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Human phase I *in vitro* liver metabolism of two bisphenolic diglycidyl ethers BADGE and BFDGE

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Highlights

- BADGE and BFDGE are subjected to phase I human *in vitro* biotransformation reactions
- Epoxides are rapidly hydrolyzed to bis-diols in a NADPH independent manner
- Oxidation led to formation of BADGE.2H₂O-OH and BADGE.H₂O-COOH from BADGE.2H₂O
- Oxidation of BFDGE.2H₂O led to the newly reported carboxylic acid BFDGE.H₂O.COOH

Abstract

Root canal sealers are commonly used to endodontically treat teeth with periapical infections. Some root canal sealers based on epoxy resin contain bisphenol A diglycidyl ether (BADGE) and bisphenol F diglycidyl ether (BFDGE). The presence of these chemicals is of concern due to the close contact to the blood stream at the apex and the long setting times of up to 24 hours. These chemicals, or any of their degradation products or metabolites, can then exert their toxic effects before being excreted. This study aimed to identify the phase I *in vitro* biotransformation products of BADGE and BFDGE using human liver microsomes. During incubation with microsomal fractions, the epoxides were rapidly hydrolysed in a NADPH independent manner resulting in the formation of BADGE.2H₂O and

BFDGE.2H₂O. Further, oxidative reactions, such as hydroxylation and carboxylation, generated other BADGE metabolites, such as BADGE.2H₂O-OH and BADGE.H₂O.COOH, respectively. For BFDGE, further oxidation of BFDGE.2H₂O led to the newly reported carboxylic acid, BFDGE.H₂O.COOH. In total, three specific metabolites have been identified which can serve in future human biomonitoring studies of BADGE and BFDGE.

Keywords: *In vitro* metabolism; human liver microsomes; liquid chromatography-mass spectrometry; BADGE; BFDGE

1. Introduction

When commensal bacteria in the human mouth invade the tooth and cause an infection in the centre of the tooth, a dental procedure called root canal treatment is necessary to save the tooth to prevent extraction. During this treatment, the pulp tissue (nerves + vascular and connective tissue) of the tooth is removed before cleaning and sealing the root canal. During an earlier study in which we investigated the composition of different dental materials, the presence of two bisphenolic ingredients was confirmed in an epoxy resin based root canal sealer AH Plus: bisphenol A diglycidyl ether (BADGE) and its structural analogue bisphenol F diglycidyl ether (BFDGE).(1)

Both for BADGE and BFDGE, and their hydrolysis products, possible endocrine disrupting effects which have been widely studied before, are debatable. Ramilo *et al* found both BADGE and BFDGE were able to induce *in vitro* morphological changes in Caco-2 cells, cell detachment from the substratum and to inhibit cell proliferation, in a time and dose-dependent manner. (2) van Leeuwen *et al* found that BADGE mainly exhibited *in vitro* anti-androgenic activity with comparable potencies as BPA.(3) Punt *et al* tried a quantitative *in vitro*-to-*in vivo* extrapolation (QIVIVE) of the estrogenic and anti- androgenic potencies of BADGE analogues. They concluded that although BADGE showed *in vitro* potencies higher than BPA, the estimated *in vivo* potencies based on the oral equivalent doses are one to two orders of magnitude lower than BPA because of fast detoxification of BADGE due to rapid hydrolysis of the epoxides to diols by epoxide hydrolases.(4) In 2002, the European Commission concluded that BADGE does not induce toxic effects on the reproduction or development.(5) In addition, EFSA reported in 2004 that several *in vitro* studies found no affinity for BADGE to estrogenic receptors in concentrations $\leq 10^{-4}$ M, nor did any harmful effects on fertility & post-natal development in rats. Oral developmental toxicity studies in rats and rabbits showed no teratogenic effects or adverse effects on embryonal and foetal development.(6) However, Olea *et al* observed a proliferative effect at a concentration of 10 μ M on MCF7 cells.(7) Perez *et al* observed the same effect, but as the cell yields were below 60% of 17 β -estradiol, they concluded BADGE is only a partial estrogen agonist at this concentration.(8)

Given the debatable disrupting effects for BADGE and the limited data for BFDGE, the presence of these chemicals in a dental root canal sealer is of concern. Due to the closer contact with the blood stream at the apex of the root and the long setting times of up to 24 hours for epoxy-based root canal sealers, BADGE and BFDGE may leach to the patients' blood stream after dental restoration. After uptake, these chemicals can potentially exert their disrupting effects before being excreted.(9) Subsequently, they will undergo biotransformation reactions leading to more polar metabolites for which information about their biological effects is hardly available.

To date, little is known about the human metabolism of BADGE and BFDGE. Earlier studies have investigated the inactivation and distribution of glycidyl ethers in various tissues of rats, mice and humans, but always tended to focus on the hydrolysis products of the epoxide moieties, BADGE.2H₂O and BFDGE.2H₂O.(10-12) Given the fact that the hydrolysed derivatives of BADGE and BFDGE can be easily formed when in contact with aqueous and acidic foodstuff, they can readily be present in canned foods. In addition they have also been detected in indoor dust and as a result cannot be solely used to monitor human exposure to BADGE and BFDGE.(13-15)

Climie *et al* have investigated the metabolic profiles of BADGE after dermal and oral exposure in mice.(16) Identification of metabolites in mouse, rat and rabbit liver preparations revealed hydrolytic ring opening of the two epoxide groups resulting in the formation of the bis-diol was the most important, followed by further oxidative reactions.(17) To the best of our knowledge, no studies have been performed to detect specific (human) metabolites of BFDGE.

This study aimed to identify human phase I liver metabolites of the two identified ingredients of the root canal sealer AH Plus, BADGE and the structural analogue BFDGE using accurate mass spectrometry. Identified *in vitro* biotransformation products could serve in the future to monitor the short- and long-term exposure of patients to these compounds, either through root canal sealer treatment, or other sources of exposure to these compounds.

2. Materials and methods

2.1. Chemicals and reagents

Human liver microsomes (HLMs, mixed gender, n=50) were acquired from Tebu-Bio (Boechout, BE). Human liver cytosol (HLCYT, mixed gender, n=150), theophylline (anhydrous, > 99 %), 2,5-uridinediphosphate glucuronic acid (UDPGA), adenosine-3'-phosphate 5'-phosphosulfate (PAPS, > 60 %) lithium salt hydrate, alamethicin (neat, > 98 %), dimethyl sulfoxide (DMSO), 4-nitrophenol (4-NP) and BADGE (analytical standard) were obtained from Sigma-Aldrich (Missouri, US). BFDGE (90%, racemic mixture of diastereomers) was acquired from Toronto Research Chemicals (North York,

Canada). NADPH tetrasodium salt hydrate (> 96 %) was purchased from Acros (Geel, BE). Acetonitrile (ACN, HPLC-grade) and methanol (MeOH, ≥ 99.9 % LC-MS grade) were acquired from Fisher Chemical (Loughborough, GB), formic acid (> 98 %) and hydrochloric acid (37%) from Merck KGaA (Darmstadt, DE). A 100 mM TRIS-buffer was prepared by dissolving 12.11 g Trizma base (Janssen Chimica, Beerse, BE) and 1.02 g MgCl₂ (Merck KGaA, Darmstadt, DE) in 1 L ultrapure water. The pH was adjusted to 7.4 by adding 1 M HCl solution. Ultra-pure water was produced in-house with a PURELAB-purifier system of Elga Labwater (Tienen, BE).

2.2. *In vitro* liver metabolism assay

This study employed an *in vitro* assay optimized and used in previous studies (18-20). An overview of the experimental setup can be found in Figure 1. All tested sample sets contained three replicates.

Phase I biotransformation products were generated using pooled HLMs. A reaction mixture containing 945 µL of TRIS-buffer (pH 7.4, 100 mM), 25 µL of HLMs (20 mg/mL in 250 mM sucrose in water) and 10 µL of substrate stock solution (0.5 mM in MeOH) was incubated in a 1.5 mL Eppendorf tube at 37 °C. 10 µL of NADPH (0.1 M in TRIS-buffer) was added after 5, 60, and 120 min to replenish NADPH levels. During incubation, the total volume of organic solvent did not exceed 1 % to avoid any effects on the microsomal activity (21). Method blanks (without parent compound) and negative controls (without HLMs or NADPH) were prepared in parallel. The reaction was stopped after one or three hours by the addition of 250 µL ice-cold ACN containing 1 % formic acid. The internal standards, theophylline and valsartan, were prepared in the ice-cold ACN with 1 % formic acid at a concentration of 5 µg/mL. A positive control for phase I experiments was included by incubating 10 µL phenacetin (5 µg/mL in ultrapure water). The formation of two phase I biotransformation products of phenacetin - N-(4-hydroxyphenyl)-acetamide (P1) and N-(4-ethoxy-2-hydroxyphenyl)-acetamide (P2) - was monitored.

2.3. LC-QTOF-MS analytical method

Extracts were analysed using an Agilent 1290 Infinity UPLC hyphenated to an Agilent 6530 QTOF (Agilent, Santa Clara, USA). Chromatographic separation was performed on a Kinetex Biphenyl column (100 x 2.1 mm; 2.6 µm particle size, Phenomenex, Torrance, USA) using a mobile phase composed of ultra-pure water with 1 mM ammonium fluoride (A) and methanol with 1 mM ammonium fluoride (B) with a flow of 0.4 mL/min. The injection volume was 1 µL. All samples were analysed in positive and negative ionization mode. The eluent was directed to the waste during the first minute of each run to protect the ion source from extensive contamination. For all analyses, the same chromatographic gradient was used. The run started in an isocratic way at 2% B for 1 min. Next, the % B was increased to 95% over a time span of 12 min. These conditions were kept for 3 min before returning to start

conditions and letting the system equilibrate for 5 min before starting the next injection. The column temperature was kept constant at 40 °C.

The QTOF-MS instrument was operated in the 2 GHz (extended dynamic range) mode, providing a Full Width at Half Maximum (FWHM) resolution of approximately 5100 at m/z 118.0862 and 10000 at m/z 922.0098. The ions m/z 121.0508 and 922.0097 for positive mode and m/z 119.0363 and 940.0009 for negative mode were selected for constant recalibration throughout the chromatographic run to ensure high mass accuracy. Drying gas temperature and flow were at 325 °C and 8 L/min, respectively. The sheath gas temperature was 325 °C at a flow of 11 L/min. Nebulizer pressure was set at 40 psig. Capillary, nozzle and fragmentor voltages were at 3500 V, 0 V and 125 V, respectively. The acquisition parameters were set for a m/z range from 80 to 1000 amu at a scan rate of 5 scans/s and 6.67 scans/s for MS and MS/MS spectra, respectively. Collision energies were applied at 10 and 30 V. Signals were detected using a data-dependent acquisition method. An active exclusion of 0.10 min was applied to prevent repetitive acquisition of MS/MS spectra for the same ion. All data were stored in centroid mode.

2.4. Data analysis

The acquired data were analyzed using a suspect screening approach. First, a suspect list was created *in silico* using two different software packages, Meteor Nexus (v2.2, Lhasa Limited, Leeds, UK) and the online tool BioTransformer. (22) In Meteor Nexus, all human redox and non-redox biotransformations were selected for Phase I metabolism. Parameters in BioTransformer were set to predict Phase I (CYP 450) transformations. The output from both approaches was exported as CSV and combined to one list where duplicates were removed by hand. The suspect list was augmented with retention times if standards were available in-house.

The resulting suspect list was then used to detect predicted biotransformation products using the Targeted Feature Extraction algorithm in MassHunter Profinder (version B.08 SP3, Agilent Technologies, Santa Clara, USA). Match tolerances for mass and retention time accuracy were set to 10 ppm and 0.15 min respectively. A feature had to be present in at least two out of three replicates of at least one sample group in order to be retained. Integration of resulting features was curated by hand. Data was treated separate for both polarities and results were joined per compound in one table before being imported in R for further analysis. In addition, MS/MS spectra were extracted using MassHunter Qualitative Analysis (version B.7, Agilent Technologies, Santa Clara, USA). This information, together with spectra from reference standards or online databases was used to define the confidence level of identification according to Schymanski *et al.*(23)

3. Results

A summary of all identified biotransformation products can be found in Table 1. A schematic representation for the suggested biotransformation pathway of BADGE and BFDGE can be found in Figure 2 and Figure 3 respectively.

3.1. Metabolism BADGE

BADGE was detected in the LC-QTOF-MS mostly as an NH_4 -adduct (m/z 358.2013), at a retention time of 12.96 min. When selecting the ammonium adduct for fragmentation, the same product ions can be observed as earlier reported by Gallart-Ayala *et al.*(24) In the negative control samples where HLM were omitted, the concentration of BADGE appeared to be relatively stable over the 3h exposure time, with only a small decrease from 1 to 3h of exposure, which can be linked to an increase of the intensity of $\text{BADGE.H}_2\text{O}$. In samples where HLM were present (HLM samples and negative controls without cofactors), the signal of BADGE was around 8 times lower at the zero-hour time point. In addition, the intensity of the signal of BADGE decreased over time in the HLM samples. In the negative controls without cofactor, BADGE was undetectable after 3h exposure, in the HLM samples, this was already after 1h of exposure.

$\text{BADGE.2H}_2\text{O}$ was mainly detected as an NH_4 -adduct (m/z 394.2226) at a retention time of 10.05 min. Upon fragmentation, the product ions with m/z 135.0790 and 107.0482 were observed, which are common fragments with BADGE. The product ion with m/z 209.1172 differs in 18 amu from the fragment ion 191.1050 observed in BADGE, confirming the hydrolysis with addition of H_2O of the epoxide ring structure. The level of identification of this biotransformation product reached level 1 by injecting a reference standard.(23) $\text{BADGE.2H}_2\text{O}$ was detected in all HLM samples and negative controls without cofactor from 0h to 3h, but it was not present in the method blanks and negative controls without HLM. In the negative controls without cofactor, the abundance of $\text{BADGE.2H}_2\text{O}$ was stable from 0h to 3h, but in the HLM samples, the relative area decreases over time as shown in Figure 4.

$\text{BADGE.H}_2\text{O}$ was identified in the negative control samples without HLMs, and absent in the other sample groups. The area of $\text{BADGE.H}_2\text{O}$ increased in linear from 0h to reach a maximum after 3h of exposure. It was detected as an NH_4 -adduct (m/z 376.2117) at a retention time of 11.61 min. Upon fragmentation, the product ions with m/z 135.0790 and 107.0790 corresponded to those seen when fragmenting BADGE. The presence of product ions with m/z 191.1050 and 209.1157 confirmed that both the epoxide ring side chain and the hydrolyzed variant exist in this compound and resemblance of the observed fragment ions to those reported by Gallart-Ayala *et al* resulted in a level 2a identification.(23, 24)

In the HLM samples, a chromatographic peak at 8.26 min was detected with an m/z of 410.2176. This feature was not present in any of the negative controls or method blanks. The feature was absent at the 0h time point but showed a steady increase to a maximal relative area after 3h (Figure 4). The database search identified this feature as an ammonium adduct of a hydroxylation biotransformation product from BADGE.2H₂O, with a molecular formula of C₂₁H₂₈O₇ and a mass difference from the theoretical mass of 1.48 ppm. Upon fragmentation, a fragment ion with m/z 225.1146 was observed. This differed by 16 amu from the 209.1157 fragment observed in BADGE.2H₂O, suggesting the presence of an extra O-atom, originating from an oxidative biotransformation reaction. The presence of a fragment ion with m/z 207.1009 resulting from the loss of water suggested that the hydroxylation occurred on an aliphatic position. Also, a specific fragment ion with a m/z value of 165.0571 was observed for this biotransformation product for which a formula of [C₉H₉O₃]⁺ could be generated pointing out the hydroxylation occurred on the dihydroxypropyl moiety. The fragment ion with 107 amu was shared with all other observed features and confirmed that the hydroxylation did not occur on a phenolic position.(20)

Finally, the carboxylic acid BADGE.H₂O.COOH, derived from the oxidation of BADGE.2H₂O could be identified in ESI negative at a retention time of 8.81 min. Because it could only be detected in very low amounts in the 3h samples, no MS/MS could be recorded at first. During a targeted reinjection, MS/MS spectra could be acquired. Upon fragmentation, a loss of C₃H₅O₃ (corresponding to the carboxylated C₃-chain) from the parent ion led to the product ion with m/z 301.1469. Further loss of the second C₃-chain led to the product ion with m/z 227.1078, characteristic for BPA. At the higher collision energy, a secondary characteristic BPA product ion with m/z 133.0671 was observed. However, the acquired MS/MS spectra did not allow for locating the exact position of the carboxylic acid moiety.

3.2. Metabolism BFDGE

BFDGE was detected in positive ionization as an ammonium adduct (m/z 330.1686) at a retention time of 12.52 min. Upon fragmentation, the product ions earlier reported by Gallart-Ayala *et al* could be observed for the different structural isomers.(24) In the negative control samples where HLM were omitted, a decrease of the BFDGE signal could be observed over time, starting already at the 1h time point. As observed for BADGE, the signal of BFDGE in samples where HLM were present (HLM samples and negative controls without cofactors) was several times lower at the 0-h time point than in the NC samples. In addition, the signal decreased over time resulting in undetectable amounts of BFDGE in these samples after 1 h.

BFDGE.2H₂O was identified in all samples where microsomal liver fractions were present from starting at the 0h time point up to 3h. BFDGE.2H₂O was detected as a NH₄-adduct at a retention time of 9.48 min. The product ion m/z 181.0846 can be assigned to a structure where C₉H₁₂O₃ has been lost from the protonated parent molecule. A secondary characteristic product ion for BFDGE.2H₂O, m/z 107.0484 could be assigned to a fragment formula [C₇H₇O]⁺. Both ions have previously been reported by Gallart-Ayala *et al*, leading to a level of identification of L2a.(23, 24)

In contrary to BADGE.2H₂O, no clear trend could be observed for BFDGE.2H₂O in the HLM samples over time, possibly due to a higher spread of the intensities in the samples. Signal intensities in the HLM samples were comparable to negative control sample where the cofactor was left out. In negative controls without HLM and method blanks, BFDGE.2H₂O could not be detected.

BFDGE.H₂O was identified in the negative control samples without HLM as an ammonium adduct (m/z 348.1827) with a mass error of 7.17 ppm. The area of the signal was stable from 0 to 1h, after which it declined. Upon fragmentation, a mix of product ions shared with BFDGE and BFDGE.2H₂O could be observed due to the two different propyl side chains. Product ions with m/z 163.0769 and 133.0646 confirmed the presence of the epoxide side chain while the product ion with m/z 181.0850 confirmed the presence of the side chain resulting from the hydrolysis of the epoxide. Finally, the product ion with m/z 107.0496 is shared with BFDGE, further confirming the identity of BFDGE.H₂O to level 3.

In negative ionisation, a chromatographic peak at 7.98 min could be detected with a m/z of 361.1294. This feature was identified by suspect screening as the carboxylic acid biotransformation product resulting from the oxidation of BFDGE.2H₂O, with a mass difference of 0.42 ppm. This carboxylic acid was only present in the HLM samples after 1 and 3h of exposure, with a maximal relative area at the 3h time point (Figure 5). Fragmentation of the parent ion led to two main fragment ions: m/z 199.0758 and m/z 93.0357. Both can be linked to a bisphenol F core structure, but neither could pinpoint the exact location of the carboxylic acid moiety.

4. Discussion

In this study, we could observe a significant decrease in the signal for BADGE when adding HLM to the reaction mixture. In addition, BADGE.2H₂O was readily present in these samples, but not when no HLM were present. These data suggest a rapid hydrolysis of BADGE to BADGE.2H₂O when exposed to microsomal fractions. This confirms earlier findings by Bentley *et al*, Boogaard *et al* and Climie *et al*.(10, 11, 17) The hydrolysis appears to be NADPH independent as it occurred also in the negative control samples where the cofactor NADPH had been omitted. Boogaard *et al* stated that this hydrolysis was

catalyzed by epoxide hydrolase, an enzyme class which does not need a cofactor, which agrees to the findings of our study.(11, 25)

We observed the similar behavior for the formation of BADGE.2H₂O and BFDGE.2H₂O from respectively BADGE and BFDGE which was in accordance to earlier study by Wang *et al*, who suggested the hydration of these chemicals in humans given the more frequent detection of the hydration products in human specimens (adipose, blood, urine).(12)

In this study, the human phase I liver metabolism reactions were studied, catalyzed by cytochrome P450 enzymes. Although most of these reactions are oxidations, CYP450s can catalyze an array of reactions including reductions, ester cleavage, dehydration among others.(26) For both BADGE and BFDGE, only an oxidative reaction could be observed, resulting in the formation of an hydroxylated or carboxylic acid biotransformation product respectively.

The absence of O-dealkylation reactions, which could lead to the formation of BPA and BPF from respectively BADGE and BFDGE or their metabolites, confirmed earlier results from Bentley *et al*. They observed O-dealkylation only occurred when BADGE was either presented in a high dose, or in low dose when in combination with an inhibitor for epoxide hydrolase.(11)

For both BADGE and BFDGE, the monohydrolysis product (.H₂O) was present in the negative control samples (without HLM) from the start. During incubation, the concentration for BADGE.H₂O rose from 0h to reach a maximal area after 3h of incubation, while the area of BFDGE.H₂O remained stable during the first hour of incubation and then declined, suggests a different behaviour for these compounds. The decrease of BADGE in the NC samples during 3h of exposure appeared to be linked to the steady increase of BADGE.H₂O over time. In contrary, the area of BFDGE also declined over time, but this did not result in an increase of BFDGE.H₂O after 3h of exposure. This suggests that the BFDGE.H₂O degrades more rapidly to BFDGE.2H₂O than is the case for the BADGE analogue.

Subsequently, after the formation of the BADGE.2H₂O or BFDGE.2H₂O hydrolysis products, further oxidative biotransformations occur. For BADGE, this resulted in the hydroxylation of BADGE.2H₂O and also the formation of the carboxylic acid BADGE.H₂O.COOH, while for BFDGE only the carboxylic acid was formed resulting from the oxidation of BFDGE.2H₂O.

The biotransformation of BADGE showed similarities to these of the dental monomer bisphenol A glycidyl methacrylate (BisGMA) which has been previously studied by our group.(20) Upon hydrolysis of the ester bond in the dimethacrylate BisGMA, both methacrylic acid moieties are removed leading to the formation of BADGE.2H₂O. In both studies, subsequent oxidative biotransformation pathways

have been observed leading to the hydroxylated (BADGE.2H₂O-OH) and carboxylated (BADGE.H₂O.COOH) biotransformation products. As with BisGMA, observed MS/MS fragment ions suggested the hydroxylation to occur on an aliphatic position and not on one of the aromatic rings.(20)

Although the acquired MS/MS fragments did not help to elucidate the exact position of the carboxylic acid in the identified in BADGE.H₂O.COOH and BFDGE.H₂O.COOH, the metabolism of other chemicals with a 1,2-propanediol moiety could help confirm the location. Ruddick *et al* have studied the metabolism of 1,2-propanediol and observed the formation of lactic acid.(27) In addition, Maurer *et al* and Vandenheuvel *et al* have studied the metabolism of guaifenesin (3-(2-methoxyphenoxy)-1,2-propanediol), which has structural resemblances to both chemicals in this study. They identified beta-(2-methoxyphenoxy)lactic acid as the major urinary metabolite.(28, 29) These studies suggest that the carboxylic acid moiety in both biotransformation products will be located at the terminal carbon. However, *in vivo* studies must be carried out in order to confirm the formation of these carboxylic acids.

BADGE.2H₂O and BFDGE.2H₂O have been detected in human urine, blood plasma and adipose tissue samples.(12, 13, 30) However, as these compounds can be easily formed through non-specific hydrolysis reactions, one can never be sure that the detected amounts of these compounds solely derive from (human) metabolism.(31-33) For example, Losada *et al* have identified the formation of the hydrolysis products of BADGE, BADGE.H₂O and BADGE.2H₂O, in different food simulants.(33) In addition, the measured hydrolysis products could also be a result from degradation during sample preparation as was demonstrated by Liu *et al*, leading to a specific water-free sample preparation to eliminate this risk.(13) The specific metabolites BADGE.2H₂O-OH, BADGE.H₂O.COOH, and BFDGE.H₂O.COOH identified in this study could potentially help in eliminating possible errors in human biomonitoring of BADGE and BFDGE due to the reasons above.

Future studies could either target these specific biotransformation products or combine them with quantification of the hydrolysis products and determine and monitor ratios between the different metabolites to confirm human metabolism. However, this would require reference standards of BADGE.2H₂O-OH, BADGE.H₂O.COOH and BFDGE.H₂O.COOH, but these are not commercially available. In any case, *in vivo* studies should first qualitatively confirm the presence of BADGE.2H₂O-OH, BADGE.H₂O.COOH and BFDGE.H₂O.COOH in human urine.

5. Conclusions

We have applied an adapted version of our in-house human *in vitro* liver metabolism assay to study the phase I metabolism of BADGE and BFDGE. Exposure of both diglycidyl ethers to the microsomal fractions led to the hydrolysis of both epoxides in a NADPH-independent manner, leading to the formation of BADGE.2H₂O and BFDGE.2H₂O. Incubation of both tested compounds with microsomal fractions and cofactors led to further oxidation of the hydrolysed compounds. For BADGE, this resulted in the formation of a hydroxylated and carboxylated biotransformation product, respectively BADGE.2H₂O-OH and BADGE.H₂O.COOH. For BFDGE, the carboxylated biotransformation product BFDGE.H₂O.COOH could be identified, which has never been reported before.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Conflicts of interest

The authors declare they have no conflict of interest.

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Tables and figures

Table 1: Overview of identified compounds with their molecular formula, measured mass, mass deviation (expressed as ppm difference), fragment ions from MS2 and confirmation level according to Schymanski et al.(23)

	Compound	Formula	RT (min)	Mass measured	Δ ppm	Fragment Ions	Confirmation level
BADGE	BADGE	C ₂₁ H ₂₄ O ₄	12.96	340.1679	1.35	191.1050; 161.0948; 135.0790; 107.0482	L1
	BADGE.H ₂ O	C ₂₁ H ₂₆ O ₅	11.61	358.1784	1.04	209.1157; 191.1050; 161.0961; 135.0790; 107.0482	L2a
	BADGE.2H ₂ O	C ₂₁ H ₂₈ O ₆	10.05	376.1888	0.57	209.1172; 135.0790; 107.0482	L1
	BADGE.2H ₂ O-OH	C ₂₁ H ₂₈ O ₇	8.26	392.1841	1.48	225.1146; 207.1009; 177.0922; 165.0571; 135.0790; 107.0482	L3
	BADGE.H ₂ O.COOH*	C ₂₁ H ₂₆ O ₇	8.81	390.1684	1.39	301.1469; 227.1078; 133.0671	L3
BFDGE	BFDGE	C ₁₉ H ₂₀ O ₄	12.52	312.1352	-3.05	295.1305; 277.1206; 189.0896; 163.0745; 145.0644; 133.0644; 107.0488	L1
	BFDGE.H ₂ O	C ₁₉ H ₂₂ O ₅	11.00	330.1491	7.17	181.0850; 163.0769; 133.0646; 107.0496	L3
	BFDGE.2H ₂ O	C ₁₉ H ₂₄ O ₆	9.48	348.1571	-0.64	181.0846; 107.0484	L2a
	BFDGE.H ₂ O.COOH*	C ₁₉ H ₂₂ O ₇	7.98	362.1367	0.42	199.0758; 93.0357	L3

*: Compounds which have been identified in negative polarity (ESI-).

Figure 1: Schematic overview of in vitro human liver biotransformation assay used in this study

Figure 2: Suggested biotransformation pathway for BADGE

Figure 3: Suggested biotransformation pathway for BFDGE

Figure 4: Signal intensity of BADGE.2H₂O (A) and BADGE.2H₂O-OH (B) expressed as area of signal, plotted over time for different samples.

Figure 5: Signal intensity of BFDGE.H₂O.COOH (expressed as area of signal) plotted over time for different samples