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S-Arylcysteine−Keratin Adducts as Biomarkers of Human Dermal Exposure to Aromatic Hydrocarbons

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To measure biomarkers of skin exposure to ubiquitous industrial and environmental aromatic hydrocarbons, we sought to develop an ELISA to quantitate protein adducts of metabolites of benzene and naphthalene in the skin of exposed individuals. We hypothesized that electrophilic arene oxides formed by CYP isoforms expressed in the human skin react with nucleophilic sites on keratin, the most abundant protein in the stratum corneum that is synthesized de novo during keratinocyte maturation and differentiation. The sulfhydryl groups of cysteines in the head region of the keratin proteins 1 (K1) and 10 (K10) are likely targets. The following synthetic *S*-arylcysteines were incorporated into 10-mer head sequences of K1 [GGGRFSS(*S*-aryl-C)GG] and K10 [GGGG(*S*-aryl-C)GGGGG] to form the predicted immunogenic epitopes for antibody production for ELISA: *S*-phenylcysteine-K1 (SPK1), *S*-phenylcysteine-K10 (SPK10), *S*-(1-naphthyl)cysteine-K1 (1NK1), *S*-(1-naphthyl)cysteine-K10 (1NK10), *S*-(2-naphthyl-)cysteine-K1 (2NK1), and *S*-(2-naphthyl)cysteine-K10 (2NK10). Analysis by ELISA was chosen based on its high throughput and sensitivity, and low cost. The synthetic modified oligopeptides, available in quantity, served both as immunogens and as chemical standards for quantitative ELISA. Polyclonal rabbit antibodies produced against the naphthyl-modified keratins reacted with their respective antigens with threshold sensitivities of 15–31 ng/mL and high specificity over a linear range up to 500 ng/mL. Anti-*S*-phenylcysteine antibodies were not sufficiently specific or sensitive toward the target antigens for use in ELISA under our experimental conditions. In dermal tape-strip samples collected from 13 individuals exposed to naphthalene-containing jet fuel, naphthyl-conjugated peptides were detected at levels from 0.343 ± 0.274 to 2.34 ± 1.61 pmol adduct/μg keratin but were undetectable in unexposed volunteers. This is the first report of adducts of naphthalene (or of any polycyclic aromatic hydrocarbon) detected in the exposed intact human skin. Quantitation of naphthyl−keratin adducts in the skin of exposed individuals will allow us to investigate the importance of dermal penetration, metabolism, and adduction to keratin and to predict more accurately the contribution of dermal exposure to systemic dose for use in exposure and risk-assessment models.

Introduction

Low molecular weight aromatic hydrocarbons are important industrial chemicals with significant potential for human dermal exposure (*1*). A number of the lower molecular weight aromatics are carcinogenic to humans and laboratory rodents and have been associated with various disorders in humans, including hemolytic anemia and cataracts (*2*). Although there is significant potential for toxicity through dermal exposure, there has been little investigation of possible dermal penetration and uptake, metabolism to reactive intermediates, and mechanisms of action of observed toxicity. Quantitation of the individual dermal dose and determination of a mechanism of action hinge upon identification of appropriate biomarkers of exposure that can discriminate between acute and chronic exposure and also on development of quantitative analytical procedures. The only method for quantitation of dermal dose of aromatic hydrocarbons reported to date (*3*) detects the unchanged parent compound on the surface of the skin, which can be attributed to recent exposure only. Adducts bound to keratin would be appropriate biomarkers for chronic exposure, as the bioactivation required to generate adducts from aromatic hydrocarbons occurs only in the suprabasal level of the epidermis, where keratin is synthesized de novo. Approximately 20 days are required for differentiation and maturation of suprabasal keratinocytes (*4*) to migrate from the basal layer to form the stratum corneum. Thus, periodic noninvasive sampling by tape stripping can be used to determine prior and cumulative chronic exposure and to provide some measure of individual variation in metabolic capacity.

Cytochrome P450 isoforms observed in human skin (reviewed in ref *5*) include CYP1A1, CYP1A2, and CYP2E1, which metabolize benzene and naphthalene as well as other polycyclic aromatic hydrocarbons (*6, 7*) to arene oxides (*8*). These reactive

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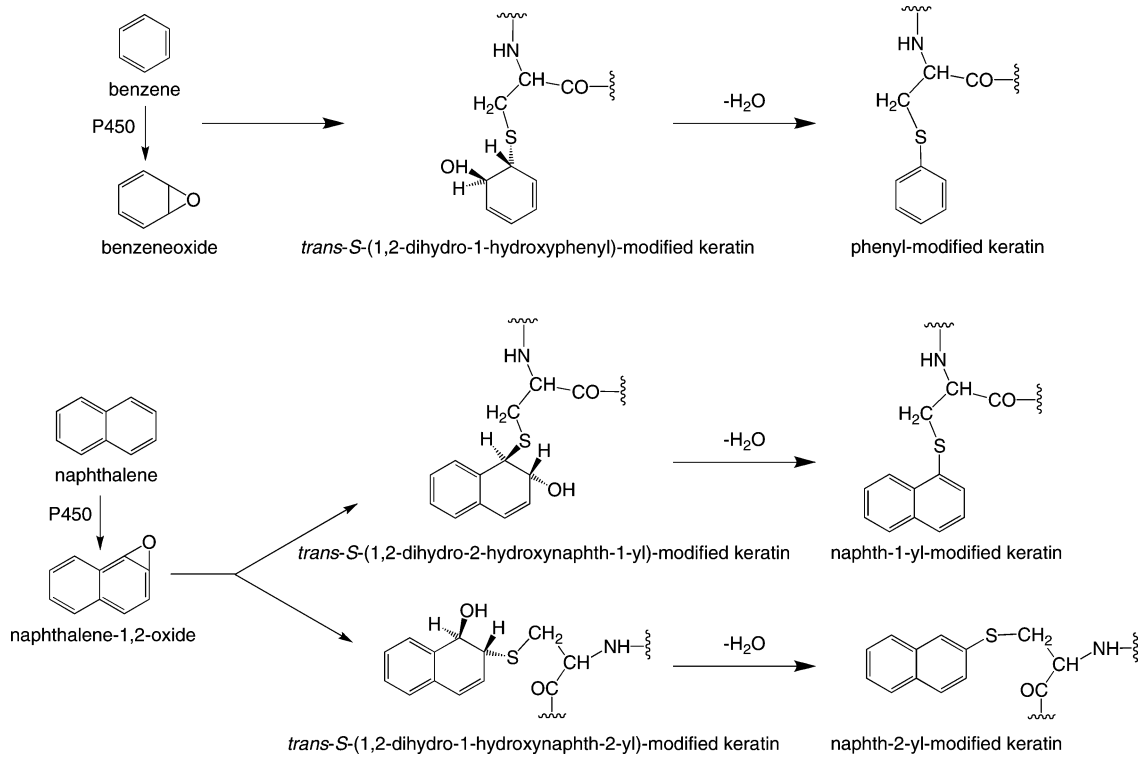

Figure 1. Putative pathway for benzene and naphthalene metabolism and adduction of keratin.

electrophiles (Figure 1) can form protein adducts with the nucleophilic sulfhydryl group of cysteine residues. Thus, systemic exposure to benzene gives rise to adducts of benzene oxide at the cysteinyl residues of albumin and globin in rats and humans (*9–14*), while protein adducts of naphthalene-1,2-oxide at cysteine residues have been demonstrated in mice exposed via intraperitoneal injection (*15, 16*). Protein adducts of bioactivated sulfonamides have been detected in cultured human keratinocytes by qualitative ELISA (*17*). We hypothesized that dermal exposure to benzene and naphthalene would lead to the formation of adducts at cysteine sulfhydryl groups in keratinocyte proteins through activation by CYP isoforms in the suprabasal layer where keratin is synthesized de novo and xenobiotic metabolism most likely occurs during differentiation and maturation. We further hypothesized that the most accessible nucleophilic targets would be within the N-terminal head regions of type II keratin K1 and type I keratin K10 proteins. K1 and K10 are present in abundance in the stratum corneum (*18*) where they are associated as 1:1 heterodimers in intermediate filaments with the N-terminal domains protruding from the filament surface (*18*). We considered that adduction would occur predominantly at cysteine sulfhydryl groups, although other highly nucleophilic residues, particularly arginine residues close to the N terminus of K1, could also be targets. Arene oxide adducts formed at the cysteine residues of newly synthesized K1 and K10 would be of interest for quantitation as biomarkers of dermal exposure at steady rates of xenobiotic metabolism and as initial events leading to possible toxic reactions (e.g., delayed type IV hypersensitivity).

We selected cysteine-containing 10-mers in the head sequences of K1 and K10 closest to the protein N terminus as likely sites for adduct formation prior to keratin folding and compaction in the corneocyte (*19*): the K1 10-mer head sequence comprised of residues 42–51 (GGGRFSSC*GG) (*20*) and the K10 10-mer head sequence comprised of residues 21–30 (GGGGC*GGGGG), where *C represents the modified cysteine (*21*). A similar strategy was applied to develop immunochemical analyses for sulfur mustard adducts at glutamine and asparagine residues of K1, K5, and K14 head groups (*22*). However, this report is distinct from our study in that the sulfur mustard adducts are formed by direct reaction of electrophiles that do not require metabolic activation.

On the basis of studies of nucleophilic addition of the sulfhydryl group to arene oxides (*16, 23, 24*), the cysteine residues would be predicted to react with benzene oxide to give initially the vicinal *trans*-*S*-(1,2-dihydro-1-hydroxy-2-phenyl)-cysteine adduct and to react with naphthalene-1,2-oxide predominantly at *C*2 (*16, 23, 24*) to yield initially the *trans*-*S*-(1,2-dihydro-1-hydroxynaphth-2-yl)cysteine adduct, although the regioisomer from attack at C1 has been reported in significant proportion from the reaction of naphthalene-1,2-oxide with glutathione (*8*), hemoglobin, and albumin (*13*). Initially formed vicinal *S*-(dihydrohydroxyaryl)cysteine adducts of arene oxides undergo facile dehydration to the aromatized *S*-aryl cysteines (*25*). In the physiologically normally acidic environment of the skin at pH 4–5 (*26, 27*), the *S*-aryl adducts were deemed the form most likely to be present and detected in our analysis. Hence, our synthetic targets were the *S*-phenyl-, *S*-naphth-1-yl-, and *S*-naphth-2-yl-cysteine-modified keratin sequences. Availability of chemically defined haptens allowed development of a quantitative ELISA, in contrast to previous studies (*17*). We incorporated the appropriately modified 9-fluorenylmethoxycarbonyl (Fmoc)-protected cysteines into the 10-mers by solid-phase peptide synthesis as the most practical scheme to obtain the putative haptens in quantity. We prepared the pure, rigorously characterized *S*-aryl haptens by a procedure published by our laboratory (*28*) for conjugation to carrier protein (keyhole limpet hemocyanin, KLH) and production of rabbit polyclonal antibodies for ELISA development. The affinity-purified polyclonal antibodies were tested for their sensitivity and specificity to detect keratin adducts formed by the benzene and naphthalene oxides in the skin samples collected from occupationally exposed workers. The sampling strategy was based on an adaptation of a quantitative tape-strip method developed for

Table 1. Keratin Head Sequences Modified at Cysteine by Arene Oxides

modified K1 10-Mers		modified K10 10-Mers	
SPK1	GGGRFSS(*S*-phenyl-C)GG	SPK10	GGGG(*S*-phenyl-C)GGGG
1NK1	KGGRFSS[*S*-(1-naphthyl)-C]GG LOS,[a] 31.25 ng/mL	1NK10	KGGG[*S*-(1-naphthyl)-C]GGGGG LOS,[a] 31.25 ng/mL
2NK1	KGGRFSS[*S*-(2-naphthyl)-C]GG LOS,[a] 15.63 ng/mL	2NK10	KGGG[*S*-(2-naphthyl)-C]GGGGG LOS,[a] 31.25 ng/mL

[a] LOS, limit of sensitivity of ELISA using polyclonal antibodies against the specific antigen.

noninvasive dermal sampling, which we have used to measure dermal deposition of acrylates (*29*), isocyanates (*30, 31*), and naphthalene (*3, 32–35*).

Experimental Procedures

Modified Oligopeptides. Synthesis and characterization of the Fmoc-protected *S*-aryl cysteines and the modified K1 and K10 sequences have been described previously (*28*). The specific targets for synthesis were the 10-mer head sequence comprised of residues 42–51 GGGRFSS(*S*-aryl-C)GG for K1 (*20*) and 10-mer head sequence comprised of residues 21–30 GGG(*S*-aryl-C)GGGGG for K10 (*21*). For ease of conjugation of the modified peptides to KLH, *S*-(1-naphthyl)cysteine-modified K1 and K10 peptides (1NK1, 1NK10) and *S*-(2-naphthyl)cysteine-modified K1 and K10 peptides (2NK1, 2NK10) were synthesized with lysine substituted for the N-terminal glycine (Table 1). The purity of the peptides was determined to be >98% by HPLC analysis.

Production of Polyclonal Antibodies. The *S*-aryl cysteine-modified peptides were conjugated to KLH (Pierce Chemical, Rockford, IL) by the glutaraldehyde cross-linking procedure. Equal weights of peptide and KLH in phosphate-buffered saline (PBS) were mixed by vortexing with the addition of glutaraldehyde (Sigma-Aldrich, St. Louis, MO) to a final concentration of 0.25%. Rabbits (two Flemish Giants per antigen; Bethyl Laboratories, Inc., Montgomery, TX) were immunized by subcutaneous injection into four separate sites with 100 μg of KLH-conjugated peptide dissolved in PBS (0.3 mL) and emulsified in an equal volume of complete Freund's adjuvant. Two booster immunizations at 2 week intervals with peptide−KLH complex in incomplete Freund's adjuvant completed the immunization. Seven days after the final booster, the animals were anesthetized, exsanguinated, and euthanized following standard protocols approved by the Bethyl Laboratories' Institutional Animal Care and Use Committee. Sodium azide was added to antisera as an antimicrobial. Antibody titers were verified using ELISA by coating peptide antigen onto microtiter wells and reacting with dilutions of antisera (dilution range 1:200 to 1:114670), followed by antirabbit IgG/horseradish peroxidase and 3,3′,5,5′-tetramethyl benzidine (TMB) substrate. Antisera were stored at −20 °C until purification.

Immunoaffinity Purification of Antibodies. Antisera were purified using Econo-Pac DEAE Blue (Bio-Rad Laboratories, Hercules, CA) and affinity column chromatography at room temperature. The DEAE with Cibacron Blue resin was used to remove serum albumin and other serum proteins. The column was washed with regeneration buffer (1.4 M NaCl, 0.1 M acetic acid, pH 3.0, and 40% v/v isopropanol) and equilibrated with application buffer (0.028 M NaCl and 0.020 M Tris-HCl, pH 8.0). Each antiserum was diluted with an equal volume of the application buffer and then loaded onto the column, and the fall-through was collected. The column was washed with elution buffer (1.4 M NaCl and 0.020 M Tris-HCl, pH 8.0) followed with regeneration buffer. The column was equilibrated with application buffer before reuse. The fall-through material from the DEAE Blue purification step from multiple aliquots of the same antiserum was pooled and concentrated with an iCON concentrator (Pierce). Eight antigen-specific immunoaffinity columns were prepared by solid-phase synthesis of the peptides SPK1, SPK10, 1NK1, 2NK1, 1NK10, 2NK10, unmodified K1, and unmodified K10 on an amino-containing resin (ToyoPearl AF-Amino-650M, Tosoh Bioscience, Montgomeryville, PA). Individual immunoadsorbants where each peptide was linked at its carboxyl terminus to the resin via an uncleavable linker were prepared. Before use, each column was equilibrated with binding buffer containing Tris-buffered saline (Bio-Rad) with 0.5 M sodium chloride (Sigma-Aldrich). The concentrated DEAE fall-through was loaded onto the corresponding column followed by the binding buffer containing Tris-buffered saline. The antibody fraction was eluted from the column with 5% glacial acetic acid (Fisher Scientific, Pittsburgh, PA). Each fraction exhibiting absorbance at 280 nm (UA-6 absorbance detector, ISCO, Lincoln, NE) was collected and neutralized with 1 M Tris base solution (Boehringer Mannheim Corp., Indianapolis, IN) and then concentrated with an iCON concentrator. The affinity column-purified antibodies were cross-absorbed onto a K1 or K10 column to remove K1 or K10 cross-reactivity, respectively. The fall-through was collected and dialyzed against PBS overnight at 4 °C using 10 mm dialysis tubing (MW 12000–14000; Spectrum Spectra/Por, Spectrum Laboratories, Inc., Rancho Dominquez, CA). Antibodies were concentrated with the iCON concentrator and quantitated using a bicinchoninic acid (BCA) protein assay (Pierce).

Enzyme-Linked Immunosorbent Assay (ELISA). After optimization of each antigen-specific ELISA, the general procedure for ELISA was as follows. A polystyrene 96 microwell plate (Nalgene Nunc International, Rochester, NY) was coated by addition of 100 μL of a serial dilution of an antigen standard or a potential cross-reactive antigen (K1, K10, and KLH) or bovine serum albumin (BSA, an unrelated antigen), starting at 500 ng antigen/mL to no antigen in Voller's buffer (100 mM sodium bicarbonate) to a well (in replicate), sealed, and incubated at 4 °C overnight. The plates were washed four times with 250 μL of 0.1% Tween 20 in PBS (PBST) and incubated for 1 h with 150 μL of blocking buffer (5% skim milk in PBST) at 37 °C to prevent nonspecific binding of subsequent reagents. The plates were washed as described above, and 100 μL of each affinity-purified antibody with the final optimized concentration of 0.8 (for 1NK1) or 7 μg/mL (for SPK1, SPK10, 1NK10, 2NK1, and 2NK10) in PBST was added to the appropriate plate and incubated for 1 h at 37 °C. The plates were washed again, and 100 μL of a 1:4000 dilution of goat antirabbit horseradish peroxidase (HRP) conjugate was added. The plates were incubated for 1 h and washed, and 100 μL of TMB-ELISA substrate (Pierce) was added and allowed to react at room temperature for 30 min. The reaction was stopped by adding 100 μL of 2 M sulfuric acid, and absorbance was determined at 450 nm (Emax, Molecular Devices, Sunnyvale, CA).

Skin Samples from Occupationally Exposed Workers and Unexposed Controls. Dermal tape-strip samples were obtained from 13 active duty U.S. Air Force fuel-cell maintenance workers who were routinely exposed to naphthalene- and benzene-containing jet fuel while performing their duties. Dermal samples were also collected from four individuals who declared that they had not knowingly been exposed to naphthalene (exposure control). This study was approved by the Institutional Review Board in the Office of Human Research Ethics at The University of North Carolina at Chapel Hill. The average age of the subjects was 22 ± 3.9 years, and 12 were male. The median naphthalene concentration in the dermal samples was 53 μg/m^2 (range, 3–5086 μg/m^2). Exposure conditions, sample collection, and analysis have been described previously (*3*). In brief, tape-strip samples were collected with adhesive tape strips (2.5 cm × 4.0 cm, Cover-Roll, Beiersdorf AG, Germany) from the volar region of the right arm after the work shift. Tape was applied to the skin surface with a constant pressure; after 1 min, it was removed at approximately a 45° angle. Three tape strips were collected sequentially from the exposed site. The tape was rolled with the adhesive side facing out and placed into a 2 mL cryovial. Samples were stored at –80 °C until the collected

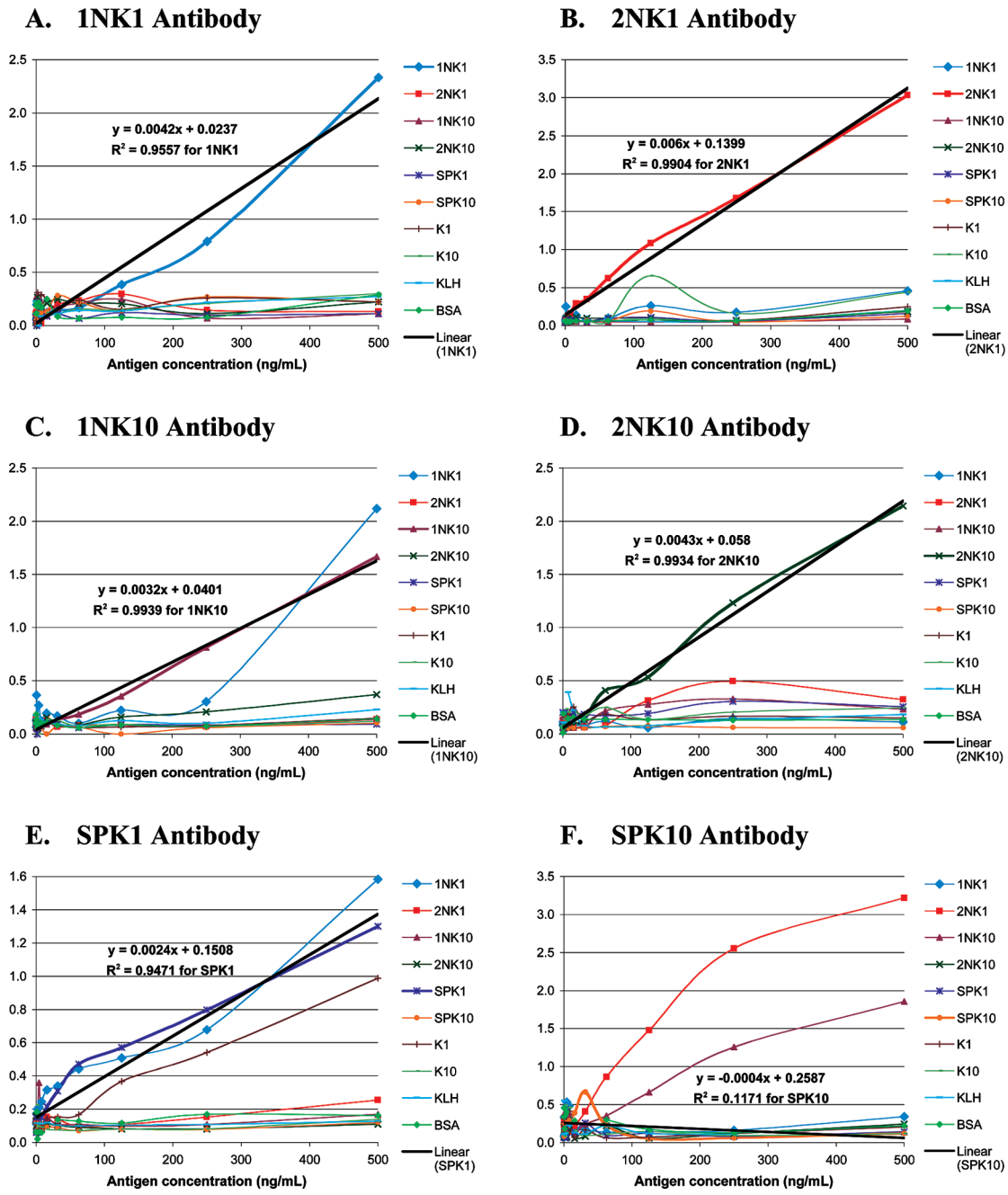

Figure 2. Sensitivity and specificity of purified antibodies against (A) 1NK1, (B) 2NK1, (C) 1NK10, (D) 2NK10, (E) SPK1, and (F) SPK10. Each purified antibody (0.8 μg/mL of 1NK1; 7 μg/mL of 1NK10, 2NK1, 2NK10, SPK1, or SPK10) was reacted against 0–500 ng/mL serial dilutions of its intended target antigen and other potential cross-reactive antigens.

protein was extracted and quantified by Bradford assay and keratin adduct quantified by ELISA (*36*).

Protein Quantification. Three sequential tapes collected from each of the exposed sites were pooled and treated with 3 mL of extraction solution (8 M urea, 50 mM Tris, 0.1 M β-mercaptoethanol, and 0.1% sodium azide), vortexed for 15 s, and placed on a shaker overnight at 150 rpm. Sample (100 μL) was placed into a cuvette, and 1 mL of Bradford reagent (Amresco Inc., Solon, OH) was added to each, vortexed, and read at 595 nm (Cary 100, Varian Instrument Co, Palo Alto, CA) (*37*). The keratin concentration of each sample was determined by interpolation and reference to a standard curve constructed from serial dilutions (0−500 μg/mL) of human epidermal keratin dissolved in urea (30 mg/mL) (Sigma-Aldrich). Our published report has established that keratins 1 and 10 are the major proteins removed from the human stratum corneum by tape stripping (*36*).

Keratin Adduct Quantification in the Skin Samples Collected from Occupationally Exposed Workers and Unexposed Controls. ELISA was performed as described above using the antigens SPK1, SPK10, 1NK1, 2NK1, 1NK10, and 2NK10 as standards. Each individual standard antigen (in duplicate) was diluted 1:2 in Voller's buffer per well across the plate starting at 500 ng/mL (0–500 ng/mL). Each exposed individual's sample was coated onto a separate well on the same plate in triplicate, along with a separate control sample (exposure control), composed of a pooled tape-strip sample of keratin extract collected from four individuals who had not knowingly been exposed to naphthalene. For antigen adsorption to the well, the plates were incubated overnight, washed, and blocked. Affinity-purified rabbit polyclonal antibodies (against 1NK1, 1NK10, 2NK1, and 2NK10 haptens) at dilutions of 0.8 (1NK1) or 7 μg/mL (1NK10, 2NK1, and 2NK10) were used as the primary antibodies for adduct detection. The plates were incubated and washed as described above. Secondary antibody (1:4000 dilution) goat antirabbit HRP-conjugate was added to each well, and the plates were washed and incubated with TMB-ELISA substrate. The reaction was terminated, and the absorbance was

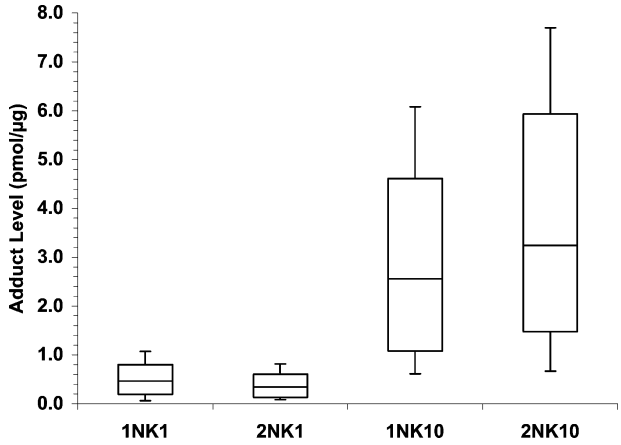

Figure 3. Quantitation of naphthyl−keratin adducts in tape-strip samples collected from 13 workers occupationally exposed to jet fuel. The top error bar represents the maximum value, while the bottom error bar represents the minimum value. The top of the box represents the 75th percentile, while the bottom represents the 25th percentile. The line in the box represents the median.

read at 450 nm. The amount of each keratin adduct in each sample was determined based on the standard curve constructed using each synthetic antigen, adjusted for the reading of the exposure control samples [i.e., average background of unexposed samples was subtracted]. The adduct level was then normalized based upon the amount of keratin recovered from each tape-strip sample. The limit of detection was determined using a 2-fold increase relative to the mean of the Voller's buffer negative control.

Results and Discussion

Sensitivity and Specificity of the Purified Antisera. The six hapten-specific affinity-purified polyclonal antibodies produced (see Table 1) were tested for sensitivity against their specific target antigens as well as for specificity against other target antigens and the four potential cross-reactive antigens K1, K10, KLH, and BSA (an unrelated antigen) (Figure 2A−F). The results showed that the three-step purification produced polyclonal antibodies specific to antigens 1NK1, 2NK1, and 2NK10 with threshold sensitivities of 31.25, 15.63, and 31.25 ng/mL, respectively, over a linear range up to 500 ng/mL (R^2 = 0.96, 0.99, and 0.99, respectively). The three affinity-purified antinaphthyl-keratin antibodies also showed low cross-reactivity against all other antigens (Figure 2A,B,D). The affinity-purified anti-1NK10 antibody had a sensitivity of 31.25 ng/mL against 1NK10; however, it also cross-reacted with antigen 1NK1 (Figure 2C). This antibody preparation reacts specifically against the *S*-(1-naphthyl) moiety but cannot discriminate between an adduct on K1 or K10. Nevertheless, it can still be used to detect adducts of 1-naphthyl-modified K10 by subtracting the reading for 1NK1 from the total.

The purified antibody to SPK1 showed high specificity to synthetic SPK1, but it also cross-reacted with antigens K1 and 1NK1 (Figure 2E), indicating the significant presence of antibodies to other epitopes that were not removed by purification. The purified SPK10 antibody also recognized antigens 1NK10 and 2NK1 while exhibiting low sensitivity toward its target antigen SPK10 (Figure 2F), suggesting an epitope specificity problem similar to that of SPK1. Consequently, neither of the antibodies to SPK1 or SPK10 produced under the reported conditions is suitable for detection and quantification of *S*-phenylcysteine-containing adducts. The strategy and protocol for production of the *S*-phenylcysteine-modified keratins will be reevaluated and additional studies performed to produce either monoclonal or polyclonal antibodies for the synthetic epitopes.

Keratin Adduct Quantification in the Skin Samples Collected from Occupationally Exposed Workers and Unexposed Controls. Keratin protein quantities in the tape-strip skin samples collected from the 13 occupationally exposed workers ranged from 16.8 to 236.4 μg/mL, with a mean value ± standard deviation of 115.0 ± 70.0 μg/mL. The variation in the amount of keratin recovered between individuals may be due to the difference in perspiration and other individual factors affecting adhesion and removal of the tape. Hence, quantification of adducts is adjusted for the quantity of keratin removed in each skin sample. The four naphthyl−keratin adducts were detected in the tape-stripped skin samples at levels from 0.047 to 6.85 pmol/μg keratin (Figure 3). The highest levels of adduct were observed for 2NK10 and the lowest for 2NK1. The mean naphthyl−keratin adduct levels ± standard deviations were 0.343 ± 0.274 (1NK1), 1.77 ± 1.13 (1NK10), 0.343 ± 0.494 (2NK1), and 2.34 ± 1.61 (2NK10) pmol adduct/μg keratin. No correlation was observed between naphthyl−keratin adduct concentrations and dermal naphthalene levels measured in this sample population (*3*) (data not shown). This finding is not unexpected because dermal naphthyl−keratin adduct concentrations reflect both long-term exposure and interindividual variation of CYP metabolic activity, whereas dermal naphthalene levels reflect only the most recent exposure (i.e., exposure received on the day of sampling). Mean levels of the total naphthyl-bound K1 (1NK1 + 2NK1) were significantly lower than the total naphthyl-bound K10 (1NK10 + 2NK10) using the Student's *t* test ($p < 0.001$). Down-regulation of type II keratins (K1) and up-regulation of type I keratins (K10) were observed in human keratinocyte cell cultures exposed to JP-8 jet fuel (*38*), although the mechanism leading to the altered keratin expression levels is currently unknown and we have not verified the effect in vivo. While evidence supports the basic unit of the intermediate filament to be a type I:type II keratin heterodimer, effects of the imbalance in K1:K10 levels on filament structure cannot be ruled out (*18*) and are also currently unknown. It is therefore not apparent whether the difference in adduct levels represents a direct reflection of an increased level of monomeric K10, the result of a difference in cysteine sulfhydryl accessibility attributable to structure in the intermediate filaments or simply less accessibility of the target cysteine of K1 as a result of location further from the N-terminal of the protein or competitive adduction at an arginine-rich region of K1 closer to the N-terminal. Significant differences were found between the mean levels of 1NK10 and 2NK10 ($p < 0.0001$) but not between 1NK1 and 2NK1 using the Student's *t* test ($p = 0.1155$). This finding is intriguing but should not be overinterpreted as the sample size is limited and binding levels to K1 are low. No adducted keratins were observed in unexposed (negative control) individuals.

In summary, we have developed methods for specific antigen synthesis, antibody purification, and ELISA for quantitation of naphthyl−keratin adducts as biomarkers of dermal exposure to naphthalene. This is the first report of the detection and quantitation of polycyclic aromatic hydrocarbon adducts formed in the skin of exposed humans in vivo and is consistent with our observation of adduct formation in vitro with reconstructed skin (unpublished). Previous reports only addressed adducts formed by direct-acting chemicals or adducts formed in vitro. Our approach also allows for direct quantitation of adduct levels, as opposed to qualitative assessment of intensity of staining or fluorescence (*17*). Quantitation of keratin adducts obtained from

the stratum corneum of exposed individuals will allow us to investigate the importance of dermal penetration, metabolism, and adduction to keratin as well as to make accurate prediction of the contribution of dermal exposure to the systemic dose for inclusion in exposure- and risk-assessment models. This study confirms that our approach of chemical synthesis of predicted epitope-specific antigens combined with sensitive ELISA should be adaptable for use with other low and high molecular weight aryl hydrocarbons or other electrophilic species, whether direct-acting or formed as a result of bioactivation. By studying the binding occurring in the viable epidermis and presented in the stratum corneum available for noninvasive sampling, we will be able to investigate both external and internal factors contributing to keratin protein adduction during dermal exposure, including exposure dosages, use of personal protective equipment, and individual differences in xenobiotic metabolism. The ability to quantitate keratin adducts as biomarkers of dermal exposure will enable us to distinguish systemic dose resulting from dermal exposure from other routes of exposure. Correlation of individual adduct levels with the CYP isoform single nucleotide polymorphisms (SNPs) among individuals in a sample population will contribute toward establishing the role of SNPs in interindividual susceptibility to exposures.

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