

## Detection of benzo[*a*]pyrene diol epoxide-DNA adducts in human placenta

(fluorescence spectroscopy/gas chromatography/mass spectroscopy/polycyclic aromatic hydrocarbons/molecular epidemiology)

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**ABSTRACT** Human placenta is a readily available organ that responds to maternal environmental insult and has been previously used to investigate metabolism and bioactivation of procarcinogens, for example, benzo[*a*]pyrene. HPLC in combination with synchronous fluorescence spectroscopy was used to examine 28 placentas for the presence of benzo[*a*]pyrene diol epoxide-DNA adducts, and 10 of these were found to be positive. DNA samples from these placentas were subsequently pooled and subjected to partial enzymatic digestion to oligonucleotide fragments. Concentration of those DNA fragments containing benzo[*a*]pyrene diol epoxide-DNA adducts was achieved by immunoaffinity chromatography with polyclonal antibodies raised against these adducts. Column eluates were hydrolyzed under mild acid conditions and extracted with an organic solvent. The presence of benzo[*a*]pyrene-7,10/8,9-tetrahydrotetrol residues in the extracts was determined by HPLC and synchronous fluorescence spectroscopy and was confirmed by GC/MS. The results unequivocally confirm bioactivation and formation of DNA adducts from benzo[*a*]pyrene in human placenta *in vivo* and establish a methodological approach to direct measurement of carcinogen-DNA adducts that are formed as a result of human environmental exposure.

Exposure to potentially mutagenic and carcinogenic chemicals is widespread in human populations, and it has been proposed that chemical damage of DNA may be a useful molecular dosimeter in epidemiological investigations of exposed populations (1-4). The placenta may be a potentially important source of material for molecular epidemiological studies in humans because it is large, readily available, and responsive to maternal exposures to environmental pollutants. Previous studies have shown that the placenta contains a mixed-function oxidase system (cytochrome P-450) that is induced by maternal cigarette smoking and other xenobiotic exposures [e.g., dietary (5-8)]. Furthermore, microsomes from human placentas will catalyze the production of mutagenic metabolites (9) and DNA adducts (10) from various polycyclic aromatic hydrocarbon (PAH) carcinogens *in vitro*. More recently, evidence that human placental DNA contains adducts has been presented, but the identity of the compounds responsible is unclear (11-13).

In 1968, Welch *et al.* (5) demonstrated that human placenta contains monooxygenase systems whose activities toward benzo[*a*]pyrene (BP), a widespread environmental contaminant, were induced in response to maternal cigarette smoking. We now present direct physicochemical evidence that BP is bioactivated and forms DNA adducts *in vivo* in human placenta. Our approach combines synchronous fluorescence spectroscopy (SFS), a highly sensitive and specific technique

for detection and identification of PAHs (14-16), with HPLC and immunoaffinity chromatography and provides a methodological basis for epidemiologic studies in selected human populations.

### MATERIALS AND METHODS

**Chemicals.** Racemic ( $\pm$ )-*r*-7,*t*-8-dihydroxy-*t*-9,10-epoxy-7,8,9,10-tetrahydro[1,3-<sup>3</sup>H]BP (<sup>3</sup>H]BPDE) was obtained from the National Cancer Institute Chemical Carcinogen Reference Standard Repository (Bethesda, MD). Nonlabeled authentic *r*-7,*t*-8,*t*-9,*c*-10-tetrahydro-BP (BP-7,10/8,9-tetrol; see ref. 17 for nomenclature) and *r*-7,*t*-8,*t*-9-trihydroxy-7,8,9,10-tetrahydro-BP (BP-7/8,9-triol) were also obtained from this source. Antibody isolation columns (IgM/IgG Quick-Sep) were obtained from Isolab (Akron, OH), and CNBr-activated Sepharose 4B was from Pharmacia. Calf thymus DNA was obtained from Sigma and was repurified before use by phenol extraction and ethanol precipitation. Redistilled phenol was purchased from Bethesda Research Laboratories. Tri-Sil/bovine serum albumin in silylation grade pyridine was obtained from Pierce. Isoamyl alcohol (analytical grade) was procured from Mallinckrodt (Paris, KY). Tetrahydrofuran was obtained from Aldrich. Water and methanol (both HPLC grade) were obtained from Baker. Trimethylchlorosilane and hexamethyldisilazane were obtained from Aldrich, and pyridine (analytical grade) was obtained from FSA Laboratory Supplies (Loughbrough, UK).

**Human Placental DNA.** Placentas from term, uncomplicated pregnancies were collected at delivery at University Hospital, Denver. Maternal histories and blood samples were obtained on the first postpartum day. Smoking histories included use of tobacco and marijuana. Active smoking was defined as daily use of cigarettes. Women who smoked less often or who quit smoking during pregnancy were excluded from the study.

Placental nuclei were prepared in two fractions by using the technique described by Resendez-Perez *et al.* (18): nuclear fraction IV (NF-IV; ref. 18) containing knotted nuclei from syncytiotrophoblast and nuclear fraction III (NF-III) containing free nuclei from syncytiotrophoblast plus nuclei from other placental cell types as well as from contaminating maternal and fetal leukocytes. For each placenta studied, nuclear fractions were prepared from  $\approx$ 40 g of freshly obtained placental villous tissue. Nuclear fractions were

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Abbreviations: BP, benzo[*a*]pyrene; NF-III, nuclear fraction III; NF-IV, nuclear fraction IV; PAH, polycyclic aromatic hydrocarbon; SFS, synchronous fluorescence spectroscopy; TMS, tetramethylsilyl; BPDE, *r*-7,*t*-8-dihydroxy-*t*-9,10-epoxy-7,8,9,10-tetrahydro-BP; BP-7/8,9-triol, *r*-7,*t*-8,*t*-9-trihydroxy-7,8,9,10-tetrahydro-BP; BP-7,10/8,9-tetrol, *r*-7,*t*-8,*t*-9,*c*-10-tetrahydro-BP.

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treated with RNase A (100  $\mu\text{g}/\text{ml}$ ) in buffer containing sodium dodecyl sulfate (SDS) (0.6%), EDTA (10 mM), and Tris-HCl (10 mM; pH 7.5) at 37°C for 60 min. Proteinase K (Sigma) was added (100  $\mu\text{g}/\text{ml}$ ), and the mixture was incubated for an additional 120 min. The digest was extracted with equal volumes of phenol, phenol/chloroform, 1:1 (vol/vol), and chloroform; DNA was precipitated by the addition of 2.5 vol of ethanol in the presence of NaCl (100 mM). Extractions were repeated until the  $A_{260}/A_{280}$  ratios were  $\geq 1.8$ .

**BPDE-Modified DNA.** Preparation of BPDE-DNA was performed by using calf thymus DNA and [ $^3\text{H}$ ]BPDE as described previously by Tierney *et al.* (19) and Pulkrabek *et al.* (20). Briefly, [ $^3\text{H}$ ]BPDE was dissolved in tetrahydrofuran (1 mg/ml), and the solution was made up to 5 ml with ethanol. The diol epoxide solution was mixed with 10 ml of a DNA solution (1 mg/ml in Tris buffer at pH 7.4). The mixture was incubated at room temperature overnight and was extracted the next day with an equal volume of water-saturated diethyl ether (eight times) and water-saturated isoamyl alcohol (four times) to remove unreacted PAH residues. The DNA was precipitated by the addition of sodium chloride (100 mM) and ethanol (2.5 vol). The precipitated DNA was then washed in 70% ethanol and redissolved in water. The adduct level was determined to be 1.3% (36 pmol of BPDE per  $\mu\text{g}$  of DNA) by UV absorption spectroscopy ( $\epsilon_{347} = 29 \times 10^3$ ) and liquid scintillation counting.

**Immunoaffinity Chromatography.** Three immunoaffinity columns were prepared as a batch from rabbit polyclonal anti-BPDE-DNA antibodies (21) as described by Tierney *et al.* (22). One column was used for calibration with [ $^3\text{H}$ ]BPDE-DNA. The other two were reserved for use with human placental DNA exclusively to avoid potential contamination with standard materials. DNA samples were digested with DNase I under the conditions described by Tierney *et al.* (22). Up to 2 mg of digested DNA was applied to a single column at one time. Each placental DNA sample was loaded onto the columns with 10 void volumes (2 ml) of phosphate-buffered saline and eluted with 10 void volumes of NaOH (50 mM). It was necessary to repeat this cycle five times for each DNA sample (2 mg) loaded in order to recover all of the DNA that was applied. Approximately 3% of the DNA applied was eluted in NaOH. Columns were washed extensively with phosphate-buffered saline between applications of DNA to achieve neutral pH. Column eluates were collected in 2-ml fractions, and DNA contents were determined spectrophotometrically ( $A_{260}$ ). DNA fragments bound by the antibodies (i.e., eluted with NaOH) were pooled, adjusted to pH 1.5 with HCl, hydrolyzed (90°C for 3 hr), and subjected to organic solvent extraction prior to HPLC/SFS as described below.

**Hydrolysis to Tetrahydrotetrols.** Previous experience with SFS in biological samples suggests the presence in human DNA of complex mixtures of fluorophores (23, 24). To resolve BP-derived signals from these mixtures, putative diol epoxide-DNA adducts were acid hydrolyzed to the corresponding tetrahydrotetrols, extracted with isoamyl alcohol, dried *in vacuo*, redissolved in water, and subjected to HPLC as previously described (25). Briefly, DNA from individual placentas (500  $\mu\text{g}$  of NF-III or NF-IV) was acidified by addition of HCl to a final concentration of 100 mM and heated at 90°C for 3 hr. In subsequent experiments, acidified immunoaffinity column eluates were also subjected to this treatment. Each sample was then extracted three times with an equal volume of isoamyl alcohol. The organic phases were then washed with an equal volume of water and evaporated to dryness under reduced pressure, and the residues were redissolved in 600  $\mu\text{l}$  of water. Under these conditions, recovery of radioactivity from a [ $^3\text{H}$ ]BPDE-DNA standard has been found to be  $>99\%$  (25).

**HPLC.** Reverse-phase HPLC was conducted at room temperature with a Vydac C-18 column (25 cm  $\times$  4.6 mm). Samples were eluted with linear methanol/water gradients (30–60% over 10 min, followed by 60–100% over 5 min) at a flow rate of 1 ml/min. Eluates were collected in 0.5-ml fractions. Labeled BP-tetrol that was extracted from hydrolyzed [ $^3\text{H}$ ]BPDE-modified DNA eluted in a major peak at 14.5–15.0 min with a final recovery of 60% of the radioactivity (24).

**SFS.** This technique is highly sensitive and specific for analysis of relatively simple mixtures of fluorescent compounds, especially PAHs. SFS is described in detail elsewhere (14, 16) and has previously been used to assay for BPDE-DNA adducts in humans (23, 26). Two types of SFS analysis were used for these studies: simple two-dimensional SFS with a Perkin-Elmer MPF-66 fluorescence spectrophotometer (Perkin-Elmer, Rockville, MD) with a fixed wavelength difference of 34 nm and three-dimensional SFS, where a total of 30 synchronous spectra were generated for each sample in a Perkin-Elmer 650-40 fluorescence spectrophotometer by incrementing the fixed wavelength difference by 4 nm beginning at 10 nm and finishing at 126 nm.

Quantitative estimates of SFS "signature" signals for the tetrahydrotetrols of BP were based on comparisons with standard curves generated from known concentrations of either [ $^3\text{H}$ ]BPDE-DNA or authentic BP-tetrols that were measured by spectrophotometry ( $\epsilon_{347} = 42 \times 10^3$ ).

**GC/MS.** Placental DNA extracts that had been subjected to HPLC/SFS either with or without immunoaffinity chromatography were analyzed by GC/MS under the following conditions. Each sample was divided into two equal portions; half was sent frozen to the MRC Toxicology Laboratories in Carshalton, and the other half was sent to the University of Colorado Gas Chromatography/Mass Spectrometry Laboratory in Denver. Samples sent to Carshalton were converted to tetratrimethylsilyl (TMS) derivatives by treatment with trimethylchlorosilane/hexamethyldisilazane/pyridine, 1:3:9 (vol/vol, 60°C for 30 min) and analyzed by selected ion monitoring for the presence of the molecular ion  $m/z$  608<sup>+</sup> and a base peak  $m/z$  404<sup>+</sup>. Splitless injection was used to introduce materials onto the column (OV-1, 25 m  $\times$  0.25 mm), which was eluted with a temperature gradient of 70°C–290°C (30°C/min). Ions were detected by using a Vacuum Generators (VG) analytical 70-SEQ GC/MS instrument in the electron-ionization mode. The samples that were received in Denver were evaporated under nitrogen, and a known volume of methanol was added. An aliquot of BP-7/8,9-triol (final concentration of 100 pg/ $\mu\text{l}$ ) was added as an internal standard, and the samples were silylated in a total volume of 100  $\mu\text{l}$  of Tri-Sil/bovine serum albumin (2.5 milliequivalents per ml) for 2 hr in a sand bath at 100°C. The treated samples were evaporated under nitrogen and redissolved in 20  $\mu\text{l}$  of bistrifluoroacetamide/toluene, 1:10 (vol/vol). Portions of these samples (2  $\mu\text{l}$ ) were isothermally chromatographed on a 30-m DB-1 capillary column (J & W Scientific, Folsom, CA) at 280°C by using helium as carrier. The column effluent was directed into the source of a VG MM-16 low-resolution mass spectrometer. Ions were formed by using a 20-eV (1 eV =  $1.602 \times 10^{-19}$  J) electron ionizer and characteristic geminal diol [ $m/z$  191<sup>+</sup>;  $(\text{CH}_3)_3\text{-Si-O-CH}_2\text{-CH}_2\text{-O-Si-(CH}_3)_2$ ] fragment ions were detected with a VG 2000 system in the selected ion monitoring mode.

## RESULTS

**HPLC/SFS.** The data in Fig. 1 show examples of three types of synchronous fluorescence signals (at a fixed wavelength difference of 34 nm) that were generated from human placental DNA samples following HPLC. Each of the spectra shown were generated from samples having a retention time

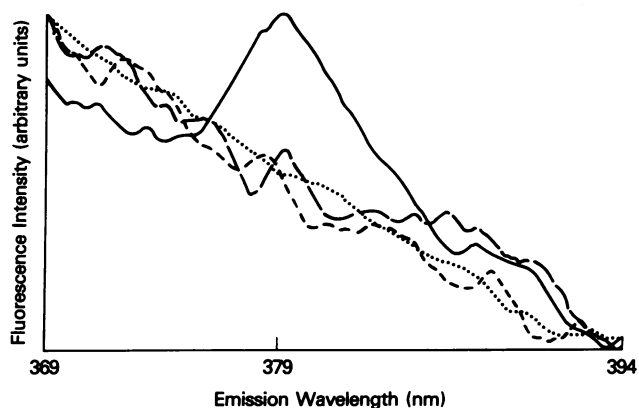


FIG. 1. Synchronous fluorescence spectra of HPLC eluates of acid hydrolysates of human placental DNA. —, BPDE-modified DNA; ···· and ---, human placental nuclear DNA; ·····, a negative sample.

that was identical to an authentic BP-7,10/8,9-tetrol standard [i.e., the fraction that was collected between 14.5 and 15.0 min (25)]. Placental samples were considered to be positive, equivocal, or negative according to the appearance of the fluorescence signals in this specific HPLC fraction. Examples of three types of signal are shown: the dotted line is negative, the line of short dashes is equivocal since the emission peak is not at 379 nm but is within 2 nm of this value, the line of long dashes suggests a positive signal, and the solid line is the spectrum generated by an HPLC-purified hydrolysate of [ $^3\text{H}$ ]BPDE-DNA. DNA samples (NF-IV) from 28 placentas, 15 from active cigarette smokers and 13 from nonsmokers, were analyzed by SFS and HPLC/SFS. In eight of these cases, NF-III fractions were also analyzed. In no case was a synchronous fluorescence signature signal for pyrene recognized prior to HPLC. However, positive signature signals for the pyrene fluorophore ( $\lambda_{\text{max}}$  emission = 379 nm) were observed in HPLC eluates (14.5–15.0 min) for 10 of the 28 placental DNA samples, but there was no correlation with smoking history. When eight placental NF-III fractions were analyzed, it was found that three were positive after HPLC. In each of these, the NF-IV fractions had also been positive. In no case was an NF-III fraction positive without the NF-IV fraction also being positive. These results are consistent with the hypothesis that DNA adducts are concentrated in knotted syncytiotrophoblast nuclei.

**Immunoaffinity Chromatography.** Immunoaffinity chromatography concentrates of DNase I-digested individual human placental DNA samples (1 mg) were found to yield complex three-dimensional SFS signals in fractions eluting with NaOH (data not shown). Furthermore, a pyrene signature was present, but it was not dominant prior to HPLC. This observation is consistent with reports that show that anti-BPDE-DNA antibodies recognize other PAH-DNA adducts (25, 27, 28) and suggests that the material concentrated by immunoaffinity chromatography contains other as yet unidentified fluorescent adducts.

**Extraction of BP-Tetrol from Placental DNA.** Corroborative evidence for the presence of BPDE-DNA adducts in human placental samples was sought by GC/MS. To concentrate enough material for these analyses, DNA (2–4 mg) was pooled from each of eight placental samples that were considered to be positive by HPLC/SFS. This pool of DNA (30 mg) was divided, and half of it (15 mg) was digested with DNase I. Fig. 2 shows the results of monitoring these placental DNA samples by two-dimensional SFS during concentration by immunoaffinity chromatography and fractionation by solvent extraction and HPLC. In each case the fluorescence spectra were obtained by driving the excitation and emission monochromators synchronously with a fixed wavelength difference of 34 nm. A highly complex spectrum that lacked a recognizable signature signal was obtained for the pooled DNA that had not undergone any preparative chromatography (Fig. 2A). A less complex spectrum containing a recognizable but imperfect signature peak was obtained following immunoaffinity chromatography (Fig. 2B). This signal was improved considerably following HPLC fractionation (Fig. 2C) and was superimposable upon an authentic reference spectrum prepared for BP-7,10/8,9-tetrol (Fig. 2D). From the intensity of these signals and a linear standard curve (data not shown), the total recovery of BP-7,10/8,9-tetrol was determined to be 2280 fmol (713 pg). By assuming complete hydrolysis, an extraction efficiency of >99% and 60% recovery from HPLC, the level of BPDE-DNA adducts in the original placental sample can be calculated to be 0.25 fmol of BPDE per  $\mu\text{g}$  of DNA, which is equivalent to 1 adduct in  $10^7$  nucleotides. In fact, the fluorescence spectral data presented in Fig. 2B and C suggest that the yield in this purification was only 18.2%. However, since the complexity of the spectrum shown in Fig. 2B is probably due to constituents in this sample, other than BP-tetrol, that may contribute to the fluorescence emissions, it is doubtful whether this comparison is strictly correct. It should also be noted that with increasing purification of the

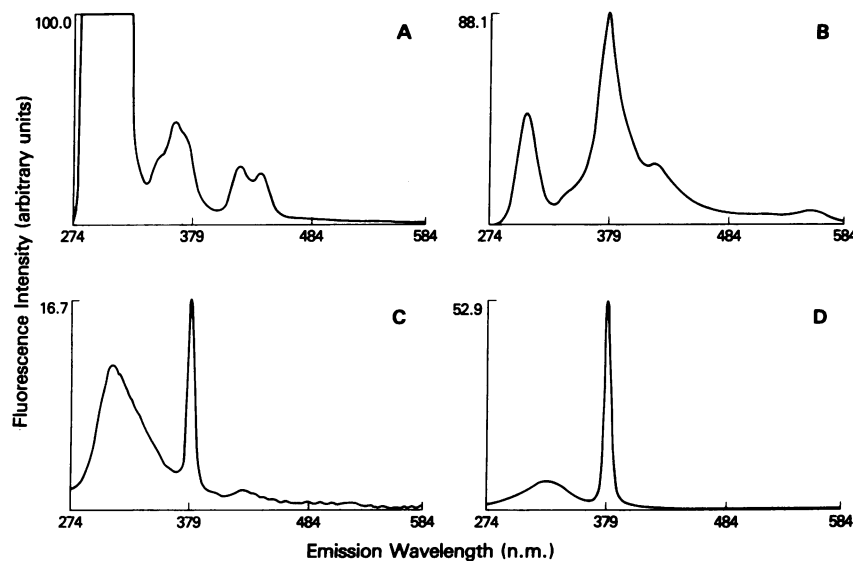


FIG. 2. Synchronous fluorescence spectra (at a fixed wavelength difference of 34 nm). (A) Acid-hydrolyzed isoamyl alcohol extracts of DNA (15 mg) pooled from eight placentas positive for BP-tetrol signature signals according to the HPLC/SFS methodology outlined in the text. (B) Acid-hydrolyzed isoamyl alcohol extracts of DNA fragments concentrated from a second pool of placental DNA (15 mg) by immunoaffinity chromatography. (C) Isoamyl alcohol extract of immunoaffinity column eluates (from B) after resolution (14.5–15.0 min fraction) by HPLC. (D) Authentic sample of BP-7,10/8,9-tetrol.

sample the fluorescence intensity at 379 nm actually increases (compare *A* and *B* in Fig. 2); this is probably due to quenching effects and chromophore interactions. When a placental DNA sample pool (15 mg) that had been purified by HPLC (retention time 14.5–15.0 min) but had not been concentrated by immunoaffinity chromatography was analyzed by SFS, the expected fluorescence spectrum was observed (data not shown); however, the signature peak was less intense than that shown in Fig. 2*C*.

**GC/MS.** After SFS analysis, samples were coded so that their identities were unknown to the investigators in the MS laboratories. Each sample set contained a solvent control, the HPLC fraction from the immunoaffinity concentrated sample (the fluorescence spectrum for which is shown in Fig. 2*C*), a corresponding HPLC-purified sample that had not been subjected to concentration or purification by immunoaffinity chromatography, and a sample containing authentic BP-7,10/8,9-tetrol. Fig. 3 shows the results of GC/MS selected ion monitoring at  $m/z$  404<sup>+</sup> (Fig. 3*A*) and  $m/z$  608<sup>+</sup> (Fig. 3*B*) for the TMS-derivatized placental DNA hydrolysates. Traces for an authentic BP-7,10/8,9-tetrol standard (100 pg) are shown in Fig. 3*C* and *D*. Clear signals for both the molecular ion ( $m/z$  608<sup>+</sup>; Fig. 3*B*) and the base peak, which is thought to be formed by loss of  $(\text{CH}_3)_3\text{-Si-O-CH-CH-O-Si-(CH}_3)_3$  from the molecular ion ( $m/z$  404<sup>+</sup>; Fig. 3*A*), were found with retention times identical to those for the TMS derivative of the authentic BP-7,10/8,9-tetrol in the human placental sample that had been purified by immunoaffinity chromatography and HPLC. Only the smaller of these fragments ( $m/z$  404<sup>+</sup>) was found in an identical sample that had been purified by HPLC but not immunoaffinity chromatography. The data shown in Fig. 4 were obtained by GC/MS selected ion monitoring analysis in order to detect a smaller fragment ( $m/z$  191<sup>+</sup>) of the TMS derivative of the placental DNA hydrolysates, which is thought to be characteristic of a geminal diol  $[(\text{CH}_3)_3\text{-Si-O-CH}_2\text{-CH}_2\text{-O-Si-(CH}_3)_2]$ . The quantity of BP-7,10/8,9-tetrol in placental samples was determined from a standard curve (Fig. 4*D*) that was generated for the authentic

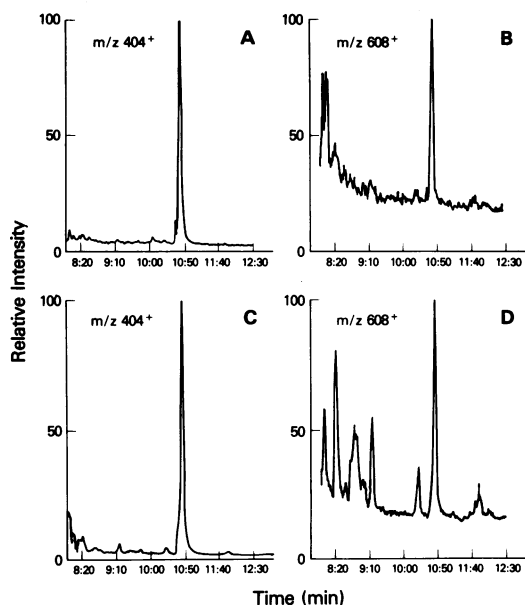


FIG. 3. GC/MS analyses. (A) TMS derivative of a human placental DNA extract following immunoaffinity chromatography and HPLC ( $m/z$  404<sup>+</sup>; the base peak). (B) TMS-derivative of a human placental DNA extract after immunoaffinity chromatography and HPLC ( $m/z$  608<sup>+</sup>; the molecular ion). (C) One hundred picograms of the TMS derivative of authentic BP-7,10/8,9-tetrol ( $m/z$  404<sup>+</sup>). (D) One hundred picograms of the TMS derivative of authentic BP-7,10/8,9-tetrol ( $m/z$  608<sup>+</sup>).

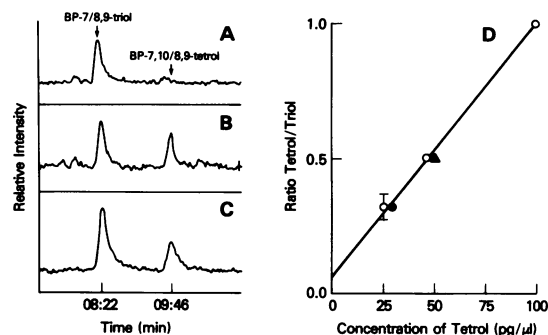


FIG. 4. GC/MS analyses. (A) Solvent control with the addition of BP-7/8,9-triol. (B) TMS derivative of a human placental DNA extract to which BP-7/8,9-triol had been added following both immunoaffinity chromatography and HPLC ( $m/z$  191<sup>+</sup>). (C) Fifty-four picograms of the TMS derivative of authentic BP-7,10/8,9-tetrol and BP-7/8,9-triol ( $m/z$  191<sup>+</sup>). (D) Calibration curve ( $m/z$  191<sup>+</sup>) for the TMS derivative of authentic BP-7,10/8,9-tetrol. Experimental points are shown by solid symbols.

BP-7,10/8,9-tetrol (50–500 pg), and BP-7/8,9-triol was used as an internal standard. The total amount of BP-7,10/8,9-tetrol that was found to be present in the sample that had been purified by immunoaffinity chromatography and HPLC was determined from the standard curve (Fig. 4*D*) to be 580 pg.

## DISCUSSION

Development of techniques for the detection of ultralow levels of adducts has facilitated the detection of BPDE–DNA adducts in simple model systems without the requirement for a radiolabeled procarcinogen. A combination of preparative techniques and physicochemical methods (immunoaffinity chromatography, HPLC/SFS, and GC/MS) were used to extract BPDE–DNA adducts from human placenta and subsequently identify and quantitate the BP-tetrol derivatives that were formed.

The data are consistent with growing evidence for damage to human DNA by BP and other environmental carcinogens (29, 30). Previously SFS has been used to detect BPDE–DNA adducts in peripheral leukocytes from coke oven workers (23, 25, 26). In some studies, corroborative enzyme immunoassays have also provided evidence for the presence of BPDE–DNA adducts in industrially exposed individuals (23, 26). Evidence for BPDE–DNA adducts in human placenta has previously been equivocal. Everson *et al.* (11, 12) analyzed placental DNA for PAH-derived adducts by immunoassay and <sup>32</sup>P-postlabeling. Both polyclonal and monoclonal antibodies raised against BPDE–DNA detected putative BPDE–DNA adducts at levels comparable to those levels determined for placentas from smoking and nonsmoking women that are presented in this report. However, analyses of these same DNA samples by Everson *et al.* (11, 12) by using <sup>32</sup>P-postlabeling nucleotide chromatography failed to detect BP-derived adducts or any known PAH–DNA adduct.

The major DNA adduct of BP that was detected in human placenta appears to be formed through metabolism of the parent hydrocarbon to BPDE. This finding is consistent with current models of oxidative metabolism involving human cytochrome P<sub>1</sub>-450 (17, 31–36) and is predicted by previous studies demonstrating cross-reactivity of monoclonal antibodies against rat hepatic PAH-inducible cytochrome P-450 and cigarette smoke-inducible human placental monooxygenases (8, 37), as well as by sequence similarities between mouse and human cytochrome P<sub>1</sub>-450 (38). Adduct formation is clearly a multifactorial process that will depend upon relative contributions of numerous genetic and environmental factors that previous studies have shown to be operative

in human placenta (8, 39, 40). It remains to be seen whether other BP adducts are formed in placental DNA and to what extent individual variation in placental metabolism of BP determines adduct formation.

The environmental origin of BP that is metabolized to form adducts with DNA in placenta is unknown. BP-tetrol was extracted from samples from both smoking and nonsmoking women. This observation is consistent with other studies that have failed to detect differences between smokers and nonsmokers in the levels of BPDE-DNA adducts (2, 30, 41) and also with the fact that human exposure to BP appears to occur primarily through the diet (42, 43). Widespread exposure to BP is not unexpected, and it is possible that adducts accumulate in knotted syncytial nuclei. The origin and relative concentrations of, as yet unidentified, human carcinogen-DNA adducts that have been observed in these studies (three-dimensional SFS) and in <sup>32</sup>P-postlabeling studies (11, 12, 44) await further scrutiny. BPDE-DNA adducts may be common and may not be dependent on cigarette smoking; consequently, measurement of their levels could serve as a useful reference against which to measure other carcinogen-DNA adducts in comparative studies.

The analytical techniques that have been combined to detect BPDE-DNA adducts in placenta will likely prove useful in further investigations of DNA adduct formation in humans. A particular advantage of SFS is that it provides chemically specific information without the destruction of the sample, thereby enabling serial purification of the signal and corroborative analytical techniques (e.g., GC/MS). With placenta as a source of DNA, milligram quantities of sample are readily available. Thus, by providing access to large samples of DNA, placental studies not only provide a basis for epidemiologic investigation but should also enable purification and identification of as yet uncharacterized carcinogen-DNA adducts. Moreover, it is also possible to isolate components of placental bioactivation, detoxication, and DNA repair systems *in vitro* and to correlate these activities on an individual basis with DNA damage by specific chemicals.

The results of this investigation represent a significant advancement in that they confirm the predictions of many previous studies in animal and human tissues with regard to exposure to BP and at the same time provide the technical foundation for further direct investigation of carcinogen damage to DNA in human populations.

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