Chemoprevention of tobacco-smoke lung carcinogenesis in mice after cessation of smoke exposure

Hanspeter Witschi1, Dale Uyeminami, Dexter Moran and Imelda Espiritu

Institute of Toxicology and Environmental Health and Department of Molecular Biosciences, School of Veterinary Medicine, University of California, One Shields Avenue, Davis, CA 95616, USA

1To whom correspondence should be addressed
Email: hrwitschi@ucdavis.edu

Male strain A/J mice were exposed for 6 h per day, 5 days per week to a mixture of 89% cigarette sidestream smoke and 11% mainstream smoke. Total suspended particulate concentrations were 137 mg/m3. In experiment 1, animals were exposed for 5 months to tobacco smoke and given a 4 month recovery period in air. Lung tumor multiplicity was 2.4 and incidence 89%. Animals exposed to filtered air had 1.0 tumor per lung (65% incidence). In animals kept for 5 months in smoke, removed into air and then fed a diet containing a mixture of myoinositol and dexamethasone, tumor multiplicity was 1.0 and incidence was 62%. These values were significantly (P < 0.01) lower than in animals exposed to smoke and identical to values seen in controls. In animals fed a diet containing 250 mg/kg each of phenethyl isothiocyanate and benzyl isothiocyanate during the entire 9 months, lung tumor multiplicity was 2.1 and incidence 96%, not significantly different from animals exposed to smoke and fed control diet. In experiment 2, animals were exposed for 5 months to smoke, followed by a 4 month recovery period in air and were fed during the entire period a diet containing either D-limonene or 1,4-phenylenebis(methylene)selenoisocyanate (p-XSC). In animals exposed to tobacco smoke and fed control diet, lung tumor multiplicity was 2.8, whereas in the animals fed D-limonene it was 2.6 and in the animals fed p-XSC it was 2.4. The differences to the controls were statistically not significant. It was concluded that myoinositol–dexamethasone successfully prevents the development of tobacco smoke-induced lung tumors even if administered when the animals have ‘quit’ smoking. On the other hand, agents otherwise shown to prevent lung tumor formation following administration of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone or benzo[a]pyrene were ineffective against tobacco smoke.

Introduction

The incidence and death rates of lung cancer in the USA have decreased in men. In females the annual increase appears to have slowed down. There is some concern, however, that changing smoking habits of teens and the substitution of cigarettes by cigars may possibly reverse this trend (1). In other countries of the world, particularly in Asia, the lung cancer epidemic continues to spread and tobacco eventually will kill millions of people (2–4). Complete cessation of smoking would be the most effective way to prevent this burden on the public health. It is recognized that a substantial number of smokers is unable to quit. Chemoprevention might be a possible way to mitigate the impact of smoking (5–10). Chemopreventive agents, such as drugs or naturally occurring constituents of the diet, might provide some protection to active smokers. Perhaps more importantly, they might help to reduce further the risk of developing lung cancer in individuals who have quit smoking or in individuals exposed to tobacco smoke in their environment.

During the last few years, a substantial number of agents has been examined for their possible effects on lung cancer chemoprevention. Practically all experiments used animals treated with lung-tumor-producing chemicals, most often tobacco-specific nitrosamines or polycyclic aromatic hydrocarbons (PAHs). Lung tumors in strain A/J mice have become the preferred test system. (11). These tumors resemble in many ways human lung adenocarcinoma (12), a tumor that during the past decades has been found with increasing frequency in man (13,14). The experimental model is attractive as a screening tool because a substantial overlap exists between man and mouse in the genetic alterations thought to be responsible for lung tumorigenesis (15). Already it has been firmly established that lung tumorigenesis in strain A/J mice can effectively be prevented by a large variety of chemicals. The carcinogenic action of tobacco-specific nitrosamines or PAHs can be counteracted by isothiocyanates (16), non-steroidal anti-inflammatory agents (17), green and black tea (18), organoselenium compounds (19), glucocorticoid hormones (20,21), perillyl alcohol (22) and others.

On occasion, some doubts have been raised whether studies with model compounds may substitute exposure to the full and complex mixture of tobacco smoke (15,23). We have addressed this problem in a series of experiments in which we examined the effects of chemopreventive agents on lung tumors induced in strain A/J mice by a mixture of 89% sidestream smoke and 11% mainstream smoke (24,25). So far we found that green tea, acetylsalicylic acid and N-acetyl cysteine were ineffective. Phenethyl isothiocyanate (PEITC) had a possible marginal, but statistically not significant effect. The only agent that was found to be highly effective against tobacco smoke was a combination of dietary myoinositol and dexamethasone (25). In the present investigation we examined three additional chemopreventive agents, D-limonene, 1,4-phenylenebis (methylene) selenoisocyanate (p-XSC) and a mixture of PEITC and benzyl isothiocyanate (BITC). We also examined whether it would be possible to achieve chemoprevention with myoinositol–dexamethasone in ex-smokers, i.e. in mice that were only fed the chemopreventive diet once they were removed from the smoke atmosphere.

Materials and methods

Animals

Male strain A/J mice, 6–8 weeks old, were purchased from Jackson Laboratories, Bar Harbor, ME. Randomly chosen animals were sent to the Comparative

Abbreviations: BITC, benzyl isothiocyanate; NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; p-XSC, 1,4-phenylenebis(methylene)selenoisocyanate; PEITC, phenethyl isothiocyanate.
Pathology Laboratory, UC Davis, for a standard rodent health surveillance screen. No evidence for infectious disease (pathogenic agents) or presence of parasites or ova in pelage and cecum were reported. Histopathology was not processed since no significant lesions were noted. Serology was negative for mouse hepatitis virus, Sendai virus, Reovirus type 3, pneumonia virus, parvo, ectromelia and mycoplasma pulmonis. The animals were housed, four to a cage, in polypylene cages with tightly fitting wire screen lids on conventional bedding material. At all times during the experiment, including during smoke exposure, water and the test diets were provided ad libitum. The animals were monitored daily and weighed weekly.

Materials
Kentucky 1R4F reference cigarettes were purchased from the Tobacco Research Institute, University of Kentucky, Lexington, KY. PEITC, BITC, myoinositol, dexamethasone, d-limone and corn oil were obtained from Sigma Chemical Co., St. Louis, MO. Anhydrous acetonitrile and α,ω-debromo-p-xylene were obtained from Aldrich Chemicals, Milwaukee, WI, and KSeCN from Acracos Organics, Fisher Scientific, Pittsburgh, PA. All reagents were of the highest available commercial grade. The AIN-76A test diet was purchased in powdered form from Dyets, Bethlehem, PA, and consisted of 20% casein, 0.3% niacin, 15% corn starch, 5.5% sucrose, 5% cellulose, 3.5% mineral mix, 1% vitamin mix and 0.2% choline bitartrate.

The organoselenium compound, p-XSC, was synthesized as has been described by et al. (26). After recrystallization, the melting point of the final product was 156°C. Purity was >99.9% based on reverse-phase HPLC analysis. The identity of the product was confirmed by both mass spectrometry and NMR spectroscopy. Major ions in the El mass spectrum were at 150 (M-SeCN) and 104 (M-SeC). Isotopic abundance at 313–317 was consistent with the presence of 25e. The NMR spectrum, taken in CDC13, yielded a singlet at 7.41 p.p.m. (related to aromatic protons) and another singlet at 4.3 p.p.m. (related to methane protons).

Test diets containing the chemopreventive agents were prepared fresh every other week by adding the appropriate amounts of the ingredients plus 50 ml of corn oil per kg of diet. The diets were mixed thoroughly in a Hobart blender. The amounts of the chemopreventive agents added to 1 kg of diet were: PEITC and BITC, 250 mg for each agent; d-limone, 6.5 g; myoinositol–dexamethasone, 10 g and 0.5 mg, respectively; p-XSC, 20 mg (i.e. 10 p.p.m. of Se). All diets were stored at 4°C until use.

Experimental design
After an acclimatization period, the 10-week-old animals were assigned at random to the different treatment groups. Two experiments were conducted. In experiment 1, three groups were formed: animals fed the control diet throughout while being exposed for 5 months to tobacco smoke, followed by a 4 month recovery period in air. A second group was placed on the control diet while being exposed to tobacco smoke. The moment they were removed into air, they were fed the myoinositol–dexamethasone diet until the end of the experiment. A third group was fed the PEITC/BITC diet through the entire experiment. Control groups were kept on the identical dietary schedule, except that they were exposed to filtered air.

In experiment 2, animals were exposed for 5 months to tobacco smoke, followed by a 4 month recovery period in air and fed either a control diet or a diet containing d-limone or p-XSC during the entire 9 months. Controls received the same diets but were exposed to filtered air. In this experiment, a positive control group was included to ascertain that d-limone and p-XSC were as effective in our laboratories as had been described previously by others. Animals were at random assigned to three groups and fed control diet AIN-76A, d-limone or p-XSC diet. Two weeks later, all animals received four i.p. injections of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) at a dose of 50 mg/kg in weekly intervals. Feeding of the three diets then continued until 4 months after the last NNK injection when the animals were killed.

In all experiments, the animals were placed, within their cages, into stainless steel inhalation chambers or into chambers of similar size ventilated with filtered air. In this experiment, all animal cages were periodically rotated so that each case occupied at least one possible location within the exposure chambers. A third group was fed the PEITC/BITC diet through the entire experiment. Control groups were kept on the identical dietary schedule, except that they were exposed to filtered air.

In experiment 2, animals were exposed for 5 months to tobacco smoke, followed by a 4 month recovery period in air and fed either a control diet or a diet containing d-limone or p-XSC during the entire 9 months. Controls received the same diets but were exposed to filtered air. In this experiment, a positive control group was included to ascertain that d-limone and p-XSC were as effective in our laboratories as had been described previously by others. Animals were at random assigned to three groups and fed control diet AIN-76A, d-limone or p-XSC diet. Two weeks later, all animals received four i.p. injections of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) at a dose of 50 mg/kg in weekly intervals. Feeding of the three diets then continued until 4 months after the last NNK injection when the animals were killed.

In all experiments, the animals were placed, within their cages, into stainless steel inhalation chambers or into chambers of similar size ventilated with filtered air. All chambers were kept on a 12 h light–dark cycle and controlled for temperature and humidity. Exposure to tobacco smoke was 6 h per day, 5 days per week. Tobacco smoke concentration was gradually increased during the first 5 weeks until the final target concentration of ~140 mg/m3 of total suspended particulates (TSP) was reached (Table I). After 5 months, all mice were removed from the tobacco smoke or filtered air chambers to a conventional animal holding facility with controlled environment (20–21°C, 40–70% relative humidity and 12 h light–dark cycle).

Exposure system
The tobacco smoke exposure system was identical to the one described by Teague et al. (27) and used before (25,28). Briefly, mice were exposed to a mixture of 89% sidestream and 11% mainstream smoke generated from burning Kentucky 1R4F reference cigarettes. Chamber atmospheres were monitored for nicotine, CO and TSP (Table I). Within the exposure chambers, all animal cages were periodically rotated so that each case occupied at least one possible location within the exposure chambers.

Tissue preparation
Animals were killed by pentobarbital overdose. For analysis of tumor incidence and multiplicity, the lungs were manually expanded to inspiratory volume by intrathoracic instillation of Tellyesnicki’s fluid and fixed for at least 24 h. The number of tumor nodules visible on the lung surface was counted and the results were expressed as tumor incidence, i.e. percentage of animals with one or several lung tumors, and as tumor multiplicity, the average number of tumors per lung, including non-tumor bearing animals. All procedures have been described in detail before (28,29).

Statistical analysis
All numerical data were calculated as means and SD or SE. Comparisons of tumor multiplicity between tobacco-smoke-exposed and air-exposed controls were made by parametric and non-parametric ANOVA, followed by the Turkey–Kramer multiple comparison post-test. Tumor incidences were compared using the exact test. A P-value <0.05 was considered to be significant.

Results
Exposure to tobacco smoke did not cause mortality, regardless of the diet fed to the animals. However, there were some differences in weight gain (Figure 1). In experiment 1 we found that feeding the myoinositol–dexamethasone diet, initiated after month 5 of the experiment, slowed down additional weight gain. In animals kept in air, the final weight was 94% of that reached by animals fed control diet throughout the experiment; the difference was statistically not significant. Animals that
Chemoprevention of carcinogenesis in mice

had been kept in tobacco smoke for the first 5 months of the experiment and that were placed on the myoinositol–dexamethasone diet (10 g and 0.5 mg/kg diet, respectively, fed from removal from tobacco smoke until the end of the experiment); PEITC/BITC, 250 mg/kg each per kg of diet and fed during the entire experiment.

In experiment 2 it was found that p-XSC had a substantial


dsignificant effect on weight gain in both animals exposed to tobacco smoke and in animals kept in filtered air (Figure 2). At the end of the experiments, body weights were only 85% of controls, a statistically significant reduction (P < 0.01). On the other hand, D-limonene was well tolerated. Evaluation of the carcinogenic response showed that D-limonene or the organoselenium compound p-XSC had no significant effect on lung tumor multiplicity or incidence. The two agents produced no significant effect on lung tumor multiplicity or incidence in animals kept in air.

In a previous experiment we had shown that in our laboratory PEITC was highly effective against NNK-induced lung tumors in strain A mice (24). It was important to make sure that D-limonene and particularly p-XSC, a compound synthesized in our laboratory, were as effective as had been described previously by others (19,21). Table IV shows that both agents significantly suppressed NNK carcinogenesis; D-limonene

### Table II. Lung tumor data from experiment no. 1

<table>
<thead>
<tr>
<th>Treatmenta</th>
<th>Lung tumor multiplicityb</th>
<th>Lung tumor incidencec</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tobacco-smoke-exposed animals</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AIN-76A</td>
<td>2.4 ± 0.3 (28)</td>
<td>25/28 (89%)</td>
</tr>
<tr>
<td>Myo</td>
<td>1.0 ± 0.2 (26)d</td>
<td>16/26 (62%)e</td>
</tr>
<tr>
<td>PEITC/BITC</td>
<td>2.1 ± 0.2 (27)</td>
<td>26/27 (96%)</td>
</tr>
<tr>
<td>Air-exposed animals</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AIN-76A</td>
<td>1.0 ± 0.1 (54)d</td>
<td>35/54 (65%)f</td>
</tr>
<tr>
<td>Myo</td>
<td>1.0 ± 0.2 (29)d</td>
<td>17/29 (59%)f</td>
</tr>
<tr>
<td>PEITC/BITC</td>
<td>1.1 ± 0.2 (28)d</td>
<td>19/28 (68%)</td>
</tr>
</tbody>
</table>

aAIN-76A, control diet; Myo, myoinositol–dexamethasone diet (10 g and 0.5 mg/kg diet, respectively, fed from removal from tobacco smoke until the end of the experiment); PEITC/BITC, 250 mg/kg each per kg of diet and fed during the entire experiment.

bAverage number of tumors per lung, including non-tumor bearing animals.

cData are given as means ± SE with the number of animals in parentheses.

dNumber of tumor-bearing animals per total number of animals at risk.

Fig. 2. Weight gain in animals exposed to tobacco smoke (TS) for 5 months and then allowed to recover in air for 4 months (closed symbols) and in animals kept in filtered air (FA; open symbols). The animals were fed t-limonene or p-XSC diet during the entire 9 months of the experiment. Data with an asterisk to their right are significantly different from animals fed the control diet and kept in air (P < 0.01 by ANOVA).

### Table III. Lung tumor data from experiment no. 2

<table>
<thead>
<tr>
<th>Treatmenta</th>
<th>Lung tumor multiplicityb</th>
<th>Lung tumor incidencec</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tobacco-smoke-exposed animals</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AIN-76A</td>
<td>2.8 ± 0.2 (38)</td>
<td>38/38 (100%)</td>
</tr>
<tr>
<td>D-limonene</td>
<td>2.6 ± 0.4 (36)</td>
<td>34/36 (94%)</td>
</tr>
<tr>
<td>p-XSC</td>
<td>2.4 ± 0.3 (38)</td>
<td>34/38 (89%)</td>
</tr>
<tr>
<td>Air-exposed animalsd</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AIN-76A</td>
<td>0.9 ± 0.2 (30)</td>
<td>18/30 (60%)</td>
</tr>
<tr>
<td>D-limonene</td>
<td>1.1 ± 0.2 (29)</td>
<td>23/29 (79%)</td>
</tr>
<tr>
<td>p-XSC</td>
<td>1.2 ± 0.3 (28)</td>
<td>18/28 (64%)</td>
</tr>
</tbody>
</table>

aAIN-76A, control diet; D-limonene, 6.3 g/kg of diet; p-XSC, 20 mg/kg diet (10 p.p.m. Se). Both diets were fed to the animals during the entire duration of the experiment.

bAverage number of tumors per lung, including non-tumor-bearing animals.

dataNumber of tumor-bearing animals per total number of animals at risk.

cData are given as means ± SE with the number of animals in parentheses.

dNumber of tumor-bearing animals per total number of animals at risk.

### Table IV. Positive controls

<table>
<thead>
<tr>
<th>Treatmenta</th>
<th>Lung tumor multiplicityb</th>
<th>Lung tumor incidencec</th>
</tr>
</thead>
<tbody>
<tr>
<td>AIN-76A and NNK</td>
<td>13.0 ± 1.2 (21)</td>
<td>21/21 (100%)</td>
</tr>
<tr>
<td>D-limonene and NNK</td>
<td>7.8 ± 0.8 (21)d</td>
<td>21/21 (100%)</td>
</tr>
<tr>
<td>p-XSC and NNK</td>
<td>2.6 ± 0.4 (22)d</td>
<td>21/22 (95%)</td>
</tr>
</tbody>
</table>

aAll animals placed on diet and, beginning 2 weeks later, given 4 weekly i.p. injections of 50 mg/kg of NNK; animals were killed 4 months after the last injection.

bAverage number of tumors per lung, including non-tumor-bearing animals.

cData are given as means ± SE with the number of animals in parentheses.

dNumber of tumor-bearing animals per total number of animals at risk.

eSignificantly different from animals fed AIN-76a diet, as determined by Fisher’s exact test.


diminished lung tumor multiplicity by 40% and p-XSC was highly effective in decreasing lung tumor multiplicity by 80%.

In summary, the results of the two experiments showed that three of the four chemopreventive agents had some effects on weight gain, but that only one of them, a mixture if myoinositol--dexamethasone, produced a significant reduction in lung tumor multiplicity and incidence, even when administered only after cessation of smoke exposure.

Discussion

We have shown before that a diet containing myoinositol--dexamethasone effectively will prevent the development of lung tumors in tobacco-smoke-exposed mice (25). The decision to use the two compounds at the dose levels that were selected and in combination was based on previous experiments reported by Wattenberg and Estensen (30). They reported that feeding the two agents in combination gave better reduction in pulmonary adenoma formation (71%) than feeding either compound alone. In this study we have found that administration of the chemopreventive diet after removal of the animals from smoke reduces lung tumor multiplicity and incidence to control levels (Table II). This is the first experiment to show that it is possible to inhibit the development of tobacco-smoke-induced lung cancer with chemopreventive agents in animals after they have been removed from the smoke atmosphere. It will be important, in future studies, to examine the effects of the two agents separately and to explore more in depth possible dose--effect relationships.

The experimental design used in this study may resemble to some extent ‘quitting’ from smoking. In earlier studies, Wattenberg and Estensen (30,31) had shown that in mice treated with NNK or benzo[a]pyrene, myoinositol or dexamethasone were highly effective in preventing lung tumor development when given in the post-initiation period, whereas they had no significant effect when given during carcinogen exposure. Other agents found to be effective during the post-initiation period are the Bowman–Birk protease inhibitor (32,33), the synthetic glucocorticoid budesonide (34) and lycopene (35). To discover agents that are effective even if administered for the first time after exposure to tobacco-specific nitrosamines or of PAHs is important. Many of the most effective agents against these particular carcinogens, particularly the isothiocyanates, need to be present during carcinogen exposure in order to have a protective effect (6,16). They might thus be less efficient in ‘quitters’.

The observation that myoinositol--dexamethasone was highly effective in mice that had ‘quit’ smoking might be of some practical importance. Attempts to prevent lung cancer development in smokers should never be considered to be an alternative to complete abstention of smoking (16). Epidemiological studies show, however, that for a few years after quitting cigarette smoking, there is actually an increased risk of developing lung cancer (36–38). This has been attributed to the fact that people who quit do most likely so because they do not feel well or are plagued by chronic cough. However, often at this moment there is not yet evidence for lung cancer that would call for aggressive treatment. Chemoprevention administered at this moment might help to reduce this temporary increase in risk. It also might help to prevent the development of second primary tumors, often seen in patients treated for lung cancer (8). Availability of a proven chemopreventive regimen would be a valuable addition to stop-smoking programs.

The mechanisms underlying the protective effect by myoinositol--dexamethasone will have to be worked out. A legitimate question is whether the observed reduction in weight gain might be a contributing factor. It has been repeatedly described that reduced weight gain reduces incidence of lung tumors in mice (39–42). Specifically, in strain A mice, underfeeding can greatly reduce lung tumor incidence and lung tumor multiplicity (43). However, our data do not support the hypothesis that it was reduction in weight gain that was responsible for the protective effect of myoinositol--dexamethasone. Figures 1 and 2 show that in smoke-exposed animals two other chemopreventive regimens produced a significant decrease in final body weight: the mixture of PEITC/BITC and p-XSC. Yet only myoinositol--dexamethasone produced a significant reduction in lung tumor multiplicity and incidence, whereas the other two agents, despite their effects on weight gain, had no such effect (Tables II and III). The decreased weight gain seen in animals kept in air and fed p-XSC (85% of control weight) did also not affect spontaneous tumor multiplicity or incidence compared with animals fed the control diet. Should reduced weight gain to ~85–90% of control body weight indeed be a critical factor in reducing lung tumor incidence and multiplicity, then all three diets should have had an effect of similar magnitude. They did not.

The results with the three test diets containing agents usually effective against carcinogenesis induced by NNK were somewhat disappointing. In previous studies, we had failed to observe a protective effect on tobacco smoke carcinogenesis by PEITC alone, N-acetylcysteine, salicylic acid or green tea (24,25). All these agents had been shown to afford protection against selected individual agents found in tobacco smoke, most often NNK (16,18,44–46). Against full tobacco smoke they are not effective. This raises the provocative question to what extent nitrosamines and PAHs are the driving elements in tobacco-smoke carcinogenesis. Evidence to support this hypothesis is extensive and has been critically analyzed and discussed (47). There is also some evidence to the contrary. In a previous study, we have shown that filtered smoke is as effective a lung carcinogen in strain A mice as is full smoke (29), an observation made also previously by Leuchtenberger and Leuchtenberger (48,49). Although most of the PAHs and quite substantial quantities of nitrosamines had been removed by the filter, the same number of tumors developed as did in animals exposed to the full smoke (29). This led to the speculation that there may be other, as yet unidentified, potent carcinogens in the gas phase.

In all experiments, mice kept in filtered air were used as controls and were fed the diets containing the chemopreventive agents during several months. Tables II and III show that in these animals the feeding of the chemopreventive agents did not result in a significant increase in spontaneous lung tumor multiplicities or incidences. When the usual criteria for a strain A/J mouse lung tumor assay are applied (50), it can be concluded that these agents do not have carcinogenic potential.

In summary, we have shown that it is possible to completely prevent the development of lung tumors in mice exposed to tobacco smoke with myoinositol and dexamethasone, even when the animals are placed on the chemopreventive diet once they have ‘quit’ smoking. On the other hand, d-limonene, p-XSC and a mixture of PEITC/BITC have joined a list of compounds that are highly effective in the strain A mouse.
Chemoprevention of carcinogenesis in mice

assay when tested against selected carcinogens, but are ineffective against tobacco smoke per se.

Acknowledgements

The help in identification and characterization of the p-XSC by the following is gratefully acknowledged: Alan R. Buckpitt, Metabolism and Analytical Core of the Center for Environmental Health Sciences (ES05707), Jeff S. deRooij, NMR Facility (NIH 1S10-RR04759 and NSF BBS88-04739), and R. Mercer, Facility for Advanced Instrumentation, University of California, Davis. This publication was made possible by grant numbers ES07908 and ES07499 from the National Institute of Environmental Health Sciences (NIEHS). The contents of this paper are solely the responsibility of the authors and do not necessarily represent the official views of the NIEHS, NIH.

References


Received November 1, 1999; revised January 21, 2000; accepted January 31, 2000.