Supporting Information

Comparative Tissue Distribution, Biotransformation and Associated Biological Effects by Decabromodiphenyl Ethane and Decabrominated Diphenyl Ether in Male Rats After a 90 Day Oral Exposure Study

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Chemicals. BDE-209 and DBDPE (both ≥ 99% purity) were obtained from Shanghai Jiachen Chemical Co., Ltd.. Corn oil was purchased from Sigma–Aldrich (St. Louis, MO). PBDE standards, including BDE-28, 47, 66, 99, 100, 138, 153, 154, 183, 196, 197, 202, 203, 206, 207, 208, and 209, were purchased from AccuStandard (New Heaven, CT). DBDPE solution (25 μg/ml) was obtained from Wellington Laboratories (Canada). $^{13}C_{12}$-PCB-141 and $^{13}C_{12}$-BDE-209 (recovery standards), and $^{13}C_{12}$-PCB-208 (internal standard) were purchased from Cambridge Isotope Laboratories Inc. (MA). BDE-77, 181 (recovery standards) and BDE-118, 128 (internal standards) were obtained from AccuStandard. Before we selected these BDE congeners as recovery and internal standards, various types of samples (sediment, air, soil, house dust, fish, and bird) were analyzed from Chinese environment to check if there are these congeners in environmental samples. Only trace of BDE-77 was found in some samples from the highly contaminated sites (such as e-waste sites), and no other three congeners (BDE-118, BDE-128 and BDE-181) were detectable in samples.

Animals. Male SD rats (21-day-old) were housed individually and maintained in a mass air-displacement room with a 12-hour light–dark cycle at 20-26°C and a relative humidity of 50-70%. In the first month, rats accessed to feed and water ad libitum, and after 1 month acclimation, rats were separated into three groups (control, DBDPE and BDE-209 treatments) ranked by body weight (n = 12 rats per treatment). The doses of 10, 100, 500, and 1500 mg/kg BDE-209 was administrated per day for male mouse from postnatal days 21 and 70 (*1*). The results indicated that the effects of BDE-209 were observed in the 500 and 1500 mg/kg/d groups. Therefore, the BDE-209 and DBDPE groups were consecutively fed 100 mg/kg body weight /d BDE-209 or DBDPE suspended in corn oil orally via gavage for 90 days. Control rats received the same amounts of feed and corn oil in our study.

Only trace amounts of BDE-209, at 1.67 ± 0.6 ng/g, were present in feed with concentrations lower than were added. The amounts of BDE-209 in original corn oil are un-detectable. No DBDPE was detected in original corn oil and feed. All rats were sacrificed by cervical dislocation at the end of 90 days experiment. Blood was collected from heart after anesthesia using carbon dioxide and

centrifuged at 2000 × g at 4 °C for 15 min. Animal handling protocol was approved by the Institute of Zoology, Chinese Academy of Sciences Institutional Animal Care Committee.

DBDPE photolytic degradation. The technical DBDPE product for UV irradiation was purchased from the Albemarle Corporations (Saytex8010, USA). 0.002 g DBDPE was first dissolved in 10 mL acetone (ACE), tetrahydrofuran (THF), and toluene mixed solvent (20:30:50%) using ultrasonic treatment, then evaporated to 1 mL under a stream of nitrogen and finally filled with THF to 100 mL in volumetric flask, stored in darkness before irradiation.

A GGZ-125 125W high-pressure mercury lamp (Yaming Lighting Co., Shanghai, China) was housed on the top of reactor to serve as an irradiation source. A Branson 1200 mode ultrasonic cleaner (Branson Ultrasonic Corporation, USA) operating at a frequency 0f 47 KHz and an output power of 30 W was used as the ultrasonic source. One milliliter of DBDPE solution was sealed into a glass vial (Chromatographic Specialties INC, C223682, Canada) for irradiation experiments. The vials were put at the bottom of the reactor around the irradiation source on the top for 30 minutes.

Irradiation of the DBDPE solution under UV lamp produced a number of peaks, identified as hepta- to nona-BDPE congeners by their GC/EI-MS full scan mass spectra (Fig. S1). The dominating fragments in DBDPE and its debrominated products were ion fragment clusters corresponding to the cleavage of the ethyl bond. This result suggested DBDPE debrominated to lower brominated BDPEs in the photodegradation experiment.

Sample extraction and analysis. Briefly, subsamples (1-2 g) were spiked with recovery standards ($^{13}C_{12}$-PCB-141, BDE-77, BDE-181, and $^{13}C_{12}$-BDE-209), and Soxhlet extracted with 1:1 hexane: acetone (V/V) for 48 h. Lipid content was determined on an aliquot of extract gravimetrically. The rest of the extract was further purified with gel permeation chromatography and a 2-g silica gel solid-phase extraction column (Isolute, International Sorbent Technology, UK). The PBDEs and DBDPE were eluted from the silica column with 6.5 mL of 60:40 hexane/dichloromethane following

the first fraction (3.5 mL of hexane). The extract was further concentrated to 200 μL and spiked with internal standards (BDE-118, BDE-128, and $^{13}C_{12}$-PCB-208).

Samples were analyzed with a Shimadzu model 2010 gas chromatograph-mass spectrometer (GC/MS) (Shimadzu, Japan) under electron capture negative ionization (ECNI) in the selected ion monitoring (SIM) mode using a DB-5HT capillary column (15 m length, 0.25 mm diameter, 0.10 μm thickness). The instrumental conditions are presented elsewhere (*2*) Ion fragments m/z 79 and 81 ($[Br]^-$) were monitored for PBDEs, DBDPE, and their possible degradation/ metabolic products. For BDE-209 and $^{13}C_{12}$-BDE209, m/z 486.7, 488.7 and m/z 494.6, 494.6 were respectively recorded. Fragments monitored for surrogate $^{13}C_{12}$-PCB-141 and internal standard $^{13}C_{12}$-PCB208 are m/z 372, 374, 376, and 474, 476, 478, respectively.

The solution from photolytic degradation experiment and extractions of selected tissue samples from DBDPE exposed rats were also analyzed by a Shimadadzu 2010 GC-MS and a Thermo TRACE GC coupled to a DSQII MS operating in the full-scan in EI (electron impact) ionization mode. The GC column and oven temperature program were identical to those used in ECNI analysis. In EI mode the electron energy was 70 eV with an ion source temperature of 230°C and a scan time of 0.8 s. Full-scan EI mass spectrums from m/z 50 to 1050 were obtained.

QA/QC and data analysis. Procedural blanks covering the whole procedure were run in parallel with the samples on each bath of extraction. Blind triplicate samples, triplicate spiked blanks, and triplicate spiked matrices were performed throughout the study. BDE-209 was found in procedural blanks, but the levels were significantly ($p < 0.05$) lower than the levels in the samples from the control rats. BDE-209 concentrations in samples were corrected from background concentrations of BDE-209 by subtracting three times the mean BDE-209 level in the blanks. The relative standard deviations (RSD) among triplicates samples were on average 3- 9.6% for all targets. Recovery of recovery standards averaged from 66 to 102% in all the samples. The limit of detection (LOD) was established from laboratory blanks as three times the standard deviation of the mean, or, for congeners

not detected in the blanks, LOD was defined as the instrumental limit of quantification (signal/noise = 5). Typical LODs ranged from 0.0023 to 0.0923 ng/g lw for tri- to hepta- BDEs, from 0.0001 to 4.3413 ng/g lw for octa- to nona-BDEs, from 0.0179 to 2.1516 ng/g lw for BDE-209, from 0.0062-1.0426 ng/g lw for DBDPE, depending on the sample size.

Thyroid hormone and serum parameters analysis. Standard spectrophotometric methods for the HITAC7170A automatic analyzer were used to measure the following serum parameters: total bilirubin (T-Bil), serum alanine aminotransferase (ALT), total bile acids (TBA), alkaline phosphatase (ALP), albumin (ALB), aspartate aminotransferase (AST), creatine kinase (CK), urea nitrogen (BUN), creatinine (Cr), high density lipid-cholesterol (HDL-C), total cholesterol (T-CHO), triglyceride (TG), low density lipid-cholesterol (LDL-C), and blood glucose (Glc). Differences between the control and the treatment groups were determined using a one-way analysis of variance (ANOVA). The calculation was based on discrimination significance between classes at the level of $p < 0.05$.

cDNA preparation and condition of real time polymerase chain reaction. cDNA was prepared with a reverse transcription system, which was performed using oligo-(dT)15 primer (Promega, Madison, WI) and M-MuLV reverse transcriptase (Promega, Madison, WI) according to the manufacturers' instructions. A Stratagene Mx3000P Real Time PCR (Polymerase chain reaction) apparatus (Stratagene, Cedar Creek, USA) was used to monitor real-time PCR amplification and detection by SYBR Green I technology. Each 25 μL reaction mixture was composed of 11.25 μL of SYBP Premix Ex Taq with ROX II dye(Tiangen, China), forward and reverse primers (10 μM, 0.5 μL each), the cDNA sample (1 μL), and 10.75 μL of nuclease-free water. β-actin was used as a housekeeping gene for data analysis. The PCR amplification protocol was as follows: 95°C for 2 min followed by 40 cycles of 94°C for 5 s, 56°C for β-actin (54°C for CYP1A2, 53°C for CYP2B1, 56°C for CYP2B2, 50°C for CYP2C6, 47°C for CYP3A2) for 15 s, and 72°C for 10 s.

Literature cited

1. Tseng, L. H.; Lee, C. W.; Pan, M. H.; Tsai, S. S.; Li, M. H.; Chen, J. R.; Lay, J. J.; Hsu, P. C. Postnatal exposure of the male mouse to 2, 2', 3, 3', 4, 4', 5, 5', 6, 6'-decabrominated diphenyl ether: Decreased epididymal sperm functions without alterations in DNA content and histology in testis. *Toxicology* **2006**, *224*, 33–43.
2. Hu, G. C.; Luo, X. J.; Dai, J. Y.; Xu, M. Q.; Mai, B.X. Brominated flame retardants, polychlorinated biphenyls, and organochlorine pesticides in captive Giand Panda (*Ailuropoda melanoleuca*) and Red Panda (*Ailurus fulgens*) from China. *Environ*. *Sci*. *Technol*. **2008**, *42*, 4704–4709.

Table S1. Sequences of primers used for real-time RT-PCR amplification.

Target gene	GeneBank accession no.	5'-3' primer sequences [b]	Product length (bp)	Tm (°C)
β-actin	NM_031144	FW: TCGTGCGTGACATTAAAGAG	134	56
		RW: ATTGCCGATAGTGATGACCT		
CYP1A2	NM_000761	FW: GTGGAATCGGTGGCTAAT	105	54
		RW: CACAAAGTCCTTGCTGCTC		
CYP2B1	NM_001134844	FW: GCCTCCTCAATTCCTTCA	99	53
		RW: TGTCTGTCCCACATAGCAT		
CYP2B2	XM_001062335	FW: AGGAGAAGTCGAACCACCAC	82	56
		RW: GAGCAGGAAACCATAGCG		
CYP2C6	XM_001066767	FW: TGTAGAGTTTCAGGGATGG	94	50
		RW: AGCAGTGAGATTGGGAAG		
CYP3A2	NM_153312	FW: GTCTCATAAAGCCCTGTC	81	47
		RW: CTGCTGGTGGTTTCATAG		

[a] GeneBank accession number (http://www.nvbi.nlm.nih.gov) used to design the primers.

[b] FW: forward primer; RW: reverse primer.

Table S2 Body weight, absolute and relative liver and kidney weight of rats exposed to 100 mg/kg/d DBDPE and BDE-209 for 90 days

	Control	DBDPE (100 mg/kg/d)	BDE-209 (100 mg/kg/d)
Body weight (g)	460.83 ± 5.01	498.5 ± 6.32	494.67 ± 10.49
Absolute liver weight (g)	13.69 ± 0.10	13.97 ± 0.68	15.51 ± 0.82
Absolute kidney weight (g)	3.24 ± 0.15	3.30 ± 0.12	3.42 ± 0.16
Relative liver weight (%)[a]	2.98 ± 0.05	3.00 ± 0.09	2.80 ± 0.09
Relative kidney weight (%)[a]	0.70 ± 0.03	0.67 ± 0.02	0.65 ± 0.02

Data are given as mean ± SEM from six rats per group. [a]Percentage of total body weight.

Table S3. Effects of DBDPE or BDE-209 on selected clinical chemistry parameters

Parameters	Control	DBDPE (100 mg/kg/d)	BDE-209 (100 mg/kg/d)
ALT (U/L)	50.33 ± 3.65	49.50 ± 1.34	57.67 ± 7.03
AST (U/L)	112.83 ± 10.48	93.50 ± 8.20*	101.83 ± 3.68
ALB (g/L)	39.10 ± 0.40	38.28 ± 0.52	38.32 ± 0.95
ALP (U/L)	164 ± 22.59	130.83 ± 10.73*	148.17 ± 14.74
CK (U/L)	2137.33 ± 154.13	2171.83 ± 166.39	2157.67 ± 130.89
T-CHO (mmol/L)	1.40 ± 0.06	1.43 ± 0.04	1.72 ± 0.12*
TG (mmol/L)	1.24 ± 0.16	1.23 ± 0.17	1.18 ± 0.11
HDL-C (mmol/L)	1.06 ± 0.04	1.13 ± 0.04	1.34 ± 0.10*
LDL-C (mmol/L)	0.22 ± 0.01	0.19 ± 0.02	0.25 ± 0.02
Glucose (mmol/L)	7.79 ± 0.22	7.68 ± 0.13	8.20 ± 0.21
BUN (mmol/L)	5.32 ± 0.22	5.23 ± 0.16	5.30 ± 0.21
Cr (μmol/L)	23.33 ± 1.54	17.67 ± 0.67*	21.83 ± 1.47
TBA (μmol/L)	7.6 ± 0.39	11.00 ± 1.28*	15.03 ± 1.38*
T3 (ng/ml)	0.53 ± 0.07	0.8 ± 0.041*	0.59 ± 0.08
T4 (ng/ml)	71.73 ± 5.37	85.52 ± 3.75	75.82 ± 3.04

Data are given as mean ± SD from six rats per group serum. * Significant difference from control, $p < 0.05$.

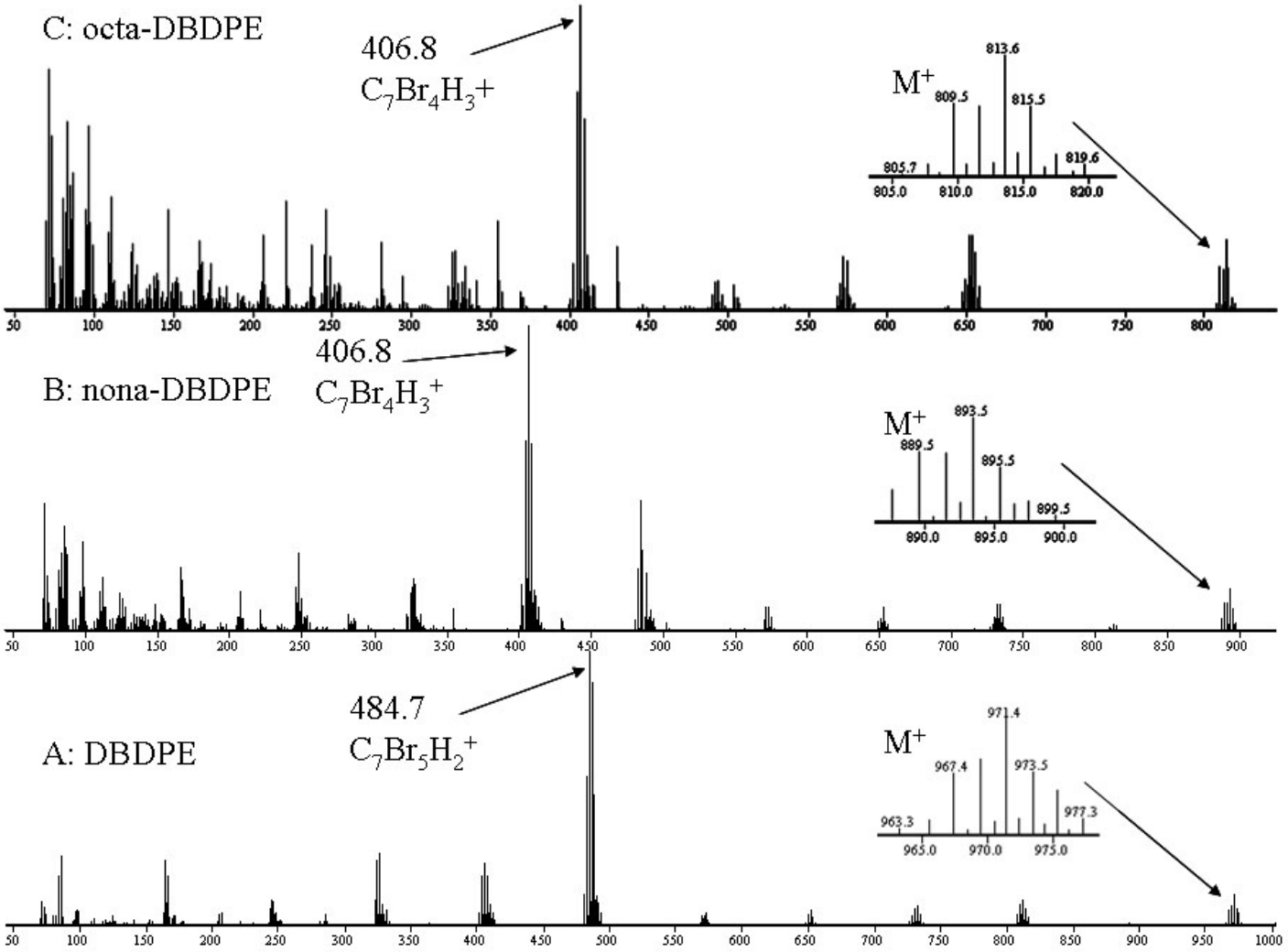

Figure S1. . Mass spectra for DBDPE (A) nona-BDPE (B) and octa-BDPE (c) in GC/EI-MS recorded from UV irradiation experiment samples.

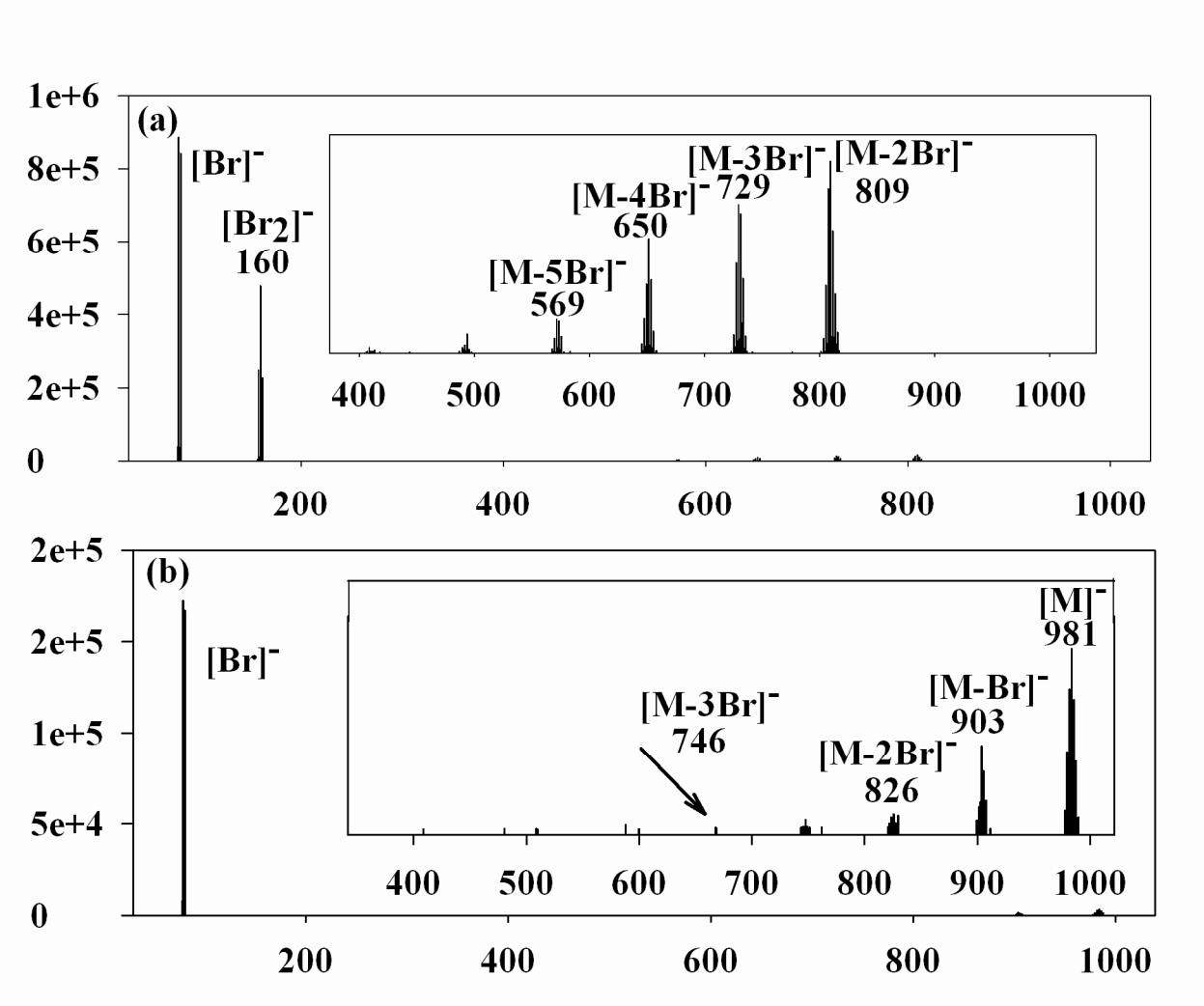

Figure S2. GC/ECNI-MS full-scan (m/z, 100-1000) spectra of two DBDPE metabolites corresponding to the peaks 3* and 7* tentatively proposed, (a) $MeSO_2$-BDPE and (b) $EtSO_2$-BDPE.

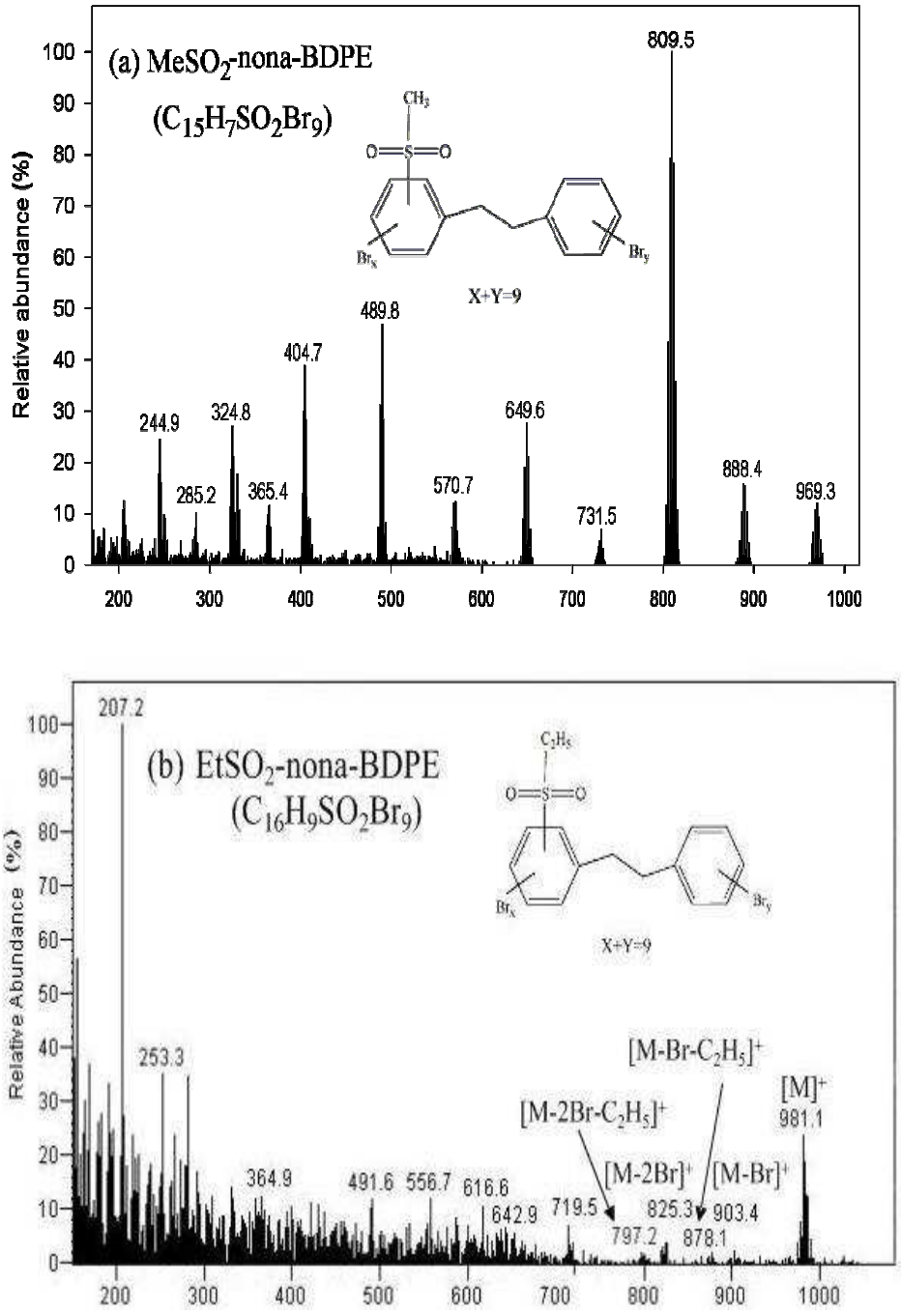

Figure S3. GC/EI-MS full-scan (m/z, 100-1050) spectra of two DBDPE metabolites corresponding to the peaks 3* and 7* tentatively proposed, (a) $MeSO_2$-BDPE and (b) $EtSO_2$-BDPE. The spectra were recorded by a Shimadadzu 2010 GC-MS.