Distinguishing PCB Isomeric Congeners with their Gas Chromatographic and Mass Spectrometric Ortho Effects using Comprehensive Gas Chromatography

Lantis Osemwengie, U.S. EPA, NERL, Las Vegas, NV

Introduction

The 209 polychlorinated biphenyl (PCB) congeners and associated nine isomeric groups
(nine groups of PCBs with the same degree of chlorination) have been long recorded as high endocrine disrupting chemicals in the environment. Difficult analytical problems exist, in those frequent cases where several chromatographically coeluting (traversing the separation column simultaneously) PCB isomers or congeners are present in environmental samples. Analysts usually report final results in the form of “total PCB concentrations,” which only reflect a compromised or average value. In a case where a toxic PCB isomer coelutes with a non-toxic PCB isomer in environmental sample (a co- planar and non-coplanar, within isomeric group), total concentration of both isomers is reported because the toxic isomer could not be separated from the non-toxic one. This leads to enormous environmental remediation cost in hot spots for industries. Remediation may be avoided altogether if the toxic PCB isomers can be separated from the non toxic ones, and the actual concentration of the toxic PCB isomer ascertained to be below actionable level. This research work solved this problem by using two approaches.
(1) Where the observable ortho effects or internal chemistry of the PCB isomers in question is used to distinguish one PCB isomer from the other. (2) The use of a newly introduced two-dimensional gas chromatography instrument for the front-end separation of several PCB congeners in ways never before possible. The issue of declorinated high mass PCB congeners, coeluting with low mass PCB congeners, is also resolved. This tool may be applied to other complex mixtures consisting of congeners and isomers, e.g., polybrominated biphenyls and toxaphene.

Research Goal

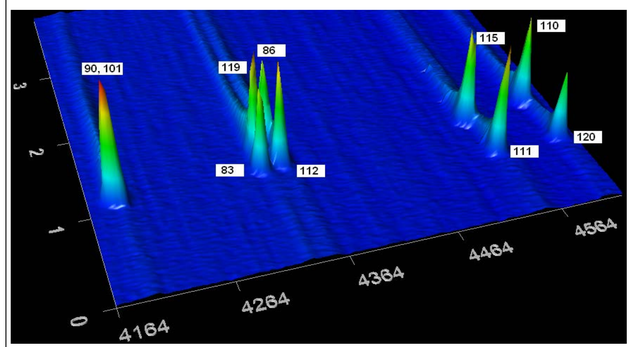
- Develop rapid, robust, and cost effective analytical method capable of distinguishing all PCB congeners and their coeluting isomers in environmental monitoring of PCBs.
- Facilitate the abatement of PCBs present in the environment by rapid screening of the class (number of chlorine substitutions) and type (coplanar and non-coplanar) of congeners present or absent in superfund sites.
- Provide invaluable information for the identification and quantification of PCB congener specific analysis and consequently, more accurate data for the US. EPA toxicity studies.

Methods/Results

Experimental

Certified solutions of individual chlorinated biphenyls (International Union of Pure and Applied Chemistry [IUPAC] # 1 through 209) were used for this study and were purchased from AccuStandard, Inc., (New Haven, CT, USA). Each isooctane solution contained 100 ng/µL of an individual chlorinated biphenyl congener and was diluted 5-fold with 99.9% *n*-hexane (B&J GC2 grade, Burdick and Jackson, Muskegon, MI, USA). To compensate for the generally decreasing sensitivity of the TOFMS for more highly chlorinated congeners, the concentrations of the injected congener solutions were between 0.20 ng/µL and 4.25 ng/µL. The concentration adjustments yielded approximately equal signal intensities upon analysis by GCxGC/TOFMS. The first column used in this study was a nonpolar phased Rtx-PCB GC column (Restek, Bellefonte, PA, USA), of 40 meters length, 0.18 mm ID, and 0.18 µm film thickness, while the second column was a polar phased GC column, DB-17 (Agilent Technologies, Palo Alto, CA, USA), of 1 meter length, 0.10 mm ID, and 0.10 µm film.

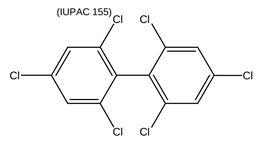
GCxGC-TOFMS Separation of Known GC Co-Eluting Pentachlorobiphenyl Isomers



Surface view showing the use of GCxGC-TOFMS in the separation of four pairs of polychlorinated biphenyl isomers with IUPAC numbers beginning from the right of (110 + 120), (115 + 111), (86 + 112), and (119 + 83). The PCB isomers (left pair) numbers (90 + 101), were inseparable under the chromatographic conditions used. However they exibited different ortho effects (42% and 29% respectively).

PCB Isomer Identification by GC Ortho Effect

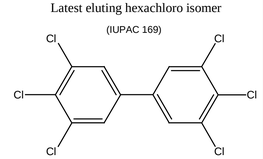
Earliest eluting hexachloro isomer (IUPAC 155)



2,4,6,2',4',6'-Hexachlorobiphenyl

(Rings actually perpendicular)

Latest eluting hexachloro isomer (IUPAC 169)

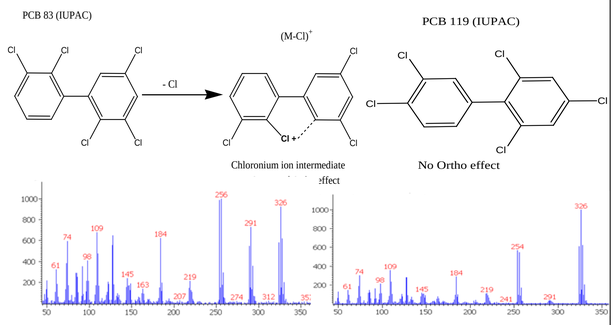


3,4,5,3',4',5'-Hexachlorobiphenyl

(Nearly planar)

Within the hexachlorobiphenyl congener series, there are 42 hexachlorobiphenyl isomers. Elution time of the hexachlorobiphenyl isomers increased as the number of ortho chlorines decreased. The 246:246 Hexachloro (4 ortho chlorine substitution) was observed to elute the GC column first, while the 345:345 Hexachloro (no ortho chlorine substitution) eluted last.

PCB Isomer Identification by MS ***Ortho Effect***



Congeners and Some PCB Coeluting Isomers with their Percent “*Ortho Effects*”IUPAC Numbers of Coeluting Isomers (“*Ortho Effects*”)

Tri	28(5%) + 33(5%) + 21(6%)
Tri	39(5%) + 38(7%)
Tetra	64(4%) + 40(82%)*
Tetra	65(7%) + 62(5%) + 47(26%)*
Tetra	67(3%) + 58(4%)
Hexa	163(4%) + 129(56%)*
Hepta	182(32%) + 175(44%)
Octa	201(4%) + 204(2%)

* This isomer can be distinguished from other coeluting isomers by the “ortho effect.”

Summary

The *ortho effect* was determined to be a reliable way of distinguishing the isomers having no ortho chlorines, one ortho chlorine, two ortho chlorines on the same ring, or four ortho chlorines from isomers with 2,2’- or 2,2’,6- chlorine substitutions, which have been shown to have unique health effects of their own. By far, the largest number of distinguishable isomers was produced from the retention characteristics. However, the *ortho effect* could help align orders of elution in cases, including environmental analysis, where structural assignments might be ambiguous, or when only limited numbers of congeners were present, or available. Use of the *ortho effect* also proved to be of value for recognizing incorrectly assigned chemical structures of standards and of library entries.

Conclusion

This study took advantage of the separation potential of GCxGC-TOFMS, aided by GC and MS “ortho effect” distinctions, to develop a robust analytical method to rapidly and accurately distinguish those PCB isomers that pose greater degree of difficulties in separation, in a cost effective approach. A journal article of this research is now available in the International Journal of Mass Spectrometry 352 (2013) 51- 64.
http://dx.doi.org/10.1016/j.ijms.2013.07.003

Contributors/Collaborators

NERL: Christian Daughton and Nellie Dujua

LECO CORP: Mark Libardoni

References

L.I. Osemwengie, G. W. Sovocool, “Evaluation of comprehensive 2D gas chromatography-time-of- flight mass spectrometry for 209 chlorinated biphenyl congeners in two chromatographic runs,” *Chromatographic Research International*, vol. 2011, Article ID 675920, pp.1-14, 2011, doi:10.4061/2011/675920.

G. W. Sovocool, R. K. Mitchum, and J. R. Donnelly, Use of the ‘ortho effect’ for chlorinated biphenyl and brominated biphenyl isomer identification,” *Biomedical and Environmental Mass Spectrometry*, vol. 14, no. 10, pp. 579-582, 1987.

L. A. Levy, and E. O. Oswald, “The effect of ortho substitution on the mass spectral fragmentation of polychlorinated biphenyls,” *Biomedical Mass Spectrometry*, vol.3, no. 2, pp.88-90, 1976.