Male strain A/J mice were exposed for six hours a day, five days a week for six months to either full tobacco smoke or to tobacco smoke drawn through a HEPA filter that removed more than 99% of particulate matter. After another four months in air, the animals were sacrificed and lung tumors were counted for calculation of multiplicities and incidences. Analysis of the chamber atmospheres showed that in the filtered smoke the concentrations of polycyclic aromatic hydrocarbons and tobacco smoke specific nitrosamines were reduced to from below 18% to even nondetectable levels of the original values measured in the unfiltered smoke. Aldehydes and other volatile organic compounds such as 1,3-butadiene, benzene, or acrolein were reduced to about 50 to 90% of the concentrations found in unfiltered smoke. Some potentially carcinogenic metals reached levels in filtered smoke ranging from 77% to less than 1% found in full smoke. The mice exposed to the filtered smoke atmosphere had practically identical lung tumor multiplicities and incidence as had the animals exposed to full smoke, significantly higher than in air exposed controls. Diets containing 0.5% \( \beta \)-carotene or 0.4% N-acetylcysteine afforded some chemoprevention. It was tentatively concluded that 1,3-butadiene might be an important contributor to lung tumorigenesis in this mouse model of tobacco smoke carcinogenesis.

Key Words: tobacco smoke; mice; strain A/J mice; lung tumors; 1,3-butadiene.

The first evidence that inhalation of tobacco smoke caused lung cancer was obtained in human studies, during the 1930’s and 1940’s in Germany and in the 1950’s in Great Britain and the U.S. (Doll, 1998). Evidence for the carcinogenicity of tobacco smoke in experimental animals was more difficult to obtain. Only a few inhalation studies were conclusive, the most convincing ones being large experiments with hamsters where tobacco smoke produced laryngeal tumors, but no tumors in the lower respiratory tract (IARC, 2004). On the other hand, skin painting studies in mice with tobacco smoke condensate produced early on evidence that the "tar" or particulate phase of tobacco smoke was carcinogenic. From then on, it was generally assumed that the carcinogenic potential of tobacco smoke was mostly, if not exclusively, associated with the particulate phase and its polycyclic aromatic hydrocarbons and nitrosamines.

Interestingly, some dissenting opinions were aired already in 1961: ‘Nearly all investigations on the tobacco problem have been started with the assumption that 1) the carcinogenic agents are produced only in the process of smoking and 2) that higher aromatic hydrocarbons, especially benzpyrene compounds, are the main causative substances. This however, seems to be a prejudice’ (Druckrey, 1961). Nevertheless, during the ensuing years considerable efforts were made to mitigate adverse health effects caused by smoking. It was anticipated that the development of low tar–low nicotine filter cigarettes would have a beneficial effect. Unfortunately, this does not seem to have been the case. Changes in cigarette design and manufacturing failed to benefit public health and no evidence of reduced risk could be found (NCI, 2001). On the other hand, Lee and Sanders (2004) concluded, after an extensive analysis of the available epidemiological literature, that the smoking of filter and low tar cigarettes carried 23 to 36% less risk than the smoking of unfiltered ones. They still considered the risk to develop lung cancer as being high.

While thus the putative role in carcinogenesis of the tobacco smoke tar phase, particularly of its polycyclic aromatic hydrocarbons and tobacco specific nitrosamines, was widely studied, much less attention was paid to the gas phase. The gas phase of cigarette smoke contains some 400 to 500 compounds (Hoffmann and Wynder, 1999). The bulk of gases are oxygen, nitrogen, carbon dioxide, and carbon monoxide. Some well-known carcinogens such as benzene, formaldehyde, acetaldehyde, and 1,3-butadiene are also present. Initial attempts to establish the carcinogenic potential of the gas phase were negative in mice; in hamsters, where full smoke produces laryngeal tumors, no such lesions were observed after gas phase inhalation only (IARC, 2004). However, in 1974 studies in Snell’s mice showed unequivocally that tobacco smoke gas phase was as active in producing benign and malignant lung tumors as was full tobacco smoke (Leuchtenberger and Leuchtenberger, 1971, 1974). This was later confirmed in strain A mice where tumor development was correlated with a partial analysis of the chamber atmospheres for polycyclic aromatic hydrocarbons and nitrosamines (Witschi et al., 1997b). In the present experiments,
we reproduced the previous findings and expanded analysis of chamber atmospheres. Analysis covered 100% of the gas phase carcinogens listed by Hoffmann and Wynder (1999), 70% of the carcinogenic inorganic compounds, 80% of carcinogenic polycyclic aromatic hydrocarbons present in more than 5 ng quantity per cigarette and four of the most important tobacco specific nitrosamines. In addition, we reexamined the effects of two putative chemopreventive agents on the carcinogenicity of cigarette smoke gas phase.

MATERIALS AND METHODS

Materials. Kentucky 2R4F reference cigarettes were obtained from the Tobacco Research Institute, University of Kentucky (Lexington, KY). They deliver on average 0.81 mg of nicotine and 11.6 mg of particulate material per cigarette. Diets AIN-93G and AIN-93M were purchased from DYETS (Bethlehem, PA). The β-carotene diet with gelatine beadlets containing 10% β-carotene was premixed by the supplier. Diets containing N-acetylcysteine (Sigma, St. Louis, MO) were prepared weekly by mixing in a Hobart blender. Test diets were stored at 4°C until used.

Animals. Male strain A/J mice, 5 to 6 weeks old were purchased from Jackson Laboratories, Bar Harbor, ME. After arrival, a few animals were selected at random for a standard rodent health surveillance screen by the Comparative Pathology Laboratory, UC Davis. No evidence for infectious disease (pathogenic agents) or presence of parasites or ova in pelage and cecum were reported. Due to the absence of significant macroscopic lesions in all animal examined, no histopathology was processed. Serology was negative for mouse hepatitis virus, Sendai virus, Reovirus type 3, pneumonia virus, parvo, ectromelia, and Mycoplasma pulmonis. After a two-week acclimatization period, the mice were assigned at random to the different treatment groups. They were housed in a conventional animal facility and killed 18 weeks later.

Exposure system. The tobacco smoke exposure system was the one used in our previous studies on the effects of full smoke and filtered smoke (Witschi et al., 1997a,b). Briefly, two Hinners-type stainless steel inhalation chambers (volume 0.44 m³) were used. In chamber 1, mice were exposed to a mixture of tobacco smoke. In chamber 2, the TS was drawn before entering the chamber through a HEPA filter that removed 99.8% of particulate material.

Experimental design. All protocols had been approved by the UC Davis Animal Use and Care Administrative Advisory Committee. Animals were exposed to full or filtered tobacco smoke 6 h a day, five days a week, for a total of 25 weeks. Animals in the full smoke chamber were kept on AIN-93G/M diet throughout. In the filtered smoke chamber, animals were divided into three groups: Ain-93G/M diet alone, AIN93-G/M diet containing 0.5% β-carotene and AIN-93G/M diet containing 0.4% N-acetylcysteine (NAC). Control animals, fed the same diets, were housed in inhalation chambers ventilated with filtered air. After 25 weeks, all animals were removed into a conventional animal facility and killed 18 weeks later.

Analysis of chamber atmospheres. Total suspended particulates, CO, and nicotine were measured in both chambers during the entire TS exposure period. CO concentrations were monitored every 30 min with a model 880 non-dispersive-infrared (NDIR) analyzer and total suspended particulate matter (TSP) was measured three times a day in the full smoke chamber and once a day in the filtered smoke chamber by weighing material collected on Pallflex filters. Nicotine was measured once a week by drawing 10 l of air through XAD4 cartridges and subsequent analysis of the extraction liquid with gas chromatography/thermonic selective detector.

Tissue preparation. After killing the animals by pentobarbital overdose, the lungs were manually expanded to total lung capacity through instillation of Telynesiczyk’s fluid. After at least 24 h of fixation, the number of tumor nodules visible on the lung surface was counted under a magnifying glass. The results were expressed as tumor incidence, i.e., percentage of animals with one or several lung tumors and as tumor multiplicity. Tumor multiplicity was calculated as average number of tumors per lung of all animals, including non-tumor bearing ones (Stoner and Shinkin, 1985). For histopathological evaluations, lung lobes containing tumors were dehydrated, embedded in paraffin, sectioned at 5 μm and stained with hematoxylin and eosin (H&E).

Statistical analysis. All numerical data were calculated as mean and SD or SEM. Comparisons of body weights and of tumor multiplicity between tobacco smoke-exposed and air-exposed controls were made by ANOVA followed by Tukey-Kramer’s or Dunnetts post test. Tumor incidences were compared using Fisher’s exact test. A p value of 0.05 or less was considered to be significant.

RESULTS

Exposure conditions for the two chambers are given in Table 1. The insertion of a HEPA filter between chamber 1 and chamber 2 effectively lowered the concentrations of total suspended particulate matter to 0.12% of chamber 1 and of nicotine to 16%, whereas CO was only reduced to 86%. In both chambers, the animals tolerated the tobacco smoke exposure well and only one death was observed during the exposure period. During the exposure to filtered and unfiltered smoke, the animals failed to gain weight as rapidly as did control animals kept in air.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Chamber 1 Full tobacco smoke</th>
<th>Chamber 2 Filtered tobacco smoke (gas phase)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Relative humidity</td>
<td>74 ± 8 (106)</td>
<td>73 ± 8 (103)</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>22 ± 1 (106)</td>
<td>22 ± 1 (106)</td>
</tr>
<tr>
<td>CO (ppm)</td>
<td>386 ± 48 (106)</td>
<td>331 ± 41 (106)</td>
</tr>
<tr>
<td>Nicotine (mg/m³)</td>
<td>9.6 ± 2.3 (20)</td>
<td>1.5 ± 0.3 (20)</td>
</tr>
<tr>
<td>TSP (mg/m³)</td>
<td>158 ± 26 (103)</td>
<td>0.2 ± 0.3 (106)</td>
</tr>
</tbody>
</table>

Note. All data given as means ± SD with number of determinations in parenthesis.
After the six months exposure, the body weights of the animals kept in full smoke was significantly lower than those of all other groups, indicating that inhalation of the gas phase appeared to be less stressful than exposure to full smoke, although it still reduced body weight gain to a significant extent in comparison with controls kept in air. At the end of the experiment, the weights of the animals exposed to filtered or unfiltered tobacco smoke were not significantly different from the weight of control animals kept in air throughout.

Tables 2 and 3 show the results of analysis of the chamber atmospheres for several of the known carcinogens in tobacco smoke. Filtering the smoke resulted in a reduction of several polycyclic aromatic hydrocarbons, among them benzo(a)pyrene, to below detection levels. The concentrations of others were reduced to less than 10%, with the exception of naphthalene and methyl-naphthalene which were reduced to only 69% resp. 51% of the full smoke atmosphere. Tobacco specific nitrosamines declined to 18% for 4-(methyl-nitrosamino)-1-(pyridyl)-1-butane (NNK) and to 5% for N-nitrosornicotine (NNN), with N-nitrosoanabasine (NAB) and N-nitrosoanatabine (NAT) becoming undetectable. Concentrations of the volatile organic compounds decreased much less and fell only to between 50 and 90% of the values measured in unfiltered smoke. The exception was formaldehyde where a bigger reduction was seen (to 17%). Metal concentrations were in general lower in filtered smoke compared to full smoke, but also were quite variable in the unfiltered smoke chamber where, with the exception of Cd and Ni, they were not detected in all samples.

Lung tumor data are shown in Table 4. In animals exposed to air and fed control diet or diets with β-carotene or NAC, lung tumor multiplicities ranged from 0.9 to 1.1. Full tobacco smoke produced a significant increase in both lung tumor multiplicities and lung tumor incidence. Filtered smoke had exactly the same effect: lung tumor multiplicities and incidences were practically identical to the ones found in animals exposed to the full tobacco smoke for six months and then removed into air. *Significantly different (p < 0.01) from animals exposed to filtered tobacco smoke; **significantly different (p < 0.01) from all other groups.

![FIG. 1. Weigh gain of mice exposed to unfiltered and filtered tobacco smoke for six months and then removed into air. *Significantly different (p < 0.01) from animals exposed to filtered tobacco smoke; **significantly different (p < 0.01) from all other groups.](image)

### Table 2

<table>
<thead>
<tr>
<th>Agent</th>
<th>Chamber 1</th>
<th>Chamber 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzo(a)pyrene (2A)</td>
<td>0.38 ± 0.12</td>
<td>N.D.*)</td>
</tr>
<tr>
<td>Benzo(c)pyrene (3)</td>
<td>0.31 ± 0.10</td>
<td>N.D.*)</td>
</tr>
<tr>
<td>Benzo(α)antracene (2A)</td>
<td>0.73 ± 0.47</td>
<td>N.D.*)</td>
</tr>
<tr>
<td>Benzo(k)fluoranthene (2B)</td>
<td>0.14 ± 0.04</td>
<td>N.D.*)</td>
</tr>
<tr>
<td>Chrysene (3)</td>
<td>2.02 ± 0.54</td>
<td>N.D.*)</td>
</tr>
<tr>
<td>Naphthalene (2B)</td>
<td>255 ± 43</td>
<td>177 ± 32 (69%)$^b$</td>
</tr>
<tr>
<td>2-Methyl-naphthalene (N.E.)</td>
<td>192 ± 46</td>
<td>98 ± 25 (51%)</td>
</tr>
<tr>
<td>Fluorene (3)</td>
<td>9.13 ± 0.05</td>
<td>0.82 ± 0.04 (9%)</td>
</tr>
<tr>
<td>Phenanthrene (3)</td>
<td>12.08 ± 3.53</td>
<td>0.95 ± 0.19 (8%)</td>
</tr>
<tr>
<td>Anthracene (3)</td>
<td>4.13 ± 1.22</td>
<td>0.31 ± 0.07 (8%)</td>
</tr>
<tr>
<td>Pyrene (3)</td>
<td>2.27 ± 0.61</td>
<td>0.16 ± 0.25 (7%)</td>
</tr>
<tr>
<td>Fluoranthene (N.E.)</td>
<td>2.99 ± 0.78</td>
<td>0.05 ± 0.01 (2%)</td>
</tr>
<tr>
<td>NNK (4-(methyl-nitrosamino)-1-(3-pyridyl)-1-butane) (2B)</td>
<td>7.12 ± 2.16</td>
<td>1.30 ± 0.43 (18%)</td>
</tr>
<tr>
<td>NNN (N-nitrosornicotine) (2B)</td>
<td>1.89 ± 0.42</td>
<td>0.09 ± 0.02 (5%)</td>
</tr>
<tr>
<td>NAT (N-nitrosoanabasine) (3)</td>
<td>0.85 ± 0.22</td>
<td>N.D.*)</td>
</tr>
<tr>
<td>NAT (N-nitrosoanatabine) (N.E.)</td>
<td>0.16 ± 0.04</td>
<td>N.D.*)</td>
</tr>
</tbody>
</table>

**Note.** All data given as $\mu g/m^3 \pm SD$; *n = 5 throughout. Numbers and letters in parenthesis behind each chemical’s name denote IARC carcinogen classification; N.E., not evaluated.

$^a$N.D. = not detected; reporting limit 0.008 $\mu g/m^3$.

$^b$Percentage of concentration in chamber 1.

$^c$N.D. = not detected; detection limit 0.02 $\mu g/m^3$ for NAB and 0.03 $\mu g/m^3$ for NAT.

### Table 3

<table>
<thead>
<tr>
<th>Agent</th>
<th>Chamber 1</th>
<th>Chamber 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,3-Butadiene (2A)</td>
<td>3620 ± 545</td>
<td>2860 ± 971 (79%)$^a$</td>
</tr>
<tr>
<td>Acetaldehyde (2B)</td>
<td>6900 ± 2680</td>
<td>3586 ± 3943 (52%)</td>
</tr>
<tr>
<td>Acrolein (3)</td>
<td>3860 ± 1240</td>
<td>3480 ± 1555 (90%)</td>
</tr>
<tr>
<td>Benzene (1)</td>
<td>3480 ± 295</td>
<td>2780 ± 811 (77%)</td>
</tr>
<tr>
<td>Formaldehyde (1)</td>
<td>354 ± 68</td>
<td>62 ± 43 (18%, ND 1)$^b$</td>
</tr>
<tr>
<td>Antimony (trioxide: 2B)</td>
<td>0.94 ± 0.84 (ND 1)</td>
<td>1.53 ± 1.77 (162%; ND 2)</td>
</tr>
<tr>
<td>Arsenic (1)</td>
<td>0.14 ± 0.32</td>
<td>0.06 ± 0.9 (8%; ND 3)</td>
</tr>
<tr>
<td>Cadmium (1)</td>
<td>1.96 ± 0.95</td>
<td>0.36 ± 0.64 (18%; ND 2)</td>
</tr>
<tr>
<td>Chromium (hexavalent: 1)</td>
<td>0.42 ± 0.56 (ND 2)</td>
<td>0 (ND 5)</td>
</tr>
<tr>
<td>Lead (2B)</td>
<td>0.57 ± 0.48 (ND 1)</td>
<td>0.44 ± 0.88 (77%; ND 2)</td>
</tr>
<tr>
<td>Mercury (3)</td>
<td>1.12 ± 0.91 (ND 1)</td>
<td>0.01 ± 0.091 (1%; ND 3)</td>
</tr>
<tr>
<td>Nickel (1)</td>
<td>1.33 ± 0.90</td>
<td>0.29 ± 0.53 (22%)</td>
</tr>
<tr>
<td>Selenium (3)</td>
<td>0.19 ± 0.16 (ND 1)</td>
<td>0.01 ± 0.01 (5%; ND 4)</td>
</tr>
</tbody>
</table>

**Note.** All data given as $\mu g/m^3 \pm SD$; *n = 5 throughout except for Sb, Hg, and Pb, where *n = 4. Numbers and letters in parenthesis behind each chemical’s name denote IARC carcinogen classification.

$^a$Percentage of concentration in chamber 1.

$^b$Denotes how many out of the five samples were below the detection limit.
Tobacco smoke, control diet 1.8
6
Air, NAC diet 1.1
6
b
ing rather than by inhalation. Since the gas phase has as much
the animals and carcinogens are absorbed orally through groom-
b
umbers of several carcinogens in the chamber atmospheres before
Although there were substantial differences in the concentra-
carcinogens seems unlikely.
Histopathological evaluation of more than 50% of all the
grossly visible tumors revealed in about one-fourth signs of
malignancy: carcinomatous foci arising within an adenoma or
malignant features through the entire tumor, as described in
detail before (Witschi et al., 1997a,b). The tumor type distribu-
tion was also similar to previous findings where control animals
had 20 to 23% and tobacco smoke exposed animals 13 to 17%
malignant tumors There was no indication that the chemopre-
vention regimens would result in a shift in tumor type. Such
shifts are usually seen in studies that examine progression of
lesions in the presence of chemopreventive agents (Estensen
et al., 2004), an experimental design not adopted in the current
study.
Our observations raise the question what agents are respon-
sible for tumor development in the lungs of A/J mice. The gen-
eral consensus appears to be that, at least in man, polycyclic
aromatic hydrocarbons, particularly benzo(a)pyrene, and
tobacco smoke specific nitrosamines are the main causative
agents of lung cancer (Hecht, 1999). By inference, it is thought
that this applies to animals, too. Our data show that, with the
exception of naphthalene and methyl-naphthalene, many poly-
cyclic aromatic hydrocarbons were no longer detectable in the
filtered smoke. Others were reduced to concentrations of less
than 10% of the full smoke atmosphere. This makes it unlikely
that PAHs were major contributors. Concentrations of NNK and
NNN also fell below 20%. We have previously discussed how
dosimetric calculations do not allow us to assume that NNK is a
major carcinogen in strain A/J mice exposed to tobacco smoke
(Witschi et al., 1997b).

**DISCUSSION**

The present results confirm earlier findings (Leuchtenberger
and Leuchtenberger, 1971, 1974; Witschi et al., 1997b).
Although there were substantial differences in the concentra-
tions of several carcinogens in the chamber atmospheres before
and after HEPA filtration of cigarette smoke (a mixture of 11%
cigarette mainstream and 89% sidestream smoke), tumor
response was not affected. This might have some implications
for experimental design. Most studies demonstrating enhanced
lung tumor development by tobacco smoke and underlying
mechanisms have been conducted by whole-body exposure of
mice (De Flora et al., 2003). It has on occasion been suggested
that under such conditions smoke particles deposit on the fur of
the animals and carcinogens are absorbed orally through groom-
ing rather than by inhalation. Since the gas phase has as much
carcinogenic activity as has whole smoke, oral exposure to
carcinogens seems unlikely.

**TABLE 4**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Lung tumor multiplicity(^a)</th>
<th>Lung tumor incidence(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Air/control diet</td>
<td>0.9 ± 0.2 (24)</td>
<td>13/24 (54%)</td>
</tr>
<tr>
<td>Air, β-carotene diet</td>
<td>1.0 ± 0.2 (14)</td>
<td>11/14 (79%)</td>
</tr>
<tr>
<td>Air, NAC diet</td>
<td>1.1 ± 0.3 (13)</td>
<td>8/13 (62%)</td>
</tr>
<tr>
<td>Tobacco smoke, control diet</td>
<td>1.8 ± 0.2 (25(^f))</td>
<td>24/25 (96%)(^f)</td>
</tr>
<tr>
<td>Filtered smoke, control diet</td>
<td>2.1 ± 0.3 (25(^f))</td>
<td>23/25 (92%)(^f)</td>
</tr>
<tr>
<td>Filtered smoke, β-carotene diet</td>
<td>1.3 ± 0.2 (26(^d))</td>
<td>19/26 (73%)</td>
</tr>
<tr>
<td>Filtered smoke, NAC diet</td>
<td>1.3 ± 0.2 (30(^e))</td>
<td>22/30 (72%)</td>
</tr>
</tbody>
</table>

\(^a\)Average number of tumors per lung, including nontumor bearing animals. Data are given as means ± SEM, with the number of animals in parenthesis.
\(^b\)Number of tumor bearing animals per total number of animals at risk.
\(^c\)Significantly higher (\( p < 0.05\)) when compared to animals kept in air.
\(^d\)Significantly lower (\( p < 0.05\)) when compared to animals exposed to filtered smoke.

**TABLE 5**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total number of tumors(^a)</th>
<th>Benign</th>
<th>Malignant</th>
<th>% Malignant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Air, control diet</td>
<td>13 (59%)</td>
<td>10</td>
<td>3</td>
<td>23%</td>
</tr>
<tr>
<td>Air, β-carotene, diet</td>
<td>9 (64%)</td>
<td>7</td>
<td>2</td>
<td>22%</td>
</tr>
<tr>
<td>Air, NAC, diet</td>
<td>10 (71%)</td>
<td>7</td>
<td>3</td>
<td>30%</td>
</tr>
<tr>
<td>Tobacco smoke, control diet</td>
<td>23 (51%)</td>
<td>21</td>
<td>2</td>
<td>9%</td>
</tr>
<tr>
<td>Filtered smoke, control diet</td>
<td>30 (57%)</td>
<td>23</td>
<td>7</td>
<td>23%</td>
</tr>
<tr>
<td>Filtered smoke, β-carotene diet</td>
<td>21 (62%)</td>
<td>14</td>
<td>7</td>
<td>33%</td>
</tr>
<tr>
<td>Filtered smoke, NAC diet</td>
<td>23 (59%)</td>
<td>18</td>
<td>5</td>
<td>22%</td>
</tr>
<tr>
<td>All air exposed</td>
<td>32 (71%)</td>
<td>23</td>
<td>8</td>
<td>25%</td>
</tr>
<tr>
<td>All smoke exposed</td>
<td>97 (75%)</td>
<td>76</td>
<td>21</td>
<td>22%</td>
</tr>
</tbody>
</table>

\(^a\)Total number of tumors evaluated; percentage of total tumors in group in parenthesis.

*Note. Histological diagnosis of tumors were divided into two groups: benign tumors (adenomas) and malignant tumors (adenomas with carcinomatous foci or frank adenocarcinomas).*
Potentially carcinogenic volatile organic compounds (IARC classes 1, 2a, and 2b) were reduced much less in the filtered smoke. Data on the pulmonary carcinogenicity in mouse lungs of some of these agents are available and may be compared to our exposure concentrations as measured in filtered smoke. When strain A/J mice inhaled for 20 weeks an atmosphere of 157 mg/m³ of naphthalene (IARC 2B), only 11% of the mice developed lung tumors and multiplicities were 0.4 (Adkins et al., 1986). In a two-year study with B6C3F1 mice, the same concentration of naphthalene produced only a weak tumorigenic response (Abdo et al., 1992). The level of naphthalene measured in our study (0.177 mg/m³) was thus far below the one expected to produce tumors. Similar considerations apply to some of the other potentially carcinogenic volatiles (IARC class 1 and 2) such as acetaldehyde, formaldehyde, and benzene. After a 16-week exposure of CBA mice to an airborne benzene concentration of 957 mg/m³, 36% developed 18 months later pulmonary adenomas (Farris et al., 1993). After long term exposure of mice to 17.5 mg/m³ to formaldehyde, only nasal tumors were found (Kerns et al., 1983). No tumor data are available for mice following inhalation of acetaldehyde. In rats, exposure for 28 months to 1350 mg/m³ caused only nasal tumors (Woutersen et al., 1986). The airborne concentrations of these agents measured in our experiments did not nearly approximate such values.

As far as acrolein is concerned (IARC class 3), oral administration to rats or mice failed to produce tumors (IARC, 1995). Considering metals, inhaled Cd is not a pulmonary carcinogen in mice (Oberdorster et al., 1994) and oral or subcutaneous exposure to Cd compounds have given conflicting results (Waalkes and Rehm, 1994). No Cr was detected in filtered smoke. Data on the pulmonary carcinogenicity in mouse lungs of these different hypotheses.

There is one agent that by itself might account for the carcinogenicity of full tobacco smoke or filtered tobacco smoke in strain A/J mice: 1,3-butadiene. A two-year inhalation study in C57Bl/6 × C3H F1 mice found increased neoplasms of the lung after exposure to 1,3-butadiene at concentrations as low as 13.8 mg/m³ (6.25 ppm) (Melnick et al., 1990). Strain A/J mice are more prone to develop chemically induced alveolar tumors than other strains and this probably also applies to 1,3-butadiene. The two exposure concentrations that yielded a tumorigenic response in our experiment are reasonably close to each other (3.6 mg/m³ for full smoke or 2.9 mg/m³ for filtered smoke) and not much lower than 13.8 mg/m³. 1,3-Butadiene itself has been shown to inhibit its own metabolism to the highly active and carcinogenic diepoxide (Dahl and Henderson, 2000). It is conceivable that other organic volatiles might interfere with 1,3-butadiene metabolism. Partial removal of them by the filter might thus enhance butadiene activation and carcinogenesis. Since mice are much more sensitive to the toxic and carcinogenic action of 1,3-butadiene than are rats and even primates (Henderson, 2001), it is difficult to extrapolate lung tumor data induced by cigarette smoke from mouse to man based on 1,3-butadiene. Furthermore there is no evidence that 1,3-butadiene produces lung cancer in occupationally exposed humans (IARC, 1999). Nevertheless, it is interesting to note that 1,3-butadiene present in tobacco smoke has been shown to enhance the development of arteriosclerotic plaques in animal models of arteriosclerosis, whereas the tar phase does not seem to have such an effect (Penn et al., 1996; Penn and Snyder, 1996).

β-Carotene and NAC are two agents who, among other activities, have strong antioxidant properties. In many experimental systems they have chemopreventive action and are capable to significantly reduce tumor development. Multiple mechanistic studies with biomarkers of effect have suggested that both agents should provide chemoprevention against tobacco smoke (De Flora et al., 1999, 2001). Experiments to show this have not been fully successful. In strain A mice, β-carotene and NAC had no chemopreventive action against full cigarette smoke (Obermueller-Jevic et al., 2002; Witschi et al., 1998). In SWR mice, a small, but statistically not significant effect of NAC was observed (de Flora et al., 2003). The protective effect of both agents against the gas phase was thus unexpected. It would seem reasonable to assume that the oxidative stress placed onto the lung by filtered tobacco smoke is as intense as it would be with full smoke. If the main carcinogenic stimulus in filtered smoke was oxidative stress, a plausible explanation for the protective effects of the two antioxidants could be postulated. However, this makes it difficult to explain why neither β-carotene nor NAC were effective (in strain A mice) against full tobacco smoke. It has been discussed before that two main classes of carcinogens found in full smoke, polycyclic aromatic hydrocarbons and nitrosamines, might not be as important contributors to carcinogenesis as is generally assumed. This allows the speculation that the full smoke contains some other as of yet unidentified carcinogen(s) that could overcome any beneficial effects of β-carotene or NAC. It also has been postulated that polycyclic aromatic hydrocarbons found in tobacco tar act mostly as promoting agents (Rubin, 2001). Such processes could counteract or even nullify critical protective mechanisms of β-carotene and NAC against full smoke. It is also conceivable that some elements of full tobacco smoke may inactivate or interfere with the bioavailability of the two chemopreventive agents. Initial studies with cell culture systems will allow to test these different hypotheses.

As surprising as they are, the present findings probably will not have much practical impact on chemoprevention of lung cancer in man, given the disappointing results of major clinical trials (Omenn, 1998; van Zandwijk et al., 2000). On the other hand, both β-carotene and NAC have been found to mitigate biomarkers of exposure and possibly of effect in phase I and phase III clinical trials (de Flora et al., 2003; IARC, 1998). Given
the right circumstances, β-carotene and NAC may yet have some beneficial effects. Our observations may lead to future experiments where mechanistic events that relate to chemoprevention can be separated from events that are inconsequential.

In conclusion, experimental evidence shows that the gas phase of tobacco smoke, containing both combustion products and volatile agents extracted from the tobacco, produces lung tumors in strain A mice. In human smokers, the reduction of PAH and TSN in cigarettes were not necessarily as effective in lowering lung cancer rates as was originally hoped for. The evaluation of cytotoxic, mutagenic, and carcinogenic properties of cigarette mainstream and sidestream smoke or of potentially less active cigarettes are often done with cigarette smoke condensate (Eclipse Expert Panel, 2000; IARC 2004; Stratton et al., 2001). The present investigation suggests that additional evaluations should include the gas phase of tobacco smoke.

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REFERENCES


