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Analysis of the Benzene Oxide-DNA Adduct 7-Phenylguanine by Liquid Chromatography-Nanoelectrospray Ionization-High Resolution Tandem Mass Spectrometry-Parallel Reaction Monitoring: Application to DNA from Exposed Mice and Humans

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Abstract

Benzene oxide, the initial metabolite of the human carcinogen benzene, reacts with DNA producing 7-phenylguanine (7-PhG) and other products. We developed a highly sensitive liquid chromatography-nanoelectrospray ionization-high resolution tandem mass spectrometry-parallel reaction monitoring method for the analysis of 7-PhG in DNA. Accuracy and precision of the method were established and the detection limit was about 8 amol of 7-PhG injected on the column and less than 1 adduct per 10^9 nucleotides in DNA. 7-PhG was detected in calf thymus DNA reacted with 1 μM to 10 mM benzene oxide. The method was applied for the analysis of DNA isolated from bone marrow, lung, and liver of B6C3F$_1$ mice treated by gavage with 50 mg/kg benzene in corn oil 5 times weekly for 4 weeks. 7-PhG was not detected in any of these DNA samples. The method was applied to DNA from mouse hepatocytes exposed to 100 μM benzene oxide and human TK-6 lymphoblasts exposed to 100 μM, 1 mM, and 10 mM benzene oxide. 7-PhG was only detected in TK-6 cell DNA from the 10 mM exposure. The method was also applied to leukocyte DNA from 10 smokers and 10 nonsmokers. 7-PhG was detected in only one DNA sample, from a nonsmoker. The results of this study do not support the hypothesis that the benzene oxide-DNA adduct 7-PhG is involved in carcinogenesis by benzene.

Keywords

Benzene; benzene oxide; 7-phenylguanine; DNA adducts; mass spectrometry

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Conflict of Interest statement:
The authors declare that there are no conflicts of interest.

1. Introduction

Benzene is considered “carcinogenic to humans” by the International Agency for Research on Cancer and is ranked as a “known human carcinogen” by the U.S. government [1,2]. It causes acute myeloid leukemia and has also been implicated as a cause of acute lymphocytic leukemia, multiple myeloma, and non-Hodgkin’s lymphoma. Some studies indicate that benzene may be a cause of lung cancer, and human breath contains benzene. These epidemiologic observations are supported by studies in rats and mice which demonstrate that benzene is carcinogenic in multiple tissues [1,2].

Benzene requires metabolism to exert its carcinogenic effects [3]. The first step in benzene metabolism is the formation of benzene oxide - oxepin, catalyzed by cytochromes P450, especially CYP2E1 (Scheme 1). This metabolite has been identified in the blood of rats treated with benzene and has an estimated half-life of about 34 min in aqueous medium and 8 min when added to rat blood [3]. Benzene oxide easily rearranges to phenol, which is a major metabolite of benzene. It also reacts with glutathione, catalyzed by glutathione-*S*-transferases, resulting in the excretion of *S*-phenylmercapturic acid (SPMA) in human urine. Most human urine samples contain SPMA, which is considered a biomarker of benzene uptake and metabolism [4].

Many studies have investigated mechanisms of carcinogenesis by benzene [5]. Multiple hypotheses have been proposed and examined, but there is no single, widely accepted mechanism at the present time. Carcinogen-DNA adducts are crucial in carcinogenesis by various well-established carcinogens such as polycyclic aromatic hydrocarbons, nitrosamines, heterocyclic aromatic amines, 1,3-butadiene, and aflatoxin B_1 [6]. All of these compounds are converted by cytochrome P450 enzymes to electrophiles, including epoxides, which produce well-characterized adducts in DNA, which in turn cause miscoding and mutations in critical growth control genes. When the reactions resulting in DNA adduct formation are blocked or decreased, the carcinogenicity of these compounds decreases accordingly.

Benzene oxide is known to react with DNA to produce 7-phenylguanine (7-PhG) following the dehydration of the transient hydroxycyclohexadienyl intermediate shown in Scheme 1[7,8]. Other mechanisms of DNA adduct formation from benzene have also been investigated [9–12]. To our knowledge, 7-PhG has never been identified in laboratory animals or humans exposed to benzene. Therefore, we have developed a highly sensitive, accurate, and precise method for the analysis of 7-PhG in DNA, using liquid chromatography-nanoelectrospray ionization-high resolution tandem mass spectrometry-parallel reaction monitoring (LC-NSI-HRMS/MS-PRM). The method was applied for the analysis of calf thymus DNA reacted with benzene oxide, DNA isolated from mice treated with benzene, DNA from cells exposed to benzene oxide, and DNA from human leukocytes.

2. Materials and methods

2.1. Chemicals

7-PhG was synthesized in 3 steps by protection of the N^2 position of guanine, reaction with phenylboronic acid, and deprotection, as described previously [13]. [D_5]7-PhG was similarly synthesized with [D_5]phenylboronic acid (Cambridge Isotope Laboratories, Inc.). Both compounds were purified by collection from HPLC and identified by their spectral properties, consistent with those reported [13]: 7-PhG: ^{1}H NMR (DMSO-D_6) δ 10.97 (s, 1H, NH), 8.29 (s, 1H, C8-H), 7.48 (m, 5H, PhH), 6.34 (s, 2H, NH_2); MS (positive ESI) *m/z* 228, $[M + H]^+$; UV (65% 15 mM NH_4OAc in 35% aq CH_3CN) λ_{max} 230, 292 nm; [D_5]7-PhG: ^{1}H NMR (DMSO-D_6) δ 10.85 (s, 1H, NH), 8.23 (s, 1H, C8-H), 6.21 (s, 2H, NH_2); MS (positive ESI) *m/z* 233 $[M + H]^+$; UV (65% 15 mM NH_4OAc in 35% aq CH_3CN) λ_{max} 230, 292 nm.

Benzene oxide-oxepin was synthesized as described previously [14] in 96% purity with 4% degradation to phenol. ^{1}H NMR (300 MHz, CD_3CN) δ 6.28 (m, 2H, Cα-H), 5.95 (dd, *J* = 3 Hz, 6 Hz, 2H, Cβ-H), 5.20 (d, *J* = 6 Hz, 2H, Cγ H).

2.2 Reaction of benzene oxide with calf thymus DNA

Calf thymus DNA (20 mg, Worthington Biochemical Corporation) was dissolved in 4.9 mL pH 7.4 phosphate buffer at 37 °C. Benzene oxide was diluted in 0.1 mL phosphate buffer and added to achieve final concentrations of 10 nM - 10 mM, and the mixture was stirred for 2 h at 37 °C. The DNA was precipitated with ice cold isopropyl alcohol and washed twice with ethanol.

2.3 Treatment of mice with benzene

This study was approved by the University of Minnesota Institutional Animal Care and Use Committee. One hundred male B6C3F_1 mice, age 6 weeks, were obtained from Charles River Laboratories and housed four mice per cage under standard conditions (20 – 24 °C temperature, 29 – 32% relative humidity and 14/10 light/dark cycle) in the Research Animal Resources facility, University of Minnesota. They were given Purina Lab Chow 5001 diet and tap water *ad libitum* and allowed to acclimate for 2 weeks before treatment. Fifty mice were treated by gavage 5 times weekly on weekdays for 4 weeks with 50 mg/kg benzene in corn oil (5 mL/kg), and fifty control mice were given corn oil only (5 mL/kg). These doses are based on those used by the National Toxicology Program [15] which resulted in tumors and malignant lymphomas but no other toxicity. The mice were euthanized by CO_2 overdose and blood, liver, lung, and bone marrow were collected. All tissues were collected the same day as the final dose of benzene, ranging from 1 to 8 h after exposure.

2.4 Treatment of cells with benzene oxide

Mouse hepatocytes were purchased from XenoTech and thawed according to the included protocol. The hepatocytes were resuspended in Hepatocyte Incubation Media (XenoTech) and 4 mL (4 million cells) were transferred to each of five 25 mL flasks. Benzene oxide was diluted in H_2O, then added to four flasks to obtain a final concentration of 100 μM. The cells were incubated at 37 °C and lysed after 1, 2, 4, or 8 h. H_2O was added to the final flask as a

negative control and was incubated for 8 h before lysis. Human TK-6 lymphoblasts (ATCC) were cultured in RPMI medium 1640 (Life Technologies) with 15% horse serum. The cells (5 mL, 5 million cells) were transferred to each of four 25 mL flasks. Benzene oxide was diluted in H_2O and added to each of three flasks to obtain final concentrations of 100 μM, 1 mM, and 10 mM. The fourth flask was treated with H_2O as a negative control. TK-6 cells were incubated at 37 °C for 1 h before lysis. DNA was isolated from all cells according to a modified QIAamp DNA isolation kit (Qiagen).

2.5 Samples from smokers and non-smokers

This study was approved by the University of Minnesota Institutional Review Board. Ten smokers (range: 10–25 cigarettes per day) and ten nonsmokers provided 20 mL blood samples obtained by routine venipuncture. Smoking status was confirmed by measurement of exhaled CO. Greater than 125 mL of urine were collected from the same subjects. Buffy coat DNA was isolated as described below and urine was analyzed for SPMA as described [16].

2.6 Isolation of DNA from mouse and human samples

DNA was isolated from human blood samples using chemical solutions and a standard protocol adapted from the Puregene protocol available through Qiagen. Briefly, the buffy coat was treated with RBC Lysis Solution for 5 min at room temperature, the samples were centrifuged and the supernatant discarded. The pellet was resuspended and incubated in Cell Lysis Solution at room temperature for 1 h. Proteinase K was added and the samples were incubated at room temperature overnight with shaking before addition of RNase A solution for a final incubation of 2 h. Protein Precipitation Solution was added, the samples were centrifuged, and the supernatant was poured into ice cold isopropyl alcohol. The DNA precipitate was removed and washed twice with ethanol. Animal tissue samples were processed in a similar manner, with tissue homogenization as an initial step where appropriate.

To assess adduct stability during the DNA isolation procedure, an experiment was performed wherein the incubation time was varied. Duplicate samples of mouse liver, mouse lung, and calf thymus DNA reacted with benzene oxide were each incubated for 1, 4, 7, or 16 hours before protein precipitation and subsequent DNA isolation.

7-PhG was released from the isolated DNA by neutral thermal hydrolysis. The DNA sample was dissolved in 10 mM sodium cacodylate buffer (1 mL, pH 7) and [D_5]7-PhG internal standard (50 fmol) was added. The sample was heated at 100 °C for 1 h and an aliquot (50 μL) was removed for acid hydrolysis (80 °C, 1 h, pH 1–2) and subsequent guanine quantitation. Guanine was quantified by HPLC-UV, based on a linear calibration curve of guanine standard. The remaining solution was filtered through a 30,000 MW filter (Millipore) and purified on a Strata-X reversed-phase solid-phase extraction (SPE) cartridge (Phenomenex). The cartridge was washed with H_2O and 20% CH_3OH/H_2O (1 mL each). The analyte was eluted with 80% CH_3OH/H_2O (1 mL), and the eluent was evaporated to dryness *in vacuo*.

2.7 Analysis of 7-PhG in DNA by LC-NSI-HRMS/MS-PRM

The hydrolyzed DNA samples were reconstituted in 20 μL H_2O and 3 μL were injected for analysis by LC-NSI-HRMS/MS-PRM. The column was a 75 μm internal diameter, 10 cm, 15 μm orifice capillary column hand-packed with Luna C18 (5 μm) bonded separation media (Phenomenex). The analysis was performed on an LTQ Orbitrap Velos instrument (Thermo Scientific, Waltham, MA) with resolution set at 30000. The parent ion was fragmented by higher-energy collisional dissociation (HCD) and the monitored mass transitions (0.5 amu isolation width) were *m/z* 228.0 → 104.0493, 129.0445, 141.0446, 169.0395, 186.0661, 211.0614, and 229.0719 for 7-PhG and *m/z* 233.0 → 109.0807, 134.0761, 146.0761, 174.0710, 191.0976, 216.0929, and 234.1034 for [D_5]7-PhG. Positive and negative controls were included with the processing of each set of DNA samples. The positive controls consisted of calf thymus DNA which had been allowed to react with benzene oxide, and the negative controls were sodium cacodylate buffer without DNA and unmodified calf thymus DNA. The calibration curves were constructed from six dilutions of synthetic standards and had excellent linearity ($R^2 \geq 0.996$, $N = 7$). The concentrations used were 10, 20, 40, 80, 160, and 320 amol/μL 7-PhG, and all standards contained 1.0 fmol/μL [D_5]7-PhG. The samples were quantitated by isotope dilution mass spectrometry wherein the area ratio of 7-PhG to [D_5]7-PhG was measured and the known amount of [D_5]7-PhG added to each sample was used to calculate 7-PhG.

3. Results

For the analysis of DNA, [D_5]7-PhG was synthesized and used as the internal standard. 7-PhG was enriched by size exclusion filtration and SPE then quantified by LC-NSI-HRMS/MS-PRM. Accuracy and precision of the method were evaluated by adding known amounts of 7-PhG to calf thymus DNA and performing the analysis in triplicate for each level. The results, which are summarized in Table 1, demonstrate good agreement between the added and detected amounts of 7-PhG. Precision was excellent at each level of added 7-PhG. The on-column limit of detection (LOD) was determined by diluting a standard solution of 7-PhG until a 3:1 signal-to-noise ratio was observed. The LOD was determined to be 8 amol on column. The limit of quantitation in DNA matrix was estimated to be 40 amol on column (>10:1 signal-to-noise ratio) starting with 0.1–5.0 mg of calf thymus DNA; better signal was obtained starting with larger amounts of DNA. Accuracy was 105.5% and precision (average coefficient of variation) was 2.7%.

For the analysis of DNA samples reacted with benzene oxide, we tested the efficiency of neutral thermal hydrolysis (10 mM sodium cacodylate buffer, pH 7.0, 100 °C, 1 h) vs. acid hydrolysis (0.1 N HCl, pH 1–2, 80 °C, 1 h). Neutral thermal hydrolysis produced levels of 7-PhG that were 1.4 times higher than acid hydrolysis and produced cleaner chromatograms by LC-NSI-HRMS/MS-PRM, so this hydrolysis method was adopted.

3.1 Reactions of calf thymus DNA with benzene oxide

Calf thymus DNA was allowed to react with a range of concentrations of benzene oxide (10 nM to 10 mM), and 7-PhG was quantified by LC-NSI-HRMS/MS-PRM (Figure 1). These reactions were used to determine the minimum concentration of benzene oxide necessary to

form detectable levels of 7-PhG. As outlined in Table 2, we were able to detect 7-PhG when DNA was exposed to 1 μM benzene oxide or greater under physiological conditions.

3.2 Studies of mice treated with benzene

We did not detect 7-PhG in DNA from tissues of mice treated with benzene. For both the treated and control mice groups, 7-PhG was below the LOD in bone marrow, lung, and liver DNA samples. These tissue samples gave chromatograms which had considerably more chemical noise than either the *in vitro* reaction with purified calf thymus DNA or the human leukocyte DNA samples discussed below. Additionally, there was lower apparent recovery based on the area of the internal standard, possibly due to signal suppression from the sample matrix. We estimate loss of signal due to matrix suppression to be ~30–70%, depending on the individual DNA sample. To address these issues, the DNA sample matrix was mixed with 7-PhG standard, and we could reliably detect 90 amol on-column in the sample matrix. We estimate the LOD to be ~50 amol on-column for DNA samples isolated from mouse lung and liver, which is higher than the LOD in human leukocytes or calf thymus DNA (8 amol on-column). However, the animal tissues yielded larger amounts of DNA (0.4–1.2 mg DNA) than the human blood samples (0.1–0.3 mg), so the LOD in animal tissues corresponds to approximately 0.24–0.6 adducts per 10^9 nucleotides while the LOD in calf thymus and human DNA is 0.15–0.45 adducts per 10^9 nucleotides, depending on the sample size of DNA.

We considered the possibility that the overnight incubation step during DNA isolation may have caused loss of the analyte. The DNA isolation protocol was repeated on mouse lung, mouse liver, and calf thymus DNA reacted with benzene oxide, testing various incubation times. Based on the quantitation of 7-PhG in calf thymus DNA, the DNA isolation procedure reduces levels of analyte to approximately 1/3 of the amount in samples which were not subjected to the isolation conditions. This loss of analyte, which occurs during the first 4 h of incubation, was incorporated into the LOD calculation above.

3.3 Studies in cell culture

Our reactions with calf thymus DNA show that we can detect 7-PhG formation above 1 μM concentrations of benzene oxide, yet we were not able to detect 7-PhG formation in mouse tissue. We considered the possibility that 7-PhG was being formed *in vivo*, but was repaired before the tissue was isolated. To address this, two types of cells (mouse hepatocytes and human TK-6 lymphoblasts) were exposed to 100 μM benzene oxide for 1–8 h. When the DNA (1–22 μg) was analyzed, we could not detect 7-PhG formation at any time point. The TK-6 cells were also exposed to 1 mM and 10 mM benzene oxide for 1 h, and only the 10 mM concentration yielded detectable levels of 7-PhG. This supports the hypothesis that benzene oxide does not react efficiently with DNA *in vivo*, consistent with the results in mice.

3.4 Studies of smokers and nonsmokers

Leukocyte DNA from 10 smokers and 10 nonsmokers was analyzed for 7-PhG. All samples but one were indistinguishable from the negative control blanks, and the one positive sample was from a nonsmoker (Figure 2A–C). The level of 7-PhG was near the LOD for human

DNA samples: 10 amol on-column from 0.12 mg DNA, which corresponds to approximately 0.75 fmol/μmol guanine or 0.48 adducts per 10^9 nucleotides. The presence of 7-PhG in the positive sample was confirmed by matching, to within 3 ppm, 6 of the 7 ion fragments which are characteristic of the 7-PhG fragmentation pattern (Figure 2B and SI Table). However, we cannot fully exclude the possibility that this one positive sample could have arisen from cross-contamination.

Urinary SPMA from the same subjects was quantified by LC-MS/MS, as described previously [16]. Levels of SPMA were significantly higher ($P < 0.05$) in urine from smokers (mean = 0.83 ng/mL urine) than from nonsmokers (0.19 ng/mL). Four of the ten nonsmokers had SPMA levels below the LOD (0.02 ng/mL), including the subject whose leukocyte DNA was positive for 7-PhG.

4. Discussion

We describe a highly sensitive and accurate method for the quantitation of 7-PhG in DNA. The use of nanoelectrospray ionization coupled with high resolution MS and parallel reaction monitoring produced an on-column detection limit of about 8 amol 7-PhG and less than 1 adduct per 10^9 nucleotides in DNA, with excellent accuracy and precision for the analysis of DNA samples to which 7-PhG had been added (Table 1). This method provides a level of sensitivity and accuracy expected to be adequate both for the analysis of 7-PhG in reactions of DNA with benzene oxide and for the analysis of DNA from laboratory animals, cells, and humans exposed to benzene. In one previous study, Micova and Linhart described the use of LC-ESI-MS/MS for the analysis of 7-PhG as the only adduct detected in reactions of benzene oxide and calf thymus DNA [8]. It is not clear whether their method would have had sensitivity adequate for analysis of samples produced from benzene exposure *in vivo*.

Multiple problems were encountered and eventually overcome during the development of the MS method. The amount of DNA per sample was optimized, with the ideal amount being 1–5 mg. Starting with larger amounts of DNA produced greater matrix suppression but also provided a more concentrated sample for LC/MS injection. The benefits of the concentrated analyte outweighed the drawbacks of matrix suppression, thus larger amounts of DNA generally yielded better results. We also optimized the monitored product ions from 7-PhG fragmentation. The normalized HCD collision energy was set to 80 (actual 33 eV) such that seven fragment ions were observed. The quantitation of 7-PhG was consistent for each of the fragment ions, and the sum of all seven ions was used to yield the highest signal. Finally, the isolation width was set at 0.5 amu to decrease chemical noise and yield cleaner HRMS/MS spectra. These changes provided us with an excellent on-column LOD in the single-digit attomole range.

Applying our method to calf thymus DNA reacted with benzene oxide, we were able to confirm the observations of Micova and Linhart [8] that 7-PhG is produced after treatment of the DNA by either acid or neutral thermal hydrolysis. Our results are consistent with theirs in demonstrating a rather low reactivity of benzene oxide with calf thymus DNA, but direct comparison is difficult because different conditions were used. They used a higher concentration of benzene oxide (53 mM), a different buffer system, and a longer reaction

time (24 h) compared to our study. We were able to detect 7-PhG in our reactions at concentrations of benzene oxide as low as 1 μM.

The animal study reported here was based on the results of the U.S. National Toxicology Program bioassay of benzene in male B6C3F$_1$ mice in which a daily dose of 50 mg/kg benzene in corn oil, administered by gavage for 103 weeks, produced significant incidences of malignant lymphomas and lung tumors as well as tumors at other sites, without apparent toxicity or loss of body weight [15]. We hypothesized that 7-PhG would be observed in DNA from bone marrow, lung, and liver in our mice that were treated with the same dose of benzene for 4 weeks. Our results clearly do not support this hypothesis, as 7-PhG was below the limit of detection of ~0.6 adducts per 10^9 nucleotides in all samples analyzed. In a previous study, the concentration of benzene oxide in the blood of rats treated with a single dose of benzene (400 mg/kg) remained constant at approximately 90 nM over a 9 h period [17]. Based on these results, it seemed plausible that local levels of benzene oxide in cells or tissues of our mice might reach a level of 1 μM, which was the lowest concentration at which we detected adducts in reactions of benzene oxide with calf thymus DNA. Apparently this was not the case, or, if N^7-phenyldeoxyguanosine were formed in DNA, it was lost by depurination or repair to levels below our limit of detection, because we found no evidence for its presence in mouse tissue DNA. Collectively, these results do not support the overall hypothesis that benzene oxide-DNA adduct formation is important in carcinogenesis by benzene in B6C3F$_1$ mice, although it is recognized that adducts from other benzene metabolites such as *o*- or *p*-benzoquinone could still be involved. An adduct of *o*-benzoquinone, 3-(3,4-dihydroxyphenyl)adenine, has been detected in the urine of mice exposed to benzene [18].

The smokers in our study had significantly higher levels of SPMA in their urine than did the nonsmokers, in agreement with previous studies [4]. These results are consistent with the higher exposure to benzene of cigarette smokers than of nonsmokers. Typical values for benzene in mainstream cigarette smoke range from 15–59 μg per cigarette [19]. Nevertheless, we did not detect 7-PhG in leukocyte DNA from any of the 10 smokers in this study; the detection limit was less than 0.5 fmol/μmol guanine (< 0.45 adducts per 10^9 nucleotides) in human blood samples. We did obtain convincing evidence for the presence of 7-PhG in leukocyte DNA of 1 nonsmoker. As the exposure of both smokers and nonsmokers to benzene would be considerably lower than in our mouse study, these results are not surprising, but the finding of one positive sample is intriguing. This particular nonsmoker had SPMA levels below our limit of detection. Interindividual differences in benzene metabolism to benzene oxide, clearance, or in DNA repair capacity may influence 7-PhG adduct levels in human leukocyte DNA and this deserves further study.

The major factor leading to the relatively low levels of 7-PhG in DNA both *in vitro* and *in vivo* in the studies reported here is probably the ease of its rearrangement to phenol. Previous studies have shown that benzene oxide is relatively unreactive with sulfur nucleophiles and that this pathway occurs at low efficiency compared to rearrangement to phenol at pH 7 [14]. This is also consistent with the studies by Micova and Linhart and with our data indicating the relatively low yields of 7-PhG in reactions of benzene oxide with DNA [8].

In summary, we have developed a highly sensitive and accurate LC-NSI-HRMS/MS-PRM method for the analysis of the benzene oxide adduct 7-PhG in DNA. We demonstrated the formation of 7-PhG in DNA reacted with benzene oxide, but did not detect this adduct in DNA isolated from bone marrow, liver, or lung of mice treated for 4 weeks with benzene at a daily dose which induces cancer when given for 103 weeks. We also did not detect 7-PhG in DNA from cells exposed to 100 μM or 1 mM benzene oxide, but we could detect 7-PhG at 10 mM exposure. We detected 7-PhG in one leukocyte DNA sample from a nonsmoker, while samples from 9 other nonsmokers and 10 smokers were negative. Collectively, these data indicate that 7-PhG is formed rather inefficiently, or not at all, in DNA upon exposure to benzene.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Highlights

- The human carcinogen benzene can form the DNA adduct 7-phenylguanine.
- A sensitive mass spectrometry method can detect 7-phenylguanine in DNA.
- 7-Phenylguanine was detected in calf thymus DNA reacted with benzene oxide.
- 7-Phenylguanine was not detected in DNA from tissues of mice treated with benzene.
- 7-Phenylguanine was detected in 1 of 20 human leukocyte DNA samples.

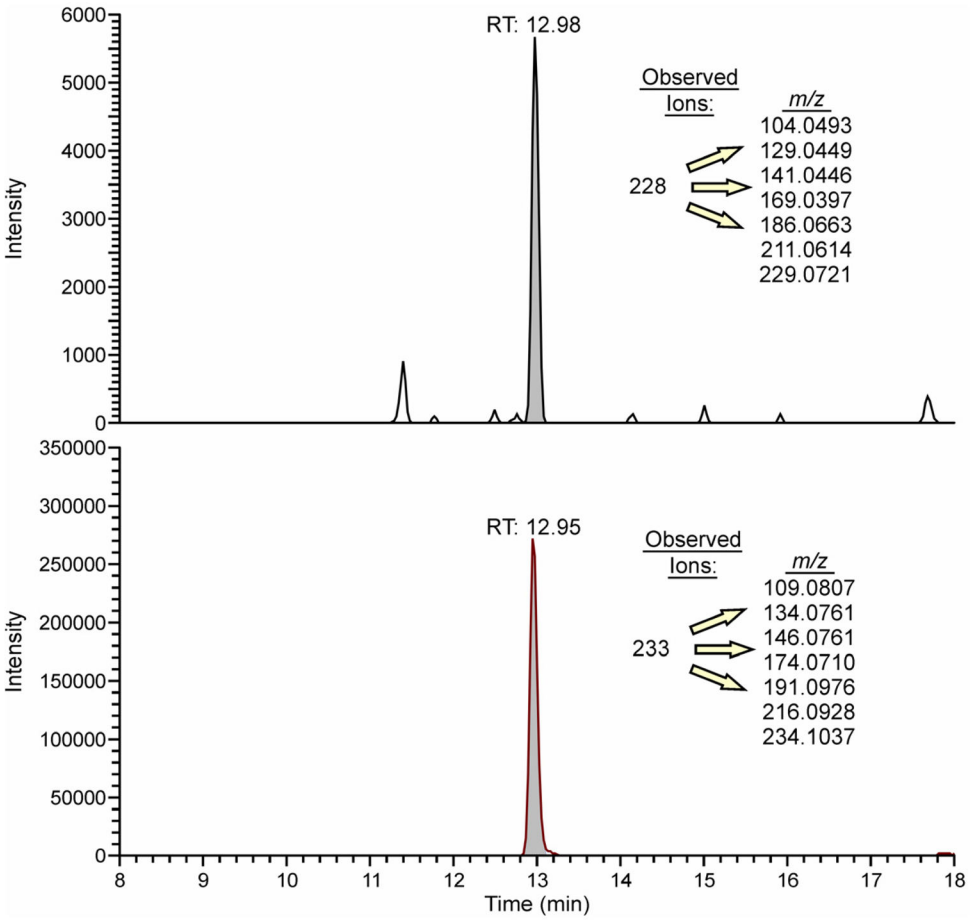


Figure 1.

A representative chromatogram from the *in vitro* reaction of benzene oxide (100 μM) and calf thymus DNA. Top chromatogram: the monitored mass transitions for 7-PhG. Bottom: those of [D_5]7-PhG. The Y-axis is labeled with absolute intensity.

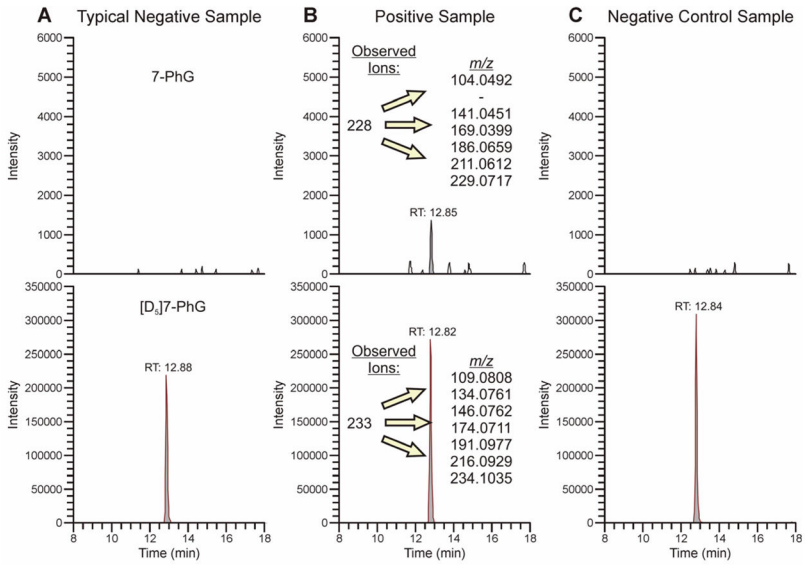
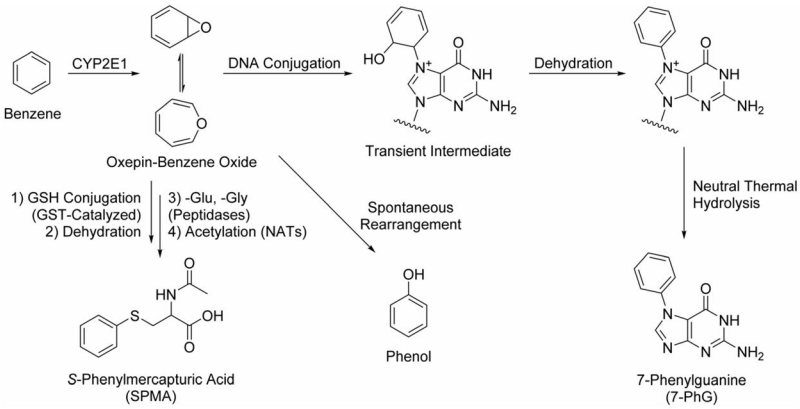


Figure 2.

Three representative chromatograms with equivalent absolute intensity to Figure 1. Top row: the monitored mass transitions for 7-PhG. Bottom row: those of [D_5]7-PhG. (A) typical negative sample from human leukocyte DNA; (B) positive human leukocyte sample including a list of observed product ions; (C) typical chromatogram from a negative control sample (sodium cacodylate buffer, no DNA).



Scheme 1.

Overview of benzene metabolism to benzene oxide-oxepin and the resulting formation of 7-PhG or SPMA.

Table 1

Accuracy and precision data from triplicate samples of calf thymus DNA spiked with increasing amounts of 7-PhG. Detected amounts are mean ± standard deviation.

7-PhG Added (fmol)	7-PhG Detected (fmol)	Coefficient of Variation (%)
1.0	0.97 ± 0.03	3.2
2.0	2.44 ± 0.04	1.5
4.0	4.39 ± 0.19	4.3
8.0	8.56 ± 0.23	2.7
16.0	14.7 ± 0.28	1.9

Table 2

Formation of 7-PhG from the reaction of calf thymus DNA and benzene oxide at 37 °C for 2 h. Data are presented as fmol adduct per μmol guanine. LOD (below the limit of detection).

[Benzene Oxide] (μM)	7-PhG (fmol/μmol guanine)
10000	330
1000	63
100	8
10	0.6
1	0.1
0.1	LOD
0.01	LOD