Lung disease is the fourth leading cause of morbidity and mortality in the U.S. population. Although death rates from heart disease, cancer, and cerebrovascular disease have decreased in the past 10 years, death rates from chronic lower respiratory disease have not. Although tobacco use and traffic-related pollutants account for a portion of disease statistics, the precise etiology of lung disease remains unknown. A significant problem in assessing the contribution of individual chemicals or mixtures of chemicals to lung disease is related to the uncertainty of the use of animal models for human lung disease. Numerous chemicals, including naphthalene (Plopper et al. 1992), trichloroethylene (Odum et al. 1992), and styrene (Green et al. 2001), cause differential toxicity in two common rodent models, mice and rats, raising the question of relevance of these rodent models for human risk assessment.

Naphthalene administration results in highly selective necrosis of nonciliated bronchiolar epithelial cells of mice regardless of the route of administration. Inhaled concentrations of 2–5 ppm for 4 hr, well below the current occupational standard, cause detectable toxicity (West et al. 2001). In contrast, injury to airway epithelium of the rat does not occur at concentrations ≤ 100 ppm (West et al. 2001). The nasal epithelium of both rats and mice is highly sensitive to naphthalene administered either parenterally or by inhalation (Lee et al. 2005; Plopper et al. 1992). Recent studies have demonstrated sensitivity of rat olfactory epithelium at concentrations similar to those in urban atmospheres (0.3 ppm) (Dodd et al. 2010).

The species-, tissue-, and site-selective injury caused by naphthalene has been used to identify mechanisms associated with toxicity (for review, see Buckpitt et al. 2002). Naphthalene undergoes metabolism by the cytochrome P450 monooxygenases to reactive metabolites that become bound covalently to tissue proteins. The primary P450 involved in metabolism in the mouse lung and rat nose appears to be cytochrome P450 2F (CYP2F2); this protein has high affinity for naphthalene as a substrate (K_m = 4 μM) with high catalytic turnover (104/min) (Shultz et al. 1999). CYP2F2 orthologs found in the lungs of nonhuman primates (CYP2F5; Baldwin et al. 2005) and humans (CYP2F1; Nhamburo et al. 1990) had little or no catalytic activity when expressed as recombinant enzyme (Lanza et al. 1999). This was consistent with the finding that lung microsomes from monkeys and humans metabolized naphthalene to water-soluble metabolites at very low rates (Buckpitt and Bahnsen 1986; Buckpitt et al. 1992) and that dissected monkey airways generated metabolites very slowly (Boland et al. 2004). Because the initial step in the turnover of naphthalene is critical to toxicity, these data would indicate that monkeys and humans would be considerably less susceptible to naphthalene exposure than rodents. However, more recent data have shown that, although the rates of water-soluble metabolite formation are low, reactive metabolite protein binding occurs at similar levels in sensitive rodent tissues (Cho et al. 1994) and in dissected airways of monkeys (Boland et al. 2004).

Human exposure to naphthalene is nearly universal; the recent National Health and Nutrition Examination Survey studies demonstrated detectable levels of 1-naphthol glucuronide in the urine of all 2,785 subjects tested (Li et al. 2008). However, epidemiologic studies have failed to establish a relationship between naphthalene exposure and any long-term health consequences in the respiratory tract. Either humans are not as susceptible to the compound as rodents are, or respiratory diseases are multifactorial in origin and epidemiologic studies have not been sufficiently powered to detect subtle contributions of naphthalene to the overall incidence of disease.

In the present study we focused on protein-bound metabolites in nasal epithelium of...
rats and rhesus macaques. In contrast to previous work showing 10- to 100-fold higher rates of naphthalene metabolism by dissected mouse airways compared with the rhesus airway (Boland et al. 2004; Plopper et al. 1991), the present study shows that the rates of formation of covalently bound metabolites in rhesus nasal epithelium are similar to those in rat nasal epithelium, a target for naphthalene toxicity. This study also demonstrates some concordance in the adducted proteins identified in rat and monkey nasal epithelial incubations. We identified proteins involved in the unfolded protein response in incubations with rat but not monkey nasal epithelium. Proteins identified in the present work could serve as potential biomarker targets for studies in exposed human populations.

**Materials and Methods**

**Animals.** All animal work was conducted under protocols approved by the University of California–Davis (UCD) Animal Use and Care Committee. Animals were treated humanely and with regard to alleviation of suffering. Male Sprague-Dawley rats (200–225 g body weight) were obtained from Harlan Laboratories (Indianapolis, IN) and housed in the UCD vivarium for at least 1 week before experimentation. Animals were euthanized with an overdose of pentobarbital, and olfactory and septal turbinates were removed. Ethmoturbines and maxillo-turbines were obtained from eight male and seven female rhesus macaques through the California National Primate Research Center [animal characteristics are available in Supplemental Material, Table 1 (doi:10.1289/ehp.0901333)]. All rhesus macaque tissue was from animals euthanized for normal colony maintenance at the center.

**Reagents.** We obtained [1,4,5,8-14C] naphthalene (specific activity 58 mCi/mmol) from Moravek Radiochemicals (Brea, CA) and checked purity (99%) by high-performance liquid chromatography. Protease inhibitor cocktail III, nondetergent sulfobetaine-195 (NDSB-195), benzamidine, 1,10-phenanthroline, aprotinin, pepstatin, and leupeptin were purchased from CALBIOCHEM (La Jolla, CA). Rhinohide acrylamide/ bisacrylamide was from Invitrogen (Carlsbad, CA), and Bio-Rad Protein Assay solution was purchased from Bio-Rad (Hercules, CA). We obtained immobilized pH gradient (IPG) buffers and narrow-range IPG Immobiline dry strips (18 cm) from GE Health Care (Piscataway, NJ). Unless otherwise stated, all reagents were molecular biology grade or better.

**Tissue incubation and preparation.** Tissue was incubated in oxygenated Waymouth’s medium deficient in sulfur amino acids (glutathione, cysteine, methionine) containing 250 µM naphthalene or 250 µM 14C-naphthalene at a specific activity of 1.10 × 105 disintegrations per minute per nanomole. Incubations were performed for 2 hr at 37°C in a shaking water bath. For protein identification, we incubated tissue with unlabeled substrate.

After the incubation, tissues were rinsed with cold 5% dextrose and homogenized in lysis solution (2 M thiourea, 7 M urea, 4% wt/wt 3-(3-cholamidopropyl)-dimethylammonio propane sulfonate (CHAPS), 0.5% Triton X-100, 1% dithiothreitol, and 2% protease inhibitor cocktail) using glass–glass homogenizers. Tissues were allowed to sit at room temperature for 1 hr after homogenization to allow further solubilization and denaturation of proteins. Insoluble proteins were removed by centrifugation (100,000 × g for 1 hr).

The tough, bony structure of primate turbinates dictated the use of modified procedures for protein recovery. Briefly, after the incubation, the incubation medium was transferred to a clean centrifuge tube. The tissue was washed with 5% dextrose to remove buffer salts, and fractions of the aqueous phase were combined. These samples were centrifuged to recover any tissue/cells removed, and the pellets were combined with the remaining cartilage containing the epithelium. Lysis buffer containing protease inhibitors was added, and the tube was agitated on a rotating shaker to dislodge cells.

**Comparison of covalent binding levels.** A portion (~1 mg protein) of each sample (from rat or monkey nasal epithelium) incubated with 14C-naphthalene was dialyzed in 0.1% sodium dodecyl sulfate, 1 mM disodium EDTA at 4°C [3,500 molecular weight (MW) cutoff Slide-A-Lyzer; Pierce, Rockford, IL].

The dialysis solution was replaced with fresh solution until the counts per minute was less than twice the background level. Finally, we determined the specific activity of adducted protein for each sample by scintillation counting and a protein assay (Bradford 1976).

**Separation and identification of adducted protein.** Procedures for the separation, localization, and identification of adducted proteins have been described in detail previously (Lin et al. 2005). Briefly, samples were not pooled, and separate gels were run for each incubation with labeled (for imaging) and unlabeled (for identification) naphthalene.

Proteins were resolved by two-dimensional (2D) gel electrophoresis on 18-cm narrow-range pl gel (isoelectric point) strips for 4.5–5.5 and 5.5–6.7 pl. Strips were rehydrated with 300 µg protein for rat samples or 600 µg protein for monkey samples. Gels containing nonradioactive samples were silver stained and imaged on a 16-bit grayscale scanner. 14C-Labeled sample gels were electroblotted to Sequa-Blot polyvinylidene fluoride membrane (0.2 mm; Bio-Rad), and these were placed against storage phosphor screens for 60 days. The patterns of 14C-labeled adducted proteins on the 2D gels were visualized using the GE Typhoon imager (GE Health Care). After exposing protein spots of interest from nonradioactive gels, the gel plugs were destained, washed, and incubated in 1:1 (vol/vol) acetone:nitric acid:50 mM ammonium bicarbonate followed by 100% acetonitrile. Tryptic digestion and matrix-assisted laser desorption ionization mass spectrometry (MALDI tandem mass spectrometry (MS/MS) identification was carried out as described by Lin et al. (2006).

We used GPS Explorer Workstation software (version 3.5; Applied Biosystems, Foster City, CA) and MASCOT (Matrix Sciences, Boston, MA) to search protein databases in the International Protein Index (IPD) rat database (http://www.ebi.ac.uk/_ipi/), the National Center for Biotechnology Information primary database (NCBI; http://www.ncbi.nlm.nih.gov/), and the Uniprot database (http://www.ebi.ac.uk/uniprot/). Databases were searched for a precursor tolerance of 150 ppm, MS/MS fragment tolerance of 0.2 Da, a maximum of two missed cleavages, and variable modifications for carbamidomethylation and methionine oxidation. An identification with a probability-based MOWSE score > 60, obtained through MASCOT, was considered statistically significant (p < 0.05).

**Results**

The specific activities of adducted proteins (nanomoles bound per milligram protein) generated in vitro incubations of nasal epithelium from rats or rhesus macaques varied from 1.0 to 1.5 nmol/mg protein; we noted no statistically significant difference in the data obtained from rat olfactory and septal epithelium compared with rhesus ethmoturbines and maxilloturbines (Figure 1). Compared with incubations of target airway subcompartments (minor daughter and distal airway) from the mouse (Cho et al. 1994), the overall levels of adduct generated in vitro are slightly lower in rat and monkey nasal epithelial mucosal incubations.

Representative images from the phosphor-imaging screens and complementary silver-stained gels/phosphor images indicating the spot locations chosen for MALDI dual time of flight (TOF/TOF) MS analysis are available in Supplemental Material, Figures 1 and 2 (doi:10.1289/ehp.0901333).

In general, more adducts were apparent on the images from incubations of rat nasal epithelium than on those from rhesus macaques, but the patterns of major adducts were similar. Spots were matched using the alignment function of Progenesis SameSpots and Progenesis PG220 (version 2006; Nonlinear Dynamics, Durham, NC) matches were manually verified. For identification, we used only those
features that could be aligned with the storage phosphor images and selected from the gel with high confidence.

We identified a large number of adducted proteins after incubation of rat nasal olfactory epithelium with naphthalene (Table 1). These included a number of redundant identifications of structural proteins (actin and tubulin), catalytic proteins (some of which are involved in energy metabolism such as ATP synthase), and a number of proteins involved in the unfolded protein response, including one of the master regulators of that response, glucose regulated protein (heat shock 70 kDa protein 5). In contrast, fewer features were identified from the gels of monkey ethmoturbinate, representing only three non-redundant proteins (Table 2). Two of these were structural proteins (actin and tubulin), and the third was a protease inhibitor with a myriad of functions.

**Discussion**

The chronic bioassays conducted by the National Toxicology Program on the potential carcinogenicity of naphthalene in mice (Abdo et al. 1992) and rats (Abdo et al. 2001) have raised concerns about the possible effects of this compound on human health. In mice, an increased incidence of bronchioloalveolar adenoma was observed in females at the highest concentration tested (30 ppm) (Abdo et al. 1992), and in rats, the nasal olfactory epithelium was a prominent target after 105 weeks of exposure (Abdo et al. 2001). Long et al. (2003) observed dose-dependent increases in adenomas of the respiratory epithelium and a significant increase in olfactory epithelial neuroblastomas in male and female rats, respectively. In the olfactory epithelium, the incidence of hyperplasia and chronic inflammation was nearly 100%, even at the lowest concentration tested (10 ppm). Overall, the high degree of hyperplastic and inflammatory effects and the relative susceptibility of areas of the nasal epithelium after long-term exposures correlate well with the susceptible sites for acute toxicity (Lee et al. 2005) and raise the possibility that the tumorigenic effects of naphthalene observed in animals are related to repeated cycles of injury and repair (North et al. 2008).

The published assessments by regulatory bodies on the potential carcinogenicity of naphthalene in humans differ (e.g., International Agency for Research on Cancer 2002; National Toxicology Program 2004). Although the toxicologic data in animals are very clear, human epidemiologic studies in populations of exposed workers are uninformative. In most short-term mutagenicity assays, naphthalene is negative (reviewed by Brusick 2008). Recent evidence for the formation of DNA adducts comes both from in vitro studies with naphthalene metabolites (Saed et al. 2007) and from skin-painting studies using both naphthalene and naphthalene metabolites (Saed et al. 2009). The skin-painting studies demonstrated the presence of both depurinating and stable adducts, suggesting that this may be a possible mechanism for tumorigenesis associated with naphthalene in rodent models.

Previous studies demonstrating 10- to 100-fold differences in the rates of pulmonary microsomal glutathione conjugates of naphthalene between rodents and human/nonhuman primates (Buckpitt et al. 1992; Buckpitt and Bahnsen 1986) and in dissected airways of rodents and nonhuman primates (Boland et al. 2004; Buckpitt et al. 1995) suggested that humans would be considerably less susceptible to naphthalene than are rodents. Although the dramatic differences in susceptibility to naphthalene of airway epithelium in the mouse and rat appear to be related to metabolism of naphthalene by CYP2F, recent work showing that several human CYPs can metabolize naphthalene with high efficiency suggests that other CYP enzymes may catalyze the turnover of this substrate in humans (Cho et al. 2006; Fukami et al. 2008). In particular, CYP2A13, which has been reported in both lung and nasal epithelium of humans (for review, see Ding and Kaminsky 2005), shows a K_{m} of 36 µM and high catalytic efficiency with naphthalene. In addition, our finding of similar levels of covalent protein binding in nasal epithelium of the rodent and monkey (Figure 1), as well as observations that some (but not all) of the proteins adducted by reactive metabolites of naphthalene are the same in dissected airways and nasal epithelium of rodents and nonhuman primates (Lin et al. 2005, 2006; see also Tables 1 and 2), supports the need for additional studies on the possible sensitivity of humans to naphthalene exposure.

The concept that posttranslational modification of proteins by electrophiles begins a series of events leading to cellular necrosis was proposed by Brodie et al. (1971) some 40 years ago, and was supported by studies showing correlations between overall electrophile protein adduct formation and toxicity (for a recent review, see Hanzlik et al. 2009). The advent of new MS techniques coupled with 2D gel electrophoresis has resulted in the identification of numerous protein targets for cytotoxic agents that require metabolic activation; a current database of these targets shows many commonalities (Fang et al. 2009). Despite these efforts, a final unifying hypothesis tying the addition of specific proteins with toxicity has not emerged, and additional work is needed to answer several questions. In those cases where adduction occurs to an abundant protein, is this protein simply acting as a sink to trap electrophiles? Does the formation of an adduct with an individual protein alter the function of that protein directly, or is the deleterious effect mediated by protein-interacting partners? These will clearly be challenging questions but, if solved, could greatly enhance our ability to more quickly and precisely assess the human risk of exposure to agents that undergo metabolic activation to protein-reactive electrophiles.

As noted above, much of the progress in identifying proteins targeted by reactive metabolites has derived from the application of 2D gel electrophoresis and more rapid MS-based identifications. Although 2D gel electrophoresis arguably provides the greatest resolving power for whole proteins of any separations technique available, there is still the potential of selecting a spot that contains two unseparated proteins, one that is low abundance and adducted and one that is much higher abundance and identified by MS. Although we cannot exclude this as a possibility in the present study, we used IPG strips with very narrow ranges to lessen the potential for misidentification. In only one case that we are aware of—with thiobenzamide—were the investigators able to show that the features removed from the gel were adducted by using isotope ratios with equal
Table 1. Adducted proteins identified from incubation of rat olfactory ethmoturbinate with 14C-naphthoic acid.

| Gel spot | Protein name (abbreviated) | MWSE scorea | Peptide countb | Protein score CIsc | Accession no. d | Protein MW (Da) | Protein pI | Summary of function |
|----------|----------------------------|-------------|----------------|-------------------|-----------------|----------------|-----------|--------------------|
| N1       | Soricin (P306B6)           | 133         | 5              | 100               | SORCN CHILOe | 22017.4        | 5.32     | Calcium-binding protein |
| N4       | Golgi autoantigen          | 90          | 24             | 99.951            | GI: 80868f    | 37240.9        | 4.96     | May participate in forming intercisternal cross bridges of the Golgi complex |
| N7       | pyruvate dehydrogenase     | 69          | 7              | 99.596            | ODP6_RATg | 39,336         | 5.94     | Glycolysis |
| N8       | mu-crystallin              | 135         | 4              | 100               | IPI0021444h  | 33704.1        | 5.34     | Ammonia elimination |
| N9       | pyrophosphatase            | 67          | 7              | 99.283            | IPI0037195i  | 33206.3        | 5.26     | Signal transduction |
| N12      | creatinine kinase          | 543         | 20             | 100               | IPI0047028j  | 42683.4        | 5.39     | Energy transduction |
| N13      | Sec 1413 protein           | 131         | 6              | 100               | IPI0020893k  | 46435.5        | 5.64     | Hydrophobic ligand binding |
| N19      | creatinine kinase          | 299         | 10             | 100               | IPI0047028j  | 42683.4        | 5.39     | Energy transduction |
| N23      | actin                      | 259         | 10             | 100               | IPI0036961l  | 59162.6        | 5.67     | Structural |
| N24      | euakaryotic translation    | 129         | 14             | 100               | IPI0036961l  | 46352.6        | 5.32     | Protein synthesis initiation |
| N25      | beta actin                 | 447         | 10             | 100               | GI: 62897625f | 41737.8        | 5.37     | Structural |
| N26      | actin, cytoplasmic         | 420         | 15             | 100               | IPI0076759i  | 42108.9        | 5.31     | Structural |
| N27      | actin                       | 753         | 17             | 100               | IPI0019408j  | 42334.4        | 5.23     | Structural |
| N29      | actin, cytoplasmic         | 198         | 6              | 100               | IPI0076501k  | 59162.6        | 5.57     | Structural |
| N32      | actin, cytoplasmic         | 164         | 4              | 100               | IPI0076501k  | 59162.6        | 5.57     | Structural |
| N33      | actin, cytoplasmic         | 186         | 5              | 100               | IPI0076501k  | 59162.6        | 5.67     | Structural |
| N41      | ATP synthase               | 919         | 21             | 100               | IPI0055181l  | 56318.5        | 5.19     | ATP catalysis |
| N43      | ATP synthase               | 160         | 17             | 100               | IPI0055181l  | 56318.5        | 5.19     | ATP catalysis |
| N47      | heat shock protein 1A      | 220         | 27             | 100               | IPI0033914m  | 61088.4        | 5.91     | Stress-induced chaperone; protein folding |
| N51      | (chaperonin) (Hsp 60)       | 419         | 9              | 100               | IPI0036326n  | 74,097         | 5.97     | Protein folding; protein export; binds ATP, nucleotides, and proteins |
| N53      | heat shock 70 kDa 9 A (predicted) | 64     | 5              | 98.428            | IPI0085838o | 74098.9        | 5.97     | Protein folding; protein export; binds ATP, nucleotides, and proteins |
| N55      | Vomeromodulin              | 87          | 8              | 99.992            | IPI0020978p  | 79527.3        | 5.92     | Putative pheromone and odorant transporter |
| N56      | heat shock 70 kDa, protein 5 | 621     | 15            | 100               | IPI0020662q  | 72473.5        | 5.07     | Assembly of multimeric protein complexes |
| N57      | heat shock 70 kDa, protein 5 | 896     | 27            | 100               | IPI0020662q  | 72473.5        | 5.07     | Assembly of multimeric protein complexes |
| N61      | tyrosine 3-monooxygenase    | 243         | 12             | 100               | IPI0020383r  | 28,456         | 4.8      | Adapter protein; signal transduction |
| N63      | tubulin                    | 485         | 12             | 100               | IPI0019879s  | 50787.9        | 4.94     | Structural |
| N64      | tubulin                    | 174         | 16             | 100               | IPI0033916t  | 50119.6        | 4.94     | Structural |
| N65      | tubulin                    | 148         | 14             | 100               | IPI0033916t  | 50119.6        | 4.94     | Structural |
| N66      | tubulin                    | 258         | 23             | 100               | IPI0040057u  | 49,789         | 4.79     | Structural |
| N67      | tubulin                    | 464         | 24             | 100               | IPI0019757v  | 49,639         | 4.78     | Structural |
| N68      | protein disulfide isomerase| 272         | 17             | 100               | IPI0019888w  | 56915.7        | 4.82     | Cell redox homeostasis |
| N69      | protein disulfide isomerase| 139         | 15             | 100               | IPI0019888w  | 56915.7        | 4.82     | Cell redox homeostasis |
| N70      | protein disulfide isomerase| 227         | 25             | 100               | IPI0019888w  | 56915.7        | 4.82     | Cell redox homeostasis |
| N73      | ubiquinol-cytochrome c     | 162         | 16             | 100               | IPI00471577  | 57815.4        | 5.57     | Mitochondrial electron transport |
| N74      | ubiquinol-cytochrome c     | 78          | 19             | 99.339            | IPI00471577  | 52815.4        | 5.57     | Mitochondrial electron transport |
| N75      | chloride intracellular      | 212         | 14             | 100               | IPI00421995  | 29638.8        | 5.09     | Macrophage activation |
| N76      | tubulin                    | 166         | 12             | 100               | IPI0018975x  | 5103.6         | 4.94     | Structural |
| N77      | tubulin                    | 126         | 19             | 100               | IPI0040057u  | 49,789         | 4.79     | Structural |

*aThe probability-based MWSE score from MASCOT. bPeptides used for matching within the database. cReflects the probability that this same match would be made in a different database. dAccession numbers are from the IPI rat database except where noted. eFrom the Swiss-Prot database searching mammals. fFrom the NCBI database searching mammals.
showing that S-glutathionylation of cysteine-374 of actin reduces the rate of polymerization of this structural molecule (Dalle-Donne et al. 2003) and that glutathionylation of this protein leads to decreased affinity of its partner protein tropomyosin (Chen and Ogur 2006). Both naphthalene and diethyl maleate substantially deplete glutathione levels in airway epithelium, which may result in increased levels of glutathionylated actin. Reactive metabolites from naphthalene produce a double hit on actin and tropomyosin, resulting in both thiolation reactions (which are reversible) and covalent arylation reactions (which presumably are not). Ongoing work in our laboratory is evaluating the protein targets of diethyl maleate as a mechanism for distinguishing adducts that may be critical to cellular injury from those that are not.

We observed a potentially important group of protein targets in nasal olfactory epithelial incubations from the rat but not from the monkey; these proteins are responsible for protein folding and repair in the cell. Glutathione depletion is a necessary but insufficient prelude to naphthalene cytotoxicity. The loss of this key thiol leaves the cell vulnerable to intracellular reactive oxygen species that, with diethyl maleate, can be corrected by chaperones, protein disulfide isomerase, peroxidoxins, and possibly several other proteins involved in antioxidant protection and protein folding. With naphthalene, several of these proteins, including the master regulator of the unfolded protein response, BiP/GRP78 (Townsend 2007), as well as the heat shock proteins HSP60 and HSP70, are adducted, thus potentially altering their ability to repair proteins undergoing thiol oxidation and unfolding during the early stages of naphthalene toxicity. This is consistent with findings showing marked up-regulation of the HSPs in mouse lung at early times after naphthalene treatment (Williams et al. 2003). An interesting but unproven link in possible mechanisms comes from recent work demonstrating that proteins involved in the unfolded protein response are substantially up-regulated in smokers compared with former smokers and nonsmokers (Kelsen et al. 2008).

The present study provides a list of potential protein targets and shows that some of these are similar in nasal epithelium of the rat (a known target for naphthalene toxicity) and that of the rhesus macaque. We derived several other adducted proteins from monkey nasal epithelial incubations, but these yielded unreliable identifications. We are working on methods for concentrating adducted proteins to provide greater sensitivity for these analyses. In the future, it will be important to gain additional insight regarding critical versus noncritical protein adduction. Part of this information will come from studies such as that published by Meier et al. (2007), which showed that metabolites of BHT alkylate key antioxidant enzymes and that the alkylation resulted in a decrement in enzyme function that, in turn, was consistent with the biological responses after BHT treatment.

In the present study we focused on protein adducts that are most likely related to acute cellular injury. Whether repeated cycles of injury and repair with subsequent hyperplasia are related to naphthalene tumorigenesis will need additional study. Finally, the possibility that exposure to naphthalene and other similar, volatile polycyclic aromatic hydrocarbons (PAHs) could produce lung disease other than cancer should be explored. In this regard, limited studies have reported correlations between serum levels of naphthalene and pyrene, but not other PAHs, with asthma incidence in children (Al-Daghri 2008). Moreover, there is a clear association of exposure to traffic-related pollution and asthma (Kim et al. 2005) and naphthalene is the most prevalent PAH in this mixture (Lu et al. 2005). Recent work showing that polymorphisms that result in alterations in enzymatic activities for two key enzymes involved in the metabolism of PAHs (microsomal epoxide hydrolase and glutathione transferase pi) lead to increased incidence of asthma in children living near freeways (Salam et al. 2007) provide an interesting but unsubstantiated link between PAHs in motor vehicle exhaust and asthma. The understanding of target proteins and their relationship to the pathology from naphthalene in animals should improve our ability to assess the possible risks of this compound in exposed human populations.

### Conclusion

This study shows that the amounts of covalent adduct generated from naphthalene in incubations of rhesus nasal epithelium are similar to those formed using rat olfactory and septal nasal epithelium, two sites known to be susceptible to the cytotoxicity associated with naphthalene. Many of the adducted proteins identified are the same as those observed in incubations with other target tissues. Our results are consistent with the view that nasal epithelium of the rhesus macaque may be susceptible to naphthalene cytotoxicity.

### Table 2. Adducted proteins identified from incubations of monkey ethmoturbinate with 14C-naphthalene.

| Gel spot | Protein name (abbreviated) | MOWSE score a | Peptide count b | Protein score CF c | Accession no. d | Protein MW (Da) | Protein pl | Function |
|----------|---------------------------|----------------|----------------|-------------------|-----------------|----------------|------------|----------|
| M1       | actin                     | 333            | 22             | 100               | P60709          | 40978.4        | 5.56       | Structural |
| M2       | actin                     | 628            | 25             | 100               | G1: 62897625    | 41737.8        | 5.37       | Structural |
| M3       | actin                     | 526            | 23             | 100               | P60709          | 40978.4        | 5.56       | Structural |
| M4       | tubulin                   | 95             | 17             | 99.992            | G1: 11462722    | 7398.6         | 4.72       | Structural |
| M5       | actin                     | 107            | 11             | 100               | P68133          | 37799.8        | 5.39       | Structural |
| M6       | actin                     | 468            | 18             | 100               | Q8W6W5          | 40477.2        | 5.78       | Structural |
| M7       | actin                     | 690            | 23             | 100               | G1: 4501883     | 41981.8        | 5.23       | Structural |
| M8       | actin                     | 479            | 20             | 100               | G1: 4501983     | 41981.8        | 5.23       | Structural |
| M9       | actin                     | 181            | 13             | 100               | G1: 4885049     | 41981.9        | 5.23       | Structural |
| M10      | actin                     | 147            | 10             | 100               | G1: 149758067   | 37799.8        | 5.39       | Structural |
| M11      | fibrinogen                | 162            | 12             | 100               | G1: 109075971   | 36492.6        | 5.61       | Various    |
| M12      | protease inhibitor        | 514            | 22             | 100               | G1: 109084715   | 46669.9        | 5.55       | Various    |
| M13      | actin                     | 528            | 23             | 100               | G1: 15277503    | 40194.1        | 5.55       | Structural |
| M14      | actin                     | 388            | 26             | 100               | G1: 62897671    | 41633.7        | 5.29       | Structural |
| M15      | tubulin                   | 455            | 25             | 100               | G1: 18088719    | 49639.9        | 4.75       | Structural |
| M16      | tubulin                   | 253            | 20             | 100               | G1: 23958133    | 49868.8        | 4.83       | Structural |
| M17      | actin                     | 226            | 15             | 100               | G1: 14250401    | 40978.9        | 5.56       | Structural |
| M18      | tubulin                   | 338            | 20             | 100               | G1: 11462722    | 47398.9        | 4.72       | Structural |
| M19      | actin                     | 491            | 19             | 100               | G1: 4885049     | 41991.9        | 5.23       | Structural |
| M20      | tubulin                   | 314            | 15             | 100               | G1: 10909648    | 42214.1        | 4.94       | Structural |
| M21      | protease inhibitor        | 108            | 11             | 100               | G1: 10396945    | 42214.1        | 5.08       | Various    |

aThe probability-based MOWSE score from MASCOT. bPeptides used for matching within the database. cReflects the probability that this same match would be made in a different database. dFrom the NCBI database searching mammals.
**Correction**

In Tables 1 and 2 of the manuscript originally published online, information for several proteins was incorrect. The tables have been corrected here.

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