Identification of bromo/chloro dibenzo-p-dioxins by negative atmospheric pressure chemical ionization: A resurgent application of APGC

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Polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), are two highly toxic groups of chemicals.

Up to eight chlorines can be substituted onto the three-ring structure yielding 75 possible dioxin and 135 possible furan congeners.

2,3,7,8-substituted congeners are the most toxic due to their strong affinity for the aryl hydrocarbon receptor. The toxicities of the various congeners can differ by 10–10,000 fold!

Inclusion of Br increases the number of potential mixed halogenated dioxin congeners (PXDDs) to 1550 PXDD and PXDFs to 3050. Only a fraction of these (2,3,7,8-substituted) are expected to be toxic.
Why is GC-HRMS considered the “gold standard” for dioxin analysis?

- **Selectivity**: Magnetic deflection (HRMS) instruments are routinely operated at 10,000 RP (10% valley).

- High mass resolution is often sufficient to distinguish most isobaric interferences that cannot be separated chromatographically.

- GC separation is **crucial**: the EI spectra of PCDD isomers are virtually identical.

- **Sensitivity**: Detection of 10fg or lower required.

- In 1970, HRMS was the only technique with any hope of achieving the 1ppt detection limits prescribed to eliminate risk to dioxin exposure.

Electron ionization (EI) cannot differentiate dioxin isomers

2378-TCDD (Most toxic congener)

1368-TCDD (Not toxic)

1234-TCDD (Closest eluting isomer)
Analysis of mixed halogenated dibenzo-p-dioxins and dibenzofurans (PXDD/PXDFs) in soil by gas chromatography tandem mass spectrometry (GC–MS/MS)

Anne L. Myers a,*, Scott A. Mabury a, Eric J. Reiner a, b

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b Ontario Ministry of the Environment, 125 Resources Road, Toronto, Ontario, Canada M9P 3V6
GC×GC is one possible technique for PXDD/F separation.

Schematic of a GC × GC

Retention Time \( t_R \)

PM = modulation time
D = dimension

GC separation (40m rtx-dioxin2)

Plastimet extract
2015-03-24-BrCIDX-045

GC×GC separation (40m rtx-dioxin2 x rtx-17SIL)
Reactions with $O_2$ can distinguish 2378-TCDD from its isomers

The Mass Spectrometry of Polychlorinated Dibenzo-$p$-Dioxins

J. Ronald Hass† and Marlin D. Friesen‡
Environmental Biology and Chemistry Branch, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina 27709, USA

Michael K. Hoffman
Washington University Medical School, St Louis, Missouri, USA

The negative ion chemical ionization mass spectra of polychlorinated dibenzo-$p$-dioxins using oxygen, methane and methane/oxygen are reported together with their methane positive ion chemical ionization mass spectra and conventional electron impact spectra. The methane/oxygen negative ion chemical ionization mass spectra proved to be the most useful of the negative ion spectra for structure determination.

\[ \begin{align*}
O_2, e^- & \rightarrow Cl_x \begin{array}{c}
\text{O}^- \\
\text{Cl}_{y-1}
\end{array} \\
O_2, e^- & \rightarrow Cl_x \begin{array}{c}
\text{O}^- \\
\text{Cl}_{y}
\end{array} + \begin{array}{c}
\text{O}^- \\
\text{Cl}_{x}
\end{array}
\end{align*} \]
Dioxin analysis was an early application of GC-APCI

Capillary Gas Chromatography/Atmospheric Pressure Negative Chemical Ionization Mass Spectrometry of the 22 Isomeric Tetrachlorodibenzo-p-dioxins

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Department of Health and Human Services, Food and Drug Administration, National Center for Toxicological Research, Jefferson, Arkansas 72079

D. L. Stalling

United States Department of Interior, Fish and Wildlife Service, Columbia National Fisheries Research Laboratory, Columbia, Missouri 65201

Figure 1. GC/NIAP/MS inlet schematic: (1) injector; (2) glass cap adaptor; (3) Teflon shrink tubing; (4) 20-m capillary column; (5) to metal tubing seal; (6) Pt/fc capillary; (7) quartz injector tub source volume; (9) 60Ni foil; (10) 50-μm aperture; (11) v housing; (12) GC oven wall, (13) makeup gas inlet; (14) overfic outlet.
1990s witnessed the widespread adoption of commercial LC-MS

John Fenn

Micromass Quattro Ultima (2002)
APCI⁻ mass spectrum of 23-Br-78-Cl dibenzo-\(\rho\)-dioxin

50ul/min hexane flow injection APCI⁻
Waters Quattro Ultima

Structure diagnostic reactions!
The Universal Mass Spectrometry System

Waters Xevo G2-XS Q-TOF

System Attributes

- **Mass range m/z 20 – 4000 (Q-limited)**
  Covers most environmental contaminants

- **Maximum Acquisition rate 30 Hz**
  Instrument is capable of LC, GC and GCxGC experiments

- **Mass Resolution 25,000 – 35,000 FWHM**
  Slightly better than an HRMS instrument tuned for dioxin analysis.
  Opens the door to mass defect analysis.

- **Mass Accuracy < 1mDa**
  That’s equivalent to ~2 electrons!

- **Full Scan data acquisition** –

Non-targeted analysis!
Atmospheric Pressure Gas Chromatography (APGC)

GC inlet and ionization

- Transfer line heated to 300 °C - 360°C
- Corona pin initiates ionization.
- Ionization similar to processes observed for APCI in LCMS
- Positive ionization usually occurs by charge exchange with $N_2^{+}$
- $H_2O$ and other gases can be introduced to modify the ionization process. (proton transfer e.g.)
- Negative ions may be generated by electron capture, but other mechanisms may also occur.
The Waters Xevo G2-XS Q-TOF mass spectrometer

Scan Modes:

- Full Scan TOF-MS.
- Target Enhanced Mode
- MS/MS (CID, Neutral loss etc.)
- TOF-MRM
Differentiation of TCDD isomers by GC-APCI-

Absence of peri Cl enhances ether cleavage (Observed by Hass et al.)
Similar reactions obtain for Br and Br/Cl analogues.
Reactions with $O_2$ improves selectivity

Analysis of PBDDs is often performed using a short, thin film GC column. APCI- improves separation between key TCDD and TBDD isomers.
## Summary of O₂ reactions with available standards

<table>
<thead>
<tr>
<th>Compound</th>
<th>Ether Cleavage 1</th>
<th>Ether Cleavage 2</th>
<th>Molecular ion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1234-TCDD</td>
<td>245.86 (Cl₄) (0.03%)</td>
<td>ND</td>
<td>300.92 (M-Cl+O)</td>
</tr>
<tr>
<td>1378-TCDD</td>
<td>175.94 (Cl₂) (35%)</td>
<td>ND</td>
<td>300.92 (M-Cl+O)</td>
</tr>
<tr>
<td>2378-TCDD</td>
<td>175.94 (Cl₂) (75%)</td>
<td>ND</td>
<td>300.92 (M-Cl+O)</td>
</tr>
<tr>
<td>1Br-DD</td>
<td>ND</td>
<td>ND</td>
<td>277.99 (M+O)</td>
</tr>
<tr>
<td>27/28Br-DD</td>
<td>ND</td>
<td>ND</td>
<td>276.95 (M-Br+O)</td>
</tr>
<tr>
<td>237Br-DD</td>
<td>265.84 (Br₂) (1%)</td>
<td>ND</td>
<td>356.86 (M-Br+O)</td>
</tr>
<tr>
<td>1234TBDD</td>
<td>423.66 (Br₄) (0.005%)</td>
<td>ND</td>
<td>434.77 (M-Br+O)</td>
</tr>
<tr>
<td>1378TBDD</td>
<td>265.84 (Br₂) (15%)</td>
<td>ND</td>
<td>434.77 (M-Br+O)</td>
</tr>
<tr>
<td>2378TBDD</td>
<td>265.84 (Br₂) (20%)</td>
<td>ND</td>
<td>434.77 (M-Br+O)</td>
</tr>
<tr>
<td>12478B-DD</td>
<td>265.84 (Br₂) (1%)</td>
<td>343.75 (Br₃) (1%)</td>
<td>514.68 (M-Br+O)</td>
</tr>
<tr>
<td>12378B-DD</td>
<td>265.84 (Br₂) (1%)</td>
<td>343.75 (Br₃) (1%)</td>
<td>514.68 (M-Br+O)</td>
</tr>
<tr>
<td>Heptabromo-DD</td>
<td>343.75 (Br₃) (0.02%)</td>
<td>423.66 (Br₄) (0.03%)</td>
<td>672.50 (M-Br+O)</td>
</tr>
<tr>
<td>Octabromo-DD</td>
<td>423.66 (Br₄) (0.02%)</td>
<td>ND</td>
<td>750.41 (M-Br+O)</td>
</tr>
<tr>
<td>7B,23C-DD</td>
<td>175.94 (Cl₂) (10%)</td>
<td>185.93 (Br₁) (1%)</td>
<td>266.96 (M-Br+O)</td>
</tr>
<tr>
<td>2B,378C-DD</td>
<td>175.94 (Cl₂) (10%)</td>
<td>221.89 (ClBr) (20%)</td>
<td>300.92 (M-Br+O)</td>
</tr>
<tr>
<td>23Br, 78Cl-DD</td>
<td>175.94 (Cl₂) (10%)</td>
<td>265.84 (Br₂) (30%)</td>
<td>346.87 (M-Br+O)</td>
</tr>
<tr>
<td>2B,1378C-DD</td>
<td>175.94 (Cl₂) (1%)</td>
<td>255.85 (BrCl₂) (5%)</td>
<td>380.83 (M-Cl+O)</td>
</tr>
</tbody>
</table>

**Peri Cl/Br absent – 2,3,7,8-substituted**

**Base peak**

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<thead>
<tr>
<th>Compound</th>
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<th>Ether Cleavage 2</th>
<th>Molecular ion</th>
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</thead>
<tbody>
<tr>
<td>1234-TBDD</td>
<td>423.66 (Br₄) (0.005%)</td>
<td>ND</td>
<td>434.77 (M-Br+O)</td>
</tr>
<tr>
<td>1378-TBDD</td>
<td>265.84 (Br₂) (15%)</td>
<td>ND</td>
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<tr>
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<td>265.84 (Br₂) (20%)</td>
<td>ND</td>
<td>434.77 (M-Br+O)</td>
</tr>
<tr>
<td>12478B-DD</td>
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<td>343.75 (Br₃) (1%)</td>
<td>514.68 (M-Br+O)</td>
</tr>
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<td>Heptabromo-DD</td>
<td>343.75 (Br₃) (0.02%)</td>
<td>423.66 (Br₄) (0.03%)</td>
<td>672.50 (M-Br+O)</td>
</tr>
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<td>Octabromo-DD</td>
<td>423.66 (Br₄) (0.02%)</td>
<td>ND</td>
<td>750.41 (M-Br+O)</td>
</tr>
<tr>
<td>7B,23C-DD</td>
<td>175.94 (Cl₂) (10%)</td>
<td>185.93 (Br₁) (1%)</td>
<td>266.96 (M-Br+O)</td>
</tr>
<tr>
<td>2B,378C-DD</td>
<td>175.94 (Cl₂) (10%)</td>
<td>221.89 (ClBr) (20%)</td>
<td>300.92 (M-Br+O)</td>
</tr>
<tr>
<td>23Br, 78Cl-DD</td>
<td>175.94 (Cl₂) (10%)</td>
<td>265.84 (Br₂) (30%)</td>
<td>346.87 (M-Br+O)</td>
</tr>
<tr>
<td>2B,1378C-DD</td>
<td>175.94 (Cl₂) (1%)</td>
<td>255.85 (BrCl₂) (5%)</td>
<td>380.83 (M-Cl+O)</td>
</tr>
</tbody>
</table>

**Peri Cl/Br absent – 2,3,7,8-substituted**

**Base peak**
In general, a preference for Br/O exchange is observed.

What about the furans?
No ether cleavages, but some structure information is obtained.
What about the furans?
No ether cleavages, but some structure information is obtained

- Exchange of peri halogen takes priority
Under what conditions do these reactions occur?

➢ Evaluate the effects of higher oxygen content in the APGC source.

- Four gas inputs into the source region:
Under what conditions do these reactions occur?

- Evaluate the effects of higher oxygen content in the APGC source.

- Four gas inputs into the source region:
Increased $O_2$ concentration decreases yield of ions

- 0.05% and 1% $O_2$ (from $N_2$ generator) decreases yield of ions.
- Dry, compressed air results in intense $[M+O]^-$ signal.

Compressed Air

Nitrogen
Gas flow optimization is critical

- High chemical noise observed at low cone gas flow

Cone Gas @100L/hr

Cone Gas @175L/hr

ECP trace at m/z 265.84

S/N 15

S/N 100
<table>
<thead>
<tr>
<th>Compound</th>
<th>Neg (M-X+O)$^-$</th>
<th>Pos (M$^{+}$)</th>
<th>I.D.L. (fg/µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>ECP 1</td>
<td>ECP 2</td>
</tr>
<tr>
<td>1234-TCDD</td>
<td>300.92 (M-Cl+O)</td>
<td>321.89</td>
<td></td>
</tr>
<tr>
<td>1378-TCDD</td>
<td>300.92 (M-Cl+O)</td>
<td>321.89</td>
<td>383</td>
</tr>
<tr>
<td>2378-TCDD</td>
<td>300.92 (M-Cl+O)</td>
<td>321.89</td>
<td>248</td>
</tr>
<tr>
<td>1Br-DD</td>
<td>277.99 (M+O)</td>
<td>261.96</td>
<td></td>
</tr>
<tr>
<td>27/28Br-DD</td>
<td>276.95 (M-Br+O)</td>
<td>341.87</td>
<td></td>
</tr>
<tr>
<td>237Br-DD</td>
<td>356.86 (M-Br+O)</td>
<td>419.78</td>
<td>680</td>
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<tr>
<td>1234TBDD</td>
<td>434.77 (M-Br+O)</td>
<td>499.69</td>
<td></td>
</tr>
<tr>
<td>1378TBDD</td>
<td>434.77 (M-Br+O)</td>
<td>499.69</td>
<td>143</td>
</tr>
<tr>
<td>2378TBDD</td>
<td>434.77 (M-Br+O)</td>
<td>499.69</td>
<td>132</td>
</tr>
<tr>
<td>12478B-DD</td>
<td>514.68 (M-Br+O)</td>
<td>577.60</td>
<td>495</td>
</tr>
<tr>
<td>12378B-DD</td>
<td>514.68 (M-Br+O)</td>
<td>577.60</td>
<td>660</td>
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<tr>
<td>Heptabromo-DD</td>
<td>672.50 (M-Br+O)</td>
<td>735.42</td>
<td></td>
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<td>Octabromo-DD</td>
<td>750.41 (M-Br+O)</td>
<td>815.33</td>
<td></td>
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<tr>
<td>7B,23C-DD</td>
<td>266.96 (M-Br+O)</td>
<td>331.88</td>
<td>132</td>
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<tr>
<td>2B,378C-DD</td>
<td>300.92 (M-Br+O)</td>
<td>365.84</td>
<td>152</td>
</tr>
<tr>
<td>23Br, 78Cl-DD</td>
<td>346.87 (M-Br+O)</td>
<td>409.79</td>
<td>141</td>
</tr>
<tr>
<td>2B,1378C-DD</td>
<td>380.83 (M-Cl+O)</td>
<td>399.80</td>
<td>166</td>
</tr>
</tbody>
</table>
The reactivity of the PBDDs and PXDDs towards $O_2^-$ follows essentially the same scheme proposed by Hass et al for the PCDDs.

(i) $O_2^-$ reacts with PBDDs and PXDDs by cleaving the C-O bonds, essentially breaking the dioxin into two. The reaction also results in the formation of pseudo-molecular ions $[M-X+O]^-$ (where X=Br/Cl).

(ii) The number of halogens per benzene ring can be determined by monitoring the ether cleavage product ions.

(iii) The yield of ether cleavage product ions is high for 2378-substituted congeners, which are the most toxic.

(iv) Formation of $[M-X+O]$ is preferred over ether cleavage for compounds which have at least one peri position (1,4,6,9) occupied by a halogen. If a peri halogen is not present, Br/O exchange is preferred over Cl/O exchange.
- 400+ tonnes of PVC and polyurethanes stored on site burned.
- The fire burned for 3 days before it was finally extinguished.
- Nearby residents were very concerned about their health.
- Several firefighters developed serious health problems.
- Chrome plating on some fire engines came off in the months following the fire due to atmospheric HCl.
APCI of HxCDDs: Improved separation

[a] 1 2 3 4 5 6+7

(b) 1 2 3 4 6

(c) 2 3 4 5 7

(d) 1 2 3 4 6
APCI\(^{-}\) of BrCl\(_5\)DDs : Improved separation

1. Improvement in separation of BrCl\(_5\)DDs using APCI\(^{-}\) mode.
2. Identification of peaks 1, 2, 3, 4, 5, 6, 7, and 8.
3. Comparison with APCI\(^{+}\) mode for [M\(^{+}\)] and [M-Cl+O\(^{-}\)].
APCI$^-$ of Br$_2$Cl$_2$DDs: Identification of 2:2 congeners

[a] APCI +
MRM trace [$M^+ \rightarrow (M-\text{COBr})^+$]

[b] APCI -

2-Br, 3,7,8-CDD
Distribution of Br/Cl atoms in PXDDs generated by Plastimet fire

Environmental implications

- Myers et al. reported PXDD concentrations that were ~10% of the PCDDs.
- Brominated chemicals are increasingly used in manufactured products.
- PXDD and PXDF analysis will become increasingly important.
Analytical implications

- The sensitivity of GC-APCI⁻ (low fg IDLs) is equivalent to positive mode ionization, but with substantially reduced chemical noise.
  - Unlike traditional NCI, dissociation into Cl⁻ and Br⁻ appears to be a minor process. APCI⁻ generates pseudomolecular ions (M-X+O)⁻.

- APCI⁻ is highly selective. Reactions with O₂ result in structure diagnostic reactions:
  - Identification of unknown congeners. There are 1550 PXDD congeners, of which few analytical standards exist.
  - Enable chromatographic separation of PXDDs that would otherwise be impossible.
  - Enable use of a shorter GC column for screening a wider range of dioxin related compounds.

- All experiments shown here can be performed using standard setup.
What other Br/Cl compounds were generated in the fire?

Plastimet extract
2015-03-24-BrCIDX-045
1: TOF MS AP+
TIC
1.76e8

2,3,7,8-tetrachlorodibenzo-p-dioxin
Kendrick mass defect plot reveals tentative structure proposals

There are potentially thousands of isomers!

APGC reduces interferences and GC×GC can resolve them

- **OCDPEs**
- **13C-HxCDFs**
- **HxCDFs**

Quechers Dioxin 135591 4s 300ms

- M-Cl₂
- OCDPE**

15-07-27_GCxGC_030 28612 (21.432) (373.824) 1.40e6

- 373.825
- 377.819
- 389.820 445.760
- 441.766
- 449.755

- 443.764
- 447.758
- 449.755

- 375.822
- 377.819
- 389.820 445.760
- 441.766
- 449.755
GC×GC can enhance sensitivity

500 fg 2378-TCDD

1D GC (2 Hz)
FWHM = 5 s

1st D: 30 m RTX-5 0.25 mm x 0.25 μm
2nd D: 2 m RTX 50 0.18 mm x 0.18 μm
10 second modulation period

Goal: a routine, automated, non-targeted analysis

(1) Mass defect filtering reveals halogenated ions

(2) Software deconvolution of both target and non-target compounds.

(3) Interpretation of full-scan high resolution mass spectra leads to structure proposal:
Target and non-target analysis in a single injection

The TEQ is increased 10x when 2,3,7,8-TBDD/F are included!
Identifying unknowns using APGC: EI libraries are still useful!

Soft ionization leads to revised structure proposal
Take home message:
APGC is a powerful and versatile tool for environmental analysis

Co-Authors/Collaborators/Contributors

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Jack Cochran

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Frank Dorman, Kari Organtini

Rhys Jones, Adam Ladak, Doug Stevens