# Chromatographic and Ionization Properties of Polybrominated Diphenyl Ethers Using GC/High-Resolution MS with Metastable Atom Bombardment and Electron Impact Ionization

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**The chromatographic and ionization properties of 35 polybrominated diphenyl ether (PBDE) congeners were investigated using GC/HRMS with metastable atom bombardment (MAB) and electron impact (EI) ionization. A multiple linear regression model based on bromine substitution patterns and MOPAC calculated physical properties was developed to predict relative GC retention times of individual PBDE congeners. Although five different sources of metastable rare gas atoms (He, $N_2$, Ar, Xe, and Kr) were investigated with MAB ionization, only MAB-$N_2$ provided adequate ionization efficiency and predictability. Because of reduced background noise to the MS detector, MAB-$N_2$ had a lower limit of detection for tetra- and penta-BDEs than EI, despite having a lower sensitivity. Using MAB-$N_2$, the molecular ion was always the base peak, with little fragmentation taking place. Conversely, using EI ionization, the $[M - nBr]^+$ peak (where $n = 1-4$, depending on the number of Br substituents) was the dominant ion for all PBDE congeners. Multiple linear regression models representing the molecular ion response of PBDE congeners analyzed by GC/HRMS with MAB-$N_2$ and EI ionization were also developed using the number and type of Br substituents and ionization potentials. A significantly higher level of predictability was obtained for the MAB-$N_2$ response model than for EI.**

Polybrominated diphenyl ethers (PBDEs) are substances used as additive flame retardants in polymeric materials.[1] They are produced in large quantities (~70 000 tons/year in 1999),[2] are

lipophilic and bioaccumulate in a variety of matrixes,[1] and are potential endocrine disrupters.[3] These compounds have been detected in all environmental compartments (sediment, air, water, and biota) and in human tissues at the nanograms-per-gram–micrograms-per-gram levels,[1,4–7] and levels are increasing exponentially in arctic regions.[4] There are 209 possible PBDE congeners and three major technical mixtures: DeBDE (97–98% deca-BDE), OcBDE (43–44% hepta-BDE, 31–35% octa-BDE; e.g., Bromkal 79-8DE), and PeBDE (24–38% tetra-BDE, 50–62% penta-BDE; e.g., Bromkal 70-5DE). However, current analytical methods and a lack of authentic standards allow identification and quantitation of only a limited number of PBDE congeners. Because of their prevalence and toxicology, there is much interest in developing reliable analytical methods for all 209 congeners.

The use of gas chromatography (GC) coupled with electron-impact (EI) and electron-capture (EC) mass spectrometry (MS) for the analysis of PBDEs has been previously reported.[1,8–11] Electron-capture negative ionization (ECNI), although generally more sensitive and less costly than other ionization methods for PBDE analysis, does not provide information on the molecular ion cluster (as required for qualitative identification), is more subject to brominated interferences, and does not allow the use of $^{13}$C-labeled standards for quantitation.[11–14] Conversely, EI

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(1) de Boer, J.; de Boer, K.; Boon, J. P. 1999. In Paasivirta, J., Ed.; *The Handbook of Environmental Chemistry: New Types of Persistent Halogenated Compounds.* Springer-Verlag: New York, pp 62–95.
(2) Arias, P. A. Brominated flame retardants – an overview, Proceedings of The Second International Workshop on Brominated Flame Retardants, Stockholm, Sweden, May 14–16, 2001; pp 3–4.

(3) Meerts, I. A. T. M.; van Zanden, J. J.; Luijks, E. A. C.; van Leeuwen-Bol, I.; Marsh, G.; Jakobsson, E.; Bergman, A.; Brouwer, A. *Tox. Sci.* **2000**, *56*, 95–104.
(4) Ikonomou, M. G.; Rayne, S.; Addison, R. F. *Environ. Sci. Technol.* **2002**, *36*, 1886–1892.
(5) Ikonomou, M. G.; Rayne, S.; Fischer, M.; Fernandez, M. P.; Cretney, W. *Chemosphere* **2002**, *46*, 649–663.
(6) Hooper, K.; McDonald, T. A. *Environ. Health Perspect.* **2000**, *108*, 387–392.
(7) Rahman, F.; Langford, K. H.; Scrimshaw, M. D.; Lester, J. N. *Sci. Total Environ.* **2001**, *275*, 1–17.
(8) Alaee, M.; Sergeant, D. B.; Ikonomou, M. G.; Luross, J. M. *Chemosphere* **2001**, *44*, 1489–1495.
(9) Thomsen, C.; Lundanes, E.; Becher, G. *J. Sep. Sci.* **2001**, *24*, 282–290.
(10) Haglund, P. S.; Zook, D. R.; Buser, H. R.; Hu, J. W. *Environ. Sci. Technol.* **1997**, *31*, 3281–3287.
(11) Eljarrat, E.; Lacorte, S.; Barcelo, D. *J. Mass Spectrom.* **2002**, *37*, 76–84.

methods suffer from fragmentation of the molecular ions, creating difficulties in both identification and quantitation of congeners in full-scan and single-ion-monitoring (SIM) modes, respectively. For example, loss of Br atoms from PBDE congeners during EI ionization may lead to incorrect identification of the parent ion as a lower brominated congener. In addition, the relatively unpredictable fragmentation during EI or EC restricts the utility of applying relative response factors (RRFs) of one congener for which an analytical standard is available (e.g., BDE47) to other members of its homologue group (e.g., tetra-BDEs). This can result in either under- or over-estimating concentrations of congeners for which analytical standards are not available, which can be a serious problem for particularly toxic congeners, for which accurate knowledge of environmental concentrations is critical. Such issues have created difficulties in the environmental analysis of coeluting PCBs,[15−17] and hence, further efforts should be invested to minimize similar problems with PBDE analysis.

Among the promising analytical tools for PBDEs is metastable atom bombardment (MAB), which has been shown to offer a high degree of ionization and fragmentation selectivity for a variety of analytes,[18−20] including halogenated aromatics. Such selectivity results from the variation and quantization of the energy transferred upon ionization, allowing a range of 8−20 eV to be transferred, depending on the gas (He, Ne, Ar, Kr, Xe, or $N_2$) used to generate the metastable beam.[18,19] Within the MAB source, Penning ionization is the major ionization process taking place.[19] This involves the electrophilic reaction of a metastable species (A*) with the analyte (BC). The reaction leads to the cationic state of the analyte (BC$^{+\bullet}$), the ground state of the metastable species (A), and an ejected electron (e$^−$). When the ionization potential (IP) of BC is only slightly less than the excitation energy of A*, reaction 1 below predominates. Increasing the excitation energy of A* shifts the process in favor of reaction 2, where the transferred energy is sufficient to induce bond cleavage.

$$A^* + BC \rightarrow A + BC^{+\bullet} + e^- \tag{1}$$

$$A^* + BC \rightarrow A + B^+ + C + e^- \tag{2}$$
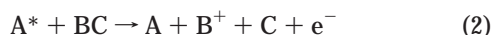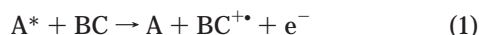
The selectivity of fragmentation offered by the MAB source stems from the nature of the energy transfer when a metastable atom collides with an organic molecule.

$$E_{int} = E^* - IP - E_k \tag{3}$$

Here, $E_{int}$ is the internal energy acquired by the ion formed, E* is the excitation energy of the metastable state that depends on the gas used, IP is the ionization potential of the organic molecule, and $E_k$ is the translational energy taken by the electron.[19] Nitrogen gas ($N_2$) is particularly intriguing, because its four usable metastable states (E*) range from 8.5 to 11.9 eV, an energy range where the ionization potentials (IPs) of most organic compounds are found.[20] As a result, MAB with $N_2$ gas as the metastable (MAB-$N_2$) should result in preferential formation of molecular ions, because the residual energy remaining once the analyte's IP is subtracted from the MAB-$N_2$ energy should be minimal, not sufficient to induce significant fragmentation.

Because analytical standards for most PBDE congeners are not available, models for estimating the GC retention time and MS detector response of unknown congeners are needed. We sought to develop such models on the basis of bromine substitution patterns and calculated physicochemical properties for mono- through hexa-BDEs that would help facilitate future screening of environmental samples and technical mixtures to determine if previously unknown PBDE congeners belonging to these homologue groups are present. The mono- through hexabrominated congeners examined in the present study typically make up >90% of total PBDEs in environmental samples, with the exception of BDE209, which may be present in high concentrations in sediments.[21,22] In addition, we have a distinct analytical method for hepta- through decabrominated PBDEs using a shorter GC column, less refined temperature program, and EI ionization[4,5] because these compounds have proven difficult to analyze in the same protocol as the lower brominated congeners. MAB-$N_2$ was used for the limited number of hepta- through decabrominated congeners for which analytical standards were available; however, these congeners provided a poor detector response and were not subjected to further investigation and method optimization. In addition, MAB-$N_2$ ionization was compared to that of EI to see if any general trends were apparent between molecular structure and instrument response and if MAB-$N_2$ offered a more accurate and selective tool for PBDE identification and quantitation.

## MATERIALS AND METHODS

**Gas Chromatography.** PBDE standards obtained from Cambridge Isotope Laboratories (CIL, Andover, MA) were analyzed by GC/HRMS using a VG-Autospec high-resolution mass spectrometer (Micromass, Manchester, U.K.) equipped with a Hewlett-Packard model 5890 series II gas chromatograph and a CTC A200S autosampler (CTC Analytics, Zurich, Switzerland). The GC was operated in the splitless injection mode, and the splitless injector purge valve was activated 2 min after sample injection. The volume injected was 1 $\mu$L of sample plus 0.5 $\mu$L of air. A 30-m DB-5 column (0.25-mm i.d. × 0.25-$\mu$m film thickness) was used with UHP-He at 90 kPa and the following temperature program: hold at 100 °C for 1 min; 2 °C/min to 150 °C; 4 °C/min to 220 °C; 8 °C/min to 330 °C; and hold 1.2 min. The splitless injector port, direct GC/HRMS interface, and the HRMS ion source were

(12) Covaci, A.; de Boer, J.; Ryan, J. J.; Voorspoels, S.; Schepens, P. *Anal. Chem.* **2002**, *74*, 790−798.

(13) Thomsen, C.; Haug, L. S.; Leknes, H.; Lundanes, E.; Becher, G.; Lindstrom, G. *Chemosphere* **2002**, *46*, 641−648.

(14) Vetter, W. *Anal. Chem.* **2001**, *73*, 4951−4957.

(15) Larsen, B.; Cont, M.; Montanarella, L.; Platzner, N. *J. Chromatogr., A* **1995**, *708*, 115−129.

(16) Schwartz, T. R.; Tillitt, D. E.; Feltz, K. P.; Peterman, P. H. *Chemosphere* **1993**, *26*, 1443−1460.

(17) Deboer, J.; Dao, Q. T. *J. High Resolut. Chromatogr.,* **1991**, *14*, 593−596.

(18) Faubert, D.; Mireault, P.; Bertrand, M. J. Analytical Potential of the MAB Source for Routine Analysis of Organic Compounds, Proceedings of the 43rd ASMS Conference on Mass Spectrometry and Allied Topics, Atlanta, GA, 1995; p 154.

(19) Faubert, D.; Paul, G. J. C.; Giroux, J.; Bertrand, M. J. *Int. J. Mass Spectrom. Ion Processes* **1993**, *124*, 69−77.

(20) Faubert, D.; Mousselmal, M.; Vuica, A.; Bertrand, M. J. Use of Nitrogen as a Gas for Metastable Atom Bombardment (MAB), Proceedings of the 45th ASMS Conference on Mass Spectrometry and Allied Topics, Palm Springs, CA, 1997; p 1207.

(21) Sellstrom, U.; Kierkegaard, A.; de Wit, C.; Jansson, B. *Environ. Toxicol. Chem.* **1998**, *17*, 1065−1072.

(22) Booij, K.; Zegers, B. N.; Boon, J. P. *Chemosphere* **2002**, *46*, 683−688.

maintained at 300, 275, and 315 °C, respectively, and the splitless injector purge valve was activated 2 min after sample injection. All sample injections were performed using the CTC A200S autosampler.

**Mass Spectrometry.** The HRMS was a sector instrument of EBE geometry coupled to the GC via a standard Micromass GC/MS interface. A standard VG Analytical EI ion source and a commercially available MAB ion source from DEPHY Technologies (Québec City, PQ, Canada) were used for all experiments. Details on the configuration of the MAB system have been discussed elsewhere.[19] For EI ionization in SIM mode, the accelerating voltage was 8000 V, the ionization energy (IE) was 35 eV, and the mass resolution was 10 000. MAB was tested using a number of different rare gases (He, Ne, Ar, Kr, Xe, and $N_2$). For MAB-$N_2$ ionization in SIM mode, the IE provided by the $N_2$ metastable was $\sim$6.17$-$11.88 eV, and the mass resolution was 1000. UHP-$N_2$ was used as the metastable source at a pressure of 160$-$170 mbar, the discharge current was 13$-$16 mA, the deflector voltage was maintained between 500 and 700 V, and the ion source temperature was 200 °C. For both MAB-$N_2$ and EI in SIM mode, the two most abundant isotopic peaks for each ion cluster were monitored (see Supporting Information for details on specific ions monitored, ion types, and isotope ratio control limits). The HRMS was tuned to 5000 resolving power for the full-scan experiments using EI MAB ionization. Other instrument settings remained constant in moving from SIM to full-scan mode. All mass spectral data were treated with a VG-OPUS data system.

**Molecular Modeling and Data Treatment.** The physicochemical properties of the PBDE congeners were calculated using CS Chem3D Ultra v.6.0 (CambridgeSoft, Cambridge, MA). Congener structures were first optimized using the Molecular Mechanics 2 (MM2) energy minimization program and minimized once again using the MOPAC2000 MNDO-PM3 program. The dipole moments and ionization potentials were then calculated using the MOPAC2000 MNDO-PM3 program.[23] Data were treated using Microsoft Excel 2002 (Redmond, WA), and multiple linear regression models were developed using KyPlot v.2.0 b. 9 (Tokyo, Japan).

## RESULTS AND DISCUSSION

**PBDE Chromatographic Properties.** Trace analytical methods using GC/HRMS with MAB and EI ionization were developed for the separation and quantitation of 35 individual PBDE congeners. The relative retention times (RRTs) of individual PBDE congeners were predicted using a multiple linear regression model. The linear predictive equation was based on bromine substitution patterns and computer-calculated physicochemical properties for 35 congeners and may help elucidate as yet unidentified congeners in environmental matrixes.

The multiple regression model for the RRTs of the 35 PBDE congeners from mono- through hexabrominated shown in Table 1 was based on the calculated dipole moments ($\mu$), the number of *ortho*-, *meta*-, and *para*-Br substituents, and the square of the total number of Br substituents (no. Br)$^2$. RRTs were calculated relative to the retention time of 2-BDE1, the first eluting PBDE. All other congeners from di- through hexabrominated were examined as potential bases for RRT calculation. In addition,

**Table 1. Identities and Retention Times for the 35 PBDE Congeners, from Mono- through Hexa-Brominated, under Consideration in This Study**

| congener | RT (min) |
|---|---|
| 2-BDE1 | 8.88 |
| 3-BDE2 | 9.28 |
| 4-BDE3 | 9.77 |
| 2,6-BDE10 | 16.27 |
| 2,4-BDE7 | 18.80 |
| 3,3′-BDE11/2,4′-BDE8 | 19.83 |
| 3,4-BDE12 | 20.47 |
| 3,4′-BDE13 | 20.72 |
| 4,4′-BDE15 | 21.48 |
| 2,4,6-BDE30 | 25.25 |
| 2,4′,6-BDE32 | 27.25 |
| 2,2′,4-BDE17 | 27.98 |
| 2,3′,4-BDE25 | 28.15 |
| 2′,3,4-BDE33/2,4,4′-BDE28 | 28.85 |
| 3,3′,4-BDE35 | 29.43 |
| 3,4,4′-BDE37 | 30.07 |
| 2,4,4′,6-BDE75 | 33.22 |
| 2,2′,4,5′-BDE49 | 33.75 |
| 2,3′,4′,6-BDE71 | 33.85 |
| 2,2′,4,4′-BDE47 | 34.55 |
| 2,3′,4,4′-BDE66 | 35.30 |
| 3,3′,4,4′-BDE77 | 36.42 |
| 2,2′,4,4′,6-BDE100 | 38.40 |
| 2,3′,4,4′,6-BDE119 | 38.78 |
| 2,2′,4,4′,5-BDE99 | 39.52 |
| 2,3,4,5,6-BDE116 | 39.70 |
| 2,2′,3,4,4′-BDE85 | 41.30 |
| 2,2′,4,4′,6,6′-BDE155 | 41.62 |
| 2,2′,4,4′,5,6′-BDE154 | 42.33 |
| 2,2′,4,4′,5,5′-BDE153 | 43.47 |
| 2,2′,3,4,4′,6′-BDE140 | 44.05 |
| 2,2′,3,4,4′,5-BDE138/2,3,4,4′,5,6-BDE166 | 44.72 |

combinations of low and higher brominated congeners were examined as potential RRT bases (e.g., BDE15/BDE99), as has been reported for the development of PCB retention time models[24] in which the RRT of a congener is calculated as relative to the average retention time of a low and a higher brominated congener. However, there was no increase in the power of the RRT model using either another individual congener or a combination of two congeners as a basis for calculating RRTs. As shown in Table 1, good separation was obtained among the individual congeners, with the exception of the three coeluting congener groups (BDE11/8, BDE33/28, BDE138/166). The minimum separation was 6 s between BDEs 49 and 71; the maximum separation was 6.5 min between BDEs 3 and 10, with an average separation of 1.2 min among the 35 congeners. The resulting RRT model generated by multiple linear regression (Figure 1) exhibited a strong correlation (predicted RRT = 0.9972(observed RRT) + 0.0094; $R^2$ = 0.997) between predicted and observed RRTs using the following equation:

$$RRT = -0.4102 + 1.211(\text{no. } ortho\text{-Br}) +$$
$$1.391(\text{no. } meta\text{-Br}) + 1.503(\text{no. } para\text{-Br}) -$$
$$0.08110(\text{no. Br})^2 + 0.1084(\mu)$$

Numerous other potential independent variables were considered in the development of this RRT model (e.g., polarizabilities,

(23) Stewart, J. J. P. *J. Comput.-Aided Mol. Des.* **1990**, *4*, 1$-$45.

(24) Frame, G. M. *Fresenius' J. Anal. Chem.* **1997**, *357*, 714$-$722.
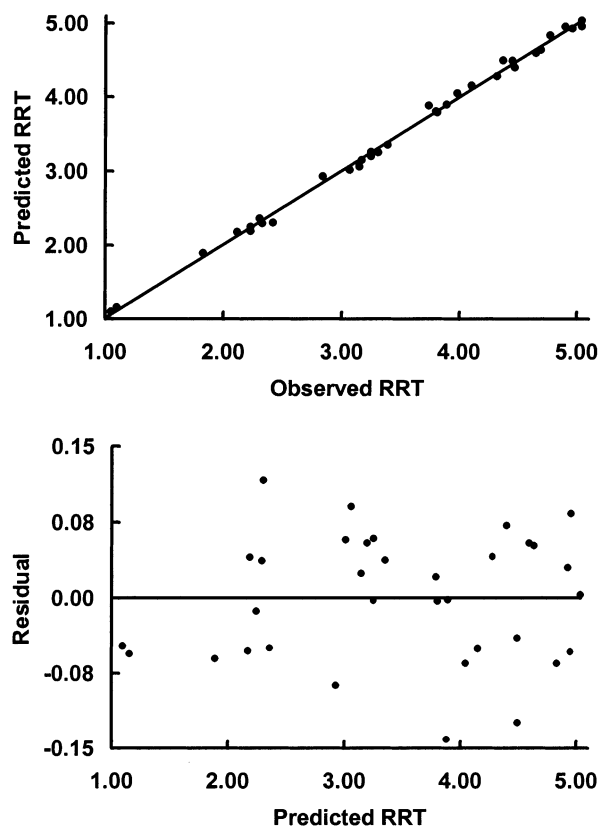
**Figure 1.** Plot of measured versus predicted RRTs for mono-through hexa-BDEs and a residual plot for the RRT model.

ionization potential, Connolly accessible surface area, Connolly solvent excluded volume, etc.). Correlation analysis was first performed using a matrix of all potential independent variables and the retention time data. Variables correlated with each other were excluded as potential candidates to minimize multicollinearity in the final model. In addition, variables that did not have a significant relationship to retention time were also excluded. This process resulted in the reduced set of optimum variables presented above.

The number and position of Br substituents were important predictors in this model, ranging from 99 to >99% significance (no. *meta*-Br, 99%; no. *ortho*-Br, >99%; no. *para*-Br, >99%; and (no. Br)$^2$, >99%). Dipole moment was a less important predictor at 95%. The residual plot shown in Figure 1 indicated that the errors were uniformly distributed across the range of predicted values. These results are those sought in multiple linear regression models. The slope of the regression output against the observed RRTs was close to unity ($m = 0.9972$), and the $y$ intercept was 0.085 min ($b = 5.1$ s), suggesting that predicted RRTs closely approximated observed values. The coefficient of variation (CV) for the model represented by eq 4 was 2.0%, and the $F$ value was 2051, further suggesting reasonable RRT predictions.

Three major forms of interaction forces determine how long a molecule remains dissolved in the stationary phase during a gas chromatographic analysis. These include dispersion (London), induction (Debye), and orientation (Keesom) forces.[25] From these, Ong and Hites showed that GC retention is adequately modeled as a linear function of molecular polarizability ($\alpha$), ionization

potential (IP), and the dipole moment ($\mu$).[26] Among the variables used in the present model, the number of bromine substituents squared is strongly correlated with the calculated molecular polarizability ($R^2 = 0.9616$). The number and location of Br substituents also strongly influences the IP and $\mu$; hence, our model incorporates these three major interaction forces. As a molecule travels down a GC column, it is continuously dissolving in the stationary phase and vaporizing into the mobile phase. Thus, the greater the attraction of a molecule to the stationary phase, the longer the retention time for that compound.

The predictive model presented above contains a positive relationship between each of the independent variables and the retention time, with the exception of the number of bromine substituents squared, (no. Br)$^2$. The reason for the negative correlation between (no. Br)$^2$ and retention time is unclear, and this weak negative correlation may be a statistical artifact required to reduce any inherent curvilinear behavior of the residuals, as noted elsewhere for PCB retention models.[27] Numbers of *ortho*-, *meta*-, and *para*-Br substituents help define the molecular shape and volume. These variables, although not necessarily overall predictors of retention time, assist (along with $\mu$) in organizing the retention behavior among congeners having the same number of Br substituents.

Similar linear relationships between GC-RRTs and observed and calculated physicochemical properties have been reported for polychlorinated biphenyls (PCBs),[26-28] polychlorinated diphenyl ethers (PCDEs),[29] polychlorinated dibenzo-*p*-dioxins[26,30] and dibenzofurans (PCDD/Fs),[26,31,32] and polybrominated dibenzo-*p*-dioxins (PBDDs).[33] Results using the MAB-N$_2$ and EI GC programs were identical; only the results for the MAB-N$_2$ program are shown in Figure 1. Overall, the utility of such RRT models based on calculated physicochemical properties resides in the identification of previously unobserved congeners in environmental samples. Because there are only a limited number of PBDE congener standards available, having a model to estimate RRTs greatly assists in elucidating whether such congeners are present in environmental samples. The RRT model presented above is not intended to predict the absolute retention time of an unknown congener without corroborating evidence. Rather, the model will help estimate the retention time of an unknown congener and will accurately predict the elution order of the congeners. Only 4 of the 35 congeners had predicted elutions times that were outside the observed order. This may indirectly allow the identification of unknown congeners (i.e., if three remaining tetra-BDE peaks require identification, the RRT model could be used to predict the elution order of the unidentified peaks and, hence, their identity).

(25) Vernon, F. *Dev. Chromatogr.* **1978**, *1*, 1−39.

(26) Ong, V. S.; Hites, R. A. *Anal. Chem.* **1991**, *63*, 2829−2834.
(27) Hasan, M. N.; Jurs, P. C. *Anal. Chem.* **1988**, *60*, 978−982.
(28) Robbat, A.; Xyrafas, G.; Marshall, D. *Anal. Chem.* **1988**, *60*, 982−985.
(29) Nevalainen, T.; Koistinen, J.; Nurmela, P. *Environ. Sci. Technol.* **1994**, *28*, 1341−1347.
(30) Liang, X.; Wang, W.; Wu, W.; Schramm, K. W.; Henkelmann, B.; Kettrup, A. *Chemosphere* **2000**, *41*, 923−929.
(31) Liang, X.; Wang, W.; Schramm, K. W.; Zhang, Q.; Oxynos, K.; Henkelmann, B.; Kettrup, A. *Chemosphere* **2000**, *41*, 1889−1895.
(32) Hale, M. D.; Hileman, F. D.; Mazer, T.; Shell, T. L.; Noble, R. W.; Brooks, J. J. *Anal. Chem.* **1985**, *57*, 640−648.
(33) Liang, X.; Wang, W.; Wu, W.; Schramm, K. W.; Henkelmann, B.; Oxynos, K.; Kettrup, A. *Chemosphere* **2000**, *41*, 917−921.

**PBDE Ionization Properties.** The ionization properties of 35 PBDE congeners were examined using EI and MAB sources. Six different sources of metastable rare gas atoms (He, Ne, Ar, Kr, Xe, and $N_2$) were investigated with MAB ionization. Results for Ne, Ar, Kr, and Xe were unsatisfactory and are not reported here. MAB-$N_2$ gave the best results in terms of ionization efficiency and minimum fragmentation, whereas the response from He was not of sufficient magnitude to warrant serious discussion. The use of He as a potential MAB source was not ruled out, however, and could be a viable ionization source provided sufficient time was spent in optimizing the method. Our preliminary data on the detector response of the PBDE congeners using MAB-He is available in the Supporting Information.

Using EI, full-scan experiments at 5000 resolution were first performed to determine the fragmentation patterns for the analytes of interest. For all PBDE congeners analyzed by EI, only $[M - nBr]^+$ ion clusters (where $n = 0-4$, depending on the number of Br substituents; $n = 0$ is the molecular ion cluster) were observed, with no other fragmentation (e.g., loss of HBr, aryl−ether bond cleavage) taking place. Penta- and hexa-BDE congeners did not show loss of more than four bromine substituents through fragmentation using EI. Hence, SIM experiments were performed by EI at 10 000 resolution to quantify the intensity of the two most abundant isotopic peaks of the $[M - nBr]^+$ clusters for each of the 35 congeners. Because no other fragmentation was observed by full-scan experiments, the SIMs quantitated only the two most abundant isotopic peaks of the $[M - nBr]^+$ clusters. Using MAB-$N_2$, full-scan experiments at 5000 resolution were first performed to determine which fragmentation patterns, if any, existed. No fragmentation other than $[M - nBr]^+$ ion clusters (where $n = 0-4$, depending on the number of Br substituents) was observed using MAB-$N_2$ for any of the PBDE congeners in full-scan mode. Penta- and hexa-BDE congeners did not show loss of more than four bromine substituents through fragmentation using MAB-$N_2$. When compared to EI, the fragmentation induced by MAB-$N_2$ ionization was minimal and always resulted in the molecular ion cluster forming the base peak (discussed in more detail below). Thus, because the MAB-$N_2$ ionization process is very selective, the mass spectrometer need not be operated in the high-resolution mode, and subsequent MAB-$N_2$ SIM experiments were performed at 1000 resolution for quantitation of the $[M - nBr]^+$ ion clusters. The response for the sum of the two most abundant isotopic peaks of each $[M - nBr]^+$ ion cluster observed using EI and MAB-$N_2$ are reported in the Supporting Information.

Under the optimized operating conditions reported above, EI produced a greater intensity (measured in counts-per-second per picomole (cps/pmol) analyte) than MAB-$N_2$ (Figure 2). Both EI and MAB-$N_2$ responses decay considerably in moving from low to high congener numbering (see Table 1 for relationship between congener number and bromine substitution pattern). Among the congeners, those with *ortho*-Br substituents produce a greater EI response than similar non-ortho congeners (see Supporting Information for details). This result is consistent with observations for PCB analysis via EI-MS.[34] Using MAB-$N_2$, this relationship between detector response and Br substitution patterns is weaker,
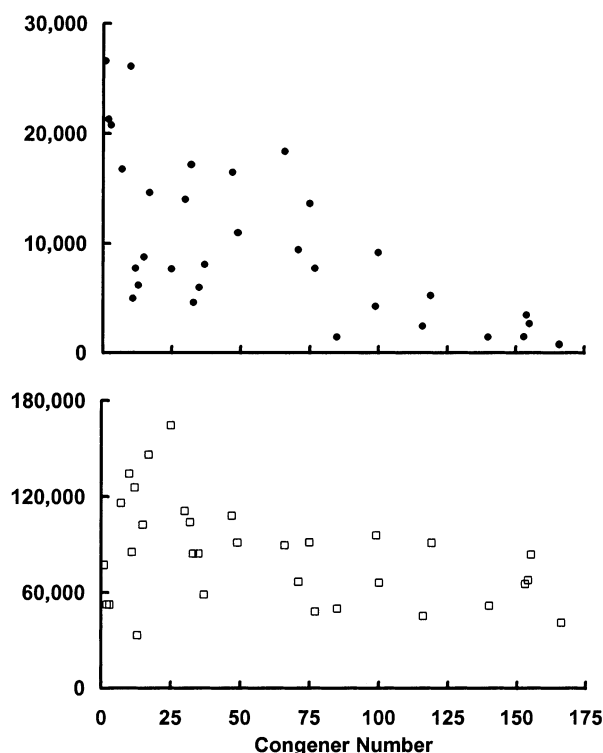


**Figure 2.** Plot of MS detector response for the sum of all ion clusters using MAB-$N_2$ (●) and EI ionization (□).

although still evident. However, the pattern of the response decay differs between the two ionization methods. With EI, the response decay is weakly linear ($y = -247.38x + 99019$; $R^2 = 0.168$), whereas that of MAB-$N_2$ is best approximated as an logarithmic decay function ($y = -3996 \ln(x) + 24089$; $R^2 = 0.530$), with a weaker fit observed when the MAB-$N_2$ response is modeled as a linear decay ($y = -91.89x + 15595$; $R^2 = 0.440$). The apparent exponential decay of the MAB-$N_2$ response with increasing congener number (and hence, increasing molecular weight) may result from the IE of the MAB-$N_2$ source being in the range of IPs for the PBDEs of interest. Thus, there is a stronger (but still weak) dependence on IP with MAB-$N_2$ response ($y = -237368 \ln(x) + 541067$; $R^2 = 0.274$) than with EI ($y = -15609x + 230162$; $R^2 = 0.006$). This weak logarithmic decay relationship between MAB-$N_2$ total ion response and IP suggests the existence of a "threshold" above which ionization is not so closely restricted by IP (i.e., where the slope of the logarithmic fit begins to increase more rapidly at IP $\approx 9.2$ eV). Mono-BDEs have sufficiently low IPs (9.07−9.20 eV) to allow more complete ionization (i.e., above the IE "threshold" provided by MAB-$N_2$), whereas the di- through hexa-BDEs have IPs (9.23−9.77 eV) high enough to restrict ionization (see below for further discussion on the IE available from MAB-$N_2$). This also points at the decreasing utility of MAB-$N_2$ for the analysis of higher brominated congeners, as we observed with hepta- through deca-BDEs, which provided a negligible response with no reproducibility. Conversely, the relatively constant response of EI with increasing IP shows that this ionization source likely remains superior for the quantitative analysis of higher brominated PBDEs. Additionally, the ratio of the maximum and minimum congener responses for MAB-$N_2$ is much higher than with EI (35 vs 5), further illustrating the stronger dependence on molecular structure with MAB-$N_2$ than

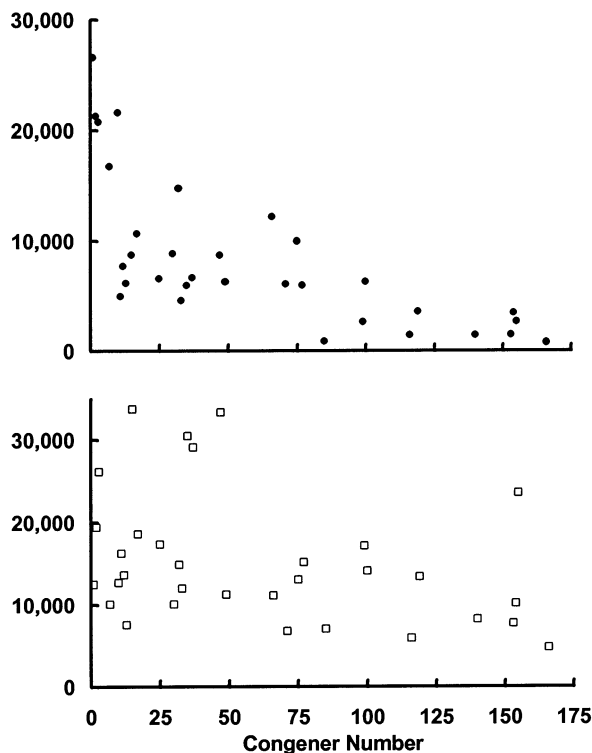(34) Lepine, F. L.; Milot, S. M.; Reimer, M. L. J.; Mamer, O. A. *Org. Mass Spectrom.* **1992**, *27*, 1311−1316.

**Figure 3.** Plot of the molecular ion cluster response using MAB-N$_2$ (●) and EI ionization (□).



**Figure 4.** Plot of the ratio of the molecular ion response to the intensity of the sum of all observed ions (as a percent) using MAB-N$_2$ (●) and EI ionization (□).



**Figure 5.** Plot of the base peak ion response using EI ionization.

with EI and the unsuitability of MAB-N$_2$ for the quantitative trace analysis of higher brominated PBDEs given their poor response.

When the intensity of the molecular ion cluster (sum of the two most abundant isotopic peaks) is considered, the sensitivity of the EI technique is greatly reduced by a factor of ~2−11 (Figure 3, with EI response poorly approximated as $y = -53.485x + 18512$; $R^2 = 0.1255$). There is no apparent pattern in this sensitivity reduction related to molecular structure. Conversely, the MAB-N$_2$ molecular ion cluster response retains the same logarithmic shape ($y = -4256.3 \ln(x) + 23336$; $R^2 = 0.715$) as in Figure 2, because the molecular ion cluster is the dominant peak for all PBDE congeners analyzed using MAB-N$_2$ (see below). EI induces significant fragmentation, and this results in a large loss of sensitivity with regard to the molecular ion cluster. Under these optimized conditions for both ionization sources, MAB-N$_2$ and EI produce approximately the same molecular ion cluster intensity for mono- through di-BDEs (ratio EI:MAB-N$_2$ = 0.5−3.9), although EI offers significantly greater sensitivity for tri- through hexa-BDEs (ratio EI:MAB-N$_2$ = 0.9−8.9). The significant fragmentation induced by EI makes identification of new PBDE congeners difficult, because homologue groups appear to coelute, as suggested by our analyses of as yet unidentified PBDEs in environmental samples. Thus, a higher brominated congener may mistakenly be identified as a lower brominated congener because of EI fragmentation. The absence of significant fragmentation with MAB-N$_2$ makes this ionization source superior for the identification of novel congeners, since the molecular ion cluster is always dominant (Figure 4). For all PBDE congeners, the molecular ion cluster dominates with from 53 to 100% of the detector response residing in this cluster. In contrast, the molecular ion cluster makes up only 9−50% of the detector response with EI. There is
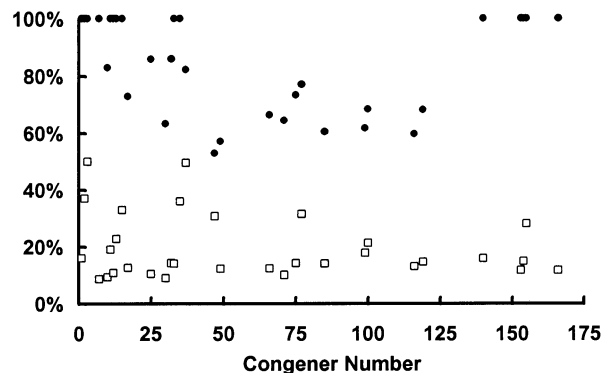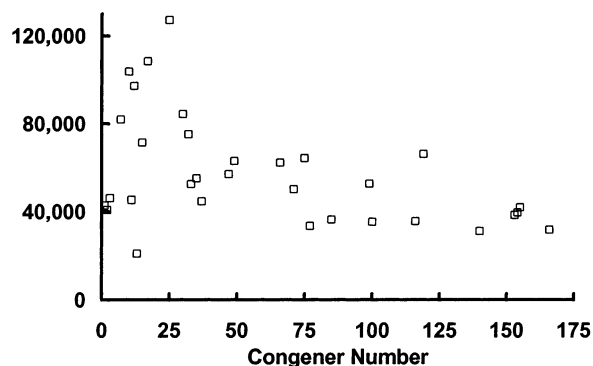
no observable pattern in the ratio of the molecular ion cluster response related to molecular structure for either MAB-N$_2$ or EI.

An alternative to quantitation by the molecular ion cluster is to use the base peak resulting from a fragmentation ion cluster. For all of the PBDE congeners using MAB-N$_2$, the molecular ion cluster is the base peak (Figure 4); however, using EI, only BDEs 3, 8/11, 13, 15, 35, and 77 had the base peak as the molecular ion cluster. In Figure 5, a weak linear decay is observed between the base peak ion cluster response and congener number for EI ($y = -223.3x + 71103$; $R^2 = 0.2174$). Because the base peak is the molecular ion cluster for all PBDE congeners, the analogous plot of Figure 5 for MAB-N$_2$ is in Figure 3 and is not presented here again. Figure 5 shows that using the base peak ion cluster for monitoring recovers some of the sensitivity lost by EI-induced fragmentation of the molecular ion. Ratios of base peak ion cluster response between EI and MAB-N$_2$ range from 1.6 for mono-BDEs to 42 for hexa-BDEs. Thus, EI and MAB-N$_2$ have approximately equal sensitivity for lower brominated homologue groups, but EI is significantly more sensitive for the quantitation of higher brominated congeners. It is important to note that the lower available energy with MAB-N$_2$ ionization reduces the effects of column bleed, impurities in the sample matrix, and instrumental noise (see Figure 7 for an example of the "cleaner" spectra resulting from MAB-N$_2$). Thus, a greater signal-to-noise (S/N) ratio is observed with MAB-N$_2$ as evidenced by its lower detection limit (S/N = 3) for tetra- and penta-brominated congeners (40 fg/$\mu$L and 20 fg/$\mu$L for EI and MAB-N$_2$ at 10 000 and 1000 resolution, respectively), using the two most abundant isotopic peaks of the molecular ion cluster for MAB-N$_2$ and the [M − 2Br]$^+$ ion cluster for EI.
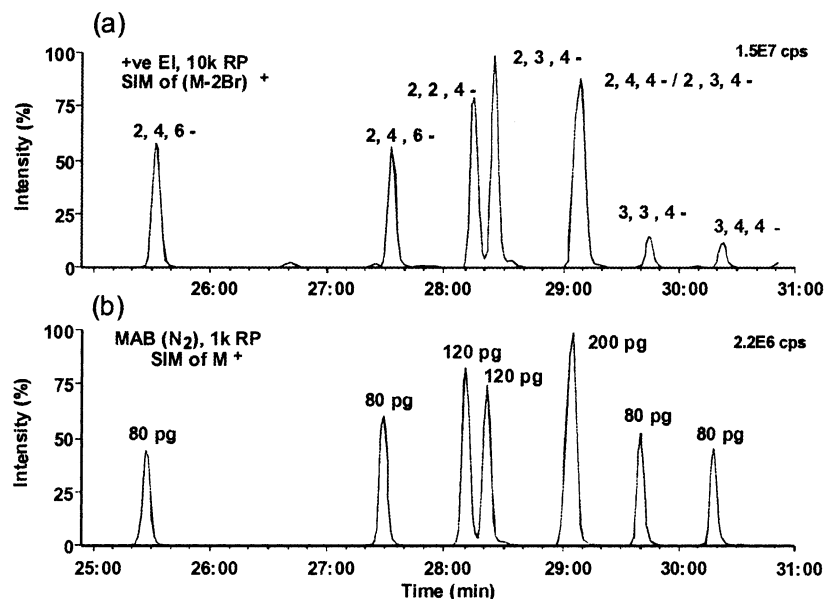
**Figure 6.** Sample GC traces for the analysis of tri-BDEs using EI and MAB-$N_2$. Intensity of the base peak ion cluster is provided at the right of each chromatogram.
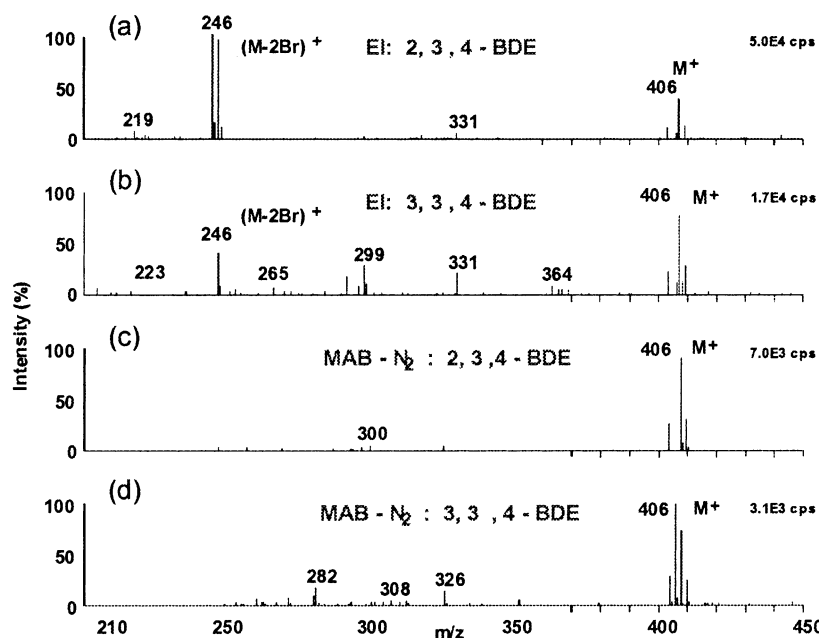


**Figure 7.** Mass spectra for 2,3′,4-BDE25 and 3,3′4-BDE35 using EI and MAB-$N_2$. Intensity of the base peak ion cluster is provided at the right of each spectrum.

A sample GC trace for tri-BDEs is shown in Figure 6 and illustrates that MAB-$N_2$ provides a higher level of consistency in base peak ion cluster response for homologue equivalent congeners. For the seven tri-BDEs examined here, MAB-$N_2$ provided a mean response of 8282 cps/pmol with a RSD = 42.0%, versus a mean and RSD of 70139 cps and 58.5% for EI. Thus, MAB-$N_2$ has an ~50% increase in RRF consistency within the tri-BDE homologue group. This is important in estimating the quantities of congeners for which analytical standards are not available, because wide deviations in congener response within a homologue group are more likely to lead to errors when estimating the RRF of new congeners. An example of the reduced fragmentation of MAB-$N_2$ is presented in Figure 7, which shows the mass spectra in full-scan mode of BDEs 25 and 35 using EI and MAB-$N_2$ at 10 000

and 5000 resolution, respectively. MAB-$N_2$ not only results in little or no fragmentation of the molecular ion cluster, but also produced a "cleaner" spectrum with fewer artifacts resulting from ionization of column bleed components and impurities in the sample matrix. Thus, MAB-$N_2$ is superior to EI for the qualitative identification of PBDE congeners.

Using the MAB-$N_2$ and EI results for the various PBDE congeners, we sought to develop a model to help explain the molecular ion cluster response (in cps/pg) based on known and calculated physicochemical properties. Using the 35 congeners identified above, the following RRF models were constructed by multiple linear regression (Figure 8): MAB-$N_2$ response = 49.51 + 115.1(1/no. Br) − 8.051(1/IP) + 5.509(no. *ortho*-Br) and EI response = −322.7 + 66.14(1/no. Br) + 36.90(1/IP) −
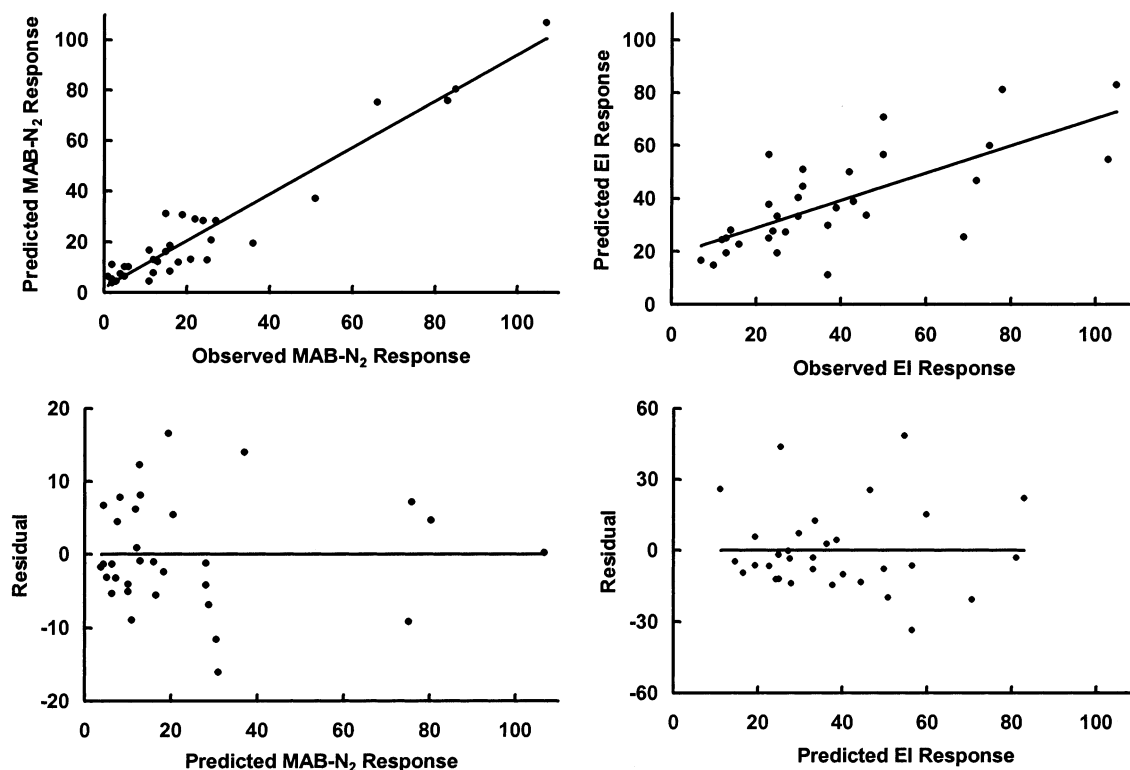
**Figure 8.** Plot of measured versus predicted molecular ion responses using MAB-$N_2$ ($y = 0.9213x + 1.8713$; $R^2 = 0.9213$) and EI ionization ($y = 0.5181x + 18.418$; $R^2 = 0.5181$) for mono- through hexa-BDEs. Residuals plots for the MAB-$N_2$ and EI models are also shown.

6.382 (no. *ortho*-Br). For both models, the IP was calculated using the MNDO-PM3 method, because the Austin method 1 (AM1) method provided poor correlation with both MAB-$N_2$ and EI responses. A significantly higher degree of predictability was noted for the MAB-$N_2$ response (predicted MAB-$N_2$ response = 0.9213- (observed MAB-$N_2$ response) + 1.8713; $R^2 = 0.9213$) versus those from EI ionization (predicted EI response = 0.5181(observed EI response) + 18.418; $R^2 = 0.5181$). All three of the variables had a higher level of significance using MAB-$N_2$ (1/no. Br, >99%; 1/IP, >99%; and no. *ortho*-Br, >98%) than with EI (1/no. Br, >94%; 1/IP, >60%; and no. *ortho*-Br, >97%). Residual plots are also presented in Figure 8 and show an even distribution of error over the range of predicted responses for both the MAB-$N_2$ and EI models. Multicollinearity was not observed among the three independent variables.

The decrease in predictability for EI may be the result of the excess energy available under EI conditions (35 eV), as compared to that required for ionization of the various congeners used in the model (8.88−9.64 eV). In contrast, the potential available MAB-$N_2$ energy (6.17−11.88 eV)[20] is in the range required for ionization, and hence, the model is more sensitive to the IP. Among the various metastable states of $N_2$, only the $a^1\Pi_g$ (8.67 eV) through $A^3\Sigma_u^+$ (6.17 eV) states have sufficiently long lifetimes[35] to effect consistent ionization. Since these states have less energy than the IPs of the PBDE congeners, it is likely that ionization took place through associative complexes, which has been reported to be possible when the IP is greater than that provided by Penning

ionization.[36−38] Alternatively, since the effective MAB-$N_2$ energy likely resides in the range from 6.17 to 8.76 eV,[20] below the IP of all PBDE congeners, we see a strong negative dependence on IP (i.e., MAB-$N_2$ response positively correlated with 1/IP) resulting from the minor contributions of the more energetic $N_2$ metastable states. For EI, there is a positive correlation between the response and IP, which is difficult to explain, because the detector response should decrease with increasing IP (as we see for MAB-$N_2$). Hence, the excess energy available from EI may be overwhelming the model's sensitivity to individual variables, such as the IP, as evidenced by the lower significance of 1/IP in moving from EI (60%) to MAB-$N_2$ (>99%).

A model representing the molecular ion cluster response of a PBDE congener as a multiple regression equation comprising the inverse of the number of Br substituents (1/no. Br), the inverse of the ionization potential (1/IP), and the number of *ortho*-Br substituents is consistent with the phenomena that take place within a mass spectrometer. The individual congeners must be ionized to be detected. Such a process is dependent on the IP of a molecule and thereby also consistent with our MAB-$N_2$ response results, which show that as the IP of congeners increase, the detector response decreases. The reverse trend observed between IP and the EI response is discussed in more detail below. As well, the intensity of the molecular ion cluster is dependent on the stability of the resulting parent ion. Previous work with PCBs has shown that the stability of the molecular ion cluster decreases with an increase in the number of chlorine substituents,[39,40] and it is likely that this relationship extends to PBDEs as well; hence,

(35) Faubert, D.; Mousselmal, M.; Vuica, A.; Bertrand, M. J. *Use of Nitrogen as a Gas for Metastable Atom Bombardment.* Proceedings of the 45th ASMS Conference on Mass Spectrometry and Allied Topics, Palm Springs, CA, June 1−5, 1997; p 1207.

(36) Hotop, H.; Niehaus, A.; Schmeltekopf, A. L. *Z. Phys.* **1969**, *229*, 1.

(37) Herman, Z.; Cermak, V. *Collect. Czech. Chem. Commun.* **1966**, *31*, 649.

(38) Herman, Z.; Cermak, V. *Nature* **1963**, *199*, 588.

the positive correlation we observe between molecular ion cluster response and $1/$no. Br for both MAB-$N_2$ and EI, because an increase in the number of Br substituents favors fragmentation and a resulting decrease in the response of the molecular ion cluster.

Finally, the number of *ortho*-Br is also an important determinant in the detector response. *Ortho*-Br substituents on the diphenyl ether system are likely more readily ionized than *meta*- and *para*-Br. For example, the bromine atom Mulliken population (calculated via MNDO-PM3) in 2-BDE1 (*ortho*-Br) is 6.9625 e$^-$, versus 6.9615 e$^-$ in 3-BDE2 (*meta*-Br) and 6.9550 e$^-$ in 4-BDE3 (*para*-Br). Thus, *ortho*-Br substituents tend to have higher electron populations (and hence, lower $\delta$-positive charges) than *meta*- and *para*-Br and are consequently more likely to eject an electron than other Br substituents to form the molecular ion cluster. Consequently, we see a positive correlation between the number of *ortho*-Br substituents and the MAB-$N_2$ response. As well, the C–Br bond length in 2-BDE1 (*ortho*-Br) is 1.8958 Å versus 1.8898 Å in 3-BDE2 (*meta*-Br) and 1.8888 Å in 4-BDE3 (*para*-Br). Since bond length is inversely correlated to bond strength in this case, it is evident that C–Br bond strength in PBDEs follows the general order para > meta > ortho. These calculations provide a rational basis for why ortho-substituted PBDEs tend to fragment more readily upon ionization than do their non-ortho analogues (see below). In this case, because the response model is examining the molecular ion cluster response, the effects of electron ejection from an *ortho*-Br appear to outweigh the potential fragmentation resulting from loss of an *ortho*-Br using MAB-$N_2$ ionization. If loss of an *ortho*-Br dominated electron ejection from an *ortho*-Br, we would expect a corresponding decrease in molecular ion cluster response with increasing numbers of *ortho*-Br, and hence, a negative correlation in the response model, as is observed using EI ionization. The excess energy provided by EI allows *ortho*-Br fragmentation to occur and to dominate electron ejection from an *ortho*-Br. With MAB-$N_2$, there is insufficient energy remaining after electron ejection for *ortho*-Br fragmentation to occur.

As discussed previously, although MAB-$N_2$ provided adequate sensitivity for the lower brominated congeners (mono- to hexa-BDE), there was a significant decrease in response for unidentified higher brominated congeners (data not shown). This decrease in sensitivity does not arise from an increase in the IP of congeners with seven Br substituents, because these congeners are within the range of IPs for lower brominated congeners (9.04–9.77 eV). In addition, the higher brominated congeners generally have greater numbers of *ortho*-Br compared to lower brominated congeners, which should increase their response. Thus, on the basis of our model, the response decrease is most likely a result of the increased number of Br substitutents, leading to substantial instability of any ions and fragments formed upon initial ionization.

Furthermore, because the IPs of the PBDE congeners are near the MAB-$N_2$ energy, only ionization can take place, after which there is little energy remaining for fragmentation. Thus, for all PBDE congeners analyzed using MAB-$N_2$, the molecular ion cluster was dominant, with little fragmentation taking place. Conversely, using EI ionization, the $[M - 2Br]^+$ ion cluster was dominant for all PBDE congeners with $\geq 2$ bromines, with the exceptions of 8/11, 13, 15, 35, and 77 (see above and Supporting Information for details). None of these congeners (with the exception of BDE8, which coelutes with the non-ortho BDE11) have an *ortho*-Br, and thus, we see the strong requirement for an *ortho*-Br to be present for PBDE fragmentation to take place by EI. Where 2 *ortho*-Br substituents are present, the production of the $[M - 2Br]^+$ ion cluster likely results from loss of these two *ortho*-Br. Where only 1 *ortho*-Br is present, loss of 2Br likely takes place sequentially with the initial loss of the *ortho*-Br substituent to form a Br$^\bullet$ radical, which subsequently abstracts a second Br atom from elsewhere on one of the aromatic rings. Although fragmentation was much reduced using MAB-$N_2$, similar patterns of fragmentation were observed, consistent with the rationale presented above.

Our results suggest the second Br abstraction using EI, and to a limited extent using MAB-$N_2$, takes place preferentially when other Br atoms are present on the same aromatic ring as the initial lost *ortho*-Br (e.g., BDE 30 vs BDE32); that is, closer Br substituents are preferentially abstracted. As well, *para*-Br appear to be more difficult to abstract than *meta*-Br, whether they are on the same or opposite ring as the initial lost *ortho*-Br. Such results could be attributed to proximity factors (*para*-Br are always more distant than *meta*-Br), bond strength factors (*para*-Br bonds are stronger than those of *meta*-Br), or both. Molecular modeling using MOPAC on several congeners suggests a general bond strength order of para > meta > ortho for PBDEs (see above), providing a rationale for why *para*-Br are more difficult to abstract than *meta*-Br. For some of the congeners with four Br substituents, loss of $[M - 4Br]^+$ was observed, albeit at very low intensities relative to the $M^+$ and $[M - 2Br]^+$ ion clusters. Thus, it appears energetically unfavorable for PBDEs to lose more than two Br substituents during EI or MAB-$N_2$ ionization, despite significant excess available energy from EI.

In general, PBDEs with *ortho*-Br substituents are more susceptible to fragmentation via EI ionization than are non-ortho PBDEs (e.g. 2,4-BDE7 vs 3,4-BDE12 and 2,3',4-BDE25 vs 3,3',4-BDE35; Figure 7). As well, increasing the number of *ortho*-Br decreases the stability of the molecular ion cluster (e.g., 2,6-BDE10 vs 2,4-BDE7 and 2,4,6-BDE30 vs 2,4,4'-BDE28). Congeners with equal numbers of *ortho*-Br are also more unstable if the *ortho*-Br are on the same aromatic ring (e.g. 2,4,4',6-BDE75 vs 2,2',4,4'-BDE47), likely as a result of their close proximity, as compared to two or more *ortho*-Br atoms on different aromatic rings separated by the ether linkage. This larger variance in EI response among homologue congeners is a problem in trace analyses, because average RRFs, resulting from the analyses of a limited number of authentic standards, cannot be used to calculate the concentrations of the remaining congeners in the homologue series. Furthermore, higher brominated congeners within coeluting homologue series cannot be reliably identified, since the ion monitored as the molecular ion cluster of one series can also be the $[M - nBr]^+$ ion cluster from a higher homologue group.

In conclusion, we have examined the qualitative and quantitative chromatographic and mass spectrometric properties of PBDEs using EI and MAB-$N_2$ ionization. Quantitative models describing the chromatographic and mass spectroscopic properties of PBDEs have also been developed. Between the two MS ionization sources

(39) Plomley, J. B.; Lausevic, M.; March, R. E. *Mass Spectrom. Rev.* **2000**, *19*, 305–365.
(40) Lausevic, M.; Splendore, M.; March, R. E. *J. Mass Spectrom.* **1996**, *31*, 1244–1252.

examined, MAB-$N_2$ ionization has been shown to induce significantly less fragmentation of the molecular ion than with EI. MAB-$N_2$ provides the specificity required to identify the degree of bromination of unknown mono- through hexa-BDEs where fragmentation varies substantially within the same homologue series and where there may be elution time overlap between two or more homologue groups. Hence, coeluting higher substituted congeners will not interfere in the mass channels of lower substituted congeners with MAB-$N_2$, especially when combined with information from the GC retention time model. However, MAB-$N_2$ provides insufficient detector response for the analysis of hepta- through deca-BDEs, and EI is still the method of choice for these homologue groups, despite any problems resulting from fragmentation Furthermore, the quantities of unknown PBDE congeners can be better estimated using average RRFs obtained from the MAB-$N_2$ analyses of available authentic standards of the same series in combination with predictions made by the MAB-$N_2$ molecular ion response model.

## SUPPORTING INFORMATION AVAILABLE

Details on specific ions monitored, ion types, and isotope ratio control limits is available as Supporting Information. This material is available free of charge via the Internet at http://pubs.acs.org.