Measurement of N7-(2′-Hydroxyethyl)guanine in Human DNA by Gas Chromatography Electron Capture Mass Spectrometry

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An improved method is presented, based on gas chromatography−electron capture mass spectrometry (GC-EC-MS), for measuring N7-(2′-hydroxyethyl)guanine (N7-HEG) in DNA from an in vivo sample. The method was used to detect this adduct in amounts of human DNA ranging from 0.07 to 11.5 μg isolated from granulocytes. In this method, the DNA is spiked with a stable isotope internal standard (N7-HEG-d_4) and heated in water to release the adduct in a nucleobase form. After the adduct is extracted into 1-butanol, it is purified by reverse phase HPLC and derivatized with HONO, pentafluorobenzyl bromide, and pivalic anhydride. Further purification by silica solid phase extraction and reverse phase HPLC is done prior to injection into a GC-EC-MS. Relatively clean GC-EC-MS chromatograms result, contributing to the high sensitivity that is observed. In the samples tested, from 1.6 to 240 N7-HEG adducts in 10^7 nucleotides were observed, a 150-fold range.

Introduction

N7-(2′-Hydroxyethyl)guanine (N7-HEG)[1] is the major DNA adduct produced by in vitro exposure of DNA to ethylene oxide, an animal carcinogen (*1*). Considering this and that humans are exposed to ethylene oxide from both endogenous and exogenous sources, as has been summarized or reviewed (*2*−*4*), it is important to measure this DNA adduct in human samples.

In one study of potential human exposure to ethylene oxide, operators of hospital sterilizers, along with control subjects, were studied (*5*). External exposure was assessed based on measurement of airborne ethylene oxide in the relevant work areas, while taking into account the time that the individuals spent in these areas. To assess internal exposure, blood samples were collected, and N(2-hydroxyethyl)valine hemoglobin adducts in erythrocytes, along with sister chromatid exchanges (SCEs) and micronuclei in lymphocytes, were measured. Both the hemoglobin adducts and the SCEs correlated with external exposure (*5*). In a follow-up study of these same samples, the glutathione S-transferase T1-null genotype was found to be associated with the hemoglobin adduct (*6*).

The results of these two studies have prompted an interest in measuring N7-HEG in these same blood samples. Unfortunately, all that remained was small amounts of residual granulocytes. We isolated the DNA from these samples and found that the amounts ranged from 0.07 to 11.5 μg. This meant that a very sensitive assay would be needed for the measurement of N7-HEG in these samples.

While many methods have been published for quantifying N7-HEG in DNA, none of them was considered to be acceptable for analysis of the above samples derived from granulocytes. While this adduct can be measured with high sensitivity by ^{32}P-postlabeling (*7*, *8*), it is difficult to control accuracy with this technique as others have discussed in general (*9*). N7-HEG has been measured by HPLC with electrochemical detection (*10*), but calibration was based on an external standard curve, which can compromise accuracy. A method involving HPLC with postcolumn fluorescence reaction detection was applied to a 100 mL amount of blood, and 7-methylguanine was used as the internal standard (*11*). HPLC with detection by electrospray ionization mass spectrometry was employed in three studies, but its sensitivity was reported to be too low for monitoring endogenous N7-HEG in human tissues (*1*); the smallest amount of DNA tested (as a spiked sample without use of an internal standard) was 35 μg (*12*), and the smallest amount of blood tested was 10 mL relying on an external standard (*13*).

A method that we introduced for N7-HEG based on gas chromatography electron capture mass spectrometry (GC-EC-MS) (*14*), which has been adopted by others (*1*, *15*−*19*), also was not sensitive enough. This is because it gave a noisy chromatographic baseline even when applied to 25 μg of DNA from human lymphocytes with use of a high-resolution mass spectrometer (*1*).

Here, we describe an improved, more sensitive method for measuring N7-HEG that we developed by modifying our prior procedure based on GC-EC-MS (*14*). Its application to the above samples has already been reported (*20*).

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[1] Abbreviations: N7-HEG, N7-(2′-hydroxyethyl)guanine; PFBzBr, pentafluorobenzylbromide; PA, pivalic (trimethylacetic) anhydride; DMAP, 4-(dimethylamino)pyridine; TFA, trifluoroacetic acid; MeOH, methanol; EtOH, ethanol; BuOH, 1-butanol; EtOAc, ethyl acetate; ACN, acetonitrile; GC-EC-MS, gas chromatography−electron capture mass spectrometry.

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Materials and Methods

Chemicals. Ethylene oxide (99.5+%), pentafluorobenzylbromide (PFBzBr) (99+%), K_2CO_3 (99.99%), *tert*-butylnitrite pivalic (trimethylacetic) anhydride (PA) (99%), 4-(dimethylamino)-pyridine (DMAP) (99+%), trifluoroacetic acid (TFA) (99%), 1,1,1,3,3,3-hexamethyldisilazane (99.9%), HCl (37 wt % in water, 99.999%), ethanol (EtOH) (99.5%), 1-butanol (BuOH) (99.8%), ethyl acetate (EtOAc) (99.8%), hexane (95%), and acetonitrile (ACN) (99.9%) were purchased from Sigma-Aldrich (Milwaukee, WI). DMAP was recrystallized from methanol (MeOH) three times and stored at 4−8 °C up to 1 week before use. K_2CO_3 was stored at 60 °C; PFBzBr, TFA, and 1,1,1,3,3,3-hexamethyldisilazane were stored at 4−8 °C; and the other chemicals were stored at room temperature. All chemicals, unless indicated otherwise, were used within 6−8 months after opening. Fisherbrand heavy-wall borosilicate glass tubes (12 mm × 75 mm), borosilicate pasteur pipets (5.75 and 9 in.), and MeOH (99.9%) were obtained from Fisher (Pittsburgh, PA). All organic solvents were HPLC grade. The QIAamp Blood Kit was purchased from Qiagen, Inc. (Chatsworth, CA). The internal standard, N7-HEG-d4, was prepared as described (*14*). No plastic ware was used in the procedure, and all glassware, except for the tubes used to collect from HPLC-1, was gas phase silanized with 1,1,1,3,3,3-hexamethyldisilazane at 200 °C for 24 h. (Silanization was not done for the indicated tubes because the collected samples after evaporation were subjected to an acidic reaction in these tubes.) Ultrahigh purity nitrogen (99.999%), methane (99.99%), and helium (99.999%) were purchased from Med-Tech Gases (Medford, MA). Hamilton syringes (Reno, NV) 80331 (10 μL) and 80665 (100 μL) were used for GC and HPLC injections, respectively.

Instrumentation. GC-EC-MS was carried out on an Agilent (Wilmington, DE) 6890 mass spectrometer equipped with an MSD 5973 Chem-Station data system. Methane (mass flow controller at 40% from 40 psi) and helium (80 psi) were used as the reagent and carrier gases (flow rate, 2 mL/min), respectively. Injections were made in the on-column mode, and the oven was programmed from 100 to 250 °C at 20 °C/min, ramped again from 250 to 300 °C at 70 °C/min, and then held at 300 °C for 5 min. An Agilent Ultra-2, 5% diphenyl/95% dimethyl siloxane capillary column (25 m × 0.2 mm i.d., 0.11 μm film thickness) was used. Source and quadrupole temperatures were 250 and 106 °C, respectively. The triode gauge read 1.9×10^{-4} Torr. Single ions were monitored at *m/z* 459 and 463 with a dwell time of 100 ms. Two satellite HPLC systems were used as follows: model 515 HPLC pump from Waters (Milford, MA) with a Waters Spherisorb S5 ODS1 2.0 mm × 150 mm Microbore Column for HPLC-1; model CC-60-S pump from Eldex Laboratories (Napa, CA) with a Varian Microsorb C18 5 μm 4.6 mm × 150 mm column (Walnut Creek, CA) for HPLC-2. Both systems used a model 7725 injector from Rheodyne (Cotati, CA). A home-built column heater was used to control the column temperature for each. A Reacti-Vap (Pierce, Rockford, IL) was used for evaporation, and all evaporation was done under nitrogen.

N1,N3-Bis-[2′,3′,4′,5′,6′-pentafluorobenzyl]-N7-[2″-(trimethylacetoxy)ethyl]xanthine (*1*). To a stirred solution of 1 mg of DMAP in 1 mL of ACN at room temperature was added N1,N3-bis(2′,3′,4′,5′,6′-pentafluorobenzyl)-N7-[2′-(hydroxy)ethyl]-xanthine, prepared as described (*21*), followed by 150 μL of pivalic anhydride. After 16 h of stirring, evaporation gave a light-yellow solid, which was partly purified by TLC on silica (EtOAc:hexane, 80:20, v/v) and then by HPLC on a Waters Symmetry column (5 μm, 7.8 mm × 100 mm) at 1 mL/min in ACN:water, 70:30, v/v). ^{1}H NMR (500 MHz, CD_3CN:D_2O, 9:1, v/v): δ 7.8 (s, 1H), 5.30 (s, 2H), 5.20 (s, 2H), 4.51 (t, 2H), 4.37 (t, 2H), 1.03 (s, 9H). MS (MALDI-TOF) *m/z* 641 (MH^+). A stock solution of the compound in CD_3CN:D_20, 90:10, v/v was quantified by NMR.

DNA Extraction from Granulocytes. DNA was extracted from granulocytes using a QIAamp kit but with all steps at room temperature. About 200 μL of granulocyte suspension was mixed with 20 μL of QIAGEN Protease (20 μg/μL stock) and 20 μL of RNaseA (20 μg/μL) followed by vortexing for 15 s. Two hundred microliters of lysis buffer was added and vortexed for 15 s before gently shaking the solution on a rocking plate at room temperature for 2 h, followed by addition of 200 μL of EtOH and vortexing for 15 s before applying it to a QIAmp spin column. Centrifugation was done at 6000*g* for 1 min. Five hundred microliters of washing buffer 1 (supplied in QIAmp kit) was added followed by centrifugation at 6000*g* for 1 min; next, 500 μL of washing buffer 2 was added followed by centrifugation at 20000*g* for 3 min. DNA was eluted with 200 μL of water at 6000*g* for 1 min, and its concentration was measured with a Hoechst dye-binding assay.

Solid Phase Extraction (SPE). SPE columns were prepared using disposable 5.75 in. borosilicate Pasteur pipets firmly plugged (using a 9 in. Pasteur pipet) with a small piece of silanized glass wool (about 0.3 cm bed length) (J. T. Baker, Phillipsburg, NJ). Two hundred millilgrams of silica gel (60 Å pore, 40 μm irregular particles; J. T. Baker) was the packing material. All washing and elution solvents were pushed to the upper bed surface with nitrogen, 1 psi (about 1 min).

Step 1. Neutral Thermal Hydrolysis (NTH). One hundred microliters of 0.1 M HCl containing 10 pg of N7-(2′-hydroxyethyl-d_4)guanine (N7-HEG-d_4, internal standard) was evaporated under nitrogen in a 12 mm × 75 mm glass test tube in a Reacti-Vap. Twice, 100 μL of water was added and evaporated. An aliquot of a DNA sample in water (45−310 μL) was transferred to the tube and hydrolyzed at 100 °C for 15 min; 1 cm of the lower tip of the tube was under the hot water surface. The sample was cooled on ice for 10 min.

Step 2. BuOH Extraction and HPLC-1. Five hundred microliters of BuOH was added, and each tube was vortexed briefly and centrifuged for 5 min at 300*g*. Most of the supernatant was transferred to a new tube. Another 500 μL of BuOH was added to each original tube followed by gentle shaking for 1 h and centrifugation. The pooled supernatant liquid (1.1 mL) was evaporated under slow nitrogen without heating (6 h). The dried sample was redissolved in 100 μL of mobile phase (MeOH/0.1%TFA−water, 5:95, v/v) and injected using a 200 μL loop into satellite HPLC-1 having an isocratic flow rate of 0.3 mL/min at 25 °C (see below for column preparation). A 2 min window (3−5 min) was collected for the analyte and evaporated. Also, the 1−3 and 5−7 min windows were collected and stored at 4 °C as a precaution (although later they were always discarded). Twenty minutes after each injection, 100 μL of mobile phase was injected, and then, 20 min later, the next sample was injected. The syringe was flushed 10 times with mobile phase before each injection.

Before the samples were injected into HPLC-1, N7-methylguanine (10 ng) was injected into this column and its retention time was noted (5.9 min). Generally, it was observed that this retention time was 1 min lower after injection of eight of the granulocyte-derived DNA samples, and the pressure increased from 78 to 85 bar. At this point, the column was washed with 100% ACN for 20 h at 0.1 mL/min and then with mobile phase for 1 h at 0.3 mL/min before reinjecting N7-methylguanine. In about half of the cases, this washing procedure needed to be repeated a second time to restore the original retention time of N7-methylguanine. At this point, the UV detector was disconnected, 100 μL of mobile phase was injected, and the first sample was injected 20 min later.

Step 3. Nitrous Acid Oxidation and Electrophore Derivatization. Each tube (evaporated sample from HPLC) was placed in an ice bath, and 50 μL of 6 M HCl and 25 μL of *tert*-butylnitrite were added. After they were shaken at 0 °C for 4 h, the samples were evaporated. Each residue was subjected to liquid−liquid extraction using 100 μL of EtOAc and 100 μL of water, and the separated aqueous layer was evaporated. To the residue was added 15−18 mg of K_2CO_3 (stored as a power at 60 °C) followed by 300 μL of a solution of 25 μL of PFBzBr in 2 mL of ACN prepared just before addition. The sample was shaken for 20 h at room temperature and evaporated. Each tube then received 300 μL of the following solution freshly prepared before

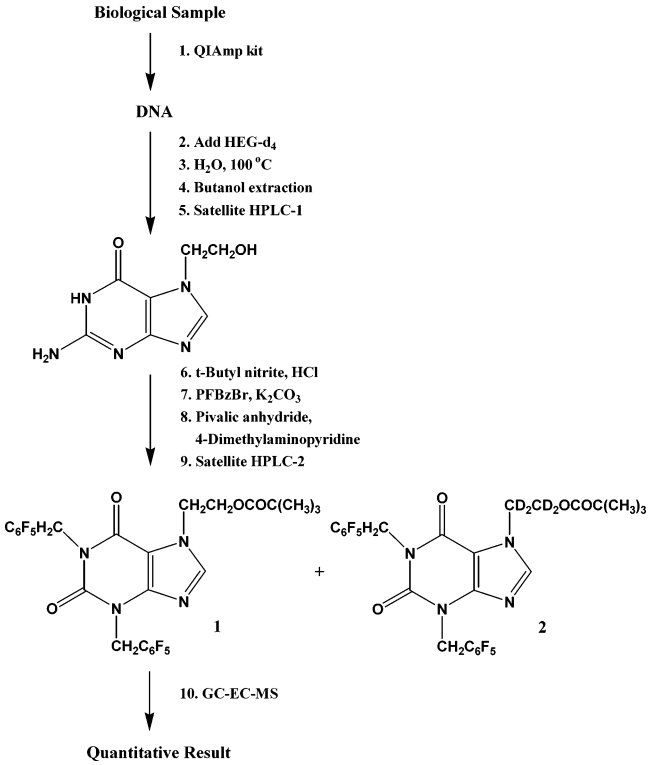

Figure 1. Scheme for the measurement of N7-HEG based on GC-EC-MS, in which this analyte is converted to a final electrophoric derivative (**1**), and the stable isotope is similarly converted to **2**.

addition: 2 mg of DMAP, 100 μL of PA, and 2 mL of ACN. After it was shaken for 20 h at room temperature, the sample was dried under nitrogen. Five hundred microliters of water and 500 μL of hexane were added followed by 30 s of vortexing and 5 min of centrifugation at 300*g*. Most of the hexane layer was collected, and the aqueous layer was hexane-extracted again. The combined hexane extract was transferred to a silica SPE column (see above), followed by washing (4.5 mL of hexane, then 6 mL of 10% EtOAc in hexane) and elution (2 mL of EtOAc). The EtOAc eluate was evaporated.

Step 4. HPLC-2 and Detection. Prior to purifying each batch of 16 samples by HPLC-2, 2 pg of **1** (see Figure 1) was injected, and the above three fractions within the 8.5−14.5 min window were collected. Following this, 100 μL of mobile phase was injected and similarly collected. These six fractions were then evaporated and redissolved in 10 μL of toluene, and 1 μL was injected into the GC-EC-MS. The recovery (relative to the peak area observed when 1 pg of **1** was injected) ranged from 85 to 95%, and no carryover was encountered in HPLC-2.

Each dried sample was dissolved in 100 μL of mobile phase (ACN/water, 70:30, v/v) and injected using a 200 μL loop into satellite HPLC-2 having a flow rate of 1 mL/min at 28 °C, and a 2 min window (10.5−12.5 min) was collected and evaporated for the analyte (see below for column preparation). Similar to HPLC-1, the 8.5−10.5 and 12.5−14.5 min windows were collected, stored, and later discarded. The time between injections, injection of mobile phase, and syringe washing was the same as for satellite HPLC-1. The evaporated analyte fraction was dissolved in 100 μL of toluene followed by 10 s of vortexing (to rinse the tube wall), evaporation, and storage at −20 °C. The evaporated sample was dissolved in 10 μL of toluene and vortexed, and 1 μL was immediately injected into the GC-EC-MS. Before each series of 16 injections of samples into the GC-EC-MS, 15 cm of the front of the column was cut off, and the GC oven was set to 300 °C overnight.

Results and Discussion

Description and Development of the Method. Our improved analytical procedure for the measurement of N7-HEG in DNA based on its derivatization to product **1** for injection into GC-EC-MS is presented in Figure 1. As seen, we have presented it as 10 steps. Most of these steps (1−3, 6, 7, and 10) have been reported before (*14*). The mass spectrum of compound **1** by GC-EC-MS shows a single peak, aside from isotopic peaks, at *m/z* 459 (loss of a pentafluorobenzyl radical from the molecular anion radical). Injection of 10 fg (22 amol) of **1** into the GC-EC-MS with selected ion monitoring at *m/z* 459 gives S/N = 6 (data not shown).

In this method, once the DNA is isolated from a biological sample (from granulocytes in this study using a QIAMP kit as step 1), a stable isotope internal standard (N7-HEG-d_4) is added (step 2), and the N7-HEG is released from the DNA by NTH (step 3). The adduct is then extracted with BuOH from the residual DNA in step 4. Previously (*14*), we isolated the N7-HEG instead by precipitating and centrifuging the DNA in cold, 0.1 M HCl. The prior acidic conditions had the advantages of low cost and simplicity. Also, N7-HEG is very soluble in acid. However, because DNA depurinates in acid, these conditions, even though conducted rapidly, potentially were compromising the overall sensitivity of the method by increasing the background of free purines.

Originally, during the development of our prior method, we attempted to isolate the N7-HEG from residual DNA, after NTH, by ultrafiltration. This technique was abandoned because it gave a poor recovery of N7-HEG at the low femtomole level. More recently, Swenberg and coworkers reported that ultrafiltration gave a good recovery of N7-HEG at the picomole level (*1*). However, they continued to rely instead on the original technique of cold, acid precipitation of the DNA, since ultrafiltration added noise to the final GC-EC-MS chromatograms.

The conditions for the butanol extraction step were set up by initially extracting nanomole amounts of N7-HEG so that recovery could be assessed conveniently by UV detection. In this way, we established conditions that recovered 74% of N7-HEG, with a double extraction by butanol at this level that had been spiked into a sample of DNA and subjected to NTH. The conditions were also found to give good results in the overall procedure at lower levels of spiked N7-HEG, as demonstrated below. Other ways of accomplishing the extraction of N7-HEG were studied at the nanomole level but were rejected because of losses of N7-HEG at lower levels, incomplete removal of DNA, or inconvenience. These other ways were small scale dialysis, hydroxylapatite chromatography, SPE on an OASIS cartridge, anion exchange chromatography, and ultrafiltration under basic conditions (data not shown).

In step 5, the N7-HEG is purified further by satellite HPLC-1 (the first of two satellite HPLC separations). As we have described (*22*), the basic concept of this technique is to set up an inexpensive HPLC system fitted with a low-cost isocratic pump and usually lacking a detector. Detection is accomplished, as needed, by moving the column to a fully equipped, "parent HPLC", thereby keeping the injector of the satellite HPLC unexposed to the higher level samples injected into the parent HPLC to avoid analyte carryover in the satellite HPLC. Because of the low cost, it is practical to dedicate such a system to a given step in a given method, only injecting trace level samples, to control analyte carryover and general chemical noise. In this case, the satellite HPLC for step 5 was fitted with a UV detector, and

N7-methylguanine was injected regularly at a UV detectable level in order to calculate the retention time of N7-HEG. When the granulocyte samples were tested, we also collected not only the fraction calculated to contain N7-HEG but also the adjacent fractions before and after this one as a precaution. As it turned out, it was never necessary to analyze these adjacent fractions. Analyte carryover can also be controlled by bypass HPLC (*23*).

The strategy and conditions for steps 6 and 7 of Figure 1 were discussed before (*14*), but step 8 is new, in which the residual hydroxyalkyl group is esterified with pivalic anhydride, yielding the final derivative, compound **1**. Previously, the hydroxyalkyl group was pentafluorobenzylated in the presence of 1 M KOH and tetrabutylammonium bisulfate (*14*). However, the stronger conditions provided by these latter reagents probably increase background noise by causing more degradation of the overall sample followed by derivatization of these degradation products. The mild conditions of step 7, in which the acidic ring nitrogens of N7-HEG are pentafluorobenzylated in the presence of sodium carbonate, minimize electrophoric background noise in the sample. Because the N7-HEG already is made intensely electrophoric by pentafluorobenzylation of the ring nitrogens, a nonelectrophoric group like pivalyl, which can be attached under mild conditions, is a good choice for capping the residual hydroxyalkyl moiety of the intermediate, bis-pentafluorobenzylated N7-HEG. The importance in general of employing mild conditions in sample derivatization for detection by GC-EC-MS has been discussed before (*24*). As for our prior tris-pentafluorobenzyl derivative (*14*), the electron capture mass spectrum of compound **1** shows a single peak loss of a pentafluorobenzyl radical. For **1**, this peak is observed at *m/z* 459.

Step 9, in which the fully derivatized N7-HEG is purified by HPLC, is conducted by satellite HPLC-2, using a completely different HPLC column than the one for step 5. However, in this case, a trace amount of the authentic, fully derivatized N7-HEG is injected regularly to establish which fraction to collect for the real samples. The retention time of the authentic compound is established by collecting fractions in the expected retention region and injecting them, after evaporation and redissolving, into the GC-EC-MS. As above, it was never necessary to analyze the fractions collected before and after the fraction calculated to contain the analyte. Finally, the collected real samples from granulocytes were injected (one-tenth of the final volume) into the GC-EC-MS as step 10.

Evaluation of the Method. Prior to applying the method shown in Figure 1 to the granulocyte samples of interest in this study, we evaluated the method with standards including spiked DNA samples. Detection of 200 fg (313 amol) of product **1** by GC-EC-MS is shown in Figure 2A. When a sample consisting of 10 pg each of N7-HEG and N7-HEG-d_4 as standards was subjected to the procedure in triplicate and the relative peak areas were compared, the value for the mean ± SD was 1.10 ± 0.03. Two samples of calf thymus DNA (90 μg each) were each spiked with 108 pg of N7-HEG-d_4, and the absolute recovery of **2** was 25 and 27%. Background N7-HEG was detected in these samples, and the level, calculated using the internal standard, was 4.6 N7-HEG adducts in 10^6 nucleotides for each sample. When two samples of calf thymus DNA (10 μg each) were each spiked with 11 pg of N7-HEG-d_4, the absolute yield of **2** was 29 and 31%.

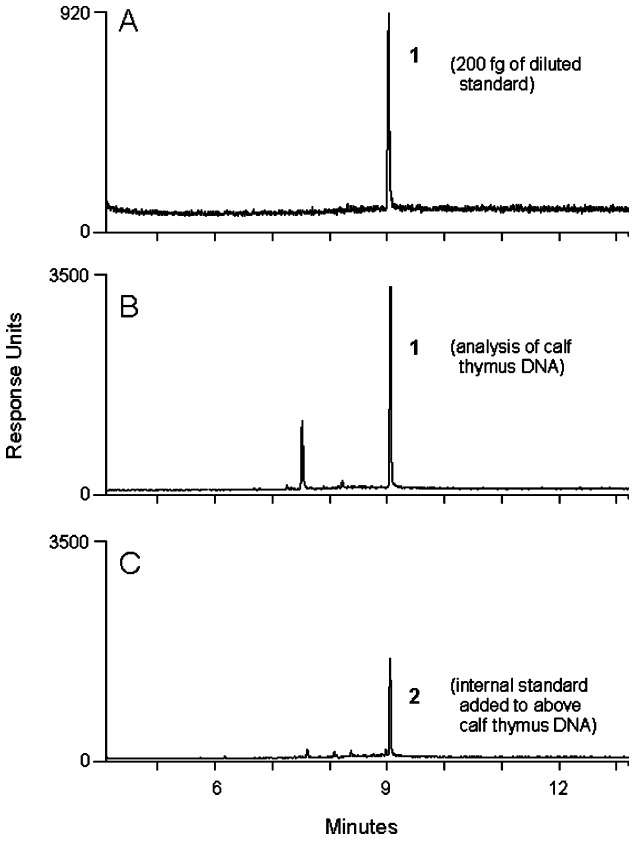

Figure 2. Representative selected ion GC-EC-MS chromatograms showing the detection of (A) 200 fg (313 amol) of **1** as a diluted standard; (B) **1** as a product obtained by spiking 10 μg of calf thymus DNA with 11 pg of N7-HEG-d_4 and conducting the procedure shown in Figure 1; and (C) **2** from the same injection used to form chromatogram B. The peaks for **1** in B and **2** in C correspond to injected amounts of 671 and 318 fg, respectively, and 1 μL of a final sample volume of 10 μL was injected.

The background level of N7-HEG in these latter samples was found to be 4.3 and 4.6 adducts in 10^6 nucleotides, consistent with the measurements on the 90 μg samples. The selected ion mass chromatograms for detection of **1** and **2** in one of these latter samples are shown in Figure 2B,C, respectively.

Analysis of Granulocyte Samples. Representative, selected ion mass chromatograms for the 65 granulocyte DNA samples that we analyzed (of which one was a blinded duplicate; see below) are shown in Figure 3. The chromatograms in Figure 3A,B (for detection of analyte and internal standard, respectively) came from the sample furnishing the smallest amount of starting DNA (0.07 μg of DNA). The sample was from a nonsmoker in the exposed group, and the adduct level was 11 in 10^6 nucleotides. In Figure 3C,D is shown the data from the sample (2.5 μg of DNA) that had the highest concentration of N7-HEG (24 in 10^6). The individual who donated this sample was a former smoker in the exposed group. Finally, the chromatograms in Figure 3E,F came from a sample (11 μg of DNA) that had the lowest concentration of N7-HEG (1.6 in 10^7). This sample came from a nonsmoker in the nonexposure group. For the duplicate sample, which came from a never smoker in the low exposure group, the values were 1.1 and 1.5 in 10^6 (data not shown).

While our procedure is tedious, often this is the nature of definitive analysis of difficult, trace analytes in real

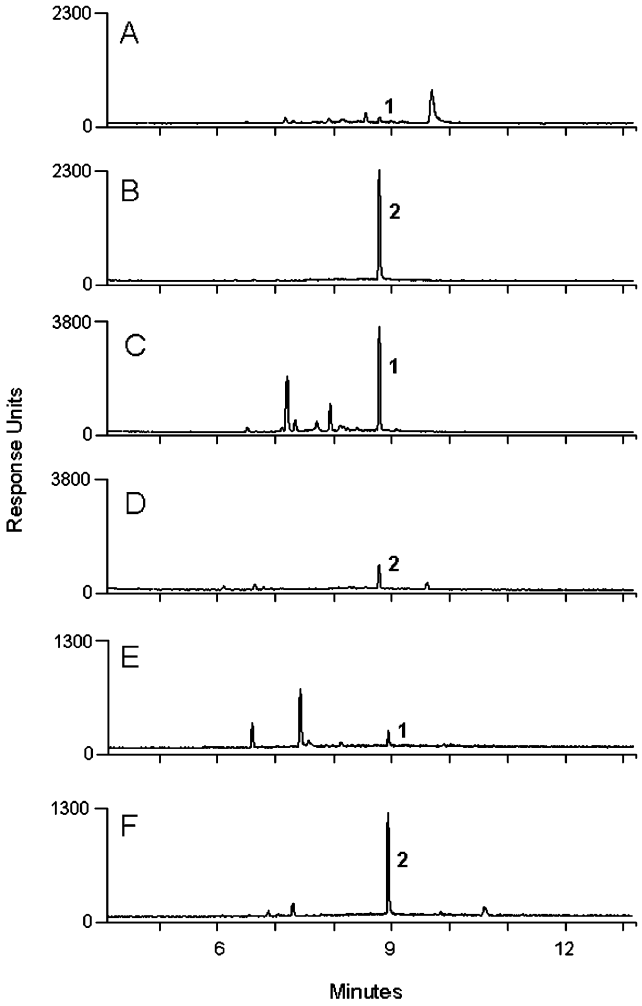

Figure 3. Detection of N7-HEG in three human DNA samples isolated form blood-derived granulocytes by GC-EC-MS following the procedure shown in Figure 1. In each case, the amount of internal standard (N7-HEG-d_4) spiked into the sample was 10 pg. The amounts of DNA tested were as follows: 0.07 μg (A,B: chromatograms for detection of **1** and **2**, respectively), 2.5 (C,D), and 11.5 (E,F).

samples. We measured 16 samples per run, and each run took 15 days. As seen in Figures 2 and 3, the chromatograms are relatively free of extraneous peaks, which is important for reliability since only a single ion is being monitored for the analyte. Note that a low resolution MS (conventional quadrupole) is employed. We did not evaluate the degree to which each of the new steps (4, 5, 8, and 9) contributed individually to the low level of noise in these chromatograms.

After the first eight samples derived from granulocytes were injected into HPLC-1, the back pressure increased from 75 to 85 bar, and the retention time of N7-methylguanine decreased from 5.9 to 4.9 min. We restored the column to its original back pressure and retention time for N7-methylguanine, by extensive washing with 100% ACN at a low flow rate. This pattern was repeated throughout the analysis of all of the samples. Potentially, we could have conducted the overall analysis without this periodic washing and just collected a different window for N7-HEG based on the shift in retention time for N7-methylguanine. However, we needed to be extra cautious since the samples could not be replaced, and the amounts were too small to permit an analysis in duplicate.

Comparison with Other Methods. By ^{32}P-postlabeling, the following ranges of HEG adducts per 10^8 nucleotides have been reported in human blood cells: 2.1−8.1 [leukocytes, eight nonsmokers; (*7*)]; 2.8−9.7 [leukocytes, 11 smokers; (*7*)]; 7−106 [lymphocytes, 34 nonsmokers, (*3*)]; 3−9 [leukocytes; five smokers, (*4*)]; and 3.7 [leukocytes, one nonsmoker, (*4*)]. Using GC-EC-MS, Wu et al. (*1*) reported a corresponding range of 23−185 [lymphocytes, 23 individuals, smoker vs nonsmoker not defined, 23−185 is calculated from an actual reported range of 90−740 in 10^8 guanines]; and using HPLC with fluorescence detection, Bolt et al. (*11*) reported 65−180 (leukocytes, five individuals, smoker status not defined; actual range reported was 2.1−5.8 pmol in 1 mg of DNA). Per 10^8 nucleotides, the range of values reported here is 16−2400, which is larger than any range reported before. Ours is the first study of this adduct in hospital workers exposed to ethylene oxide sterilization rooms and the only one that specifically tested granulocytes. Given the small numbers of samples along with different kinds of cells and exposures and lack of a reference sample to compare the methods, there is little one can say about the differences in these values or ranges. N7-HEG is considered to be an adduct that can arise from both endogeneous and exogenous sources (*19*).

Conclusion. A sensitive method is reported for quantifying the DNA adduct, N7-HEG. The amounts of DNA tested correspond to what can be isolated from a finger prick of blood. Potentially, in future work, the method could be simplified, especially if a high-resolution mass spectrometer were available. Future work also might extend this method to include more of the adducts that can be harvested from DNA by NTH.

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