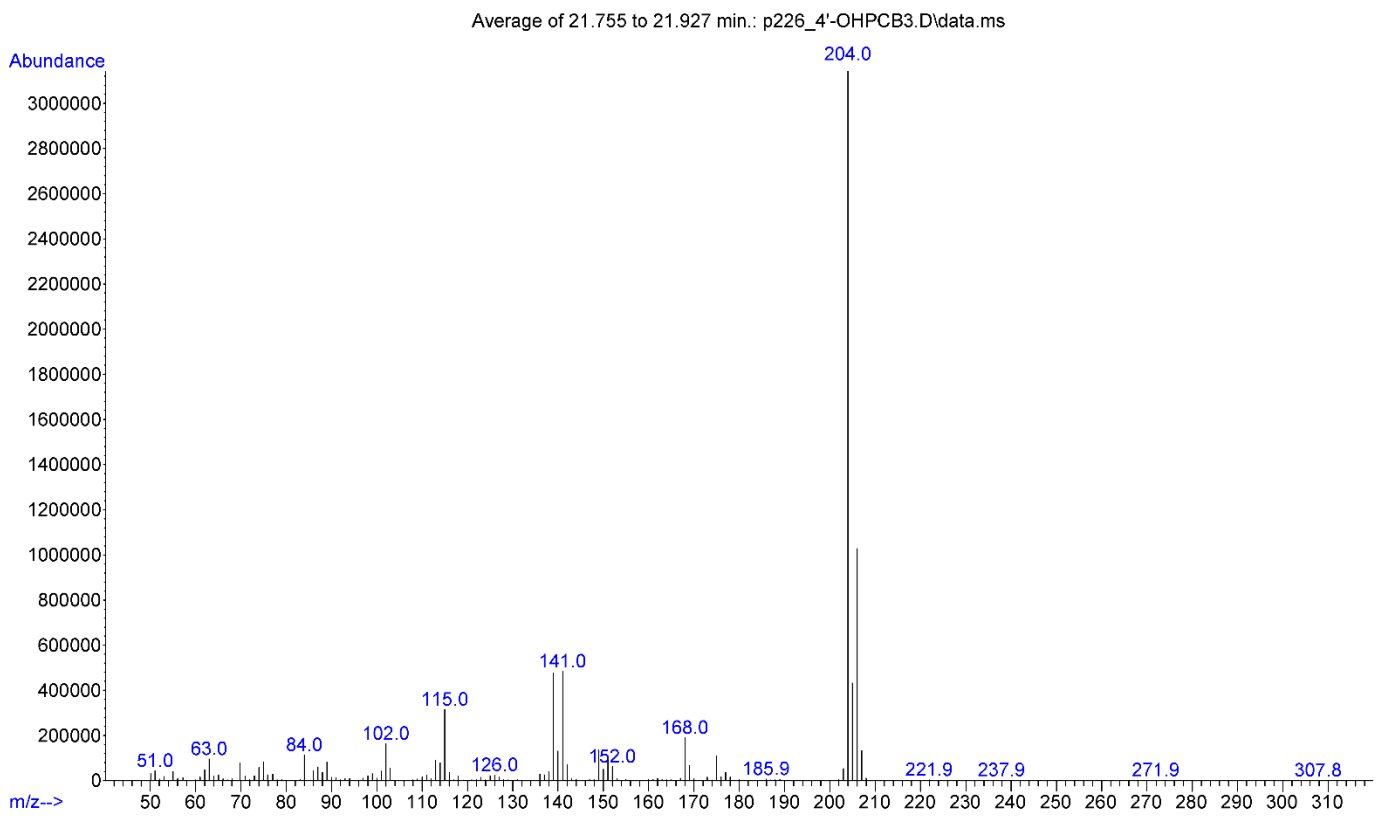
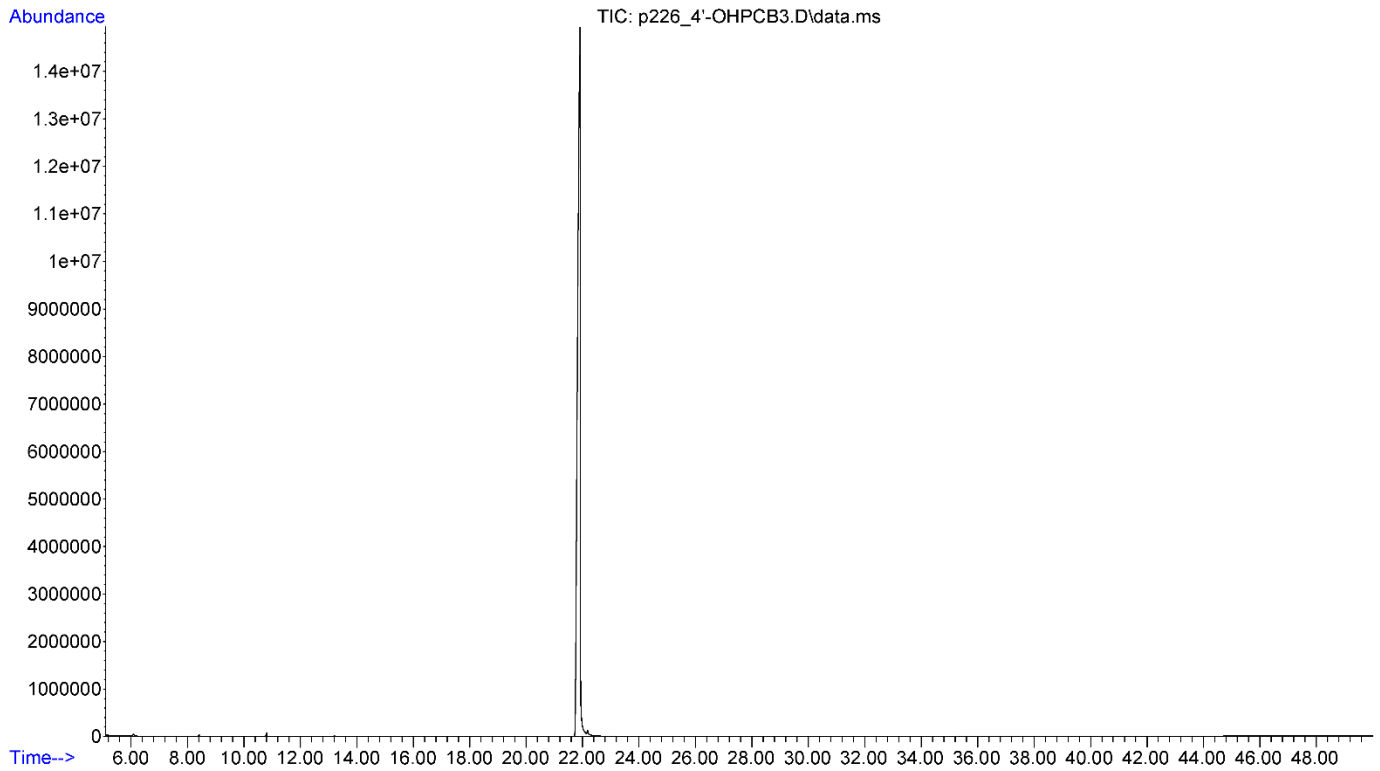
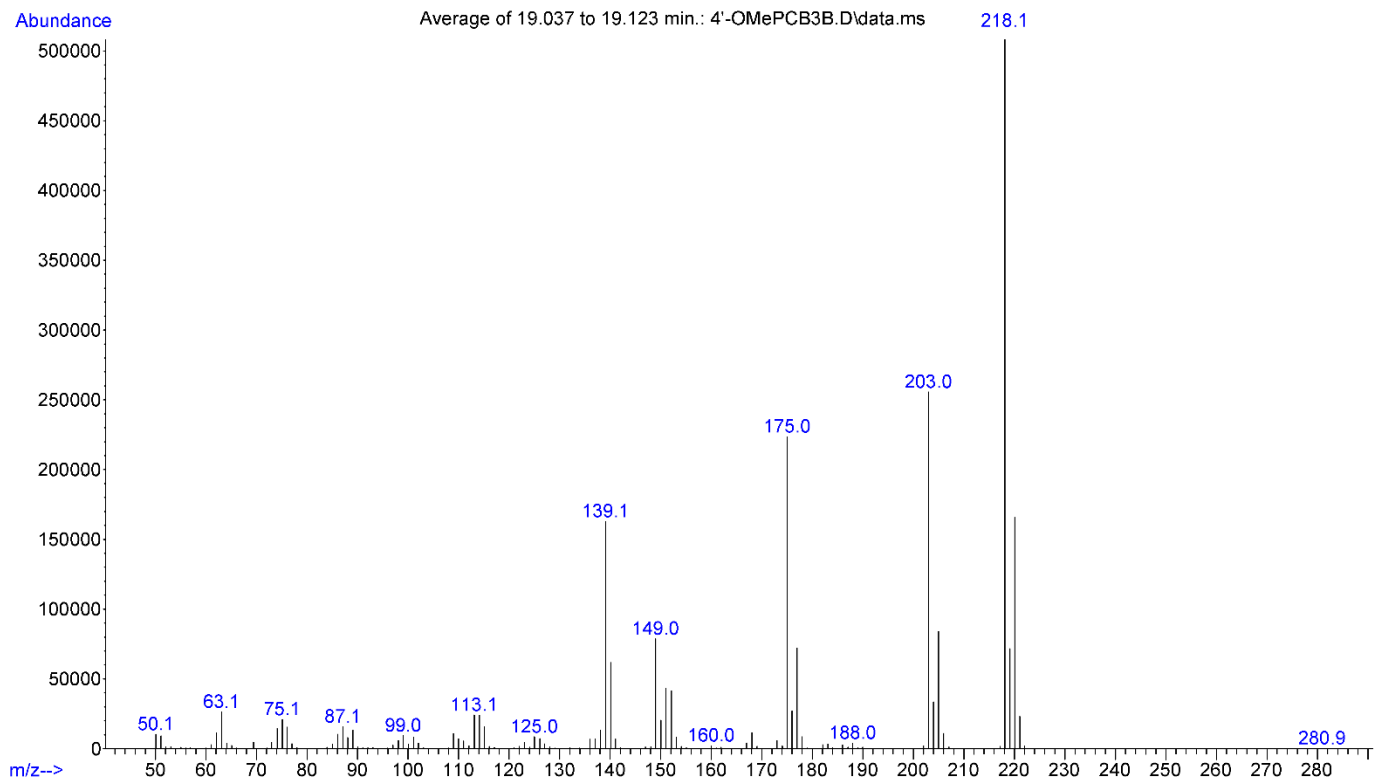
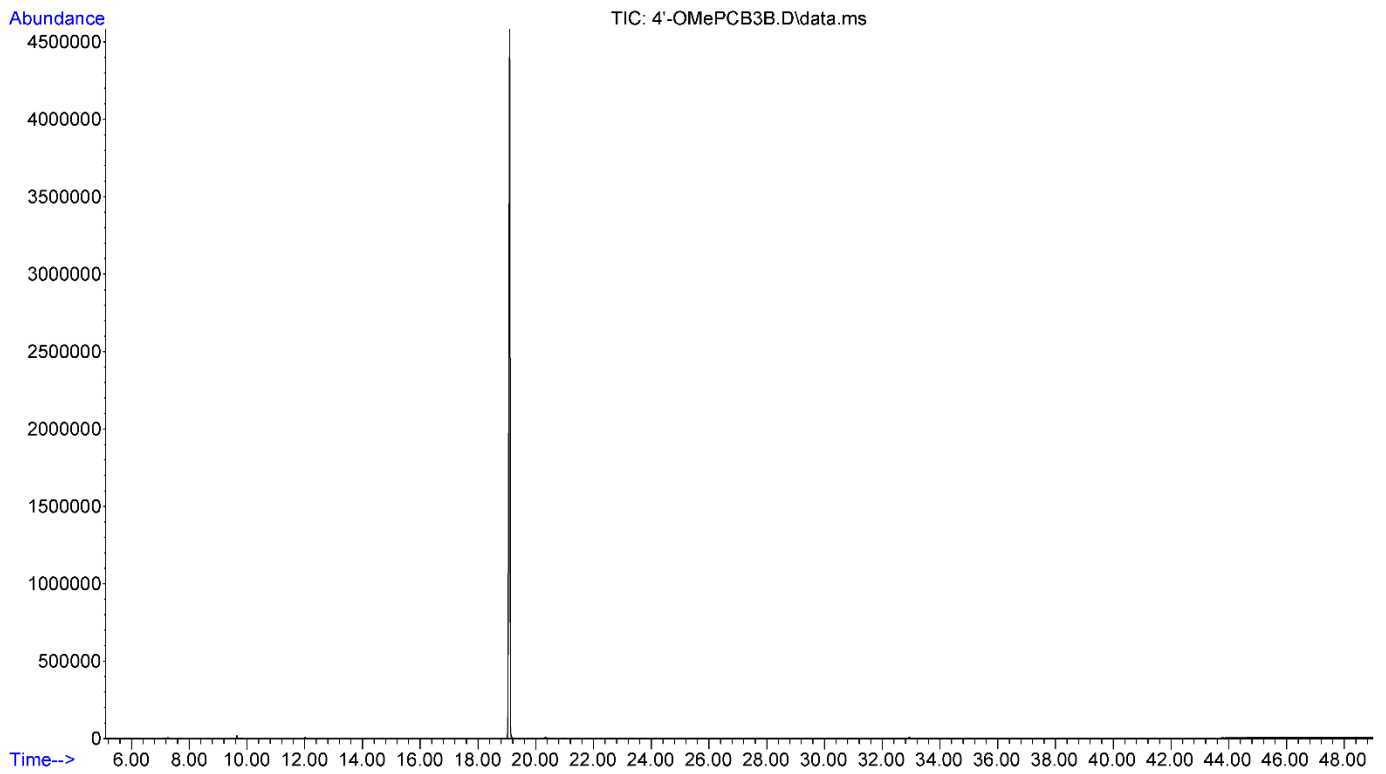
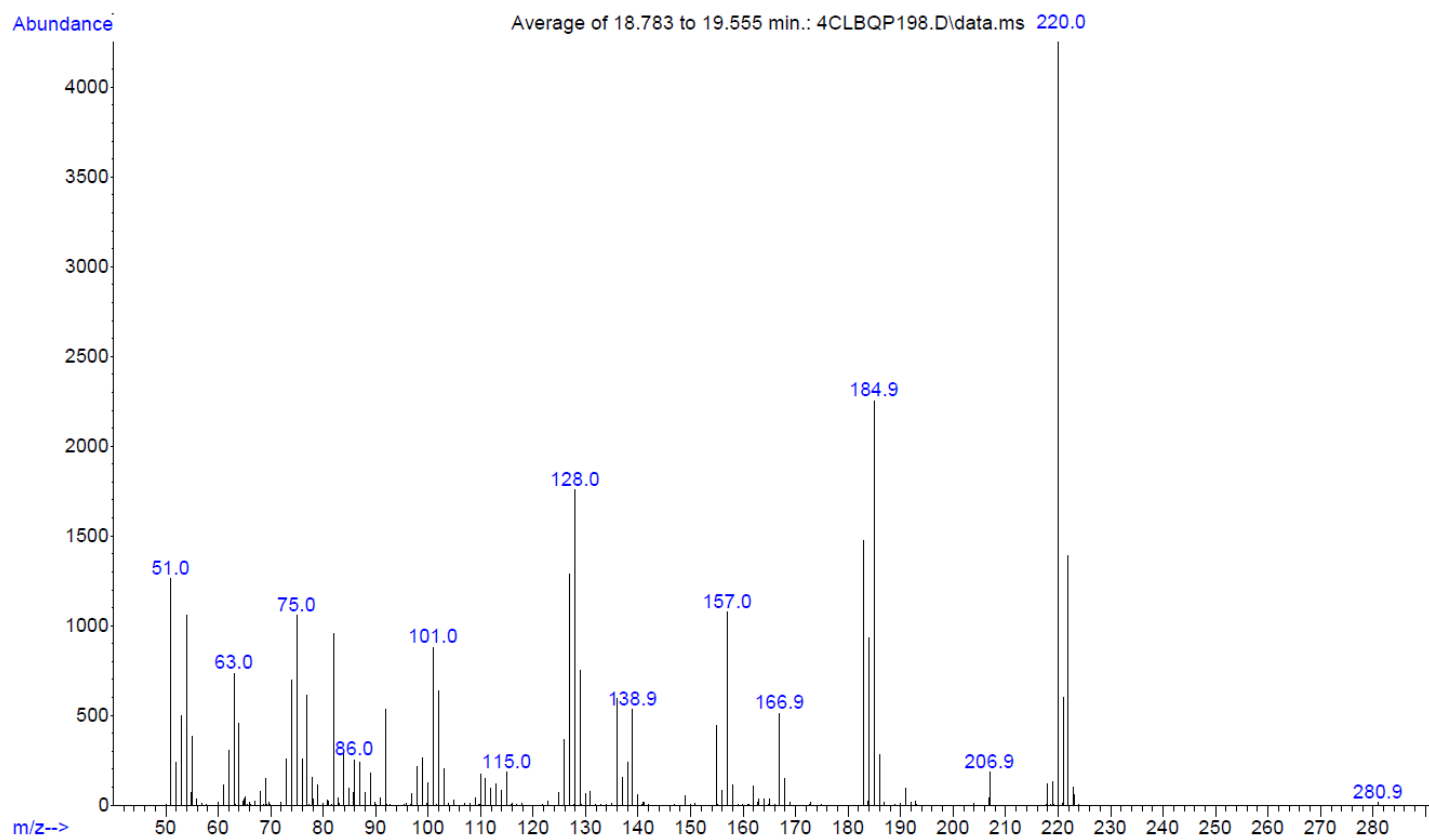
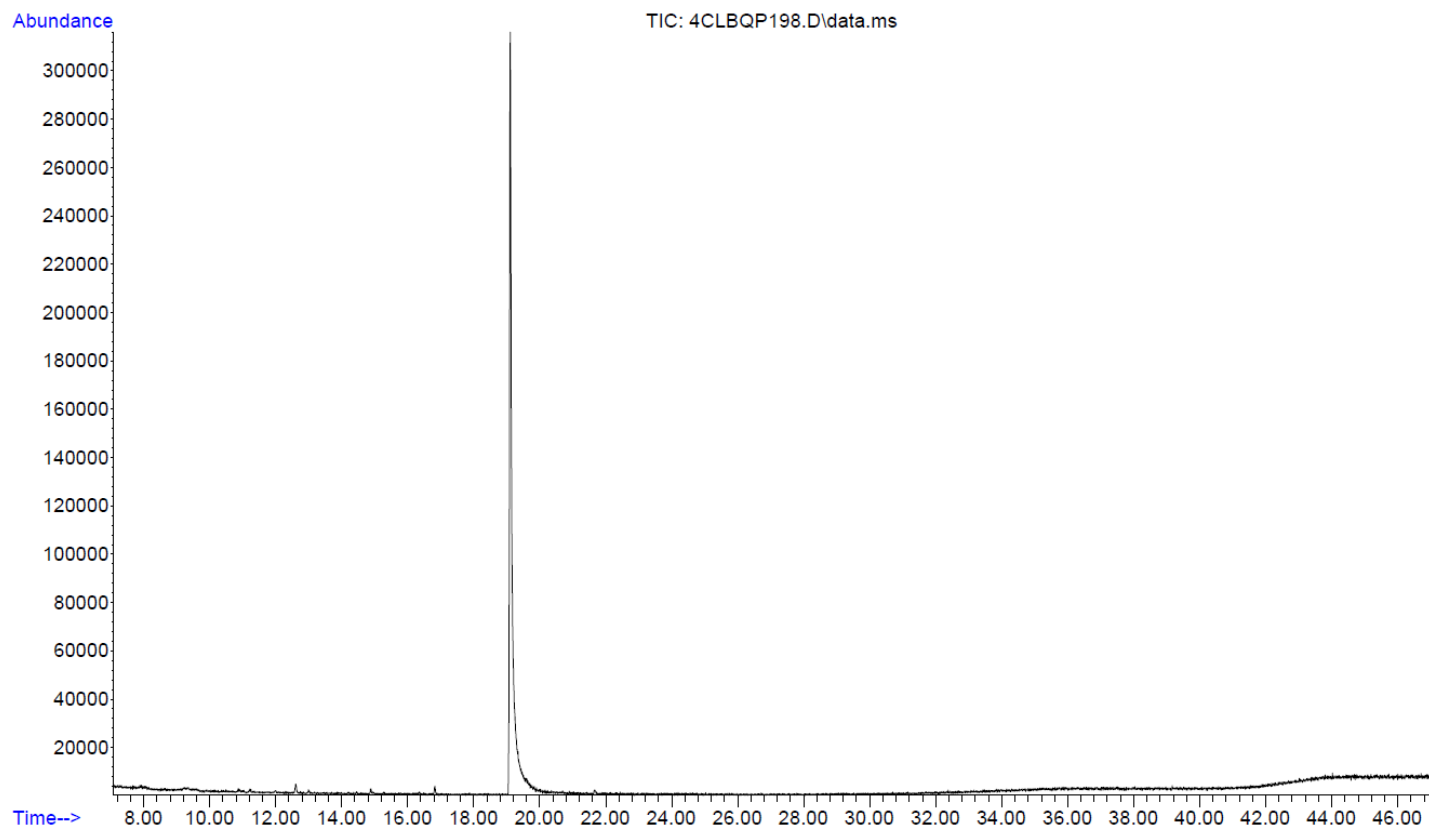


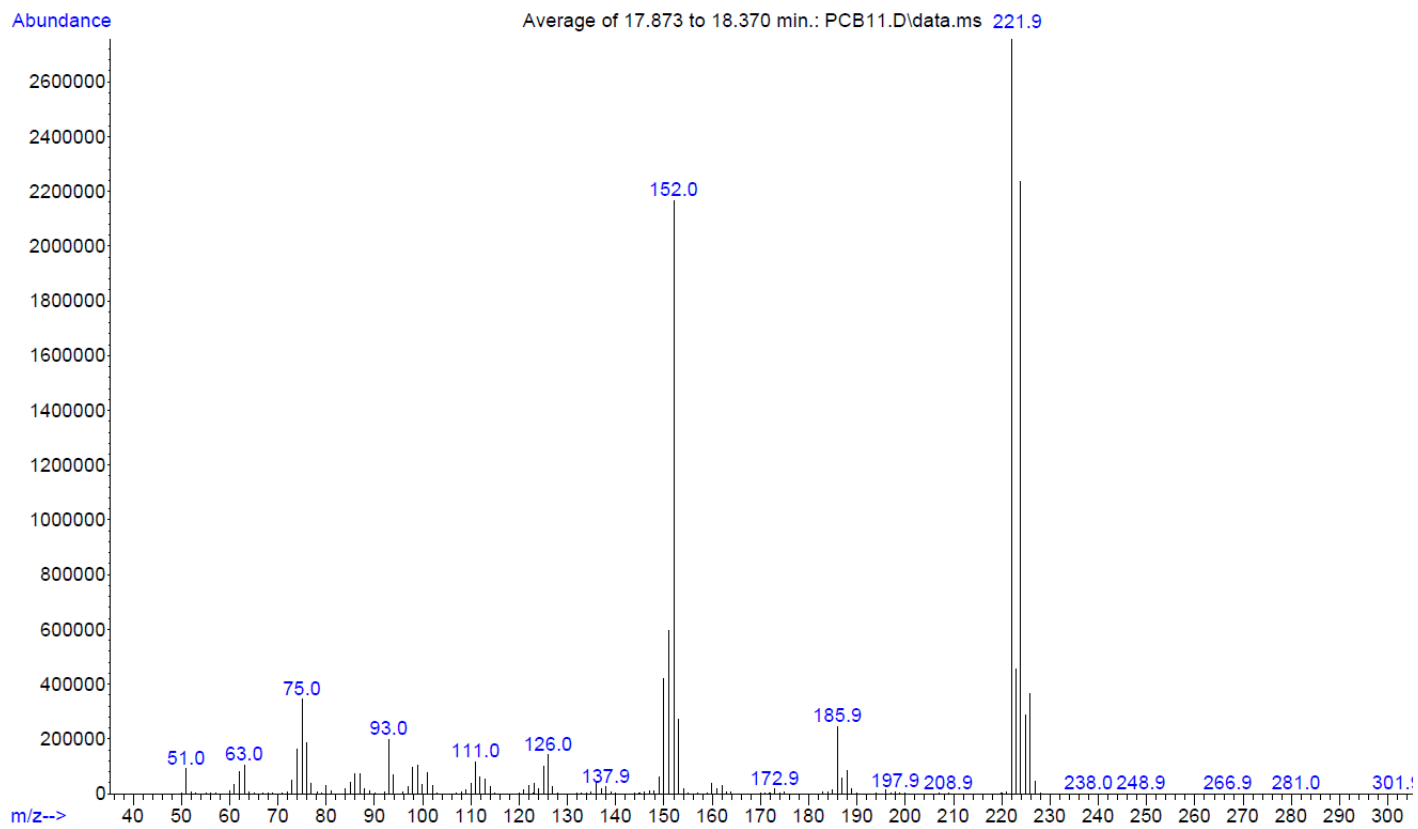
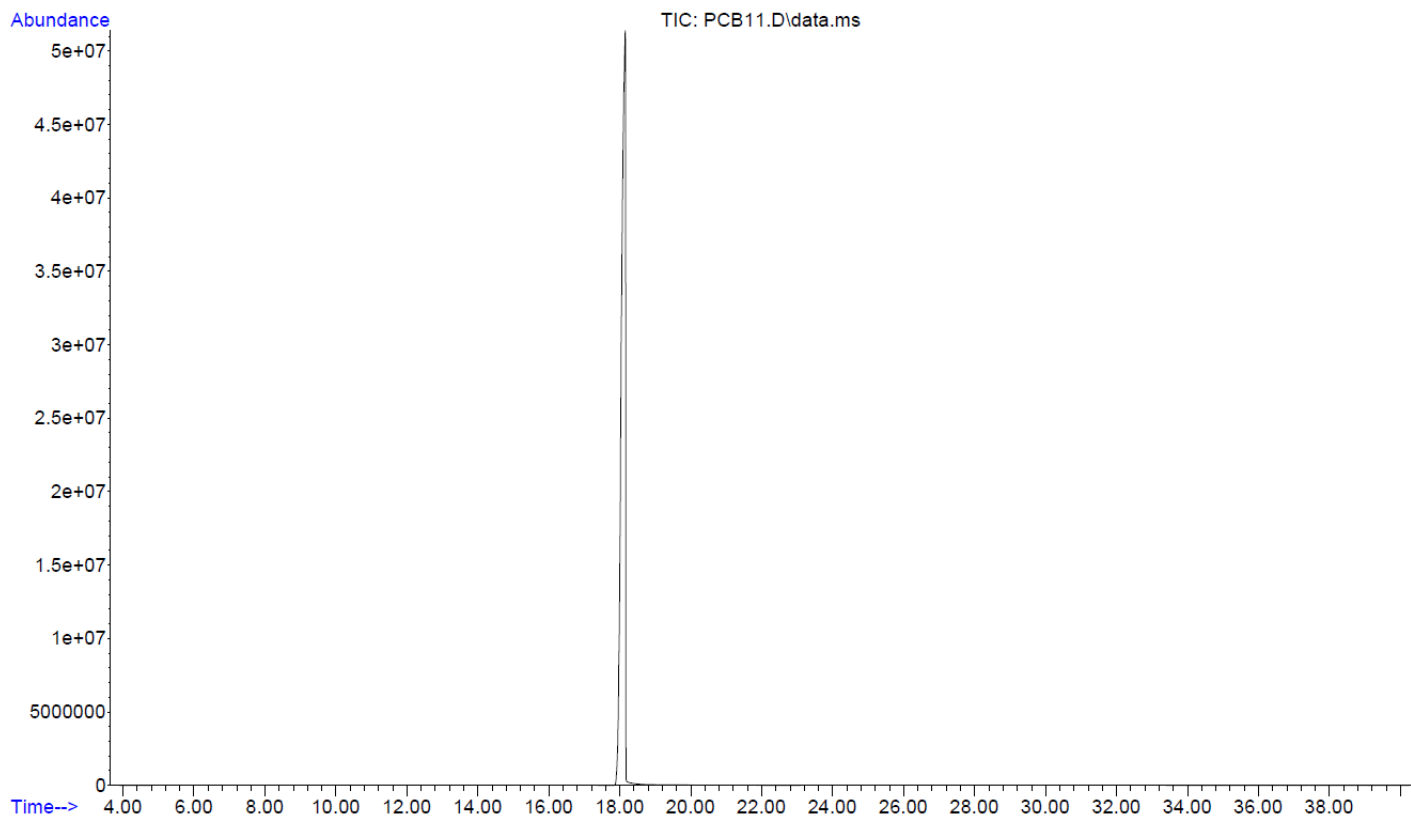
Fig. S4. Gas chromatogram and mass spectrum of 4-chlorobiphenyl-4'-ol (4'-OH PCB 3).



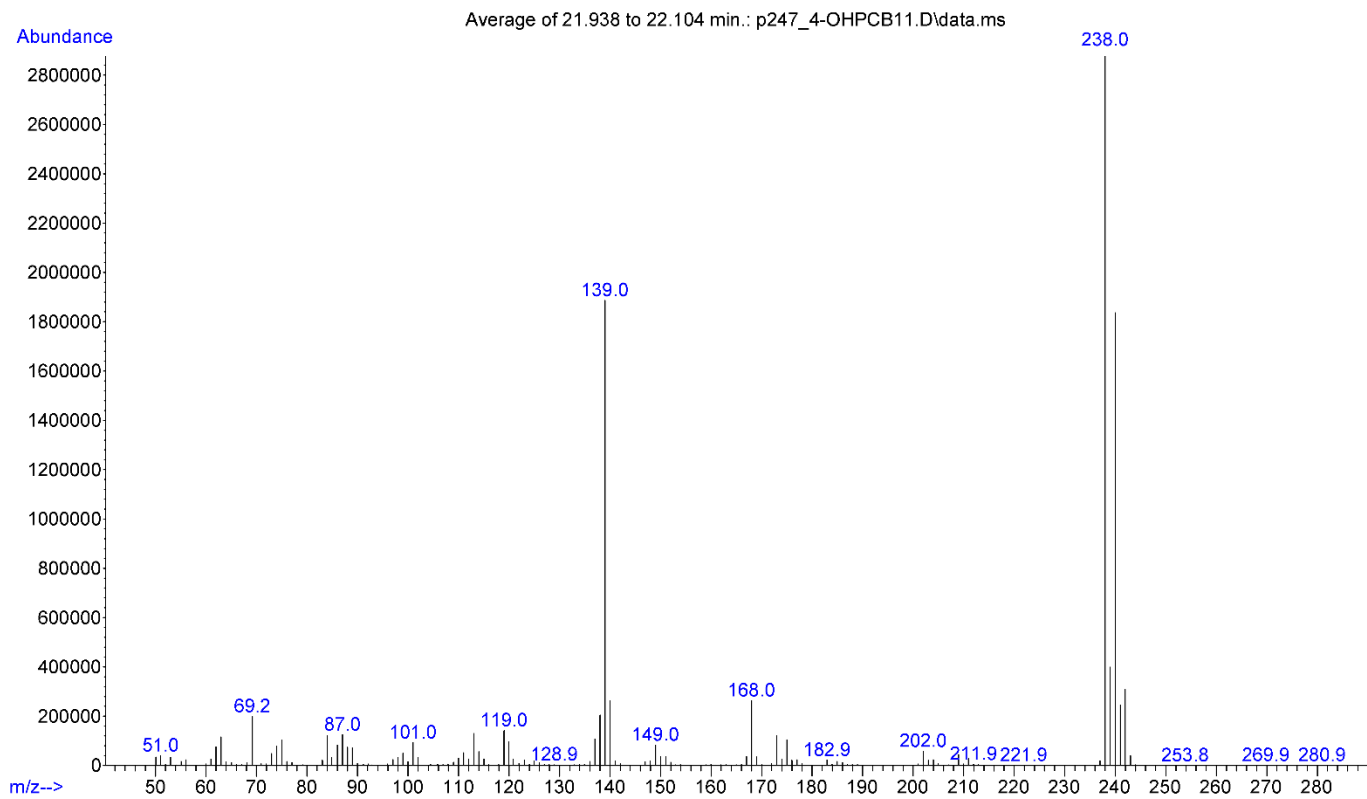
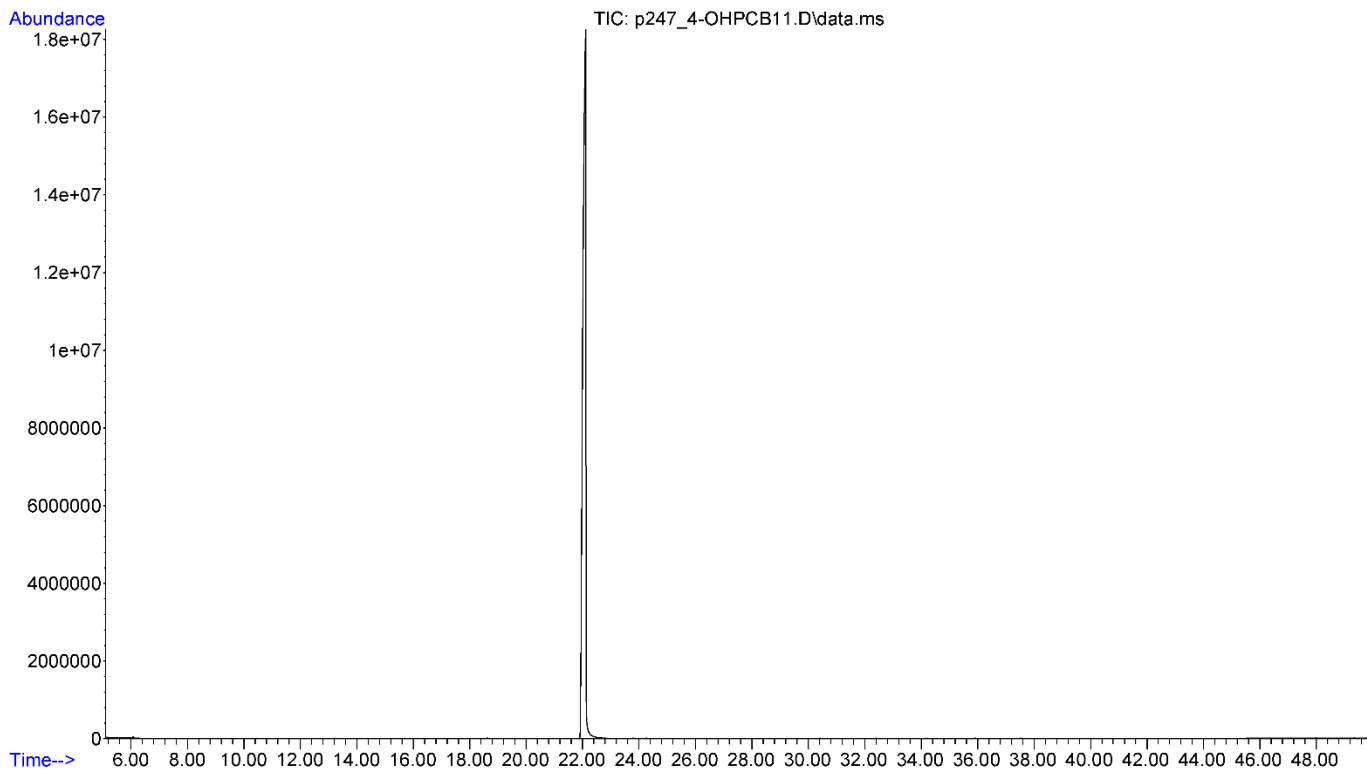
**Fig. S5.** Gas chromatogram and mass spectrum of 4'-Methoxy-4-chlorobiphenyl (4'-OMe PCB 3).



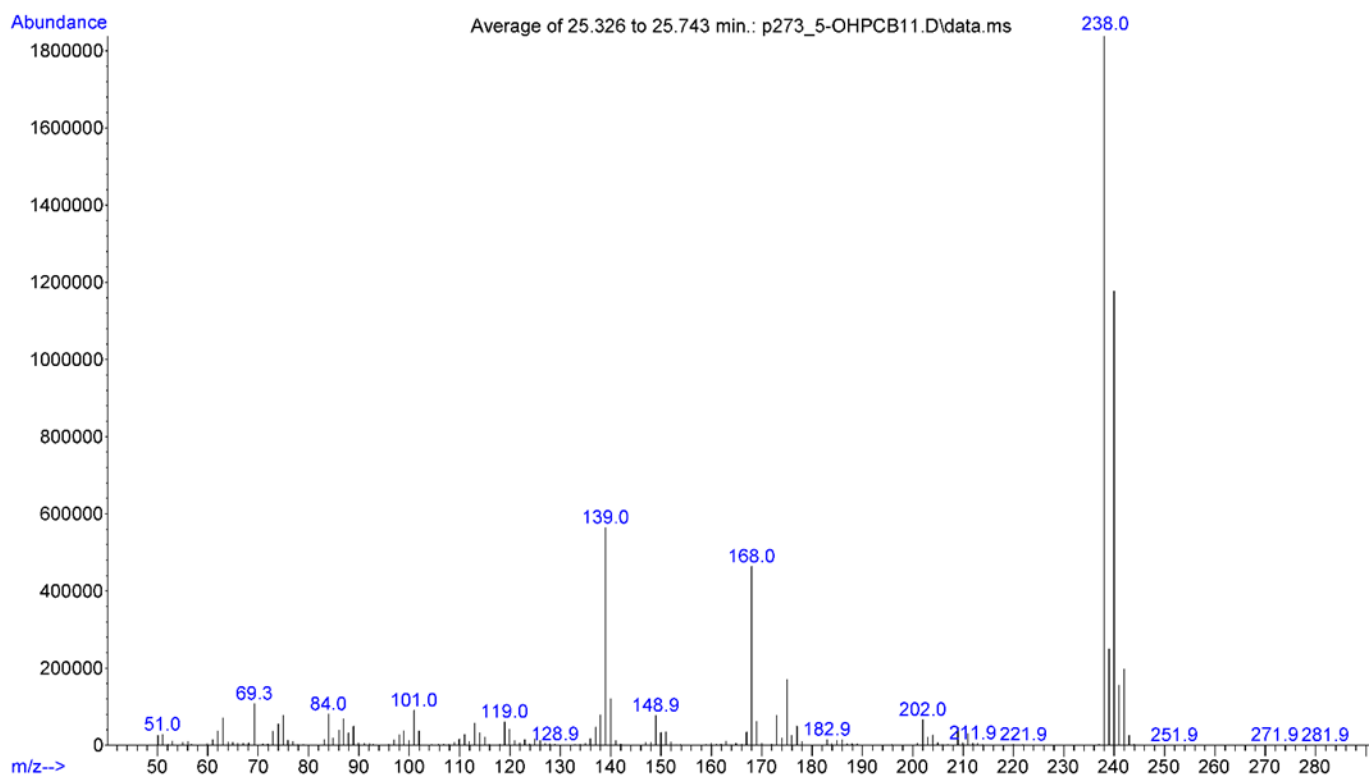
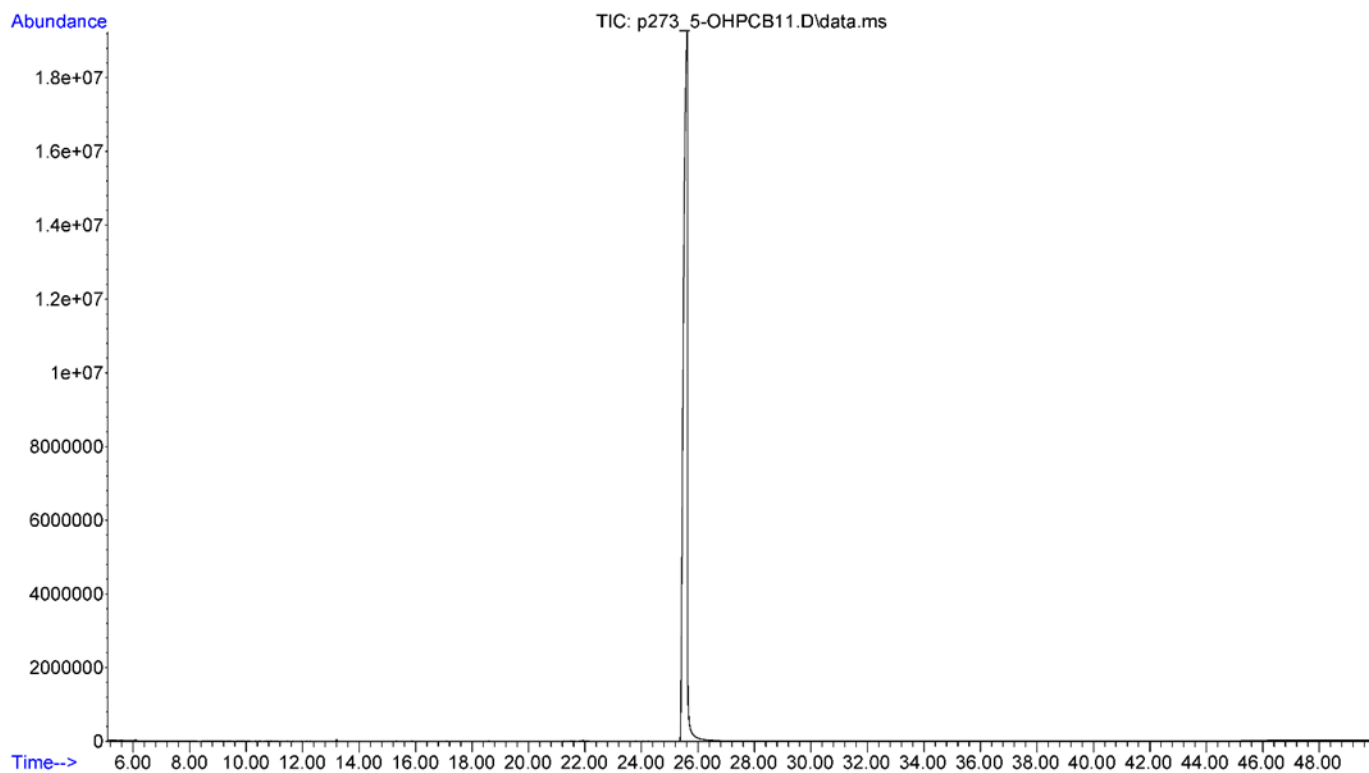
**Fig. S6.** Gas chromatogram and mass spectrum of 2-(4-chlorophenyl)benzo-2,5-quinone (4-Cl BQ).



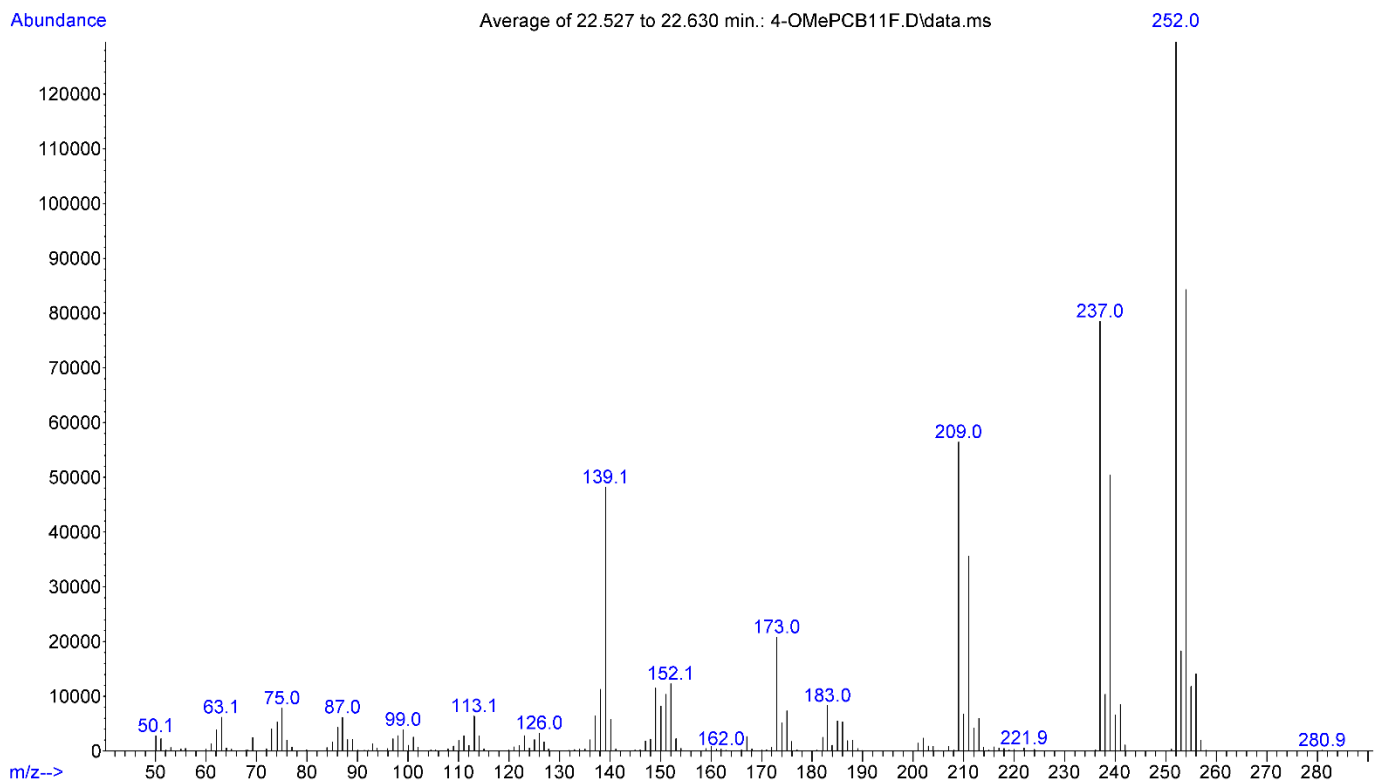
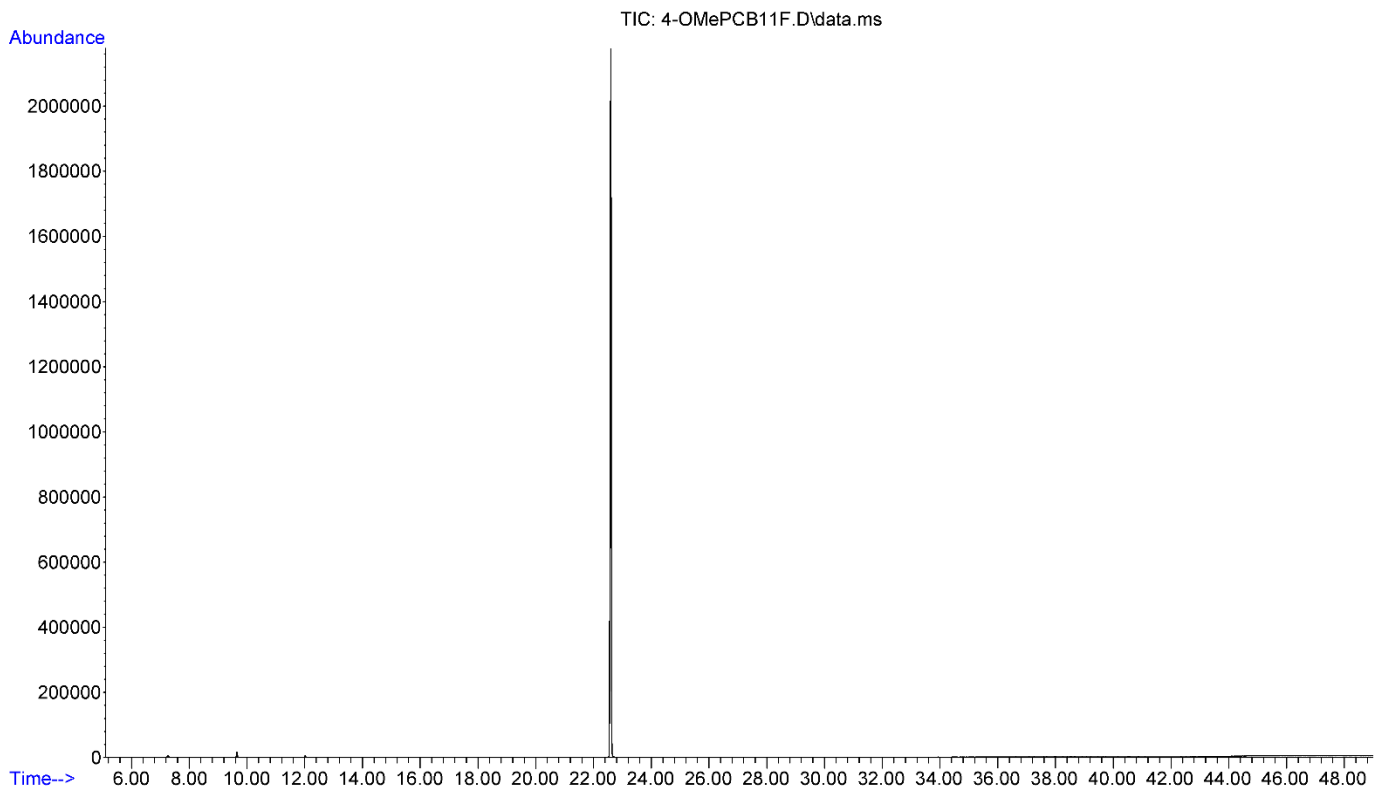
**Fig. S7.** Gas chromatogram and mass spectrum of 3,3'-dichlorobiphenyl (PCB 11).



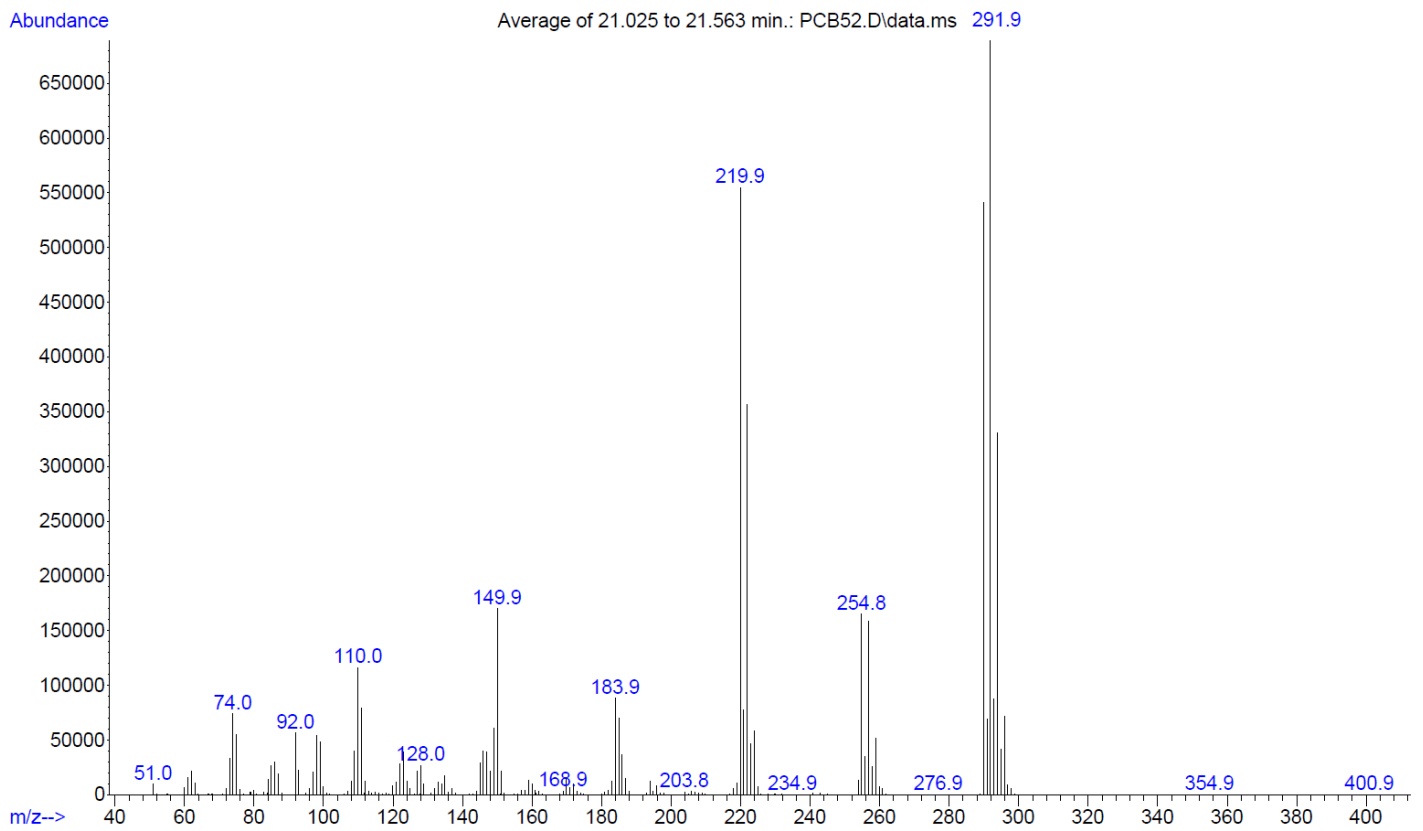
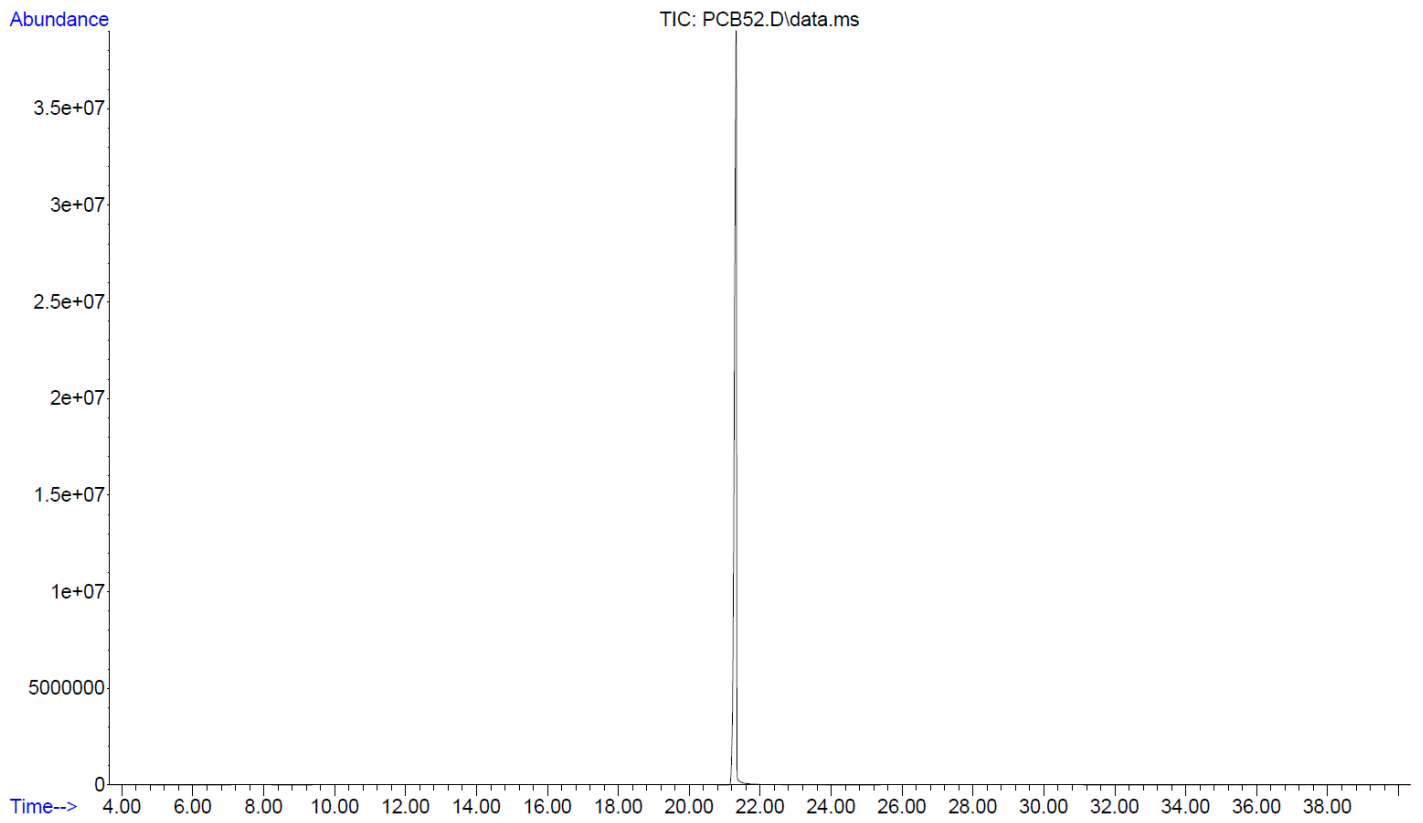
**Fig. S8.** Gas chromatogram and mass spectrum of 3,3'-dichlorobiphenyl-4-ol (4-OH PCB 11).



**Fig. S9** Gas chromatogram and mass spectrum of 3,3'-dichlorobiphenyl-5-ol (5-OH PCB 11)

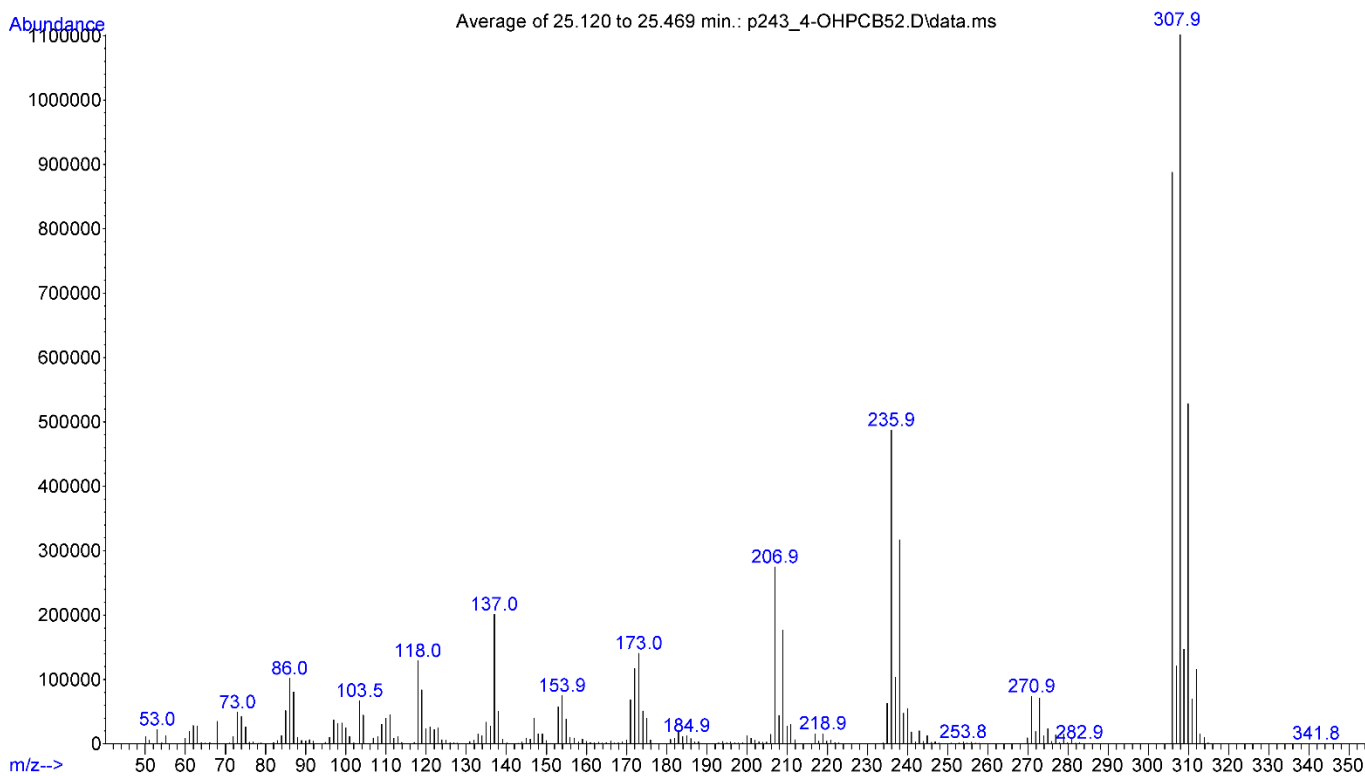
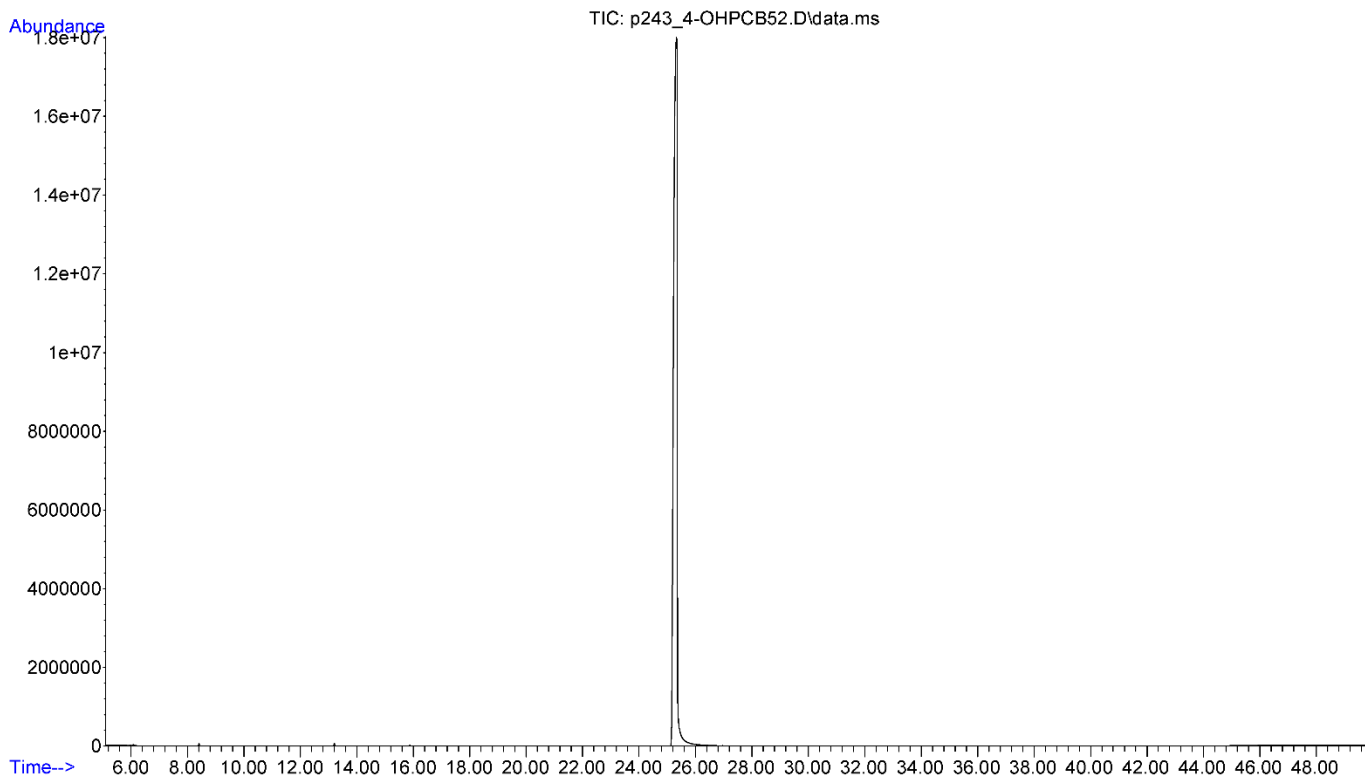


**Fig. S10.** Gas chromatogram and mass spectrum of 4-methoxy-3,3'-dichlorobiphenyl (4-OMe PCB 11).

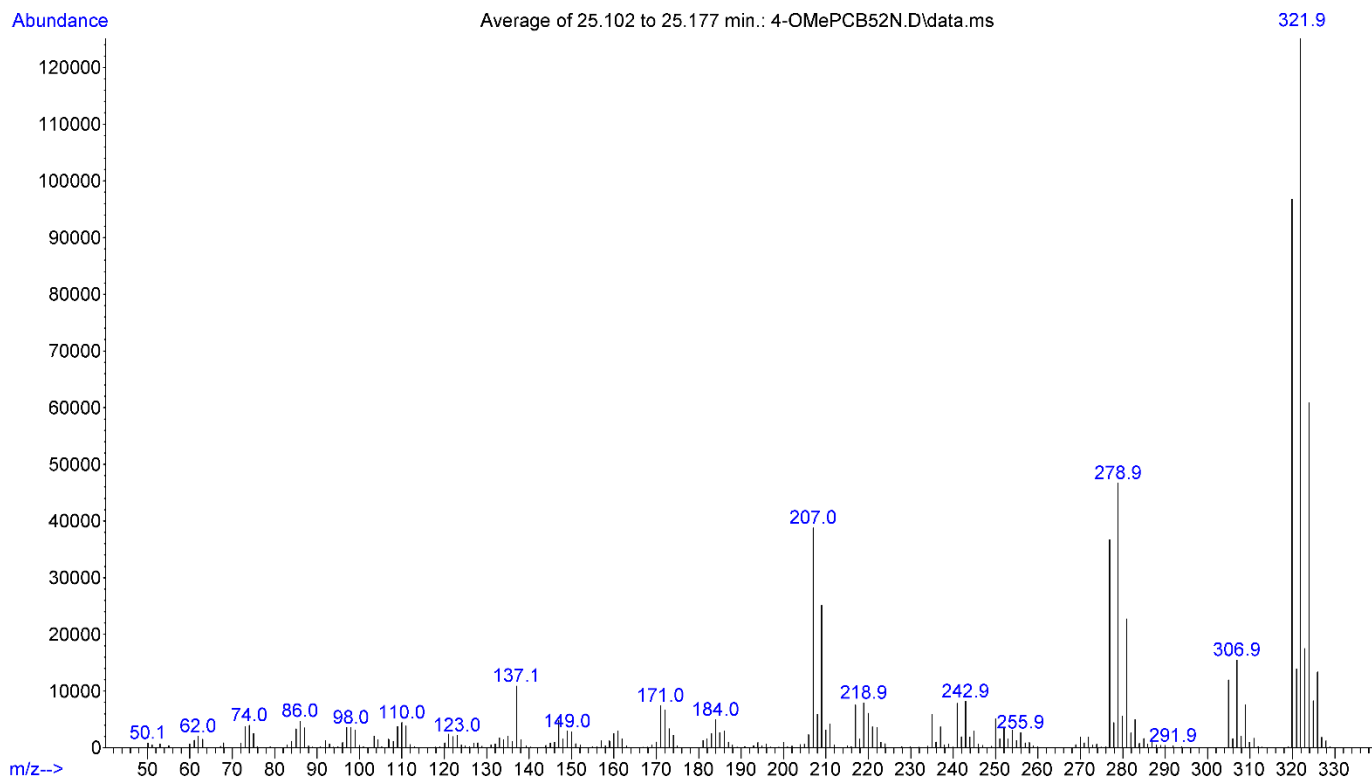
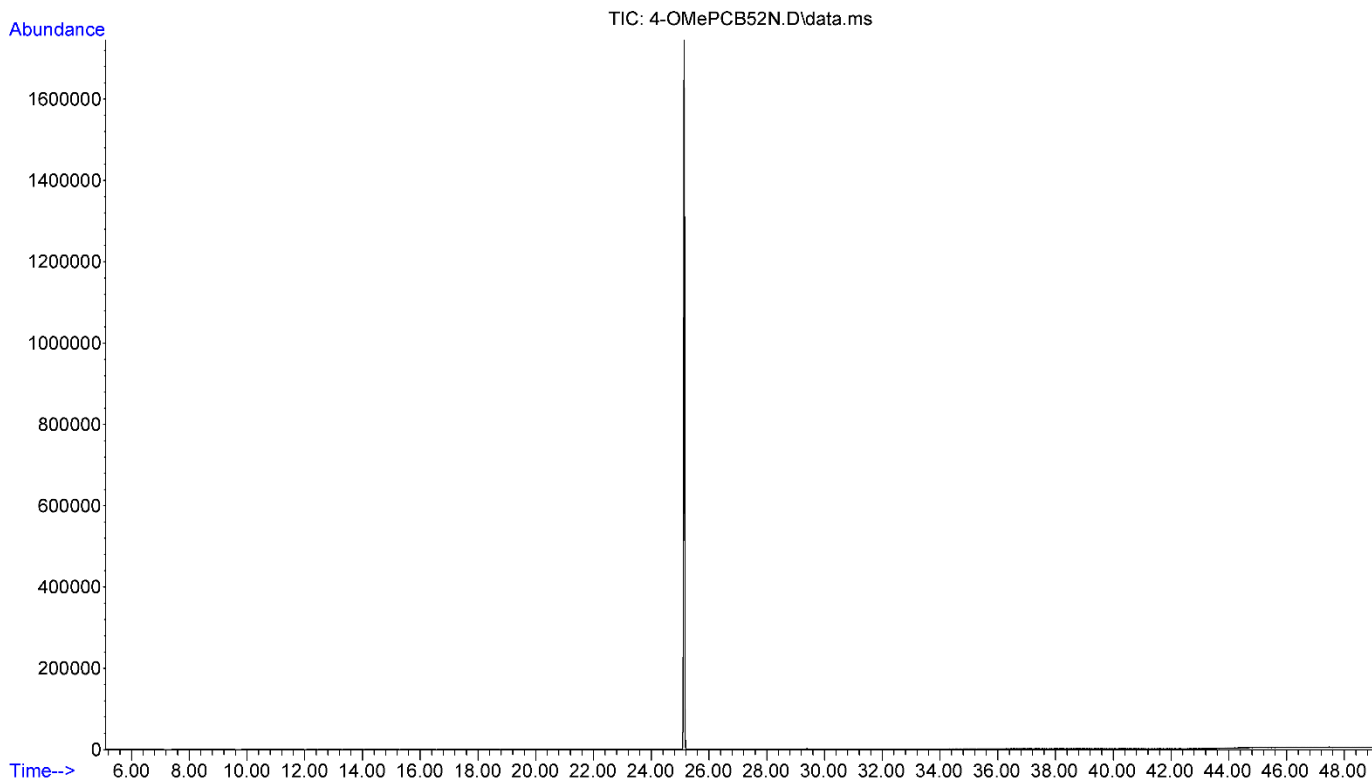


**Fig. S11.** Gas chromatogram and mass spectrum of Gas chromatogram and mass spectrum of 2,2',5,5'-tetrachlorbiphenyl (PCB 52).

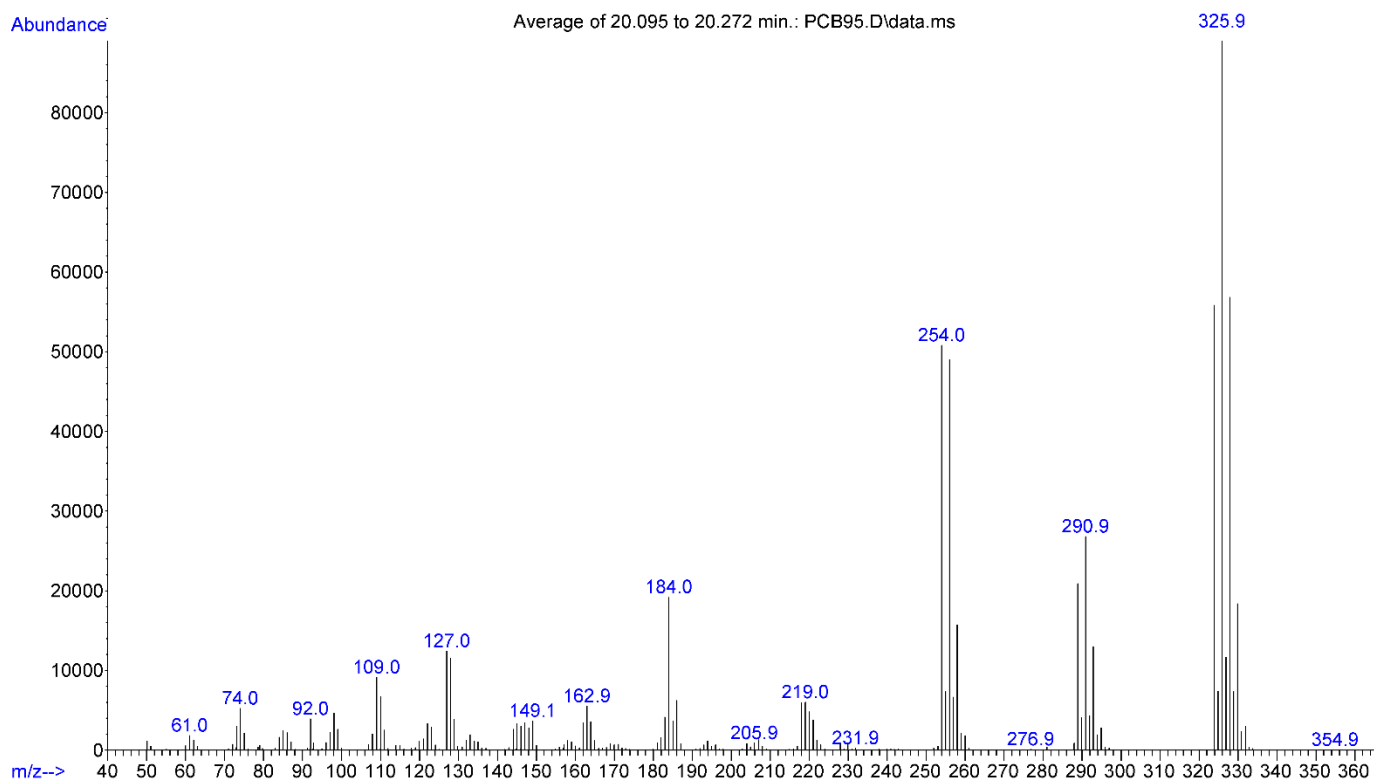
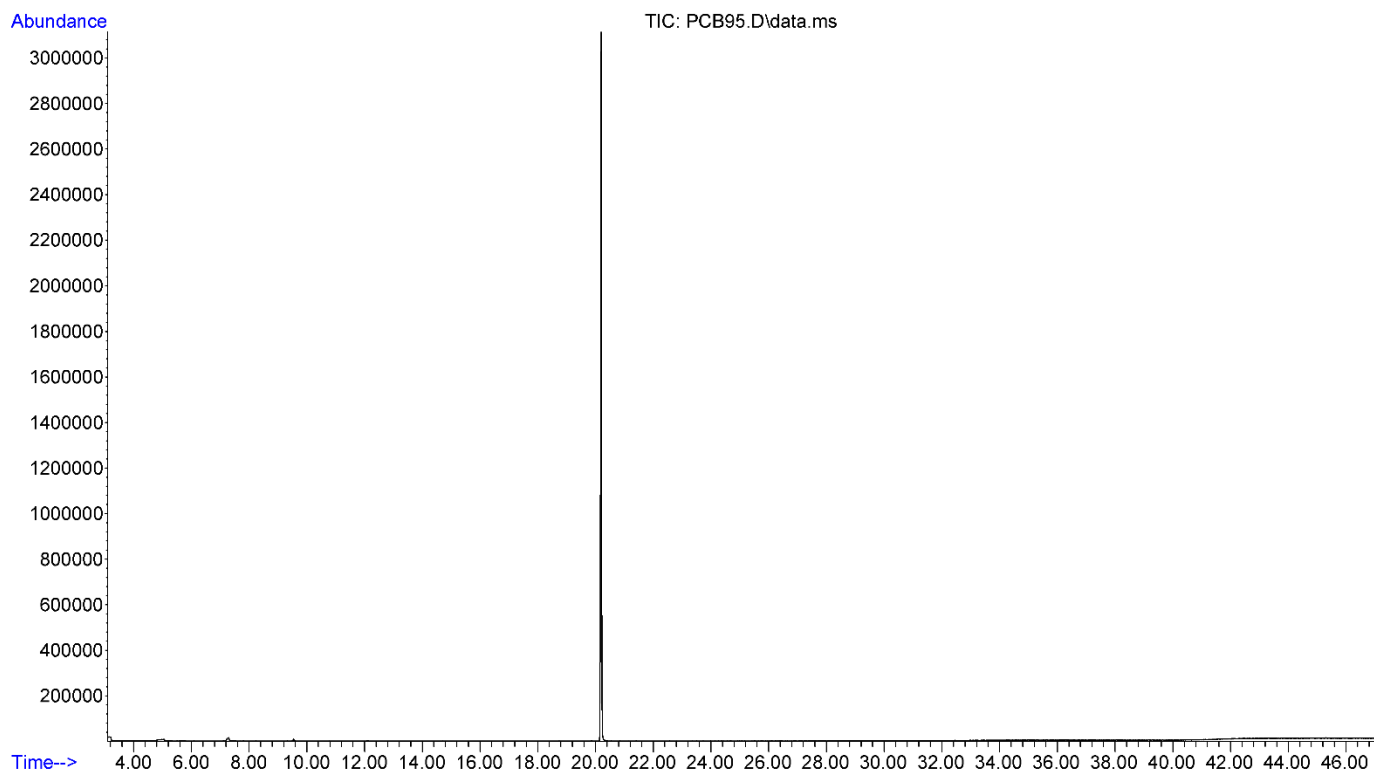




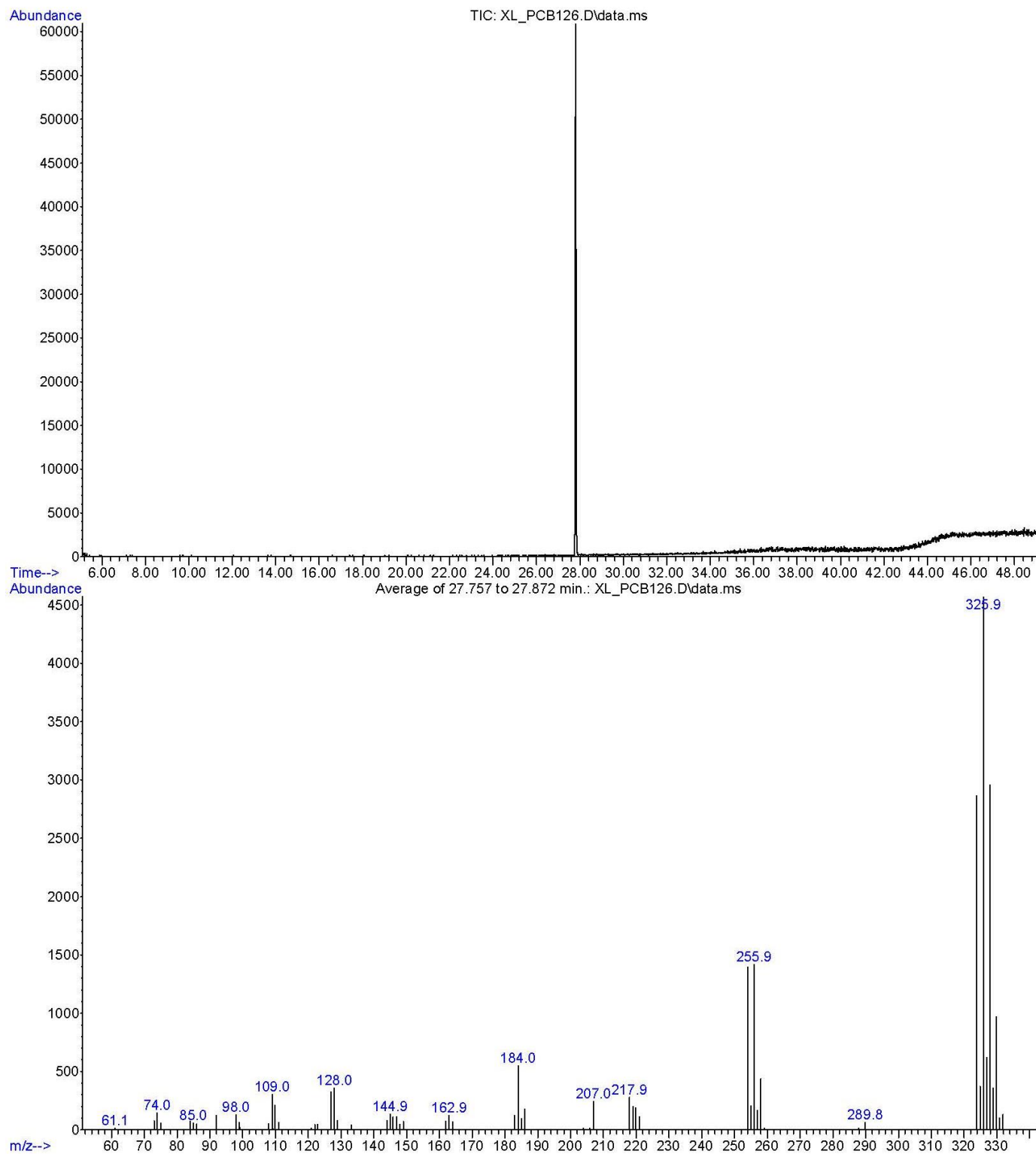
**Fig. S12.** Gas chromatogram and mass spectrum of 2,2',5,5'-tetrachlorbiphenyl-4-ol (4-OH PCB 52).



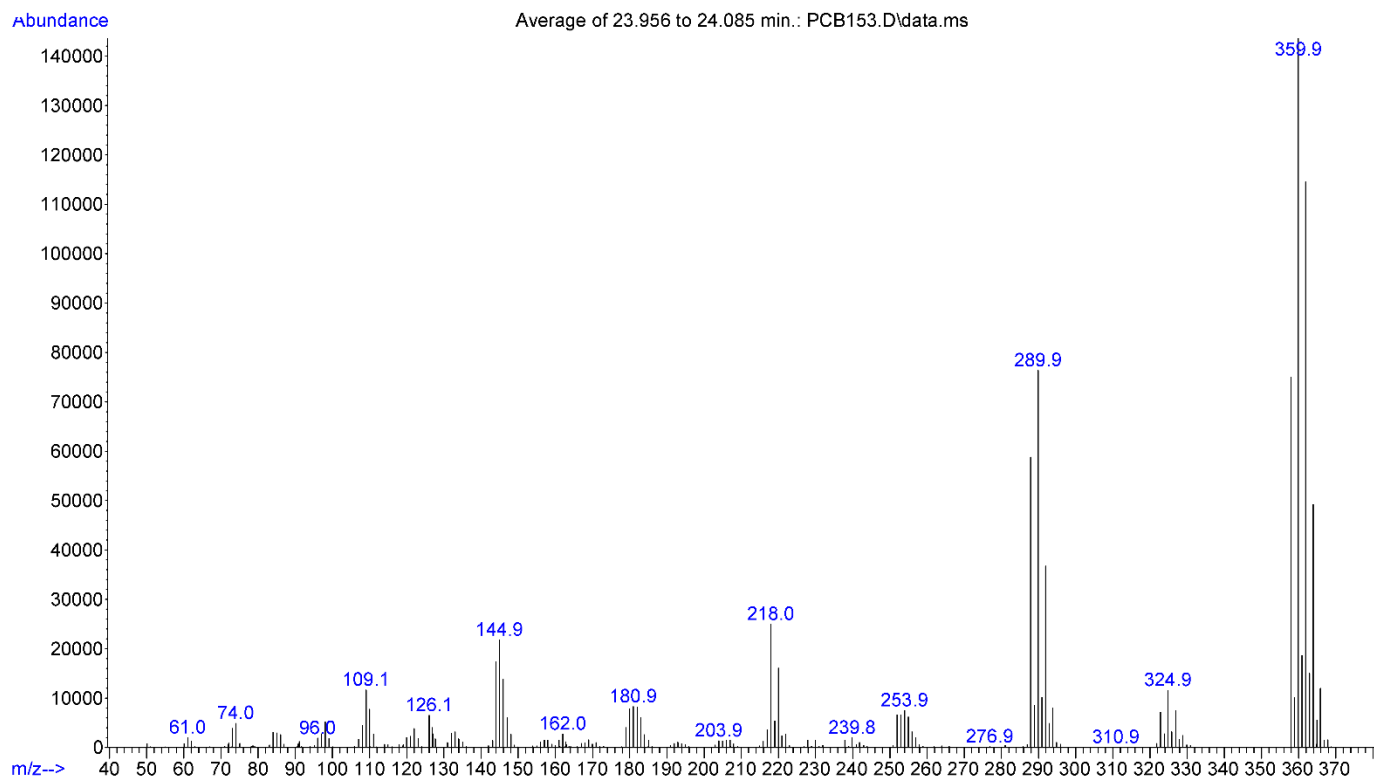
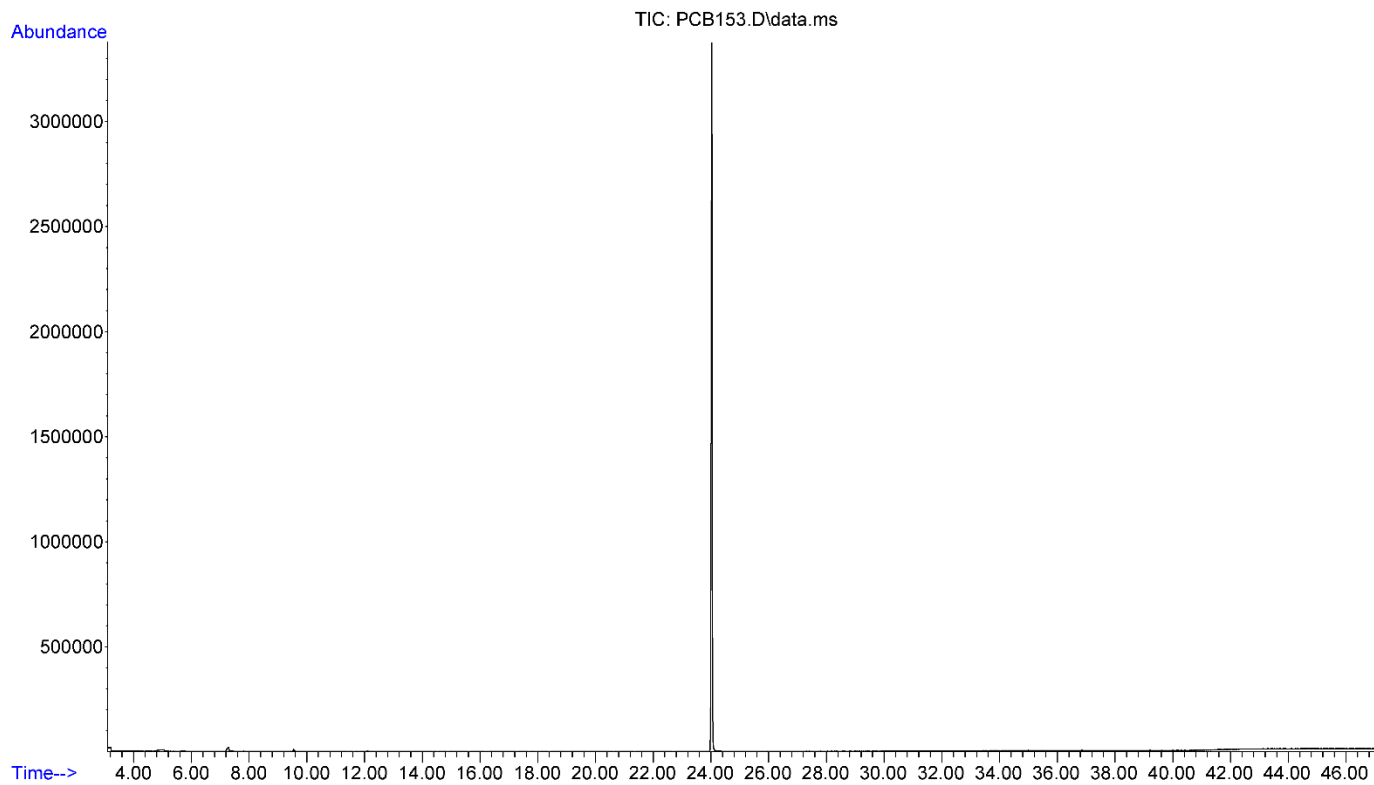
**Fig. S13.** Gas chromatogram and mass spectrum of 4-methoxy-2,2',5,5'-tetrachlorobiphenyl (4-OMe PCB 52).



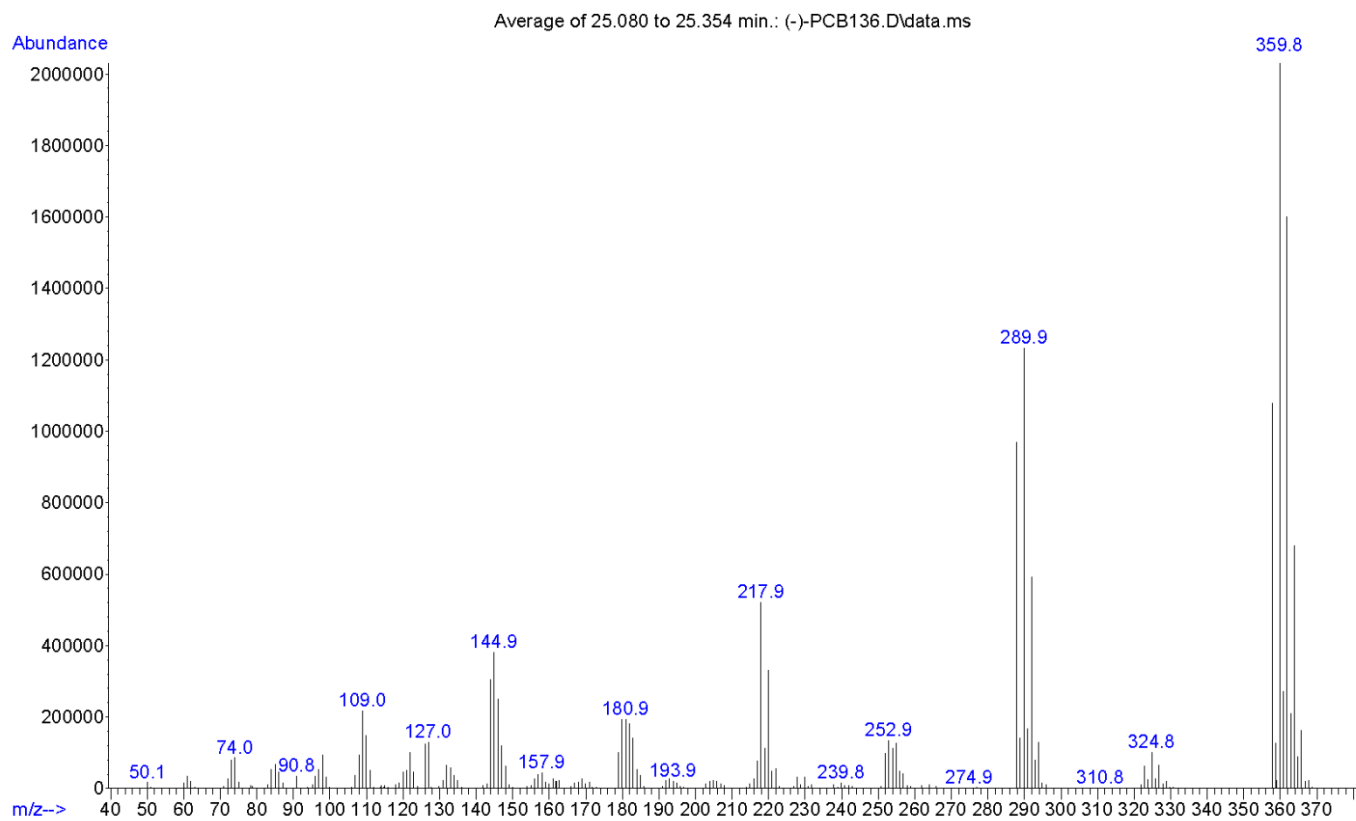
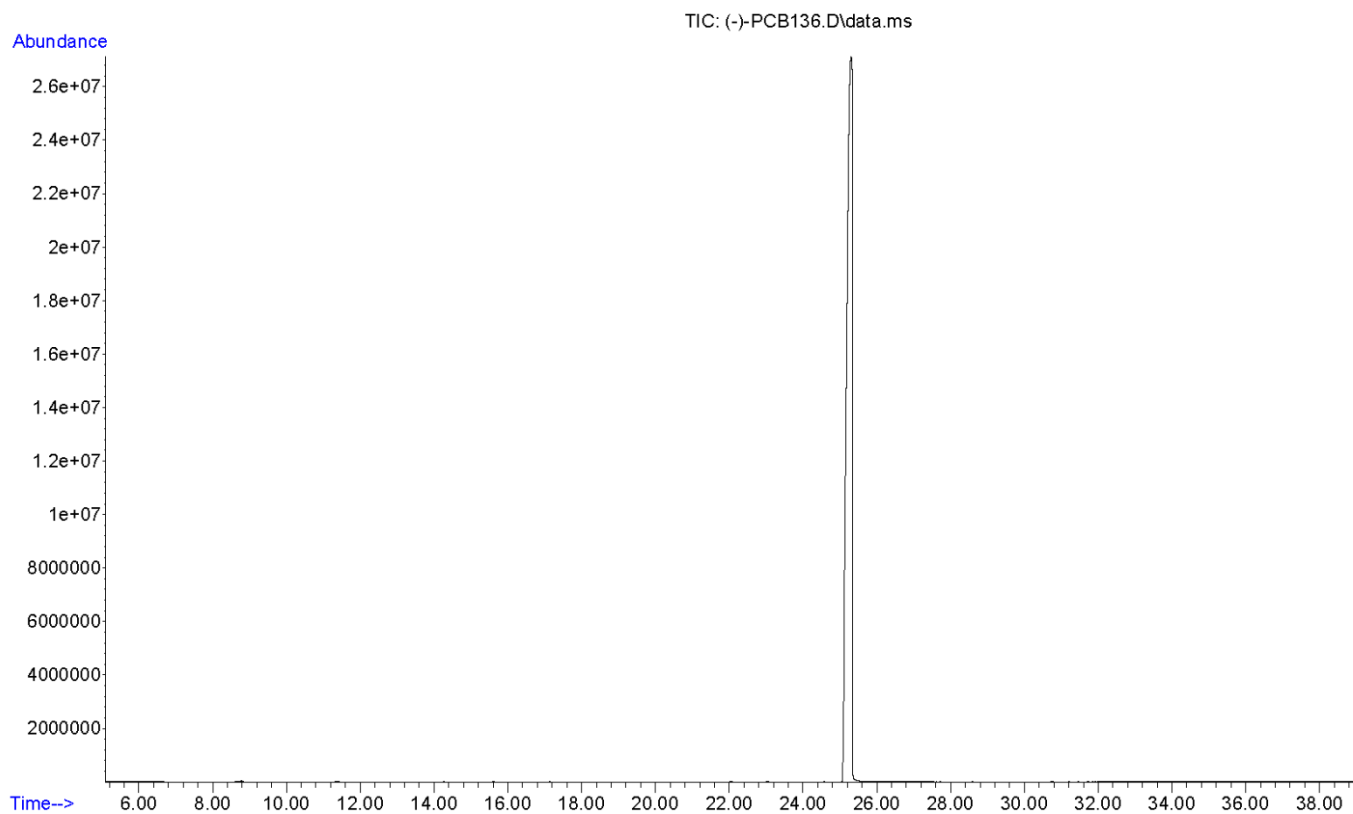
**Fig. S14.** Gas chromatogram and mass spectrum of 2,2',3,5',6-pentachlorobiphenyl (PCB 95).



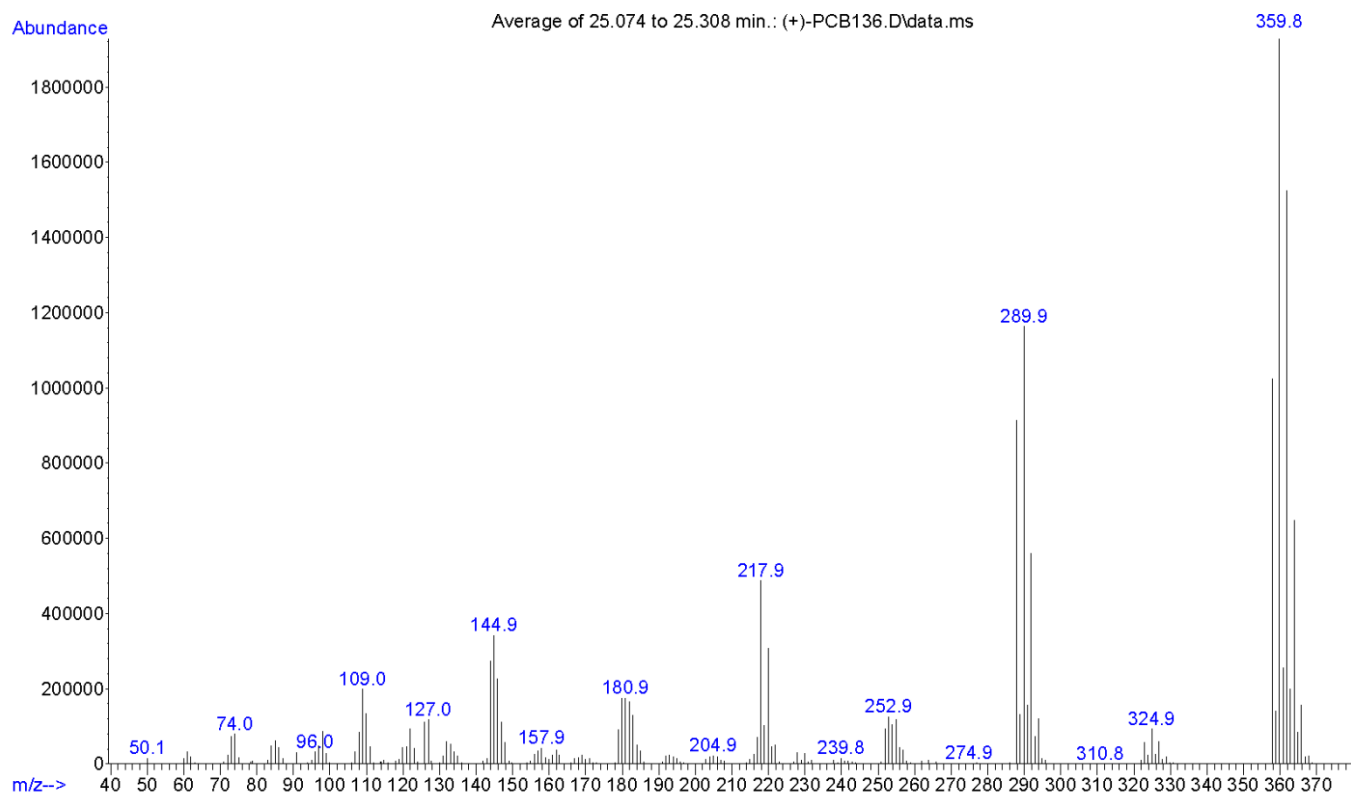
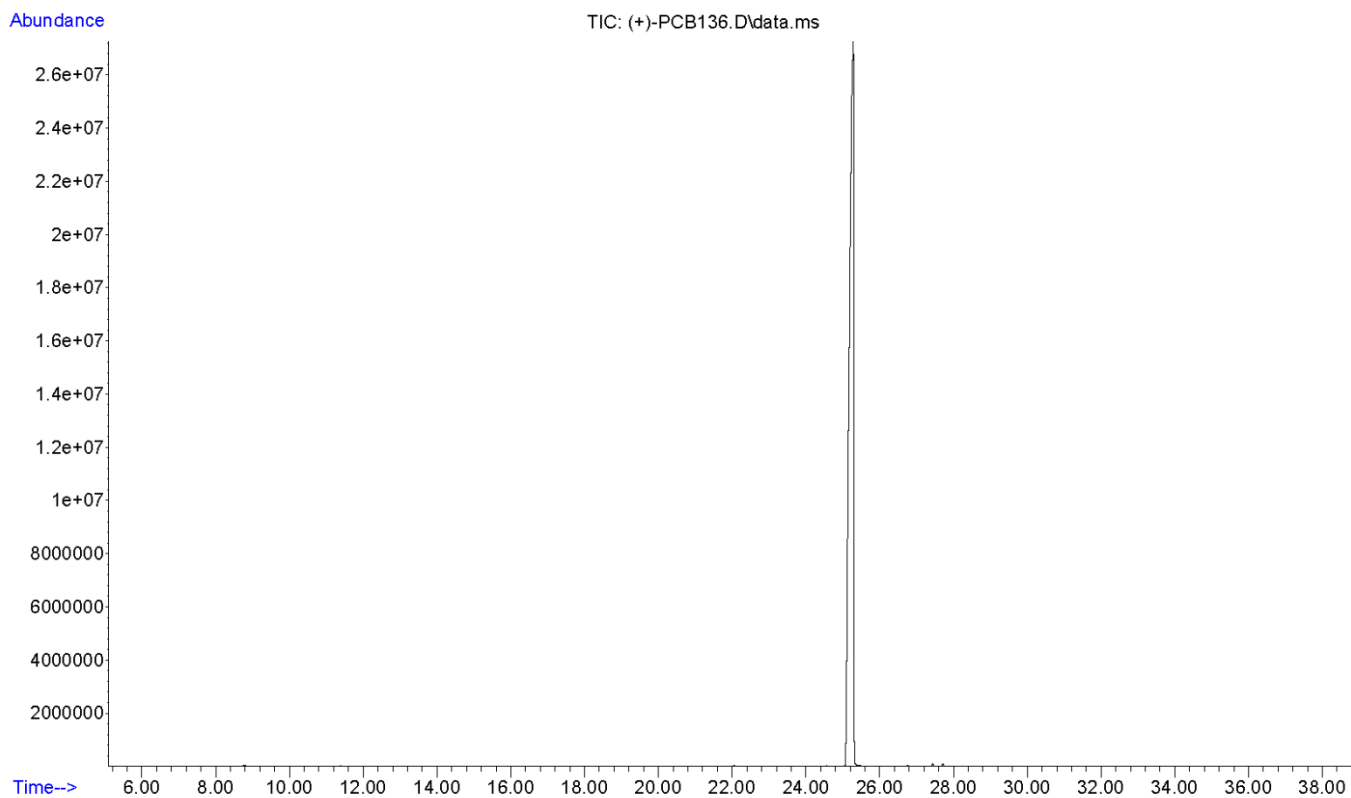
**Fig. S15.** Gas chromatogram and mass spectrum of 3,3',4,4',5-pentachlorobiphenyl (PCB 126).



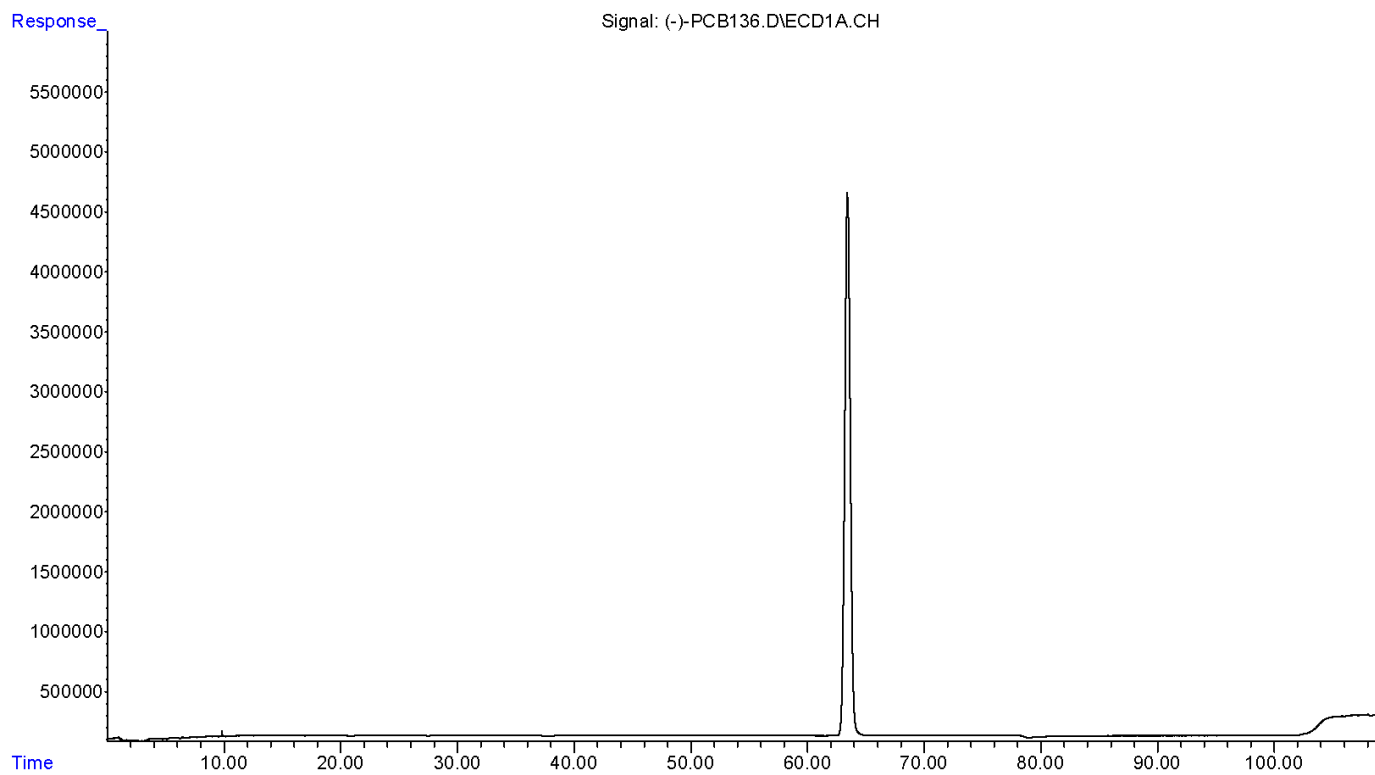
**Fig. S16.** Gas chromatogram and mass spectrum of 2,2',4,4',5,5'-hexachlorobiphenyl (PCB 153).



**Fig. S17.** Gas chromatogram and mass spectrum of (-)-2,2',3,3',6,6'-hexachlorobiphenyl ((-)-PCB 136).

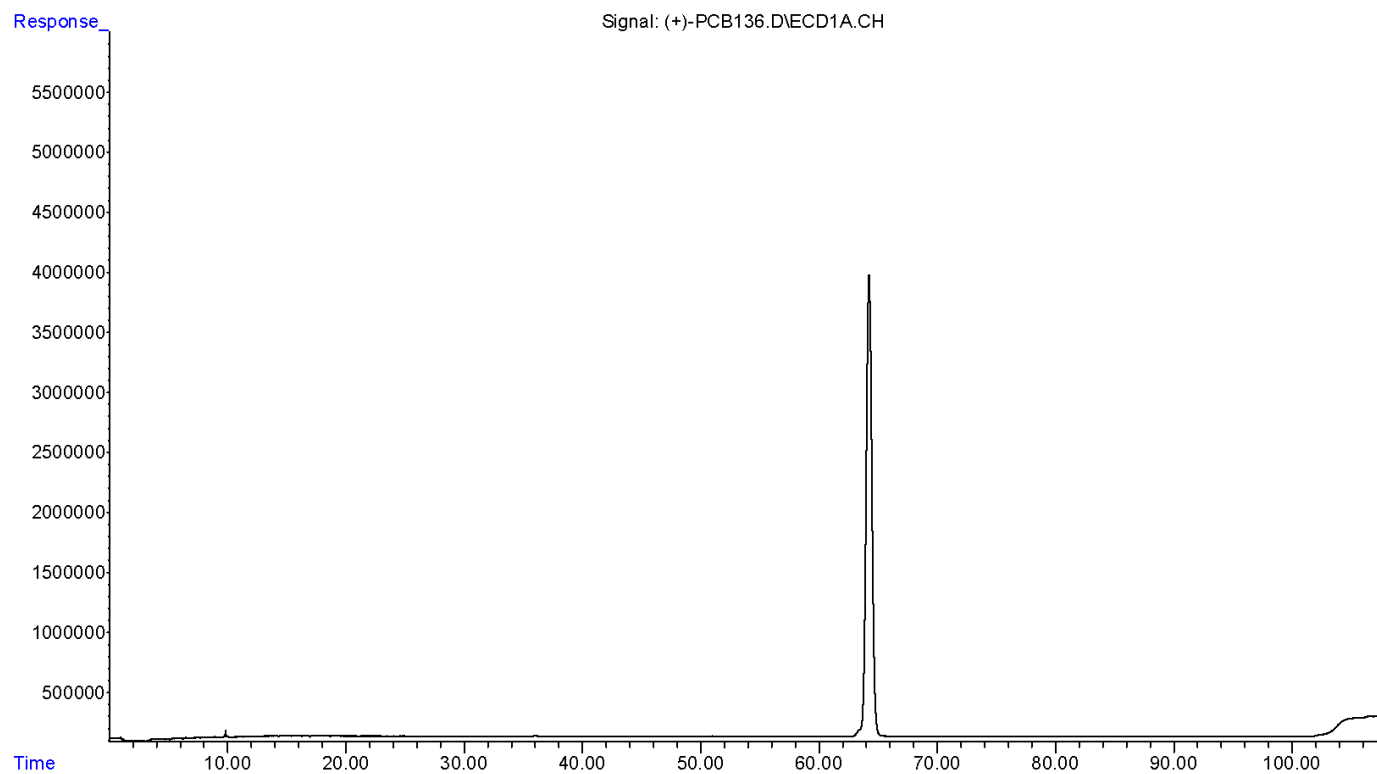


**Fig. S18.** Gas chromatogram and mass spectrum of (+)-2,2',3,3',6,6'-hexachlorobiphenyl ((+)-PCB 136)).



**Fig. S19.** Chromatogram of (-)-PCB 136 on enantioselective column.





**Fig. S20.** Chromatogram of (+)-PCB 136 on enantioselective column.

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