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Sunlight Irradiation of Highly Brominated Polyphenyl Ethers Generates Polybenzofuran Products That Alter Dioxin-responsive mRNA Expression in Chicken Hepatocytes

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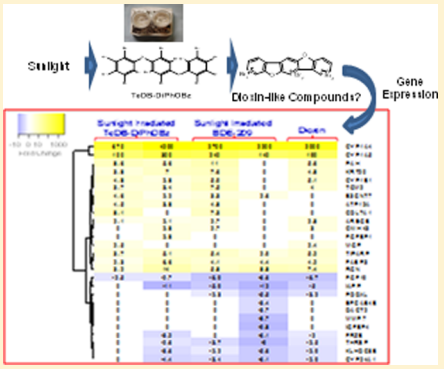
Supporting Information

ABSTRACT: We report on two highly brominated polyphenyl ether flame retardants, tetradecabromo-1,4- diphenoxybenzene (TeDB-DiPhOBz) and 2,2′,3,3′,4,4′,5,5′,6,6′-decabromodiphenyl ether (BDE-209), that formed photolytic degradation products in tetrahydrofuran (THF)/hexane solvent after 21 days of natural sunlight irradiation (SI). These degradation products of SI-TeDB-DiPhOBz and SI-BDE-209 included the numerous polybrominated homologue groups of polybenzofurans and dibenzofurans, respectively. Formation of similar polybenzofuran and dibenzofuran products was also observed following a 3 month exposure of the solid powder forms of TeDB-DiPhOBz and BDE-209 to natural SI. These resulting degradation product mixtures were administered to chicken embryonic hepatocytes (CEH) to determine effects on mRNA expression levels of 27 dioxin-responsive genes. For the solvent-based SI study, equivalent concentrations of 1 or 25 μM of SI-TeDB-DiPhOBz or 1 or 10 μM of SI-BDE-209 resulted in gene expression profiles that were similar to those of the most potent dioxin-like compound, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. In addition, a concentration-dependent induction of *CYP1A4* and *CYP1A5* mRNA was observed following exposure to SI-TeDB-DiPhOBz and SI-BDE-209. Based on $EC_{threshold}$ values for *CYP1A4/5* mRNA expression, relative potency (ReP) values were 1×10^{-6} and 1×10^{-5} for SI-TeDB-DiPhOBz and SI-BDE-209, respectively. The SI TeDB-DiPhOBz and BDE-209 powder degradation product mixture also significantly induced *CYP1A4* mRNA levels in CEH. Our findings clearly show that the environmental stability of TeDB-DiPhOBz and BDE-209, and possibly other highly brominated polyphenyl ethers, is of great concern from a dioxin-like degradation products and toxicity perspective.



INTRODUCTION

Tetradecabromo-1,4-diphenoxybenzene (TeDB-DiPhOBz, also known as 4′-PeBPO-BDE208 and SAYTEX 120, CAS No: 58965−66−5) and 2,2′,3,3′,4,4′,5,5′,6,6′-decaBDE (BDE-209, CAS No: 1163−19−5) are additive flame retardants (FRs). TeDB-DiPhOBz is generally used in solid plastic and wire/cable products, and as an alternative FR to BDE-209, which is used in a variety of polymeric applications.[1] Information on the volume produced or environmental distribution of TeDB-DiPhOBz is not currently available. Albemarle corporation stated that manufacture of SAYTEX-120 was discontinued as of January 2011,[2] but neither mandatory nor voluntary regulations for the production of TeDB-DiPhOBz exist. In fact, a number of suppliers around the world (particularly in Asia) are currently marketing TeDB-DiPhOBz or TeDB-DiPhOBz-containing products,[3] and it is present in several commercial technical formulations.[4−6] In addition, Chen et al. recently reported the detection of several methyoxylated polybrominated diphenoxybenzenes (MeO-PB-DiPhOBzs) in herring gull (*Larus argentatus*) eggs from the Laurentian Great Lakes of North America, and, given their extremely similar structures, it

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is hypothesized that these contaminants were degradation products and sourced from TeDB-DiPhOBz.[7,8]

BDE-209 is the major congener of currently produced deca-BDE products (generally >90%)[9] that are commercially available in some districts around the world. Due to the large volume of BDE-209 produced and used,[10] it has been frequently detected in various environmental matrices. For example, between 1982 and 2013 increases in concentrations of BDE-209 have been observed in the eggs of herring gulls from the Laurentian Great Lakes.[11−13] Although deca-BDE was voluntarily phased out by U.S. manufacturers in 2013, its environmental effects might persist[14] given the continued use of products containing BDE-209,[15] persistence in various environmental matrices[12] and generally unrestricted use in Asian districts.[16]

The structure of TeDB-DiPhOBz contains three fully brominated aromatic rings connected by two oxygen atoms, and BDE-209 consists of two fully brominated aromatic rings connected by one oxygen atom. Their similar chemical structures mean that they exhibit some common properties, i.e. low volatility, high log K_{ow} and low bioavailability, and thus they are predicted to not be bioaccumulative in the environment.[2,17] Hardy et al. stated that TeDB-DiPhOBz is stable and unlikely to undergo environmental biodegradation.[2] However, when these two FRs were dissolved in different organic solvents, they are photolytically unstable and rapidly degraded via stepwise, reductive debromination.[18−21] A recent and extremely important finding that we made was that the degradation of TeDB-DiPhOBz and BDE-209 in solution by natural sunlight generated degradation products that altered mRNA expression, especially one gene for the aryl hydrocarbon receptor (AhR)-mediated *CYP1A4* (fold changes >1000), in a chicken embryonic hepatocyte (CEH) assay.[22] However, there are still knowledge gaps as to the identity of components of these complex mixtures of photodegradation products and especially from TeDB-DiPhOBz. Also unknown is whether the products in the complex mixtures that constitute the SI-TeDB-DiPhOBz and SI-BDE-209 can alter other dioxin-responsive genes in addition to CYP1A4.

The present study examined (1) profiles of expression of mRNA of 27 genes that are responsive to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in CEH following administration of sunlight irradiated (SI) degradation product mixtures of SI-TeDB-DiPhOBz or SI-BDE-209; (2) an evaluation of whether SI-TeDB-DiPhOBz and SI-BDE-209 mixtures induce *CYP1A4/5* mRNA in a concentration-dependent manner; (3) the identity of degradation products of photolysis that induce the observed AhR activation; and (4) an investigation of whether alteration in expressions of mRNA occur following exposure to pure solid (powder) forms of SI-TeDB-DiPhOBz and SI-BDE-209 as well as in organic solution.

EXPERIMENTAL SECTION

Chemicals and Reagents. To the best of our knowledge, pure standards for TeDB-DiPhOBz or its possible products of photolysis are not yet commercially available. Technical SAYTEX-120 (TeDB-DiPhOBz; Lot# 0GN01-$I0) and BDE-209, in solid powder form, were kindly supplied by Wellington Laboratories (Guelph, ON, Canada). Purity of BDE-209 was reported to be greater than 98% by its supplier. Organic solvents used in this research were provided by Caledon Laboratories Ltd. (Georgetown, ON, Canada) with the exceptions of dimethyl sulfoxide (DMSO) and tetrahydrofuran (THF), which were purchased from Sigma-Aldrich (St. Louis, MO).

Sample Preparation. Detailed information on solar irradiation of solvent-dissolved TeDB-DiPhOBz and BDE-209 has been published previously,[22] and is provided in Text S1 of the Supporting Information. For comparison, TeDB-DiPhOBz or BDE-209 solid powder was also exposed to natural sunlight (Figure S1). In brief, solid powder of the two chemicals was transferred into quartz weighing bottles and the exact weights were 0.01458 g (TeDB-DiPhOBz) and 0.00896 g (BDE-209). Samples of powder were distributed as thinly as possible across the bottom of quartz bottles. Quartz caps were placed on top of bottles, sealed with plastic paraffin film, and placed on a platform on the roof of a house located in the City of Ottawa, Canada. The powders were exposed continuously to solar irradiation over a three month period during the summer; July 1 to October 8, 2014. Following the exposure period, the irradiated powder (SI_P) was dissolved in DMSO (SI_P-TeDB-DiPhOBz and SI_P-BDE-209) for subsequent administration to CEH or for instrumental analysis.

Regarding the concentration units for SI-TeDB-DiPhOBz and SI-BDE-209 in the present study, "μM" does not represent the actual concentrations of products of photolysis. They are the "complex" (equivalent) concentration of products relative to the initial concentration of their precursor nonirradiated (NI) compounds, NI-TeDB-DiPhOBz or NI-BDE-209. For in vitro experiments, DMSO stock solutions of 5000 μM and 10 000 μM were prepared for SI-TeDB-DiPhOBz and SI-BDE-209, respectively, 380 and 1800 μM for NI-TeDB-DiPhOBz and NI-BDE-209, respectively, and 1000 and 10000 μM for SI_P-TeDB-DiPhOBz and SI_P-BDE-209, respectively. Stock and serial dilutions in DMSO were administered to CEH such that the DMSO concentration was 0.5% in the aqueous medium.

Chicken Embryonic Hepatocyte Assay. Methods for culture of CEH have been described previously,[23,24] and are also provided in Text S2 of the Supporting Information. The final nominal concentration ranges for the SI-brominated flame retardants (BFRs) in the medium were 0.001−25 μM (SI-TeDB-DiPhOBz), 0.001−50 μM (SI-BDE-209), 0.015−5 μM (SI_P-TeDB-DiPhOBz), 0.15−50 μM (SI_P-BDE-209) (n = 3 replicate wells/treatment). Exposure of CEH to solutions of parent TeDB-DiPhOBz or BDE-209 were not included in our current study because essentially no gene alterations were observed following exposure to NI-TeDB-DiPhOBz or NI-BDE-209 in our previous study.[22]

RNA Isolation and Complementary DNA (cDNA) Synthesis. Total RNA was isolated from CEH in each well using RNeasy 96 kits (Qiagen, Mississaguga, Ontario, Canada) according to the manufacturer's protocol with a slight modification described elsewhere.[25] The quality and concentration of extracted RNA was determined using a NanoDrop 2000 (Thermo Scientific, Wilmington, DE) and 200 ng of total RNA was reverse-transcribed following the protocols of the QuantiTect Reverse Transcription kit (Qiagen, Valencia, CA). cDNA was placed on ice until PCR array or real-time reverse transcription polymerase chain reaction (RT-PCR) processing.

Aryl Hydrocarbon Receptor-Custom PCR Array. Samples of products of photolysis, SI-TeDB-DiPhOBz and SI-BDE-209, were assayed at two concentrations, the greatest noncytotoxic dose (25 μM of SI-TeDB-DiPhOBz and 10 μM of SI-BDE-209; cell survival rate >85%)[22] and 1 μM. The custom chicken RT^2 Profiler PCR Array was built by SABiosciences (Qiagen, Valencia, CA) according to our specifications. Details

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of the custom design and TCDD-responsive gene selections are described elsewhere.[26] Each 96-well array contained three identical sets of 27 target genes and 5 control genes (Table S1), allowing three technical replicates to be screened per plate. The 27 target genes were identified as potential biomarkers of TCDD exposure based on a previous study in which CEH were exposed to 0.03 nM and 1.0 nM TCDD.[26] The five control genes included two internal control genes, a positive PCR control, a reverse transcription control, and a well to test for genomic DNA contamination. The cDNA was added directly to the RT^2 SYBR Green Mastermix (Qiagen, Valencia, CA) and 25 μL of this mixture was added to each well containing a set of primers at preoptimized concentrations. All arrays were run using the Stratagene MX3005P PCR system (Agilent Technologies, Santa Clara, CA) with the following thermal profile: 95 °C for 10 min followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min and ending with a dissociation curve segment of 95 °C for 1 min, 55 °C for 30 s, and 95 °C for 30 s.

Real-Time Reverse Transcription Polymerase Chain Reaction (Real-Time RT-PCR). A previously described multiplex real-time RT-PCR[27] was employed to simultaneously measure *CYP1A4*, *CYP1A5*, and *β-actin* (normalizer gene) mRNA abundance in CEH exposed to DMSO, SI-TeDB-DiPhOBz, SI-BDE-209, SI$_p$-TeDB-DiPhOBz or SI$_p$-BDE-209. Reactions were performed using Brilliant Multiplex QPCR Mastermix kits (Agilent Technologies, Santa Clara, CA). Each 25 μL reaction mixture contained primers and probes for *CYP1A4*, *CYP1A5*, and *β-actin*, 1× Multiplex QPCR master mix, 30 nM reference dye (ROX) and 5 μL of cDNA. The thermal profile for all reactions was 10 min at 95 °C, followed by 40 cycles of 95 °C for 30 s and 60 °C for 1 min. All samples were assayed in duplicate. SI-TeDB-DiPhOBz and SI-BDE-209 were assayed at six (0.001, 0.01, 0.1, 1, 10, and 25 μM) and five (0.001, 0.01, 0.1, 1, and 10 μM) concentrations, respectively. Both SI$_p$-TeDB-DiPhOBz and SI$_p$-BDE-209 were assayed at six concentrations (SI$_p$-TeDB-DiPhOBz: 0.015, 0.05, 0.15, 0.5, 1.5, and 5 μM; SI$_p$-BDE-209:0.15, 0.5, 1.5, 5, 15, and 50 μM). Amplification of gene expression was not detected in controls containing no reverse transcriptase or no template for any of the assays, which confirmed the absence of any gene-responsive contamination. *β-actin* mRNA expression was consistent across all treatment groups (not shown) and was therefore considered to be an appropriate control gene.

Characterization of AhR-Related Activities of Eight Fractions of SI-TeDB-DiPhOBz or SI-BDE-209 Using a Luciferase Reporter Gene (LRG) Assay. Fractionation of SI-TeDB-DiPhOBz and SI-BDE-209 solutions was performed using gel permeation chromatography (GPC) consisting of a 5.0 mL injection loop. The GPC was performed on an Autoprep 2000 (O.I. Analytical, College Station, TX) with a S-X3 column (50 × 3 cm i.d.). Aliquots of 40 and 80 μL of SI-TeDB-DiPhOBz and SI-BDE-209 solutions were diluted into 5 mL of 1:1 dichloromethane (DCM)/hexane solvent, and injected into the GPC. The GPC was set to a flow rate of 5 mL/min of DCM/hexane (1:1) and fractions were collected one per 10 min with a total time period of 80 min. The collected fractions were evaporated to dryness under a gentle stream of nitrogen and reconstituted with 200 μL of DMSO for further analysis by high performance liquid chromatography-atmospheric pressure photoionization(−)-quadrupole-time-of-flight mass spectrometry (LC-APPI(−)-Q-TOF-MS) or AhR-mediated reporter gene expression. Fractions prepared from SI-TeDB-DiPhOBz and SI-BDE-209 were compared to TCDD by using a luciferase reporter gene (LRG) assay that measures AhR-mediated transcriptional activity.[28] A nominal concentration of 300 nM TCDD was included as a positive control. The concentration of the stock TCDD solution was determined by isotope dilution following EPA method 1613 (U.S. EPA, 1994) by high-resolution gas chromatography high-resolution mass spectrometry, and its actual concentration was 72.9 μg/mL as described elsewhere.[29]

A detailed description of transfection methods and the LRG assay is provided elsewhere.[28] Briefly, monkey kidney cells (COS-7) were transiently transfected with 8 ng of chicken AHR1 expression construct, 1.55 ng of cormorant ARNT and 7.5 ng of pGL4-ccCYP1A5 (both were kindly provided by Dr Hisato Iwata -Ehime University),[30] 0.75 ng of *Renilla* luciferase vector (phRL-CMV; Promega), and 32.2 ng of salmon sperm DNA (Invitrogen). Transfected cells were incubated for 5 h prior to treatment with DMSO or DMSO solutions of SI-TeDB-DiPhOBz (40 and 400 μM) and SI-BDE-209 (40 and 400 μM) fractions. Luciferase activity was measured after 20 h of incubation.

LC-APPI(−)-Q-TOF-MS Analysis of Products Formed during Solar Irradiation of Flame Retardants. Characterization of congeners of brominated homologue groups of the formed dibenzofurans or polybenzofurans was carried out using an Agilent 1200 LC system, coupled with an Agilent 6250A (Q-TOF)-MS (Agilent Technologies, Mississauga, ON, Canada). The LC system was equipped with an Xterra Phenyl column (2.1 × 100 mm, 3.5 μm particle size) (Waters, Mississauga, ON, Canada). The mobile phase (A, water; B, 1:1 isopropanol/methanol) flow rate was 0.3 mL/min and the following gradient was employed: 5% B ramped to 100% B in 5 min (linear) and held for 20 min, followed by a change to 5% B and held for 15 min for the next injection. Toluene was introduced into the Q-TOF at a flow rate of 0.02 mL/min by a Series 200 Micro pump (PerkinElmer, Woodbridge, ON, Canada) and via a T connector after the LC system. The MS was operated using atmospheric pressure photoionization in the negative ion mode (APPI(−)), and its capillary voltage was 5.0 kV. Nitrogen was used as the drying and nebulizing gas and helium was used as the collision gas. Other parameters for MS were optimized as follows: gas temperature, 300 °C; drying gas, 10 L/min; neubilizer, 30 psi; fragmentor voltage, 200 V; skimmer voltage, 60 V. The Q-TOF instrument was tuned and calibrated with tuning calibration solution (G1969-85000, Agilent Technologies). The TOF-MS was operated at resolution (R) > 20 000 at m/z 601.9790 and within 3 ppm mass error in mass range m/z 50−1700. For each run, 2 μM purine (m/z 119.0363) and 50 nM HP-0921 (m/z 805.9854) were introduced into the Q-TOF with toluene as reference masses.

Data Analysis. Analyses of data from PCR arrays were conducted using MxPro v4.10 software (Agilent Technologies, Santa Clara, CA) and the cycle threshold (Ct) was set to 0.1. The fold change of target gene mRNA abundance relative to the vehicle control was calculated using the $2^{-\Delta\Delta Ct}$ method and significant differences in fold change compared to the DMSO vehicle control were determined using one-way ANOVA. For visualization, nonsignificant fold changes ($p > 0.05$) and those less than 2 were set to 0 to minimize noise, and the gene expression clustering was performed on R 3.0.2 version using "gplots" package. $EC_{threshold}$ (EC_{thr}) values, which represent the concentration of a compound that elicits a significantly greater alteration of *CYP1A4/5* mRNA expression than DMSO-treated cells, were determined from concentration−response curves

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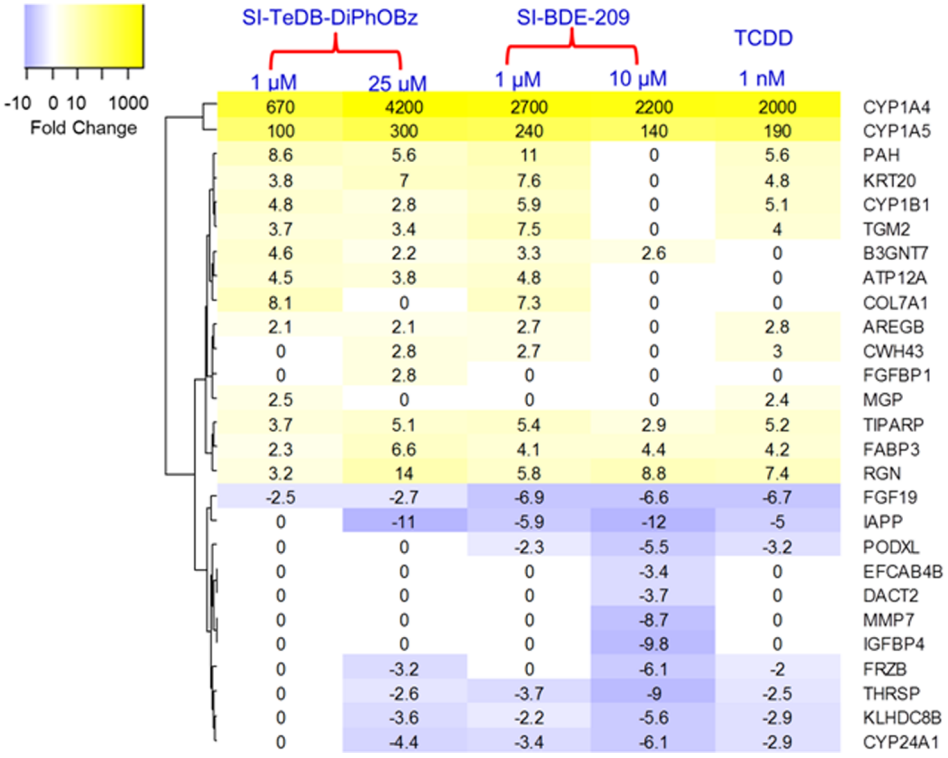

Figure 1. Transcription of 27 dioxin-responsive genes on a custom-designed PCR array following exposure to SI-TeDB-DiPhOBz (1 μM, 25 μM), SI-BDE-209 (1 μM, 10 μM) and 1 nM TCDD. The data for TCDD was cited from a previous publication.[26] Hierarchical clustering was conducted based on mRNA expression fold-changes derived from a mean of three replicates. Genes with a $p > 0.05$ (ANOVA, Dunnett's Test) or fold-change less than 2 were set to 0 to minimize noise. Blue-white-yellow represents down-, no- and up-regulation. Unprocessed fold-change data for expression of mRNA are available in Table S2 for all treatment groups.

using a one-way ANOVA ($p < 0.05$) with a Dunnett's posthoc test ($p < 0.05$), and three technical replicates per concentration of SI-TeDB-DiPhOBz and SI-BDE-209 were included.

RESULTS AND DISCUSSION

SI-TeDB-DiPhOBz and SI-BDE-209 Altered Expression of TCDD-Responsive Genes. Fifteen, 20, 20, and 17 of 27 TCDD-responsive genes (Table S1), which were selected based on the results of microarray analysis,[26,31] were altered in CEH following exposure to SI-TeDB-DiPhOBz (1 or 25 μM) or SI-BDE-209 (1 or 10 μM), respectively (Figure 1 and Table S2). Hierarchical clustering of the 27 TCDD-responsive genes was conducted based on levels of expression of mRNA, and two genes, *CYP1A4* and *CYP1A5*, were separated into an independent group from all other genes due to their extremely large fold-changes. The remaining 25 TCDD-responsive genes (Table S1) were further classified into two very clear subclusters that exhibited consistent up-regulation (*PAH KRT20 CYP1B1 TGM2 TIPARP FABP3 RGN ATP12A AREGB B3GNT7 COL7A1 CWH43 FGFBP1MGP*) or down-regulation (*GFG19 IAPP PODXL DACT2 EFCAB4B MMP7 IGFBP4 FRZB THRSP KLHDCBB CYP24A1*) following exposure to SI-TeDB-DiPhOBz and SI-BDE-209. SI-TeDB-DiPhOBz and SI-BDE-209 altered these genes in a similar manner as TCDD (Figure 1). Furthermore, specific fold changes of the 27 TCDD-responsive genes in CEH exposed to SI-TeDB-DiPhOBz, SI-BDE-209 or TCDD were significantly correlated based on the Spearman correlation model (Spearman $r > 0.77$, $p < 0.001$, Table S3), which strongly suggesting that natural sunlight irradiation of TeDB-DiPhOBz or BDE-209 in solution generates AhR agonists.

In terms of specific biological functions, the 27 TCDD-responsive genes on the PCR array can be grouped into 17 biological processes (e.g., phase I and phase II metabolism, lipid metabolism and oxidation, bone development, calcium homeostasis, apoptosis) (Table S1). Exposure to two concentrations of SI-TeDB-DiPhOBz resulted in alteration of expression of genes associated with 15 pathways; the exceptions were the apoptosis and wnt gene signaling pathways. Similarly, SI-BDE-209 altered genes from 16 pathways with the exception of carrier protein (*FGFBP1*).

Five genes were of interest because they were not significantly altered by 1 nM TCDD CEH administration but were significantly (fold change >4) dysregulated by SI-TeDB-DiPhOBz or SI-BDE-209 exposure (Figure 1 and Table S2). These genes included *IGFBP4* (−9.8-fold, induced by 10 μM of SI-BDE-209), *MMP7* (−8.8-fold, by 10 μM of SI-BDE-209), *COL7A1* (8.1-fold, by 1 μM of SI-TeDB-DiPhOBz), *ATP12A* (4.7-fold, by 1 μM of SI-BDE-209), and *B3GNT7* (4.6-fold, by 1 μM of SI-TeDB-DiPhOBz). *IGFBP4*, insulin-like growth factor binding protein 4, was down-regulated in CEH exposed to 10 μM SI-BDE-209 and is associated with the lipid metabolism and oxidation pathway and can bind both insulin-like growth factors (IGFs) I and II. Previous studies observed the down-regulation of *IGF1* in CEH following exposure to various contaminants, that is, bisphenol S,[32] triphenyl phosphate,[24] extracts of herring gull eggs[33] as well as these two SI-BFRs.[22] *MMP7* is a member of the matrix metalloproteinase (MMP) family, which is thought to enhance tumor metastatic potential.[34] Overexpression of MMP7 facilitates cancer invasion and angiogenesis by degrading extracellular matrix macromolecules and connective tissues,

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and thus up-regulation of *MMP7* has generally been observed in the luminal surface of dysplastic glands in human colorectal cancers,[35] human liver carcinoma[34,36] or endometrial stromal and epithelial cells.[37] In the present study, significant down-regulation of *MMP7* was observed in CEH following exposure to 10 μM SI-BDE-209 (Figure 1), but the underlying mechanism is unknown. Similarly, the mechanism underlying the up-regulation of *COL7A1* in CEH following exposure to 1 μM SI-TeDB-DiPhOBz or 1 μM SI-BDE-209 is also unknown, although this gene encodes the alpha chain of type VII collagen. *ATP12A* (nongastric H+/K+ATPase) plays a role in kidney function and is located in the luminal plasma membrane of renal epithelial cells where it reabsorbs potassium from the filtrate in exchange for H+, which acidifies the urine and helps to main ion balance.[38,39] Interestingly, a previous study found that *ATP12A* can be significantly altered in the developing kidney of mice exposed to TCDD.[40] In the present study, we did not observe *ATP12A* dysregulation in CEH exposed to TCDD, but it was up-regulated by 1 or 25 μM of SI-TeDB-DiPhOBz or 10 μM of SI-BDE-209 (Figure 1).

BFR Products of Photolysis and Concentration-Dependent Induction of *CYP1A4/5*. Given the relatively large fold-change inductions observed for *CYP1A4/5* genes (Figure 1 and Table S2) on response of the PCR array following exposure to SI-TeDB-DiPhOBz (1 or 25 μM) and SI-BDE-209 (1 or 10 μM), a more complete concentration-dependent response curve was generated. Upregulation of expression of *CYP1A4/5* mRNAs occurred in a concentration-dependent manner by SI-TeDB-DiPhOBz and SI-BDE-209 in CEH, and *CYP1A4* had a greater maximal induction than *CYP1A5* for both SI-TeDB-DiPhOBz and SI-BDE-209 (Figure 2A, B, D, and E). Maximal levels of *CYP1A4* induction were 5200- and 2300-fold following exposure to SI-TeDB-DiPhOBz and SI-BDE-209, respectively. These values were comparable to those from the PCR array (i.e., 4200 and 2200-fold, respectively). In fact, the maximal *CYP1A4* fold-change following exposure to SI-TeDB-DiPhOBz was not only greater than SI-BDE-209, but also greater than TCDD from several previous studies that used the same CEH bioassay system.[29,41,42] Maximal levels of *CYP1A5* mRNA expression were 310-fold and 320-fold following exposure to SI-TeDB-DiPhOBz and SI-BDE-209, respectively.

As shown in previous studies with avian hepatocytes, *CYP1A4* and *CYP1A5* mRNA expression can be used to predict relative potency (ReP) of dioxin-like compounds (DLCs).[29,42,43] Because a plateau was not reached for any of the curves at the concentrations tested (Figure 2), the ReP values of SI-TeDB-DiPhOBz and SI-BDE-209 were calculated based on EC_{thr} values. Compared to previously reported EC_{thr} values for TCDD (0.001 nM) based on *CYP1A4/5* mRNA expression in CEH,[29,42] EC_{thr} values were 1 and 0.1 μM for SI-TeDB-DiPhOBz and SI-BDE-209, respectively. Thus, ReP_{ECthr} values for SI-TeDB-DiPhOBz and SI-BDE-209 were approximately 1×10^{-6} and 1×10^{-5}. Collectively, these results clearly demonstrate that sunlight irradiation can transform TeDB-DiPhOBz and BDE-209 (dissolved in hexane/THF) into potentially toxic photoproducts with ReP_{ECthr} values 30 and 3-fold lower than those of dioxin-like, mono-ortho-PCB congeners (PCB-105, -114, -118, -123, -156, -157, -167, and -189).[44,45]

Characterization of Products in Relation to Observed AhR-Related Activities. A number of studies have reported that after irradiation with sunlight[20,46] or as a result of heating

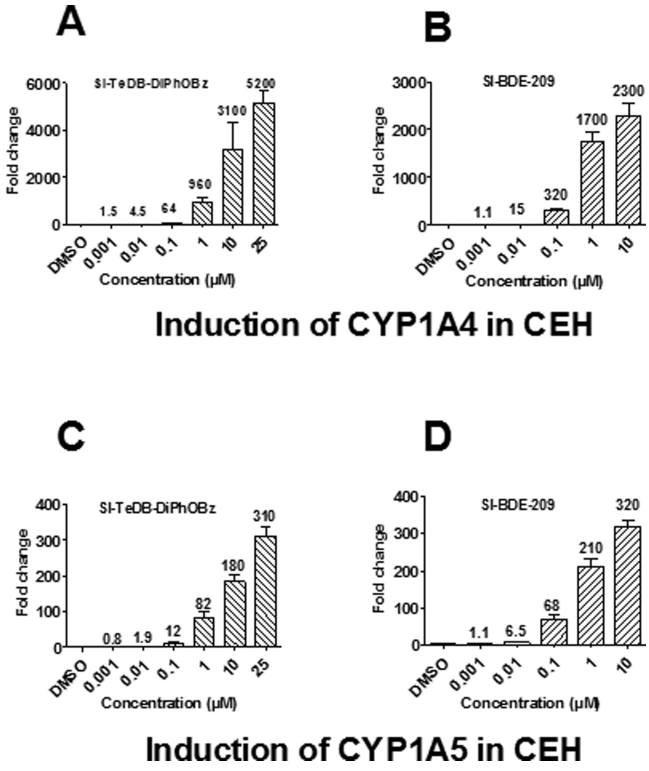

Figure 2. Concentration-dependent effects of SI-TeDB-DiPhOBz and SI-BDE-209 on expression of *CYP1A4* and *CYP1A5* mRNA in chicken embryonic hepatocytes (CEH) exposed for 36 h (panels A–D). Error bars for each point represent the standard derivation (SD).

to higher temperatures,[47] BDE-209 can be transformed into polybrominated dibenzofurans (PBDFs) that can bind to the AhR, activate AhR-mediated signaling pathways, and elicit typical dioxin-like toxic and biological responses.[48] Most importantly, in mammalian systems these PBDFs generally have exhibited very similar ReP values relative to those of their respective dioxin-like polychlorinated dibenzofuran (PCDF) analogues or at least within 1 order of magnitude.[48] It is plausible that in the present study, PBDFs or perhaps polybrominated dibenzo-*p*-dioxins (PBDDs) were formed from BDE-209 after sunlight irradiation, and it was thus hypothesized that these products would be responsible for the observed dioxin-responsiveness.

Before we performed any further experiments, 19 individual standards of PBDE congeners, containing from 1 to 10 bromine atoms, were used to generate LC-APCI(−)-Q-TOF-MS and LC-APPI(−)-Q-TOF-MS reference mass spectra for the polybrominated furan structures (Table 1 and Figure S4). APCI(−) and APPI(−) generated similar patterns of ions of PBDE congeners, although APPI(−) produced greater ion intensities. LC-APPI(−)-Q-TOF-MS analysis showed that the $[M\text{-}Br+O]^-$ fragment ion was of greatest abundance for most of the PBDE congeners with the exception of BDE-208 and four other PBDE congeners with less than three bromine atoms. For example, BDE-208 produced $[C_6OBr_5]^-$ as the most abundant fragment ion rather than $[M\text{-}Br+O]^-$, and PBDE congeners with less than three bromine atoms showed no response in APPI source. Regardless of the PBDE congener, other less-abundant ions included $[M\text{-}H]^-$, $[M\text{-}Br]^-$, $[M\text{-}Br+O]^-$, $[M\text{-}Br+2O-H]^-$, $[M\text{-}2Br+O+H]^-$, $[M\text{-}2Br+2O-H]^-$, $[M\text{-}2Br+O-H]^-$, $[M\text{-}3Br+O]^-$, $[M\text{-}3Br+2O-H]^-$, and

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Table 1. Formation of Ions of 19 Individual PBDE Congeners (Concentration: 5 μg/mL) As a Result of APPI(−)-Q-TOF-MS Analysis[f]

PBDEs	R[a]	Ions[b]	AM[c] (Da)	TM[d] (Da)	MD[e] (ppm)
BDE-209	1	M-Br+O	894.2472	894.2478	-0.7
	2	C_6OBr_5	486.5831	486.5843	-2.5
	3	M-3Br+O	734.4126	734.4131	-0.7
	4	M-2Br+O+H	816.3367	816.3364	0.4
BDE-208	1	C_6OBr_5	486.5831	486.5843	-2.5
	2	M-Br+O	816.3367	816.3373	-0.7
	3	M-Br	800.3418	800.3423	-0.6
	4	C_6OBr_4H	408.6725	408.6735	-2.4
	5	M-H	878.2523	878.2522	0.1
	6	M-3Br+O	656.5021	656.503	-1.4
	7	M-2Br+2O-H	750.4075	750.4075	0.0
	8	M-2Br+O+H	736.4283	736.4237	6.2
	9	M-3Br+2O-H	671.4892	671.4902	-1.5
BDE-207	1	M-Br+O	816.3367	816.338	-1.6
	2	C_6OBr_5	486.5831	486.5844	-2.7
	3	C_6OBr_4H	408.6725	408.6735	-2.4
	4	M-3Br+O	656.5021	656.5035	-2.1
	5	M-2Br+O+H	736.4283	736.4272	1.5
BDE-197	1	M-Br+O	736.4291	736.4283	1.1
	2	C_6OBr_4H	408.6725	408.6736	-2.7
	3	M-3Br+O-2H	576.5936	576.5944	-1.4
	4	M-2Br+O+H	658.5177	658.5161	2.4
	5	M-Br+2O-H	751.4158	751.4157	0.1
BDE-194	1	M-Br+O	736.433	736.4283	6.4
	2	M-2Br+O-H	656.5044	656.5021	3.5
	3	C_6OBr_4H	408.6725	408.6732	-1.7
	4	M-Br+2O-H	751.4158	751.4153	0.7
BDE-180	1	M-Br+O	658.5177	658.5202	-3.8
	2	M-Br+2O-H	673.5043	673.5053	-1.5
	3	M-2Br+O-H	576.5936	576.5975	-6.8
BDE-170	1	M-Br+O	658.5177	658.5202	-3.8
	2	M-Br+2O-H	673.5043	673.5058	-2.2
	3	M-2Br+O-H	576.5936	576.5965	-5.0
BDE-154	1	M-Br+O	578.6093	578.6106	-2.2
	2	M-2Br+O-H	498.6831	498.6846	-3.0
	3	M-Br+2O-H	593.5964	593.597	-1.0
BDE-153	1	M-Br+O	578.6093	578.6114	-3.6
	2	M-2Br+O-H	498.6831	498.6862	-6.2
	3	M-Br+2O-H	593.5964	593.5969	-0.8
BDE-99	1	M-Br+O	500.6988	500.7009	-4.2
	2	M-2Br+O-H	418.7746	418.7755	-2.1
	3	M-Br+2O-H	515.6859	515.6866	-1.4
BDE-85	1	M-Br+O	500.6988	500.7004	-3.2
	2	M-Br+2O-H	515.6859	515.687	-2.1
	3	M-2Br+O-H	418.7746	418.7756	-2.4
BDE-66	1	M-Br+O	420.7903	420.7914	-2.6
	2	M-Br+2O-H	435.7774	435.7783	-2.1
BDE-47	1	M-Br+2O-H	435.7774	435.7783	-2.1
	2	M-Br+O	420.7903	420.7911	-1.9
BDE-28	1	M-Br+O	342.8798	342.8801	-0.9
	2	M-Br+2O-H	357.8669	357.8673	-1.1
BDE-17	1	M-Br+O	342.8798	342.8803	-1.5
	2	M-Br+2O-H	357.8669	357.8675	-1.7

[a]"R" means "response ranking", and the number 1 fragment showed the highest instrumental response. [b]When bromine number is even, molecular mass was calculated with the highest-response peak. When bromine number is odd, there should be two highest-response peaks, and the molecular mass was calculated with the lower-mass (left) one. [c]"AM" means "actual mass" that is observed in APPI-QTOF. [d]"TM" means "theoretical mass" that is calculated based on the specific molecular weight of atoms. [e]"MD" means "mass defect" that is the difference between actual and theoretical m/z masses. [f]Detailed mass characteristics are provided in Figure S4.

$[C_6OBr_4H]^-$. Generation of $[M\text{-}3Br+O]^-$ and $[M\text{-}2Br+O-H]^-$ ions, which could have m/z ratios coincidental with those of polybrominated furan structures, could lead to "false-positive" assignments of peaks as furan structures.

Given that (highly brominated) PBDE congeners could be analyzed by LC-APPI(−)-Q-TOF-MS, we analyzed the SI-BDE-209 and SI-TeDB-DiPhOBz solution using the same instrumental parameters, with the assumption that an abundant ion generated by APPI(−) would also be $[M\text{-}Br+O]^-$. Any potential "false-positive" furan-like structures were reduced by comparing their retention times with the debrominated products of BDE-209 or TeDB-DiPhOBz. In other words, there had to be clear retention time differences between any detected structures of polybenzofuran and debrominated products from the photodegradation of TeDB-DiPhOBz. Furthermore, the measured m/z value had to be within 10 ppm of its calculated value and there had to be agreement between the predicted and observed peaks in terms of the specific mass spectra characteristics of any organic brominated compounds (i.e., molecular mass, peak response ratio and peak number).

Under these strict criteria, LC-APPI(−)-Q-TOF-MS analysis of the SI-BDE-209 solution showed four peaks in extracted ion chromatograms for the most abundant fragment ion $[M\text{-}Br+O]^-$ (M: molecule formula), which eluted between 7 and 8 min (Figure S2 and Table S4). Considering the isotopic patterns and the m/z ratios in the corresponding mass spectra, these spectra indicated the presence of at least two Br_3-PBDFs and two Br_4-PBDFs (Figure S2). It is possible that other peaks in the EICs may have been coeluting PBDF isomers in the same homologue groups and products containing three and four bromine atoms. The Br_3- and Br_4-PBDFs in the SI-BDE-209 solution possess molecular planar structures that are necessary to elicit dioxin-like activity and responsiveness.

To our knowledge, other than products of debromination of TeDB-DiPhOBz during solar irradiation,[21,22] any other products of photolysis are presently unknown. Our initial hypothesis was that sunlight irradiation caused ether bond cleavage to produce PBDE products, which are further transformed into PBDFs. However, LC-APPI(−)-Q-TOF-MS or GC-MS(ECNI) analysis of the present SI-TeDB-DiPhOBz solution showed no detectable peaks attributable to PBDE or PBDF congeners (data not shown). This suggested that the photolytic formation of PBDF from TeDB-DiPhOBz did not occur in the present experiments, and thus PBDF products were not responsible for the observed dioxin-like gene expression for the SI-TeDB-DiPhOBz solution. This result led to a second hypothesis: TeDB-DiPhOBz is photodegraded to form polybrominated polybenzofuran products. Such products would have to retain the planar molecular backbones to elicit AhR-mediated dioxin-like activity, which has been demonstrated for known[49,50] or newly emerging[51,52] dioxin-like

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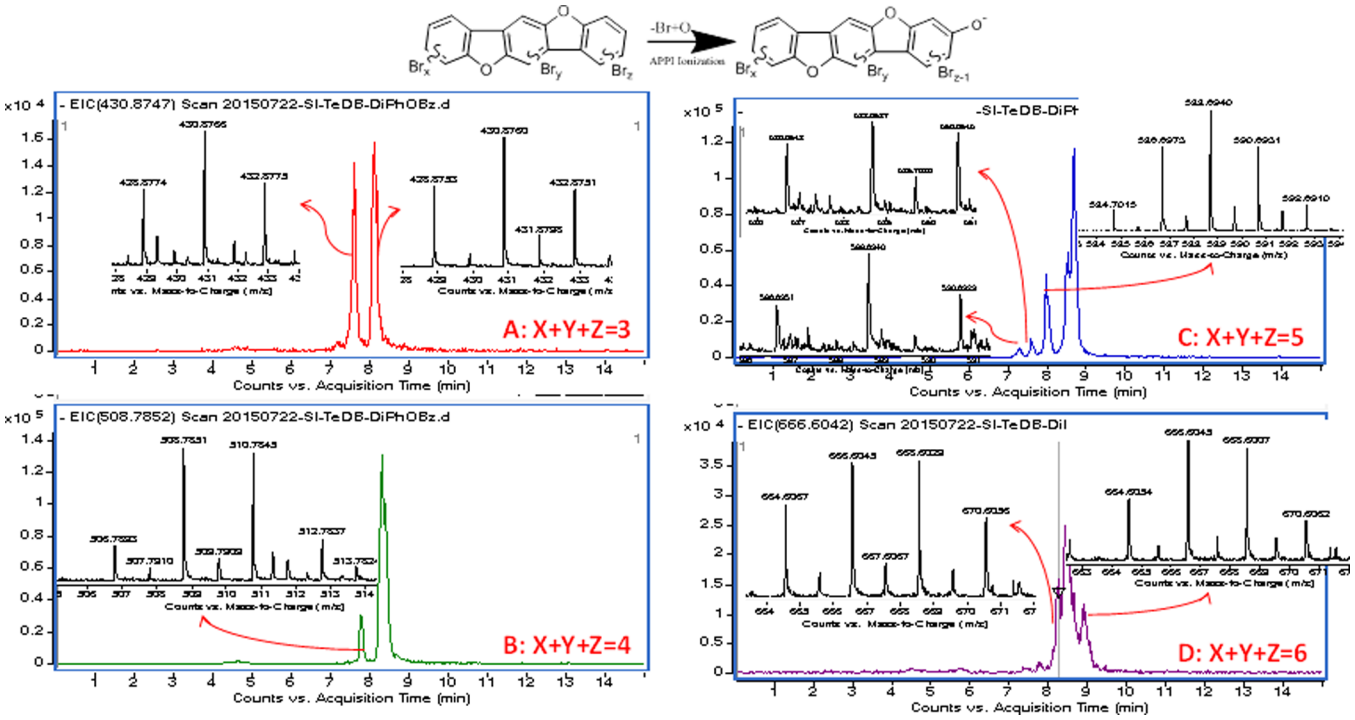

Figure 3. Liquid chromatography- atmospheric pressure photoionization(−)-time-of-flight mass (LC-APPI(−)-TOF-MS) spectra extracted ion chromatograms (EICs) of solutions of sunlight irradiated-TeDB-DiPhOBz. The mass spectral peak assignments are based on their specific mass spectrum characteristic (mass, peak response ratio, peak number) and retention time on the LC system. The bromine number is 3, 4, 5, and 6 in Figure 3A, 3B, 3C, and 3D, respectively.

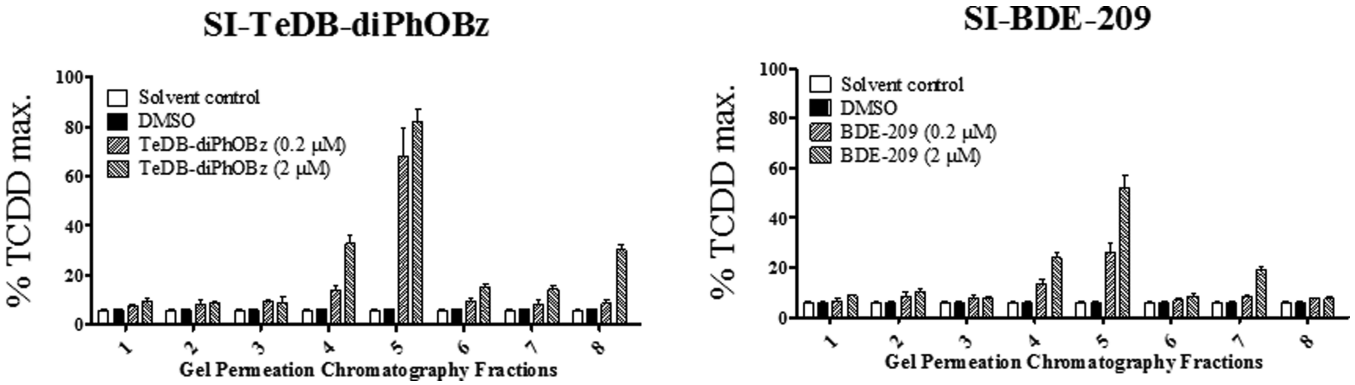

Figure 4. Percent response relative to a 300 nM TCDD positive control of eight fractions of 0.2 and 2 μM SI-TeDB-DiPhOBz and SI-BDE-209 on AHR1-mediated luciferase reporter gene activity in COS-7 cells. A full-length chicken AHR1 construct was transfected into COS-7 cells with cormorant ARNT, CYP1A5 reporter construct, and *Renilla* luciferase reporter vector. Each data point represents the mean positive control-normalized luciferase ratio (firefly luminescence/*Renilla* luminescence) determined from four wells; bars represent SE of the mean values.

compounds. To address this second hypothesis, the SI-TeDB-DiPhOBz solution was initially analyzed by GC-MS(ECNI), LC-ESI(±)-Q-TOF-MS, LC-APCI(±)-Q-TOF-MS and LC-APPI(±)-Q-TOF. Mass spectral peaks were observed by use of only LC-APCI(−)-Q-TOF-MS and LC-APPI(−)-Q-TOF-MS. Based on the same criteria for LC-APPI(−)-Q-TOF-MS analysis, EIC peaks for two Br_3, one Br_4, three Br_5 and two Br_6 polybenzofuran structures were observed in the SI-TeDB-DiPhOBz solution (Figure 3 and Table S4).

For the Br_3- and Br_4-PBDFs in the SI-BDE-209 solution, such polybenzofurans in SI-TeDB-DiPhOBz, possess planar molecular structures that could elicit dioxin-like activity via AhR mediated gene expression. To confirm this, solutions of SI-TeDB-DiPhOBz and SI-BDE-209 were fractionated using gel permeation chromatography (GPC) into 8 fractions based on molecular size, and the collected fractions were examined for AhR-mediated transcriptional activity by using a luciferase reporter gene (LRG) assay (Figure 4). The greatest responses in the LRG assay were observed in the fourth and fifth GPC fractions from the SI-TeDB-DiPhOBz and SI-BDE-209 product mixtures, and these two GPC fractions from the SI-TeDB-DiPhOBz and SI-BDE-209 also contained the most detectable polybenzofuran and dibenzofuran structures, respectively.

Effects of Products from Sunlight Irradiation of TeDB-DiPhOBz and BDE-209 Powder on *CYP1A4* mRNA Expression. In the present study, we also investigated the sunlight irradiation and photolytic degradation of TeDB-DiPhOBz and BDE-209 in their solid powder forms. SI_p-TeDB-DiPhOBz and SI_p-BDE-209 were administered to CEH and resulted in greater mRNA gene expression changes than

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their respective precursors. Fold changes in *CYP1A4* up-regulation induced by SI_P-TeDB-DiPhOBz or SI_P-BDE-209 (Figure 5) were clearly lower than those elicited by SI-TeDB-DiPhOBz or SI-BDE-209 irradiated in solution (Figures 1 and 2), respectively. However, concentration-dependent induction was observed and concentrations >1.5 μM of both compounds elicited significant up-regulation of *CYP1A4* (Figure 5). Specifically, SI_P-TeDB-DiPhOBz and SI_P-BDE-209 elicited maximal fold-change response of 5.3 ± 2.1 and 66 ± 6.9, respectively. Previous results showed that NI-TeDB-DiPhOBz did not significantly alter expression of *CYP1A4* mRNA at concentrations up to 1.9 μM (greatest concentration that could be dissolved in the solvent medium), and NI-BDE-209 only slightly altered expression of *CYP1A4* at concentrations up to 9 μM (greatest concentration that could be dissolved in medium).[22]

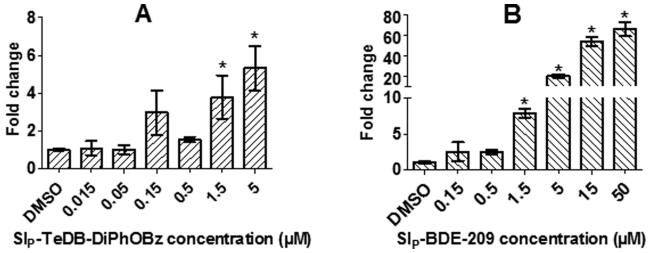

Figure 5. Concentration-dependent effects of SI_P-TeDB-DiPhOBz (Panel A) and SI_P-BDE-209 (Panel B) (generated in solid form by sunlight irradiation for 3 months) on *CYP1A4* mRNA expression in chicken embryonic hepatocytes (CEH) exposed for 36 h. Error bars for each point represent the standard deviation of three replicates. Treatments that were significantly (ANOVA, Dunnett's Test, $p < 0.05$) different from DMSO controls are identified by *.

Studies of photolysis of BDE-209 have been conducted in organic solvents such as THF/methanol/water mixtures[53] and toluene,[20] as well as in house dust[19] and sediment.[20] Although the rates of degradation of BDE-209 varied among matrices, the various matrices had little effect on the ultimate degradation process.[20] In a previous study, we found that degradation products of BDE-209 in three solvents (methanol, hexane, and hexane/THF) after solar irradiation generally showed comparable EC_{50} values determined using a luciferase reporter gene (LRG) assay, which indicated that solvents used did not play a role in the BDE-209 degradation outcome.[22] Collectively, the results of the present study demonstrate that expression of dioxin-responsive genes was greater for TeDB-DiPhOBz and BDE-209 irradiated in their solid forms. TeDB-DiPhOBz and BDE-209 are additive flame retardants used in polymeric products that are used outdoors and can end up in waste facilities and landfills where irradiation by sunlight would be extensive and longer-term. Therefore, instability of TeDB-DiPhOBz and BDE-209 and possibly other highly brominated polyphenyl ethers, is of concern.

TeDB-DiPhOBz and BDE-209 are major components of the flame retardants SAYTEX-120 and deca-BDE. Results of the present study show that solar irradiation of TeDB-DiPhOBz and BDE-209 (in solution and as solid powder) can generate products that dysregulate expression of mRNA for genes in CEH to a similar fashion to the AhR agonist, TCDD, which is the most potent dioxin-like compound. Significant findings/implications are as follows: (1) the environmental instability of TeDB-DiPhOBz and BDE-209, and possibly other highly brominated polyphenyl ethers, is of concern; (2) new chemical structures, polybrominated benzofurans, were identified for the first time and represented products of photolysis of TeDB-DiPhOBz, which potentially modulate expression of genes that result in adverse outcomes similar to those of known halogenated benzofuran contaminants; and (3) the formation of dibenzofurans from brominated polyphenyl ether structures should be considered in future FR development.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.5b04939.

Further details are given on the dioxin-response genes in the custom chicken PCR array and significance of change in gene dysregulation, and LC-MS/MS mass chromatograms and mass spectra identifying polybenzofuran degradation products due to sunlight irradiation, as noted in the text (PDF)

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Notes

The authors declare no competing financial interest.

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Supporting Information:

Sunlight Irradiation of Highly Brominated Polyphenyl Ethers Generates Polybenzofuran Products That Alter Dioxin-responsive mRNA Expression in Chicken Hepatocytes

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Supporting Text:

Text S1: Sample Preparation

Detailed information on solar irradiation of solvent-dissolved TeDB-DiPhOBz and BDE-209 has been published previously[1]. In brief, solid powder of the two target chemicals was dissolved in 30 % THF/n-hexane, which was then exposed for 21 days to natural sunlight irradiation (SI) in the natural outside environment. The same volume of 30% THF/n-hexane was used as the solvent control. Irradiation with sunlight was conducted from December 24, 2013 to January 14, 2014 in Ottawa (ON, Canada), and the location coordinates were 45°40'06"N and 75°74'22"W.

Text S2: Chicken Embryonic Hepatocyte Assay

Two independent CEH cultures were prepared for the following chemical exposure experiments: 1) products of photolysis by sunlight in solvent, and 2) products of photolysis by irradiation of their solid forms. Methods for culture of CEH have been described previously[2, 3]. Briefly, fertilized, unincubated white leghorn chicken (*Gallus gallus domesticus*) eggs were obtained from the Canadian Food Inspection Agency (Ottawa, ON, Canada) and incubated for 19 days (37.5 °C, 60 % relative humidity). At day 19, embryos were euthanized by decapitation and livers were removed, pooled and treated with Percoll (GE Healthcare, Little Chalfont, UK) and DNase I (Roche Applied Science, Penzberg, Upper Bavaria, Germany). The resulting cell pellets were suspended in a volume of Medium 199 (Life Technologies, Burlington, Canada), supplemented with 1 µg/mL insulin (Sigma Aldrich) and thyroxine (Sigma Aldrich), equal to 32 times its mass. Twenty-five µL of the cell suspension was distributed into 48-well plates containing 500 µL of fresh supplemented medium, and incubated for 36 h (37.5 °C and 5 % CO_2) prior to dosing. The final nominal concentration ranges for the SI-brominated flame retardants (BFRs) in the medium were

0.001-25 μM (SI-TeDB-DiPhOBz), 0.001-50 μM (SI-BDE-209), 0.015-5 μM (SI_P-TeDB-DiPhOBz), 0.15-50 μM (SI_P-BDE-209) (n=3 replicate wells/treatment). Exposure of CEH to solutions of parent TeDB-DiPhOBz or BDE-209 were not included in our current study because essentially no gene alterations were observed following exposure to NI-TeDB-DiPhOBz or NI-BDE-209 in our previous study[1].

Table S1. List of TCDD-responsive genes and two housekeeping genes on the custom chicken PCR array.

Biological Process	Gene symbol	GenBank accession	Official full name
Phase I and Phase II Metabolism	CYP1A4	NM_205147	Cytochrome P450 1A4
	CYP1A5	X99454	cytochrome P450 1A5
	CYP1B1	XM_419515	PREDICTED: Gallus gallus cytochrome P450, family 1, subfamily B, polypeptide 1
	CYP24A1	NM_204979.1	Gallus gallus cytochrome P450, family 24, subfamily A, polypeptide 1
Calcium homeostasis	RGN	NM_204729.1	Gallus gallus regucalcin
	EFCAB4B	XM_003642738.2	PREDICTED: Gallus gallus EF-hand calcium-binding domain-containing protein 4B-like (LOC100859805), mRNA
Organization of keratin filament	KRT20	NM_001277981	Gallus gallus keratin 20
phenylalanine catabolism	PAH	NM_001001298.1	Gallus gallus phenylalanine hydroxylase
apoptotosis, prolifieration, cell spreading, cell-cell adhesion,	TGM2	NM_205448	Gallus gallus transglutaminase 2 (C polypeptide, protein-glutamine-gamma-glutamyltransferase)
	PODXL	NM_001271894	Gallus gallus podocalyxin-like, transcript variant 1
alter protein function	TIPARP	XM_422828	PREDICTED: Gallus gallus TCDD-inducible poly (ADP-ribose) polymerase
Collagen biosynthesis	COL7A1	XM_425157	PREDICTED: Gallus gallus collagen alpha-1(VII) chain-like
Ion channel transport	ATP12A	NM_001030909.1	Gallus gallus ATPase, H+/K+ transporting, nongastric, alpha polypeptide
proliferation, migration, differentiation, angiogenesis, apoptosis	MMP7	NM_001006278.1	Gallus gallus matrix metallopeptidase 7 (matrilysin, uterine)
bone development	MGP	NM_205044.1	Gallus gallus matrix Gla protein (MGP)
	FRZB	NM_204772.2	Gallus gallus frizzled-related protein
Lipid Metabolism and Oxidation	AREGB	NM_001031537.1	Gallus gallus amphiregulin B
	IGFBP4	NM_204353.1	Gallus gallus insulin-like growth factor binding protein 4
	FGF19	NM_204674.1	Gallus gallus fibroblast growth factor 19
	FABP3	NM_001030889.1	Gallus gallus fatty acid binding protein 3, muscle and heart (mammary-derived growth inhibitor)
	CWH43	XM_004936041.1	PREDICTED: Gallus gallus cell wall biogenesis 43 C-terminal homolog (S. cerevisiae)
Keratan sulfate/keratin metabolism	B3GNT7	XM_422738	PREDICTED: Gallus gallus UDP-GlcNAc:betaGal beta-1,3-N-acetylglucosaminyltransferase 7
Wnt signaling pathway	DACT2	XM_419602	PREDICTED: Gallus gallus dapper, antagonist of beta-catenin, homolog 2
carrier protein	FGFBP1	XM_420773	PREDICTED: Gallus gallus fibroblast growth factor binding protein 1
thyroid hormone pathway	THRSP	NM_213577.1	Gallus gallus thyroid hormone responsive
protein-protein intraction	KLHDC8B	XM_414393	PREDICTED: Gallus gallus kelch domain containing 8B
Glucose and fatty acid metabolism	IAPP	NM_205397.1	Gallus gallus islet amyloid polypeptide
Housekeeping genes	EEF1A1	NM_204157	Eukaryotic translation elongation factor 1 alpha 1
	RPL4	NM_001007479	Ribosomal protein L4

Table S2. Fold changes and p-values of 27 dioxin-responsive genes on the PCR array following exposure of chicken embryonic hepatocytes to 1 or 25 µM of SI-TeDB-DiPhOBz, and 1 or 10 µM of SI-BDE-209 and 1 nM of TCDD, respectively. The fold change represents the mean value of three replicates and significant differences in fold change compared to the DMSO vehicle control were determined using a one-way ANOVA. The numbers in red signify that the mRNA levels were significantly altered (fold change ≥ 2.0, $p<0.05$).

Genes	SI-TeDB-DiPhOBz (1 µM)		SI-TeDB-DiPhOBz (25 µM)		SI-BDE-209 (1 µM)		SI-BDE-209 (10 µM)		TCDD (1 nM)	
	p value	Fold change	p value	Fold change	p value	Fold change	p value	Fold change	p value	Fold change
ATP12A	0.007	4.5	0.016	3.8	0.001	4.7	0.097	2.1	0.711	-1.2
B3GNT7	0.008	4.6	0.035	2.1	0.026	3.3	0.008	2.6	0.940	-1.1
CWH43	0.079	1.5	0.008	2.8	0.007	2.7	0.037	-1.9	0.017	3
CYP1A4	<0.001	670	<0.001	4200	0.002	2700	<0.001	2200	<0.001	2000
CYP1A5	<0.001	100	<0.001	300	<0.001	240	<0.001	140	<0.001	190
CYP1B1	<0.001	4.8	0.001	2.8	<0.001	5.9	0.006	1.9	0.003	5.1
FGF19	0.010	-2.5	0.009	-2.7	0.003	-6.9	0.003	-6.6	<0.001	-6.7
FGFBP1	0.892	1	0.001	2.8	0.684	1.1	0.381	-1.2	0.004	1.6
FRZB	0.070	-1.4	<0.001	-3.2	0.013	-1.4	<0.001	-6.1	0.008	-2
KLHDC8B	0.096	-1.5	0.007	-3.6	0.021	-2.2	0.005	-5.6	0.003	-2.9
KRT20	<0.001	3.8	<0.001	7	<0.001	7.6	0.004	1.5	0.001	4.8
MGP	0.004	2.5	0.390	2.3	0.005	1.9	0.344	-1.2	0.028	2.4
MMP7	0.296	1.2	0.482	1.1	0.095	1.3	<0.001	-8.7	0.607	1.1
PAH	0.004	8.6	<0.001	5.6	<0.001	11	0.014	1.9	0.009	5.6
RGN	<0.001	3.2	<0.001	14	<0.001	5.8	<0.001	8.8	<0.001	7.4
TGM2	0.019	3.7	0.001	3.4	<0.001	7.4	0.945	-1	0.040	4
TIPARP	<0.001	3.7	<0.001	5.1	0.001	5.4	0.005	2.9	0.011	5.2
IAPP	0.030	-1.8	0.002	-11	0.003	-5.9	0.002	-12	<0.001	-5
COL7A1	0.001	8.1	0.063	2.9	0.004	7.3	0.134	2.4	0.204	1.8
EFCAB4B	0.105	1.5	0.263	-1.6	0.887	1.1	0.018	-3.4	0.029	-2.1
AREGB	0.001	2.1	<0.001	2.1	<0.001	2.7	0.600	-1.1	0.055	2.8
CYP24A1	<0.001	-1.9	<0.001	-4.4	<0.001	-3.4	<0.001	-6.1	0.001	-2.9
DACT2	0.353	-1.2	0.721	-1	0.632	1	0.001	-3.6	0.304	1.3
PODXL	0.001	-1.9	<0.001	-1.7	<0.001	-2.3	<0.001	-5.5	0.017	-3.2
FABP3	0.006	2.3	<0.001	6.6	0.001	4	<0.001	4.4	0.014	4.2
IGFBP4	0.781	-1	0.062	-6.9	0.191	-2	0.051	-9.8	0.035	-2.1

THRSP	0.046	-1.8	0.012	-2.6	0.009	-3.7	0.004	-9	0.005	-2.5

Table S3. Spearman correlations between SI-TeDB-DiPhOBz and TCDD or SI-BDE-209 and TCDD

Parameter	1 uM SI-TeDBDiPhOBz vs TCDD	25 uM SI-TeDBDiPhOBz vs TCDD	1 uM SI-BDE-209 vs TCDD	10 uM SI-BDE-209 Vs TCDD
Number of XY Pairs	27	27	27	27
Spearman r	0.7749	0.8858	0.8949	0.8343
95% confidence interval	0.5515 to 0.8946	0.7575 to 0.9483	0.7755 to 0.9525	0.6584 to 0.9238
P value (two-tailed)	< 0.0001	< 0.0001	< 0.0001	< 0.0001
P value summary	***	***	***	***
Exact or approximate P value?	Gaussian Approximation	Gaussian Approximation	Gaussian Approximation	Gaussian Approximation
Is the correlation significant? (alpha=0.05)	Yes	Yes	Yes	Yes

Table S4. Detection of ions of dibenzofurans and polybenzofurans in photolytic degradation products of TeDB-DiPhOBz and BDE-209 as a result of APPI(-)-Q-TOF-MS analysis. Detailed mass characteristics are provided in Figure 3 and Figure S2.

	RT (min)	Molecular Formula	[M-Br+O]		Mass Defect (ppm)
			Therotical (Da)	Observed (Da)	
Dibenzofurans	7.3	$C_{12}O_1Br_3H_7$	340.8641	340.8641	<0.1
	7.8	$C_{12}O_1Br_3H_7$	340.8641	340.8643	0.6
	7.3	$C_{12}O_1Br_4H_6$	418.7746	418.7748	0.5
	7.4	$C_{12}O_1Br_4H_6$	418.7746	418.7749	0.7
Polybenzofurans	7.6	$C_{18}Br_3O_2H_{11}$	430.8747	430.8766	4.4
	8.1	$C_{18}Br_3O_2H_{11}$	430.8747	430.876	3.0
	8.4	$C_{18}Br_4O_2H_{10}$	508.7852	508.7851	0.2
	7.3	$C_{18}Br_5O_2H_9$	588.6937	588.694	0.5
	8.0	$C_{18}Br_5O_2H_9$	588.6937	588.694	0.5
	7.6	$C_{18}Br_5O_2H_9$	588.6937	588.693	1.2
	8.3	$C_{18}Br_6O_2H_8$	666.6042	666.6045	0.5
	9.0	$C_{18}Br_6O_2H_8$	666.6042	666.6045	0.5

a "M" in [M-Br+O] means molecular formula;
e Mass defect represents the difference between observed and theoretical m/z masses.

Figure S1. Equipment and Location for the sunlight irradiation of TeDB-DiPhOBz and BDE-209 solid powder.

Figure S2. Liquid chromatography-atmospheric pressure photoionization(-)-time-of-flight mass (LC-APPI(-)-TOF-MS) spectra extracted ion chromatograms (EICs) of solutions of sunlight irradiated-BDE-209. The mass spectral peak assignments are based on their specific mass spectrum characteristic (mass, peak response ratio, peak number) and retention time on the LC system. The bromine number is 3 and 4 in upper and lower Figures, respectively.

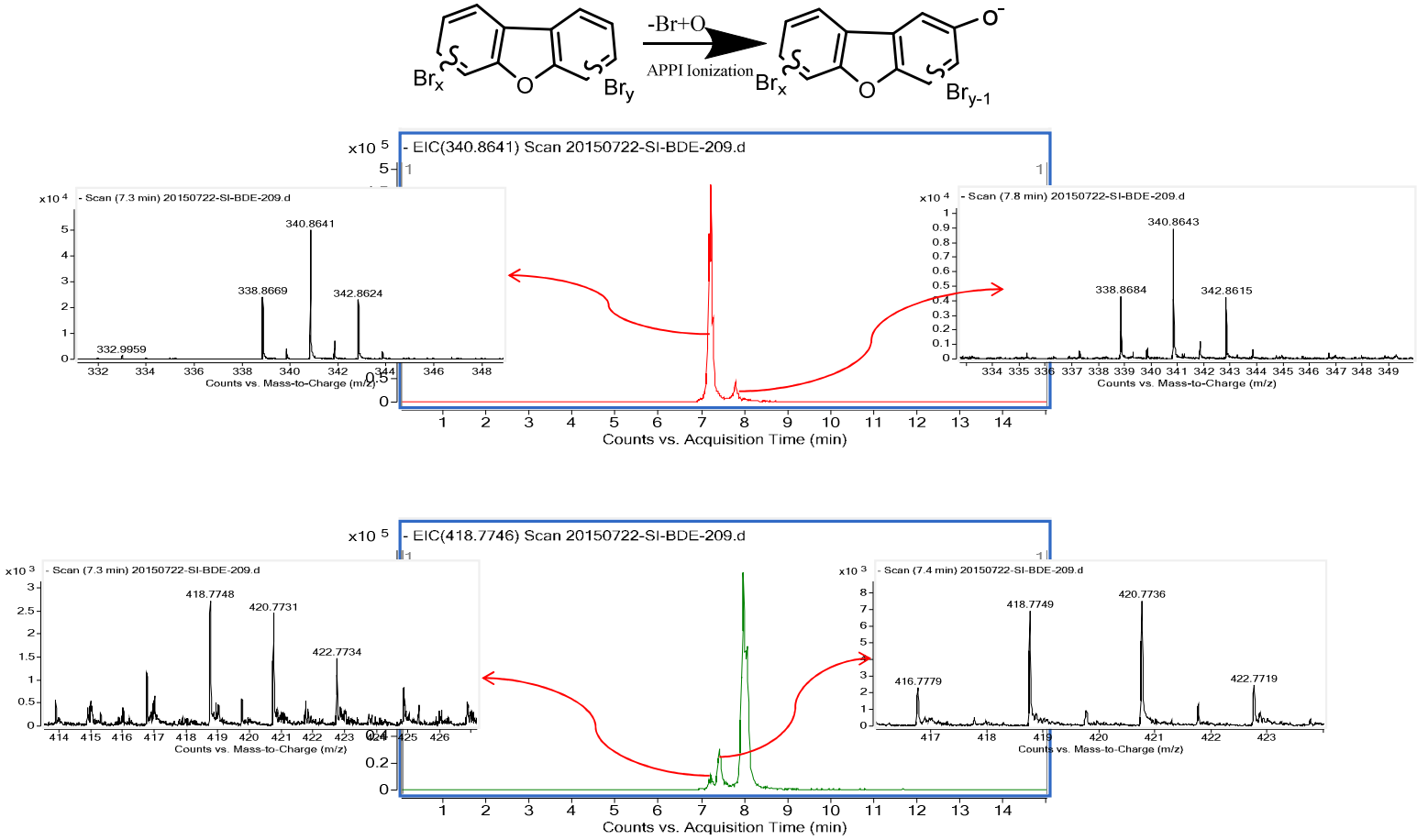


Figure S3. Schematic flow diagram of identification of furans in SI-BDE-209 and SI-TeDB-DiPhOBz solutions.

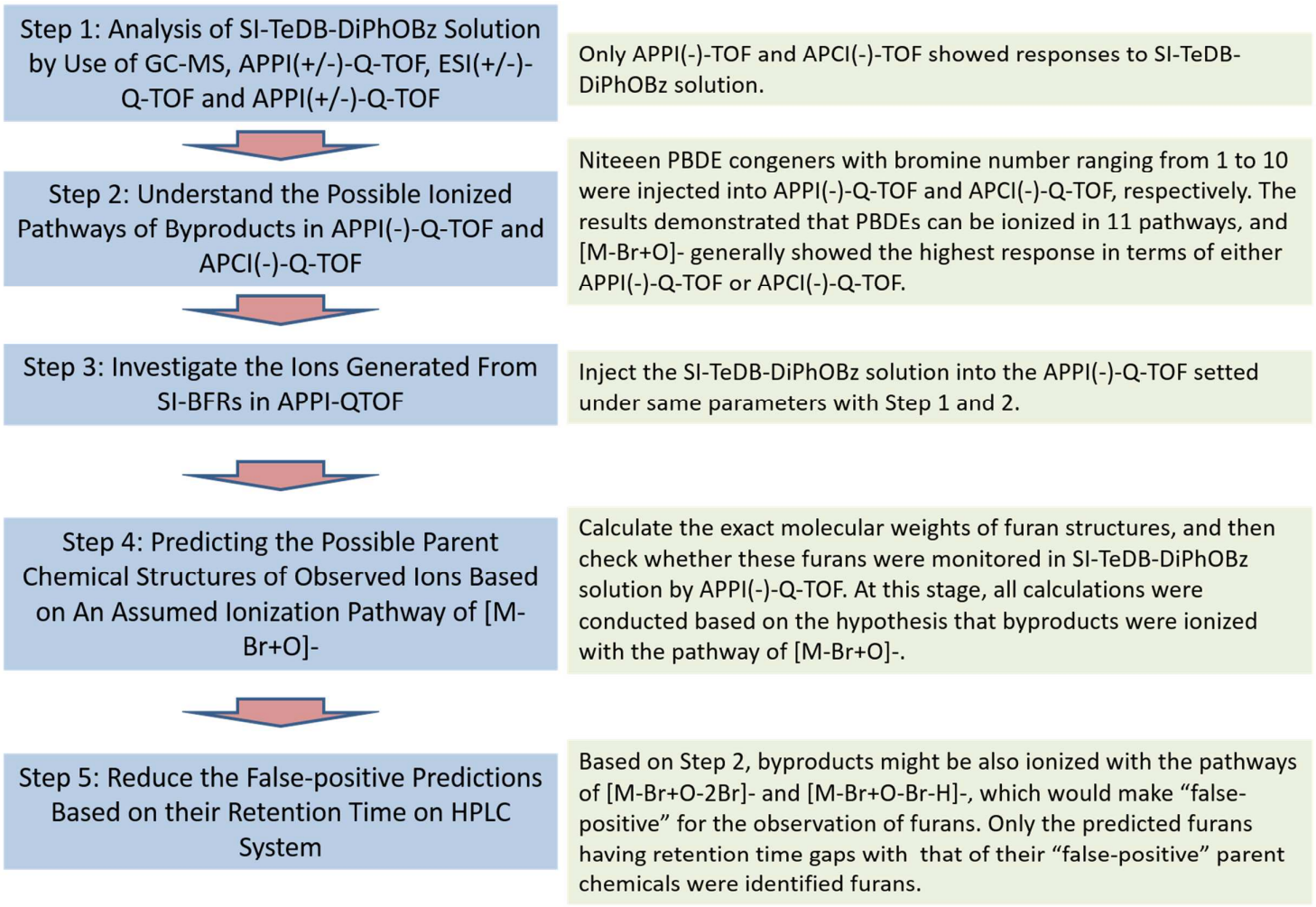


Figure S4. Observed Ionized Fragments of 19 PBDE Congeners After Injection into APPI-QTOF (Detailed fragment molecular formula or actual/theoretical masses are provided in Table 1.).

Figure S4A: BDE-209 (Br10)

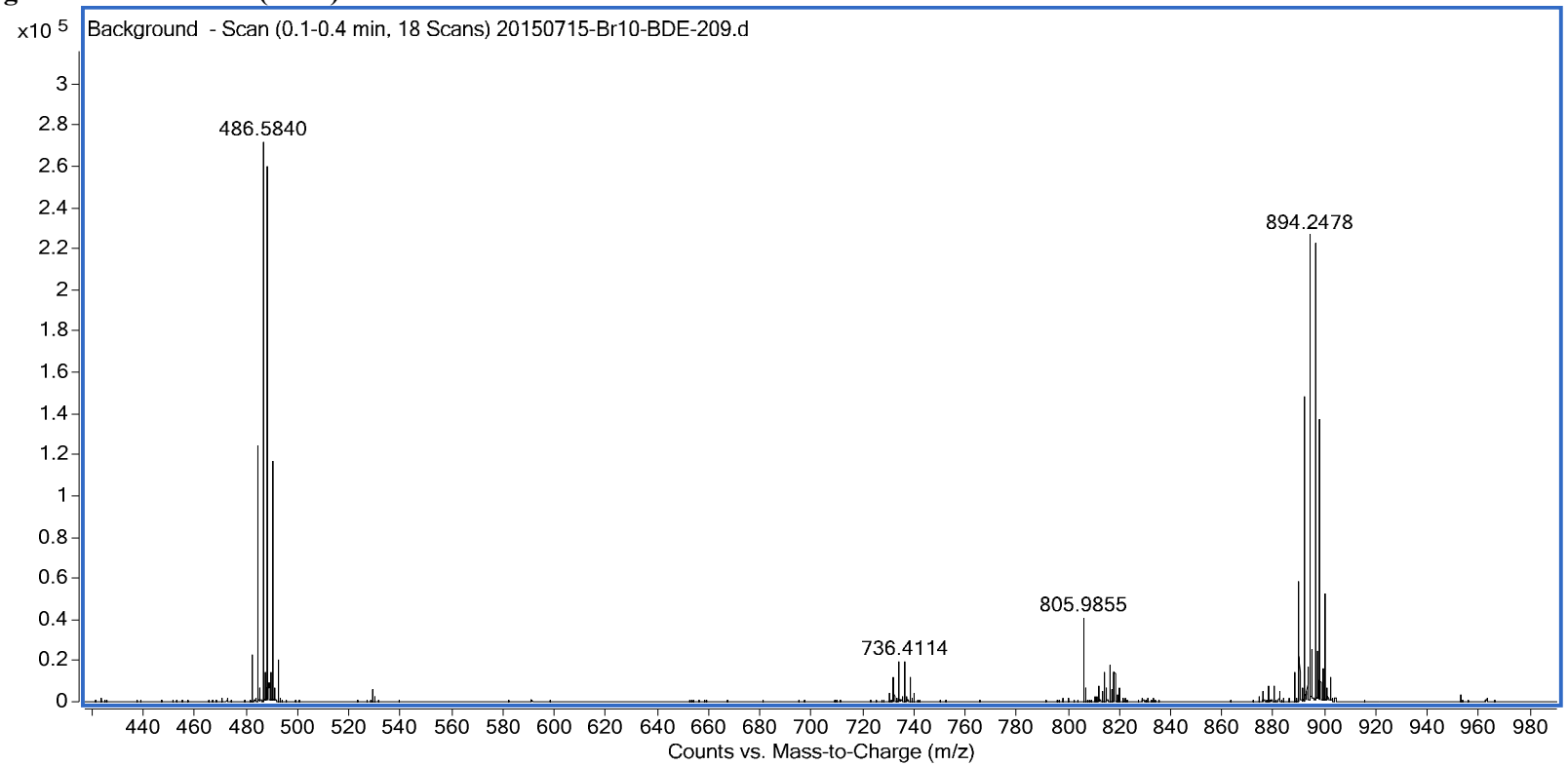


Figure S4B: BDE-208 (Br9)

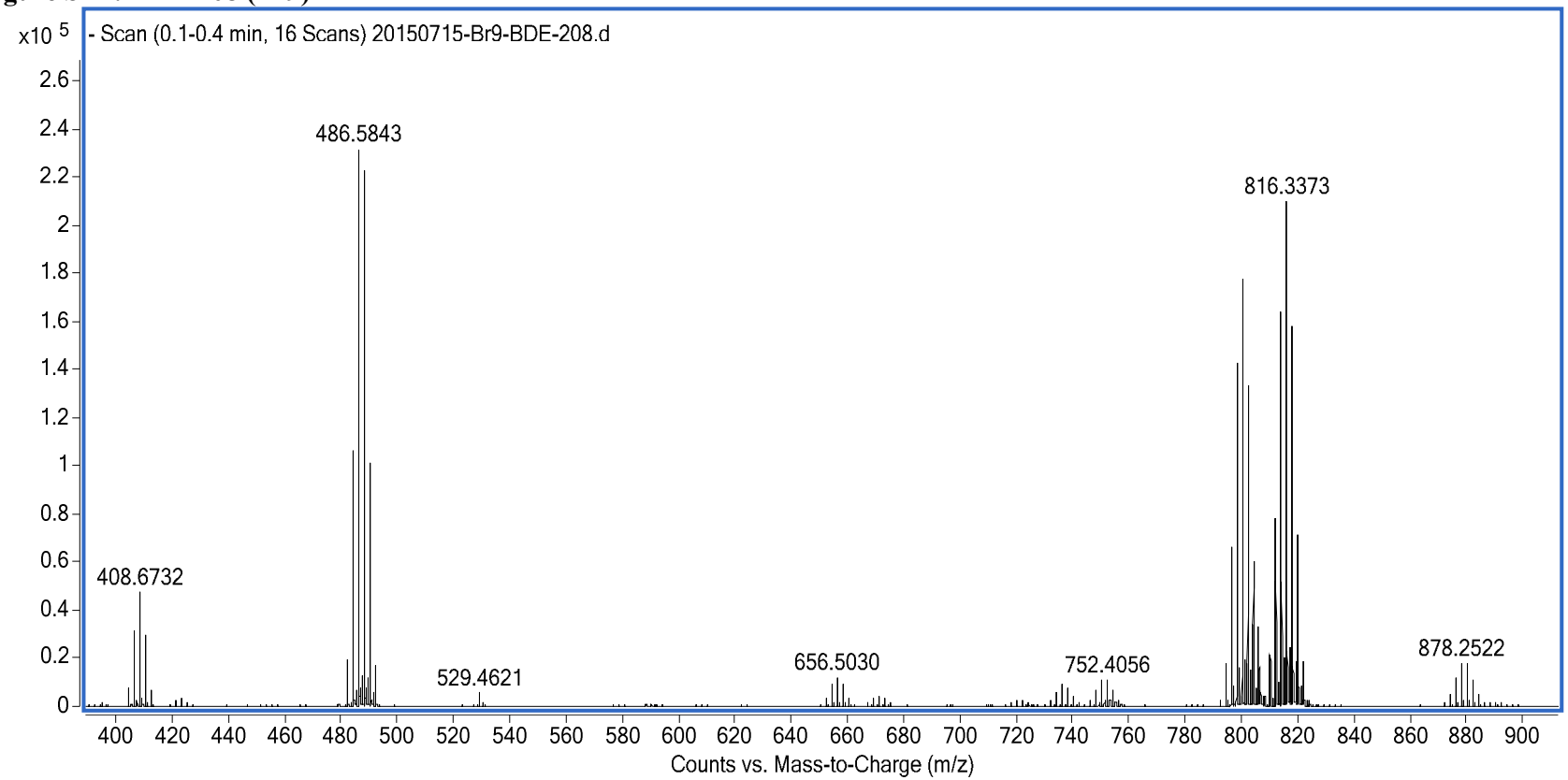


Figure S4C: BDE-207 (Br9)

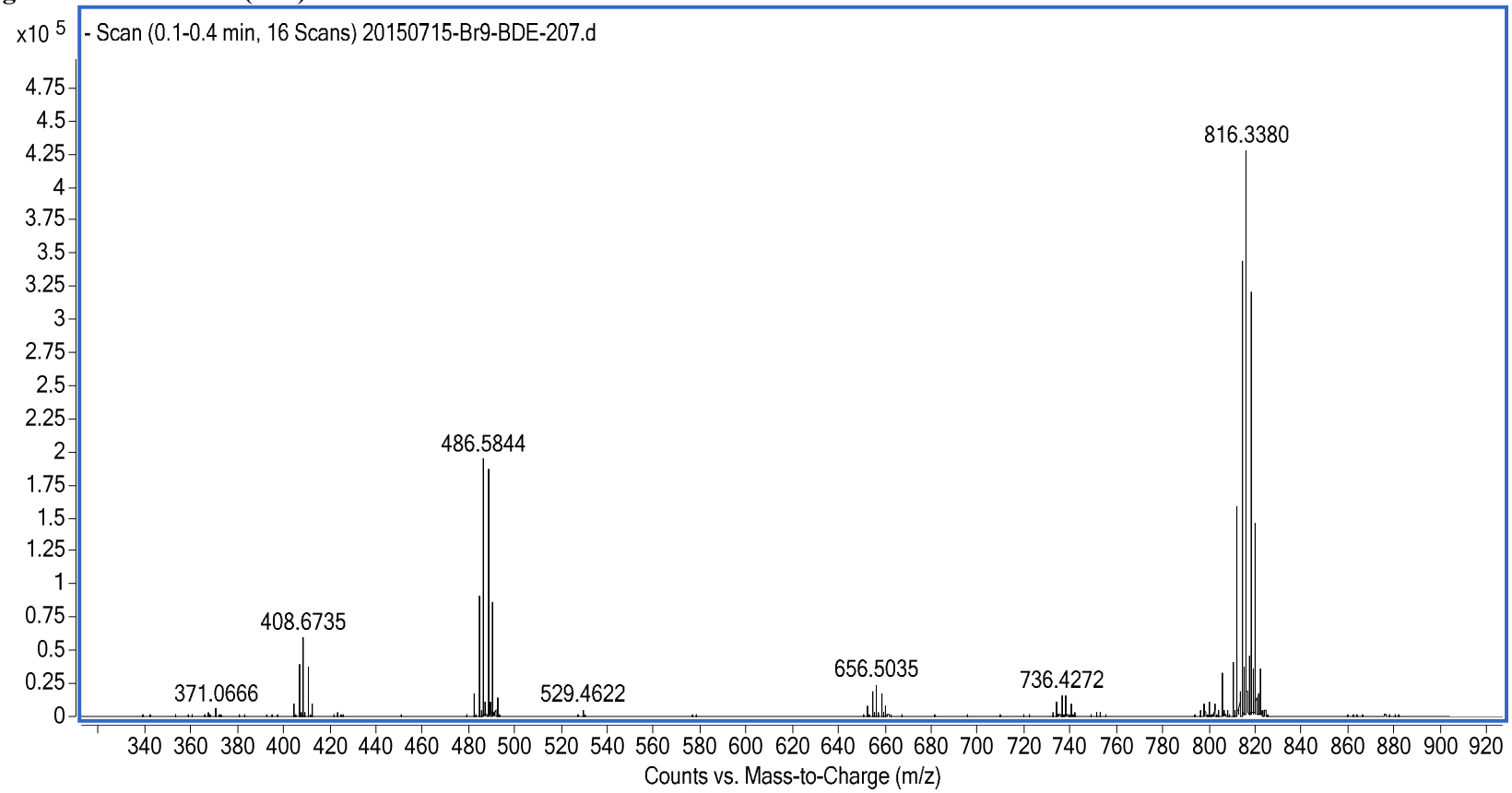


Figure S4D: BDE-197 (Br8)

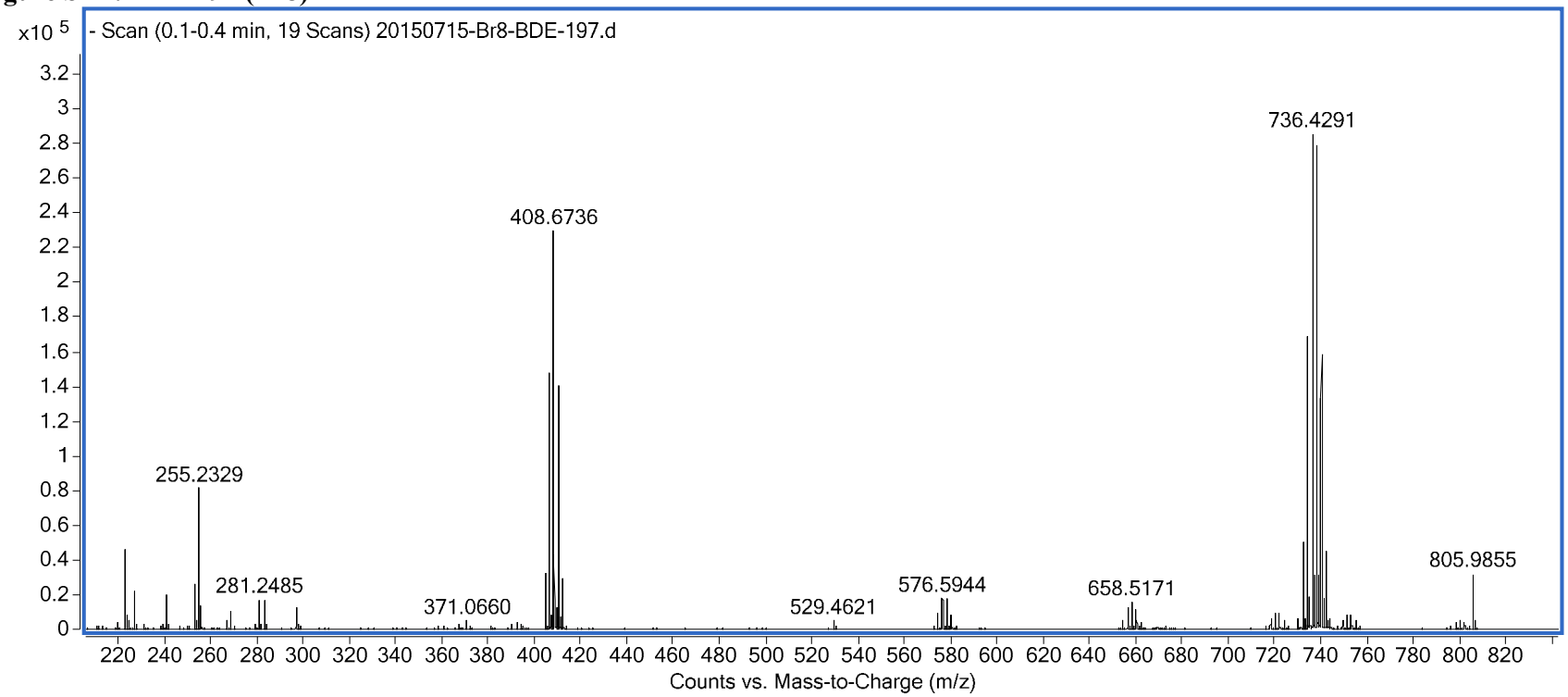


Figure S4E: BDE-194 (Br8)

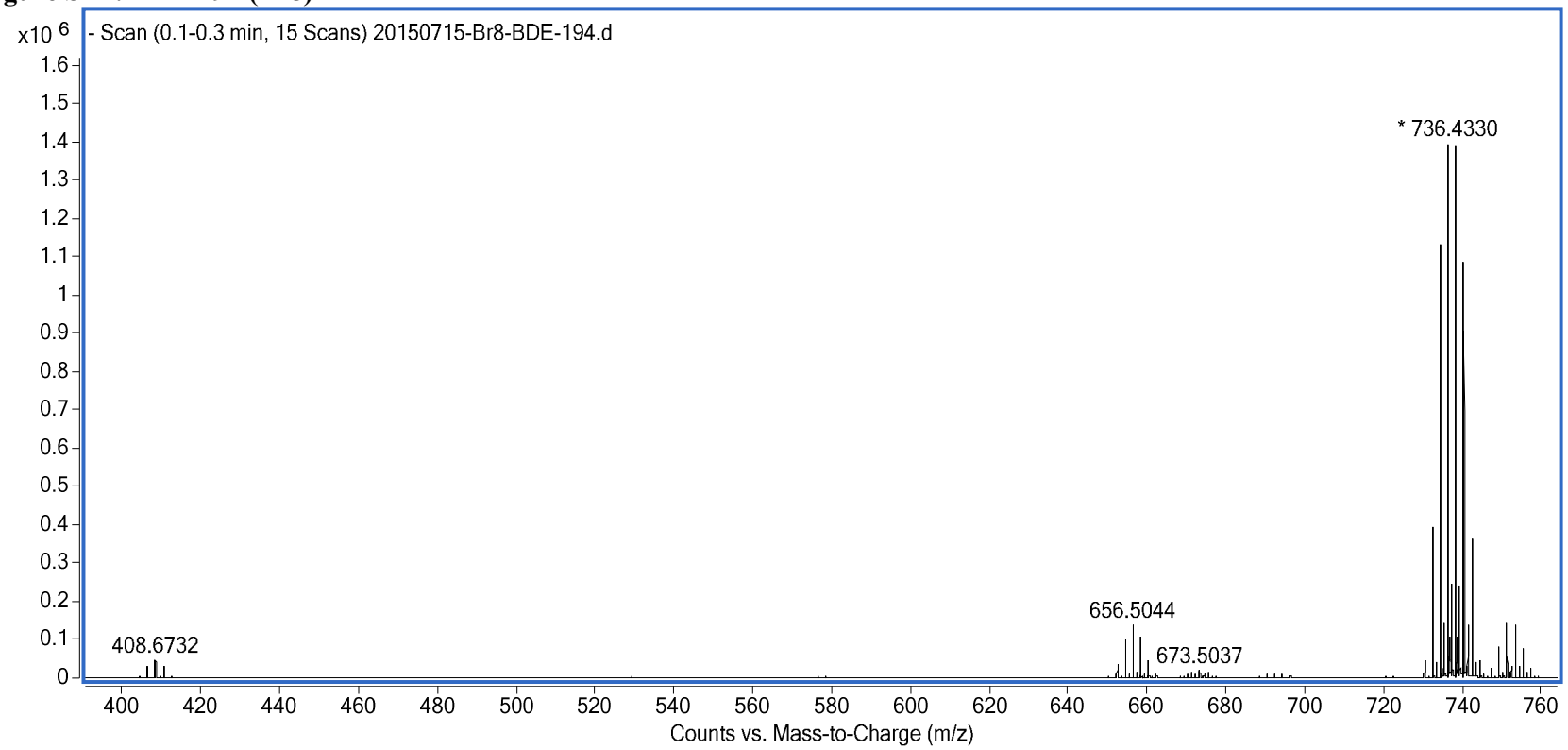


Figure S4F: BDE-180 (Br7)

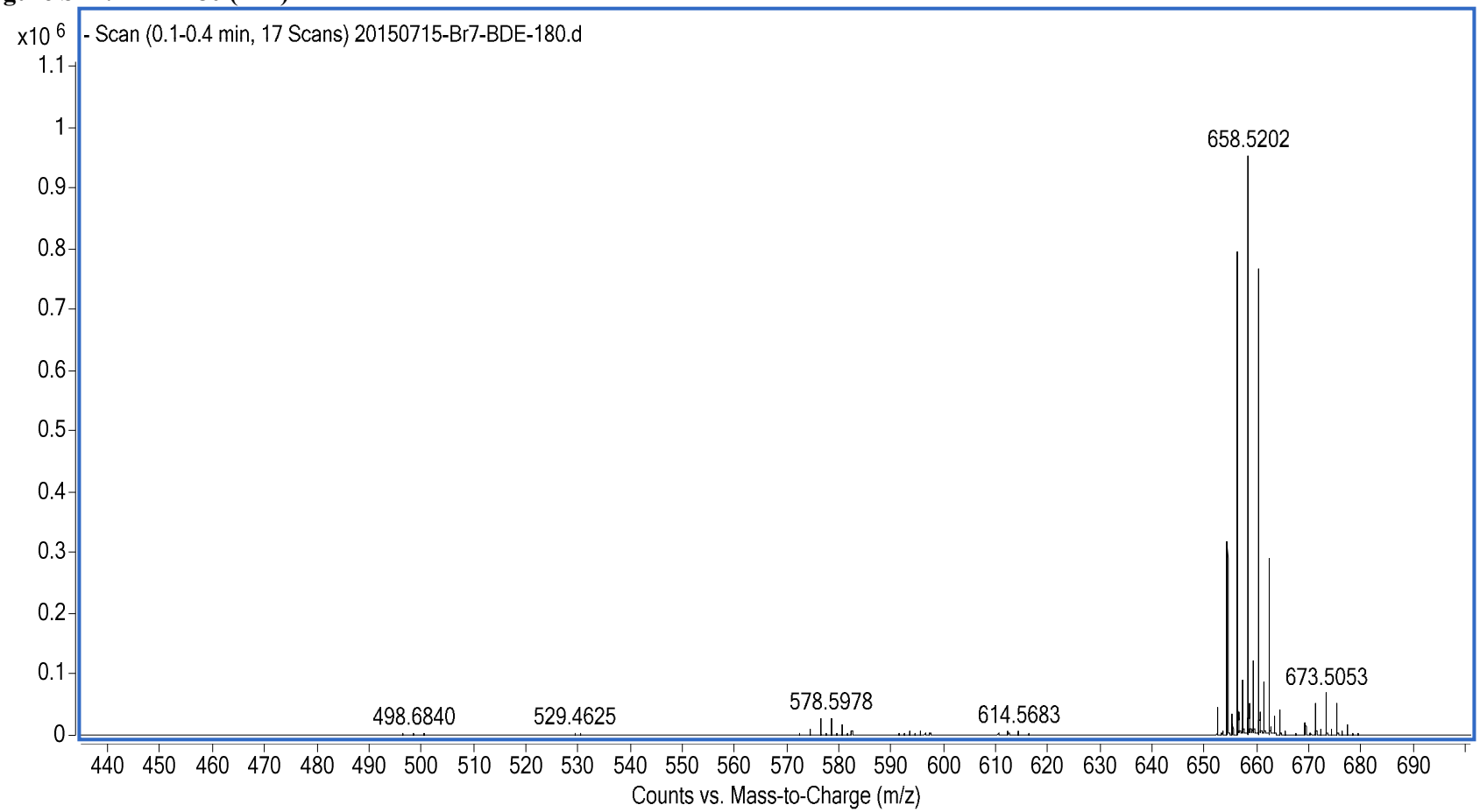


Figure S4G: BDE-170 (Br7)

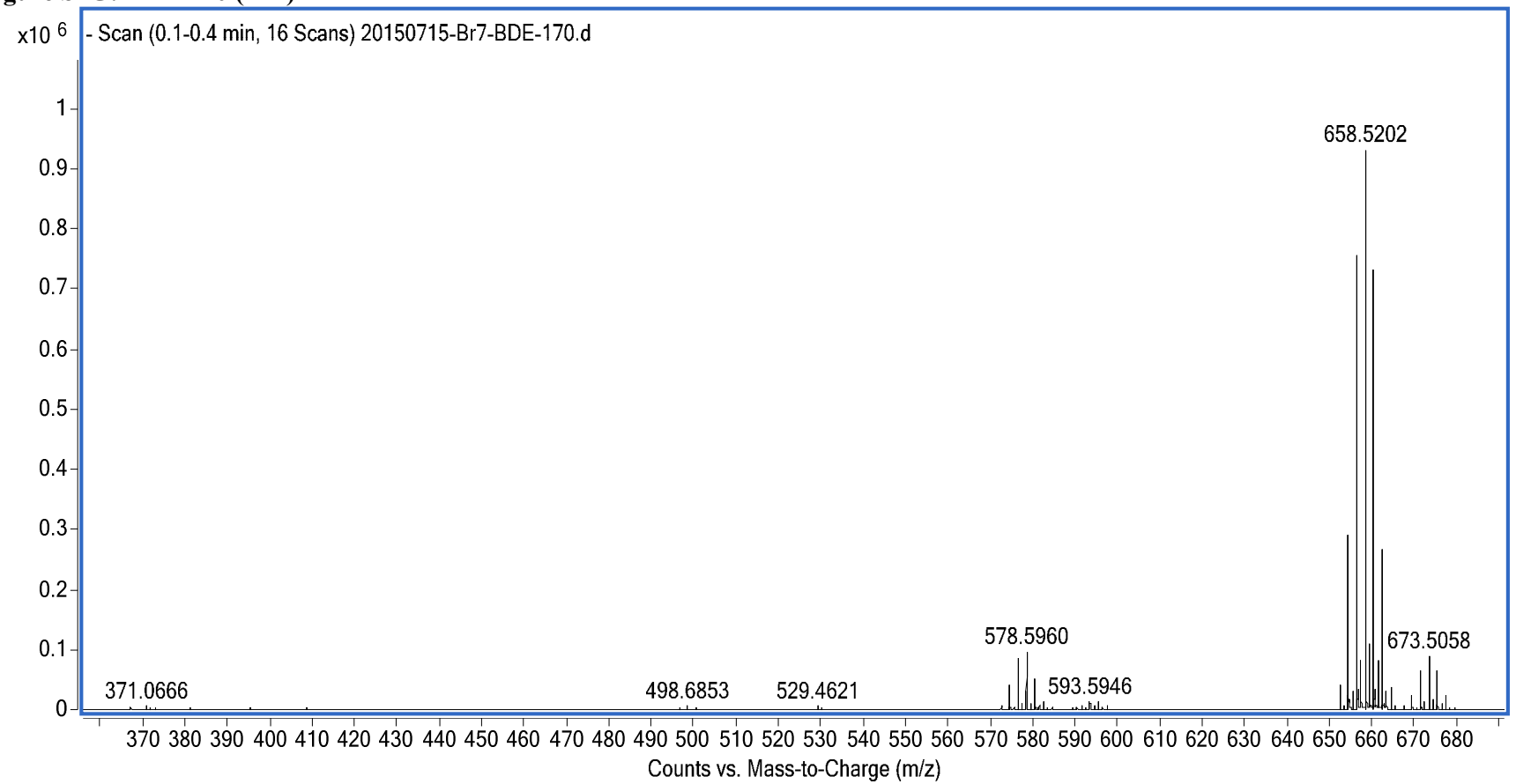


Figure S4H: BDE-154 (Br6)

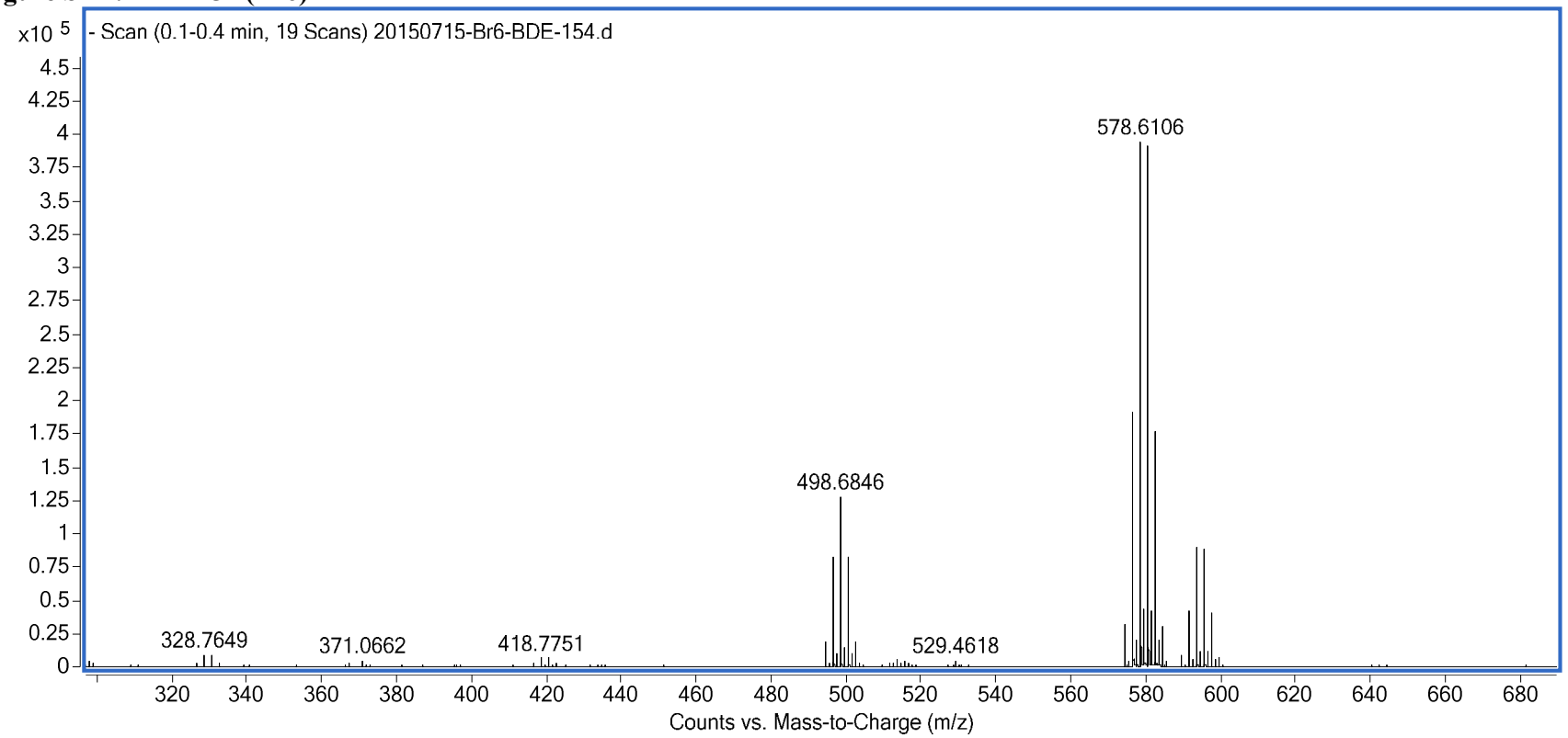


Figure S4I: BDE-153 (Br6)

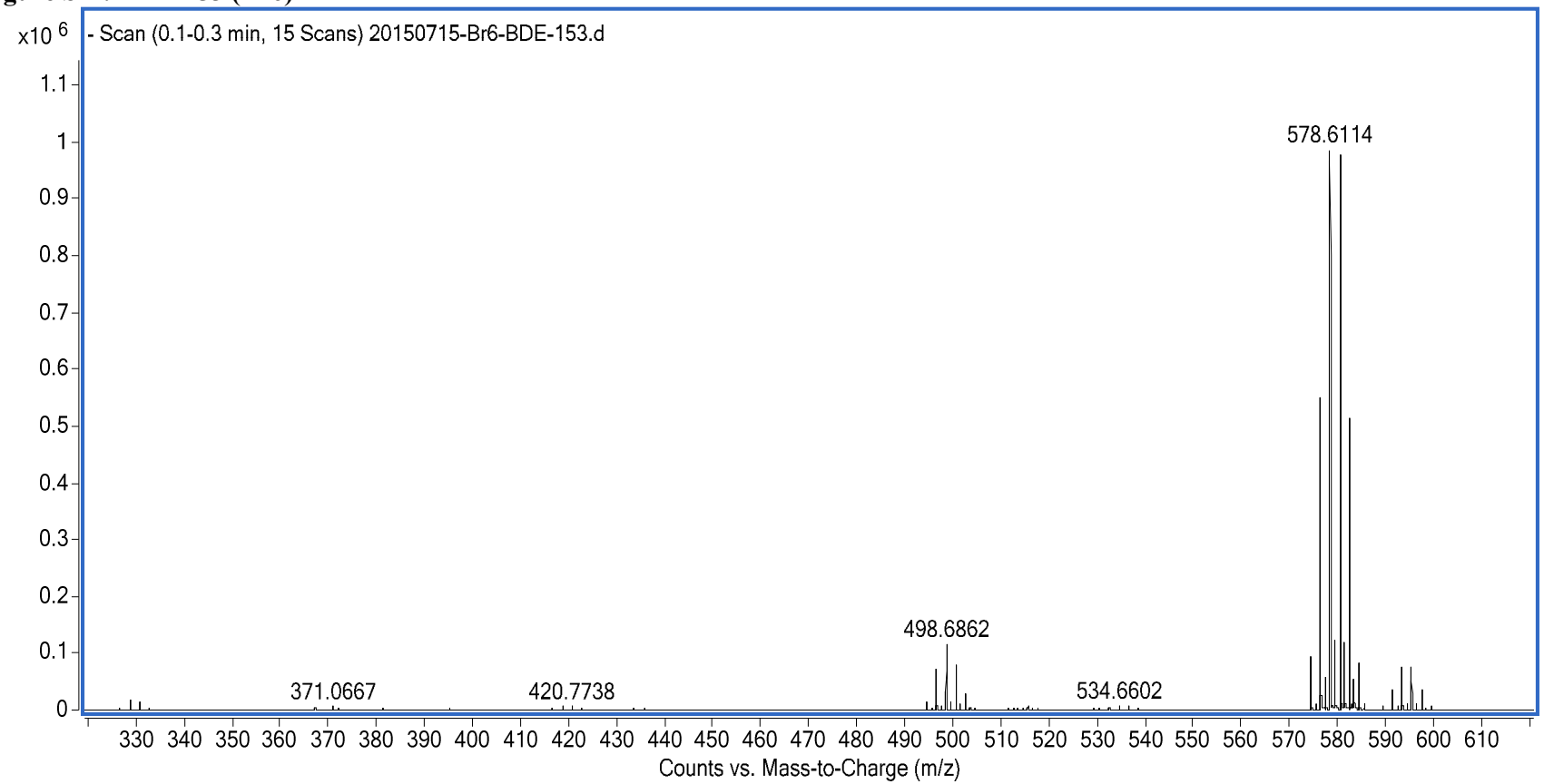


Figure S4J: BDE-99 (Br5)

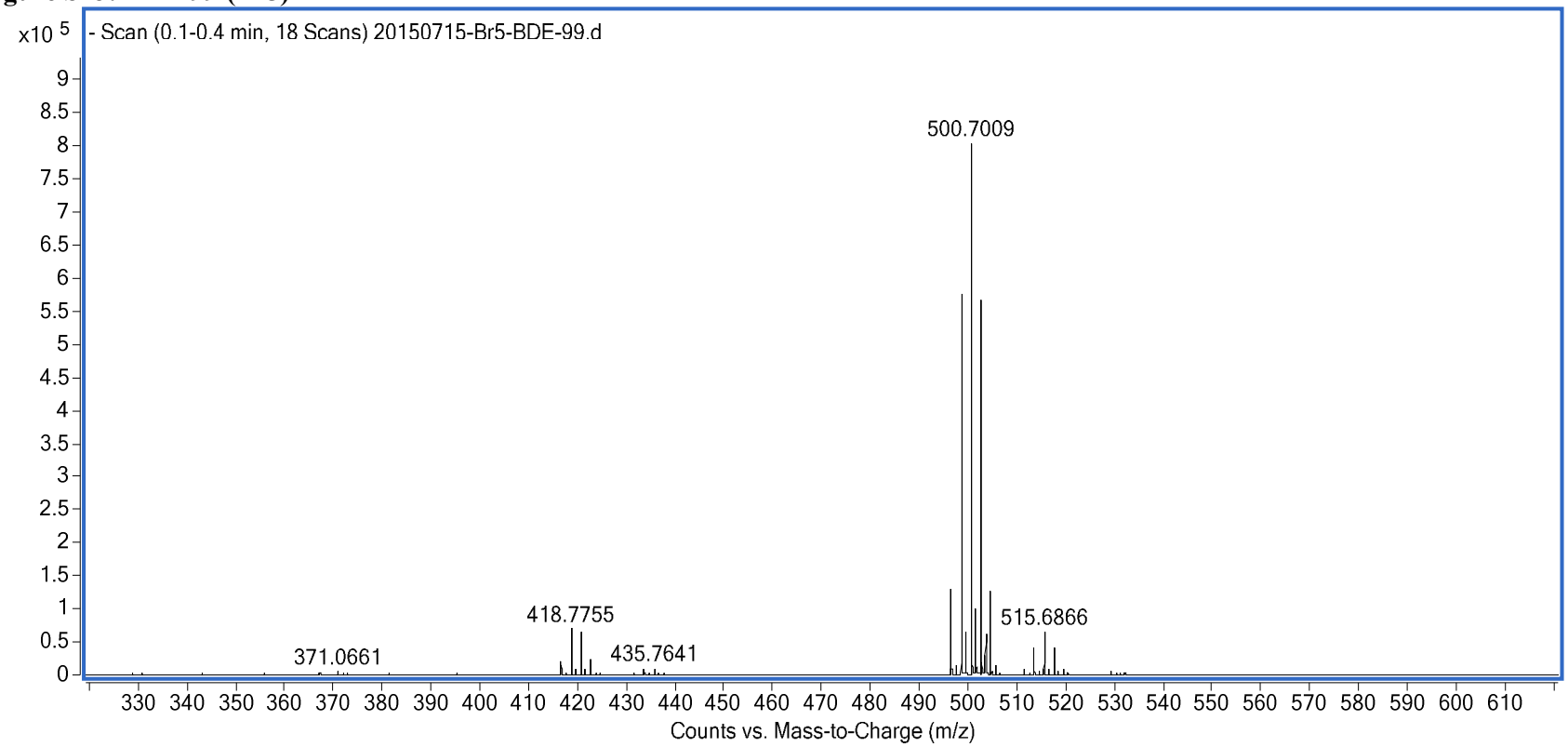


Figure S4K: BDE-85 (Br5)

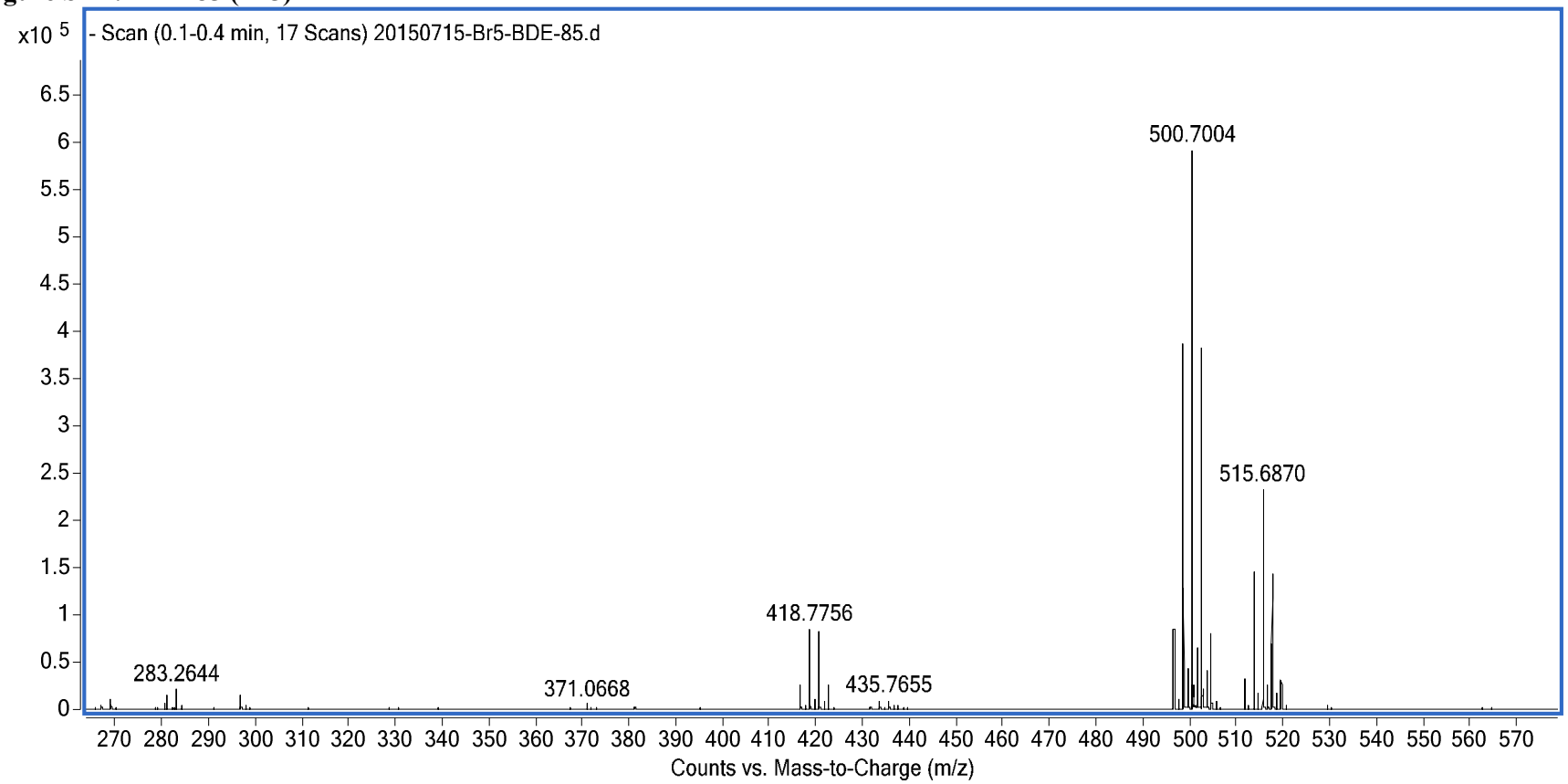


Figure S4L: BDE-66 (Br 4)

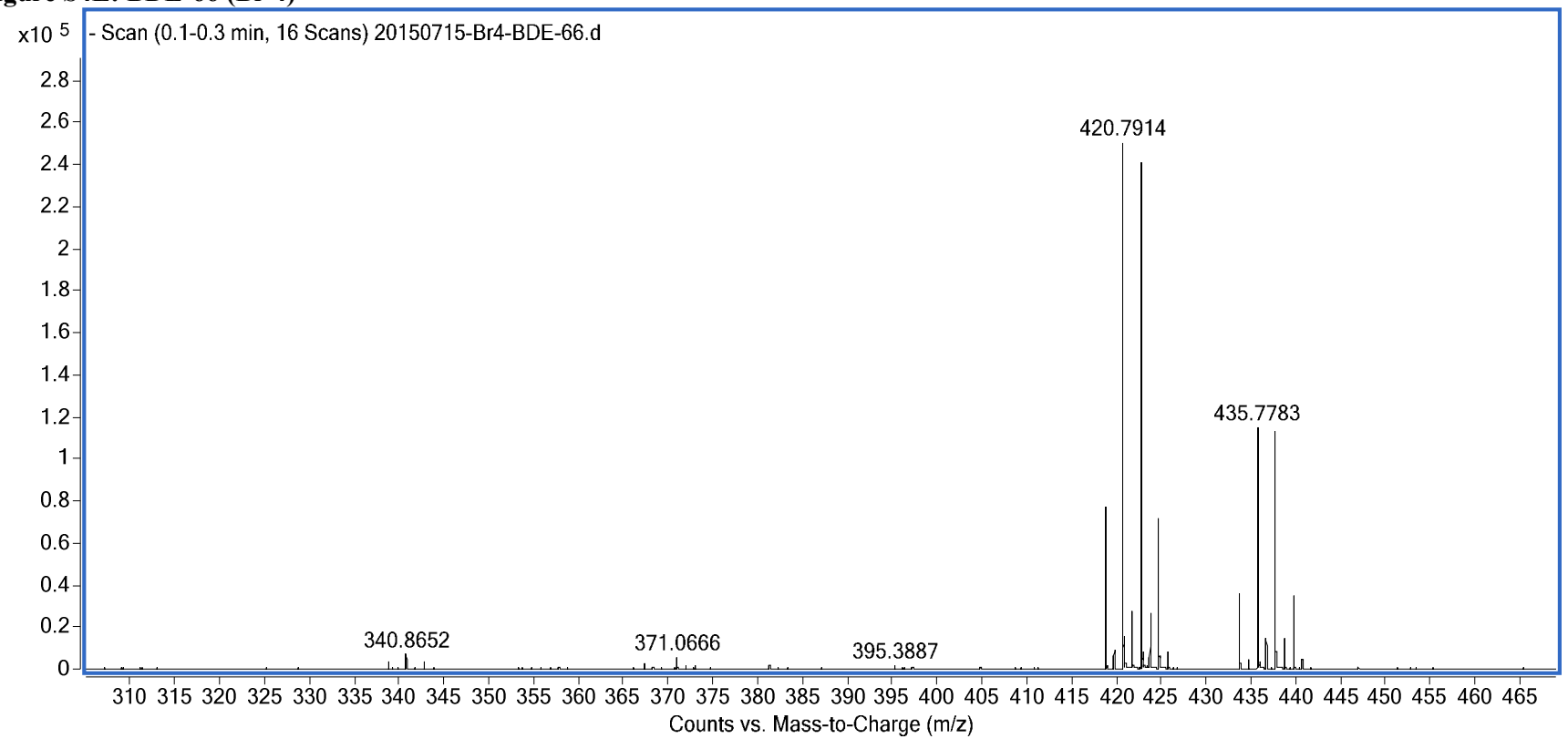


Figure S4M: BDE-47 (Br4)

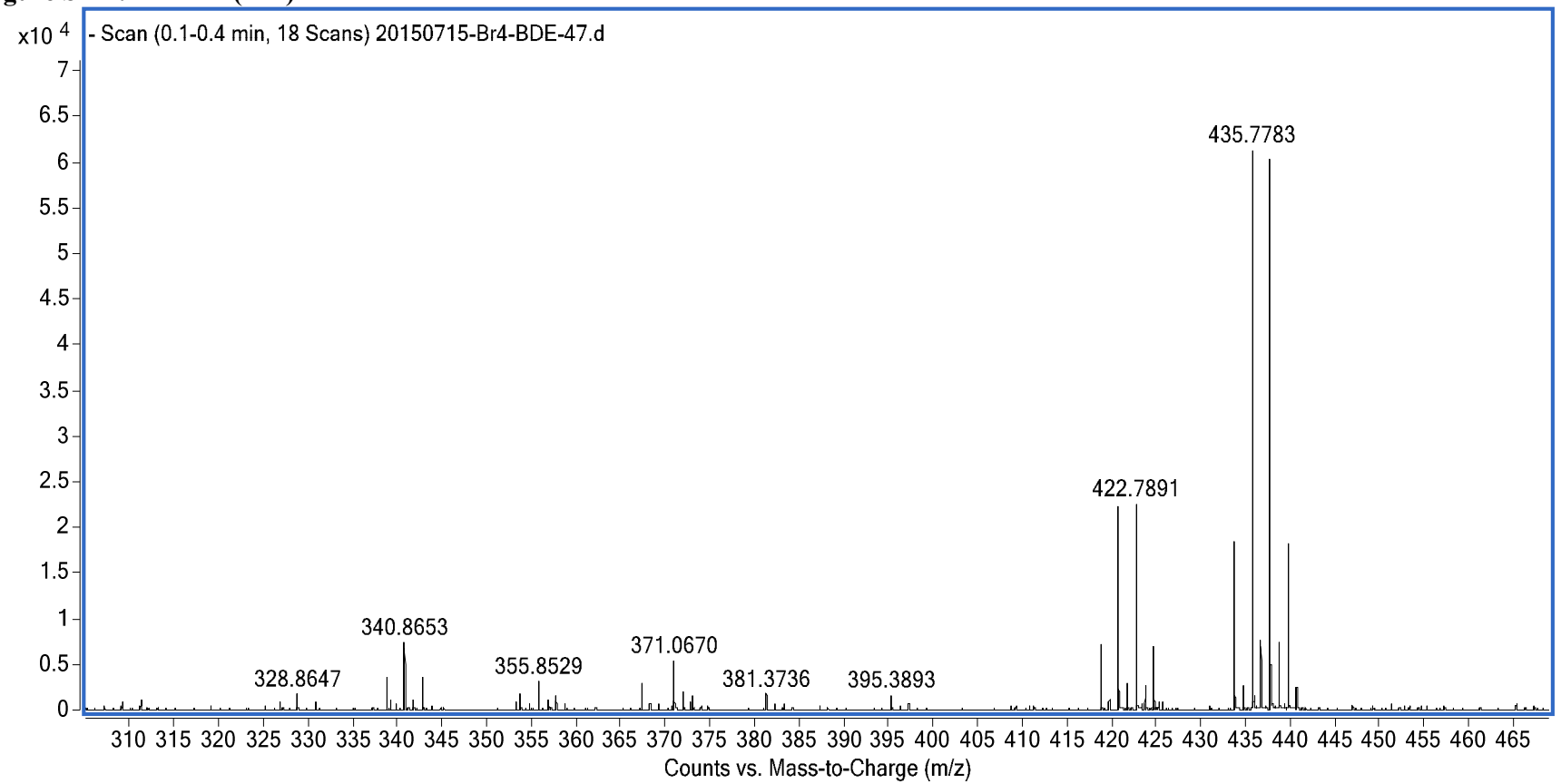


Figure S4N: BDE-28 (Br3)

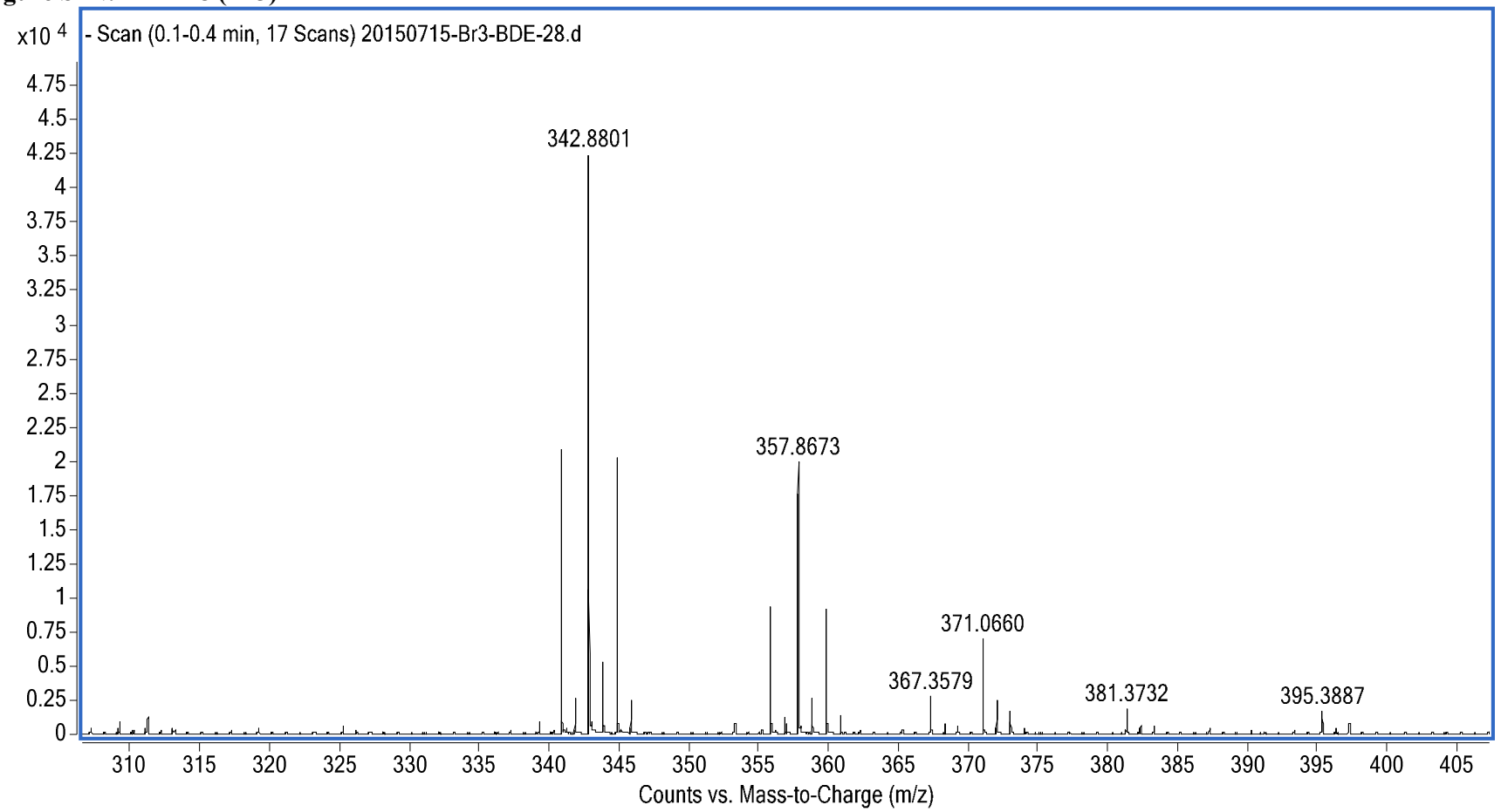
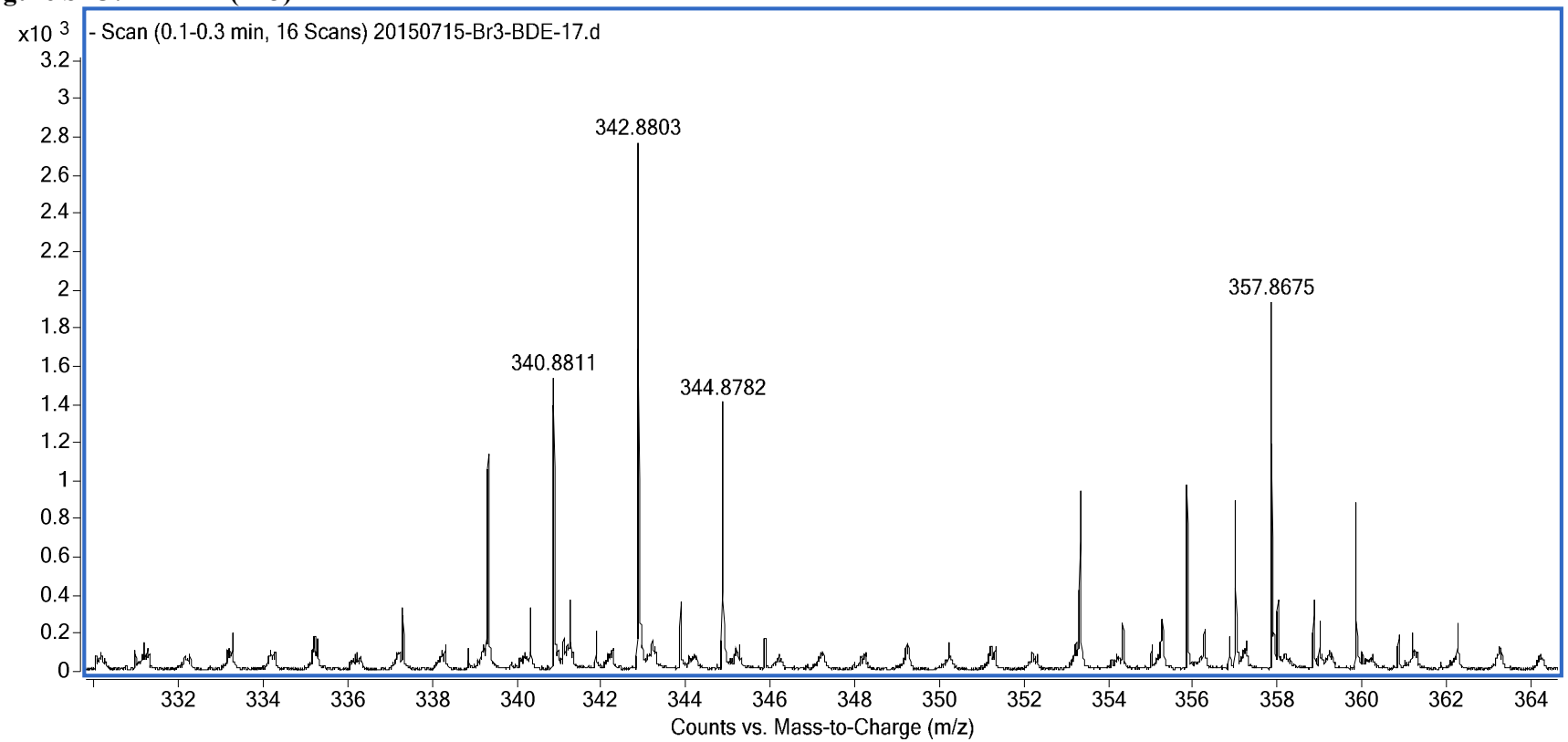


Figure S4O: BDE-17 (Br3)



1. Su, G.; Letcher, R. J.; Crump, D.; Farmahin, R.; Giesy, J. P.; Kennedy, S. W., Photolytic degradation products of two highly brominated flame retardants cause cytotoxicity and mRNA expression alterations in chicken embryonic hepatocytes. *Environ Sci Technol* **2014,** *48*, (20), 12039-46.
2. Porter, E.; Crump, D.; Egloff, C.; Chiu, S.; Kennedy, S. W., Use of an avian hepatocyte assay and the avian toxchip polymerse chain reaction array for testing prioritization of 16 organic flame retardants. *Environ Toxicol Chem* **2013,** *33*, (3), 573-582.
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