TOF-SIMS MASS SPECTROMETRY IMAGING DEMONSTRATES A SELECTIVE TROPISM OF BDE-209 RESIDUES LOCATION IN TARGET TISSUES OF RATS

Seyer A[1], Riu A[2], Debrauwer L[2], Laprévote O[1,3], Bourges N[4], Brunelle A[1], Zalko D[2].

1. Institut de Chimie des Substances Naturelles, CNRS, UPR 2301, Avenue de la Terrasse, 91190 Gif-sur-Yvette, France
2. UMR 1089 Xénobiotiques INRA-ENVT, 180 Chemin de Tournefeuille, BP93173, F31027 Toulouse Cedex 3, France
3. Laboratoire de Toxicologie, IFR 71, Faculté des Sciences Pharmaceutiques et Biologiques, Université Paris Descartes, 4, avenue de l'Observatoire, 75006 Paris, France
4. Ecole Nationale Vétérinaire de Toulouse ENVT, 23 chemin des Capelles, BP 87614, 31076 Toulouse, France

Abstract :

BDE-209 (Deca-Bromo-Diphenyl Ether, DBDE) is a major BFR for which human exposure has been demonstrated. DBDE, administered by oral route, is efficiently absorbed in rats (at least 20% of the dosage) and its residues are mainly concentrated in the liver and in two endocrine glands, namely the adrenals and the ovaries. In this study, we based on previously published work and dosed female rats 2 mg/kg BW DBDE daily over 96 hr, with the aim to investigate the possible tropism of DBDE residues in these target tissues. We took advantage of the presence of bromine atoms to check the possibility to detect DBDE residues using a novel technique of imagery: Time-of-Flight Secondary Ion Mass Spectrometry.

ToF-SIMS mass spectrometry imaging proved to be an efficient method for the study of the localisation of DBDE residues. It allowed to reveal an homogeneous repartition of DBDE residues in the livers of dosed rats. In ovaries, residues were located in several spots, possibly corresponding to corpora lutea. In addition, the study of the repartition of DBDE residues in the adrenals showed a marked cortical tropism. Due to the intrinsic toxicity of DBDE, but also because this BFR is metabolised into several BDE with a lower degree of bromination *in vivo*, part of which could be biologically active metabolites, it appears that DBDE residues repartition in the adrenals and the ovaries may be connected with the mechanisms of action by which BDE could trigger endocrine disruption in mammals.

Introduction :

DBDE (Deca-Bromo Diphenyl Ether) is one of the most efficient Brominated Flame Retardant (BFR) available on the market. Like other organic BFR that belong to the bromo diphenyl ethers (BDE) family, it shares close chemical similarities with Poly-Chlorinated Biphenyls (PCB). BFR manufacturers do generally consider that DBDE, due to its higher molecular weight, is poorly bioavailable, contrary to lower brominated BDE, and that for this reason, it is a rather safe chemical. Due to current regulations, DBDE accounts for almost all of the BDE market nowadays, which itself is estimated to represent 30 to 40% of the total BFR production. We have previously demonstrated that the bioavailability of DBDE in rat, following oral dosage, was much higher than previously expected with the highest residual concentrations found in the adrenals, the ovaries, and the liver[1].

Here, we report first data about the use of ToF-SIMS mass spectrometry imaging, with the aim to locate DBDE residues in these target tissues. Time-of-flight secondary ion mass spectrometry (TOF-SIMS) gained several years ago an increase of sensitivity with the advent of cluster ion sources such as bismuth ones. This technique allows the acquisition of mass spectrometric images of any ion present in the mass spectra with an excellent spatial resolution from several micrometres to less than 500 nm and a mass range from 0 to about 1000 Da. This method is therefore ideal to localize lipids and small molecules at the surface of tissue sections and various applications have recently been published in the fields of biology and medicine[2,3].

Material and methods

Female Wistar rats aged 12 weeks (n=3) were acclimatized under a 12/12 hr light/dark cycle for one week and were fed a standard diet with *ad libitum* access to water. Animals were force-fed a daily dose of 2 mg/kg DBDE (Sigma, Saint-Quentin Fallavier, France) dissolved in peanut oil as previously described[1], every 24 hr, during 4 days. Twenty-four hr after the latest DBDE dosage, animals were euthanized and the liver, the adrenal glands and the ovaries were immediately frozen and stored at -80°C before analysis. Sections of 10 µm thickness were prepared with a Cryostat CM3050-S (Leica Microsystems SA, Rueil - Malmaison, France), at -20°C for liver and adrenal glands and at -25°C for ovaries, and immediately deposited on silicon wafers. Samples were dried before analysis under a pressure of a few millibars for 10 minutes, without any other treatment. Optical images were recorded with an Olympus BX51 microscope (Rungis, France) equipped with 1.25x to 50x lenses and a Color View I camera and monitored by Cell^B software (Soft Imaging System GmbH, Münster, Germany). Inside the ToF-SIMS mass spectrometer, video images taken with a small field of view of 1 x 1 mm^2 were recorded with an integrated camera.

All experiments were performed on a ToF-SIMS IV commercial mass spectrometer (Ion-Tof GmbH, Münster, Germany) located at the Institut de Chimie des Substances Naturelles in Gif-sur-Yvette (France). This mass spectrometer is fitted with a Bismuth cluster ion source (Bi_3^+ ions are selected). The primary ion dose density was between $1x10^9$ and $2x10^{10}$, *i.e.* ions/cm^2, well below the static SIMS limit. The secondary ions are accelerated to 2 keV, pass through a field free region and a reflectron (ion effective way ~ 2 m) and are postaccelerated to 10 keV before hitting the detector surface. A low energy electron flood gun is activated between two primary ion pulses to neutralize the surface of the sample. Large area images (several mm^2) were recorded always with 256x256 pixels, enabling micrometer spatial resolution.

DBDE molecular ions were detected neither in positive nor in negative ion mode in sections of dosed rat organs. In addition, in the negative ion mode, the $^{79}Br^-$ isotope ion was hidden by an intense phosphate ion peak, while the $^{81}Br^-$ isotope was fortunately well detected, thanks to the high mass resolution. This ion was solely detected in organs of dosed rats, e.g. not in those of rats only dosed with the vehicle (peanut oil). Therefore this ion could be unambiguously selected for image reconstruction**.**

Results and discussion :

Images of $^{81}Br^-$ ions obtained in negative ion mode are shown in Figure 1. First line displays ion images obtained from controls rats, and the second one shows ion images obtained from dosed rats. Bromide ions are not detected in organs of control rats, for which images correspond to the background signal of the spectrum. Ion images from adrenal sections show a tropism of DBDE for the adrenocortical area, since the bromide ions are not detected in the medullar part of the adrenals. This is better seen in close-up analyses of a section roughly corresponding to one adrenal gland quarter (Figure 2.). The specific location of DBDE residues in the cortical part of the adrenal may be connected with a possible alteration of the hormonal functions of this gland. The distribution of the bromide ion in the ovary sections seems to show localization in some ovarian follicles. Finally, the $^{81}Br^-$ ion is also detected in liver sections, but its localization appears to be very diffuse compared to what is obtained with ion images of adrenals and ovaries.

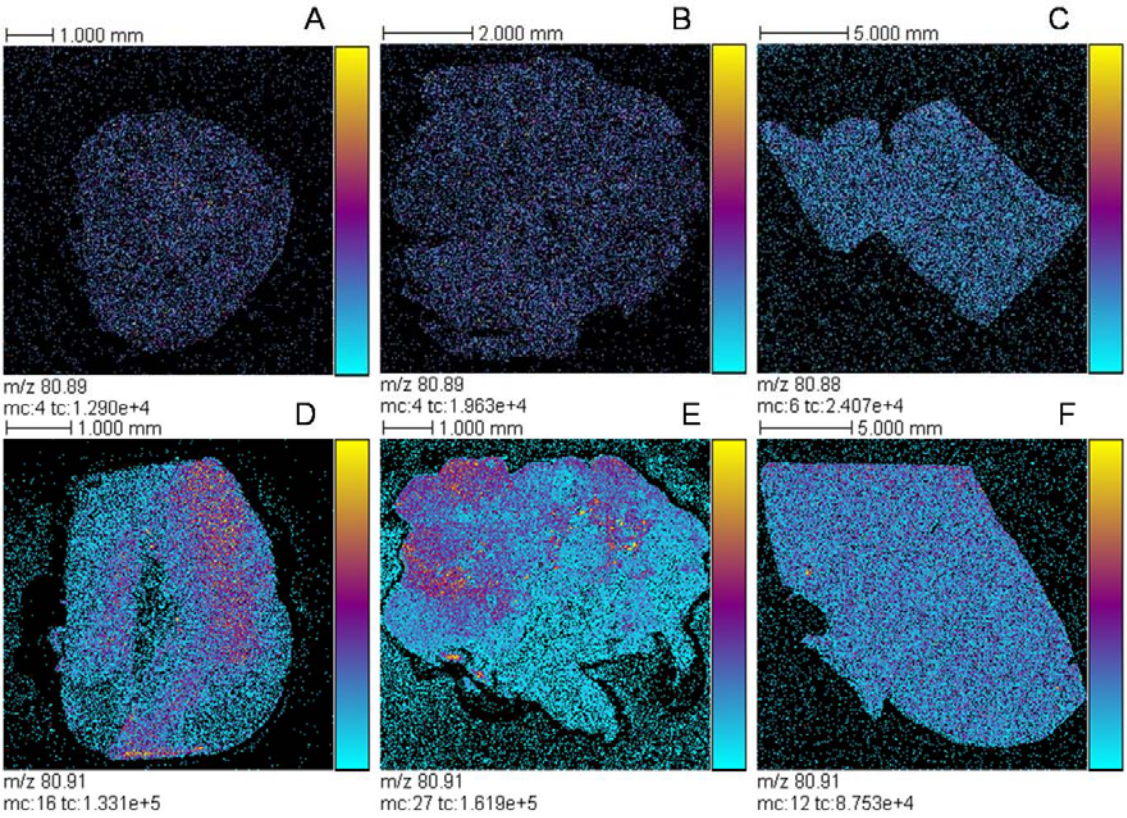

Figure 1: ToF-SIMS ion images of the $^{81}Br^-$ ion for the three different organs. The first line shows ion images obtained from control organs and the second line shows ion images of dosed rat organs. A, D: images of 5.1x5.1 mm^2 of adrenal glands; B, E: images of 6.7x6.7 mm^2 of ovaries; C, F: images of 17.8x17.8 mm^2 of livers. The amplitude of the color scale corresponds to the maximum number of counts mc and could be read as [0, mc]. tc is the total number of counts recorded for the specified m/z (corresponding to the sum of the counts of all pixels)

Several studies have demonstrated the usefulness of ToF-SIMS imaging in the detection and the localization of endogenous compounds in biological tissue[4,5,6,7]. This is particularly true for lipids. However, much fewer data has been released to date regarding xenobiotics. In addition, it is very difficult to detect and locate exogenous compounds which are present at low concentration in organs. Here, the detection of bromide ions, being the signature of the presence of DBDE, made it possible to localize unambiguously the residues of DBDE, in different organs of female rats.

Using ^{14}C-labelled DBDE and following exactly the same protocol for *in vivo* experiments, we have previously demonstrated that female rats orally dosed with DBDE presented high concentrations of DBDE residues in the liver (11 ppm, *e.g.* 11 µg of DBDE equivalent per gr of tissue)[1]. Even greater residual concentrations were detected in the ovaries (16 ppm) and in the adrenals (33 ppm). The use of a labeled compound also allowed to study the metabolic profiles of DBDE residues in tissues, leading to the identification of several other BDE ranging from hepta to nona-brominated analogues. However, the relatively low specific activity of radio-labeled DBDE would not allow to further produce an image of the corresponding tissues using radio-imaging tools. In the current study, we demonstrated that ToF-SIMS imaging could be efficiently used for this purpose.

Although SIMS ionization yielded too weak signals on molecular species, bromide ions were efficiently transmitted and detected, and were used as markers of brominated species, *i.e.* DBDE and related metabolites. The ion corresponding to the parent compound (DBDE) was not detected. Instead, we used the $^{81}Br^-$ ion. Hence, not only parent DBDE, but also its metabolites formed in rat do contribute to the recorded signal. These include hepta- to nona-BDEs, as demonstrated previously following radio-HPLC studies carried out after dosing animals with [^{14}C]-DBDE, using exactly the same protocol as in the current study[1]. In rats dosed with DBDE, bromide ions were apparently homogeneously distributed in the liver. On the contrary, images showing specific intra-tissular repartitions were obtained for the ovaries and the adrenals. For ovaries, the location of residues seems to be in corpora lutea. Equally interesting is the very pronounced localization of DBDE residues in the cortical zone of the adrenal glands.

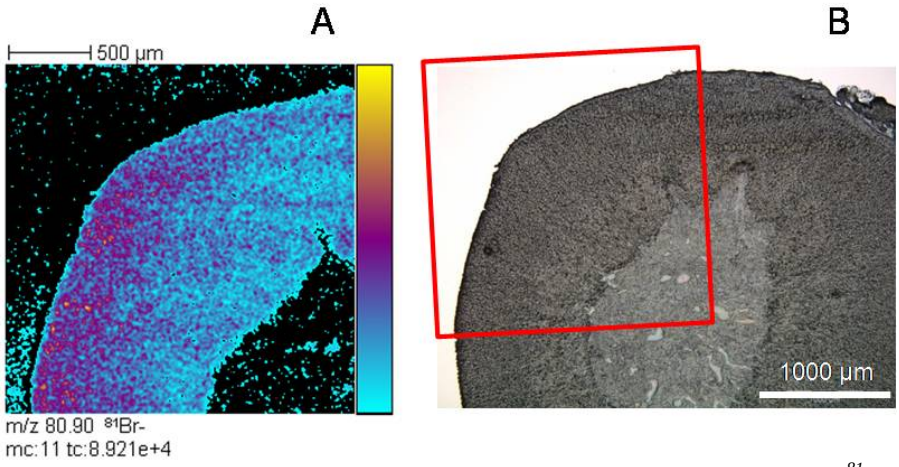

Figure 2: Image of an adrenal section from a dosed animal. A: ToF-SIMS ion image of the $^{81}Br^-$ ion. Image surface 2.1 x 2.1 mm^2, 256x256 pixels, pixel size 8.2 x 8.2 µm^2. The image was averaged and rescaled for an easier legibility. The amplitude of the color scale corresponds to the maximum number of counts mc and could be read as [0, mc]. tc is the total number of counts recorded for the specified m/z (corresponding to the sum of the counts of all pixels). B: Picture recorded just before the TOF-SIMS analysis, showing the tissue section deposited on a silicon wafer. The ion image (A) was recorded inside the red square.

Like other organic BFR that belong to the BDE family, DBDE shares close chemical similarities with PCB, which toxicity has been far more extensively documented than that of BDE. Among others, it has been demonstrated that selected PCB and their hydroxylated metabolites target the adrenals in mammals, ultimately producing an immunodeficiency, triggered by a hyperplasia of the cortical part of these glands. Some BDE have also been postulated to be bioactivated through metabolic processes, with either estrogenic or anti-thyroidal effects suggested by *in vitro* studies[8]. Not only DBDE (though it is the main residue) but also lower brominated metabolites are present both in the ovaries and in the adrenals of rats dosed with this BFR[1]. ToF-SIMS imaging clearly demonstrates a specific localization of DBDE residues, which in turn strongly suggest possible links between the suspected endocrine disruptor properties of BDEs and the preferential location of their residues in specific parts of both endocrine glands.

Acknowledgements :

AS is indebted to the Institut de Chimie des Substances Naturelles for a Ph.D. research fellowship. This work was supported by the European Union (Contract LSHG-CT-2005-518194 COMPUTIS). DZ and coworkers wish to thank AFSSET (Agence Française de Sécurité Sanitaire de l'Environnement et du Travail) for continuous support in BFR related projects (RD 2004-011, EST 08-46).

References :

1 Riu A, Cravedi JP, Debrauwer L, Garcia A, Canlet C, Jouanin I, Zalko D. Disposition and metabolic profiling. *Environ. Int.* 34(3):318-329, 2008.
2 Brunelle, A.; Laprévote, O. *Curr. Pharm. Design* 2007, 13, 3335-3343.
3 Brunelle, A.; Laprévote, O. *Anal. Bioanal. Chem.* 2009, 393, 31-35.
4 Touboul, D., Roy, S.; Germain, D. P.; Chaminade, P.; Brunelle, A.; Laprévote, O. *Int. J. Mass Spectrom*. 2007, 260: 158–165.
5 Touboul, D.; Brunelle, A.; Halgand, F.; De La Porte, S.; Laprévote, O. *J. Lipid Res.* 2005, 46, 1388-1395.
6 Tahallah, N.; Brunelle, A.; De La Porte, S.; Laprévote, O. *J. Lipid. Res.* 2008, 49, 438-454.
7 Debois, D.; Bralet, MP.; Le Naour, F.; Brunelle, A.; Laprévote, O. *Anal Chem* 2009, 81, 2823-2831.
8 Meerts IA, Letcher RJ, Hoving S, Marsh G, Bergman A, Lemmen JG, van der Burg B, Brouwer A. *Environ Health Perspect*. 2001 Apr;109(4):399-407.