Preferential glutathione conjugation of a reverse diol epoxide compared to a bay region diol epoxide of benzo[a]pyrene in human hepatocytes

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10-(N-acetylcysteinyl)-7,8,9,10-tetrahydrobenzo[a]pyrene, BPDE-10-NAC; 10-(N-acetylcysteinyl)-9-hydroxy-9,10-dihydrophenanthrene, Phe-9-hydroxy-10-NAC; 1-chloro-2,4-dinitrobenzene, CDNB; 7-(N-acetylcysteinyl)-8,9,10-trihydroxy-7,8,9,10-tetrahydrobenzo[a]pyrene, rev-BPDE-7-NAC; 4-(N-acetylcysteinyl)-1,2,3-trihydroxy-1,2,3,4-tetrahydrophenanthrene, Phe-1,2-D-4-NAC; 1-(N-acetylcysteinyl)-2,3,4-trihydroxy-1,2,3,4-tetrahydrophenanthrene, Phe-3,4-D-1-NAC; anti-BaP-7,8-diol-9,10-epoxide, BPDE; anti-BaP-9,10-diol-7,8-epoxide, rev-BPDE; benzo[a]pyrene, BaP; anti-Phe-1,2-diol-3,4-epoxide, Phe-1,2-D-3,4-E; anti-Phe-3,4-diol-1,2-epoxide, Phe-3,4-D-1,2-E; glutathione, GSH; glutathione-S-transferase, GST; liquid chromatography-electrospray ionization-tandem mass spectrometry-selected reaction monitoring, LC-ESI-MS/MS-SRM; N-Acetylcysteine, NAC; phenanthrene, Phe; polycyclic aromatic hydrocarbons, PAH
Abstract

Many studies have examined the relationship between polymorphisms in glutathione-S-transferase genes and cancer in people exposed to polycyclic aromatic hydrocarbons (PAH) such as benzo[a]pyrene (BaP), but the results to date have been modest. Missing from these studies has been an exploration of the formation of the appropriate glutathione conjugates in humans. We incubated human hepatocytes from ten donors with racemic anti-BaP-7,8-diol-9,10-epoxide (BPDE), believed to be a major ultimate carcinogen of BaP, or with the non-carcinogenic reverse diol epoxide, racemic anti-BaP-9,10-diol-7,8-epoxide (rev-BPDE). Incubations were carried out for 12 or 24h. We used high performance liquid chromatography-electrospray ionization-tandem mass spectrometry-selected reaction monitoring at m/z 464 → m/z 317 to analyze the incubation mixtures for the mercapturic acid products which would result from glutathione conjugation. The standard mercapturic acids were synthesized by reaction of BPDE or rev-BPDE with N-acetylcysteine. We obtained convincing evidence in human hepatocytes for mercapturic acid formation from rev-BPDE in all 10 samples, in amounts up to 17 pmol/ml. However, we could detect mercapturic acids from BPDE in only 1 of 10 samples (0.05 pmol/ml). Taken together with our similar previous results of analyses of phenanthrene metabolites in human hepatocytes and human urine, the results of this study indicate that conjugation of BPDE with glutathione is a minor pathway in humans, indicating that glutathione-S-transferase genotyping is not an effective method of assessing risk of PAH induced cancer in humans, at least with respect to the diol epoxide pathway of PAH carcinogenesis.
Introduction

Polycyclic aromatic hydrocarbons (PAH) are well-established environmental carcinogens that are believed to play a significant role as causes of cancer of the skin and lung in occupationally exposed humans, and of lung cancer in smokers (Dipple et al., 1984; International Agency for Research on Cancer, 1983; International Agency for Research on Cancer, 1984; International Agency for Research on Cancer, 1985; Luch, 2005; Straif et al., 2005). The prototypic PAH is benzo[a]pyrene (BaP), one of the most extensively studied of all carcinogens, and considered carcinogenic to humans by the International Agency for Research on Cancer (Straif et al., 2005). BaP and other PAH require metabolic activation to exert their carcinogenic effects (Dipple et al., 1984). One of the accepted pathways of metabolic activation of BaP proceeds through the formation of the carcinogenic “bay region diol epoxide,” anti-BaP-7,8-diol-9,10-epoxide (BPDE), illustrated in Figure 1A (Conney, 1982; Cooper et al., 1983; Dipple et al., 1984). BPDE reacts easily with DNA to form adducts that cause miscoding and mutations, and are crucial factors in its mechanism of carcinogenesis. There are opposing detoxification mechanisms, one of which is commonly believed to be conjugation with glutathione (GSH). Many epidemiologic studies have examined the relationship between polymorphisms in glutathione-S-transferase (GST) genes and the occurrence of cancer in people exposed to PAH, investigating the hypothesis that those deficient in GST activity, as determined by genotyping of variants in GSTM1 and GSTP1, should be at higher risk. Taking lung cancer as one thoroughly studied example, the results have not shown consistent associations or were null (Carlsten et al., 2008; Cote et al., 2009; Hashibe et al., 2003; Vineis et al., 2004; Ye et al., 2006).

Our approach to investigating the role of GSTs in PAH carcinogenesis started with a phenotyping strategy in which we proposed to characterize and quantify the urinary mercapturic acids resulting from GST-catalyzed detoxification of PAH diol epoxides, followed by normal metabolic processing of the initially formed GST conjugates by γ-glutamyltranspeptidase, cysteinylglycine dipeptidase, and cysteine S-conjugate N-acetyltransferase (Hecht et al., 2008). In this work we focused on phenanthrene (Phe), the simplest PAH with a bay region, a feature closely associated with PAH carcinogenicity, although Phe is generally not considered carcinogenic. Human exposure to Phe is thousands of times higher than to BaP,
thus facilitating analysis of its urinary metabolites. We analyzed human urine for the mercapturic acid 4-(N-acetylcysteinyl)-1,2,3-trihydroxy-1,2,3,4-tetrahydrophenanthrene (Phe-1,2-D-4-NAC) (Figure 1B) which would be formed upon conjugation of the bay region diol epoxide, anti-Phe-1,2-diol-3,4-epoxide (Phe-1,2-D-3,4-E). We found no evidence for the presence of this mercapturic acid in human urine, but rather identified considerable amounts of 1-(N-acetylcysteinyl)-2,3,4-trihydroxy-1,2,3,4-tetrahydrophenanthrene (Phe-3,4-D-1-NAC), the mercapturic acid resulting from the “reverse diol epoxide” anti-Phe-3,4-diol-1,2-epoxide (Phe-3,4-D-1,2-E). Reverse diol epoxides are not generally associated with strong mutagenicity or carcinogenicity, although there are some exceptions (Conney, 1982; Glatt et al., 1993). In further studies, we investigated the metabolism of Phe-1,2-diol, Phe-3,4-diol, Phe-1,2-D-3,4-E and Phe-3,4-D-1,2-E in human hepatocytes (Hecht et al., 2009). Consistent with the results of our analyses of human urine, we found that glutathione conjugation of the reverse diol epoxide, Phe-3,4-D-1,2-E was strongly favored over conjugation of the bay region diol epoxide Phe-1,2-D-3,4-E (Hecht et al., 2009). Collectively, these results were quite surprising because they seemed to contradict the hypothesis that carcinogenic bay region diol epoxides such as Phe-1,2-D-3,4-E and BPDE were detoxified by GST-catalyzed conjugation. Many studies have investigated the conjugation of BPDE with glutathione, catalyzed by GST-M1-1, GST-P1-1, and GST-A1-1, either as purified or expressed enzymes or in cellular systems engineered to overexpress these enzymes (Fields et al., 1998; Hu et al., 1999; Jernström et al., 1989; Jernström et al., 1996; Kushman et al., 2007b; Kushman et al., 2007a; Robertson et al., 1986; Romert et al., 1989; Seidel et al., 1998; Srivastava et al., 1999; Sundberg et al., 1997; Sundberg et al., 1998; Sundberg et al., 2001; Sundberg et al., 2002). All of these studies demonstrate GSH conjugation of BPDE, and most show a decrease in BPDE-DNA binding and mutagenesis in tandem with the conjugation. Indeed, these studies reasonably form the basis for the hypothesis that individuals with lower GST activity are at higher risk for cancer upon exposure to PAH. However, we are not aware of any studies in the literature that demonstrate conjugation of BPDE with glutathione in human cellular systems that have not been engineered to overexpress GSTs. Collectively, these data raised some important questions about the veracity of the BPDE GSH detoxification hypothesis. If BPDE is not
conjugated by GSTs in humans, as we have seen for Phe-1,2-D-3,4-E, then this pathway is not as important as previously thought. Therefore, in this study we examined the formation of mercapturic acids in human hepatocytes incubated with BPDE or the reverse diol epoxide \textit{anti}-BaP-9,10-diol-7,8-epoxide (rev-BPDE, Figure 1A).
Materials and Methods

Chemicals. 10-((N-Acetylcysteinyl)-9-hydroxy-9,10-dihydrophenanthrene (Phe-9-hydroxy-10-NAC) was prepared as described (Upadhyaya et al., 2006). Racemic BPDE and rev-BPDE were obtained from the National Cancer Institute Chemical Carcinogen Reference Standard Repository. N-Acetylcysteine (NAC) was purchased from Sigma-Aldrich.

7-((N-Acetylcysteinyl)-8,9,10-trihydroxy-7,8,9,10-tetrahydrobenzo[a]pyrene (rev-BPDE-7-NAC) was prepared essentially as described (Hecht et al., 2008). Briefly, a solution of racemic rev-BPDE (2.4 mg, 0.008 mmole) in 2.0 ml of dry THF was added to 2 ml of 3.0 M NAC (adjusted to pH 10.0). The mixture was stirred for 2 h and the isomers were separated by HPLC and collected using a 250 mm x 10 mm, 10µ, C-18 Vydac 210TP column (Separations Group, Hesperia, CA) eluted at 3 ml/min with 60% 30 mM NH₂HCO₃ and 40% CH₃OH, overall yield, 1.6mg, 45.3%. Isomer 1 (670 µg), retention time 29.92 min; UV λmax 202, 248, 328, 345 nm; MS (negative ion ESI) m/z 464 [M – H]⁻ (100); MS/MS of m/z 464; m/z 446 (100), 317 (51). ¹H NMR (D₂O) δ 8.19 (s, 1H, H11), 8.01 (m, 1H, H6), 7.66 – 7.45 (m, 6H, H1, H2, H3, H4, H5 and H12), 5.46 (m, 1H, H10), 4.40 (m, 1H, H9), 4.31 (m, 2H, H7 and H8), 3.95 (m, 1H, CHNH), 2.51 (s, 3H, CH₃); ¹³C NMR (D₂O) δ 128.2, 127.9, 127.3, 126.2, 125.5, 125.5 (CH1, CH2, CH3, CH4, CH5, CH12), 125.8 (CH11), 123.1 (CH6), 72.9 (CH9), 68.9 (CH8), 68.6 (CH10), 54.3 (CHNH), 49.3 (CH7), 31.8 (SCH2), 21.8 (COCH3). Isomer 2 (1.0 mg), retention time 34.56 min; UV λmax 201, 248, 328, 345 nm; MS (negative ion ESI) m/z 464 [M – H]⁻ (100); MS/MS of m/z 464; m/z 446 (100), 317 (51). ¹H NMR (D₂O) δ 8.21 (s, 1H, H11), 8.13 (m, 1H, H6), 7.87 – 7.79 (m, 3H, H1, H5 and H3), 7.69 (dd, J = 7.2, 7.2 Hz, 1H, H2), 7.60 (m, 3H, H4 and H12), 5.51 (d, J = 3.6 Hz, 1H, H10), 4.43 (m, 1H, H9), 4.34 (m, 2H, H7 and H8), 4.27 (m, 1H, CHNH), 2.95 (dd, J = 4.8, 13.2 Hz, 1H, SCH2a), 2.60 (m, 1H, SCH2b), 1.81 (s, 3H, CH3); ¹³C NMR (D₂O) δ 127.6, 127.1, 126.7, 125.9, 125.2, 125.2 (CH1, CH2, CH3, CH4, CH5, CH12), 123.5 (CH11), 122.8 (CH6), 72.5 (CH8), 70.9 (CH10), 70.8 (CH9), 54.5 (CHNH), 46.0 (CH7), 34.4 (SCH2), 21.1 (COCH3).

10-((N-Acetylcysteinyl)-7,8,9-trihydroxy-7,8,9,10-tetrahydrobenzo[a]pyrene (BPDE-10-NAC) was similarly prepared, 0.87 mg, 23.4% overall yield. Isomer 1 (254 µg), retention time 27.57 min; UV λ
.max 201, 248, 328, 345 nm; MS (negative ion ESI) m/z 464 [M – H]⁻ (100); MS/MS of m/z 464; m/z 446 (100), 335 (58); 317 (53.5); 283 (13.6); ¹H NMR (D₂O) δ 8.3 (s, 1H, H11), 8.19 (s, 1H, H6), 8.10 (m, 2H, H1 and H3), 8.01 (d, J=7.2Hz, 1H, H5), 7.87 (dd, J = 7.8, 7.2 Hz, 1H, H2), 7.83 (m, 1H, H4), 7.67 (m, 1H, H12), 5.03 (m, 1H, H10), 4.64 (m, 1H, H7), 4.52 – 4.48 (m, 2H, H8 and H9), 4.19 (dd, J = 3.6, 6.6 Hz, 1H, CH/NH), 3.17 (dd, J = 7.2, 13.2 Hz, 1H, SCH₂a), 2.89 (m, 1H, SCH₂b), 1.56 (s, 3H, CH₃); ¹³C NMR (D₂O) δ 128.3, 127.9, 127.4, 126.7, 125.9, 125.9 (CH₁, CH₂, CH₃, CH₄, CH₅, CH₁₂), 123.7 (CH₁₁), 123.8 (CH₆), 73.8 (CH₉), 70.9 (CH₈), 71.1 (CH₁₀), 71.0 (CH₇), 54.8 (CHNH), 35.0 (SCH₂), 22.0 (COCH₃). Isomer 2 (622 µg), retention time 31.25 min; UV λmax 201, 248, 328, 345 nm; m/z 464 [M – H]⁻ (100); MS/MS of m/z 464; m/z 446 (100), 335 (58); 317 (53.5); 283 (13.6); ¹H NMR (D₂O) δ 7.78 (s, 1H, H11), 7.73 (s, 1H, H6), 7.48 – 6.96 (m, 6H, H1, H2, H3, H4, H5 and H12), 4.98 (d, J = 8.4 Hz, 1H, H10), 4.73 (m, 1H, H7), 4.57 (m, 1H, H9), 4.51 (m, 2H, H8 and CH/NH), 3.29 (m, 1H, SCH₂a), 2.80 (m, 1H, SCH₂b), 1.76 (s, 3H, CH₃); ¹³C NMR (D₂O) δ 128.2, 128.8, 127.0, 126.4, 125.5, 125.5 (CH₁, CH₂, CH₃, CH₄, CH₅, CH₁₂), 126.0 (CH₁₁), 123.3 (CH₆), 73.0 (CH₉), 69.8 (CH₈), 68.7 (CH₁₀), 54.6 (CHNH), 49.2 (CH₇), 32.6 (SCH₂), 22.0 (COCH₃).

**Hepatocyte incubations.** Primary human hepatocytes were purchased from Cellzdirect (St. Louis, MO). Briefly, freshly isolated hepatocytes were plated onto 12-well plates (7 x 10⁵ cells/well) and overlaid with Matrigel 24-48 h after attachment. Cells were shipped overnight on cold preservation media and, upon receipt, media was replaced with serum-free Williams’ E media (without phenol red) and supplements as described (Hecht et al., 2009). Cells were allowed to recover from shipping for 10 h at 37 °C in an atmosphere containing 5% CO₂. Prior to incubation with substrate, the media was exchanged with 2 ml fresh media per well. Hepatocytes (approximately 0.12 mg protein per well) were incubated with BPDE or rev-BPDE, dissolved in 20 µl of DMSO, for a final substrate concentration of 10 µM, except as noted in Table 1. Aliquots (0.4 ml) of media were removed 12 h after addition of substrate. At 24 h after addition, the remaining media was collected. Samples were stored at -20 °C until analysis. Cell viability was assessed at 24 h by trypan blue exclusion staining.
Expression of GSTM1 and GSTA1 and measurement of GST activity by conjugation of 1-chloro-2,4-dinitrobenzene (CDNB) were carried out as described (Hecht et al., 2009).

**Analysis of mercapturic acids.** The internal standard Phe-9-hydroxy-10-NAC was added to a 0.2 ml aliquot of incubation medium and the mixture was applied to a Strata-X polymeric sorbent cartridge (33 µm, 30 mg/1 ml, Phenomenex) that had been activated with 1 ml of CH₃OH and 1 ml of H₂O. The cartridge was washed with 1 ml of H₂O and the analyte was eluted with 1 ml of 90% CH₃OH. The eluant was collected in a 2 ml silanized vial, and the solvents were removed on a Speedvac. The residue was taken up in 250 µl of CH₃OH and 5 µl of 4% NH₄OAc, transferred to an insert vial and concentrated to dryness. The residue was dissolved in 20 µl of 1% aqueous NH₄OAc, and 8 µl was analyzed by liquid chromatography-electrospray ionization-tandem mass spectrometry-selected reaction monitoring (LC-ESI-MS/MS-SRM) on a Thermo Finnigan TSQ Quantum Discovery MAX instrument (Thermo Electron, San Jose, CA) interfaced with an Agilent 1100 Capillary HPLC System (Agilent Technologies, Palo Alto, CA). The HPLC was equipped with a 5µm, 150 x 0.5 mm Zorbax SB-C18 column (Agilent Technologies, Santa Clara, CA) and a KrudKatcher disposable pre-column filter (Phenomenex). The column was eluted at 10 µl/min with 60% 30 mM NH₄HCO₃ in CH₃OH. The analysis was carried out essentially as described (Hecht et al., 2009), except that the transition m/z 464→ m/z 317, negative ion mode, [M – 1]⁻ → [(M – 1) – (CH₂CH(CO₂)NHCOCH₃ + H₂O)]⁻ was monitored for rev-BPDE-7-NAC or BPDE-10-NAC, and m/z 356 → m/z 209 for Phe-9-hydroxy-10-NAC.
Results

Each reaction of racemic BPDE or rev-BPDE with NAC produced a mixture of two isomers, separable by HPLC. An example from the reaction of rev-BPDE with NAC is illustrated in Figure 2A. Each isomer had a UV spectrum, $^1$H and $^{13}$C-NMR spectra, and MS consistent with the expected products, BPDE-10-NAC and rev-BPDE-7-NAC (Figure 1A). By analogy to previous work (Hecht et al., 2008), the first and second isomers probably resulted from cis- and trans- addition of NAC to the diol epoxides but further characterization was not pursued.

Demographic data for the hepatocyte donors are summarized in Table 1. Each sample was positive for expression of GSTA1 and 4 were positive for GSTM1 expression. CDNB activity averaged $65.2 \pm 41.8$ nmol conjugate/min/mg protein. These data are consistent with previous results (Hecht et al., 2009).

A chromatogram obtained upon LC-ESI-MS/MS-SRM analysis of a sample in which rev-BPDE was incubated for 24h with human hepatocytes is illustrated in Figure 2C, along with the internal standard for the analysis (Figure 2B). A clear peak corresponding to the second rev-BPDE-7-NAC isomer was observed. All samples in which rev-BPDE was added to human hepatocytes gave similar chromatograms, although the amounts of the two peaks varied. The results are summarized in Table 1. Mean levels of isomers 1 and 2 of rev-BPDE-7-NAC, respectively, in the nine samples incubated with 10 $\mu$M rev-BPDE were $0.017 \pm 0.023$ pmol/ml and $0.30 \pm 0.61$ pmol/ml in the 12h incubations, and $0.18 \pm 0.12$ pmol/ml and $3.59 \pm 5.95$ pmol/ml in the 24h incubations.

BPDE-10-NAC (isomer 2) was observed in only one of the 10 samples incubated with BPDE at levels of $0.015$ (12 h) and $0.035$ (24 h) pmol/ml.
Discussion

The results reported here demonstrate that the reverse diol epoxide rev-BPDE undergoes GSH conjugation in human hepatocytes, but the carcinogenic bay region diol epoxide, BPDE, does not (except for relatively small amounts in one sample). These results are completely consistent with our previous study of GSH conjugation of Phe diol epoxides in human hepatocytes, summarized in Figure 1B. The reverse diol epoxide, Phe-3,4-D-1,2-E, was a far better substrate for GSH conjugation, as determined by analysis of mercapturic acids in the medium, as in this study, than was the bay region diol epoxide Phe-1,2-D-3,4-E, for which only minimal conjugation was observed. Reverse diol epoxides such as rev-BPDE and their diol precursors generally have little or no carcinogenic or mutagenic activity in contrast to bay region diol epoxides (Conney, 1982; Glatt et al., 1993). Consequently, these results have substantial implications for molecular epidemiologic studies which have examined, using genotyping strategies, the relationship between GSTM1 null or GSTP1 low activity variants and cancer in people exposed to PAH. We find little or no evidence to support the assumption, inherent in many of these studies, that carcinogenic bay region diol epoxides of PAH are detoxified by GSH conjugation in unadulterated human cells.

The Jernström group was apparently the first to study conjugation of BPDE and other PAH diol epoxides by human enzymes (Robertson et al., 1986). In a series of studies using purified human GSTs (Robertson et al., 1986), cellular subfractions with GST activity (Jernström et al., 1989), recombinant GSTs and their variants (Jernström et al., 1996; Sundberg et al., 1997; Sundberg et al., 1998), and Chinese hamster V79 cells overexpressing human GSTs (Seidel et al., 1998; Sundberg et al., 2001; Sundberg et al., 2002), they clearly demonstrated that these bay region and fjord region diol epoxides can be converted to GSH conjugates by GST M1, GST P1, and GST A1, and that such conjugation was in most cases protective against DNA damage and mutagenicity. In one study using unadulterated MCF-7 human breast carcinoma cells incubated with BPDE, however, they found no activity (Romert et al., 1989). A notable finding in another study was that only about 1-2% of the rate expected for GSH conjugation of BPDE was actually observed in cells, indicating the importance of competing reactions of
BPDE (Sundberg et al., 2002). Townsend and co-workers also studied GSH conjugation of BPDE in cells overexpressing human GSTs and found protection against BPDE-DNA binding and mutagenicity (Fields et al., 1998; Kushman et al., 2007b; Kushman et al., 2007a). Singh and co-workers obtained similar results and made the interesting observation that the GSH conjugate of BPDE inhibits its own formation (Hu et al., 1999; Srivastava et al., 1999). We are not aware of any studies in the literature which report the GSH conjugation of BPDE in unadulterated human cells or in humans.

The studies by the Jernström group present a comprehensive and authoritative examination of PAH diol epoxide conjugation catalyzed by GSTs. One conclusion of their studies is that diol epoxide stereochemistry strongly affects conjugation. Our results are consistent with this. In BPDE, the 10-position of the diol epoxide, where conjugation would occur, is sterically more hindered because of its presence in the bay region than is the 7-position of rev-BPDE. The major difference between the Jernstrom studies and those presented here is that they used hamster V79 cells stably expressing different human GSTs, whereas our work was performed with human hepatocytes which were not engineered to affect GST conjugation, and this difference is undoubtedly critical in explaining the divergent results. One aspect not examined in this study however is possible differences in transport or secretion of the GST conjugates of rev-BPDE and BPDE into the medium.

In summary, the results presented here provide no support for the hypothesis that the carcinogenic bay region diol epoxide of BaP is detoxified by GSH conjugation in human hepatocytes. Therefore, a basic assumption underlying molecular epidemiology studies of GST variants and cancer in people exposed to PAH may be incorrect.
References


Robertson IG, Guthenberg C, Mannervik B, and Jernström B (1986) Differences in stereoselectivity and catalytic efficiency of three human glutathione transferases in the conjugation of glutathione with 7β,8α-dihydroxy-9α,10α-oxy-7,8,9,10-tetrahydrobenzo(a)pyrene. *Cancer Res* **46**:2220-2224.


Footnotes.

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Figure Legends.

1. Overview of (A) the question posed in this study and (B) our previous results in studies of GSH conjugation of Phe diol epoxides (Hecht et al., 2008; Hecht et al., 2009). The mercapturic acid metabolites illustrated are produced by normal metabolic processing of initially-formed GSH conjugates by successive action of γ-glutamyltranspeptidase, cysteinylglycine dipeptidase, and cysteine S-conjugate N-acetyltransferase.

2. Chromatograms obtained upon LC-ESI-MS/MS-SRM analysis of (A) products formed in the reaction of racemic rev-BPDE with NAC; (B) internal standard Phe-9-hydroxy-10-NAC for the hepatocyte analysis; and (C) medium from hepatocytes incubated with 10 µM rev-BPDE for 24h.
Table 1. Characteristics of hepatocytes and their donors and formation of mercapturic acids

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<td>0.04</td>
<td>0.14</td>
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<td>1.29</td>
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<td>1085</td>
<td>F</td>
<td>43</td>
<td>27</td>
<td>Caucasian</td>
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<td>no</td>
<td>163</td>
<td>+</td>
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<td>1088</td>
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<td>58</td>
<td>22</td>
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<td>yes</td>
<td>74</td>
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<td>1.29</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>54 ± 11</td>
<td>27 ± 4.5</td>
<td>65.2 ± 41.8</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>0.017 ± 0.23</td>
<td>0.30 ± 0.61</td>
<td>0.18 ± 0.12</td>
<td>3.59 ± 5.95</td>
</tr>
</tbody>
</table>

a BPDE-10NAC was detected in only 1 sample, #1085, 0.015 (12h) and 0.035 (24h) pmol/ml
b 1 µM rev-BPDE and BPDE were used
c Means for rev-BPDE-7-NAC are based on the 9 samples in which its concentration was 10 µM
Figure 1.

A. bay region

BPDE → human hepatocytes?

BPDE-10-NAC

rev-BPDE → human hepatocytes?

rev-BPDE-7-NAC

B. bay region

minimal in human hepatocytes or urine

Phe-1,2-D-3,4-E → Phe-1,2-D-4-NAC

observed in human hepatocytes or urine

Phe-3,4-D-1,2-E → Phe-3,4-D-1-NAC
Figure 2.