

Improve PCB Congener Identification Using the Ortho Effect

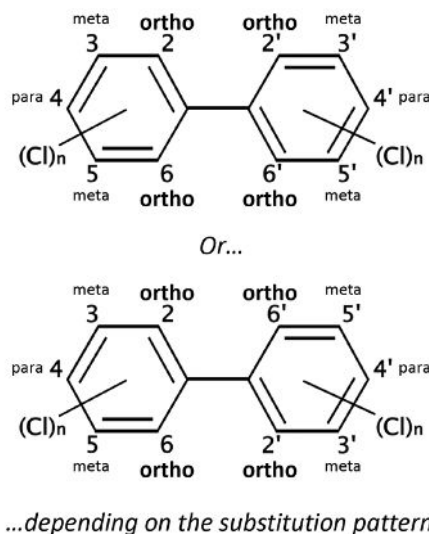
Differentiating polychlorinated biphenyl (PCB) congeners from each other is not always an easy feat, especially when examining PCBs with the same level of chlorination, even when using mass spectrometry. For example, two tetra-substituted congeners would be predicted to have the same mass spectra. If those congeners were not separated chromatographically, positive identification of the specific congeners in a sample would be difficult at best. Among the many tools available to help ensure accurate PCB congener identification, one helpful clue might slip by unnoticed depending on how data acquisition occurs during analysis. This particular clue, called “the ortho effect,” is the result of specific chlorine substitution patterns. The ortho effect stabilizes a given ion fragment, the $M-Cl+$ ion, that otherwise would be too transient to be effectively detected by the mass spectrometer. The end result is a cluster of ions for certain PCB congeners at significantly elevated abundances, which provides a strong clue to the identity of the observed PCB. We have performed our own lab work to demonstrate this effect, but the topic has also been covered very thoroughly by Osemwengie and Sovocool [1].

What Is the “Ortho Effect?”

The term “ortho effect” can refer to two different phenomena related to PCB congener identification. The one that this article focuses on is how ortho chlorine substitution patterns affect PCB fragmentation during ionization in the mass spectrometer. This is the *spectral* ortho effect. The chlorine substitution can also affect the chromatographic separation of PCB congeners, but we will not focus on this *chromatographic* ortho effect because its manifestation can be very column dependent.

The 10 possible substitution sites on the biphenyl molecule where chlorine can bond to make PCBs are each identified by both a number and a name that describes the position relative to the bond that joins the phenyl rings together (Figure 1). Chlorines that occupy the 2, 2', 6, and 6' points are in the “ortho” position but just having a chlorine in the ortho position does not mean a significant ortho effect will be observed. For example, the single monochloro congener (2-chlorobiphenyl, PCB #1) with a chlorine in the ortho position shows no ortho effect. It appears the ortho effect requires at least a disubstituted PCB, although PCBs with multiple chlorine substitution but only a single chlorine in the ortho position showed the smallest effect [1].

Figure 1: General numbering convention for PCB congener identification illustrating where chlorine substitutions can occur on a biphenyl molecule.



Experimentally, it has been shown that when a PCB congener has chlorine substitutions in the 2,2'- and 2,2',6'- positions, the greatest effect is observed. The effect is less pronounced among the 2,6- and 2,2',6,6'- substituted congeners [1].

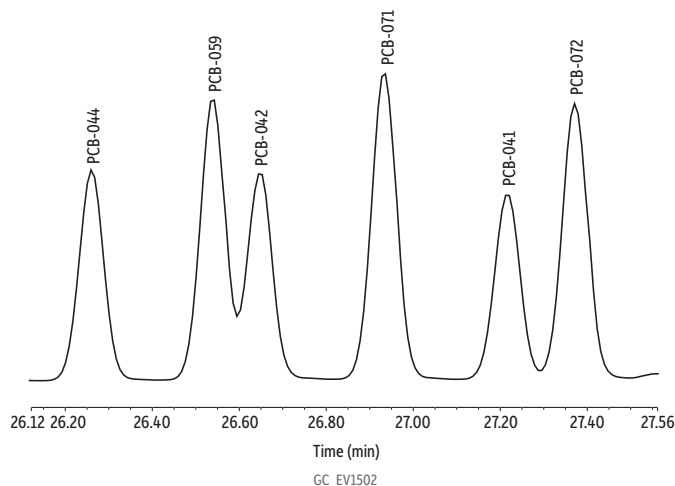
As noted above, when chlorines are in these positions, a fragment ion formed by the loss of a single chlorine atom can be stabilized. The greater stability means that the ion fragment is more likely to exist long enough to be monitored by the mass spectrometer. In fact, a stabilized M-Cl⁺ ion is sometimes present at 20-100% relative abundance compared to the base peak (i.e., the ion with the greatest abundance—often the molecular ion for PCBs). However, the telltale M-Cl⁺ ion for PCB congener identification will only be observed if a mass spectrometer is monitoring the relevant masses.

In our work, we found it possible to monitor the molecular ion as the precursor and the M-Cl⁺ as the product ion transition, as well as monitoring the M-Cl⁺ ion as the precursor and a subsequent M-2Cl⁺ ion as the product ion transition. Both were found to be viable, although the latter showed higher abundances and was selected for the following example.

An Illustration of the Spectral Ortho Effect

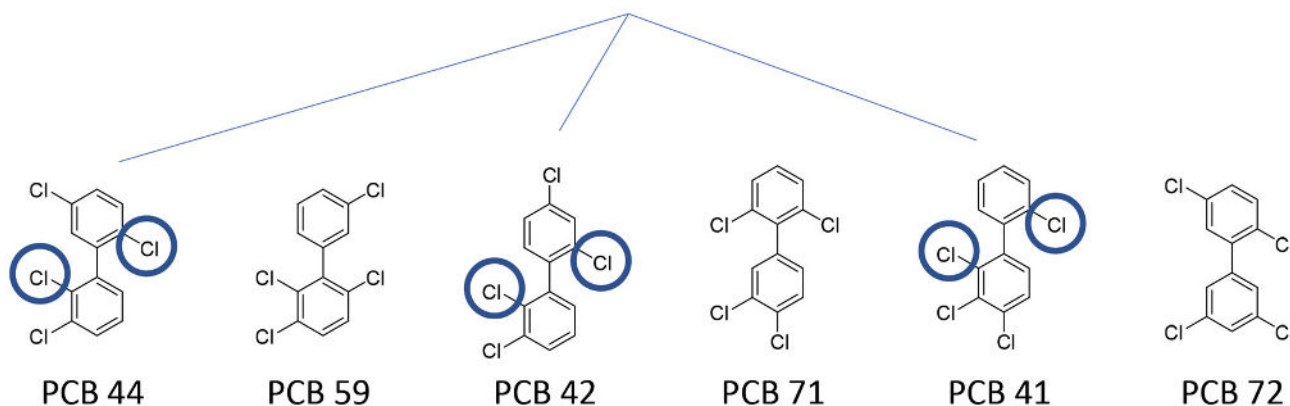
To illustrate the ortho effect, let's examine six tetrachloro-substituted PCBs: PCB #s 44, 59, 42, 71, 41, and 72, which elute in that order from an Rtx-PCB column run under the conditions given in Figure 2. From their structures, shown in Figure 3, you would predict that three of the compounds—PCB #s 44, 42, 41—would have pronounced ortho effects, exhibiting elevated M-Cl⁺ ion response compared to the other three.

Figure 2: Extracted ion chromatogram for a sequence of tetrachloro-substituted PCBs that should vary in degree of ortho effect due to differences in chlorine substitution patterns. (Quantitation ion shown is from the M⁺ → M-2Cl⁺ transition.)



Column	Rtx-PCB, 60 m, 0.18 mm ID, 0.18 µm (cat.# 41304)
Sample	AccuGrand 209 PCB standard (cat.# PCB-209-AG)
Diluent:	Isooctane
Injection	
Inj. Vol.:	2 µL splitless (hold 0.75 min)
Liner:	Topaz 4.0 mm ID single taper inlet liner w/ wool (cat.# 23303)
Inj. Temp.:	350 °C
Purge Flow:	100 mL/min
Oven	
Oven Temp.:	120 °C (hold 1.1 min) to 188 °C at 20 °C/min to 260 °C at 2 °C/min to 300 °C at 6 °C/min to 320 °C at 10 °C/min (hold 6 min)
Carrier Gas	He, constant flow
Flow Rate:	1.44 mL/min
Detector	TSQ 9000 with AEI
Transfer	
Line Temp.:	320 °C
Analyzer Type:	Quadrupole
Source Type:	AEI
Source Temp.:	350 °C
Solvent	
Delay Time:	11.01 min
Tune Type:	PFTBA
Ionization Mode:	EI
Instrument	Thermo Scientific TSQ 8000 Triple Quadrupole GC-MS

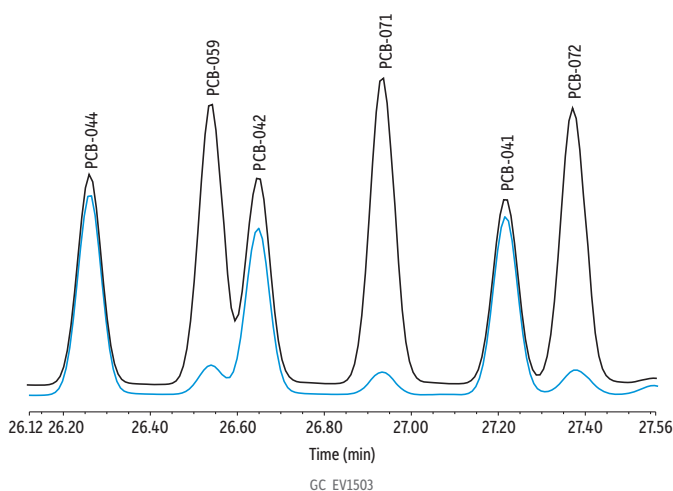
Figure 3: Of the six PCB congeners studied here, three have chlorines in the 2,2'-ortho positions (circled) and are predicted to exhibit a significant spectral ortho effect.



All six PCBs are fairly well resolved from each other, so when all six are present they can provide reference points for one another, and it is not too difficult to distinguish them, even without monitoring the telltale $M-Cl+$ ion. However, if a given sample had only a few of these particular PCBs present, some context is lost, and identification becomes more challenging. But, due to the ortho effect, confidence in a positive PCB congener identification can be increased by also using the $M-Cl+$ ion. As shown in Figure 4, the ortho effect leads to a stabilized $M-Cl+$ ion that can then be monitored as the precursor ion in the MS/MS transition, showing a pronounced signal for that transition for those PCBs that have chlorines in the 2,2'-spots.

Figure 4: The stabilized $M-Cl+$ ion for PCBs exhibiting the ortho effect creates a strong signal for a $M-Cl+ \rightarrow M-2Cl+$ transition that provides valuable confirmation of PCBs 44, 42, and 41, which could be difficult to distinguish spectrally using the $M+ \rightarrow M-2Cl+$ transition alone.

$M+ \rightarrow M-2Cl+$ Transition (m/z 289.9 \rightarrow 220)
 $M-Cl+ \rightarrow M-2Cl+$ Transition (m/z 255 \rightarrow 220)

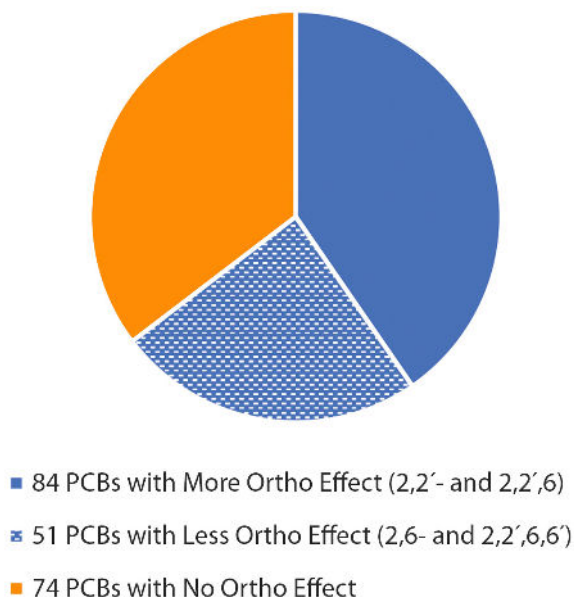


Column Rtx-PCB, 60 m, 0.18 mm ID, 0.18 μ m (cat.# 41304)
Sample AccuGrand 209 PCB standard (cat.# PCB-209-AG)
Diluent: Isooctane
Injection
 Inj. Vol.: 2 μ L splitless (hold 0.75 min)
 Liner: Topaz 4.0 mm ID single taper inlet liner w/ wool (cat.# 23303)
 Inj. Temp.: 350 °C
 Purge Flow: 100 mL/min
Oven
 Oven Temp.: 120 °C (hold 1.1 min) to 188 °C at 20 °C/min to 260 °C at 2 °C/min to 300 °C at 6 °C/min to 320 °C at 10 °C/min (hold 6 min)
Carrier Gas
 Flow Rate: 1.44 mL/min
Detector TSQ 9000 with AEI
Transfer
 Line Temp.: 320 °C
 Analyzer Type: Quadrupole
 Source Type: AEI
 Source Temp.: 350 °C
 Solvent
 Delay Time: 11.01 min
 Tune Type: PFTBA
 Ionization Mode: EI
Instrument Thermo Scientific TSQ 8000 Triple Quadrupole GC-MS

PCBs that Exhibit Significant Spectral Ortho Effects

As already noted, not all PCBs with chlorines substituted in ortho positions on the biphenyl rings will exhibit highly pronounced M-Cl⁺ stability. Predominately, congeners with the 2,2'- and the 2,2',6- positions substituted will have significant M-Cl⁺ signals. Out of the 209 total PCB congeners, 84 congeners have ortho chlorines in the 2,2'- and 2,2',6- positions only (they may have chlorines in the meta and para positions as well, of course), so about 40% of the congeners would be expected to have M-Cl⁺ responses that range from close to 20-100% [1] of the intensity of the molecular ion. Keep in mind that while the effect will not likely be as pronounced, congeners with ortho chlorines in the 2,6- and the 2,2',6,6'- substitution patterns (51 congeners in total) may still yield elevated M-Cl⁺ fragment responses compared to other congeners that do not have pronounced ortho effects. This means that out of the 209 total PCBs, approximately 64% will display some degree of ortho effect (Figure 5).

Figure 5: Sixty-four percent of all 209 PCBs exhibit some degree of ortho effect.



In summary, either directly monitoring the M-Cl⁺ ion or using it as a precursor ion in an MS/MS transition, as shown in this article, can provide valuable insight into PCB congener identification. This powerful tool is available all because of the stabilization effect that chlorine atoms in certain positions on the PCB molecule can have.

References

1. Osemwengie, G.W. Sovocool, The mass spectrometric ortho effect studied for all 209 PCB congeners, International Journal of Mass Spectrometry, 352 (2013) 51–64. <https://www.sciencedirect.com/science/article/abs/pii/S1387380613002686?via%3Dihub>

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	0.18 µm	40 m	30 to 320/340 °C	ea.	41303
	0.18 µm	60 m	30 to 320/340 °C	ea.	41304
0.25 mm	0.25 µm	30 m	30 to 320/340 °C	ea.	13223
	0.25 µm	60 m	30 to 320/340 °C	ea.	13226
0.32 mm	0.50 µm	30 m	30 to 320/340 °C	ea.	13239



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