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Human Exposure and Dosimetry of Environmental Tobacco Smoke

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Abstract

Environmental tobacco smoke (ETS) is the largest source of indoor air pollution and is also the major combustion source contributing to total human exposure to mutagens and carcinogens. This report provides specific data on human exposure to ETS using biochemical and mutagenesis bioassay measures as well as physical/chemical markers of exposure. Controlled laboratory chamber studies were used to determine ETS emission factors for mutagenic activity using three bioassays. The emission factors for alkenes (e.g., 1,3-butadiene) and aldehydes (e.g., formaldehyde), which are either known or potential carcinogens, are also reported for the first time in these papers. Human exposure concentrations and dosimetry are reported and compared under both controlled chamber and actual indoor environmental conditions. Nicotine exposure and its major metabolite, cotinine, have been found to be useful quantitative and semiquantitative measures of human exposure and dosimetry. The relationship between nicotine exposure and urinary cotinine excretion has been studied in pre-school children exposed in their homes and in adults exposed on commercial airline flights. Air monitoring studies in residences and public indoor areas using both nicotine and mutagenic activity have demonstrated that separation of smokers into separate areas does not achieve an ETS-free or genuine nonsmoking area unless there is both physical separation and separate ventilation.

TABLE OF CONTENTS

	Page
Executive Summary	1
Background and Rationale	2
Approach	5
Emissions and Exposure Characterization	5
Biological Markers of Exposure and Dose	6
Results	10
Characterization of ETS	10
Assessment of Public Exposure to Environmental Tobacco Smoke Using a Mutagenicity Bioassay of Airborne Particulate Matter	11
Evaluation of Urinary Cotinine as a Biomarker of ETS Exposure in Children	11
Variability of Measures of Exposure to Environmental Tobacco Smoke in the Home	12
Application of the Nicotine/Cotinine Exposure/Dosimetry Method for Assessment of ETS on Airline Flights	13
Questionnaire Assessment of Lifetime and Recent Exposure to Environmental Tobacco Smoke Using Urinary Cotinine	15
Summary of Major Findings	16
Exposure	16
Dosimetry	16
Disclaimer	18
List of Publications Summarized	19

EXECUTIVE SUMMARY

Environmental tobacco smoke (ETS) is the largest source of indoor air pollution and is also the major combustion source contributing to total human exposure to mutagens and carcinogens. This report provides specific data on human exposure to ETS using biochemical and mutagenesis bioassay measures as well as physical/chemical markers of exposure. Controlled laboratory chamber studies were used to determine ETS emission factors for mutagenic activity using three bioassays. The emission factors for alkenes (e.g., 1,3-butadiene) and aldehydes (e.g., formaldehyde), which are either known or potential carcinogens, are also reported for the first time in these papers. Human exposure concentrations and dosimetry are reported and compared under both controlled chamber and actual indoor environmental conditions. Nicotine exposure and its major metabolite, cotinine, have been found to be useful quantitative and semiquantitative measures of human exposure and dosimetry. The relationship between nicotine exposure and urinary cotinine excretion has been studied in pre-school children exposed in their homes and in adults exposed on commercial airline flights. Air monitoring studies in residences and public indoor areas using both nicotine and mutagenic activity have demonstrated that separation of smokers into separate areas does not achieve an ETS free or genuine nonsmoking area unless there is both physical separation and separate ventilation. This data provides information to Congress as mandated by the Indoor Air Program and to OAR for information needed in developing policy and guidance information which will be provided to the public, local, state, and national governmental agencies and other specific groups (e.g., ventilation engineers, etc.) on how to achieve indoor air quality.

BACKGROUND AND RATIONALE

The Environmental Tobacco Smoke (ETS) Research Program is a part of EPA's Indoor Air Research Program. The overall goal of the ETS research program is to conduct the research necessary to provide information to the public, governmental agencies, the building industry, employers, and others in order to allow them to make well informed choices regarding the control of exposure to ETS. In order to accurately assess the relationship between human exposure and the resulting risk from that exposure, one of the goals of this program is to determine the relationship between ETS exposure, uptake, and ultimate tissue dose in order to assess the relationship between exposure and risk.

Exposures to ETS have been assessed by questionnaires, air monitoring, modeling, and biological markers. The simplest and yet the least precise and reliable method of exposure assessment has been the use of simple questionnaires (e.g., "If you are a nonsmoker, do you live with, work with, or have regular contact with persons who are smokers?"). Such questions have been the basis for classifying individuals into broad categories of exposure, however there are serious difficulties in developing uniform questions that elicit unambiguous and correct replies, and even more difficult problems in using these replies to make quantitative estimates of exposure. Questionnaires are particularly difficult to use to estimate an integrated exposure over many years, yet this is the primary method which has been used to approximate such long-term exposures. The NAS Committee on ETS (1986) recommended that future epidemiologic studies should incorporate into their design several different exposure assessment methods in order to assess exposure to ETS more accurately and to estimate dose.

Personal exposure and dosimetry of ETS is dependent upon so many factors that the optimal assessment should theoretically be measured directly through the use of biological markers that accurately indicate uptake and/or dose in physiological fluids, tissues or cells. Several chemicals found in body fluids of active smokers have been evaluated as biological markers of exposure to ETS.

Nicotine and its metabolite, cotinine, measured in saliva, blood or urine have been the most useful biological markers of recent ETS exposure since they are derived virtually exclusively from tobacco products. Urinary cotinine levels have been shown to increase in non-smokers with increasing number of smokers in the home for all age groups (infants, children and adults). Currently there is difficulty in interpreting the relative cotinine levels in nonsmokers compared to smokers because of the reported slower clearance of cotinine in nonsmokers and the lack of good uptake and clearance data for nonsmokers of different ages, sex and genetic background. The NAS Committee on ETS (1986) recommended that absorption, metabolism, and excretion of ETS constituents, including nicotine or cotinine, be carefully studied in order to evaluate whether there are differences between smokers and nonsmokers in these factors. Further epidemiologic studies using biological markers are needed to quantify exposure-dose relationships in nonsmokers.

Several other potential biological markers which have been evaluated as indicators of ETS exposure including thiocyanate, carboxyhemoglobin and exhaled CO are not sufficiently sensitive to moderate or low levels of ETS exposure to be generally useful. Since there are several other sources of CO

in the environment that equal or exceed the contribution made by ETS, this marker is even less useful. The use of nicotine and cotinine measurements in urine and possibly saliva are recommended as the best methods now available for quantifying human exposure to ETS, however, research is urgently needed to improve the quantitation and interpretation of these markers. Nicotine is not an ideal marker for all constituents of ETS. In ETS polluted environments, nicotine is present in the vapor phase as a free base, thus its uptake by the passive smoker may not be representative of the uptake of acidic and neutral smoke components from the vapor phase nor of any component in the particulate phase. Other suggested biological markers of exposure include N-nitrosoproline, nitrosothioproline, and some of the aromatic amines that are present in high concentrations in side stream smoke as well as 3-vinylpyridine, solanesol and other tobacco specific constituents. Thus, future studies should be concerned with developing techniques to measure the uptake by nonsmokers of various other types of tobacco-specific ETS components which would be representative of the particulate organic phase of ETS and the volatile acidic and neutral phases. Studies are needed to develop and apply highly sensitive methods (e.g., immunoassays or postlabelling) for measuring DNA and protein adducts of tobacco-specific chemicals.

APPROACH

Emissions and Exposure Characterization

Research is being conducted in chamber studies to determine the emission factors and reactivities of specific ETS constituents. The components currently being measured in these studies include RSP (as well as the size distribution of the aerosol), nicotine, particulate organic mass, mutagenicity, semi-volatile organics, aldehydes, alkenes (and other hydrocarbons), CO and nitrogen oxides. Future studies will expand the organic analysis to include nitrosamines and other components being considered for tracer compounds. The results from these studies are critical to efforts to model human exposure to ETS. Effects of various factors such as room size, temperature, humidity, air-exchange rate, numbers of cigarettes smoked and surface materials will be determined.

Exposure models developed as a part of EPAs indoor air research program will be employed together with data from the emission factors determined in the chamber studies to predict indoor air exposures. These models and emission factors will be evaluated in test home studies under realistic indoor exposure conditions prior to field validation studies.

Source receptor modeling procedures will be developed for apportioning RSP, mutagens and specific organics to ETS in indoor exposure conditions using the tracers nicotine, solenosol, C-32 (anteiso) HC and other candidate tracers selected as described above. RSP, mutagens and PAHs originating from other

sources (e.g., kerosene heaters, wood & coal burning, etc) would be apportioned using inorganic tracer methods previously developed as a part of other EPA air pollution research programs.

Biological Markers of Exposure and Dose

Although cotinine is currently the best biological marker of human exposure to ETS, research is needed to improve the interpretation of cotinine measurements in body fluids. Specifically, needed research is being conducted to determine the absorbtion, metabolism and excretion of nicotine and its metabolites. Specific studies either in progress or in planning stages include: (1) Determination of the dose of nicotine absorbed from ETS by simultaneous chamber exposure to ETS and infusion of dueterated-nicotine in adults. (2) Continuation of studies of adults, and children of various ages, including infants, from homes where ETS is present to determine cotinine clearance rates and to compare exposure, uptake and dosimetry using nicotine and its metabolites. (3) Establish relationships between personal air exposure to RSP, mutagens and nicotine to measured nicotine intake and nicotine metabolites in body fluids for different exposure conditions and population groups.

A workshop was convened to evaluate the biological and analytical methods for cotinine and the recommendations will be implemented in collaboration with other governmental agencies using cotinine as a biological marker for ETS exposure. A standard reference materials laboratory is being established to provide reference chemicals and standardized reference body fluids applicable

to all cotinine assays. Guidelines for a quality assurance program for cotinine analysis have been recommended and an intercollaborative comparison study designed and recommended specifically for quantitating cotinine at levels appropriate to ETS exposures (rather than smoking exposures). EPA will collaborate with CDC, NCI, and the developer of a new monoclonal immunoassay for cotinine (Langone, et al., 1986) to implement further improvements in this assay and apply the assay in collaborative and comparative studies.

Research will be conducted to evaluate candidate marker compounds for the particulate phase of ETS and possibly the acidic or neutral gaseous phase. Highest priority will be given to marker compounds which are preferably unique to ETS and if possible, genotoxic components which would form either protein (e.g., hemoglobin) or DNA adducts. Candidate compounds under consideration are the tobacco-specific nitrosamines, solanesol, polyphenols (e.g., chlorogenic acid, rutin) and other compounds for which DNA-adduct and/or hemoglobin adduct methods are already available.

Research to develop and evaluate ETS specific biological markers of exposure and dose through the use of protein and DNA adducts is being conducted. Initially, postlabelling methods developed by Randerath, et al. (1986) and Gupta et. al. (1986) are being applied to blood cells, placental tissue, buccal cells, and lung cells from smokers and ETS exposed individuals. Although standard adducts [e.g.. B(a)P-DNA adducts and tobacco specific NNK-DNA adducts] are used as reference standards, the postlabelling method allows the detection and quantification of adducts formed in DNA after exposures to complex mixtures such as ETS without knowledge of the specific adduct formed

and without human exposures to radiolabelled materials. These methods are now able to detect 1 adduct in 1010 nucleotides, the sensitivity needed to detect environmental exposures to indoor air pollutants such as ETS.

In collaboration with the UNC-EPA clinical group, we have completed a pilot study to determine the relative adduct levels for human lavaged macrophages isolated from smokers and nonsmokers. DNA adducts were detected in both groups, however, heavy smokers were shown to have higher levels when compared to nonsmokers. It is not clear whether DNA isolated from light to moderate smokers has DNA adduct levels above control nonsmokers. Due to the small number of cells isolated from nonsmokers, we are currently collecting additional samples to establish a base line level for the control group. We plan to modify the solvents used in the assay to resolve any nonpolar adducts that might otherwise go undetected using the standard procedures described in the original ^{32}P-postlabeling method.

Exposure-dosimetry studies in progress at the Frank Porter Graham Child Development Center on 40 pre-school children are designed to understand the exposure-dose-effect relationships in infants and children exposed in the home to ETS. Studies on school age children have been conducted in collaboration with Harvard and the Univ. of New Mexico to assess exposure to ETS using biological markers will be continued with the addition of improvements in cotinine and cotinine QA methods.

Studies to evaluate urinary mutagens as markers of ETS exposure under controlled exposure conditions have been completed. Although the sensitivity

of the methods used have been improved more than tenfold, the urinary concentrations are near the detection limits. It appears that an ETS specific mutagen or mutagenic fraction of the urine would have to be isolated and quantitated to provide the sensitivity and specificity required in such a biological marker.

RESULTS

Characterization of Environmental Tobacco Smoke

EPA conducted an ETS characterization study in both a human exposure chamber and an indoor environment. This study characterized both exposure concentrations and airborne yields for particulate matter and its mutagenic activity as well as nicotine, aldehydes and alkenes. Environmental tobacco smoke (ETS) was analyzed with respect to several components following smoking of research cigarettes in the chamber. Parameters analyzed and their airborne yield per cigarette (shown in parentheses) included: particulate matter (10 mg) and its mutagenic activity in a Salmonella bioassay, carbon monoxide (67 mg), nitrogen oxides (2 mg), nicotine (0.8-3.3 mg), formaldehyde (2 mg), acetaldehyde (2.4 mg), acrolein (0.56 mg), benzene (0.5 mg) and several unsaturated aliphatic hydrocarbons (e.g., 1,3-butadiene) of which isoprene (3.1 mg) had the highest yield. ETS from commercial cigarettes were likewise analyzed in the experimental chamber and at a public location. The relative component composition for ETS is similar when generated from either research or commercial cigarettes. All components analyzed were present at concentrations above the background concentrations. Isoprene might be utilized as a tobacco smoke tracer for unsaturated aliphatic hydrocarbons. This study provided documentation that the chamber ETS exposure was comparable to that which people would encounter in indoor environments where tobacco is being smoked. Additional chemical analyses and subsequent studies were conducted to relate the chamber ETS components to the analysis of ETS in an indoor environment.

Assessment of Public Exposure to Environmental Tobacco Smoke Using a Mutagenicity Bioassay of Airborne Particulate Matter

Airborne particulate matter has been collected by personal samplers in public indoor areas and travel situations with environmental tobacco smoke pollution. Following extraction, the samples were assayed for mutagenicity in the presence of S9 with a sensitive microsuspension test using Salmonella TA98. The mutagenic responses of indoor air from public areas were much higher than those of ambient outdoor air. Depending on the circumstances, the mutagenic response varied in trains and airplanes but the results show that physical separation of non-smoking sections from smoking sections is necessary in order to achieve genuine non-smoking areas. Chemical fractionation and mutagenicity assay of the basic fraction show that Salmonella mutagenicity of airborne particulate matter might be used as a tobacco smoke-specific indicator, as the basic fraction of environmental tobacco smoke contains a large part of the mutagenic activity. This is not the case for outdoor ambient airborne particulate matter and many other combustion emissions.

Evaluation of Urinary Cotinine as a Biomarker of ETS Exposure in Children

The extent of correlation between urine cotinine and home air nicotine levels was examined in 27 children who attended a research day care program where they were not exposed to ETS during the daytime hours. Average concentrations of nicotine in home air were determined by active air sampling during the evening and night hours on two consecutive days. Urine samples for cotinine and creatinine determinations were collected before, during, and after the two sampling periods. In addition, four sequential weekly urine

samples for cotinine were obtained from study children to determine the extent to which single determinations of cotinine were representative for individual children. Fifteen children resided in homes with smokers and 12 in homes without smokers. Urine cotinine consistently distinguished most exposed and unexposed children. However, three exposed children had urine cotinines which clustered routinely around the criterion (30 ng cotinine/mg creatinine) which best distinguished exposed and unexposed children. In children exposed to ETS in the home, there was a significant correlation between average home air nicotine levels and the average logarithm of urine cotinine the two mornings following the home air monitoring periods ($r = 0.67$; $p = 0.008$). In study children, urine cotinines were remarkably stable over the one month observation period. Rank correlation coefficients for sequential weekly determinations of cotinine were consistently greater than $r = 0.88$; $p < 0.0001$.

Variability of Measures of Exposure to Environmental Tobacco Smoke in the Home

The variability of four markers of environmental tobacco smoke exposure was assessed in 10 homes with 20 nonsmoking and 11 smoking household members. The study included obtaining exposure questionnaires, saliva and urine for cotinine, and air particle samples for respirable particles and nicotine on 10 sampling days: every other day over 10 days, and then one day every other week over 10 weeks. The mean concentrations of respirable particles in the 10 homes ranged from 32.4 $\mu g/m^3$ to 76.9 $\mu g/m^3$ and concentrations of nicotine ranges from 0.59 $\mu g/m^3$ to 6.85 $\mu g/m^3$. A linear regression model that included indicator variables for the number of smokers exposed to in the home and the season, and the number of hours of exposure as a continuous variable explained

nine and 6% of the variability of the respirable particle and the nicotine concentrations, respectively. The individual mean cotinine levels standardized to urinary creatinine concentration, ranged from 3.89 ng/mg Cr to 55.77 ng/mg Cr. A linear regression model that included the number of smokers exposed to in the home, the season, the age group, and the number of hours of exposure explained 8% of the variability of the urinary cotinine levels. Because of the marked variability of these measures, multiple measurements are needed to establish a stable profile of exposure to environmental tobacco smoke in a particular home or individual. Furthermore, detailed questions to quantitate exposure offered little additional information beyond whether the subject was exposed or not.

Application of the Nicotine/Cotinine Exposure-Dosimetry Method for Assessment of ETS on Airline Flights

The National Cancer Institute and EPA jointly conducted a pilot research study to measure environmental tobacco smoke exposure on typical commercial flights at the request of The Surgeon General of the Public Health Service. This study was undertaken (1) to measure nicotine levels in ambient air during flights of approximately four hours' duration and urinary cotinine levels at various points during the three days after the flights, and (2) to determine if these exposure and excretion measurements correlate with each other and with acute symptoms experienced during the flights.

In-flight exposure to nicotine, urinary cotinine levels, and symptom self-reports were assessed in a study of nine subjects (five passengers and four attendants) on four routine commercial flights each of approximately four

hours' duration. Urine samples were collected for 72 hours following each flight. Exposures to nicotine measured during the flights using personal exposure monitors were found to be variable, with some nonsmoking areas attaining levels comparable to those in smoking sections. Attendants assigned to work in nonsmoking areas were not protected from smoke exposure. The type of aircraft ventilation was important in determining the levels of in-flight nicotine exposure.

The levels of ETS that occurred during the four-hour flights led to increased levels of cotinine in the urine of both passengers and attendants. Subjects who experienced the greatest in-flight nicotine exposure generally had the highest levels of urinary cotinine and continued to excrete cotinine for 72 hours after the flight. The shape and time course of the decay pattern are consistent with a first-order pharmacokinetic decay process following an initial exposure to nicotine. The peak level of cotinine excreted is related to the dosage of nicotine received over the range of exposures encountered.

Reports on dose-response data under conditions of exposure are sparse, especially for the nicotine concentration range typically encountered by nonsmokers under free-living conditions. This analysis provides estimates of the response to a bolus of ETS, delivered over a four-hour period, shown by a subsequent increase in urinary cotinine excretion over time synchronized across subjects. This study expands upon previous studies employing single-point estimates of cotinine or self-reported smoke exposure levels and provides information on the shape of the excretion curve, delay to peak, amplitude to the peak, approximate functional form, and decay time of cotinine excretion after ETS exposure.

NCI measured changes in eye and nose symptoms between the beginning and end of the flights. These symptoms were significantly related both to nicotine exposure during the flight and to the subsequent urinary excretion of cotinine. In addition, subjects' perceptions of annoyance and smokiness in the airplane cabin were also related to in-flight nicotine exposure and urinary excretion measures.

Questionnaire Assessment of Lifetime and Recent Exposure to Environmental Tobacco Smoke Using Urinary Cotinine

In a sample of 149 adult volunteers recruited in New Mexico in 1986, a study was designed to assess the reliability of questions on lifetime exposure to tobacco smoke in the home and also compared urinary cotinine level with questionnaire report of environmental tobacco smoke exposure during the previous 24 hours. The agreement of responses obtained on two occasions within six months was high for parental smoking during childhood: 94% for the mother and 93% for the father. For smoking by the spouse during adulthood, agreement was 100%. However, responses concerning amount smoked and hours smoked in the home were less reliable. For the amounts smoked by the mother and father during the index subject's childhood, the agreement between the two interviews was 52% and 39% respectively. For hours smoked in the home, the Spearman correlation coefficients also indicated only moderate reliability ($r = 0.18$ for maternal smoking and $r = 0.54$ for paternal smoking). For each set of interviews, responses concerning recent tobacco smoke exposure and urinary cotinine level were correlated to only a modest degree. It was concluded that adults can reliably report whether household members smoked during their childhood, but information on quantitative aspects of smoking is reported less reliably.

SUMMARY OF MAJOR FINDINGS

Exposure

These studies confirm that ETS is a major source of indoor air pollution. Methods were developed and applied to indoor environments to quantitate human exposure to the following pollutants from ETS:

- nicotine
- mutagenicity
- alkenes (e.g., 1,3-butadiene)
- aldehydes (e.g., formaldehyde)

Exposure concentrations of these pollutants, as well as emission rates, have been determined for use in assessment and guidance documents as well as future exposure assessment and modeling studies.

Dosimetry

Urinary cotinine, a nicotine metabolite, was demonstrated to be a useful biological marker of human exposure to nicotine from ETS. The results of an interagency workshop sponsored by EPA recommend steps that should be taken to standardize and assure the quality of cotinine data. Research in progress will significantly improve our ability to determine nicotine dosimetry from ETS exposure.

Exposure-dosimetry studies were conducted both in the normal indoor exposure environments (day care and home) and controlled chamber environments

of preschool children. A similar study was conducted on a commercial airline. These studies show that urinary cotinine is a reliable and semi-quantitative biological marker of ETS exposure in young children and adults.

DISCLAIMER

The information in this document has been funded wholly or in part by the United States Environmental Protection Agency. It has been subjected to the Agency's peer and administrative review, and it has been approved for publication as an EPA document. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

LIST OF PUBLICATIONS SUMMARIZED AND INCLUDED

1. Human Exposure to Mutagens from Indoor Combustion Sources. J. Lewtas, L. Claxton, and J. L. Mumford. Indoor Air '87, Vol. 1, B. Seifert, H. Esdorn, M. Fischer, H. Ruden, J. Wegner, Eds, Oraniendruck GmbH, Berlin, pp. 473-477 (1987).

2. A Genotoxic Assessment of Environmental Tobacco Smoke Using Bacterial Bioassays. L.D. Claxton, R.S. Morin, T.J. Hughes, and J. Lewtas. Mutation Research, 222:81-99 (1989).

3. Characterization of Environmental Tobacco Smoke. G. Lofroth, R. Burton, L. Forehand, K. Hammond, R. Seila, R. Zweidinger and J. Lewtas. Environmental Science and Technology, 23:610-614 (1989).

4. Environmental Tobacco Smoke: Mutagenic Emission Rates and Their Relationship to Other Emission Factors. J. Lewtas, K. Williams, G. Loforth, K. Hammond, B. Leaderer. Indoor Air '87, Vol. 2, B. Seifert, H. Esdorn, M. Fischer, H. Ruden, J. Wegner, Eds, Oraniendruck GmbH, Berlin, pp. 8-12 (1987).

5. Mutagenic Determination of Passive Smoking. P.I. Ling, G. Lofroth, and J. Lewtas. Toxicology Letters, 35:147-151 (1987).

6. The Effect of Solvent and Extraction Methods on the Bacterial Mutagenicity of Sidestream Cigarette Smoke. R.S. Morin, J.J. Tulis, L.D. Claxton. Toxicology Letters, 38:279-290 (1987).

7. Public Exposure to Environmental Tobacco Smoke. G. Lofroth, P.I. Ling, and E. Agurell. Mutation Research, 202:103-110 (1988).

8. Variability of Measures of Exposure to Environmental Tobacco Smoke in the Home. D.B. Coultas, J.M. Samet, J.F. McCarthy, and J.D. Spengler. Proceedings of the APCA Conference on Indoor Air, Niagra Falls, 1988.

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HUMAN EXPOSURE TO MUTAGENS FROM INDOOR COMBUSTION SOURCES

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Abstract

We have measured human exposure to mutagens, using indoor medium-volume samplers and personal samplers, in targeted field studies of homes in the U.S. The combustion sources included in these studies were woodstoves, fireplaces, gas appliances, cooking, and tobacco smoking. These studies demonstrate that the presence of environmental tobacco smoke (ETS) consistently results in human exposure to mutagens which are significantly higher than outdoor air or non-smoking indoor spaces. The mutagenic emission rates from the other indoor combustion sources (e.g., kerosene heaters) as determined in chamber studies are more variable than ETS and are dependent on the combustion source design and operation. Woodstoves and fireplaces result in higher concentrations of mutagens outdoors, which may indirectly influence the concentration of mutagens indoors.

Introduction

Combustion sources are known to emit carcinogenic chemicals and account for most of the mutagenicity associated with particulate matter in the ambient outside air (3). Apportionment studies of the mutagenicity for these sources show that automotive emissions and residential heating are the major sources of mutagens associated with ambient outdoor particulate matter (4). Time-activity studies show that we spend more than 80% of our time indoors in primarily two types of indoor environments, home and work. In order to evaluate human exposure to mutagens, it is necessary to evaluate indoor as well as outdoor sources of mutagens. The sample mass required for standard mutagenesis bioassays exceeds that amount conveniently collected in either indoor air or personal exposure studies. Recently the application of mutagenesis microassay methods has facilitated the evaluation of human exposure to mutagens. The purpose of these studies is to assess the potential human exposure to mutagens from different indoor combustion sources.

Methods

Sampling and Analysis

Two medium volume indoor air samplers have been used in these studies. The 0.22 m^3/min (8 CFM) sampling system used in the Columbus, Ohio study was equipped with a filter to collect total suspended particulate (TSP) followed by a polyurethane foam (PUF) adsorbent cartridge to collect semi-volatile organic (SVO) matter. The details of the sampling system as used

in this residential indoor air study in Columbus, Ohio are reported elsewhere (5). The 0.11 m^3/min (4 CFM) sampling system used in the woodstove study in Raleigh, NC has a PM_{10} size selective inlet (to exclude particles >10 µm) followed by a filter and an organic adsorbant cartridge usually containing XAD-2 to collect the semi-volatile organics. The details of the development of this sampling system are described by Mumford et al. (7). Studies are in progress to compare PUF and XAD-2 as adsorbants for semi-volatile mutagens indoors. The filter and PUF cartridge samples were Soxhlet extracted with methylene chloride and 5% ether/hexane respectively prior to solvent exchange into dimethylsulfoxide for bioassay analysis.

Microsuspension Mutagenicity Assay

The bioassays used in these studies both employed microsuspension modifications of established bioassay protocols in *Salmonella typhimurium* TA98 (6) and TM677 (9). The TM677 forward mutation assay modification has been described elsewhere (5). In this assay both 8-azaquanine resistant (mutant) colonies and surviving colonies are counted and the mutant frequency (MF) is the number of mutants per 10^6 surviving cells. The TA98 reverse mutation assay is a microsuspension assay described by Kado et al. (2). This assay also utilizes (8) the nitroreductase deficient strains TA98NR and $TA98DNP_6$ which are diagnostic for nitroaromatics.

The bioassays were performed with or without the addition of Aroclor 1254-induced male Sprague-Dawley rat liver S9 mixture prepared as described in the above-referenced protocols. All assays were performed at 3-5 doses and the linear slope of the dose-response curve was used to determine the mutagenicity, expressed as either revertants per m^3 air, or per µg organic mass. In all assays both solvent controls (negative) and positive controls were assayed simultaneously as described in the protocols (2, 6, 9).

Results and Discussion

The use of microsuspension bioassays has made it possible to measure the mutagenicity of indoor air as shown in Table 1. In the absence of any combustion sources, the mutagenicity associated with particulate-phase organic matter (PPOM) was similar inside and outside the homes. Across all of these homes there were elevated concentrations of semi-volatile organics (SVO) which were mutagenic, resulting in mutagenic exposures indoors which were twice as high as outdoors. Fireplaces in operation did not result in higher concentrations of PPOM mutagenicity; however, one of these three homes had higher concentrations of SVO mutagenicity. The concentration of PPOM mutagenicity per m^3 was greatest in homes with cigarette smoking and was highly correlated to the number of cigarettes smoked as we have recently reported (5).

The SVO mass and mutagenicity concentration averaged 3.5 times higher indoors when compared to outdoors. Neither the SVO mass nor mutagenicity were correlated with the number of cigarettes smoked and did not appear to be related to any of the major combustion sources. Although certain cooking activities (e.g., frying bacon) significantly increased the concentration of PPOMs, the kitchens did not show substantially higher concentrations of SVO when compared to other rooms in the house.

The influence of outdoor PPOM mutagen concentrations on indoor air is seen in Table 1. The outdoor mutagenicity of the two electric homes was significantly (14 times) less than outside the gas homes. This may account for the 10 times lower PPOM mutagenicity observed in these two electric homes. Table 2 shows the same effect in a neighborhood where woodstove emissions were shown to increase the outdoor mutagenicity at least 10 times. Under these conditions the concentration of mutagens indoors also increased but only up to five times. Alfheim and Ramdahl (1) have also shown that woodstoves and fireplaces result in increased mutagenicity outdoors.

Table 1: Influence of combustion sources on indoor air mutagenicity in a winter residential study in Columbus, Ohio

Major Combustion Source(s)	No. Homes	Particulate-Phase Organics (PPOM) I[a] (MF/m^3)	O[b] (MF/m^3)	I/O[c]	Semi-Volatile Organics (SVO) I (MF/m^3)	O (MF/m^3)	I/O	Total I/O
None[d]	2	.13	.18	0.7	28.2	11.1	2.5	2.3
Gas[e]	2	1.33	2.53	0.5	16.3	10.3	1.5	0.8
Gas/Fireplace[f]	3	.68	2.84	0.2	32.4	2.4	13.5	1.3
Tobacco Smoke								
Average	5	5.64	2.13	2.6	15.2	4.9	3.1	2.7
High[g]	1	26.70	2.73	9.8	9.3	5.6	1.7	8.4
Average	12	2.76	2.05	1.3	21.9	6.2	3.5	1.9

[a]Indoor sample; [b]outdoor sample; [c]ratio of indoor/outdoor mutant frequency (mutants/10^6 survivors)/m^3; [d]all electric homes; [e]natural gas appliances, both central heat and cooking; [f]fireplace was burning wood; [g]kitchen sample taken from 7:00 a.m. to 3:00 p.m. in a home where 58 cigarettes were smoked in a 24 hr. day.

Table 2: Exposure to PPOM mutagenicity in a woodstove impacted neighborhood in Raleigh, NC

Date	Relative Mutagenicity[a] Community Site	Outside Home	Inside Home
2/27	1.0	1.0	1.0
2/26	1.7	3.1	1.0
2/13	3.6	5.1	2.9
2/14	4.7	11.2	3.0
2/20	5.7	7.5	4.6
2/21	11.1	16.3	5.1

[a]Mutant frequency (MF) per m^3 for each location was divided by the MF/m^3 for 2/27.

In order to examine the mutagenic emission rates from appliances, we have collaborated on studies to examine the organic pollutant emissions from kerosene heaters (11). Table 3 summarizes the mutagenicity of these emissions from initial studies of two heater types, radiant and convective. Kerosene heaters have previously been reported to emit highly mutagenic nitrated polycyclic aromatic hydrocarbons (NO_2-PAHs) and dinitropyrenes in particular (10).

Table 3: Mutagenicity of kerosene heater emissions

Microsuspension Bioassay	S9	Radiant		Maltuned Convective	
		Filter	XAD-2	Filter	XAD-2
TA98 (rev/µg)	-	5.1	0.33	0.63	neg
TA98NR (rev/µg)	-	2.4	neg	0.42	neg
TA98/1,8DNP$_6$ (rev/µg)	-	0.8	neg		
TM677 (MF/µg/ml)	+	1.9	neg	0.22	0.10
Emission Rate[a]					
TA98 (rev/hr)		2,500	17,490	4,300	--
TM677 (MF/hr)		930	--	1,500	3,800

[a]Determined from GRAV emission rates given in Traynor et al., 1986 (11).

The 84% decrease in mutagenicity observed in TA98/1,8DNP$_6$ suggests that dinitropyrenes may account for much of the PPOM mutagenicity observed in the radiant heaters' emissions. The decreased activity observed in TA98NR suggests that other NO_2-PAHs (e.g., 1-nitropyrene) are also important. The nitro-compounds, including 1-nitropyrene, 3-nitrofluoranthene, 9-nitroanthracene, and 1-nitronapthalene, were detected from these heaters (11). We have also found an increase of PPOM mutagenicity in several homes with kerosene heaters (unpublished data).

Further studies are needed to measure mutagenic emission rates and human exposures to PPOM and SVO mutagens emitted from indoor combustion sources and to determine the contribution of combustion sources vented outdoors on the resulting indoor air exposures.

Disclaimer

The research described in this paper has been reviewed by the Health Effects Research Laboratory, U.S. Environmental Protection Agency and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the Agency nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

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A genotoxic assessment of environmental tobacco smoke using bacterial bioassays

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Summary

Recently, the National Research Council in the U.S.A. stated that laboratory studies of environmental tobacco smoke (ETS) should be important in identifying ETS carcinogens, their concentrations in typical daily environments, and in understanding how these compounds contribute to ETS dose–response relationships. This paper demonstrates that integrated chemical and bacterial mutagenicity information can be used to identify ETS genotoxicants, monitor human exposure, and make comparative assessments. Approximately 1/3 of the ETS constituents for which there is quantitative analytical chemistry information also have associated genotoxicity information. For example, 11 of the quantitated compounds are animal carcinogens. Work presented in this paper demonstrates that both the nonparticle-bound semi-volatile and the particulate-bound organic material contain bacterial mutagens. These ETS organics give an equivalent of ~ 86 000 revertants per cigarette. In addition, this article summarized efforts to estimate ETS bacterial mutagenicity, to use bacterial tests for the monitoring of ETS-impacted indoor environments, and to use bacterial assays for the direct monitoring of human exposure.

Environmental tobacco smoke (ETS) is the total tobacco smoke found in an environment and includes both sidestream cigarette smoke and the exhaled tobacco smoke of the smokers within the environment. The first suggestion that environmental tobacco smoke (ETS) could have detrimental health effects was a medical case report published by Rosen and Levy in 1950. This report concluded that an infant's severe astmatic symptoms were directly related to the mother's smoking of tobacco products. It was not until 31 years later that the results from epidemiological studies of passive smoking and lung cancer were available. Three studies were published in 1981. Based on a population of 91 540 nonsmoking Japanese housewives, Hirayama (1981) reported that the wives of heavy smokers (> 20 cigarettes/day) had 2.4 times the risk of developing lung cancer as the nonsmoking wives of nonsmokers. Trichopoulos et al. (1981) reported a slightly larger risk for nonsmoking Greek women whose husbands smoked

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more than 20 cigarettes per day. Although Garfinkel (1981) found a small (10–30%) increase in mortality associated with passive smoking, his study of 469 000 nonsmokers did not detect a statistically significant increase in risk of developing lung cancer. Since that time, a number of other epidemiologic studies have emerged (National Research Council, 1986). The National Research Council (1986) concluded that the summary estimate of increased risk of lung cancer ranges from 10% to 34%. They also concluded that although bias may contribute to the results, the best estimate at present for increased adjusted risk of lung cancer to nonsmokers due to passive exposure is approximately 25%. In identifying needed scientific information, the National Research Council (1986) stated: 'Laboratory studies should be important in determining the carcinogenic constituents of ETS and their concentrations in typical daily environments and in facilitating understanding of possible dose–responsive relationships.' The purpose of this paper is to partially fulfill this need by integrating chemical and bacterial genetic bioassay information concerning sidestream and indoor air (IA) tobacco smoke.

Materials and methods

Integrating known chemical and genotoxicity information for sidestream cigarette smoke

Although it is expected that most of the compounds found in mainstream tobacco smoke (MS) would also be found in sidestream smoke (SS), the purpose of this effort was to extract from the literature compounds identified specifically in SS and to associate this information with presently available genotoxicity information. As part of an earlier effort to identify airborne compounds and genotoxicity information (Graedel et al., 1986), confirmed compounds found in tobacco smoke and indoor air were cataloged. This summary provided the baseline information for this tabulation. In addition, an on-line *Chemical Abstracts* search was used to locate relevant papers published since January, 1983. The compounds identified in these papers were summarized, and the MS, SS, and/or indoor air (IA) concentrations were tabulated. Where available, the bioassay summary information as reported by Graedel et al. (1986) was paired with the identified chemicals. All computer literature searches were kindly supplied by the Resource Information Center, U.S. Environmental Protection Agency, Research Triangle Park, NC (U.S.A.) via Ms. Libby Smith. Although the search periods covered a time period extending to December 1986, the information is not meant to be exhaustive but representative of the available literature.

Methods for determining the Salmonella mutagenicity of organic extracts from sidestream tobacco smoke particles

After being lit, a generic U.S. brand of filter cigarettes was allowed to burn within a 0.04-m^3 Plexiglass® chamber into which filtered air was allowed to enter. The generated SS was continuously exhausted at a rate of 0.03 m^3/min and collected on a 142-mm Teflon®-coated glass-fiber filter. Sample preparation was done in the manner reported by Morin et al. (1987). The method can be summarized as follows. All filter samples were extracted using 2 15-min sonications (Constant Temperature, Sonicor® waterbath sonicator, Bay Shore, NY) using either dichloromethane, methanol, or acetone as the solvent. Extracted samples were concentrated to 5–10 ml using rotatory evaporation. Samples were then transferred into 15-ml volumetric tubes and concentrated to 1–2 ml using dry nitrogen. After adding 15 ml of dimethyl sulfoxide (DMSO), the remaining solvent was removed using the nitrogen purge method. Negative controls were prepared in the same manner using filters not containing ETS particles. The solvent-exchanged samples were tested using the *Salmonella typhimurium* plate incorporation assay as previously described (Ames et al., 1975; Claxton et al., 1987).

Direct comparisons of mainstream and sidestream tobacco smoke bacterial mutagenicity

In order to compare the total genotoxic potential of MS and SS it was necessary to bioassay the particle-bound, semi-volatile, and volatile compounds emitted. In order to accomplish this, separate trapping trains for MS and SS were used. Each train consisted of an ethanol bubbler solvent trap, a sand trap, and a liquid-nitrogen cold trap in sequence (Monteith et al., 1986). Cigarette sam-

ples were generated using a smoking machine. The standard conditions were 1 puff/min for each of the 30 cigarettes (1R3 Kentucky Reference), a puff duration of 2 s, and a puff volume of 35 ml. Mainstream smoke was pulled directly into the MS trapping train. In order to collect the SS, the cigarette smoking machine was enclosed within a Tedlar® bag which had a total volume of 0.12 m^3. Air from the bag was continuously drawn into the SS trapping trains. The ethanol bubbler collected primarily semi-volatile compounds, the sand trap collected primarily particulate matter, and the cold trap collected any remaining organic compounds. The sand trap and semi-volatile concentrates were bioassayed in sealed dram vials using a modified version of the preincubation protocol of Yahagi et al. (1975). Preincubation was for 15 min at 37°C while vials were shaken by a rotary shaker at 60 rpm. Tests were done using 500 μl/plate of 5% Aroclor-1254-induced male Syrian hamster liver homogenate. Volatiles from the cold trap were transferred as a gas to Tedlar® bags containing standard bioassay plates. All other aspects of the test were conducted as described by Ames et al. (1975).

Results and discussion

When one evaluates the hazard of a complex organic emission such as ETS, there are multiple and varied approaches that one may use. As stated in the introduction, several researchers have assessed ETS through epidemiological efforts (Hirayama, 1981; Trichopoulos et al., 1981; Garfinkel, 1981; National Research Council, 1986). Although such studies can demonstrate the statistical likelihood of increased risk associated with ETS, these studies were not designed to answer more specific questions concerning ETS and human health. For example, these studies do not identify which constituents within ETS emissions are carcinogens, mutagens, and other types of toxicants. Epidemiology, therefore, is unlikely to determine whether or not changes in tobacco type used, cigarette design, tar values, room ventilation rates, etc. will produce major changes in the health impact of ETS. When human epidemiological data are unavailable and unlikely to be available to answer such relevant issues, one can sometimes use whole animal studies (Stara and Kello, 1979). However, since whole animal studies for carcinogenesis and heritable mutations are expensive, lengthy processes, they too are unlikely to be useful for answering many relevant questions. Short-term test data, especially when associated with quantitative data on analytical chemistry, can be used to make comparative assessments, to identify genotoxicants, and to monitor human exposure.

After identification and quantification of the individual constituents of the mixture, the known toxicological properties of each constituent can be related to the source, for example ETS. The total potential impact of a source can then be estimated by summing the activity of the known constituents or by using some form of toxicological modeling or scaling.

Approximately 10% of the more than 3800 compounds found in MS have been identified in ETS. Table 1 lists over 100 constituents found in ETS and in MS and IA samples for which there is quantitative analytical information. The relative concentrations of the constituents vary according to whether they are measured as MS, SS, or IA components. The reasons (e.g., combustion temperature, oxygen levels, etc.) for differences in concentrations of organic components in MS and SS have been discussed previously (Sakuma et al., 1983, 1984; Baker, 1981, 1982; Klus and Kutin, 1982; IARC, 1968; National Research Council, 1986).

Table 2 lists the genotoxicity information (Graedel et al., 1986; Nesnow et al., 1987) for the compounds in Table 1. Approximately 1/3 of the compounds in Table 1 have associated genotoxicity information. Eleven of these compounds are animal carcinogens (Nesnow et al., 1987). Nineteen of the compounds are positive in at least 1 bioassay, and 10 are positive in the Salmonella bioassay (Graedel et al., 1986). This type of information provides a qualitative evaluation of potential human health impacts. For comparative risk analysis methods (Lewtas, 1985), the amount of each constituent can be associated with the bioassay activity of each constituent in order to provide a crude means of comparison between sources or components of interest. For example, using concentrations of constituents in SS and their known

TABLE 1

CONCENTRATIONS OF COMPOUNDS ASSOCIATED WITH MAINSTREAM AND SIDESTREAM TOBACCO SMOKE AND INDOOR AIR POLLUTED WITH TOBACCO SMOKE [a]

Compound	Sample type	Concentration range			Ref. [b]
		Low	High	Units	
Acetamide	MS	70.00	111.00	μg/cig	25
Acetamide	SS	86.00	156.00	μg/cig	25
Acetic acid	MS	333.00	809.00	μg/cig	23
Acetic acid	MS	272.00	475.00	μg/cig	25
Acetic acid	SS	1241.00	2187.00	μg/cig	23
Acetic acid	SS	695.00	1148.00	μg/cig	25
Acrolein	IA	0.90	1.30	ppm	2
Acrolein	IA	0.02	0.12	mg/m^3	3
Acrolein	IA	6.00	10.00	ppm	12
Acrolein	IA	0.01	0.19	mg/m^3	19
Acrolein	SS	50.00	70.00	ppm	2
Acrolein (gas only + people)	IA	130.00	190.00	μg/m^3	19
Acrolein (people absent)	IA	119.00	133.00	μg/m^3	19
Acrolein (people present)	IA	10.00	48.00	μg/m^3	19
Acrolein control air	IA	0.00	5.00	ppm	12
Aldehydes (gas only + people)	IA	1290.00	1350.00	μg/m^3	19
Aldehydes (generic)	IA	0.39	1.37	mg/m^3	19
Aldehydes (people absent)	IA	1100.00	1370.00	μg/m^3	19
Aldehydes (people present)	IA	391.00	622.00	μg/m^3	19
Alkoxyl radicals	MS	8.00×10^{15} spins/c			22
Alkoxyl radicals	SS	6.00×10^{6} spins/c			22
Ammonia	MS	79.40	131.00	μg/cig	9
Ammonia	MS	95.30	163.00	μg/g smoked	9
Ammonia (cigars)	MS	30.50	322.00	μg/g smoked	9
Ammonia (cigars)	MS	148.00	288.00	μg/product	9
Ammonia	SS	5.14	5.77	mg/cig.	9
Ammonia	SS	6.11	7.18	mg/g smoked	9
Ammonia (cigars)	SS	6.98	106.00	mg/cig	9
Ammonia (cigars)	SS	9.34	20.50	mg/g smoked	9
Anatabine	MS	2.40	20.10	μg/cig	24
Anatabine	SS	0.00	2.40	μg/cig	24
Anthanthrene	IA			Qual (ng/m^3)	1
Anthanthrene	IA	3.00		ng/m^3	15
Anthanthrene	MS	22.00		ng/cig	14
Anthanthrene	SS	39.00		ng/cig	14
Anthracene	IA			Qual (ng/m^3)	1
Anthracene	MS, P	23.60		ng/cig	16
Anthracene	MS, V	0.10		ng/cig	16
Anthracene	SS, P	670.00		ng/cig	16
Anthracene	SS, V	40.00		ng/cig	16
Benz[*a*]anthracene	IA			Qual (ng/m^3)	1
Benz[*a*]anthracene	MS, P	13.30		ng/cig	16
Benz[*a*]anthracene	MS, V	0.09		ng/cig	16
Benz[*a*]anthracene	SS, P	201.00		ng/cig	16
Benz[*a*]anthracene	SS, V	2.50		ng/cig	16

TABLE 1 (continued)

Compound	Sample type	Concentration range			Ref. [b]
		Low	High	Units	
Benz[*e*]acenaphthylene	IA			Qual (ng/m^3)	1
Benzene	IA	0.05	0.15	mg/m^3	3
Benzene (breath, non-smokers)	IA	2.50		μg/m^3	30
Benzene (breath, smokers)	IA	16.00		μg/m^3	30
Benzene (homes, non-smokers)	IA	4.40	9.20	μg/cm^3	30
Benzene (homes, smokers)	IA	4.80	16.00	μg/m^3	30
Benzo[*a*]fluorene	IA			Qual (ng/m^3)	1
Benzo[*a*]fluorene	IA	39.00		ng/m^3	15
Benzo[*a*]fluorene	MS	184.00		ng/cig	14
Benzo[*a*]fluorene	SS	751.00		ng/cig	14
Benzo[*a*]pyrene	IA			Qual (ng/m^3)	1
Benzo[*a*]pyrene	IA	7.10	21.70	ng/m^3	11
Benzo[*a*]pyrene	IA	6.20	144.00	ng/m^3	13
Benzo[*a*]pyrene	IA	22.00		ng/m^3	15
Benzo[*a*]pyrene	MS	44.00		ng/cig	14
Benzo[*a*]pyrene	MS, P	10.90		ng/cig	16
Benzo[*a*]pyrene	MS, V	0.08		ng/cig	16
Benzo[*a*]pyrene	SS	199.00		ng/cig	14
Benzo[*a*]pyrene	SS, P	103.00		ng/cig	16
Benzo[*a*]pyrene	SS, V	0.48		ng/cig	16
Benzo[*a*]pyrene control	IA	0.00	0.69	ng/m^3	11
Benzo[*b*]naphtho[2,1-d]thiophene	MS, P	2.80		ng/cig	16
Benzo[*b*]naphtho[2,1-d]thiophene	MS, V	0.21		ng/cig	16
Benzo[*b*]naphtho[2,1-d]thiophene	SS, P	50.00		ng/cig	16
Benzo[*b*]naphtho[2,1-d]thiophene	SS, V	1.10		ng/cig	16
Benzo[*b/c*]fluorene	MS	69.00		ng/cig	14
Benzo[*b/c*]fluorene	SS	251.00		ng/cig	14
Benzo[*b/j/k*]fluoranthene	IA	35.00		ng/m^3	15
Benzo[*b/j/k*]fluoranthene	MS	49.00		ng/cig	14
Benzo[*b/j/k*]fluoranthene	SS	260.00		ng/cig	14
Benzo[*e*]fluorene	IA			Qual (ng/m^3)	1
Benzo[*e*]pyrene	IA			Qual (ng/m^3)	1
Benzo[*e*]pyrene	IA	18.00		ng/m^3	15
Benzo[*e*]pyrene	IA	3.30	23.40	ng/m^3	20
Benzo[*e*]pyrene	MS	25.00		ng/cig	14
Benzo[*e*]pyrene	MS, P	6.70		ng/cig	16
Benzo[*e*]pyrene	MS, V	0.13		ng/cig	16
Benzo[*e*]pyrene	SS	135.00		ng/cig	14
Benzo[*e*]pyrene	SS, P	75.00		ng/cig	16
Benzo[*e*]pyrene	SS, V	0.74		ng/cig	16
Benzo[*e*]pyrene control	IA	3.00	5.10	ng/m^3	20
Benzo[*ghi*]fluoranthene	IA			Qual (ng/m^3)	1
Benzo[*ghi*]perylene	IA			Qual (ng/m^3)	1
Benzo[*ghi*]perylene	IA	17.00		ng/m^3	15
Benzo[*ghi*]perylene	MS	39.00		ng/cig	14
Benzo[*ghi*]perylene	MS, P	7.10		ng/cig	16
Benzo[*ghi*]perylene	MS, V	0.09		ng/cig	16
Benzo[*ggi*]perylene	SS	98.00		ng/cig	14
Benzo[*ghi*]perylene	SS, P	41.00		ng/cig	16
Benzo[*ghi*]perylene	SS, V	0.62		ng/cig	16

TABLE 1 (continued)

Compound	Sample type	Concentration range			Ref. [b]
		Low	High	Units	
Benzofluoranthenes (b + j + k)	MS, P	20.50		ng/cig	16
Benzofluoranthenes (b + j + k)	MS, V	0.22		ng/cig	16
Benzofluoranthenes (b + j + k)	SS, P	196.00		ng/cig	16
Benzofluoranthenes (b + j + k)	SS, V	1.38		ng/cig	16
Benzoic acid	MS	14.00	28.00	μg/cig	23
Benzoic acid	SS	12.00	23.00	μg/cig	23
Benzoic acid, *m*-hydroxy-	MS	8.00	64.00	μg/cig	23
Benzoic acid, *m*-hydroxy-	SS	3.00	15.00	μg/cig	23
Benzonitrile	MS	5.00	6.00	μg/cig	25
Benzonitrile	SS	33.00	57.00	μg/cig	25
Bipyridyl, 2, 3′-	MS	9.90	27.40	μg/cig	24
Bipyridyl, 2, 3′-	SS	20.00	73.00	μg/cig	24
Bipyridyl, 5-methyl-2, 3′-	MS	6.60	14.70	μg/cig	24
Bipyridyl, 5-methyl-2, 3′-	SS	6.00	14.00	μg/cig	24
Butyrolactone, gamma-	SS	40.00	103.00	μg/cig	25
Butyrolactone, gamma-	MS	11.00	22.00	μg/cig	25
Carbon monoxide	IA	2.00	23.00	ppm	3
Carbon monoxide	IA	0.00	1.20	ppm	31
Carbon monoxide (gas only + people)	IA	23.00	26.00	ppm	19
Carbon monoxide (people absent)	IA	21.00	25.00	ppm	19
Carbon monoxide (people present)	IA	18.00	22.00	ppm	19
Carbon monoxide (people present)	IA	3.70	4.20	ppm	28
Carbon monoxide control	IA	0.00	15.00	ppm	3
Carbon monoxide control	IA	0.00	0.50	ppm	31
Carbon monoxide (artificial cond.)	IA	8.00	16.00	ppm	29
Carbon monoxide (natural conditions)	IA	9.00		ppm	29
Carbon, total	IA	207.00		$\mu g/m^3$	27
Carbon, elemental	IA	11.90		$\mu g/m^3$	27
Carbon, organic	IA	195.00		$\mu g/m^3$	27
Carboxyhemoglobin (blood, passive)	IA	0.55		%	28
Carboxyhemoglobin (blood, smoker)	IA	3.38		%	28
Carboxyhemoglobin (blood, no smoking)	IA	0.57		%	28
Catechol	MS	148.00	362.00	μg/cig	23
Catechol	SS	138.00	292.00	μg/cig	23
Catechol, 2-methyl-	MS	6.00	13.00	μg/cig	23
Catechol, 2-methyl-	SS	8.00	21.00	μg/cig	23
Catechol, 3-methyl-	MS	31.00	62.00	μg/cig	23
Catechol, 3-methyl-	SS	24.00	47.00	μg/cig	23
Catechol, 4-ethyl-	MS	27.00	102.00	μg/cig	23
Catechol, 4-ethyl-	SS	19.00	68.00	μg/cig	23
Catechol, 4-methyl-	SS	25.00	55.00	$\mu g/m^3$	23
Catechol, 4-methyl-	MS	29.00	80.00	μg/cig	23
Catechol, 4-vinyl-	MS	23.00	113.00	μg/cig	23
Catechol, 4-vinyl-	SS	7.00	40.00	μg/cig	23
Catechols (all catechols)	MS	25.00	328.00	μg/cig	4
Catechols (all catechols)	SS	88.00	212.00	μg/cig	4
Coronene	IA	0.50	1.20	ng/m^3	20
Coronene control	IA	1.00	2.80	ng/m^3	20

TABLE 1 (continued)

Compound	Sample type	Concentration range			Ref. [b]
		Low	High	Units	
Cotinine (plasma, non-smoker)	IA	1.40		ng/ml	28
Cotinine (plasma, passive smoker)	IA	2.10		ng/ml	28
Cotinine (plasma, smoker)	IA	52.40		ng/ml	28
Cresol, *m*-	MS	11.00	18.00	μg/cig	23
Cresol, *m*-	MS	17.00	26.00	μg/cig	25
Cresol, *m*-	SS	13.00	24.00	μg/cig	23
Cresol, *m*-	SS	18.00	34.00	μg/cig	25
Cresol, *o*-	MS	13.00	19.00	μg/cig	23
Cresol, *o*-	SS	14.00	24.00	μg/cig	23
Cresol, *p*-	MS	30.00	37.00	μg/cig	23
Cresol, *p*-	MS	32.00	47.00	μg/cig	25
Cresol, *p*-	SS	30.00	46.00	μg/cig	23
Cresol, *p*-	SS	45.00	62.00	μg/cig	25
Cyclopentenone, 2, 3-dimethyl-2-	MS	9.00	23.00	μg/cig	25
Cyclopentenone, 2, 3-dimethyl-2-	SS	21.00	39.00	μg/cig	25
Cyclopentenone, 2-	MS	21.00	27.00	μg/cig	25
Cyclopentenone, 2-	SS	70.00	103.00	μg/cig	25
Cyclopentenone, 2-*OH*-3-methyl-2-	MS	3.00	5.00	μg/cig	25
Cyclopentenone, 2-*OH*-3-methyl-2-	SS	24.00	30.00	μg/cig	25
Cyclopentenone, 2-methyl-2-	MS	17.00	22.00	μg/cig	25
Cyclopentenone, 2-methyl-2-	SS	49.00	95.00	μg/cig	25
Dibenz[*a*, *j*]anthracene	IA	6.00		ng/m^3	15
Dibenz[*a*, *j*]anthracene	MS	11.00		ng/cig	14
Dibenz[*a*, *j*]anthracene	SS	41.00		ng/cig	14
Ethylbenzene (breath, non-smokers)	IA	0.80		μg/m^3	30
Ethylbenzene (breath, smokers)	IA	2.60		μg/m^3	30
Ethylbenzene (homes, non-smokers)	IA	3.50	5.10	μg/m^3	30
Ethylbenzene (homes, smokers)	IA	3.50	8.30	μg/m^3	30
Ethylmethylenephenanthrene	IA			Qual (ng/m^3)	1
Fluoranthene	IA			Qual (ng/m^3)	1
Fluoranthene	IA	99.00		ng/m^3	15
Fluoranthene	MS	272.00		ng/cig	14
Fluoranthene	MS, P	61.30		ng/cig	16
Fluoranthene	SS	1255.00		ng/cig	14
Fluoranthene	SS, P	669.00		ng/cig	16
Fluoranthene	SS, V	16.90		ng/cig	16
Formaldehyde	IA	1.50	2.10	ppm	2
Formaldehyde	IA	0.10	0.16	ppm	28
Formaldehyde	SS	80.00	110.00	ppm	2
Formic acid	MS	210.00	478.00	μg/cig	23
Formic acid	SS	341.00	665.00	μg/cig	23
Furaldehyde, 2-	MS	15.00	43.00	μg/cig	25
Furaldehyde, 2-	SS	113.00	290.00	μg/cig	25
Furaldehyde, 5-methyl-	MS	6.00	29.00	μg/cig	25
Furaldehyde, 5-methyl-	SS	20.00	127.00	μg/cig	25
Furfuryl alcohol	MS	18.00	65.00	μg/cig	25
Furfuryl alcohol	SS	73.00	283.00	μg/cig	25

TABLE 1 (continued)

Compound	Sample type	Concentration range			Ref. [b]
		Low	High	Units	
Furoic acid, 2-	MS	44.00	107.00	μg/cig	23
Furoic acid, 2-	SS	25.00	60.00	μg/cig	23
Glutaric acid	MS	10.00	58.00	μg/cig	23
Glutaric acid	SS	6.00	18.00	μg/cig	23
Glycolic acid	MS	37.00	126.00	μg/cig	23
Glycolic acid	SS	35.00	77.00	μg/cig	23
Guaiacol, 4-vinyl-	SS	24.00	32.00	μg/cig	23
Guaiacol, 4-vinyl-	MS	23.00	36.00	μg/cig	23
Guaiacol, 4-vinyl-	MS	16.00	30.00	μg/cig	25
Guaiacol, 4-vinyl-	SS	15.00	37.00	μg/cig	25
HCN	IA	0.01	0.08	mg/m^3	19
HCN (gas only + people)	IA	82.00	86.00	μg/m^3	19
HCN (people absent)	IA	50.00		μg/m^3	19
HCN (people present)	IA	10.00	14.00	μg/m^3	19
Hydrazine	MS	31.50		ng/cig	21
Hydrazine	SS	94.20		ng/cig	21
Hydroquinone	MS	114.00	300.00	μg/cig	23
Hydroquinone	SS	91.00	285.00	μg/cig	23
Hydroquinone, methyl-	MS	23.00	39.00	μg/cig	23
Hydroquinone, methyl-	SS	21.00	41.00	μg/cig	23
Hydroxypropionic acid, 3-	MS	2.00	31.00	μg/cig	23
Hydroxypropionic acid, 3-	SS	1.00	29.00	μg/cig	23
Indeno[1, 2, 3-*cd*]pyrene	IA			Qual (ng/m^3)	1
Indeno[1, 2, 3-*cd*]pyrene	MS, V	0.17		ng/cig	16
Indeno[1, 2, 3-*cd*]pyrene	SS, P	51.00		ng/cig	16
Indeno[1, 2, 3-*cd*]pyrene	SS, V	0.36		ng/cig	16
Indeno[1, 2, 3-*cd*]pyrene	MS, P	8.10		ng/cig	16
Isoquinoline	MS	1.60	2.00	μg/cig	24
Isoquinoline	SS	5.00	8.00	μg/cig	24
Lactic acid	MS	63.00	174.00	μg/cig	23
Lactic acid	SS	45.00	123.00	μg/cig	23
Levulinic acid	MS	29.00	56.00	μg/cig	23
Levulinic acid	SS	25.00	49.00	μg/cig	23
Limonene	MS	15.00	49.00	μg/cig	25
Limonene	SS	63.00	397.00	μg/cig	25
Lutidine, 2, 4-	SS	35.00	315.00	μg/cig	6
Lutidine, 2, 6-	MS	1.40	33.00	μg/cig	6
Lutidine, 2, 6-	SS	1.40	33.00	μg/cig	6
Lutidine, 3, 5-	MS	0.00	17.00	μg/cig	6
Lutidine, 3, 5-	SS	22.00	251.00	μg/cig	6
Methylenephenanthrene, 4, 5-	IA			Qual (ng/m^3)	1
Methylnaphthalene, 1-	SS	30.00		μg/cig	26
Methylnaphthalene, 1-	MS	1.02		μg/cig	26
Methylnaphthalene, 2-	MS	1.21		μg/cig	26
Methylnaphthalene, 2-	SS	31.60		μg/cig	26

TABLE 1 (continued)

Compound	Sample type	Concentration range			Ref. [b]
		Low	High	Units	
Methylnitrosoamino-pyridyl-butanone	MS	46.00	240.00	ng/cig	8
Methylnitrosoamino-pyridyl-butanone	SS	201.00	540.00	ng/cig	8
Methylphenanthrene, 1-	IA			Qual (ng/m^3)	1
Methylphenanthrene, 2-	IA			Qual (ng/m^3)	1
Methylphenanthrene, 3-	IA			Qual (ng/m^3)	1
Methylphenanthrene, 4/9-	IA			Qual (ng/m^3)	1
Myosmine	MS	13.10	33.00	μg/cig	24
Myosmine	SS	73.00	224.00	μg/cig	24
Naphthalene	MS	2.76		μg/cig	26
Naphthalene	SS	45.50		μg/cig	26
Neophytadiene	MS	66.00	232.00	μg/cig	25
Neophytadiene	SS	70.00	421.00	μg/cig	25
Nicotine	IA	25.00	1010.00	μg/m^3	3
Nicotine	IA	0.70	3.10	μg/m^3	17
Nicotine	IA	1.00	10.30	μg/m^3	18
Nicotine	IA	1.70	180.00	pg/m^2 min	32
Nicotine	MS	1720.00	3330.00	μg/cig	24
Nicotine	MS	1483.00	3149.00	μg/cig	25
Nicotine	SS	3210.00	5830.00	μg/cig	24
Nicotine	SS	2987.00	6588.00	μg/cig	25
Nicotine (gas only + people)	IA			Traces only	19
Nicotine (people absent)	IA	130.00		μg/m^3	19
Nicotine (people present)	IA	102.00		μg/m^3	19
Nicotine, office buildings	IA	1.70	180.00	pg/m^2 min	32
Nicotyrine	MS	4.20	20.20	μg/cig	24
Nicotyrine	MS	17.00	41.00	μg/cig	25
Nicotyrine	SS	49.00	211.00	μg/cig	24
Nicotyrine	SS	93.00	263.00	μg/cig	25
Nitrogen dioxide	IA	0.00	0.03	ppm	19
Nitrogen dioxide	IA	58.00		ppb	31
Nitrogen dioxide (gas only + people)	IA	0.01	0.03	ppm	19
Nitrogen dioxide (people absent)	IA	0.00		ppm	19
Nitrogen dioxide (people present)	IA	0.00		ppm	19
Nitrogen dioxide control	IA	27.00		ppb	31
Nitrogen oxide	IA	0.30	0.60	ppm	19
Nitrogen oxide	IA	0.00	9.00	ppb	31
Nitrogen oxide (gas only + people)	IA	0.31	0.40	ppm	19
Nitrogen oxide (people absent)	IA	0.48	0.59	ppm	19
Nitrogen oxide (people present)	IA	0.30	0.60	ppm	19
Nitrogen oxide control	IA	5.00		ppb	31
Nitrogen oxides (combined)	IA	59.00	218.00	ppb	12
Nitrosoamine, methylethyl-	MS	0.10	9.10	ng/cig	5
Nitrosoamine, methylethyl-	MS	0.00	1.80	ng/cig	7
Nitrosoamine, methylethyl-	SS	9.00	75.00	ng/cig	5
Nitrosoamine, methylethyl-	SS	0.00	27.00	ng/cig	7
Nitrosoanabasine, *N'*-	SS	15.00	40.00	ng/cig	8
Nitrosoanatidine, *N'*-	MS	82.00	167.00	ng/cig	8
Nitrosoanatidine, *N'*-	SS	61.00	220.00	ng/cig	8

TABLE 1 (continued)

Compound	Sample type	Concentration range			Ref. [b]
		Low	High	Units	
Nitrosodiethylamine	MS	0.00	4.80	ng/cig	5
Nitrosodiethylamine	SS	8.00	73.00	ng/cig	5
Nitrosodiethylamine, *N*-	MS	1.80	4.80	ng/cig	10
Nitrosodiethylamine, *N*-	SS	8.20	73.00	ng/cig	10
Nitrosodiethylamine (artificial)	IA	0.00	0.01	ng/l	29
Nitrosodiethylamine (natural cond.)	IA	0.00	0.20	ng/l	29
Nitrosodimethylamine	MS	1.70	97.00	ng/cig	5
Nitrosodimethylamine	MS	0.00	27.00	ng/cig	7
Nitrosodimethylamine	SS	680.00	1 770.00	ng/cig	5
Nitrosodimethylamine	SS	143.00	415.00	ng/cig	7
Nitrosodimethylamine	SS	460.00	1 880.00	ng/cig	8
Nitrosodimethylamine, *N*-	MS	1.70	97.00	ng/cig	10
Nitrosodimethylamine, *N*-	SS	680.00	1 040.00	ng/cig	10
Nitrosodimethylamine (artificial)	IA	0.02	0.15	ng/l	29
Nitrosodimethylamine (natural cond.)	IA	0.00	0.70	ng/l	29
Nitrosoethylmethylamine, *N*-	MS	0.00	9.10	ng/cig	10
Nitrosoethylmethylamine, *N*-	SS	9.40	30.00	ng/cig	10
Nitrosonornicotine	MS	81.00	390.00	ng/cig	8
Nitrosonornicotine	SS	110.00	390.00	ng/cig	8
Nitrosopyrrolidine	MS	2.60	52.00	ng/cig	5
Nitrosopyrrolidine	MS	1.50	29.00	ng/cig	7
Nitrosopyrrolidine	SS	204.00	612.00	ng/cig	5
Nitrosopyrrolidine	SS	28.00	143.00	ng/cig	7
Nitrosopyrrolidine	SS	80.00	500.00	ng/cig	8
Nitrosopyrrolidine, *N*-	MS	2.60	51.70	ng/cig	10
Nitrosopyrrolidine, *N*-	SS	204.00	387.00	ng/ciq	10
Octane (breath smokers)	IA	1.10		$\mu g/m^3$	30
Octane (breath, non-smokers)	IA	0.10		$\mu g/m^3$	30
Octane (homes, non-smokers)	IA	1.70	3.10	$\mu g/m^3$	30
Octane (homes, smokers)	IA	1.50	4.70	$\mu g/m^3$	30
Parvoline	MS	0.00	4.30	μg/cig	6
Parvoline	SS	10.00	145.00	μg/cig	6
Pentadien-4-olide, 2, 4-	MS	8.00	41.00	μg/cig	25
Pentadien-4-olide, 2, 4-	SS	71.00	256.00	μg/cig	25
Perylene	IA			Qual (ng/m^3)	1
Perylene	IA	11.00		ng/m^3	15
Perylene	IA	0.70	1.30	ng/m^3	20
Perylene	MS	9.00		ng/cig	14
Perylene	SS	39.00		ng/cig	14
Perylene control	IA	2.80	1.70	ng/m^3	20
Phenanthrene	IA			Qual (ng/m^3)	1
Phenanthrene	MS, P	74.80		ng/cig	16
Phenanthrene	MS, V	2.10		ng/cig	16
Phenanthrene	SS, P	2149.00		ng/cig	16
Phenanthrene	SS, V	248.00		ng/cig	16
Phenol	MS	79.00	136.00	μg/cig	23
Phenol	MS	77.00	139.00	μg/cig	25
Phenol	SS	69.00	241.00	μg/cig	23
Phenol	SS	157.00	289.00	μg/cig	25

TABLE 1 (continued)

Compound	Sample type	Concentration range			Ref. [b]
		Low	High	Units	
Phenol, 4-vinyl-	MS	18.00	45.00	μg/cig	25
Phenol, 4-vinyl-	SS	25.00	57.00	μg/cig	25
Phenols	IA	7.40	11.50	μg/m^2	20
Phenylacetic acid	MS	18.00	38.00	μg/cig	23
Phenylacetic acid	SS	11.00	30.00	μg/cig	23
Picoline, 3-	MS	12.00	22.00	μg/cig	25
Picoline, 3-	SS	90.00	166.00	μg/cig	25
Picoline, alpha-	MS	12.30	189.00	μg/cig	6
Picoline, alpha-	SS	128.00	1090.00	μg/cig	6
Pyran-4-one, 5, 6-diOH-3, 5-diOH-2-ME	MS	13.00	153.00	μg/cig	25
Pyran-4-one, 5, 6-diOH-3, 5-diOH-2-ME	SS	0.00	143.00	μg/cig	25
Pyrazine, 2, 3-dimethyl-	SS	0.00	50.00	μg/cig	6
Pyrazine, 2-methyl	MS	0.00	8.60	μg/cig	6
Pyrazine, 2-methyl-	SS	0.00	8.60	μg/cig	6
Pyrene	IA			Qual (ng/m^3)	1
Pyrene	IA	66.00		ng/m^3	15
Pyrene	IA	4.10	9.40	ng/m^3	20
Pyrene	MS	270.00		ng/cig	14
Pyrene	MS, P	43.00		ng/cig	16
Pyrene	MS, V	1.90		ng/cig	16
Pyrene	SS	1011.00		ng/cig	14
Pyrene	SS, P	466.00		ng/cig	16
Pyrene	SS, V	10.30		ng/cig	16
Pyrene control	IA	2.80	7.00	ng/m^3	20
Pyrene, 1-methyl-	IA			Qual (ng/m^3)	1
Pyrene, 2-methyl-	IA			Qual (ng/m^3)	1
Pyrene, 4-methyl-	IA			Qual (ng/m^3)	1
Pyridine	MS	32.40	648.00	μg/cig	6
Pyridine	MS	16.00	20.00	μg/cig	25
Pyridine	SS	336.00	3420.00	μg/cig	6
Pyridine	SS	187.00	262.00	μg/cig	25
Pyridine, 2-(3-pentyl)-	MS	0.00	1.50	μg/cig	6
Pyridine, 2-(3-pentyl)-	SS	0.00	143.00	μg/cig	6
Pyridine, 2-ethyl-	MS	2.60	35.00	μg/cig	6
Pyridine, 2-ethyl-	SS	2.60	35.00	μg/cig	6
Pyridine, 3-acetyl-	MS	3.80	6.40	μg/cig	24
Pyridine, 3-acetyl-	SS	9.00	11.00	μg/cig	24
Pyridine, 3-cyano-	SS	24.00	64.00	μg/cig	24
Pyridine, 3-cyano-	MS	2.40	4.20	μg/cig	24
Pyridine, 3-ethyl-	MS	4.00	6.00	μg/cig	25
Pyridine, 3-ethyl-	SS	71.00	960.00	μg/cig	6
Pyridine, 3-ethyl-	SS	21.00	36.00	μg/cig	25
Pyridine, 3-ethyl-4-methyl-	SS	6.40	34.00	μg/cig	6
Pyridine, 3-ethyl-4-methyl-	MS	0.00	1.50	μg/cig	6
Pyridine, 3-hydroxy-	MS	125.10	211.40	μg/cig	24
Pyridine, 3-hydroxy-	MS	90.00	119.00	μg/cig	25
Pyridine, 3-hydroxy-	SS	152.00	167.00	μg/cig	24
Pyridine, 3-hydroxy-	SS	157.00	191.00	μg/cig	25
Pyridine, 4-ethyl-	SS	27.00	379.00	μg/cig	6
Pyridine, 4-*t*-butyl-	MS	0.00	4.50	μg/cig	6
Pyridine, 4-*t*-butyl-	SS	17.00	287.00	μg/cig	26
Pyridine, methylvinyl-	MS	2.20	4.10	μg/cig	24
Pyridine, methylvinyl-	SS	12.00	19.00	μg/cig	23

TABLE 1 (continued)

Compound	Sample type	Concentration range			Ref. [b]
		Low	High	Units	
Pyrrole	MS	16.00	23.00	μg/cig	25
Pyrrole	SS	140.00	272.00	μg/cig	25
Styrene (breath, non-smokers)	IA	0.30		μg/m³	30
Styrene (breath, smokers)	IA	1.10		μg/m³	30
Styrene (homes, non-smokers)	IA	0.80	1.10	μg/m³	30
Styrene (homes, smokers)	IA	1.10	2.20	μg/m³	30
Succinic acid	MS	112.00	163.00	μg/cig	23
Succinic acid	SS	65.00	70.00	μg/cig	23
Succinic acid, methyl-	MS	4.00	31.00	μg/cig	23
Succinic acid, methyl-	SS	1.00	13.00	μg/cig	23
Tar radical sol in *t*-butylbenzene	MS			Qualitative	22
Tar radical sol in *t*-butylbenzene	SS			Qualitative	22
Thiocyanate (plasma, non-smoker)	IA	70.80		μmol/l	28
Thiocyanate (plasma, passive smoker)	IA	71.80		μmol/l	28
Thiocyanate (plasma, smoker)	IA	70.70		μmol/l	28
Thioethers (urine, non-smoker)	IA	6.00		mmol/ml	28
Thioethers (urine, passive)	IA	6.40		mmol/ml	28
Thioethers (urine, smoker)	IA	6.30		mmol/ml	28
Toluene	IA	0.04	1.04	mg/m³	3
Valeric acid, 3-methyl-	MS	20.00	261.00	μg/cig	23
Valeric acid, 3-methyl	SS	20.00	384.00	μg/cig	23
Vinylphenol, *p*-	MS	21.00	51.00	μg/cig	23
Vinylphenol, *p*-	SS	21.00	45.00	μg/cig	23
Xylene, *m* + *p*- (breath, non-smokers)	IA	2.10		μg/m³	30
Xylene, *m* + *p*- (breath, smokers)	IA	5.50		μg/m³	30
Xylene, *o*- (breath, non-smokers)	IA	0.80		μg/m³	30
Xylene, *m* + *p*- (homes, non-smokers)	IA	10.00	13.00	μg/m³	30
Xylene, *m* + *p*- (homes, smokers)	IA	10.00	20.00	μg/m³	30
Xylene, *o*- (breath, smokers)	IA	1.60		μg/m³	30
Xylene, *o*- (homes, non-smokers)	IA	4.00	5.20	μg/m³	30
Xylene, *o*- (homes, smokers)	IA	3.20	7.10	μg/m³	30
Xylenol, 2, 6-	MS	8.00	16.00	μg/cig	23
Xylenol, 2, 6-	SS	8.00	20.00	μg/cig	23

[a] Listings are given in alphabetical order by compound and special conditions are noted in parentheses within the column labeled 'Compound'. The sample type is categorized in the second column as IA, indoor air; SS, sidestream smoke; MS, mainstream smoke; P, associated with particulate matter; and V, associated with volatile compounds.

[b] Reference numbers are as follows: 1, Alfheim and Ramdahl, 1984; 2, Ayer and Yeager, 1982; 3, Badre et al., 1978; 4, Brunnemann et al., 1976; 5, Brunnemann et al., 1977; 6, Brunnemann et al., 1978; 7, Brunnemann et al., 1980; 8, Brunnemann et al., 1983, 9, Brunnemann and Hoffmann, 1975; 10, Brunnemann and Hoffmann, 1978; 11, Elliott and Rowe, 1975; 12, Fischer et al., 1978; 13, Galuskinova, 1964; 14, Grimmer et al., 1977a; 15, Grimmer et al., 1977b; 16, Grimmer et al., 1987; 17, Harmsen and Effenberger, 1957; 18, Hinds and First, 1975; 19, Hugod et al. 1978; 20, Just et al., 1972; 21, Liu et al., 1974; 22, Pryor et al., 1983; 23, Sakuma et al., 1983; 24, Sakuma et al., 1984a; 25, Sakuma et al., 1984b; 26, Schmeltz et al., 1976; 27, Sexton et al., 1984; 28, Sorsa et al., 1985, 29, Stehlik et al., 1982; 30, Wallace and Pellizzari, 1986; and 31, Williams et al., 1985.

genotoxicity, one may calculate, in an additive fashion, the 'total genotoxic potential' of a cigarette. Comparative human exposure could then be calculated by knowing the total genotoxic potential of a cigarette, room air volume, and the air exchange rate. If one were to make a crude estimate of the bacterial mutagenicity of 'a cigarette' by using the upper concentration ranges from Table 1 and Salmonella potency range for the 10 bacterial mutagens (1 rev/100 mg to 1 rev/0.10 μg), the calculated mutagenic potency [μg of compound/cigarette × revertants/μg compound] of a cigarette would be approximately 0.5–45 revertants per cigarette. If all of the compounds in Table 2 except the known negatives were to be shown to be Salmonella mutagens, one could estimate the mutagenic activity level to be as high as 1500 revertants/cigarette. Obviously, this method of summing the total genotoxic activity for a 'typical cigarette' is most useful when the chemical and bioassay values are quantitatively accurate, when all genotoxicants have been identified and bioassayed properly, and when synergistic and/or antagonistic interactions do not interfere. Because one would not expect this list of bacterial mutagens to be inclusive of all the mutagens and because one cannot presently rule out all possible types of interactions, this type of calculation would estimate only the lower limits of mutagenicity as will be clearly demonstrated below.

Upon testing an acetone extract of SS particles, Löfroth et al. (1983) found the extract preferentially mutagenic in *S. typhimurium* TA98 in the presence of S9. The observed response using TA98 corresponded to 15 000 revertants per cigarette. Upon using a cigarette-smoking machine in a 15-m^3 room, Löfroth and Lazaridis (1986) calculated that their results represent the equivalent of 30 000 revertants per cigarette for MS and 10 000–20 000 revertants per cigarette for SS when using *S. typhimurium* TA98 with S9 activation. In 1987, Ling et al., using the same methods, observed for strain TA98 a range of 17 200–31 300 revertants per cigarette for SS organics tested using the plate incorporation protocol and a range of 36 000–118 300 revertants per cigarette using a micro-assay preincubation protocol. In addition, they observed significant activity using TA100 without

TABLE 2

THE GENOTOXICITY OF COMPOUNDS ASSOCIATED WITH ENVIRONMENTAL TOBACCO SMOKE

Compound	CAS Number	Bioassay results [a]	
Acetamide	60-35-5	CCC	+
		CT	+
		ST	NEG
Acetic acid	64-19-7	ST	NEG
Acrolein	107-02-8	ST	+
Anthracene	120-12-7	CCC	I
		CT	NEG
		CYC	NEG
		MNT	NEG
		ST	NEG
Benz[*a*]anthracene	56-55-3	CCC	+
		CT	+
		REC	NEG
		ST	+
		V79	+
Benzene	71-43-2	CCC	+
		CYI	+
		MNT	+
		SCE	NEG
		TRM	+
Benzo[*a*]pyrene	50-32-8	CCC	+
		CT	+
		CYC	NEG
		MDR	+
		MNT	+
		MNT	+
		MST	+
		SCE	+
		SRL	+
		ST	+
		V79	+
Benzo[*b*]fluoranthene	205-99-2	CCC	+
Benzo[*e*]pyrene	192-97-2	CCC	I
		CT	NEG
		REC	NEG
Benzo[*ghi*]perylene	191-24-2	ST	+
Benzoic acid	65-85-0	ST	NEG
Butyrolactone, gamma	96-48-0	ST	NEG
Cresol, *m*-, *o*-, and *p*-	95-48-7	ST	NEG
Dibenz[*a*, *j*]anthracene	224-41-9	ST	+
Formaldehyde	50-00-0	ASPD	+
		CCC	+
		MDR	+
		NEU	+
		REC	+
		SRL	+
		SRL	+
		ST	+
Hydrazine	302-01-2	CCC	+
		ST	+

TABLE 2 (continued)

Compound	CAS Number	Bioassay results [a]	
Hydroquinone	123-31-9	ALC	+
		ST	NEG
Indeno[1, 2, 3-*cd*]pyrene	193-39-5	CCC	+
Isoquinoline	119-65-3	ST	NEG
Limonene	5989-27-5	ST	NEG
Naphthalene	91-20-3	ST	NEG
Nicotine	54-11-5	NEU	NEG
		ST	NEG
Nitrosodiethylamine, *N'*-	55-18-5	CCC	+
		CT	+
		CYC	+
		L5	+
		MDR	+
		MST	NEG
		REC	+
		SCE	+
		ST	+
		V79	+
Nitrosodimethylamine, *N'*-	62-75-9	ARA	+
		CCC	+
		CT	+
		CYG	+
		CYG	NEG
		L5	+
		MNT	+/−
		MST	NEG
		NEU	+
		SCE	+
		SRL	+
		SRL	+
		ST	+
		V79	+
		YEA	+
Nitrosonornicotine	16543-55-8	CCC	+
Nitrosopyrrolidine	930-55-2	CCC	+
Perylene	198-55-0	SCE	NEG
Phenanthrene	85-01-8	ALC	+
		CCC	I
		CT	NEG
		CYC	NEG
		ST	NEG
Phenol	108-95-2	NEU	NEG
		ST	NEG
Pyrene	129-00-0	CCC	I
		CT	NEG
		CYC	NEG
		ST	NEG
		V79	NEG
Pyridine	110-86-1	SCE	+
		ST	NEG

TABLE 2 (continued)

Compound	CAS Number	Bioassay results [a]	
Styrene	100-42-5	CCC	+
Toluene	108-88-3	SCE	NEG
		ST	NEG

[a] Bioassay information is extracted from Graedel et al., 1986. Abbreviations used for bioassay results are as follows: ALC, Allium cytogenetics assays; ARA, Arabidopsis mutagen assay; ASPH, Aspergillus mutagen assay; CCC, whole animal carcinogen assays; CT, cell transformation bioassays; CY, mammalian cytogenetic bioassays; L5, L5178Y mouse lymphoma assay; MDR, mammalian cell DNA repair assays; MNT, micronucleus assays; MST, mouse spot test; NEU, Neurospora assays; REC, DNA repair-deficient bacterial assays; SCE, sister-chromatid exchange assays; SRL, sex-linked recessive lethal assays in Drosophila; ST, Salmonella assays; TRM, Tradescantia mutagen assays; V79, V79 Chinese hamster mutation assays; and YEA, Yeast mutation tests. Results are recorded as +, positive; NEG, negative; and I, Indefinite.

activation. Ong et al. (1984) examined the mutagenicity of SS using *S. typhimurium* TA98W (an antibiotic-resistant strain of TA98) in an in situ impinger system. They stated that '... the concentration of cigarette smoke that could be detected for mutagenic activity was 0.0065 cigarette per ml.' Because the concentration of cigarette smoke in these experiments was equivalent to approximately 0.036 cigarettes/ml and the observed response was approximately 40 induced mutants/ml, they (Ong et al., 1984) observed 1111 [(40 rev/ml) · (1 ml/0.036 cig)] revertants per cigarette. However, if one assumes from their calculations that 0.0065 cigarettes would give a doubling (~ 20 induced revertants) of the spontaneous revertant number, the activity of SS is approximately 3080 [(20 rev/doubling) · (1 doubling/0.0065 cigarettes)] revertants per cigarette. The 3- to 10-fold difference between the results of the Löfroth et al. (1983) and Ong et al. (1984) is most likely due to differences in the collection and exposure systems used, although, once again, one cannot rule out the role of differences in chemical interactions. Löfroth et al. (1983) collected only particulate matter and tested the extracted organic material, whereas Ong et al. (1984), while attempting to test total SS, probably assayed primarily the

nonparticulate, semi-volatile organic material that would dissolve in the fluid medium. Although the methods of Löfroth et al. (1983) and Ong et al. (1984) did not identify the specific genotoxicants responsible for the biological activity, the direct bioassay of the emissions did provide a more accurate determination of the total mutagenic potential of a cigarette.

Knowing the amounts and bioassay activity of SS constituents or the total mutagenic activity of a 'typical cigarette' also does not necessarily indicate that one can accurately estimate human exposure to genotoxic (ETS) emissions. For both MS and SS, the amount of a specific material that is emitted from a cigarette ranges from sub-nanogram per cigarette levels to milligram per cigarette levels. The concentrations of constituents in smoke-impacted spaces range from ng/m^3 to mg/m^3; however, the calculation of ambient concentrations from cigarette emission rates is not always simple because ETS levels are functions of smoking rate, ventilation, sink rate, mixing, and volume of the space (National Research Council, 1986). Also, each individual compound may be removed from the ambient air at different rates due to these functions. For example, genotoxicant exposure levels can be approximated by measuring respirable particulate (RSP) levels and measuring the level of specific genotoxicants associated with the particles. RSP levels in a one-smoker residence can vary by 3 orders of magnitude from approximately 17 to 5000 $\mu g/m^3$ (National Research Council, 1986). Similarly, most IA concentrations (Table 1) for specific compounds span at least 2 orders of magnitude; however, the relative amounts of components with SS are often different from the relative amounts found in IA samples. It is also unlikely that all SS carcinogens have been identified. By collecting indoor air particulate matter and bioassaying the extracted organic matter using *S. typhimurium* TM677 in modified bioassay, Lewtas et al. (1987) demonstrated that particle-associated mutagenicity per cubic volume of air was greatest in the homes with cigarette smoking and correlated with the number of cigarettes smoked. The mutagenic activity per cigarette, however, could not be calculated in this study due to the presence of other potential sources of mutagens such as woodstoves, gas appliances, cooking, etc. Recently, Husgafvel-Pursiainen et al. (1986) demonstrated that indoor airborne particulate matter collected in 3 restaurants where smoking occurred gave up to 2370 revertants/m^3 air. On the basis of optical particle counting, they attributed the majority of the airborne particulate matter to cigarette smoking; however, they also did not estimate mutagenicity on a per cigarette basis. Husgafvel-Pursiainen et al. (1986), however, did show that the levels of polynuclear aromatic compounds roughly correlated with the mutagenic activity. Human exposure to tobacco-smoke genotoxicants, therefore, is highly variable and difficult to assess by evaluating individual components and/or equivalent bioassay activity levels emitted into ambient air.

In order to explore the use of bacterial bioassays in evaluating ETS, our laboratory has examined some of the alternative methods that can be used for evaluating ETS. The approaches used are briefly described in the Materials and methods section. As part of one study (Morin et al., 1987), filters used to collect SS particles were extracted with various solvents, solvent-exchanged to DMSO, and bioassayed. Depending upon the extraction conditions, tester strain, and activation conditions the revertants per cigarette ranged from 400 to 19 000 (Fig. 1). Results using TA98 with S9 of ~ 19 000 revertants/cigarette resemble data obtained by Löfroth et al. (1983).

Because the work of Ong et al. (1984) demonstrated that the semi-volatile and volatile components of SS may be mutagenic, we decided to assess the total mutagenic potential of both MS and SS. Using the sequential trapping train method described, we collected volatile, semi-volatile, and particulate-bound organics simultaneously from both MS and SS using separate trapping trains. Each of the fractions from both trains was bioassayed separately in the Salmonella mutagenicity assay. No mutagenicity was associated with the cold trap (volatile fraction) of either the MS or SS when evaluated as gases in the Tedlar dessicator system (Hughes et al., 1987). Table 3 summarizes the results for the other components of the trapping trains. Both the bubbler and sand trap samples were mutagenic for MS and SS; therefore, both the particulate and the semi-volatile components were mutagenic. The revertants/cigarette

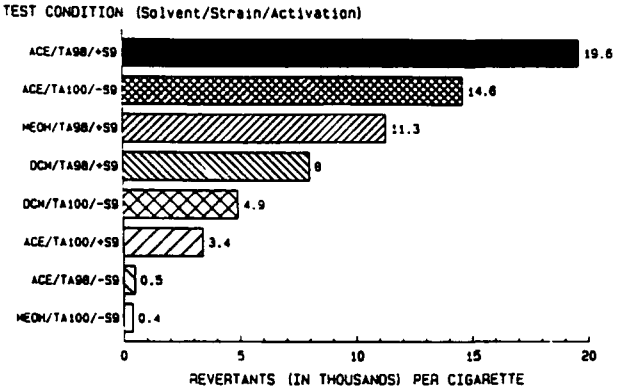

Fig. 1. *Salmonella typhimurium* mutagenicity of sidestream cigarette smoke when tested using various solvent extraction systems, strains of *S. typhimurium*, and activation conditions. Abbreviations are as follows: ACE, Acetone; DCM, Dichloromethane; MEOH, Methanol; TA98 and TA100 for respective strains of *S. typhimurium*; +S9, with exogenous activation; and −S9 without exogenous activation.

value of 35 200 is approximately twice the 19 000 and 15 000 values previously seen with particle-associated fractions; however, when the cigarette machine was not used to generate a puff mode sample and the cigarettes were allowed to smolder, this value was reduced to 23 250 revertants per cigarette. It has been demonstrated previously that the products produced during puffing and natural smoldering are somewhat different due to generated temperatures, the rate of mass transfer of oxygen to the tobacco source, properties of the cigarette paper, etc. (Baker, 1981, 1982). Therefore, it is reasonable that the mutagenicity of both MS and SS varies with the degree of puffing. The effect of burn conditions can also be noted by examining the revertants/μg of organic material values in Table 3. Compared on a per mass of organic material basis, the MS organics are 2–3 times as mutagenic as the SS organics. This demonstrates that the mainstream and sidestream combustion processes produce different relative amounts of bacterial mutagens. One also could speculate – from knowledge of the 2 types of combustion processes and differences in MS and SS chemistry – that the quantitative distribution of mutagens in MS and SS is different. Due to the mass of organic material produced, however, SS organics make a larger contribution to the total mutagenic activity of a cigarette. It is interesting to note that within our laboratory the response of benzo[*a*]pyrene (B[*a*]P) in *Salmonella typhimurium* TA98 with exogenous activation is approximately 20 revertants/μg; therefore, one can calculate that total cigarette bacterial mutagenicity is equivalent to ~2.6 mg of B[*a*]P. Approximately 70% (61 600/86 300) of the total mutagenicity is associated with the sand trap (particulate) samples. This proportion also is approximately the same for the MS and SS samples. Overall, the SS sample accounted for approximately 60% of the total mutagenicity on a per cigarette basis.

The best way presently available to assess human exposure to genotoxic ETS compounds is by the analysis of the fluids and tissues of exposed individuals. Exposure of target tissues and sites to genotoxic constituents of ETS depends on several factors, including the number of cigarettes smoked

TABLE 3

THE *SALMONELLA TYPHIMURIUM* TA98 MUTAGENICITY OF MAINSTREAM AND SIDESTREAM TOBACCO SMOKE COLLECTED IN A PUFF MODE

Fraction	Ethanol bubbler		Sand trap		Bubbler + sand
	Rev/μg [a]	Rev/cig [b]	Rev/μg	Rev/cig	Rev/cig
Main	8.1	7557	4.0	26400	33957
Side	2.7	17099	1.6	35200 [c]	52299
Total		24656		61600	86256

[a] *S. typhimurium* TA98 mean revertants per plate per μg of organic material collected using a preincubation bioassay. Determined using a linear regression model (Myers et al., 1981)

[b] *S. typhimurium* TA98 revertants per cigarette calculated from (Rev/μg)×(μg of fraction/cigarette).

[c] When puff mode was not used and the cigarettes were allowed to smolder, this value was 23253 rev/cig.

in an enclosed area, the size and nature of the area, the degree of room ventilation, breathing rates and volume, absorption of the genotoxicants, body distribution and excretion, and metabolism. The uptake of individual agents from ETS can be determined using chemical methods. For example, one can measure products such as thiocyanate, carboxyhemoglobin, nicotine and cotinine in physiological fluids. However, some of these (e.g., thiocyanate due to HCN exposure and carboxyhemoglobin from CO exposure) do not originate exclusively from SS (National Research Council, 1986). The quantitative aspects of other chemical and biochemical markers such as cotinine, hydroxyproline, *N*-nitrosoproline, and aromatic amines are still somewhat questionable (National Research Council, 1986). In addition, when one bioassays an agent individually, it may not be known whether or not different genotoxic compounds are absorbed, distributed, metabolized, and excreted at the same rate as each other. The use of a noncompound-specific measurement of exposure to genotoxicants, therefore, would be very advantageous. The monitoring of nonsmokers' urine for mutagenic potential using bacterial assays provides a possible means of evaluating in vivo exposure to ETS genotoxicants. In 1977, Yamasaki and Ames reported the presence of bacterial mutagens in the urine of smokers. As noted by IARC (1986), a number of studies have confirmed the finding of Yamasaki and Ames; however, there is a wide variation in the results of these studies. Much of this variation may be due to dietary factors (Sasson et al., 1985). The studies that have examined the urine mutagenicity of passive smokers (Putzrath et al., 1981; Bos et al., 1983; Sorsa et al., 1985) demonstrated increased mutagenicity in adult passive smokers. These studies generally examined small numbers of people and did not control for dietary factors; therefore, the results might be considered somewhat ambiguous. In spite of the shortcomings, these studies did support the use of bacterial bioassays as a screening tool for human exposure to ETS genotoxicants; however, more effort is needed in improving and standardizing the methods and in creating the proper controls for other environmental factors such as diet.

In summary, our studies support previous studies that demonstrated that ETS particle-bound organic material is mutagenic. In addition, our studies demonstrated that some semi-volatile and volatile components were mutagenic; however, the highly volatile compounds (for both MS and SS) collected in the third-stage liquid nitrogen cold trap were not mutagenic. Within these studies, the total mutagenicity was divided among 4 fractions approximately as follows: SS sand trap (particulate) fraction, 40%; MS sand trap (particulate) fraction, 30%; SS solvent (semi-volatile) fraction, 20%; and MS solvent (semi-volatile) trap fraction, 10%. Results also gave an indication that the frequency with which a cigarette is puffed affects the total amount of mutagenic material produced. Although these studies illustrate the usefulness of bacterial mutagenicity bioassays for characterizing ETS, there are also other uses (e.g., identifying specific genotoxicants) for which bacterial assays will find great utility.

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Characterization of Environmental Tobacco Smoke

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■ Environmental tobacco smoke (ETS) has been analyzed with respect to several components following smoking of research cigarettes in an experimental chamber. Parameters analyzed and their airborne yield per cigarette included particulate matter (10 mg) and its mutagenic activity in a *Salmonella* bioassay, carbon monoxide (67 mg), nitrogen oxides (2 mg), nicotine (0.8–3.3 mg), formaldehyde (2 mg), acetaldehyde (2.4 mg), acrolein (0.56 mg), benzene (0.5 mg), and several unsaturated aliphatic hydrocarbons (e.g., 1,3-butadiene) of which isoprene (3.1 mg) had the highest yield. ETS from commercial cigarettes was likewise analyzed in the experimental chamber and at a public location. The relative component composition for ETS is similar when generated from either research or commercial cigarettes. All components analyzed were present at concentrations above the background concentrations. Isoprene might be utilized as a tobacco smoke tracer for unsaturated aliphatic hydrocarbons.

Introduction

Environmental tobacco smoke (ETS), which is derived primarily from sidestream smoke emitted between puffs, is a major contributor to indoor air pollution wherever smoking occurs (*1*, *2*). ETS differs both chemically and physically from the precursor sidestream smoke, presumably due to chemical and physical transformations that occur as the mixture is diluted and aged. Chemical characterization studies have focused on mainstream and sidestream smoke (*1*). Data are lacking, however, on the presence and concentration of potentially toxic and carcinogenic components in tobacco-smoke-polluted indoor environments. An ideal ETS tracer air contaminant is not available for total ETS exposure (*2*), although nicotine is the best available tracer.

In this study we investigated the concentration of a number of genotoxic components as well as potential tracers of ETS under controlled and environmental conditions. Some of the components measured are routinely monitored air pollutants including carbon monoxide, nitrogen oxides, and particulate matter. A series of aldehydes and alkenes were measured in these studies, including several that are carcinogenic. The mutagenicity of the particulate phase was assayed in *Salmonella typhimurium*. Nicotine was measurd as an ETS tracer. Indoor chamber experiments were performed at the EPA facility at the University of North Carolina, Chapel Hill, partly in conjunction with studies on the urinary cotinine (nicotine metabolite) concentration and excretion rate in young children following exposure to sidestream cigarette smoke (*3*). Indoor measurements were also made in a tavern.

Experimental Section

Chamber and Smoking. The tests were performed in a 13.6-m^3 Plexiglas chamber (*4*) set at a ventilation rate of 3.55 air changes h^{-1}; in addition, air removed by the sampling added ~0.50 air changes h^{-1}. The air in the chamber was circulated by a fan at 1.35 m^3 h^{-1}. The temperature and the relative humidity are given in Table I.

Research cigarettes of the type 2R1 (*5*), which had been equilibrated at 22 °C at 60% relativity humidity for 48 h, were smoked by machine (RM30, Heinr. Borgwalt, Hamburg, FRG). One cigarette was lighted every 30 min and was smoked with a 35-mL puff of 2 s every minute until extinguished after ~12 min. Mainstream smoke was vented to the outside of the chamber. The cigarettes weighed ~1.2 g, of which 0.9–1.0 g was consumed.

One adult and one child were present in the chamber during the 4-h tests in the first series of nine experiments. Six additional experiments were performed with the research cigarettes smoked by machine later in a second series, including two tests with no smoking, two tests (13 and 14) similar to the first series, (one cigarette every 30 min), and two tests (15 and 16) with one cigarette every 15 min. In tests 15 and 16, decay of components in the chamber was measured. Subsequently, in a third series of chamber tests, the emissions from two different commercial cigarette brands (A and B, both low-tar and nicotine brands) were analyzed in the chamber with regular smoking by one person without any applied ventilation.

Sampling and Analysis. Particle Sampling and Analysis. Total suspended particles (TSP) were collected in duplicate on preweighed Teflon-coated glass fiber filters (Pallflex) at 1.7 m^3 h^{-1} by modified Anderson samplers consisting of the 10-mm preseparator and the backup filter. TSP was also measured continuously by an Electric Aerosol Analyzer, EAA (Thermo-System, Inc., Model 3030), with measurements taken every 9 or 10 min. Particles were also collected in triplicate with personal sampling pumps (Model P4000, Du Pont, Kennett Square, PA) at 1.7 and 3 L min^{-1}.

Nicotine. Nicotine was collected on bisulfate-impregnated filters (*6*) placed downstream from the particle filters on the personal samplers (first series) or on both Anderson and personal samplers (second series). Extraction and gas chromatography analysis of nicotine was performed as described by Hammond et al. (*6*).

Particle Mutagenicity. The filters were extracted by sonication in dichloromethane, and the extract was brought to a fixed volume. Aliquots of the solution were distributed into 4-mL vials together with 5 μL of dimethyl sulfoxide (DMSO) and then evaporated by nitrogen gas at 35 °C. The vials were kept capped at −20 °C until bioassayed.

The mutagenicity was determined by a microsuspension assay developed by Kado et al. (*7*) and modified by DeMarini et al. (in preparation) using *Salmonella* TA98 in the presence of S9 (*8*). The microsuspension was modified by using a bacterial suspension concentrated 5 times in-

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Table I. Average Chamber Concentrations (±SD) and Average Airborne Yields for Carbon Monoxide, Nitrogen Oxides, Total Suspended Particles, Particle Mutagenicity, and Nicotine during Smoking of One Cigarette Every 30 Minutes (Tests 1–9, 13, and 14) and Every 15 Minutes (Tests 15 and 16) in a 13.6-m^3 Chamber with an Air Exchange of 3.55 h^{-1}.

component	series I 1–9	series II 13 and 14	15 and 16	airborne yield per cigarette[a]
relative humidity, %	53 ± 4	29 ± 1	34 ± 0	*b*
temperature, °C	24 ± 0.5	23 ± 0	23 ± 0	*b*
carbon monoxide, mg/m^3	2.48 ± 0.29	1.79 ± 0.81	4.76 ± 0.21	67 mg
nitric oxide, μg/m^3	68.0 ± 6.0[c]	61.0 ± 2.8	139 ± 6	*b*
nitrogen oxides,[d] μg/m^3	72.8 ± 6.9[c]	65.0 ± 8.5	139 ± 9	1950 μg
total suspended particles, μg/m^3				
by mass	349 ± 39	321 ± 25	934 ± 46	10 mg
by EAA[e]	349 ± 45[f]	513 ± 47	1223 ± 84	*b*
mutagenicity, revertants/m^3				
personal sampler	628 ± 49	nd[g]	nd	17300 revertants
Anderson sampler	494 ± 58	517 ± 40	837 ± 76	13400 revertants
nicotine, μg/m^3				
personal sampler 1–9	29 ± 7	nd	nd	800 μg
personal sampler 13–16	nd	127 ± 23	228 ± 49	3300 μg
Anderson sampler 13, 14	nd	150 ± 14	nd	*b*

[a] Uncorrected for surface removal. [b] Not applicable. [c] Based on eight tests. [d] Expressed as NO from total concentration of NO and NO_2. [e] Assuming unit density. [f] Based on four tests. [g] nd, not determined.

stead of 10 times, 0.015 M phosphate-buffered saline instead of 0.15 M, no shaking during the 90-min incubation of the vials at 37 °C, and addition of histidine and biotin to the plate bottom agar instead of to the top agar.

The combined sample from the duplicate Anderson filters from each experiment was tested with six doses corresponding to 25–300 L of air in duplicate tests with duplicate vials for each dose and test. The combined sample from the personal filters from each experiment was tested with three doses corresponding to 50–200 L of air in one test, with triplicate vials for each dose. The response was calculated by linear regression using doses on the linear or almost linear part of the dose–response curve.

Carbon Monoxide and Nitrogen Oxides. Carbon monoxide (CO) was measured continuously by nondispersive infrared absorption (Bendix 8501-5), and nitrogen oxides NO_x (i.e., NO plus NO_2) were measured indirectly by chemiluminescence (Bendix 8101-B). Data points were recorded every 3 min.

Hydrocarbons. Air was collected in evacuated stainless steel canisters (*9*), and the sample was then subjected to speciated gas chromatographic analysis by the method described by McElroy et al. (*10*). Samples in the first experimental series were collected as grab samples at a peak concentration of carbon monoxide in the chamber, whereas samples in the second series were collected over the entire smoking period (4 h).

Aldehydes. Aldehydes were collected in the second series at a rate of 1.0 L min^{-1} using 2,4-dinitrophenylhydrazine-coated silica gel cartridges for collection and high-performance liquid chromatography for analysis of the hydrazone derivatives as described by Tejada (*11*).

Calculations. The average chamber concentrations were calculated as the average value between 1 h after start until the end of the experiments. When sampling included the first hour, the average concentration was calculated by normalizing to the continuous CO concentration; this correction was approximately 5%. Likewise, grab samples of hydrocarbons were normalized to the average concentration from the peak concentration, when the sample had been collected. The airborne yield, expressed as amount per cigarette, was calculated from the average concentration by using the known smoking frequency, the chamber volume, and the total air exchange rate.

Environmental Sampling. The impact of tobacco smoke was determined in two studies in a local tavern. The main room in which sampling took place had a volume of ~180 m^3 (l = 15 m, w = 4 m, and h = 3 m) and was variously occupied by 5–25 persons, many of whom were smoking.

Indoor TSP and nicotine were collected on a Teflon-coated glass fiber filter and a second bisulfate-impregnated filter, respectively, at 20 L/min by an Anderson sampler. Particulate matter was measured by taking 120-s readings each 1/2 h over the 3- or 4-h study with a piezobalance Model 3500 (TSI Inc., St. Paul, MN) both indoors and outdoors with at least two cleaning cycles per hour. Indoor and outdoor carbon monoxide was determined with two General Electric Model 15ECS3CO3 carbon monoxide detectors (Wilmington, MA) that had been calibrated at zero and 60 ppm CO. Indoor aldehydes and indoor and outdoor hydrocarbons were collected and analyzed as described for the chamber studies. The hydrocarbon sampling was performed during only 2 h in each of the two studies.

Results

The concentrations and calculated yields are given in Table I for components that were analyzed in all chamber tests in the first and second series. Carbon monoxide and nitrogen oxides were determined continuously every 3 min, and their concentrations varied in a saw-toothed form with the smoking cycle of one cigarette every 30 min. The ratio of the average maximum to the minimum concentratiuon was ~3. The average concentration of carbon monoxide was about 65–70% of the maximum concentration; similar ratios were found for nitrogen oxides.

Particle concentrations measured by EAA had the same type of variation, but the resolution was less because the analyses were performed less frequently. The average concentration of particles as measured by EAA (assuming unit density) was in good agreement with the concentration obtained by filter collection in the first series and overestimated the particle concentration in the second series under lower relative humidity. Due to the organic character of ETS, however, the density would be expected to be somewhat less than 1.0.

The nicotine concentrations and yields were lower during the first series than during the second series, with yields of 800 μg/cigarette and 3300 μg/cigarette, respectively. There were several differences in the two series. In the first series, the chamber contained more adsorbant surfaces: two persons, mother and child, television set, crib, chair, and a curtain, all of which were absent in the

Table II. Selected Hydrocarbon Concentrations ($\mu g/m^3$) in Background Air and in the 13.6-m^3 Chamber with Sidestream Cigarette Smoke, and Their Airborne Yields

	grab samples at peak concn 1 cigarette/30 min					continuous sampling						
						1 cigarette/15 min			1 cigarette/30 min			
		test					test			test		
hydrocarbon	backgrd	1	2	6	av concn[a]	backgrd	13	av concn[a]	backgrd	15	av concn[a]	av airborne yield,[b] μg/cigarette
ethene	65	116	105	136	35	42	92	50	57[c]	155	98	1200
ethane	5	63	93	69	48	16	77	61	23	161	138	1600
propene	2	74	77	64	46	3	48	45	2	107	105	1300
propane	9	54	56	50	29	12	39	27	15	84	69	830
1,3-butadiene	<1	22	24	18	14	0.5	11	11	0.6	20	19	400
isoprene	11	189	231	128	128	2	94	92	8	233	225	3100
benzene	3	35	38	29	20	4	19	15	3	40	37	500

[a] Above the background concentration. [b] Uncorrected for surface removal. [c] Estimated as ethene, as ethene and acetylene cochromatographed.

Table III. Selected Aldehyde Concentrations ($\mu g/m^3$) in Background Air and in the 13.6-m^3 Chamber with Sidestream Cigarette Smoke, and Their Airborne Yields

sample	form-aldehyde	acet-aldehyde	acrolein
test 11, no smoking	14	16	<0.2
test 12, no smoking	14	12	<0.2
test 13, 1 cigarette/30 min	80	87	18
test 14, 1 cigarette/30 min	84	94	19
test 15, 1 cigarette/15 min	170	205	49
test 16, 1 cigarette/15 min	173	233	39
av airborne yield,[a] μg/cigarette	2000	2400	560

[a] Uncorrected for surface removal.

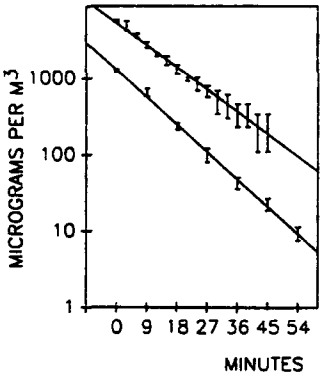

Figure 1. Decay of carbon monoxide (upper curve) and particle (lower curve) concentrations in the chamber after the end of smoking. The values represent the average of tests 15 and 16 with bars giving the highest and lowest values.

second series. The relative humidity was about 50–60% during the first series but only about 30% in the second series. Although surface area is likely to alter surface deposition, relative humidity has also been shown to effect surface deposition (B. Leaderer, personal communication).

Hydrocarbon concentrations and calculated yields are given in Table II. There was a good agreement between the two different series, the first of which comprised three grab samples at peak smoke concentration and the second with continuously collected samples. Among the hydrocarbons analyzed, ethene had an exceptionally high background, which has been previously observed (J. J. Bufalini and B. W. Gay, Jr., personal communication). The analyses of selected aldehydes are given in Table III together with the calculated yields. The four tests gave results in close agreement with each other.

The limited study of the decay of carbon monoxide and particles measured by EAA that were performed in tests 15 and 16 (Figure 1) showed that these components had a mean ventilation time of about 14 and 11 min, respectively. The ventilation time of carbon monoxide corresponds to the predetermined air exchange rate of ~4 changes/h. The disappearance of particles is ~25% faster than the disappearance of carbon monoxide under the experimental conditions used.

Table IV. Peak Concentrations of Five ETS Components from Smoking of Two Commercial Cigarette Brands and Comparison with the Average Chamber Concentrations with 2R1 Research Cigarettes

	cigarette		2R1 av chamber
component	brand A (2)	brand B (1)	concn[a]
carbon monoxide, mg/m^3	9.8	5.1	2.4
nitrogen oxides, $\mu g/m^3$	280	130	71
particles by EAA, mg/m^3	*b*	1.5	0.46
ethene, $\mu g/m^3$	300	110	45
isoprene, $\mu g/m^3$	550	280	110

[a] Recalculated from Tables I and II. [b] Above the maximum measurable concentration of the EAA.

Table V. Air Pollutant Concentrations Indoors in a Tavern and Simultaneously in the Ambient Outdoor Air

	first study (870424) 3 h		second study (870501) 4 h	
component	indoor	outdoor	indoor	outdoor
particles, $\mu g/m^3$				
TSP by weight	470	nd[a]	390	nd
by piezobalance	420	<50	320	40
mutagenicity, revertants/m^3	500	nd	600	nd
nicotine, $\mu g/m^3$	71	nd	60	nd
carbon monoxide, mg/m^3	4.4	<1	4.8	1–2
aldehydes, $\mu g/m^3$				
formaldehyde	104	nd	89	nd
acetaldehyde	183	nd	204	nd
acrolein	24	nd	21	nd
hydrocarbons,[b] $\mu g/m^3$				
ethene	110	12	56	16
ethane	180	9	68	8
propene	70	6	40	6
propane	70	6	33	7
1,3-butadiene	19	<1	11	1
isoprene	150	<1	85	2
benzene	27	6	21	8

[a] nd, not determined. [b] Only 2 h of sampling.

ETS from two commercial brands of cigarettes was analyzed following smoking of 1 or 2 cigarettes by a smoker located in the chamber. Although the airborne yields cannot be calculated, as only peak concentrations were measured, the relative proportions of the components show that the composition of the smoke is similar to that from research cigarettes (Table IV).

Finally, available portable equipment was used to sample and analyze the air in a tavern during normal smoking conditions. All ETS components that were analyzed indoors and outdoors were highly elevated in the indoor environment (Table V). Furthermore, none of the analyzed components was conspicuously much higher or much lower relative to each other than what would have been expected from the studies of the airborne yield of research cigarettes.

Discussion

This study characterizes both exposure concentrations and airborne yields for particulate matter and its mutagenic activity, as well as nicotine, aldehydes, and alkenes. This study provides documentation that the chamber ETS exposure was comparable to that which people would encounter in indoor environments where tobacco is being smoked. Additional chemical analyses and subsequent studies were conducted to relate the chamber ETS components to the analysis of ETS in an indoor environment.

ETS and other air pollutants emitted into an indoor environment can disappear by three routes: ventilation, surface deposition, and chemical reactions while airborne. The ventilation rate in most indoor environments is 0.5 air change/h or higher, which means that the time frame of interest is a few hours or less. Whereas few, if any, studies have dealt with chemical reactions of ETS components, there is good evidence that smoke particles can be removed by surface deposition (*2*, *12*), the process being dependent on surface characteristics and mixing ratio. Thus, the airborne yield of particulate tar can vary depending on the experimental conditions.

Among the gases studied, carbon monoxide is considered to be sufficiently stable to be removed only by ventilation. This is also probably the case for the low molecular weight hydrocarbons, ethene to isoprene, analyzed in the present investigation, whereas nicotine as well as the aldehydes may decay by surface adsorption or reaction. This phenomenon may account for the divergent nicotine concentrations found in the first and second series (Table I), because the chamber was different with respect to surface characteristics in these two series.

Nitric oxide (NO) is the primary nitrogen oxide formed in tobacco smoke (*13*), but it can slowly be oxidized to nitrogen dioxide, NO_2 (*14*), or species detected as NO_2. The low contribution of NO_2 to the total concentration of nitrogen oxides found in the present study (Table I) most likely reflects the high ventilation rate, which would not give sufficient time for the formation of NO_2 from NO in the chamber. In contrast, about 15–25% of the nitrogen oxides detected in the smoking of commercial cigarettes (Table IV) was in the form of NO_2, indicating that both the higher concentration and the lower ventilation rate in these tests resulted in a significant conversion.

The determined ETS airborne yields of carbon monoxide, nitrogen oxides, and nicotine (Table I) are about the same as those reported for sidestream smoke from commercial cigarettes (*13*, *15*, *16*). The ETS yield of particles is, however, lower by a factor of 2–3 than those reported for sidestream smoke from commercial cigarettes (*16*). One study of 2R1 cigarettes by Ueno and Peters (*17*) found only 6–9 mg of particulate matter/cigarette based on a sample collected with an Anderson cascade impactor and 1–2 mg/cigarette based on EAA measurements.

The mutagenic yield of particulate matter, 13 400–17 300 revertants/cigarette, is lower than the mutagenic emission, 36 500–118 000 revertants/cigarette, for sidestream cigarette smoke collected in a small hood (*18*). These differences may be due to the differences between the sample collection methods for sidestream smoke and ETS and between the emission rate and airborne yield measurements. In both cases, the loss of mutagens associated with particles is likely due to loss of the particles to surfaces.

The mutagenicity concentrations and yields determined on particle extracts from the personal sampler were consistently higher than those from the modified Anderson sampler. The personal samplers have a 2-fold lower face velocity (1.3 cm s^{-3}) compared to the Anderson sampler (2.7 cm s^{-1}). The higher face velocity of the Anderson sampler may result in the loss of the more volatile organics and mutagens from the filter. Recent studies comparing face velocities substantiate this hypothisis (K. Hammond et al., unpublished data). The mutagenic response of 400–900 revertants/m^3 of air is a range that may be encountered in moderately smoky environments and is higher than that found for ambient outdoor air (*19*).

Airborne yield is a direct measure of the components present in a particular indoor environment and will vary with the surface area and characteristics. The advantage of this measurement is that it can be directly used to estimate indoor ETS compound concentrations based on the number of cigarettes smoked and the ventilation. Emission factors for sidestream cigarette smoke have classically been determined by using a small-volume collecting device surrounding the cigarette tip (*16*), and emission factors for ETS particles and nicotine have been determined in chambers with correction of surface removal (*6*). Thus, it can be expected that airborne yields are less than emission factors for components that are significantly removed by processes other than ventilation.

The emission of aliphatic hydrocarbons in sidesteam smoke has not been assessed quantitatively previously, although there are several earlier studies on mainstream smoke that have been summarized by Elmenhorst and Schultz (*20*). The airborne yields for sidestream smoke found in the present study (Table II) are generally higher than those found for mainstream smoke. Isoprene is the predominant unsaturated hydrocarbon in sidestream smoke (Tables II, IV, and V), and the concentrations measured were well above the background concentrations. There are several other sources for isoprene: It is exhaled by man (*21*, *22*) and rodents (*23*) and thus possibly by other animals. It is emitted from vegetation, with ambient concentrations generally below 15 mg/m^3 (*24*, *25*). It is also produced during combustion, but the most likely combustion source, wood combustion, gives much less isoprene than ethene (*26*), indicating that isoprene is a minor constituent of the hydrocarbon emission. The carbonyl compounds studied (Table III) had airborne yields of a magnitude reported earlier (*15*).

The results presented in Table IV, obtained in the smoking of commercial cigarettes in the chamber, show that there is a similar relative distribution of major components from such cigarettes when compared to the airborne yields from the research cigarettes 2R1. This is also the fact for components measured in a tavern (Table V) in which a mixture of commercial cigarettes was being smoked. These comparisons between research cigarettes and commercial cigarettes show that both types of cigarettes give rise to ETS with very similar composition.

The ratio of nicotine to particles is ~160 μg/mg for the tavern samples (Table V), which is intermediate between the ratios that can be calculated for the chamber study, 80 and 330 μg/mg. This may indicate that the tavern has surface characteristics with respect to nicotine removal intermediate between the occupied and unoccupied Plexiglas chamber.

It is well-known that tobacco smoking causes cancer, and recently a series of epidemiological studies reviewed by the National Research Council (*2*) and others (*27, 28*) have reported excess lung cancer deaths in individuals exposed to ETS. Cancer from passive smoking at sites other than the lung is also a possibility (*29*).

It is not known which of the many components present in tobacco smoke and ETS are the most hazardous. It is therefore important to analyze ETS for a variety of components comprising both particulate matter and gas-phase constituents. We have in the present study determined particulate matter and used a *Salmonella* mutagenicity bioassay to measure genotoxicity (*30*). Among the numerous gas-phase compounds in ETS, aldehydes (*1*) and unsaturated aliphatic hydrocarbons (*31*) are known or potential animal carcinogens. Although these compounds have a relatively low carcinogenic potency, they might be of importance in the total evaluation because they are present in relatively high concentration. Among the unsaturated hydrocarbons, isoprene might be used as a tobacco smoke tracer considering the low background concentration of this compound. Studies are needed to examine other potential indoor sources of isoprene that could interfere with its use as a tracer.

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Registry No. Carbon monoxide, 630-08-0; nitric oxide, 10102-43-9; nitrogen oxides, 11104-93-1; nicotine, 54-11-5; ethene, 74-85-1; ethane, 74-84-0; propene, 115-07-1; propane, 74-98-6; 1,3-butadiene, 106-99-0; isoprene, 78-79-5; benzene, 71-43-2; formaldehyde, 50-00-0; acetaldehyde, 75-07-0; acrolein, 107-02-8.

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Corrections:

mm should be μm as follows:

p. 610, col. 2, para. "Sampling and Analysis. Particle Sampling and Analysis.", line 4: consisting of the 10-μm preseparator and the backup filter.

p. 613, col. 2, para. 3, line 14: [esoprene is emitted from vegetation, with ambient] ... concentrations generally below 15 $\mu g/m^3$ (24, 25). It is

ENVIRONMENTAL TOBACCO SMOKE: MUTAGENIC EMISSION RATES AND THEIR RELATIONSHIP TO OTHER EMISSION FACTORS

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Abstract

The objective of this study was to evaluate the emission rates and exposure concentrations of mutagens, nicotine, and particles from cigarettes. Studies were conducted under controlled laboratory and chamber conditions as well as in personal residences. The mutagenicity of environmental tobacco smoke (ETS) was evaluated in three bioassays using two strains of Salmonella typhimurium. Strain TA98 was used in the standard plate-incorporation and microsuspension histidine reversion assays; and strain TM677 in a microsuspension forward mutation assay. The mutagenicity, expressed either per µg particle mass or per µg nicotine, appeared to be a relatively constant factor that did not vary significantly between various cigarette brands. These data are being used to model the emissions of mutagens to predict mutagenic exposure concentrations under various conditions.

Introduction

The concentration of mutagens associated with particulate matter in indoor air is substantially increased in the presence of tobacco smoke (1, 6, 7). In a residential field study of 10 homes, we found the mutagenicity per cubic meter of air sampled to be highly correlated with the number of cigarettes smoked (4). Recent studies of the mutagenicity of ETS from cigarettes either smoked or smoldering, suggest that the mutagenicity emitted per cigarette is relatively constant (5, 7). Controlled chamber studies showed that the emission rate of nicotine and particles per cigarette from sidestream smoke is also relatively constant (2). The purpose of this study was to determine the mutagenic emission rate and its relationship to respirable particulate matter and nicotine in environmental tobacco smoke (ETS).

Methods

Environmental Tobacco Smoke (ETS) Generation, Sampling and Analysis

The smoking experiments, test chamber, and sample collection methods used are similar to those described in detail by Hammond et al. (2) and are summarized here. ETS was generated by 4 smokers smoking at the rate of 8 cigarettes per hour in a 34-m^3 chamber. In these experiments, the chamber fresh air supply rate was typically 2.6 air changes per hour with a temperature of 19°C and relative humidity of 45%. Particulate matter was collected on 37-mm Teflon-coated glass fiber filters or on 0.5-µm millipore type FH. Vapor-phase nicotine was collected downstream from the filters with a second filter impregnated with sodium bisulfate. The particulate filters were extracted with acetone by sonication and solvent exchanged into dimethyl sulfoxide for bioassay by using a stream of dry nitrogen to evaporate the acetone. The sodium bisulfate-treated filters were extracted and analyzed for nicotine as described by Hammond et al. (2). Equilibrium sampling and calculation of emission rates per gram of tobacco consumed were determined as described by Hammond et al. (2) for nicotine.

Mutagenicity Assays

The principal bioassay employed in this study was a forward mutation assay described originally by Skopek et al. (9) that uses *S. typhimurium* strain TM677. A microsuspension modification of this assay, described by Lewtas et al. (4), was used to measure mutant frequency (MF) (mutants per 10^6 surviving cells) by plating and counting both 8-azaquanine-resistant (mutant) colonies and surviving colonies. The mutagenicity in this bioassay was compared to that obtained in *Salmonella typhimurium* strain TA98 that was conducted by either (a) the plate-incorporation assay of protocol of Maron and Ames (8), which will be referred to here as the standard Ames assay, or (b) a microsuspension assay described originally by Kado et al. (3) and modified in our laboratory by using 5x cell concentration; 0.015M phosphate buffer, pH 7.4; and a reduced assay volume that will be referred to here as the microsuspension reverse assay.

All of the bioassays were performed with the addition of Aroclor 1254-induced male Sprague-Dawley rat liver S9 mixture prepared as described in the above-referenced protocols. The initial linear portion of the dose-response curve was used to determine the mutagenicity expressed as either revertants per mg of particles, per m^3 of air, or per cigarette. In all assays both solvent controls (negative) and positive controls were assayed simultaneously as described in the protocols (9, 8, 3).

Results and Discussion

The mutagenicity of ETS was compared across six brands including brands both high and low in tar and nicotine, filtered and unfiltered as shown in Table 1. Despite the significant differences in the cigarette brands, very little difference in the mutagenicity of ETS was observed when measured either per µg of total suspended particulate (TSP) or per m^3.

The mutagenic emission rates shown in Table 1 were also very similar across all six brands and ranged from 22,000-37,000 MF/g tobacco consumed. The emission rate per cigarette would range from 11,000-18,500 MF/cig., assuming that 0.5 g of tobacco is consumed per cigarette smoked.

The ratio of mutagenicity to TSP ranged from 0.7 to 1.4 MF/µg TSP. The TSP and nicotine emission rates for these six brands was determined in a similar series of experiments. The ETS nicotine emission rates (1.6-2.4 mg nicotine/g tobacco consumed) are very similar to that reported earlier by Hammond et al. (2) for four brands. Only Brand E (2.2 mg nicotine/g tobacco) is identical to Brand B (1.8 mg nicotine/g tobacco) in this earlier study. Using this nicotine data from separate experiments, the ratio of MF to nicotine in ETS ranged from 8-16 MF/µg nicotine. This agrees with subsequent experiments not reported here where we have measured both mutagenicity and nicotine on the same samples.

The blank chamber experiment was conducted without smokers present. The TSP and mutagenicity concentrations in this nonsmoking experiment are typical of outside urban air (4).

Table 1: Comparison of mutagenicity of ETS across several brands and mutagenic emission rates compared to nicotine and particles

			Emission Rates			
Cigarette Brand	TSP ($\mu g/m^3$)	Mutant Frequency[a] per m^3	Mutagenicity (MF/g)	TSP[b] (mg/g)	Ratio of MF to TSP (MF/µg)	Ratio of MF to Nicotine[b] (MF/µg)
A*	1177	1178	35,400	35.4	1.0	10
B	1200	1259	25,900	24.7	1.0	16
C†	936	1186	34,600	27.3	1.3	15
D†	905	1280	37,100	26.2	1.4	12
E	1318	907	21,900	32.0	0.7	8
F†	901	1025	30,400	26.5	1.1	-
Blank Chamber	25	91				

[a]Microsuspension forward mutation assay in TM677. Determined from five separate experiments from the linear portion of the dose-response curves.
[b]TSP and nicotine emission rates from these brands were determined in separate chamber studies using the same protocol reported here. The nicotine emission rates in mg nicotine/g tobacco consumed were 2.4 (A), 1.6 (B), 1.6 (C), 2.1 (D), and 2.2 (E).
*Unfiltered cigarette, all others are filtered.
†Low tar and nicotine

Table 2: Comparison of ETS mutagenicity per µg of TSP in different bioassays

ETS Sample	Bioassays		
	Micro TM677 (MF/µg/ml)	Standard TA98 (rev/µg)	Micro TA98 (rev/µg/ml)
Chamber Brand E[a]	0.69	2.9	-
Chamber 2R1[b] Research Cigarette	0.79	1.6	7.0

[a]ETS from studies in 34 m^3 chamber at Yale U.; other data shown in Table 1.
[b]ETS from studies in 13.6 m^3 chamber at EPA using cigarette smoking machine. Ratio of nicotine to TSP was 87 µg nicotine/mg TSP.

ETS is present in indoor air in sufficiently high concentrations to measure the mutagenicity in the standard Ames plate-incorporation assay, which requires approximately 10 times the sample mass of the two microsuspension assays. Table 2 provides a comparison of the mutagenicity of ETS per µg of TSP for several different cigarettes. Although it is possible to use the standard Ames assay to measure the mutagenicity of either sidestream cigarette smoke or ETS, there is one important advantage to using a microsuspension assay. The microsuspension assays require 3-10 times less sample, thereby permitting mutagenicity measurements on samples of less than 1 m^3 of air. These methods will facilitate evaluation of the mutagenicity of personal air samples in human exposure assessment studies.

Disclaimer

The research described in this paper has been reviewed by the Health Effects Research Laboratory, U.S. Environmental Protection Agency and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the Agency nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

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MUTAGENIC DETERMINATION OF PASSIVE SMOKING*

(Environmental tobacco smoke; ETS; *Salmonella*/microsome mutagenicity test)

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SUMMARY

The mutagenic activity of tobacco smoke has been further investigated with the plate-incorporation method and a microsuspension technique of the Ames *Salmonella* assay. The microsuspension test gives a higher response than the conventional plate incorporation test. It is possible to detect environmental tobacco smoke (ETS) in moderately smoky indoor environments by collection of particulate matter with personal low volume samplers followed by particle extraction and mutagenicity testing with the microsuspension assay.

INTRODUCTION

Environmental tobacco smoke (ETS) is a very complex mixture of compounds formed in the pyrolysis and combustion of tobacco products. Many of the components are also formed in other anthropogenic combustion processes making it difficult or impossible to discriminate between ETS and other sources if these components are used as indicators. Tobacco specific components are nicotine, some N-nitrosamines and probably also some N-heterocyclic hydrocarbons and aromatic amines [1] of which nicotine is being used for the specific determination of ETS [2,3].

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Abbreviations: DMSO, dimethyl sulfoxide; ETS, environmental tobacco smoke.

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The Ames *Salmonella* mutagenicity test has earlier been employed to show that the mutagenic response of indoor airborne particulate matter is high in the presence of habitual smoking and that the mutagenic activity mainly is associated with basic components making it possible to discriminate the activity of ETS from other combustion sources [4,5].

Although the *Salmonella* plate incorporation assay is highly sensitive, the use of low or medium volume air samplers in indoor environments may require assay methods which give higher responses. We are therefore studying the mutagenic activity of tobacco smoke in a microsuspension assay described by Kado and coworkers [6] as giving responses several times those obtained with the conventional plateincorporation test.

METHODS

Sampling

Mainstream cigarette smoke was collected on glass fiber filter with a smoking machine which was operated under normal conditions as described earlier [5]. The machine was placed in a 0.2 m^3 hood and the emitted sidestream smoke was collected on glass fiber filter with a flow rate of 10 m^3/h [5].

Environmental samples were collected with a battery-operated system (AFC 123 Personal Air Sampler, Casella London Ltd., England) on glass fiber filter (diam. 2.5 cm) with a flow rate of 2 l/min.

For the present study, personal sampling was performed in an apartment during a party representing a highly smoke-polluted environment and in an office with one smoker representing more common indoor smoke conditions.

The apartment, having a volume of about 100 m^3, was sampled for 8 h during which it was well ventilated with open windows; 82 butts were found at the end.

The office, having a volume of about 50 m^3 and a ventilation rate exceeding 5 changes per hour, was sampled for 8 h on two separate days during which 20 and 25 cigarettes were smoked, respectively.

Extraction and sample preparation

Filters were extracted by sonication with acetone.

For the preparation of samples for the plate incorporation mutagenicity assay, an aliquot of the extract was reduced to a small volume and then diluted with an appropriate amount of DMSO [4,5]. The DMSO solution was stored at $-20°C$ prior to and between mutagenicity tests.

For the preparation of samples for the microsuspension mutagenicity test, aliquots of the acetone extract were dispensed into 8 cm × 1.1 cm sample tubes together with 5 μl DMSO. The samples were then evaporated under a stream of nitrogen gas in a heating block at a temperature $<40°C$ and the tubes sealed with silicon rubber stoppers and stored at $-20°C$ until they were assayed.

Mutagenicity assays

Mutagenicity was determined with the Ames *Salmonella* tester strains TA98 and TA100 using the plate incorporation method [7] and the microsuspension method described by Kado et al. [6]. Slight differences exist between the performance of the mutagenicity assays as described by Löfroth and Lazaridis [5] and by Claxton [8] and Austin et al. [9]. The liver-S9 was obtained from Aroclor 1254-induced male Sprague–Dawley rats. Addition of S9 was generally with 10% S9 in the S9-mix, i.e. 50 μl S9 per plate for the plate-incorporation assay and 10 μl S9 per tube for the microsuspension assay.

RESULTS

The mutagenic activity of the cigarette smoke samples is given in Table I and is expressed as revertants per cigarette. The results obtained with the plate incorporation assay agree qualitatively and quantitatively with the previous study [5]. Mainstream cigarette smoke is mainly mutagenic in the presence of S9. The sidestream smoke is also active in the presence of S9, but has, in addition, mutagenic activity in the absence of S9 with the TA100 strain.

The mutagenic response in the microsuspension assay is higher and the enhance-

TABLE I

THE MUTAGENIC RESPONSE OF SIDESTREAM AND MAINSTREAM CIGARETTE SMOKE USING THE *SALMONELLA* PLATE INCORPORATION AND MICROSUSPENSION ASSAYS

Sample	Stream type	Collected particulate tar mg/cig.	Revertants per cigarette[a] Plate incorporation assay				Microsuspension assay			Ratio micro-susp./plate	
			TA98		TA100		TA98		TA100	TA98	TA100
			−S9	+S9	−S9	+S9	−S9	+S9	−S9	+S9	−S9
Swedish brand	side-	21.8	2300	31 300	23 800	34 200	16 300	81 900	118 200	2.6	5.0
	main-	17.6	< 1000	20 900	4 200	16 000	-[b]	54 300	37 300	2.6	8.9
Research brand 2R1	side-	17.0	1500	27 500	17 100	19 300	28 300	118 300	108 800	4.3	6.4
	main-	33.3	1400	52 100	4 700	21 900	13 000	137 000	67 800	2.6	14
American generic	side-[c]	10.7	-	-	-	-	-	36 500	25 000	-	-
American generic	side-[d]	10.5	< 1000	17 200	5 800	14 200	16 000	59 900	58 300	3.5	10

[a] The response has been estimated from the linear or almost linear dose-response curves with at least three doses.

[b] Not tested.

[c] This sample was collected, extracted and assayed at the Genetic Bioassay Branch.

[d] This sample was collected at the Genetic Bioassay Branch, EPA and brought to Sweden for extraction and assay; the filter was at room temperature during the 14 h transfer.

ment over the plate incorporation assay is about three-fold with TA98 in the presence of S9. The response with TA100 in the absence of S9 is also enhanced with the microsuspension assay.

The difference in the response obtained with the same type of sample from American Generic cigarettes extracted and assayed in two different laboratories is real; similar differences have been obtained with other tobacco smoke samples indicating the need for normalizing the results between laboratories.

The results of two representative indoor samples assayed by the microsuspension method are given in Table II. The sample from the highly polluted apartment was judged to contain sufficient activity for four dose series; two of these were used for assays with TA98 in the presence of S9 and two with TA100 in the absence of S9. The samples from the moderately smoky office were each only subdivided into one dose series which were assayed with TA98 in the presence of S9.

The mutagenic responses detected in these indoor environments agree with magnitudes which can be estimated from the air volume, the air exchange rate, number of cigarettes smoked and their emission factor and considering that the concentrations are modulated by surface deposition [5,10].

TABLE II

THE MUTAGENIC ACTIVITY OF INDOOR PARTICULATE MATTER COLLECTED WITH A LOW VOLUME SAMPLER IN ONE HIGHLY AND ONE MODERATELY SMOKY ENVIRONMENT AS ASSAYED WITH THE *SALMONELLA* MICROSUSPENSION TECHNIQUE

Apartment during party			Office with one smoker[a]	
Dose	Revertants per plate[b]		Dose	Revertants per plate[c]
Liter air per plate	TA98 + S9	TA100 − S9	Liter air per plate	TA98 + S9
0[d]	41	251	0[d]	65
16	89	356	64	81
32	187	483	128	114
64	271	785	256	175
128	451	1091	512	263
(rev./m³ air)[e]	(3200)	(6800)	(rev./m³ air)[e]	(400)
Positive controls				
0.5 μg benzo[a]pyrene	435	–[f]	0.25 μg 2-amino-anthracene	1660
1.0 μg sodium azide	–	1020		

[a] These samples were collected, extracted and assayed at the Genetic Bioassay Branch, EPA.
[b] Average of duplicate plates.
[c] Average of two samples from the two different days.
[d] Spontaneous control determined with three or more plates.
[e] Estimated from the linear part of the dose-response curve.
[f] Not tested.

DISCUSSION

The *Salmonella* microsuspension mutagenicity assay is sufficiently sensitive that it is possible to detect the smoking of a few cigarettes in an average apartment or house with a normal ventilation of 0.5–1 air changes per hour during a sampling period of 8 h with a personal sampler having a flow rate of 2–10 l/min.

The major part of the mutagenic activity of environmental tobacco smoke is caused by basic components [4,5], and if deemed necessary, simple fractionation and mutagenicity test of the basic fraction can show whether a sample comprises tobacco smoke. The *Salmonella*/microsome mutagenicity assay may thus be an alternative or an adjunct to nicotine as a specific indicator of environmental tobacco smoke. Further studies are, however, required to characterize indoor sources of mutagenic activity as there may exist other sources of basic mutagenic components.

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THE EFFECT OF SOLVENT AND EXTRACTION METHODS ON THE BACTERIAL MUTAGENICITY OF SIDESTREAM CIGARETTE SMOKE

(Soxhlet extraction; sonication; *Salmonella typhimurium*; dichloromethane; methanol; acetone; storage)

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SUMMARY

The mutagenic activity of sidestream cigarette smoke particles was estimated by testing sidestream cigarette smoke particles which had been collected under controlled burning conditions in the laboratory. Two different extraction methods (Soxhlet and ultrasonic agitation) and 3 different solvents (dichloromethane, methanol, and acetone) were compared for their efficiencies in the extraction of compounds from sidestream cigarette smoke particles which are mutagenic in the Ames test. The mutagenic activity of the sidestream smoke particles was estimated to be 15 000–20 000 revertants per cigarette in TA98 with metabolic activation and 12 000–17 000 revertants per cigarette in TA100 without metabolic activation. Only weak mutagenic activity was detected in TA98 without activation and in TA100 with activation. Under test conditions used, ultrasonic agitation produced the most consistent results and acetone extraction produced the highest levels of mutagenic activity.

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Abbreviations: ETS, environmental tobacco smoke.

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INTRODUCTION

Environmental tobacco smoke (ETS) is composed of sidestream tobacco smoke and exhaled tobacco smoke. Sidestream smoke enters the ambient air directly from the burning tobacco source. Exhaled tobacco smoke is any combination of tobacco smoke products (mainstream and sidestream) that have been inhaled and then exhaled by an individual. The percentage of each mainstream smoke component exhaled into the environment has been reported to range from as low as 1% to as high as 70% [1,2]. The ratios of the various smoke components in sidestream smoke compared to mainstream smoke have been reported to range from 1.2- to 46-fold [3]. A typical cigarette smoker inhales mainstream smoke for approximately 3 s, 8–10 times per cigarette. This is equivalent to a total of 24–30 s out of a total average burn time of 12 min for each cigarette [4]. Sidestream cigarette smoke, therefore, is produced during 96% of the total time a cigarette is burning.

It has been estimated that sidestream smoke forms approximately 85% of the total tobacco smoke in the ambient indoor air [5]. Therefore, both smokers and non-smokers could be exposed, especially in indoor spaces with low levels of air exchange, to significant levels of airborne pollutants.

Although a great deal of effort has been expended characterizing the harmful constituents of mainstream tobacco smoke, much less is known about the toxic components of sidestream tobacco smoke. Rickert et al. [6] tested 15 different brands of cigarettes and reported that in most cases considerably more tar, nicotine, and carbon monoxide were present in the sidestream than in the mainstream smoke. The findings from a 1983 study demonstrated that indoor air contaminated with sidestream cigarette smoke can be more mutagenic than ambient outdoor air in an urban area [7]. It was reported that the smoke from one cigarette which is diluted in a structure with a volume of 300 m^3 would result in mutagenic activity 2.5 times that found in urban ambient air.

More than 2000 compounds have been identified in mainstream tobacco smoke [8]. Since sidestream smoke would be expected to be similarly complex, it is difficult to identify the specific mutagenic constituents. A recent report by the National Research Council [9] says 'Research is needed to standardize both the collection and evaluation of ETS so that the effects of ETS can be studied in the laboratories'. They also recommend that 'Further in vitro assays of ETS are needed'. The purpose of this paper is to examine and compare appropriate methods for preparing sidestream smoke samples for mutagenicity studies.

MATERIALS AND METHODS

Collection of samples

Sidestream cigarette smoke particles were collected by allowing a generic brand (Price Breaker, Winn-Dixie Stores, Jacksonville, FL) of king-size filter cigarettes

(738.5 mg tobacco/cigarette, average of 10 cigarettes), to burn in a Plexiglas chamber measuring 32 cm × 38 cm × 29 cm (volume = 0.0353 m^3). The air inside the chamber was exhausted by a constant flow vacuum pump at a rate of 0.028 m^3/min (1 cu. ft. of air per min). The air was pulled through a 142 mm diameter Teflon-coated glass fiber filter (Pallflex Products Corp., Putnam, CT) supported in a stainless steel filter stand. Filtered air was drawn into the chamber through three 1 cm diameter openings in the bottom of the chamber.

Before sample collection, all filters were heated for 24 h at 175°C and then desiccated for 24 h. After sampling, each filter was folded to enclose the sample and rewrapped in aluminum foil. All samples were returned to the laboratory after collection, usually within 2 h. To minimize sample degradation or reaction, samples were stored on the filters in the dark at −70°C.

Sample preparation

During preparation of the samples for mutagenicity testing, all solvents were glass distilled, high purity, spectroscopic grade (Burdick and Jackson, Muskegon, MI). To reduce artifact contamination only glass, stainless steel, and Teflon equipment was used in the preparation of samples. All materials were washed with Alkonox and consequently rinsed with distilled water, 50% nitric acid, 50% sulfuric acid, distilled water, methanol, and extraction solvent.

Ultrasonic extraction. Each 142 mm diameter filter was divided into 8 replicate pieces using stainless steel scissors. Each replicate was placed in a 50 ml glass vial with a Teflon-lined screw cap along with 25 ml of solvent. One of 3 different solvents (dichloromethane, methanol, or acetone) was used to extract the organic material from the filter pieces. The vials were held in a plastic rack and placed in a water bath sonicator (Sonicor, Model 401, Clean Room Products, Bay Shore, NY) for 15 min at 25°C. Solvent was then decanted into a clean vial. The samples were reextracted for an additional 15 min with 25 ml of fresh solvent. After the second sonication, the extracts were combined to yield a sample volume of approximately 50 ml.

Soxhlet extraction. Each replicate was placed in a 500 ml extraction flask with 250 ml of solvent and extracted for 24 h at a rate of 2–3 cycles/h using a standard Soxhlet apparatus (Kontes Glassware, Vineland, NJ).

Refluxing of cigarette smoke extracts. Additional filters containing sidestream cigarette smoke particles were extracted with acetone using the sonication procedure above. The extracts were then split into 4 replicates. Three of the 4 replicates were placed in individual 500 ml extraction flasks with 100 ml of acetone. The flasks were attached to a Soxhlet extractor and refluxed for 8, 16, and 24 h respectively.

Sample concentration and solvent exchange. After each of the above procedures, the extraction solvent was removed by rotary evaporation in individual 250 ml round-bottom flasks. The samples were placed in a 40°C water bath during the evaporation procedure. Each sample was concentrated to a volume of approximate-

ly 5–10 ml and then quantitatively transferred into 15 ml volumetric tubes. The samples were concentrated to a volume of 1–2 ml by immersing the tubes in a 45°C water bath while a stream of dry nitrogen was bubbled through the sample using stainless steel needles. A known volume of DMSO was added to each sample and the remaining extraction solvent was evaporated using the nitrogen purge method. Representative samples were analyzed by gas chromatography prior to bioassay to check for adequate removal of the extraction solvent. In all cases, an unused filter from each lot was extracted to determine background levels of mutagenic activity due to the filter.

Salmonella mutagenicity testing

The *Salmonella typhimurium* plate incorporation assay was performed as described by Ames et al. [10], with 2 modifications consisting of: (1) adding a trace amount of histidine to the agar base layer instead of the overlay agar and (2) incubating the plates for 72 h instead of 48 h. All samples were tested using Ames *Salmonella typhimurium* tester strains TA98 and TA100 with and without exogenous metabolic activation (S9) unless otherwise indicated. The S9 was prepared in the manner described by Ames et al. [10] from Aroclor-induced, male CD-1 rats. Tester strains were obtained from Dr. Bruce Ames, University of California, Berkeley, CA and maintained by the Genetic Bioassay Branch, Genetic Toxicology Division, Health Effects Research Laboratory, U.S. EPA Environmental Research Center, Research Triangle Park, NC. All dose levels were run in triplicate, unless otherwise noted. Negative controls for spontaneous reversion and positive controls for each strain were performed in triplicate for each experiment. The control chemicals used were 2-aminoanthracene for both strains with metabolic activation. Without metabolic activation, 2-nitrofluorene and sodium azide were used as positive controls for TA98 and TA100 respectively. Each sample was tested at 4–5 doses (5, 10, 50, 100, 300 μl/plate). In most cases, these doses corresponded to 0.008, 0.016, 0.08, 0.16 and 0.48 cigarettes respectively.

All plates were counted using an automatic plate counter (Artek Model 880, Artek Systems Corp., Farmingdale, NY). Each plate was counted 3 times and a mean for the 3 counts was recorded. Data analysis was accomplished using the Ames test curve fitting program of Stead et al. [11]. This program is a FORTRAN program which fits a non-linear dose-response curve to the plate count data. Using the model slope obtained from the dose-response curve, an estimate of mutagenicity was calculated and expressed as the number of revertants per cigarette.

RESULTS

Effect of extraction method and solvent

Tables I and II present the bioassay results of the experiments designed to compare the efficiencies of 2 different extraction methods and 3 different extraction

TABLE I

THE EFFECT OF EXTRACTION METHOD AND SOLVENT ON THE MUTAGENIC ACTIVITY OF SIDESTREAM CIGARETTE SMOKE PARTICLES TESTED IN *SALMONELLA TYPHIMURIUM* TA98 WITH METABOLIC ACTIVATION

Average of 4 experiments, 5 doses, 3 replicates per dose, analysis by the method of Stead et al. [11].

Extraction method	Number of revertants (thousands) per cigarette: as a function of extraction solvent		
	DCM[a]	MEOH[a]	ACET[a]
Soxhlet	12.2 ± 10.2	10.9 ± 7.5	11.4 ± 5.1
Ultrasonic	8.0 ± 3.2	11.3 ± 3.7	14.6 ± 1.8

[a]DCM, dichloromethane; MEOH, methanol; ACET, acetone.

TABLE II

THE EFFECT OF EXTRACTION METHOD AND SOLVENT ON THE MUTAGENIC ACTIVITY OF SIDESTREAM CIGARETTE SMOKE PARTICLES WHEN TESTED IN *SALMONELLA TYPHIMURIUM* TA100 WITHOUT METABOLIC ACTIVATION

Average of 4 experiments.

Extraction method	Number of revertants (thousands) per cigarette: as a function of extraction solvent		
	DCM[a]	MEOH[a]	ACET[a]
Soxhlet	0.7 ± 1.0	0.7 ± 1.2	1.9 ± 3.6
Ultrasonic	4.9 ± 3.7	0.4 ± 0.2	14.6 ± 6.5

[a]DCM, dichloromethane; MEOH; methanol; ACET, acetone.

TABLE III

THE EFFECT OF REFLUXING ON THE MUTAGENIC ACTIVITY OF SIDESTREAM CIGARETTE SMOKE PARTICULATE EXTRACT

Number of hours refluxed	Number of revertants (thousands) per cigarette: *S. typhimurium* strain/activation conditions			
	TA98/+ S9	TA98/− S9	TA100/+ S9	TA100/− S9
0[a]	19.6	0.5	3.4	14.6
8	15.3	1.9	3.8	1.9
16	17.2	3.8	4.4	3.6
24	16.2	1.1	3.6	3.6

[a]Extract tested for activity without refluxing.

TABLE IV

THE EFFECT OF STORAGE ON THE MUTAGENICITY OF SIDESTREAM CIGARETTE SMOKE PARTICLES

Storage conditions and time	Number of revertants (thousands) per cigarette	
	TA98 + S9	TA100 − S9
Collected, extracted, bioassayed in less than 6 days	17.1	14.6
Extract stored frozen at −70° C in DMSO for 23 days	15.9	3.3
Extract stored frozen at −70° C in DMSO for 60 days	5.1	1.7
Particles stored frozen at −70° C in DMSO for 60 days	9.3	2.1
Particles stored frozen at −70° C in DMSO for 180 days	11.5	0.4

solvents. For TA98 results with metabolic activation (Table I), the differences between the 2 methods and 3 solvents were small. The activity in TA100 without metabolic activation (Table II) was almost equal to that in TA98 with activation, but only when ultrasonic agitation and acetone were used to extract the particles.

Effect of refluxing cigarette smoke extracts

Table III contains the mutagenicity data from the experiments conducted to determine whether or not the refluxing of sidestream cigarette smoke extracts affects their mutagenic activity. The mutagenic activity in TA98 with metabolic activation was not significantly affected by refluxing the extracts for up to 24 h. An increase in activity in TA98 without metabolic activation was seen after refluxing. Significant reductions in activity, however, were observed in TA100 without metabolic activation after the extracts were refluxed.

Effect of storage time and conditions

Sidestream cigarette smoke particles and extracts were stored for different lengths of time as either particles or as extracts in DMSO. Following storage, the samples were tested for mutagenic activity in the Ames test. The results of this experiment are contained in Table IV.

Substantial reductions in activity were observed in both TA98 with activation and in TA100 without activation. The largest reductions in activity were seen in TA100 without metabolic activation after the extracts had been frozen for 180 days in DMSO. The reduction in activity in TA98 was much less than the reduction in TA100 especially when the samples were stored as particles rather than as extracts in DMSO.

DISCUSSION

As early as 1974, Kier et al. [12] demonstrated the mutagenicity of mainstream

cigarette smoke condensate in *Salmonella typhimurium* strain TA1538 with a metabolic activation system prepared from polychlorinated biphenyl-induced rat liver tissue. Since 1974, numerous investigators have reported mutagenic activity for mainstream cigarette smoke and cigarette smoke condensates in a variety of mutagenicity test systems [14–18]. More recently, extracts of sidestream cigarette smoke particles have also been shown to be mutagenic in the Ames test [7,20]. As in previous studies [7], the highest mutagenic response in this study (15 000–20 000, revertants per cigarette) was observed in TA98 with metabolic activation (Table III).

Method of extraction

Although Soxhlet extraction is the most widely used procedure for the removal of organic compounds from airborne particulate samples, it has potential for artifact formation and is a lengthy procedure usually requiring a minimum of 24–36 h. During this extraction process there is opportunity for sample loss due to volatilization. In addition, since this method requires a substantial amount of glassware and laboratory hood space, it is often not practical when numerous samples are extracted in a short period of time. Soxhlet extraction also has been reported to result in the decomposition of some known mutagenic compounds [21–23].

Ultrasonic agitation, an alternate method for the extraction of airborne particles, has been reported to yield recoveries which are comparable to Soxhlet extraction [23–26]. Although this method requires less time, glassware, and supplies, the most important advantage of sonication may be the reduction in the opportunity for sample loss. A number of extraction solvents have been used in both methods including benzene, methanol, acetone, dichloromethane, acetonitrile, ethanol, and mixtures of 2 or more of these solvents [27].

A number of studies have compared these extraction methods for ambient air particles [24,25,28–31]. In a recent investigation, Rives [32] compared the efficiencies of sonication and Soxhlet extraction of woodsmoke particles. Using both mutagenic activity and extractable mass as the criteria for his evaluation, he concluded that either method can be used for the extraction of mutagenic compounds from woodsmoke. Despite individual preferences for one method over another, the recent literature recommends both techniques equally [26,33].

In this study (Tables I and II), the mutagenicity data indicate that the ultrasonic extraction method is as effective or more effective than the Soxhlet procedure in extracting those compounds from sidestream cigarette smoke particles which are mutagenic in the Ames test. The use of sonication also resulted in more consistent data than that obtained when Soxhlet extraction was used. These findings are in agreement with Sawicki et al. [28] who reported that ultrasonic agitation was a more reproducible extraction method than Soxhlet extraction.

We conclude that both Soxhlet extraction and ultrasonic agitation are effective in the extraction of mutagens from sidestream cigarette smoke particles. Sonication

requires less time and equipment and results in less opportunity for loss of sample during extraction. Thus, sonication would be the favored method in cases where large numbers of samples require extraction in a short period of time.

The choice of a solvent for use in any extraction procedure is usually influenced by the class or classes of compounds for which extraction is desired. Numerous solvents have been used by different investigators for the extraction of organic compounds from air particulate samples for mutagenicity testing in the Ames test [27,34]. Acetone has been widely used by several investigators for air particle extraction; however, some do not recommend its use because of its reactivity with other chemicals and its unavailability in pure form [35]. Other solvents and combinations of solvents, such as cyclohexane [36], benzene/dichloromethane mixture [37], cyclohexane, dichloromethane and acetone serially [38], methanol [39], and benzene [40] have also been used for the extraction of mutagenic material from air particles. Jungers and Lewtas [30] concluded that the preferred solvent for those studies in which the non-mutagenic and sometimes bactericidal mass is to be minimized is dichloromethane. Krishna et al. [25] compared 7 solvents or combinations of solvents and reported that sequential extraction with acetone followed by dichloromethane gave a higher response than acetone alone or acetone in combination with dichloromethane. Of the solvents tested by Krishna et al. [25], cyclohexane resulted in the lowest mutagenic response. Studies published by Jungers et al. [29] and Talcott and Wei [41] report similar findings. In a recently published study, acetone was the extraction solvent chosen by Alfheim and Ramdahl [20] in their study which investigated the mutagenicity of wood and sidestream cigarette smoke. In a comparison of 4 solvent systems, Talcott and Wei [41] using both Soxhlet and ultrasonic reported that acetone extracted more mutagens than benzene, chloroform, and methanol. Using both Soxhlet and ultrasonic extraction methods, Goto et al. [42] concluded that benzene, ethanol or methanol were the solvents of choice for the detection of ambient air particulate mutagenicity in the Ames test.

These studies (Tables I and II) suggest that acetone was the solvent-of-choice among those used for removal of organic compounds from sidestream cigarette particles. Use of methanol or dichloromethane as an extraction solvent greatly reduced the ability to detect mutagens with TA100.

Effect of refluxing

Goto et al. [42] cited the results of reflux experiments in their choice of extraction solvent. Increases or decreases in mutagenic activity were seen when several mutagenic pure compounds were refluxed in various extraction solvents. Several of the solvents evaluated by these investigators resulted in significant changes in the mutagenic activity of pure compounds when these compounds were refluxed in the extraction solvent, including dichloromethane and acetone. The results obtained in this study indicate that refluxing extracts of sidestream cigarette smoke particles does result in an apparent decrease in mutagenic activity in TA98 with metabolic

activation (Table III). Statistical analysis, however, does not demonstrate that this difference is significant. Activity in TA100 without metabolic activation, however, was significantly reduced after refluxing (Table III). These results suggest that the compounds responsible for the mutagenicity in TA100 may be compounds which are volatile or labile and, therefore, are lost during the refluxing process. Another possibility is that a chemical reaction occurs during the refluxing process which results in the transformation of the mutagenic compounds into compounds which are not mutagenic in the Ames test.

While differences are seen in the mutagenicity of replicate samples of sidestream cigarette smoke particles extracted with the 3 solvents compared in this study, these differences are generally not significant. The one significant difference is in the detection of direct-acting mutagens from sidestream cigarette smoke particles when acetone was used (Table II). Extraction using acetone resulted in higher levels of activity than seen with either dichloromethane or methanol.

Effect of storage

It has been reported that cigarette smoke condensate stored at refrigerator temperatures showed no loss of activity after 40 days; however, a 50% drop in activity in TA1538 was reported after the same length of storage time at room temperature [17]. The effect of storage of cigarette smoke particles or extracts on the mutagenicity of these substances was investigated in this study. Substantial reductions in the mutagenic activity of sidestream cigarette smoke particle extracts were observed after storage as either particles or as extracts in DMSO (Table IV). After storage for 60 days at −70°C in DMSO, the activity in TA98 with metabolic activation was reduced to 30% of the 'fresh' sample (less than 6 days storage). Greater reductions in activity were observed in TA100 without metabolic activation, as the samples contained only 12% of the activity of the 'fresh' samples. Storage as particles for 180 days at −70°C resulted in a 33% reduction of activity in TA98 with metabolic activation compared to the 'fresh' sample. These findings suggest that the effect of sample storage should be considered when attempting to evaluate the mutagenic activity of tobacco smoke particles. In general, it would seem prudent to conduct bioassay of the samples as quickly as possible after collection.

CONCLUSIONS

This study clearly demonstrates the mutagenicity of sidestream cigarette smoke particle extracts in the Ames *Salmonella* mutagenicity assay. The level of mutagenic activity of sidestream cigarette smoke particles was estimated from the slope of a dose-response curve calculated using the method of Stead et al. [11]. The number of revertants in the Ames test was estimated to be 15 000–20 000 per cigarette in TA98 with metabolic activation and 12 000–17 000 per cigarette in TA100 without metabolic activation. After refluxing acetone extracts of sidestream cigarette smoke

particles, the mutagenic activity in TA100 without metabolic activation was significantly reduced compared to extracts which were not refluxed.

Sidestream cigarette smoke particles were collected under experimental conditions by burning cigarettes in a chamber and collecting the smoke particles on glass fiber filters. The filters were extracted using either Soxhlet extraction or ultrasonic agitation and one of 3 solvents (dichloromethane, methanol, or acetone). When the combinations of extraction method and solvent were compared for their efficiency in detecting mutagenic activity in the Ames test, the differences in mutagenic activity were slight; however, ultrasonic agitation produced results which were more internally consistent than results with Soxhlet extraction. Of the 3 solvents which were compared, acetone yielded statistically higher levels of activity than the other 2 solvents (dichloromethane and methanol) in TA100 without exogenous activation. Differences in mutagenic activity in TA98 with metabolic activation among the 3 solvents, however, were small.

The effect of storing sidestream cigarette smoke particles and particle extracts on the mutagenic activity was also investigated. Activity in both TA98 with metabolic activation and TA100 without metabolic activation was found to be reduced after storage either as particles or as extracts in DMSO. Storage in DMSO at −70°C for 60 days resulted in a reduction in mutagenic activity of 70% in TA98 with activation. Activity in TA100 without activation was reduced by 88% after the same storage. When stored as particles, the reduction in activity in TA98 was less (33%); however, the decrease in activity in TA100 was over 90%.

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Public exposure to environmental tobacco smoke

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Summary

Airborne particulate matter has been collected by personal samplers in public indoor areas and travel situations with environmental tobacco smoke pollution. Following extraction, the samples were assayed for mutagenicity in the presence of S9 with a sensitive microsuspension test using Salmonella TA98. The mutagenic responses of indoor air from public areas were much higher than those of ambient outdoor air. Depending on the circumstances, the mutagenic response varied in trains and airplanes but the results show that physical separation of non-smoking sections from smoking sections is necessary in order to achieve genuine non-smoking areas. Chemical fractionation and mutagenicity assay of the basic fraction show that Salmonella mutagenicity of airborne particulate matter might be used as a tobacco smoke-specific indicator, as the basic fraction of environmental tobacco smoke contains a large part of the mutagenic activity whereas this is not the case for outdoor ambient airborne particulate matter.

Environmental tobacco smoke (ETS) is a complex mixture of gases and particulate tar matter comprising numerous compounds. ETS is one of the most common air pollutants in industrialized and urban societies as 25–40% of the adult population are smokers and much smoking takes place indoors causing pollution of the air breathed by everyone.

Among several types of pollution indices, Salmonella mutagenicity of airborne particulate matter has been used to study the contribution from ETS in office buildings (Löfroth et al., 1983), restaurants (Husgafvel-Pursiainen et al., 1986) and homes (van Houdt et al., 1984; Alfheim and Ramdahl, 1984; Löfroth and Lazaridis, 1986; Lewtas et al., 1987). In some studies (Löfroth et al., 1983; Löfroth and Lazaridis, 1986) the origin of the mutagenic activity was ascertained by fractionation in which a major part of the activity of ETS was recovered in the basic fraction.

The use of personal samplers for the collection of particulate matter coupled with a more sensitive mutagenicity test, a Salmonella microsuspension assay, was recently explored and found feasible (Ling et al., 1987). These studies have now been extended with measurements of the mutagenic response of airborne particulate matter collected during some typical situations outside home and work where involuntary exposure to ETS can occur. The chemical behavior of ETS as compared

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to outdoor ambient particulate matter with respect to the contribution from the basic fraction has also been further studied.

Materials and methods

Sampling

Indoor airborne particulate matter was collected on glass fiber filters with 2 battery-operated personal samplers (Casella AFC 123, Casella London Inc., Great Britain) using a flow rate of 2 l/min for each sampler and 25-mm sampling heads. The samplers were carried in a small bag keeping the sampling heads near the breathing zone, i.e. no more than about 25 cm below the mouth/nose region. Sampling time varied from less than 1 h to more than 6 h depending on location. The filters were stored in Al-foil at −20 °C within 2 h of the end of the sampling.

Parallel sampling of outdoor airborne particulate matter was performed with a battery-operated portable sampler (Casella HFS 800) using a flow rate of 10 l/min and a 35-mm sampling head. The sampling was made from a car parked at the nearby parking lot with the air intake of the sampler placed outside the car.

Experimental sidestream smoke was collected as described previously (Ling et al., 1987) from machine-smoked cigarettes in a small 0.3-m^3 hood. A high-volume sample of glass fiber filter-collected urban airborne particulate matter was also used for comparison.

Sample preparation

The filters were extracted within 10 days after sampling. Extraction was performed with acetone using a bath-type sonicator. The solution was filtered through a No. 4 glass filter to remove glass fiber debris.

Fractionation with respect to polarity into basic and non-basic fractions was performed by liquid–liquid extraction with diethyl ether and sulfuric acid and sodium hydroxide aqueous solutions as described earlier (Löfroth, 1981).

For the microsuspension assay, aliquots of the extract corresponding to known air volumes were transferred to 11 mm × 75 mm sterile glass tubes containing 5 μl dimethyl sulfoxide (DMSO). The acetone was evaporated by a stream of nitrogen gas with the tubes inserted in a heating block and the evaporating solution was kept at < 40 °C. The tubes were finally stoppered using sterile silicon stoppers and stored at −20 °C until they were used for the bioassay.

For the plate incorporation assay, the extract was evaporated to a small volume and then diluted with DMSO. The samples were stored at −20 °C.

Bioassay

The microsuspension technique (Kado et al., 1983) was used with minor modifications for all environmental samples in the present study. Salmonella TA98 grown for about 13 h with rapid shaking was centrifuged and then resuspended in 1/10 of the original volume in Vogel and Bonner (1956) medium E (instead of phosphate-buffered saline) giving approximately 10^{10} cells/ml of which 0.1 ml was added to the sample tubes.

The S9 was obtained from livers of Aroclor 1254-induced male Sprague–Dawley rats. Its protein content was 33 mg/ml as determined by the method of Lowry et al. (1951). The S9 mix was prepared as described by Ames et al. (1975) but with Vogel and Bonner medium E instead of 0.2 M phosphate buffer for the microsuspension assay. Each sample tube received 0.1 ml S9 mix containing 10% S9 or buffer without S9, NADP and glucose 6-phosphate.

The sample tubes, covered with sterile caps, were then immediately incubated for 90 min at 37 °C with about 175 rpm shaking after which 2 ml top agar containing histidine and biotin were added. Following Vortex mixing, the samples were poured on minimal glucose agar plates. The plates were incubated for 48 h at 37 °C. Revertant colonies were then counted manually.

The plate incorporation assay was performed as described by Maron and Ames (1983). The S9 mix contained 4 or 10% S9 and 0.5 ml was added to each plate. The S9 amounts employed were those which are routinely used for other outdoor ambient and indoor tobacco smoke samples.

Depending on the amount of sample available, each sample was tested repeatedly on several occasions with 1 plate per dose and 3–5 plates for the spontaneous control. Some samples were only available for 1 or 2 independent tests but larger

samples were always tested 3 or 4 times. Each test comprised positive control compounds. Blank filter samples have been assayed and not found to give any detectable mutagenic activity.

The dose response was evaluated with least square linear regression using all plate counts in the linear or approximately linear part of the dose–response curve.

Results

Environmental samples

During this study a number of samples of airborne particulate matter have been collected at locations or travel situations which people may experience in their daily life.

Table 1 relates the results from 2 visits to a shopping center in the northern part of the Stockholm area and 2 visits to the Stockholm Central (railway) station. The shopping center is about 12 m high and has a central 25 m × 45 m indoor plaza with four 45–65-m-long extending alleys. The Central station main hall has an indoor area of 28 m × 119 m and a height of about 15 m. Despite these spacious designs, the indoor pollution, measured as mutagenic response of particulate matter, is high and is higher than the response of simultaneously collected ambient outdoor air.

Samples collected during train travel (Table 2) were obtained in the common type of passenger cars containing 2 compartments separated by a

TABLE 1

MUTAGENIC ACTIVITY AND RESPONSE IN TA98+S9 OF AIRBORNE PARTICULATE MATTER COLLECTED IN INDOOR PUBLIC AREAS AND SIMULTANEOUSLY AT NEARBY OUTDOOR LOCATIONS

Sample location, date and duration	Indoors			Outdoors		
	Dose (l air/plate)	Counts (rev./plate)	Response (rev./m^3)	Dose (l air/plate)	Counts (rev./plate)	Response (rev./m^3)
Shopping center	0	49		0	49	
861222	25	62		75	46	
175 min	50	112	1200	150	58	< 50
	100	177		300	55	
	150	231				
Benzo[*a*]pyrene [a]	0.5 μg	301				
Shopping center	0	51		0	51	
861230	25	52		75	58	
175 min	50	94	1000	150	82	140
	100	165		300	85	
	150	192				
Benzo[*a*]pyrene	0.5 μg	345				
Central station	0	53		0	53	
870116	25	101		200	71	
235 min	50	163	2500	400	149	260
	100	330		600	236	
	200	552				
Benzo[*a*]pyrene	0.5 μg	445				
Central station	0	62		0	62	
870130	25	107		200	59	
235 min	50	178	2200	400	75	< 50
	100	335		600	77	
	200	449				
Benzo[*a*]pyrene	0.5 μg	477				

[a] Concurrent positive control.

sliding door; one for smoking with 20 seats and one for non-smoking with 60 seats. The high responses in the smoking compartment are not unexpected and a dependence on the number of cigarettes smoked is evident. Most samples collected in the non-smoking compartment have a relatively low response but there is one exception in the sample collected 870215. This train was congested and there was much passenger movement with frequent openings of the door; the smell of tobacco smoke in the non-smoking compartment was apparent.

Samples have been collected during 2 short air flights and 2 transatlantic flights (Table 3). The short flights gave only samples sufficient for 1 assay with 1 plate per dose but even with this limitation a dose-dependent increase can be observed resulting in relatively high responses. There is a substantial difference between the two transatlantic flights which may be explained by the fact that the sample of 861029 was collected in a non-smoking seat only 2 rows apart from smoking seats whereas the sample of 870516 was collected in an entire non-smoking section separated from smokers by the stewardess' areas.

Experimental tobacco smoke

The experimental cigarette sidestream smoke (Table 4) was generated from a common Swedish filter brand which in this experiment gave 29.6 mg tar particles/cigarette. This sample was used for comparing the response in the regular assay and

TABLE 2

MUTAGENIC ACTIVITY AND RESPONSE IN TA98+S9 OF AIRBORNE PARTICULATE MATTER COLLECTED DURING TRAIN TRAVEL IN SMOKING AND NON-SMOKING COMPARTMENTS

Sample, date and duration	Dose (l air/plate)	Counts (rev./plate)	Response (rev./m^3)
Smoking	0	48	
860921	71	78	
250 min	143	126	600
5 cig. smoked	286	226	
Benzo[*a*]pyrene [a]	0.5 μg	478	
Smoking	0	62	
870208	75	372	
275 min	150	705	3 500
25 cig. smoked	300	929	
Benzo[*a*]pyrene	0.5 μg	477	
Non-smoking	0	48	
861002	71	49	
250 min	143	60	100
	286	92	
Benzo[*a*]pyrene	0.5 μg	478	
Non-smoking	0	53	
870121	75	61	
270 min	150	75	100
	300	83	
Benzo[*a*]pyrene	0.5 μg	444	
Non-smoking [b]	0	53	
870215	50	79	
240 min	100	117	600
	167	147	
Benzo[*a*]pyrene	0.5 μg	427	

[a] Concurrent positive control.

[b] 2 additional non-smoking samples collected 870210 and 870219 gave responses of 100 rev./m^3.

the presently employed microsuspension assay and for studies of the response of the basic and non-basic fractions of the smoke. For comparison, a sample of urban airborne particulate matter was also investigated and fractionated simultaneously.

The total response of the sidestream smoke obtained in the present study in the microsuspension assay is higher than previously reported values (Ling et al., 1987). This is mainly due to the use of the modified assay technique. Two other experimental cigarette sidestream samples were analyzed in the course of this study and the assays gave 220 000 and 290 000 revertants/cigarette with TA98 in the presence of S9.

The much higher response of the microsuspension assay (Table 4), known from previous investigations (Kado et al., 1983, 1986; Ling et al., 1987), is evident for both the sidestream smoke and the urban particulate sample.

In the plate incorporation assay, the response in TA98 + S9 of the basic fraction of sidestream smoke is about 67% of the total response. This is in agreement with earlier studies in which 67 and 70% were obtained (Löfroth et al., 1983; Löfroth and Lazaridis, 1986). In the microsuspension assay, the response of the basic fraction is about 45% of the total response in TA98 + S9. This lesser relative response of the basic fraction in the microsuspension assay is further supported by the results obtained with 2 environmental samples. The first sample was collected in an appartment during a party and gave a response of 3200 rev./m^3 (Ling et al., 1987) and showed after fractionation that 45% was present in the basic fraction (data not shown). The second sample, collected in the non-smoking section (corner) of a coffee shop in downtown Stockholm (data not shown), had about 36% of the total response of 2200 rev./m^3 in the basic fraction. A contribution of about 400 rev./m^3 from ambient outdoor particulate matter would in this case explain the fractionation result.

Urban airborne particulate matter has very lit-

TABLE 3

MUTAGENIC ACTIVITY AND RESPONSE IN TA98+S9 OF AIRBORNE PARTICULATE MATTER COLLECTED DURING AIR TRAVEL IN NON-SMOKING SECTIONS

Sample, date, flight and duration	Dose (l air/plate)	Counts (rev./plate)	Response (rev./m^3)
Gothenburg–Oslo	0	52	
860919	25	66	
SK886	50	78	500
38 min	75	91	
Oslo–Stockholm	0	52	
860919	25	70	
SK708	50	96	1000
38 min	75	143	
Benzo[*a*]pyrene [a]	0.5 μg	467	
New York–Oslo	0	46	
861029	24	65	
SK902	48	82	800
360 min	96	117	
	192	193	
Benzo[*a*]pyrene	0.5 μg	403	
New York–Stockholm	0	68	
870516	50	87	
SK904	100	96	200
390 min	200	111	
Benzo[*a*]pyrene	0.5 μg	634	

[a] Concurrent positive control.

TABLE 4

MUTAGENIC RESPONSE IN TA98 OF UNFRACTIONATED AND FRACTIONATED EXTRACTS OF CIGARETTE SIDESTREAM PARTICULATE TAR AND AN URBAN PARTICULATE MATTER SAMPLE IN THE REGULAR PLATE INCORPORATION ASSAY AND THE MICROSUSPENSION ASSAY AND THE ACTIVITY OF CONCURRENT POSITIVE CONTROL COMPOUNDS

Sample	Unit	Plate incorporation			Microsuspension	
		−S9	+S9–4%	+S9–10%	−S9	+S9–10%
Sidestream smoke [a]	Rev./cig.					
Crude extract		2700	– [c]	37000	70000	240000
Basic fraction		1000	–	25000	26000	110000
Non-basic fraction		1500	–	5400	19000	75000
Urban particulates [b]	Rev./mg					
Crude fraction		450	520	–	3000	4200
Basic fraction		20	30	–	100	120
Non-basic fraction		460	430	–	2700	2200
Positive controls	Rev./plate					
5.0 μg quercetin		–	–	–	257	–
25.0 μg quercetin		318	–	–	–	–
0.5 μg benzo[*a*]pyrene		–	–	–	–	267
2.5 μg benzo[*a*]pyrene		–	385	207	–	–
Spontaneous control	Rev./plate	26	33	36	26	36

[a] The responses have been evaluated with 6 doses in the range 0.001–0.1 cig./plate in the plate incorporation and in the range 0.00025–0.024 cig./plate in the microsuspension assay.
[b] The responses have been evaluated with 6 doses in the range 25–1600 μg/plate for both assays.
[c] –, not tested.

tle activity in the basic fraction (Table 4) which is in agreement with earlier results (Löfroth, 1981; Löfroth et al., 1983).

Discussion

Public indoor locations

This exploratory study shows that typical public indoor locations, a shopping center plaza and a railway station waiting room, are much more polluted than the ambient outdoor air by mutagenic compounds present in airborne particulate matter. Although the sampling and analysis cannot prove the origin of the increased mutagenic activity, smoking is the only conceivable source. Concomitant with an increase of the mutagenic response there is consequently also an increase of other pollutants, such as nitrogen oxides and volatile hydrocarbons which are not detected by the mutagenicity test. The level of the indoor mutagenic response of 1000–2500 revertants/m^3 (Table 1) can be compared with an average response of 45 revertants/m^3 (range 9–162) for seventy-six 24-h samples collected at street level at various locations in Gothenburg (Sweden) and analyzed with the microsuspension assay with TA98 + S9 (Löfroth et al., unpublished results). The ambient outdoor response of < 50–260 revertants/m^3 measured simultaneously in the present study (Table 1) is of a reasonable magnitude considering the fact that these samples were collected during a few hours in the afternoon when the traffic is of more than the average 24-h intensity.

Train travel

The mutagenic responses of the air of smoking train compartments (Table 2) are of an expected magnitude. The type of compartment sampled has a volume of about 40 m^3 and assuming an efficient air mixing, the concentration of 600 and 3500 revertants/m^3 following smoking of 5 and 25 cigarettes, respectively, during 4–4.5 h may be obtained with a combined ventilation and surface removal rate (Repace and Lowrey, 1980) corresponding to about 9–12 air changes/h.

The result from the non-smoking compartments (Table 3) shows that smoke from the smoking compartment can penetrate into the non-smoking section. Although most samples only gave a small response, an activity of 100 revertants/m^3 must be judged to be above the background as the electrically powered train mostly travels through rural areas. Separation of non-smoking and smoking compartments in a train is most easily done by having an entire smoking car (or several) at the rear end of the train.

Air travel

Tobacco smoke in airplanes has recently been studied with nicotine analysis by Oldaker and Conrad (1987) who found that the average nicotine concentration in the non-smoking sections was about half of that in the smoking sections; 5.5 vs. 9.2 $\mu g/m^3$. The concentration in the non-smoking sections correponds to about 0.0013 cigarettes/m^3 using the sidestream emission of nicotine (4.1 mg/cigarette) given by Rickert et al. (1984). The mutagenic response of 200–1000 revertants/m^3 (Table 3) obtained in the present study corresponds to 0.001–0.005 cigarettes/m^3 of which the higher value best relates to the situations investigated by Oldaker and Conrad, i.e. non-smoking seats near smoking sections.

The results of this study and of the investigation by Oldaker and Conrad (1987) indicate that if smoking is permitted on airplanes, the smoking section ought to be physically separated from the non-smoking sections, e.g. by stewardess' areas.

Chemical fractionation

Using the plate incorporation assay, previous fractionation studies have shown that mutagens in tobacco tar particulates to a large extent are basic compounds responsible for more than 65% of the response. The relative activity of the basic fraction in the microsuspension assay is smaller with slightly less than 50% of the total response being recovered in this fraction (Table 4). This is, however, still significantly more than the corresponding relative response of compounds in ambient particulate matter with a very small contribution from the basic fraction. There are no published reports indicating that some commonly occurring processes generate airborne particulate matter with a high portion of the mutagenic activity in the basic fraction. A conceivable source of basic mutagenic compounds is cooking but it has only given a weak correlation to the total indoor mutagenic activity (van Houdt et al., 1984; Lewtas et al., 1987). The sensitive microsuspension assay might thus be used as a tobacco-specific analysis if part of an air particulate sample is subjected to fractionation, as has been explored in this study with analysis of a sample from a restaurant and a sample from an apartment (see Results). Such differential analyses are deemed possible for moderately tobacco smoke-polluted air with sample sizes 2–3 times larger than those used in the present study of public indoor locations.

Mutagenic activity and other tobacco smoke indicators

Nicotine has so far been the only tobacco-specific air pollutant. Its value as an indicator may, however, be limited as nicotine may not be a health issue and as it may be prone to rapid adsorption to surface materials causing an underestimate of the air pollution of other smoke components. Among other compounds emitted in the sidestream, several unsaturated hydrocarbons have high emission factors (Löfroth et al., 1987, and unpublished data). The emission of isoprene is about 2–3 mg/cigarette and this alkadiene may be utilized as a semi-specific tobacco smoke indicator although it is present at low background concentrations originating from natural sources (Gelmont et al., 1981; Lamb et al., 1986). An advantage with isoprene, which it shares with mutagens in the tar particulates, is that it is a potential mutagen and carcinogen following mammalian metabolism (Longo et al., 1985). Ultimately, a combination of Salmonella mutagenicity of particulates, isoprene and nicotine may be used for a better estimate of environmental tobacco smoke.

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VARIABILITY OF MEASURES OF EXPOSURE TO ENVIRONMENTAL TOBACCO SMOKE IN THE HOME

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We have assessed the variability of four markers of environmental tobacco smoke exposure in 10 homes with 20 nonsmoking and 11 smoking household members. We obtained exposure questionnaires, saliva and urine for cotinine, and air particle samples for respirable particles and nicotine on 10 sampling days: every other day over 10 days, and then one day every other week over 10 weeks. The mean concentrations of respirable particles in the 10 homes ranged from 32.4 ug/m^3 to 76.9 ug/m^3, and concentrations of nicotine ranged from 0.59 ug/m^3 to 6.85 ug/m^3. A linear regression model that included indicator variables for the number of smokers exposed to in the home and the season, and the number of hours of exposure as a continuous variable explained nine and 6% of the variability of the respirable particle and the nicotine concentrations, respectively. The individual mean cotinine levels standardized to urinary creatinine concentration, ranged from 3.89 ng/mg Cr to 55.77 ng/mg Cr. A linear regression model that included the number of smokers exposed to in the home, the season, the age group, and the number of hours of exposure explained 8% of the variability of the urinary cotinine levels.

We conclude that because of the marked variability of these measures, multiple measurements are needed to establish a stable profile of exposure to environmental tobacco smoke in a particular home or individual. Furthermore, detailed questions to quantitate exposure offered little additional information beyond whether the subject was exposed or not.

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Introduction

Passive smoking refers to the involuntary exposure of nonsmokers to the combination of tobacco combustion products released by the burning cigarette and smoke components exhaled by the active smoker[1,2]. The adverse health effects of passive smoking on children and adults have been described in numerous epidemiologic investigations[1,2].

Although some health effects of passive smoking have been convincingly demonstrated, many questions on the health effects of passive smoking remain unanswered. More precise description of exposure-response relationships is needed for the adverse effects on children and for lung cancer and nonmalignant effects on adults. In most epidemiologic studies on involuntary smoking published to date, exposure has been assessed with questionnaires. However, other indicators of exposure are available, including atmospheric and biologic markers, which have received increasing attention.

We have assessed the variability of four markers of environmental tobacco smoke exposure in 10 homes with 20 nonsmoking and 11 smoking household members. The markers of exposure included questionnaires, respirable particles and nicotine from air samples, and urinary cotinine.

Methods

Sample Selection

Between February and December, 1986, 149 nonsmoking volunteers, 18 years of age and older, were recruited from Albuquerque and surrounding communities to participate in a study of the accuracy of questionnaire assessment of exposure to environmental tobacco smoke[3]. From this sample, 10 subjects volunteered their households for this investigation.

Data Collection

We obtained exposure questionnaires, saliva and urine, and air particle samples on 10 sampling days: every other day over 10 days, and then one day every other week over 10 weeks. The questionnaires, and saliva and urine specimens were obtained at the end of a 24-hour air monitoring period (described below). From the questionnaires we determined the reported number of smokers and number of hours that the subjects were exposed, during the previous 24 hours, to cigarettes, cigars, and pipes at home, at work or school, in a vehicle, and in other places. Questionnaires were self-completed by the adults, and by a parent for children 13 years of age and younger. The saliva and urine specimens were frozen at -20°C until the cotinine assays were performed.

Cotinine Assay

Cotinine was quantitated by a double antibody radioimmunoassay, as described by Langone et al.[4]. A specific antiserum produced in rabbits was supplied by Dr. Helen Van Vunakis, Brandeis University. Urine samples were diluted 1:4 for the assay. The sensitivity of the assay in our hands was 36 pg/tube or 0.78 ng/ml of urine (4204 pmol/L). Urine creatinine concentrations were determined by the Jaffe reaction[5], and the cotinine concentrations were standardized to the creatinine concentrations. Assays were performed without knowledge of questionnaire responses.

Particle Measurements

In the major activity room of each home, Harvard School of Public Health impactors[6] operating at a flow rate of 4 liters/minute were used to collect respirable particles and gaseous nicotine samples. Through a timed solenoid switching valve, two impactors used a common, mass flow controlled

pump, and each impactor operated on alternate 15 minute collection cycles. Respirable particle samples were collected on teflon filters (Membrana, Inc.) and nicotine was collected on sodium bisulfate treated glass fiber filters (Millipore Corp.) to minimize its volatilization. After extraction from the filter, analysis for nicotine was done on a Shimadzu GC7A gas chromatograph with a flame ionization detector. The nicotine collection and extraction procedure is a modification of that described by Hammond et al.[7]. The recovery of nicotine by this procedure has been shown to be 98% efficient.

Data Analysis

Variability of questionnaire responses, respirable particle and nicotine concentrations, and urinary cotinine levels were assessed with univariate analyses. From the questionnaires the measures of exposure in the home were the total number of smokers, including cigarettes, cigars, and pipes, and the total number of hours exposed. During the entire sampling period there were only four days that any subject reported exposure to a cigar smoker, the predominant exposure was to cigarette smoke.

To examine determinants of the variability in the measurements, we used multiple linear regression. The dependent variables, respirable particles, nicotine, and urinary cotinine, were analyzed as continuous variables. For the independent variables, indicators were defined for house (HOUSE = 1-10), individual (INDIVIDUAL = 1-20), age group (AGE GROUP <18 years or ≥18 years), season (SEASON = March-April or May-October), and number of smokers per day (NUMBER = 0 or ≥1). The other independent variable, number of hours (HOURS) exposed per day was continuous.

Data analyses were performed with standard programs of the Statistical Analysis System[8].

Results

The 10 households included 11 cigarette smokers and 20 nonsmokers aged 1.5 to 74 years (Table I). The types of homes included, eight unattached, single family houses, one mobile home, and one apartment.

Reports on exposure to tobacco smoke in the home were obtained for all 10 sampling days from 17 subjects, and for nine days from three subjects. The reported number of cigarette smokers in the home per day did not vary widely. The median number (range) of smokers per day was one for 18 subjects (0-10), zero for one subject (0-1), and four for one subject (2-25). Greater variability was reported for the number of hours exposed to cigarette smoke in the home (Table I).

Respirable particle and nicotine concentrations were obtained for 99% of the sampling days (Figures 1 and 2). The mean concentrations of respirable particles in the 10 homes ranged from 32.4 ug/m^3 (SD = 13.1) to 76.9 ug/m^3 (SD = 32.9) and concentrations of nicotine ranged from 0.59 ug/m^3 (SD = 0.69) to 6.85 ug/m^3 (SD = 8.21). The degree of variability with sampling every other day or every other week was similar (data not shown).

For the particle and nicotine measurements we used linear regression to examine determinants of variability and of level. A model that included indicator variables for the 10 houses explained the greatest variability (Table II). Compared to the model with the house variables, other models that included specific exposure variables explained markedly lower percentages of the variability of levels. Although not statistically

significant, increases in respirable particles were associated with exposure to one or more cigarette smokers in the home and with the colder months, March and April (Table II). There was no association with the number of hours of exposure. Nicotine levels also increased with exposure to smokers in the home, but were not predicted by the season (Table II).

Urinary cotinine levels were obtained on 187 specimens from the 20 nonsmokers. The individual mean cotinine levels standardized to urinary creatinine concentration, ranged from 3.89 ng/mg Cr (SD = 6.54) to 55.77 ng/mg Cr (SD = 32.02). The mean levels and variability tended to be greater in the children compared to the adults (Figures 3 and 4). As with the atmospheric measures, the variability was comparable with sampling every other day or every other week (data not shown).

For the urinary cotinine levels, we also examined determinants of variability and determinants of concentration with linear regression. A model that included indicator variables for the 20 nonsmoking individuals explained 47% of the variability in cotinine levels (Table III). Compared to this model, other models that included specific exposure variables and age group explained much lower proportions of the variability. Cotinine levels were significantly ($p<0.05$) higher among children compared to adults (Table III). Although not significant, exposure to one or more smokers resulted in higher cotinine levels compared to no exposure. The number of hours of reported exposure and the season were not significant predictors of cotinine level.

Conclusions

In a group of volunteers, from 10 homes, we found that concentrations of respirable particles and nicotine, and urinary cotinine levels varied widely within homes and individuals, respectively. Sampling every other day or every other week did not offer any advantage for establishing a stable profile of exposure with these markers. A moderate degree of the variability of these measures was explained by variables representing houses and individuals, but more specific measures of exposure contributed little to explaining the variability.

Many factors, which we were unable to measure, may contribute to the variability of these measures of environmental tobacco smoke in the home. For the atmospheric measurements, concentrations depend on the intensity and duration of smoking, room size, ventilation, adsorption of smoke components, methods of collection, and methods for measurement. Particle concentrations are also affected by sources other than tobacco smoking. Furthermore, at a given level of nicotine exposure, urinary cotinine level is also influenced by uptake, metabolism, and excretion, which are likely to vary among individuals. Finally, the ability to predict levels of these markers is also limited by subjects' inability to comprehensively and accurately describe the extent of exposure.

The results of this investigation have several implications for the measurement of environmental tobacco smoke in epidemiologic investigations. Because of the marked variability of these measures, multiple measurements are needed to establish a stable profile of exposure in a particular home or for a particular individual. Detailed questions quantitating exposure to environmental tobacco smoke seem to offer little information beyond determining whether the subject was exposed or not. Future investigations of methods for measurement of environmental tobacco smoke must determine the importance of other factors that may contribute to the variability of

atmospheric and biologic markers, the role of personal monitoring, and the relevance of these markers to acute and chronic health effects of passive smoking.

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Table I

Description of Houses* and Subjects, and Reported Number of Hours Exposed to Cigarette Smoke in the Home per Day, New Mexico, 1986

House/Subject	Number of Rooms	Age (yrs)	Sex	Hours/day Exposed Median (range)
One	7			
1		28	F	4.5 (0-11)
2		1.5	M	3.0 (0-7)
Two	6			
3		29	F	5.5 (0-10)
4		9	F	6.3 (2-13)
5		5	M	6.5 (0-11)
Three	7			
6		37	M	3.5 (2-8)
7		4.5	M	5.0 (2-10)
Four	7			
8		35	F	4.5 (4-9)
9		13	F	7.0 (3-12)
10		4	M	8.5 (7-14)
Five	8			
11		32	F	1.0 (0-19)
12		2	F	3.0 (0-19)
Six	6			
13		49	F	4.0 (0-10)
14		22	M	0.0 (0-3)
Seven	4			
15		41	F	11.0 (0-16)
16		14	M	5.5 (0-14)
Eight	5			
17		46	F	6.0 (3-13)
18		13	M	6.0 (0-13)
Nine	6			
19		74	M	5.0 (4-7)
Ten	4			
20		63	F	5.5 (2-16)

*Houses 1-6, 8-9 are unattached, single family homes, 7 is a mobile home, and 10 is an apartment.

Table II

Linear Regression Models* Predicting Respirable Particle and Nicotine Concentrations in Air Samples from 10 Homes, New Mexico, 1986

Dependent Variable	R^2		
	Model 1	Model 2	Model 3
Respirable particles (mg/m^3)	0.34	0.08	0.09
Nicotine (mg/m^3)	0.28	0.04	0.06
	Regression Coefficients† Model 3		
	One or More Smokers	HOURS	Cold Months
Respirable particles (mg/m^3)	+17.3 (7.4 - 27.2)	+0.4 (-1.0 - 1.8)	+8.9 (-1.1 - 18.9)
Nicotine (mg/m^3)	+2.1 (-1.7 - 5.9)	+0.2 (-0.1 - 0.5)	-0.7 (-2.5 - 1.1)

*Independent Variables: Model 1 = HOUSE, Model 2 = NUMBER (0 or ≥1) + SEASON (March - April or May - October), Model 3 = NUMBER (0 or ≥1) + HOURS (continuous) + SEASON (March - April or May - October).

†95% confidence intervals in parentheses.

Table III

Linear Regression Models* Predicting
Urinary Cotinine Concentrations from 20 Nonsmokers
Exposed to Tobacco Smoke,
New Mexico, 1986

	R^2			
Dependent Variable	Model 1		Model 2	Model 3
Urinary cotinine (ng/mg Cr)	0.47		0.05	0.08
	Regression Coefficients† Model 3			
	One or More Smokers	HOURS	Cold Months	Children
Urinary cotinine (ng/mg Cr)	+5.4 (-4.8 - 15.6)	+0.8 (0.0 - 1.6)	-0.2 (-5.8 - 5.4)	+5.4 (0.0 - 10.8)

*Independent Variables: Model 1 = INDIVIDUAL, Model 2 = NUMBER (0 or ≥1) + SEASON (March - April or May - October), Model 3 = NUMBER (0 or ≥1) + HOURS (continuous) + SEASON (March - April or May - October) + AGE GROUP (<18 or ≥18 years).

†95% confidence intervals in parentheses.

Figure 1

Respirable particle concentrations and means in 10 homes.

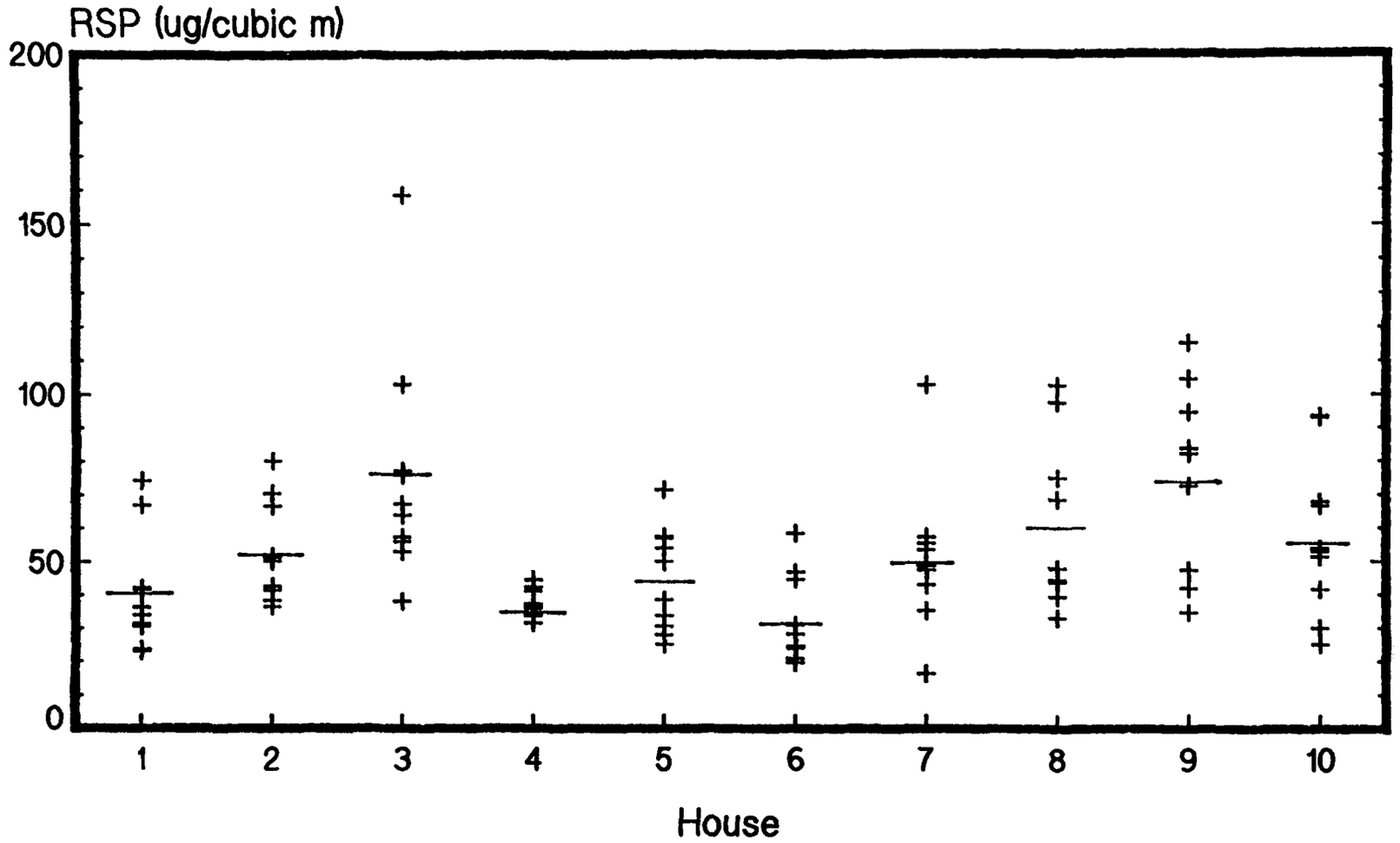


Figure 2

Nicotine concentrations and means in 10 homes.

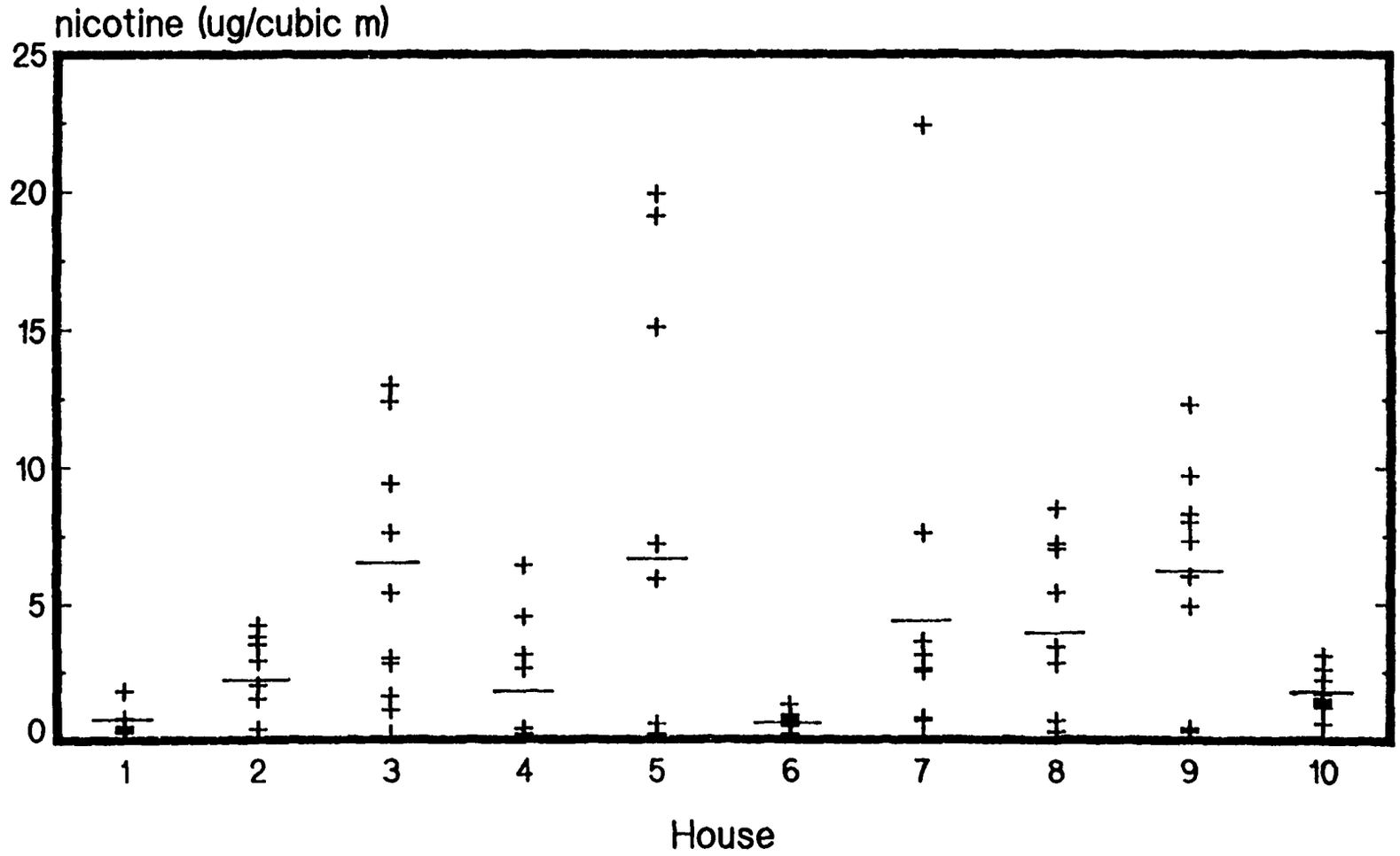


Figure 3

Urinary cotinine levels and means for children from 10 homes

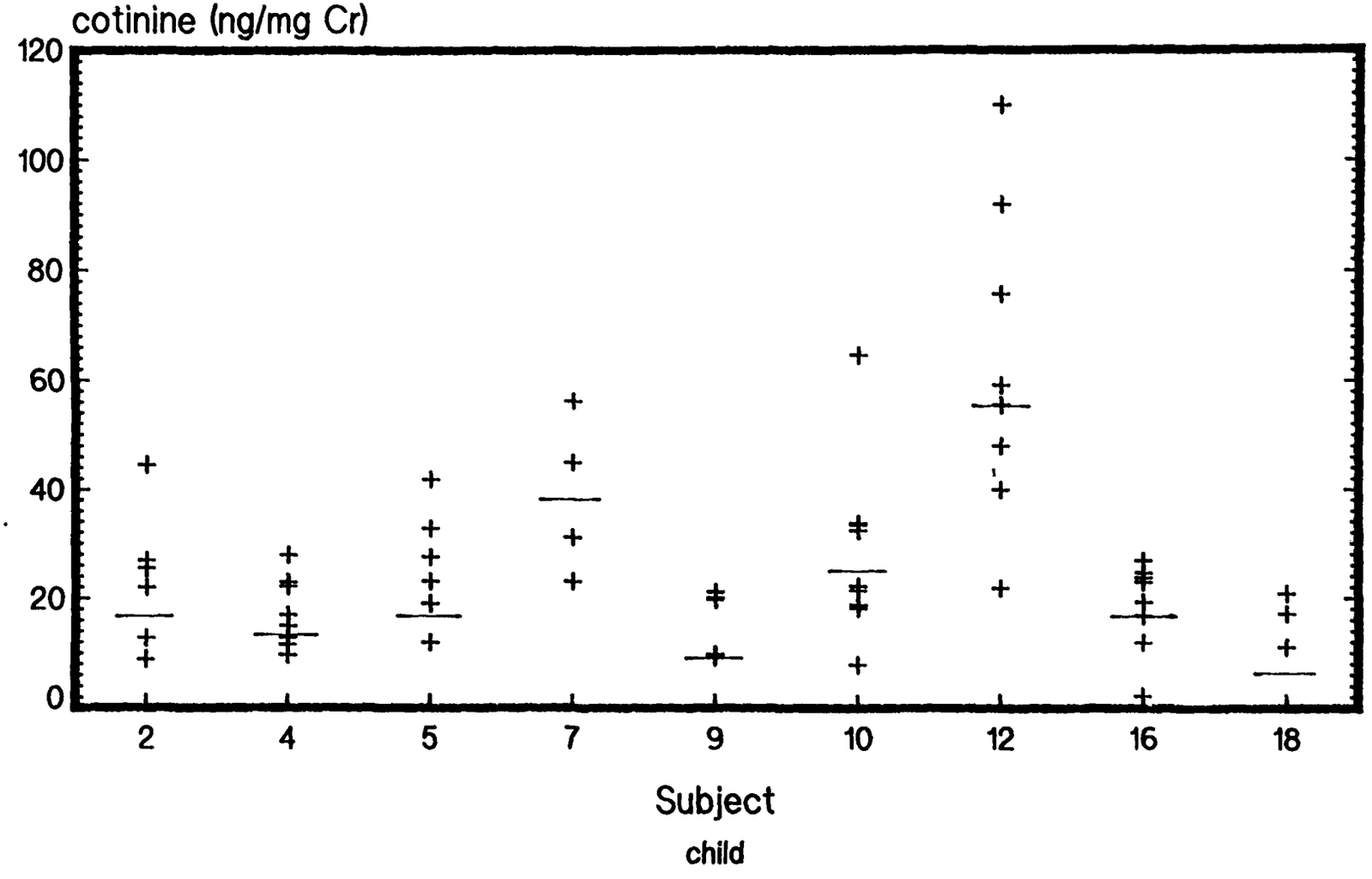
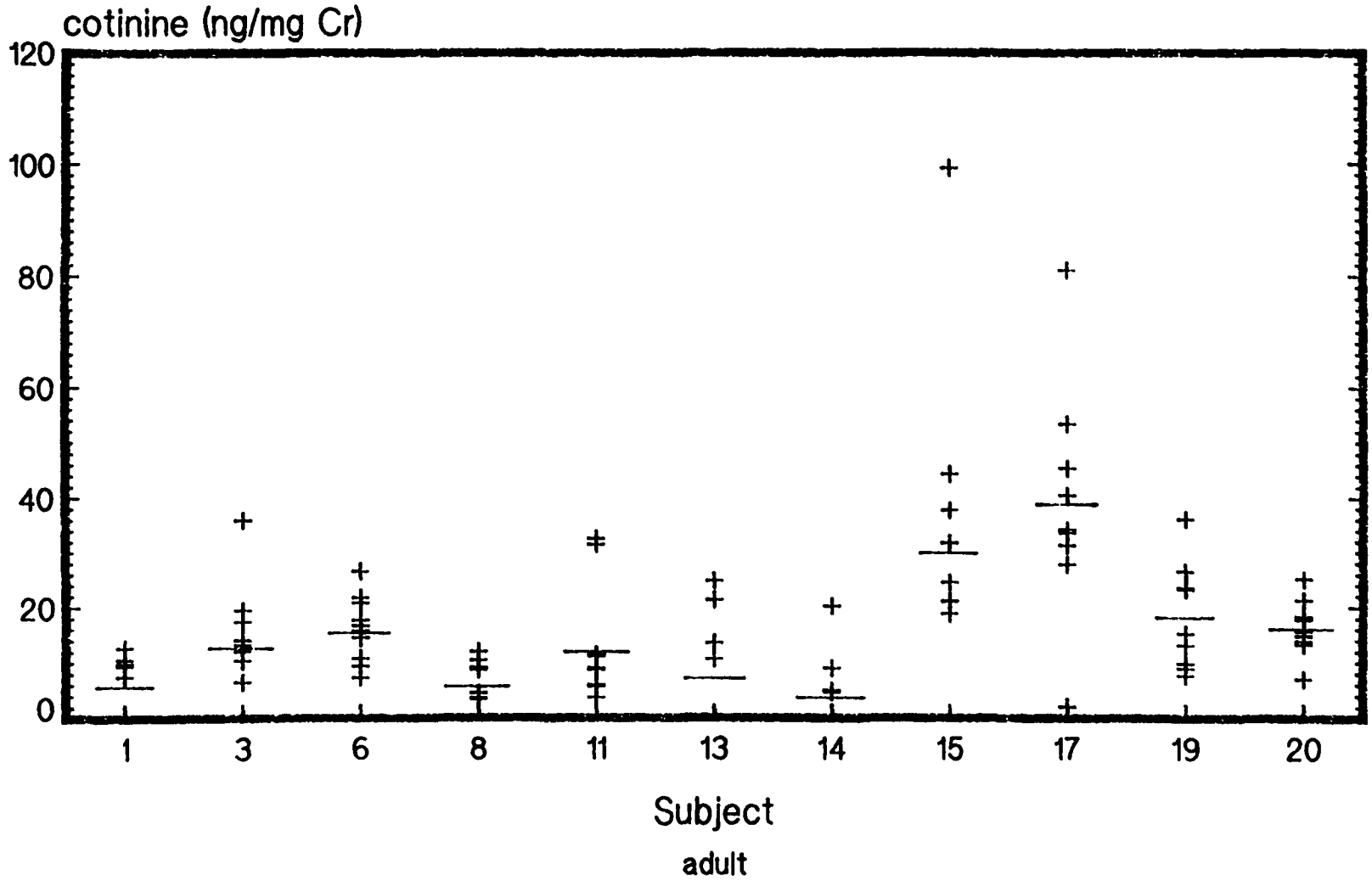


Figure 4

Urinary cotinine levels and means for adults from 10 homes.



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QUESTIONNAIRE ASSESSMENT OF LIFETIME AND RECENT EXPOSURE TO ENVIRONMENTAL TOBACCO SMOKE

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Coultas, D. B. (New Mexico Tumor Registry, Cancer Center, U. of New Mexico Medical Center, Albuquerque, NM 87131), G. T. Peake, and J. M. Samet. Questionnaire assessment of lifetime and recent exposure to environmental tobacco smoke. *Am J Epidemiol* 1989;130:338–47.

In a sample of 149 adult nonsmokers recruited in New Mexico in 1986, the authors assessed the reliability of questionnaire responses on lifetime exposure to tobacco smoke in the home. They also compared urinary cotinine levels with questionnaire reports of environmental tobacco smoke exposure during the previous 24 hours. The agreement of responses obtained on two occasions within six months was high for parental smoking during childhood: 94% for the mother and 93% for the father. For the amounts smoked by the mother and the father during the subject's childhood, the agreement between the two interviews was moderate: 52% and 39%, respectively. For the number of hours per day that each parent smoked in the home during the subject's childhood, the Spearman correlation coefficients also indicated only moderate reliability ($r = 0.18$ for maternal smoking and $r = 0.54$ for paternal smoking). For each set of interviews, responses concerning recent tobacco smoke exposure and urinary cotinine levels were correlated to only a modest degree. The authors conclude that adults can reliably report whether household members smoked during their childhood, but information on quantitative aspects of smoking is reported less reliably.

pyrrolidinones; questionnaires; tobacco smoke pollution

The term "passive smoking" refers to the involuntary exposure of nonsmokers to the combination of tobacco combustion products released by the burning cigarette and smoke components exhaled by the active smoker (1, 2). The adverse health effects of passive smoking on children and adults have been described in numerous epidemiologic investigations (1, 2). However, despite the evidence linking malignant and nonmalignant diseases with active and passive smoking, tobacco smoking remains highly prevalent worldwide (1). In the United States at present, about 30 per cent of adults are active cigarette smokers (3), so that a large proportion of nonsmokers

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in this country are involuntarily exposed to environmental tobacco smoke (1, 2).

Although some health effects of passive smoking have been convincingly demonstrated, many questions on the health effects of passive smoking remain unanswered. More precise description of exposure-response relations is needed for assessment of the adverse effects on children and the development of lung cancer. Additionally, further studies on exposure to environmental tobacco smoke in the workplace are warranted because of the high prevalence of smoking among adults and public concern about this source of exposure. In most epidemiologic studies on involuntary smoking published to date, exposure has been assessed with questionnaires; for the purposes of some investigations, the questionnaires have spanned the entire lifespans of the subjects. Questionnaires will remain the most feasible method for assessing exposure to environmental tobacco smoke in new studies. However, the reliability and validity of questionnaire measures of involuntary smoking have not been adequately characterized.

In this study, we have assessed the reliability of a comprehensive questionnaire on lifetime exposure to environmental tobacco smoke in 149 adult nonsmokers. While validity is also of interest, no appropriate standard for comparison is available for a lifetime history. Questionnaire responses with poor reliability are also likely to have poor validity. In this sample, we also examined the relation between reports of recent exposure to environmental tobacco smoke and urinary cotinine levels.

MATERIALS AND METHODS

Sample selection

Between February and December of 1986, nonsmokers aged 18 years and older were recruited from Albuquerque, New Mexico, and the surrounding communities. Recruitment was accomplished by two methods: advertisements and direct contact with subjects from a population survey (4). In both approaches, we asked for volunteers to participate in a study of indoor air quality that involved completing a questionnaire on two occasions and providing saliva and urine samples. The subjects were not informed that the study was directed specifically at exposure to environmental tobacco smoke. We attempted to stratify the sample uniformly by age and by sex but were not completely successful (table 1). Of our sample, 62 per cent were female, and only five males were aged 60 years and older.

Data collection

A structured questionnaire on lifetime and recent exposure to environmental tobacco smoke was administered by a trained interviewer to each subject on two occasions separated by approximately four to six months. Training involved familiarization and practice with the questionnaire and review of probing techniques, which were standardized. The interviews were conducted by four interviewers who completed 89.2, 5.4, 2.7, and 2.7 per cent of the first interviews and 38.2, 6.7, 54.4, and 0.7 per cent of the second interviews, respectively. We asked whether the subject's mother had smoked while pregnant with the subject, and we determined the smoking status of parents, spouses, and others from questions on whether these persons had smoked in the subject's home on a daily basis for six months or more. These questions referred to two time periods: birth to age 18 years and age 19 years to the time of the interview. These time periods were chosen to correspond to the usual ages for

TABLE 1

Age and sex distribution of 149 participants in a study of involuntary exposure to tobacco smoke, New Mexico, 1986

Age (years)	Males		Females	
	No.	%	No.	%
20–29	12	21.4	17	18.3
30–39	20	35.7	27	29.0
40–49	9	16.1	15	16.1
50–59	10	17.9	15	16.1
≥60	5	8.9	19	20.4

living in the parents' home and subsequently living outside the parents' home. In addition, for each smoker, we asked about the type(s) of tobacco smoked (cigarette, pipe, or cigar), the amount of each type smoked in the home, the number of years each type was smoked, and the number of hours of exposure per day to each type in the home. Another set of questions asked about the amount of exposure during the previous 24 hours. The questions covered the number of smokers to which the subject was exposed, the type(s) of tobacco smoked (cigarette, pipe, or cigar), and the number of hours of exposure. These questions were asked separately for exposures at home, at work, in vehicles, and at social gatherings. At the time of the interview, a urine specimen was collected and frozen at −20 C until the cotinine assays were performed.

Cotinine assay

Cotinine was quantitated by a double antibody radioimmunoassay as described by Langone et al. (5). A specific antiserum produced in rabbits was supplied by Dr. Helen Van Vunakis of Brandeis University (Waltham, MA). Urine samples were diluted 1:4 for the assay. The sensitivity of the assay in our hands was 36 pg/tube or 0.78 ng/ml of urine (4,204 pmol/liter). Urinary creatinine concentrations were determined by the Jaffe reaction (6), and the cotinine concentrations were standardized to the creatinine concentrations. Assays were performed without knowledge of questionnaire responses.

Data analysis

Reliability was assessed by comparison of the two lifetime histories for the exposure variables during the two time periods, birth to age 18 years and age 19 years to the time of the interview. Because of the small number of pipe and cigar smokers among parents ($n = 24$) and spouses ($n = 4$), we restricted our analysis to cigarette smokers. We summarized the per cent agreement between the first and second interviews for categorical variables, which included mother's smoking during pregnancy; mother's, father's, and spouse's cigarette smoking status; amount smoked, categorized as less than one pack per day, one pack per day, and more than one pack per day; and number of other cigarette smokers in the household, categorized as none, one, and two or more. To discount chance agreements between the first and second interviews, Cohen's kappa was calculated for all categorical items and tested for significance (7, 8). Spearman rank order correlation coefficients (9) were calculated for continuous variables, which included both the number of years and the number of hours per day that the subject's mother, father, spouse, and others had smoked.

For questions on exposure to tobacco smoke during the previous 24 hours, we created summary variables for cigarette smoke exposure only, because exposure to pipe and cigar smokers was infrequent. The summary variables for cigarette smoke exposure included the total number of hours of exposure and the total number of cigarette smokers in all locations. To examine the relation between measures of short term exposure to environmental tobacco smoke within and between interviews, we calculated Spearman rank order correlations (9).

Data analyses were performed with standard programs of the Statistical Analysis System (10).

Results

Of the 158 subjects enrolled for the first interview, 149 (94 per cent) also completed the second interview. Of the nine subjects who were not reinterviewed, there were seven males and two females, with mean ages of 43.6 years and 43.0 years, respectively. This report is based on responses of those 149 subjects who were reinterviewed. The age range of the 149 subjects was 21–79 years (mean = 43 years); 37.6 per cent were males and 62.4 per cent were females (table 1). The median duration between

interviews was 17 weeks, with a range of 6–35 weeks.

For the period birth to age 18 years, agreement between the first and second interviews was high for parental smoking status during childhood (table 2). The per cent agreement was similar for mother's and father's smoking during childhood and was lowest for maternal smoking during pregnancy. The percentage of unknown responses was highest for maternal smoking during pregnancy. The per cent agreement and kappa statistic for the number of other cigarette smokers in the home during childhood were 77.0 per cent and 0.47 ($p < 0.0001$), respectively.

In contrast to the high reliability of responses about parental smoking status during childhood, concordance was low for responses about the usual amount smoked in the home by the parents during childhood (table 3). The concordance was highest for the amount smoked by the mother and lowest for the amount smoked by the fa-

TABLE 2

Percentage of nonsmokers reporting exposure to parental cigarette smoking during childhood, New Mexico, 1988

Response	Maternal smoking during pregnancy (n = 149)	Maternal smoking during childhood (n = 149)	Paternal smoking during childhood (n = 149)
Yes			
First interview	20.1	36.9	55.7
Second interview	20.1	32.9	56.4
No			
First interview	67.1	62.4	43.6
Second interview	64.4	67.1	42.9
Unknown			
First interview	12.8	0.7	0.7
Second interview	15.5	0.0	0.7
Agreement			
Concordance	85.9	94.0	92.6
Kappa	0.73*	0.87*	0.85*

* $p < 0.0001$.

TABLE 3

Percentage of nonsmokers reporting exposure to various amounts of cigarettes smoked by the parents during childhood and by the spouse during adulthood, New Mexico, 1988

Amount smoked	Maternal smoking during childhood (n = 48)	Paternal smoking during childhood (n = 79)	Spousal smoking during adulthood (n = 64)
Less than one pack/day			
First interview	62.5	70.9	84.4
Second interview	50.0	35.4	40.6
One pack/day			
First interview	20.8	11.4	7.8
Second interview	22.9	32.9	31.3
More than one pack/day			
First interview	6.3	10.1	6.3
Second interview	16.7	22.8	28.1
Unknown			
First interview	10.4	7.6	1.6
Second interview	10.4	8.9	0.0
Agreement			
Concordance	52.1	39.3	43.8
Kappa	0.22*	0.04*	−0.04*

* $p > 0.05$.

ther. Compared with the first interview, the percentage of subjects reporting parental smoking of one pack per day or more was higher at the second interview.

We also examined the reliability of responses on smoking status and amount smoked by sex and by age. The findings were similar to the overall analysis within strata defined by either sex or age, above and below age 40 years.

Spearman correlations were used to describe the agreement between the first and second interviews on the reported number of years and hours per day of exposure to environmental tobacco smoke during childhood. The correlation coefficients were high for responses on the number of years the parents and other smokers in the household had smoked (table 4). However, for responses on the number of hours per day of smoke exposure in the home, the correlation coefficients were much lower (table 4).

We next examined the reliability of reported smoke exposure during adulthood (tables 3 and 5). After age 18 years, the numbers of subjects living with either their mother ($n = 8$) or their father ($n = 9$) were small. For this small group of subjects, the concordance of responses on parental smoking status was 100 per cent. Similarly, the per cent agreement on spouse's smoking status, as obtained at the two interviews, was 100 per cent ($n = 67$). For the amount currently smoked by the spouse, the concordance was lower (table 3). Agreement between responses about the number of other cigarette smokers in the household

TABLE 4

Mean years and hours per day of childhood cigarette smoke exposure reported by nonsmokers, New Mexico, 1986

Exposure variable	No.	First interview	Second interview	Spearman's *r*
Maternal smoking				
Years*	33	15.4	15.7	0.76
Hours/day†	31	5.0	6.4	0.18
Paternal smoking				
Years	57	16.1	15.4	0.75
Hours/day	55	4.8	4.8	0.54
Other household members' smoking				
Years	26	13.9	13.2	0.63
Hours/day	20	9.2	8.4	0.51

* "During the period from birth to age 18 years, for how many years did he/she smoke cigarettes?"

† "On average, during the period from birth to age 18 years, for how many hours per day were you exposed to individuals' cigarette smoke?"

TABLE 5

Mean years and hours per day of adulthood cigarette smoke exposure reported by nonsmokers, New Mexico, 1986

Exposure variable	No.	First interview	Second interview	Spearman's *r*
Spouse's smoking				
Years*	40	16.2	16.4	0.95
Hours/day†	39	5.9	5.5	0.25
Other household members' smoking				
Years	67	8.3	8.2	0.78
Hours/day	58	12.7	10.3	0.54

* "For how many years did he/she smoke cigarettes while you were sharing your home?"

† "On average, how many hours per day were you exposed to their cigarette smoke?"

was 74.0 per cent (n = 66), with a kappa value of 0.50 ($p < 0.0001$).

Correlations between responses at the two interviews were high for the number of years the spouse and other smokers in the household had smoked during the subject's adulthood, but much lower for the number of hours per day of exposure during adulthood (table 5). Because of the small number of subjects living with their parents after age 18 years, we did not calculate correlation coefficients for these variables.

Urine specimens were obtained from 98 per cent of the 149 subjects at the first interview and 95 per cent at the second interview. The median urinary cotinine levels were zero at both interviews, with mean levels of 9.2 ng/mg of creatinine at the first interview and 7.3 ng/mg of creatinine at the second interview. Cotinine levels varied widely with the total number of smokers and the total number of hours of exposure to tobacco smoke (in various situations) during the 24 hours prior to urine collection at both the first interview (figures 1 and 2) and the second interview (data not shown). The cotinine levels correlated only modestly with the questionnaire measures of exposure (table 6).

We also assessed the stability of data on exposure, as measured by questionnaire and by cotinine level (table 6). At the first

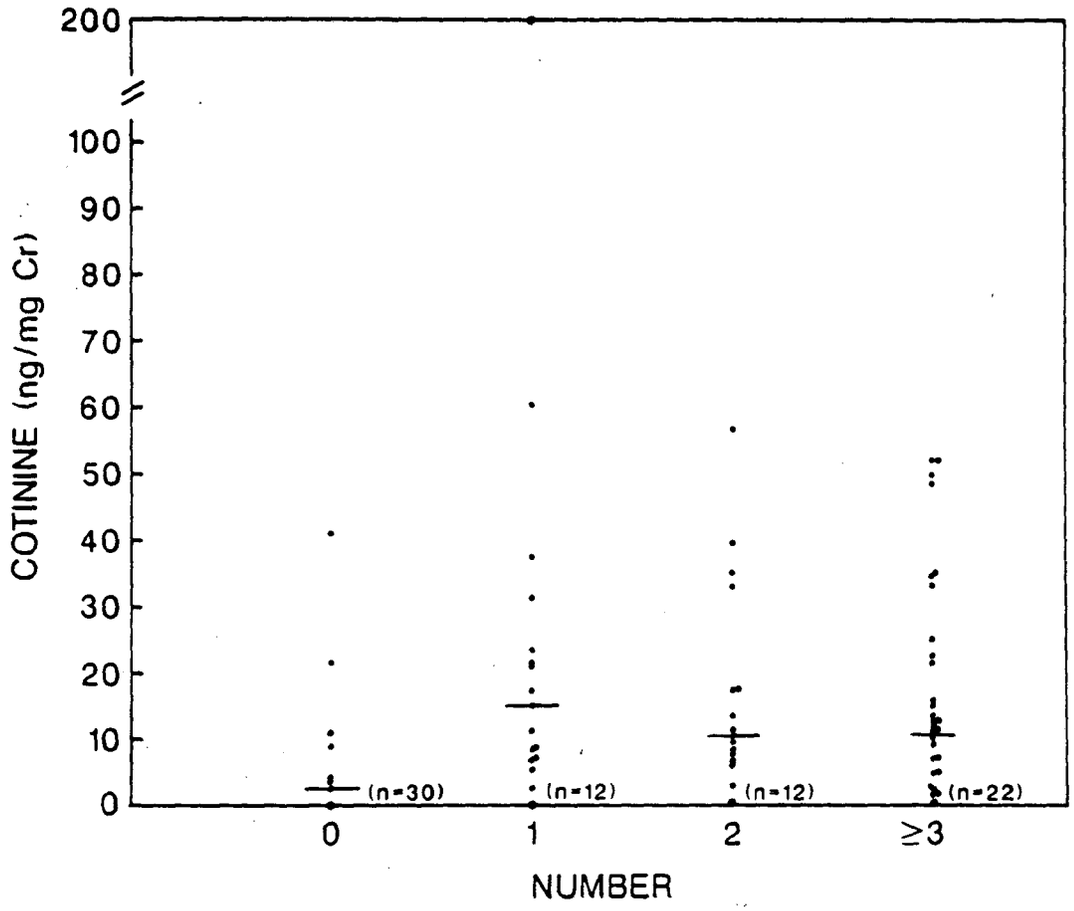

FIGURE 1. Urinary cotinine levels, standardized to urinary creatinine (Cr) concentration, among nonsmokers interviewed about tobacco smoke exposure, by the total number of cigarette smokers the subject reported being exposed to during the 24 hours prior to the first interview. Bars show the mean cotinine level for each group. Values in parentheses indicate the number of subjects with nondetectable levels of cotinine. New Mexico, 1986.

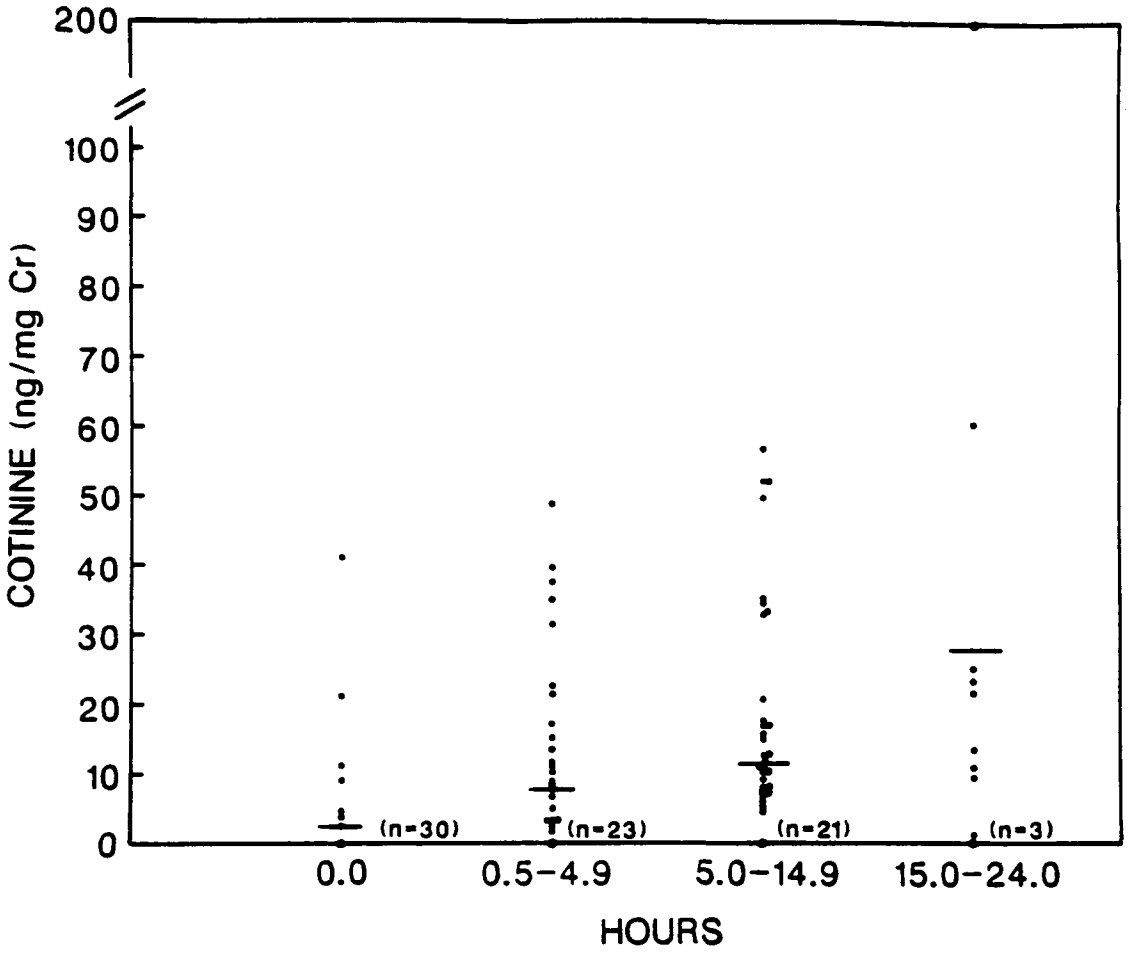

FIGURE 2. Urinary cotinine levels, standardized to urinary creatinine (Cr) concentration, among nonsmokers interviewed about tobacco smoke exposure, by the self-reported total number of hours that the subject was exposed to cigarette smoke during the 24 hours prior to the first interview. Bars show the mean cotinine level for each group. Values in parentheses indicate the number of subjects with nondetectable levels of cotinine. New Mexico, 1986.

and second interviews, the mean responses for the reported number of cigarette smokers that the subjects had been exposed to during the previous 24 hours were 2.1 and 1.8, respectively, with 20 subjects at the first interview and 22 subjects at the second interview reporting exposures in "crowds." For the total number of hours of exposure during the previous 24 hours, the mean responses at the first and second interviews were 5.1 and 4.6, respectively. Both the questionnaire variables and the cotinine data indicated a relatively stable pattern of exposure. The Spearman correlation coefficients were somewhat higher for the questionnaire-based indexes than for urinary cotinine levels.

DISCUSSION

In a group of adult nonsmokers, we found high reliability for reports on parental smoking and on smoking by others in the home (table 2) but lower reliability for semiquantitative exposure measures (tables 3–5). Mean levels of urinary cotinine increased with exposure to cigarette smoke compared with no exposure ($n = 37$) (figures 1 and 2). However, within specific levels of exposure, the cotinine levels varied widely. Across the follow-up period of sev-

TABLE 6

Spearman correlations between measures of exposure to environmental tobacco smoke during the 24 hours prior to interview, New Mexico, 1986

Exposure variable	No.	r
Total no. of smokers to which subject was exposed		
Responses at the first and second interviews	143	0.50
Response at the first interview and cotinine level	143	0.24
Response at the second interview and continine level	139	0.21
Total no. of hours that subject was exposed to cigarette smoke		
Responses at the first and second interviews	144	0.62
Response at the first interview and cotinine level	145	0.32
Response at the second interview and cotinine level	138	0.29
Cotinine level		
Levels at the first and second interviews	140	0.45

eral months, exposures to environmental tobacco smoke were relatively stable, as were urinary cotinine levels (table 6). Most subjects were able to provide responses to the questions on maternal smoking during pregnancy, parental smoking during childhood, and smoking by a spouse during adulthood (tables 2 and 3).

Several limitations of these data must be considered. Because a standard for validating a lifetime history of exposure to environmental tobacco smoke is unavailable, we used repeatability as an index of the quality of questionnaire responses. We addressed the reliability of questions on lifetime exposure at home, but not in the workplace, an important source of exposure for a substantial proportion of adults (11). Interview with a volunteer subject does not replicate the usual setting of a case-control study, the design most often used to examine lung cancer and passive smoking (1). In that setting, recall bias by ill subjects may affect reliability of questionnaire responses in comparison with a volunteer population.

Similar observations on the reliability of questionnaire data on passive smoking were recently reported by Pron et al. (12). These investigators interviewed 117 subjects, controls in a case-control study of lung cancer, on two occasions separated by an average of six months. Smoking by spouses was reported with high reliability (kappa = 0.89 for both wife and husband). Repeatability was somewhat lower for smoking by the mother (kappa = 0.76) and by the father (kappa = 0.44). As in the present study, repeatability of quantitative estimates of duration of exposure was lower than for the categorical descriptions of smoking by household members.

Although neither the investigation of Pron et al. (12) nor the present study directly addresses validity of questionnaires on lifetime passive smoking, the validity of subjects' reports on smoking by parents and spouses has been described. Sandler and Shore (13) compared responses on parents' smoking given by cases and controls with responses given by the parents or siblings of the index cases. Concordance was high for whether the parents had ever smoked, although the agreement was somewhat better for smoking by the mother than for smoking by the father. Responses concerning numbers of cigarettes smoked did not agree as highly. In a follow-up study of a nationwide sample, children's responses on smoking by their deceased parents closely agreed with the information given 10 years previously by the parents themselves (14). Other studies have shown that people generally report the smoking habits of their spouses correctly (14–19). However, people's reporting of quantitative aspects of the smoking behavior of their spouses tends to be less valid (16, 18, 19).

Smoking by parents during childhood and by a spouse during adulthood represent the most important sources of household exposure to environmental tobacco smoke. The studies of subject reports for parents and spouses indicate good validity of responses on smoking by these household

members; the study of Pron et al. (12) and the present study show that these reports are also highly reliable. Thus, exposure measures based on cigarette smoking status of parents and of spouses, as reported by an index subject, are reported with a high degree of validity and reliability, although these measures may only crudely quantitate the dose of biologically relevant tobacco smoke components. In contrast, the accuracy of more quantitative measures of smoking by these household members is lower. The resulting misclassification may explain the failure to find exposure-response relations for passive smoking and lung cancer in some studies (1, 20).

We also compared responses to questions on exposure during the previous 24 hours with urinary cotinine level. The time period for the questionnaire was limited to the previous 24 hours to reduce bias from faulty recall. However, since this period is approximately the half-life of cotinine in nonsmokers (21, 22), the cotinine level represents not only exposure during the 24 hours covered by the questionnaire but prior exposure as well.

We found modest correlations between the questionnaire-based measures of exposure and urinary cotinine levels (table 6). The level of correlation must be interpreted in the context of the different lengths of time of exposure assessed by the questionnaire and by the urinary cotinine level. Furthermore, at a given level of nicotine exposure, urinary cotinine level is also influenced by uptake, metabolism, and excretion, which are likely to vary among individuals.

Coultas et al. (23) found that questionnaire measures of household exposure were not strong predictors of salivary cotinine level. In 247 adult nonsmokers with a detectable cotinine level, the subject's age, the number of cigarettes smoked per day by the spouse, and the number of cigarettes smoked per day by other smokers in the household explained only 2 per cent of the variance in cotinine levels for females and 16 per cent of the variance for males. Even in active smokers, questionnaire responses on smoking behavior do not tightly predict cotinine concentrations in body fluids (24–27). Higher correlations between urinary cotinine levels and reported exposure to cigarette smoke have been reported for young children (28). The higher correlations in the studies of young children probably reflect the time-activity patterns in this age group (29); parental smoking in the household is generally the dominant source of exposure.

In adults, the weak relation between cotinine level and reported smoke exposure implies that a single cotinine measurement should not be used to estimate exposure for individuals (23). However, in our subjects, cotinine levels varied among exposure groups (figures 1 and 2), suggesting that cotinine measurements might be used as an index of mean exposure for members of a particular exposure group.

Nonsmokers are exposed to environmental tobacco smoke in many different environments, including the home, the workplace, and other private and public locations. Since subjects in an epidemiologic investigation cannot be expected to comprehensively describe the extent of exposure in each of these environments, misclassification of the amount of exposure to environmental tobacco smoke must be anticipated from the use of questionnaires. However, subjects can provide valid and reliable reports concerning the smoking status of household members. The combination of questionnaires and biologic markers offers a feasible approach for assessing recent exposure to environmental tobacco smoke.

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Cotinine Analytical Workshop Report: Consideration of Analytical Methods for Determining Cotinine In Human Body Fluids as a Measure of Passive Exposure to Tobacco Smoke*

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A two-day technical workshop was convened November 10-11, 1986, to discuss analytical approaches for determining trace amounts of cotinine in human body fluids resulting from passive exposure to environmental tobacco smoke (ETS). The workshop, jointly sponsored by the U.S. Environmental Protection Agency and Centers for Disease Control, was attended by scientists with expertise in cotinine analytical methodology and/or conduct of human monitoring studies related to ETS. The workshop format included technical presentations, separate panel discussions on chromatography and immunoassay analytical approaches, and group discussions related to the quality assurance/quality control aspects of future monitoring programs. This report presents a consensus of opinion on general issues before the workshop panel participants and also a detailed comparison of several analytical approaches being used by the various represented laboratories. The salient features of the chromatography and immunoassay analytical methods are discussed separately.

Introduction

Environmental tobacco smoke (ETS) has increasingly become a health concern since a series of epidemiological studies between 1981 and 1986 (*1-6*) reported an association between tobacco smoke exposure and increased risk of human lung cancer. Humble and co-workers (*7*) recently confirmed the health risk conclusions of earlier researchers and reported that people who never smoked and were married to smokers had about a 2-fold increased risk of lung cancer.

Methods for determining the degree of exposure of individuals has received much attention in recent years, and various biological markers have been studied as surrogate analytes for determining exposures. A general consensus is that the nicotine metabolite, cotinine, has the prerequisites of specificity, retention time in the body, and detectable concentration levels that make it the analyte of choice for quantifying exposures. In recent years a number of procedures have been reported for determining cotinine in human body fluids. The majority of these procedures use either a chromatographic technique or some form of immunoassay analysis.

This paper is a report from the two-day Cotinine Analytical Workshop, which was attended by invited health scientists and analytical chemists recognized for their expertise in studies of population exposure

*Chairperson: J. Lewtas, US EPA, Research Triangle Park, NC; Session chairpersons: F. Sperto, CDC. Atlanta, GA, R. Watts, US EPA, Research Triangle Park, NC; invited speaker/panel participants included: Neal Benowitz and Peyton Jacob, San Francisco General Medical Center; Colin Feyerabend, New Cross Hospital, London, England; Nancy Haley, American Health Foundation; George Knight, Foundation for Blood Research; Richard Kornfeld, Battelle Columbus Laboratories; John Langone, Baylor College of Medicine; Peter McElroy, Rosewell Park Memorial Institute; M. A. H. Russell, Maudsley Hospital, London, England; Karl Verebey, New York State Division of Substance Abuse Services; and Helen Van Vunakis, Brandeis University.

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to ETS and/or analytical methodology related to these studies. The workshop was jointly sponsored by the U.S. Environmental Protection Agency (EPA) and Centers for Disease Control (CDC) and was attended by 32 scientists who shared their expertise in immunoassay or chromatography methods for cotinine and provided guidance for developing and establishing related programs for determining passive exposures to tobacco smoke. The meeting objective was to compare the various analytical approaches to cotinine analysis and to make recommendations regarding the general aspects of establishing and conducting monitoring programs. Discussions included quality assurance/quality control (QA/QC) programs to support cotinine monitoring studies and also the possibility of conducting a future interlaboratory methods comparison study. The diverse analytical approaches represented by chromatography and immunoassay methods for cotinine were separately discussed and reported by respective work groups. The purpose of this communication is to summarize discussions from the immunoassay and chromatography work groups relevant to the aforementioned topics and to convey the workshop general consensus on other joint issues including QA/QC aspects of ETS studies.

Chromatography Group Report

The workshop participants with expertise in developing and applying chromatography methods for determining cotinine in biological fluids met in a one-day session. The goal of this session was to develop a group consensus on several key issues including *a*) general method considerations and approaches, *b*) QA/QC programs to support cotinine monitoring studies, and *c*) considerations related to conducting an interlaboratory methods comparison study. The following is a summary of the chromatography group discussions and a draft of their recommendations related to topics *a* and *c*. The QA/QC recommendations are contained in a separate section.

General Method Considerations

Sample Type. The body fluids discussed for monitoring tobacco smoke exposure included blood serum, saliva, and urine. Group consensus was that all three are generally acceptable; however, the choice of a body fluid to analyze should be predicated on the goals of the specific monitoring program. For studies that require a quantitative assessment of exposure, blood was recommended by the group as the fluid of choice (*8*). Saliva was also considered acceptable, and good correlations were reported between saliva and blood for results from the same subject (*9*). Sample collection considerations, however, resulted in the selection of blood as the sample medium of first choice. Analysis of either blood or saliva for cotinine permits an estimate of the degree of exposure to tobacco smoke in persons passively exposed at home or in the work place. While cotinine determination in urine was also recommended for estimating exposure, it was generally felt that estimation based on urinary cotinine excretion would be less reliable than estimation based on plasma or salivary levels. Cotinine excretion is variable across and within individuals depending on renal function, urine flow rate, and urine pH (*10*). Urine results may be expressed as micrograms of cotinine per milligram of creatinine in order to correct, in part, for the variable dilution effects. This correction or normalization, however, introduces additional variability since this requires another analytical determination (and opportunity for experimental error), and creatinine excretion rates for individuals are also variable. Horstmann (*11*) reported creatinine excretion rate for 56 subjects to be 1.11 ± 0.68 g/day (mean ± SD). Hoffman and Brunneman (*12*) also found 13 nonsmokers on a controlled diet to have creatinine values of 1.65 ± 0.5 g per 24 hr urine (mean ± SD). The coefficients of variation between subjects for these two studies were 61 and 30%, respectively.

Sample Collection and Handling. Chromatography procedures for cotinine generally require analysis of a 1 mL sample with an additional 1 mL volume needed for reanalysis. A total sample volume of 2.5 to 3.0 mL was therefore recommended. Glass and/or polypropylene sample tubes with screw cap closures were recommended. The polypropylene tubes were preferred to avoid breakage during shipment. Minimum size sample tubes were suggested to reduce volume losses from freeze drying during long-term storage.

Blood should be centrifuged at the field site and the serum samples frozen prior to shipment to the laboratory. Urine should be frozen soon after collection to prevent bacterial degradation of the sample. Saliva may be collected by expectoration into a sample tube; however, an alternative saliva collection procedure that uses highly adsorbent dental rolls is recommended (*13*). The subject is asked to place a dental roll in the mouth for approximately 15 min. The sample is then placed in a tube and frozen prior to shipment to the laboratory. The thawed sample is regenerated at the laboratory by placing the dental roll in a glass syringe and compressing with a glass plunger. The resultant clear liquid may then be aliquoted for analysis.

Shipment in a frozen condition with dry ice was recommended for all three sample types to prevent bacterial degradation of the sample matrix. Loss or degradation of the cotinine analyte was not considered to be a problem since participants had found this compound to be stable.

Upon receipt at the laboratory, samples should be placed in a freezer (approximately −20°C) until analyzed. Samples that will be held in excess of one year should be stored at −80°C. No cotinine degradation problems were reported for frozen samples. Precautions were recommended, however, to prevent concentration errors resulting from freeze-drying of samples stored over one year in a frost-free freezer.

Analytical Method Considerations

The group consensus was that the analytical method should permit the determination of nicotine and cotinine in a single analysis and should allow a clear separation and distinction between these and other analytes that may be present. The method should be sufficiently sensitive to give good definition of passive exposure and thereby yield analytical results which will show a distinction, for example, between a child or other nonsmoker that is exposed in the home and one that is not. Tables 1 and 2 list the range of detection limits for both chromatography and immunoassay methods.

The importance of this sensitivity consideration was supported by the 1981 report of Hirayama (*1*) and the 1987 report of Humble et al. (*7*) which showed an increased risk of lung cancer for a spouse exposed to a smoker in the home. Russell reported that cotinine levels in children's saliva averaged 0.44 ± 0.68 ng/mL where no parents are smokers, 1.31 ± 1.21 ng/mL where only the father smoked, 1.95 ± 1.71 where only the mother smoked, and 3.38 ± 2.45 ng/mL where both parents are smokers (*13*). This study used an analytical method with a detection and quantification limit of 0.1 ng/mL, which permitted classification of the lowest exposures into exposure distributions differing by only 0.1 ng/mL. Over 30% of the children from nonsmoking homes had cotinine concentrations below the 0.1 ng/mL detection limit. In the groups where one or more parents smoked, the cotinines were significantly ($p < 0.01$) elevated, and 50% of the children of the lowest exposed group had less than 1 ng/mL (when only the father smoked). Table 1 shows that several available chromatography methods have detection limits ranging from 0.1 to 0.2 ng/mL while the most sensitive immunoassay method in Table 2 reports a 0.3 ng/mL detection limit.

The question of analyte volatility losses during analysis was discussed, and it was generally agreed that if nicotine were included as an analyte, precautions would need to be taken to prevent loss during concentration steps. Acidification to convert nicotine to a salt form prevents losses during concentration.

Cotinine primary standards are used in the free base form by some analysts; however, a salt form was preferred by meeting participants, since the free base form is hygroscopic and difficult to maintain at a well-defined purity. A perchlorate salt of cotinine was recommended for preparation of 1 mg/mL stock solutions in 0.01 N HCl (*8*). This standard solution could be frozen and kept indefinitely. The group consensus was that a salt form of cotinine should be made available as a primary standard.

Chemical analysis is usually accomplished by gas chromatography with nitrogen/phosphorus thermionic detection (GC-NPD) or GC-mass spectrometry (GC-MS) using either electron impact ionization or chemical ionization. Packed columns for GC were successfully used; however, fused silica capillary columns containing a methyl silicone or methyl phenyl silicone liquid phase were recommended (see Table 1 HRGC references).

A high-performance liquid chromatography (HPLC) method using a C_{18} reversed phase column with paired ion chromatography and UV detection (at 257 nm) was also reported by McElroy where the HPLC method of Machacek and Jiang (*14*) was modified for analyzing urine samples at passive exposure levels. Further improvement in HPLC sensitivity and detection limit is required before application to the more limited sample volumes generally available for blood or saliva. HPLC was considered a very promising approach due to the highly efficient columns now available and the stability and reproducibility of response commonly obtainable by UV detection.

The final quantitation of residues in all methods was accomplished with internal standards and standard curves developed from fortified blank samples. It was recommended that standard curves be prepared daily or with each batch of samples. A variety of internal standards were used ranging from deuterated cotinine and nicotine for GC-MS to chemically similar compounds for other GC or LC detectors.

Table 1 lists the chromatography methods (*14–17*) presented at the workshop and summarizes the salient features of each. Information for this table was derived from questionnaire responses submitted by each author/participant.

Chromatography Group Recommendations

The chromatography group recommended that an interlaboratory methods comparison study be conducted prior to any large-scale monitoring efforts aimed at determining population exposure to tobacco smoke. Specific suggestions and recommendations relating to method comparison studies were as follows:

- Separate studies should be conducted for passive exposure levels and active smoker levels.
- Statisticians should be used in planning study samples.
- Blood, urine, and saliva should be included in each study.
- Immunoassay and chromatography methods should be included in each method comparison study.
- Samples should be fluids from exposed individuals and also from fortified blanks in order to look for bias from chromatography or immunoassay methods through measurement of artifacts or metabolites related to nicotine/cotinine.
- The study coordinator should supply standard reference material(s) to each participating laboratory.

Table 1. Summary of passive exposure chromatography methods.

	Machacek and Jiang (*14*)	Jacob, et al. (*15*)	Verebey et al. (*16*)	Feyerabend et al. (*17*)	Kornfeld (personal communication)
Sample type	Urine	Blood, plasma, urine	Serum	All biological fluids	Urine
Vol. analyzed, mL	6	1	0.5	1	5
Concentration step	N2 evaporated to dryness	N2 evaporated to dryness	None	N2 evaporated to dryness	N2 evaporated solvent exchange
Extraction method	SPE column Chloroform elution	Solvent extraction	Solvent extraction	Solvent extraction	Solvent extraction
Isolation step	Acid/base partition	Acid/base partition	Acid/base partition	None: plasma and saliva back extract urine	None
Determination	HPLC reverse-phase Paired ion chromatography	HRGC-NPD HRGC-MS	HRGC-NPD	GC-NPD	HRGC-MS
Quantitation	Internal standard Calibration curve	Internal standard Calibration curve	Internal standard Calibration curve	Internal standard Calibration curve	Internal standard Calibration curve
Linear range	0-500 ng	0-4000 ng/mL	40-400 ng/mL	0-15,000 ng/mL	1-500 ng/mL
Detection limit	<1 ng/mL	0.2 ng/mL	5 ng/mL	0.1 ng/mL	0.13 ng/mL
Quantification limit	1 ng/mL	0.5-5 ng/mL	5 ng/mL	0.1 ng/mL	0.25 ng/mL
CV, %	13	6.8 (3.0 ng/mL) 12 (1.0 ng/mL) 13 (0.5 ng/mL)	5.6	7.7	6
% recovery	90	107	90	90	85

Immunoassay Group Report

Participants in the workshop with expertise in the development and use of immunoassays for detecting cotinine in biological fluids met independently of the chromatography group to discuss and make recommendations regarding methodology and applications of immunoassay in monitoring passive as well as active exposure to tobacco smoke, QA/QC programs, and interlaboratory methods comparison. The following discussion presents an overview of the available immunoassay techniques for cotinine analysis, their applications with advantages and disadvantages, and the views and recommendations of the immunoassay panel members. There is notable agreement between this group and the chromatography group on most common issues outside the technical aspects specific to each methodology.

General Method Considerations

Introduction. The first radioimmunoassay (RIA) for cotinine was reported in 1973 (*18,19*). Antisera were raised in rabbits and goats immunized with a covalent conjugate prepared by linking cotinine 4′-carboxylic acid to immunogenic carrier proteins, such as bovine serum albumin and keyhole limpet hemocyanin. The radioactive tracer was prepared by labeling a tyramine derivative of cotinine 4′-carboxylic acid with ^{125}I; since then, [^{3}H]cotinine has been prepared enzymatically (*19*) from [^{3}H]nicotine and is now widely used. Another approach uses cotinine derivatized at the 1-position in the pyridine ring for preparing the immunogen and as a precursor of an ^{125}I-labeled tracer (*20*). The original assay has been used to measure cotinine levels in physiological fluids, e.g., urine, blood, saliva, amniotic fluid, and spinal fluid

Table 2. Summary of immunoassay methods.

	Langone et al. (*18*) Langone and Van Vunakis (*19*)	Haley et al. (*22*)	Knight et al. (*29*)	Bjercke et al. (*27,28*)
Sample type and volume analyzed, mL	Urine (0.02-0.05) Serum (up to 0.5) Saliva (0.02)	Urine, plasma, saliva (0.005-0.025)	Urine (0.01) Serum (0.1)	Urine, serum, saliva (0.1 in RIA; 0.01 in microtiter plate assays)
Assay type	RIA (^{125}I, ^{3}H)	RIA (^{3}H)	RIA (^{125}I)	RIA (^{125}I, ^{3}H), ELISA,[a] FIA
Quantitation	Internal standard Calibration curve	Internal standard Calibration curve	Internal standard Calibration curve	Internal standard Calibration curve
Detection limit, ng/mL	2	0.37	0.3	0.5-1.5
Quantitation limit, ng/mL	2	1	1	0.5-1.5
CV, %	6-10	10	10-15	9-14

[a]Enzyme-labeled protein A cannot be used to assay concentrated serum.

(*18,19,21-23*) of active smokers and serum, urine, and saliva (*24-26*), of passive smokers.

More recently, monoclonal antibodies specific for cotinine have been prepared and used to develop fluid phase RIAs with the ^{125}I- and ^{3}H-labeled tracers as well as enzyme-linked immunosorbent assays (ELISA) and a fluorescence immunoassay (FIA) in a microtiter plate format (*27,28*). These assays also have been used to measure cotinine levels in fluids of active (*27,28*) and passive smokers (Langone et al., unpublished results).

Test Samples and Standards. Because the original RIAs and the monoclonal antibody-based nonisotopic assays have been developed for analysis of unextracted physiological fluids, careful attention must be paid to possible nonspecific inhibition of antigen-antibody binding resulting from effects of pH or high concentrations of salts or urea, e.g., in urine. In this regard the immunoassay group agreed and strongly recommended that pooled standard samples of serum, saliva, and urine containing known amounts of added or endogenous cotinine should be made available through an agency such as the National Institute of Standards and Technology. There was general agreement that GC/MS would be the best method to establish the cotinine concentration for purposes of methods comparison and that levels should cover the range from cotinine-free through concentrations found in passive and active smokers. Essentially cotinine-free samples might be collected from a population that would represent a group with minimal exposure to tobacco smoke (e.g., Mormons in Utah). The suggestion also was made that low-level or essentially cotinine-free fluids might be treated (e.g., by absorption with XAD-2 resin or charcoal) to remove possible traces of cotinine. However, because absorption could remove other constituents that might affect the assays, it was not considered to be a firm recommendation.

Although it was suggested that urine may be the fluid of choice for RIA analysis, there was no strong consensus for priority over serum or saliva. In this regard, one participant pointed out the advantage that salivary cotinine levels determined by RIA are independent of saliva flow (Van Vunakis and Regas, unpublished results). The monoclonal antibody assays also have been used to detect cotinine in saliva and urine of passively exposed children (Langone et al., unpublished observations), and these investigators tended to favor the use of saliva. In addition to the use of dental rolls as discussed by the chromatography group, one member of the immunoassay group suggested that subjects chew a piece of Teflon tape to stimulate the flow of saliva that is then collected in a glass vial. It was pointed out that Teflon will not contaminate the sample. Regardless of which fluid is tested, it was recommended that samples be centrifuged (e.g., 2000*g* for 10-20 min or 10000*g* for 1-2 min) to sediment particulate matter before analysis. Immunoassay group participants concurred with the sample handling recommendations given in the Chromatography Group Report.

Comparison of the Assays

The original RIA and variations of it are used by the immunoassay group participants. Therefore, the discussion focused on this method and the monoclonal antibody based assays, the salient features of which are summarized in Table 2.

Reagents. The same immunogen was used to produce the rabbit, goat, or sheep antisera and the monoclonal antibodies. However, it was emphasized that cotinine 4′-carboxylic acid (and the ^{125}I-labeled derivative) is a mixture of stereoisomers giving rise to a heterogeneous population of polyclonal antibodies recognizing both natural (−)-cotinine and the (+)-enantiomer. Also, conventional antisera contain a population of antibodies that bind specifically to the linkage group that joins cotinine to the immunogenic carrier protein and to the tyramine group in the ^{125}I-labeled derivative. The practical consequences are a relatively shallow standard inhibition curve and the failure to achieve 100% inhibition of immune binding with (−)-cotinine. Although these problems are circumvented by using (−)-[^{3}H] cotinine, this assay is somewhat less sensitive, owing to the lower specific activity and counting efficiency achieved with tritium. Also, disposing of large volumes of radioactive scintillation fluid is a major concern.

Two approaches have been used with some success to improve the quality of the ^{125}I-RIA with rabbit antisera. They involve removing antibridge group antibodies by absorption with a nicotine-hemocyanin conjugate (*29*) and preparing an ^{125}I-labeled cotinine derivative with a bridging group different from that present in the immunogen (*30*). In contrast, monoclonal antibodies to cotinine were produced in a way that avoided the problems inherent in the use of polyclonal antisera (*27,28*). Although the immunogen contained a mixture of isomers, the hybridomas were screened using (−)-[^{3}H]cotinine to optimize chances of detecting antibodies that preferentially recognize the naturally occurring isomer, but not the bridging group in the immunogen. Furthermore, it was pointed out that monoclonal antibodies are preferred standard reagents for immunoassay because they are continuously available and are homogeneous in terms of binding affinity and specificity.

The specificity of any newly produced antiserum must be fully characterized one time with a battery of compounds that would include at least cotinine, nicotine, and metabolites such as nicotine N′-oxide, nornicotine, and *trans*-3′-hydroxycotinine. This recommendation holds even for new antisera prepared with a proven immunogen, since the response of individual immunized animals cannot be predicted. However, all agreed that when the properties of the antiserum had been established, it was unnecessary for each laboratory that received that antiserum to

complete a thorough reexamination of specificity, although it would be good laboratory practice to routinely compare the cotinine and nicotine inhibition curves.

Immunoassay methods have often reported using standard cotinine as the free base. However, because cotinine is hygroscopic and difficult to weigh accurately, all participants agreed that a nonhygroscopic salt of cotinine, such as the perchlorate or fumarate, would be the preferred standard.

Assay Performance. In the original RIA, antibody-bound and free-labeled cotinine were separated by the double-antibody method in which a heterologous antibody directed against the species of anticotinine was used to precipitate antigen-antibody complexes (*18*). Other techniques can be used including precipitation with ammonium sulfate or polyethylene glycol (*29*). Although the latter methods are faster and less expensive, there was some concern expressed that background radioactivity precipitated by ammonium sulfate, when normal serum is used in place of anti-cotinine, can exceed 10% of the added amount of cotinine tracer.

In contrast to the conventional fluid phase RIAs, the monoclonal antibody-based assays are carried out in a solid-phase system in which a cotinine-polylysine conjugate is passively adsorbed to the surface of 96-well plastic microtiter plates (*27,28*). Immobilized cotinine and fluid phase cotinine in the test sample compete for monoclonal anticotinine, which is detected with a variety of enzyme-labeled antiimmunoglobulin reagents including the bacterial product, protein A. Assay sensitivity can be enhanced by using a sandwich procedure in which rabbit anti-mouse immunoglobulin is added before (or along with) labeled protein A. It was emphasized that protein A reagents cannot be used to detect low levels of serum cotinine, because host IgG will bind nonspecifically to the microtiter wells giving high background binding of the enzyme-labeled protein A tracer.

Compared to times when rabbit antisera were used, assays with monoclonal antibodies were more sensitive, the standard curves were steeper, and the antigen-antibody reaction was completely inhibited by (−)-cotinine, even when the ^{125}I-labeled tyramine derivative was used in RIA (*28*). There was good agreement between the levels of cotinine found in saliva and serum of smokers determined by conventional RIA, the monoclonal antibody ELISA and GC (*27,28*). It was pointed out that high quality rabbit antisera also can be used in the solid phase nonisotopic immunoassays with titers that can be 100- to 1000-fold higher than in RIA (*27,28*).

Specificity and Sensitivity. Both polyclonal and monoclonal antibodies are specific for cotinine (*18,19,27*). Approximately 50 to 100 compounds that have been tested in the immunoassays including several nicotine metabolites, related tobacco alkaloids, and other compounds that retain structural features of either or both ring systems found in nicotine or cotinine fail to inhibit the antigen-antibody reactions at levels that would interfere in the assays.

One participant emphasized that literally thousands of serum and urine samples from both active smokers and nonsmokers had been analyzed over a period of several years and that few, if any, false positives had been reported. Although the subjects studied are mainly from the U.S. and England, these data support the view that diet or other factors such as prescription or other drugs do not interfere in the assays and are consistent with high specificity of anticotinine. It was pointed out that differences in diet or drug use must be considered when other populations are studied, or at least be aware that interference in the assays might arise from factors which have not appeared to date.

The immunoassays generally can detect cotinine down to the ng/mL level or less (Table 2), although it was emphasized that sensitivity can be affected by the need to dilute samples (e.g., urine) that may give spurious results when higher concentrations are tested. This point was discussed at some length with the participants in agreement that a sensitivity for cotinine of 0.1 ng/mL of physiological fluids could not generally be achieved with confidence using the available immunoassays. In this regard, it was pointed out that differences in sensitivity limits between chromatography and immunoassay likely reflect fundamental differences in methodology and are not strictly comparable. GC methods, for example, might extract and analyze a considerably larger portion of sample than would be analyzed by immunoassay.

Analytical Results. There was general agreement that cotinine concentration should be expressed as nanogram per milliliter. However, urine values also should be given as nanogram per milligram creatinine, as this ratio is used conventionally in the medical literature to account for differences in urine volume. Because low levels of creatinine in infants relative to adults may result in misleading values that fall into the range reported for active smokers, the need to include primary data for urine was stressed. Furthermore, experience has shown that urinary cotinine levels determined by conventional RIA generally are 30 to 50% higher than values obtained for the same samples by GC. Discussion centered on the possibility that the higher RIA values may reflect cross-reactivity of anti-cotinine with *trans*-3′-hydroxycotinine, which recently has been reported to be a major nicotine metabolite found in smokers' urine at levels up to three times higher than cotinine (*31*).

Since this meeting, synthetic *trans*-3′-hydroxycotinine (supplied by Dr. Peyton Jacob, San Francisco General Medical Center) has been shown to cross-react only 1 to 2% compared to cotinine in the monoclonal antibody based ELISA; one participant stated that he found only about 5% cross-reactivity with his rabbit antiserum in RIA. This degree of cross-reaction would not account for the discrepancy

Table 3. Summary comparison of chromatography and RIA methods.

	Chromatography methods	RIA methods
Sample type	Blood, saliva, urine	Blood, saliva, urine
Vol. analyzed, mL	0.5-6	0.005-0.5
Extraction and concentration	Yes	No
Quantification	Internal standard Calibration curve	Internal standard Calibration curve
Detection limit, ng/mL	0.1-5	0.3-2
Quantification limit, ng/mL	0.1-5	0.5-2
CV, %	5.6-13	6-15

in the urine values, and it was agreed that further research was needed to clarify the basis for the differences.

Considerations in Selecting an Analytical Technique

Table 3 shows some comparisons for RIA and chromatography methods. Apparent differences are in sample volumes used, sample work-up requirements, and limits of detection. RIA methods use less than 10% of the sample volumes required for chromatography methods, and this is a major reason that RIA detection limits are not as low as those for chromatography methods. Because RIA methods do not require sample manipulations such as extraction and concentration, they are faster, simpler, and presumably less expensive. Chromatography procedures not only have the advantage of increased sensitivities, but also are more specific and can provide quantification of both nicotine and cotinine in a single analysis. Workshop participants agreed that the choice between these two approaches would depend on the goals of a particular study. Both approaches have been found to be 100% effective in discriminating smokers from nonsmokers (*32*). This particular goal would favor the use of an RIA method. At least one participant suggested that the more sensitive chromatography methods are recommended to characterize ETS exposures for plasma or saliva concentrations when levels are less than 1 ng/mL.

A compilation of literature values for cotinine concentrations in body fluids of nonsmokers before and after documented ETS exposures is shown in Table 4. This comparison indicates a similarity between plasma and saliva concentrations, while urine values are often a factor of two or more higher. This is a primary reason that urine is often the fluid of choice when RIA methods are used in passive smoking studies.

Quality Assurance for Laboratories Assaying Cotinine

Participants in the cotinine workshop discussed the need for developing a quality assurance (QA) program for monitoring performance of laboratories assaying cotinine for the purpose of assessing exposure to ETS. When assuming many subjects, such a QA program would be essential to ensure that the conclusions reached are based on reliable data. A one-time exercise where the ability of laboratories to measure cotinine levels found in both active smoking and for passive exposure to ETS was considered as an alternative possibility. This suggestion was prompted by the realization that although published data on cotinine levels found in body fluids for active smokers show reasonable agreement, levels of cotinine reported for subjects exposed to ETS show considerable variation. Such differences might not be unexpected when measuring the low levels of cotinine found in ETS exposure, given that the detection limits for existing analytical methods approximate these cotinine levels.

To evaluate the between-laboratory variation in cotinine analyses, an international study was initiated by the Forschungsellschaft Rauchen and Gesundheit mBH in Hamburg (*32*). Eleven laboratories experienced in measuring nicotine and cotinine by RIA and/or GC participated. Serum and urine specimens from eight nonsmokers and eight smokers, and from two nonexposed nonsmokers spiked with nicotine and cotinine were distributed on dry ice to each laboratory. Results were returned and analyzed by method and by laboratory. Recoveries on both the urine and serum specimens spiked with cotinine corresponding to levels found in smokers ranged from 79 to 119%, with the exception of one laboratory with a 20% recovery (the data from this laboratory were excluded from further analysis). The interlaboratory coefficient of variation on these same samples was excellent (9-13%). The coefficient of variation on samples from smokers was fairly large, however, ranging from 18 to 45% for serum and 21 to 59% for urine. Further, cotinine levels reported for urine were about 60% higher than from those using RIA as compared to GC, suggesting a possible interfering substance in the immunoassay system. Cotinine levels reported for nonsmokers were extremely variable, and a number of laboratories could not detect cotinine in serum from exposed nonsmokers. In addition, cotinine values reported by some laboratories bore no relationship to estimated ETS exposure, or they were so high as to be unrealistic. In spite of this variability, all laboratories were able to discriminate smokers from nonsmokers with 100% effectiveness.

Table 4. Mean or median concentrations of cotinine in nonsmokers before and after exposure to environmental tobacco smoke.

Study reference	Plasma cotinine, ng/mL		Saliva cotinine, ng/mL		Urine cotinine, ng/mL	
	Before	After	Before	After	Before	After
(*33*)	0.82	2.04	0.73	2.48	1.55	7.71
(*34*)	1.1	7.3	1.5	8.0	4.8	12.9
(*9*)	0.8	1.8-2.5	0.7	2.2-2.8	1.5	6.5-9.4
(*13*)			0.4	1.3-3.4		
(*35*)	0.9-1.7	2.6-3.3	1.0-1.7	1.4-2.5	14	21-55
(*36*)					8.5	25.2
(*25*)						2.8-29.6
(*37*)			1.3-1.7	2.4-5.6		

The cotinine results reported for ETS exposure should be viewed with caution, however. A number of the participants at this conference workshop, who also were in the study, indicated that the volumes provided were insufficient for repeat analysis using GC or an assay was used which had not been optimized for measuring passive levels of cotinine. A further limitation of the study was that recovery of spiked cotinine was only assessed for smoking levels. Finally, immunoassays based on monoclonal antibodies were not included, nor were HPLC methods evaluated.

This interlaboratory study indicates the need for further information on the reliability of data provided by laboratories for study subjects exposed to ETS. A quality assurance program could provide such information, as well as an ongoing assessment of quality and a mechanism for improving performance.

QA Recommendations

Interlaboratory QA. The need for an interlaboratory quality assurance program was endorsed by most of the session participants, with some concern being expressed that the number of samples evaluated be kept to reasonable limits to minimize unnecessary assays. It was recommended that such a program should be administered by a QA coordinator laboratory. The coordinating laboratory would be responsible for monitoring the performance of participating laboratories and for providing specified samples as standards and/or controls. This laboratory should have in-house expertise or have access to laboratories having expertise in both immunoassay and high resolution gas chromatography/mass spectrometry (HRGC-MS).

Suggested objectives of the QA coordinator laboratory include:

- To provide an objective measure of the precision and accuracy of analytical methods used routinely by laboratories assaying cotinine.
- To identify preferred method(s) for measuring cotinine.
- To assess the reliability of results provided by different laboratories.
- To provide a mechanism for improving performance through knowledge of the performance of others.
- To serve as a resource center for communication and exchange of information among participants.

Recommended mechanisms for accomplishing the foregoing objectives are as follows:

Interlaboratory Quality Assurance Studies: Quality Assurance Samples. The coordinator laboratory should periodically conduct a blind or check sample study consisting of authentic biological fluids (serum, urine, or saliva) with actual or spiked levels of cotinine. Samples should be selected to represent cotinine levels typical of those found in passive and active smoking. Authentic biological samples with actual levels of cotinine are strongly recommended because only they will contain nicotine metabolites or other substances that may interfere in assays. In addition, blank samples spiked with known levels of cotinine should be distributed to evaluate recovery. Finally, samples with high levels of cotinine should be diluted with negative specimens to check for linearity. Samples should ideally have target values assigned by the QA coordinating laboratory through use of reference methods. Data returned by participants would be analyzed and reports containing results and a critique distributed.

Field Study Samples. The QC coordinating laboratory may assist organizations carrying out field studies in assessing the performance of the study laboratory on actual study subjects. The workshop considered that this could be accomplished by submission at intervals of blind duplicates: duplicates of the same study subject submitted at intervals to assess precision; split samples: sample is split with one portion being sent for analysis to the study laboratory and one portion to the QC coordinating laboratory for comparison purposes; blanks: samples that are considered free of analyte to serve as a check on environmental contamination.

Ancillary Activities of the QA Coordinating Laboratory: Primary Reference Standard(s). A strong consensus was reached that a well-characterized, pure, primary reference standard be made available. This material should be aliquoted into quantities sufficient to allow any laboratory to use

the standard for assay calibration. Handling and storage information should also be provided along with suggested methods for preparing secondary standards. It was generally agreed that cotinine should be in the form of a salt, since cotinine freebase is hygroscopic and, therefore, likely to vary in composition dependent on handling conditions. The perchlorate salt was suggested as one possibility (see chromatography group report). It was further recommended that the standard be supplied in solution to preclude errors due to dilution. The GC group made the suggestion that the National Institute of Standards and Technology might be the appropriate agency to prepare such a standard. The QC coordinating laboratory could then distribute the standard.

Biological Reference Samples. The suggestion was made that, in addition to providing a primary reference standard, the QA coordinating laboratory make available authentic biological samples from actual smokers and subjects exposed to ETS. Cotinine concentrations would be established by the QA coordinating laboratory using a reference method(s) and declared on each reference sample. Such samples would be important because the cotinine would be present in the matrix (urine, serum, saliva) actually used by analysts, thus allowing evaluation of possible matrix interference. In addition, such specimens would contain nicotine, nicotine metabolites other than cotinine, and other substances which might interfere in the assay.

The GC group also felt that blank samples, i.e., those essentially free of cotinine would be desirable. Suggested sources were bovine serum or human samples with very low exposure to ETS. Pooled specimens might be necessary because obtaining sufficient volume of biological reference samples could prove difficult. Samples would therefore be provided in restricted quantities only to allow laboratories to periodically evaluate their own method.

Reference Method. Workshop participants also discussed a reference method for establishing cotinine levels in biological samples. The consensus of the group was that GC/MS would be the ultimate reference method because of its extreme specificity. However, in the group discussion, the GC group pointed out that although the method is highly specific, it ultimately is no better than the reliability of the extraction and evaporation methods chosen to prepare samples for analysis. A further concern was that differences in assigned values may result from differences attributable to the detection method (chemical ionization or electron impact). Consequently, it appears unlikely that a gold standard will be available and acceptance of a reference method will depend ultimately on judgment of its reliability. Representatives of the National Institute of Standards and Technology indicated that their practice is to evaluate a variety of independent methods, and if sufficient agreement is reached, a certified value is provided, albeit with the understanding that confidence limits are somewhat uncertain. In the absence of agreement between various methods, NIST provides a consensus value(s) for informational purposes.

In the event that GC/MS is adopted as a reference method, the implication for immunoassayists is that their performance would be judged against this standard. Judging immunoassay results against GC/MS is not without precedent, since other immunoassays, such as those for steroids, are already compared to this method.

Postscript

A cotinine spiked, freeze-dried human urine reference material is being prepared by the National Institute of Standards and Technology (formerly the National Bureau of Standards). Three lots with different cotinine concentration levels are being prepared: *a*) an unspiked blank level (< 1 ppb), *b*) an approximately 50 ppb low level, and *c*) an approximately 500 ppb high level. This material (EPA/NIST Reference Material 8444) is planned for issue during the first quarter of 1989. The material may be ordered from: Office of Standard Reference Materials, Building 222 Room B-311, National Institute of Standards and Technology, Gaithersburg, MD 20899. Telephone 301-975-6776. Technical information may be obtained from Dr. Lane Sander, Organic Analytical Research Division, Center for Analytical Chemistry, NIST, Gaithersburg, MD 20899. Telephone 301-975-3117.

A cotinine perchlorate salt reference material is also being planned for development by NIST. A date has not been determined, however, for release of this standard.

The research described in this report has been reviewed by the Health Effects Research Laboratory, U.S. Environmental Protection Agency, and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the Agency nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

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ELIMINATION OF URINARY COTININE IN CHILDREN EXPOSED TO KNOWN LEVELS OF SIDE-STREAM CIGARETTE SMOKE

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Abstract

The establishment of a quantitative personal marker of side-stream smoke exposure in children is important in the study of potential health effects in this group. Cotinine, a metabolite of nicotine, has been shown to exhibit a dose-response relationship to side-stream smoke exposure in adults, and has been used to quantitate prior exposure. This study was undertaken to determine the dose of nicotine, the peak level of urinary cotinine, the time to peak cotinine levels, and the elimination half-life of cotinine in young children exposed to a controlled amount of side-stream smoke. With an exposure to 26.4 $\mu g/m^3$ of nicotine, nine children, averaging 2.03 years old had peak cotinine/creatinine levels of 818 ng/mg. The time to peak cotinine was 4.1 hours, with an elimination half-life of 28.7 hours.

Disclaimer

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Introduction

Increased concern has been expressed about the potential health risks associated with the exposure to side-stream smoke (21). Recent studies implicate exposure to side-stream smoke as a particular health risk in infants and young children (3,8,17,19). Research into the health effects of exposure to side-stream smoke in children would be greatly aided if a chemical marker could be used to predict the level of exposure to side-stream smoke. Several substances, isolated from tobacco smoke, or their metabolic products, have been measured in biological fluids to estimate this exposure to side-stream smoke. These substances include carboxyhemoglobin, thiocyanate, nicotine and cotinine (4,6,12,13,14,15). Cotinine, a metabolite of nicotine, has been shown to be a good indicator of the exposure to side-stream smoke (14). Studies in adults have shown that there is a dose-response relationship between the number of cigarettes smoked and the level of cotinine in the urine (9,13). The elimination half-life of cotinine in the urine and in the blood has also been reported in adults (1,2,12).

The use of cotinine as an indicator of side-stream smoke exposure in children has been studied by Greenberg et al. (6). They found a high correlation between the exposure of children at home to side-stream smoke and their levels of urinary cotinine. These results suggested that uninary cotinine may be a useful indicator of side-stream smoke exposure in infants and young children. Etzel et al. (4) provided data on the elimination half-life of cotinine in the urine of newborn infants exposed to side-stream smoke in utero. The level of exposure for both these studies came from the self-reported smoking behavior of the mother.

Information on the uptake of nicotine and the elimination of its metabolite, cotinine in young children, age 1 to 3 years of age, exposed to side-stream smoke is unavailable. This information is considerd critical because it will allow one to estimate prior exposure, rather than rely on questionnaire data. This study was undertaken to determine the exposure dose of nicotine, the peak level of urinary cotinine, the time to peak levels of cotinine, and the elimination half-life of urinary cotinine when children are exposed to a controlled amount of side-stream smoke.

Methodology

Subjects: Nine children (5 males, 4 females; mean age 2.03 yrs.; age range 9 months to 3.5 yrs.), accompanied by their parents or guardians were exposed to side-stream smoke generated by a smoking machine at the rate of two cigarettes per hour for a total of four hours. All children were individuals who had been exposed to side-stream cigarette smoke in their home environments. The parents were asked to refrain from exposing the children to side-stream smoke for three days prior to the chamber exposure, during the day of exposure, and for three days following the exposure. Urine samples were collected for cotinine analyses each morning prior to the exposure, during the exposure, the evening

following the exposure and for three days following the exposure. Once collected, the urine samples were coded, frozen, and shipped to the American Health Foundation in Valhalla, New York, for analysis. Cotinine was measured by radioimmunoassay (6,18). The concentration of cotinine in the urine was standardized by expressing it as the ratio of cotinine/ creatinine, ng/mg (18).

Prior to each study, the children received a physical examination during which respiration rate and body weight were determined. This was repeated at the end of the exposure period. This study was approved by the Committee on the Protection of the Rights of Human Subjects of the School of Medicine of the University of North Carolina at Chapel Hill.

Chamber: The experiments were performed in a chamber, 13.6 m^3, with 3.55 air changes per hour. Temperature was maintained at 24±0.3 C, with a relative humidity of 55±5 %. The air within the chamber was mixed by a small boxer fan at 50CFM.

Environmental Measurements: Air nicotine levels were measured on bisulfite-impregnated filters (7). Each exposure concentration was the average of 3-6 filters, collected over the four hour exposure period.

Total Suspended Particulate values for the study were averaged from duplicate Anderson filter samples collected during each 4 hour exposure at a flow rate of 1.7 m^3 per hour. A Thermo-Systems, Inc. Model 3030 Electronic Aerosol Analyzer made measurements every 10 minutes. Carbon monoxide (CO), NO and NOx were measured continuously and reported every three minutes. CO was measured by nondispersive infrared, (NDIR) while NO and NOx were measured by chemiluminescence.

Exposure: Research grade cigarettes, 2R1, were smoked by a smoking machine, [RM30, Heinr. Borgwaldt, Hamburg,FRG]. Prior to the study, the cigarettes were conditioned at 22°C at 60% RH for 48 hours. One cigarette was ignited every 30 minutes and burned for about 12 minutes. The cigarette was puffed once a minute, each puff lasting 2 seconds with a draw of 35 ml (10,16). All mainstream smoke was vented to the outside of the chamber.

Data Analyses: The urine half-life elimination rate of cotinine was calculated by an interactive pharmacokinetic model (11), starting from the peak urine level of cotinine.

Results

Concentrations of gases in the side-stream smoke peaked 15 minutes after ignition and cycled at 30 minute intervals. Peak levels for CO were 2.2±0.8 ppm; NO, 54.8±4.3 ppb; and NOx, 58.8±5.3 ppb. The mean value for the air concentration of nicotine was 26.4±6.6μg/m^3. The mean concentration of particulates, measured as TSP was 321.8± 20.7μg/m^3, and by electronic aerosol analyzer was 345.4±51.2μg/m^3.

The dose of nicotine that each child received was calculated from the mean concentration of air nicotine for that exposure, the average of the child's respiratory rate, and the child's tidal volume calculated from

its body weight (table 1) using the formula of Gautlier et al. (5).

The distribution of baseline levels of urinary cotinine appeared to be bimodal, indicating the possibility of exposure of several children to side-stream smoke prior to entering the study. The mean cotinine/creatinine ratio for one group was 36.5±17.3 ng/mg, while the other group was 190±18 ng/mg.

At 4.1±1.4 hours after exposure to the first cigarette, peak levels of urine cotinine/creatinine averaged 393.7±209.8 ng/mg in the first group, and 1635±489 ng/mg in the second group (table 1). The average urine elimination half-life of cotinine was 28.7±13.1 hours with a range of 14.6 to 55.1 hours.

Table 1: Summary of Nicotine and Cotinine Levels for Each Exposure

Subject	Air Nicotine µg/m^3	Nicotine dose µg	Baseline† cotinine ng/mg	Peak cotinine ng/mg	Time to peak (hours)	T1/2 hours
1	20	26.9	43[a]	175[a]	3.8[a]	38.9
2	23	20.3	190[b]	1095[b]	6.3[b]	24.2
3	28	21.8	208[b]	2048[b]	4.0[b]	14.6
4	35	23.8	21[a]	429[a]	5.3[a]	15.1
5	23	24.9	44[a]	429[a]	5.8[a]	55.1
6	23	23.9	37[a]	667[a]	3.9[a]	20.3
7	18	12.9	13[a]	238[a]	2.9[a]	33.1
8	35	28.0	172[b]	1762[b]	3.2[b]	22.1
9	33	29.5	61[a]	524[a]	1.9[a]	35.3
Mean ±S.D.	26±7	23.6 ±4.9	37±17[a] 190±18[b]	394[a] ±210 1635[b]± 489	3.9[a] ±1.5 4.5[b] ±1.6	28.7±13.1

†Cotinine/Creatinine ratio

[a]Non-exposed subjects

[b]Subjects suspected of having prior exposure to smoke in the home environment.

Discussion

This study was undertaken to describe the uptake of nicotine by young children in a controlled exposure to side-stream smoke and the events associated with cotinine elimination. The gaseous and particulate fraction of side-stream smoke reported in this study compare very favorably with the values reported by Hoffman et al. (9) when their data are adjusted for the differences in chamber size, exhaust rate, and the number of cigarettes consumed per hour.

In this study population, the distribution of baseline levels of urinary cotinine was bimodal. One group had an average value of 36.5 ng/mg which compares to reported values of 14.5 (4) and 19 ng/mg (9). We believe that the other group, with an average value of 190 ng/mg, had been exposed recently to side-stream smoke. This is supported by values of cotinine/creatinine of 459 ng/mg (9) and 117 to 780 ng/mg (12) reported in exposed children. For each child, a dose of inhaled nicotine was calculated from the average nicotine concentration for that child's exposure.

The distribution of the peak levels of cotinine also exhibited a bimodal distribution (table 1). The group of subjects with the initial lower baseline level of cotinine had a mean peak level of cotinine/creatine of 393.7 ng/mg compared to 1635 ng/mg value for the group that may have had prior exposure. The time that it took to reach peak cotinine levels was 4.1 hours for the entire group. This compares with a reported value of 2 hours by Wilcox et al. (20) and 2 to 4 hours reported by Hoffmann et al. (9). In the paper by Hoffmann et al. (9), it is interesting to note that as the exposure to side-stream smoke increases, not only does the peak level of cotinine increase, but the time to peak also increases. Our data suggest that this may have also occurred, but our data base is too limited to compare the group with the presumed prior exposure with the group that had abstained for three days. The time course of cotinine elimination as measured in the urine appears to follow exponential kinetics. The average urine half-life was 28.7 hours. This rate appears to be shorter when compared to a half-life in neonates of 68 hours reported by Etzel et al. (4) but within the 7 to 40 hours reported in the adult population (1,2,12).

Conclusion

Although the study population was small, the data suggest that the elimination half-life of urinary cotinine in very young children is similar to that reported in the literature for adults. Future work should replicate this study, while exercising control of the children's exposure to side-stream smoke during both the pre and post chamber exposure phase.

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Home Air Nicotine Levels and Urinary Cotinine Excretion in Preschool Children[1-4]

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SUMMARY We examined the extent of correlation between home air nicotine levels and urine cotinine/creatinine ratios (CCR) in 27 children who attended a research day care program where they were not exposed to environmental tobacco smoke (ETS) during the daytime hours. Average concentrations of nicotine in home air were determined by active air sampling during the evening and night hours on 2 consecutive days. Urine samples for cotinine and creatinine determinations were collected before, during, and after the two sampling periods. In addition, four sequential weekly urine samples for CCR were obtained from study children to determine the extent to which single determinations of CCR were representative for individual children. Fifteen children resided in homes with smokers, and 12 did not. Urine CCR consistently distinguished most exposed and unexposed children. However, three exposed children had urine CCRs that clustered routinely around the criterion CCR (30 ng/mg cotinine-creatinine) that best distinguished exposed and unexposed children. In children exposed to ETS in the home, there was a significant correlation between average home air nicotine levels and the average logarithm of urine CCR the two mornings after the home air monitoring periods ($r = 0.68$; $p = 0.006$). In study children, urine CCRs were remarkably stable over the 1-month observation period. Rank correlation coefficients for sequential weekly determinations of CCR were consistently greater than $r = 0.88$; $p < 0.0001$.

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Introduction

Measurement of cotinine concentrations in body fluids has received recent scrutiny for its usefulness as an indicator of tobacco smoke exposure in adults and children (1-13). Average levels of cotinine in serum, saliva, and urine differ significantly for unexposed individuals, those exposed passively to environmental tobacco smoke (ETS), and active smokers. There remain questions, however, regarding the extent to which cotinine levels in body fluids are quantitatively indicative of the intensity of ETS exposure among passive smokers. Lack of this knowledge and the absence of information regarding the extent to which single cotinine determinations are representative for individuals has limited the usefulness of cotinine measurements in epidemiologic studies of the health effects of passive smoking.

In the present study, we examined the relationship between levels of nicotine in the home air and levels of cotinine in urine of 27 children between 11 months and 5 yr of age. In addition, we obtained a midweek urine sample from study children for cotinine determination each of 4 consecutive weeks to examine the stability over time of this biologic marker of ETS exposure.

Methods

The children studied attended the research day care program of the Frank Porter Graham Child Development Center in Chapel Hill, North Carolina. The respiratory disease research conducted in this population has been described previously (14-16). Children start attending the Center between 6 wk and 3 months of age and are followed continuously to the age of kindergarten entry. They attend the day care program 8 h/day, 5 days/wk and return home each evening and on weekends. Smoking is not permitted in child care areas of the day care center or in vans that are used to transport children to and from the day care center. Twenty-seven children participated in the study: 15 from homes with smokers and 12 from homes without smokers. Three pairs of siblings were studied: two pairs from smoking homes and one pair from a nonsmoking home. The study population consisted of 15 males and 12 females; 14 black and 13 white children. Their ages ranged from 11 months to 5 yr.

Measurement of the Extent of Tobacco Smoking in the Home

Three methods were used to estimate the concentration of tobacco smoke present in home air. (*1*) A questionnaire was administered with questions that identified the persons who smoked in the home and how many cigarettes were smoked. (*2*) Parents were asked to save the unsmoked portions of all cigarettes smoked in the home over 2 consecutive monitoring days. Parents were also asked to save the remnants of cigarettes smoked in their cars while riding with their children. Separate cigarette collections were made for each study day. (*3*) Active home air sampling for nicotine was performed simultaneously with cigarette remnant collections. Air sampling pumps (Model 520; Gilian Co., Wayne, NJ) operating at 15 L/min were employed with Andersen sampling heads (Particle Fractionating Sampler; Andersen Co., Atlanta, GA). Teflon-coated glass fiber filters (75 mm, T60A20; Pallflex, Putnam, CT) impregnated with aqueous 5% sodium bisulfate were used for nicotine trapping (17). The pumps operated from 5:00 P.M. to 7:00 A.M. for each of the 2 days of monitoring. Separate air nicotine determinations were made for each study day. Sampling heads were kept in the child's main activity room during the evening hours, then moved to the child's bedroom at bedtime.

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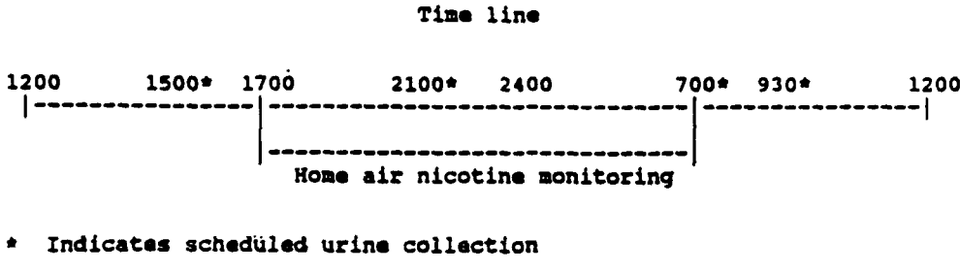


Fig. 1. Home air monitoring and urine collection schedule: time line for both study days 1 and 2.

Collection of Urine Specimens for Cotinine Determinations

Urine specimens were obtained before, during, and at the end of the period of home air nicotine monitoring (figure 1). Children who were not toilet-trained (n = 9) did not have urine specimens collected at home. These children had the 3:00 P.M. and midmorning urine specimens specified by the protocol collected while they were at the day care center. In addition, urine specimens for cotinine determination were obtained on four consecutive Thursdays after the home air monitoring period.

Measurement of Nicotine and Cotinine

Nicotine was eluted from sodium bisulfate-impregnated filters into ethanol and subsequently quantitated by gas chromatography using described methods (17). Concentrations of cotinine in urine samples were determined by competitive-inhibition radioimmunoassay using rabbit cotinine antiserum and tritiated cotinine (purchased from Helen VanVunakis, Brandeis University) (18). To adjust for urine dilution, urine cotinine concentrations were standardized to creatinine concentration and expressed as cotinine/creatinine ratios (CCR).

Results

Comparability of Estimates of Quantity of Tobacco Smoked in Home and Classification of Exposure Status

Except for one instance, there was agreement among the three measures of exposure employed: questionnaire, cigarette remnant collection, and active home air nicotine monitoring. Figure 2 shows the distribution of two consecutive home air nicotine determinations in homes where parents did or did not save cigarette remnants. Average air nicotine concentrations in homes where cigarette remnants were saved were consistently greater than 0.5 μg/m³; all children from these homes were considered exposed to ETS in the home. One family that reported smoking in the home in questionnaire responses saved no cigarette remnants. Average concentrations of nicotine in home air were greater than 0.5 μg/m³ on both monitoring days; therefore, this child was classified as exposed to ETS in the home. Otherwise, children who lived in homes where no cigarette remnants were saved were classified as not exposed to ETS in the home. The average concentration of air nicotine in the homes of unexposed children was 0.34 μg/m³ (SEM, 0.07 μg/m³) and in the homes of exposed children was 3.74 μg/m³ (SEM, 0.52 μg/m³).

Accuracy of Exposure Assignment Using Midmorning Urine Cotinine Determinations

The study protocol specified that midmorning urine specimens for cotinine be collected from study participants 6 times over a 5-wk period (two consecutive mornings after active home air nicotine monitoring and four consecutive Thursdays thereafter). A total of 153 of 162 (94%) of these urine specimens were successfully obtained and assayed for cotinine and creatinine concentrations. The distribution of CCR for these specimens is plotted in figure 3 in relation to exposure status. A urine CCR criterion level of 30 ng/mg resulted in the highest rate of agreement in exposure assignment. Sixty-six of 69 (95%) midmorning urine specimens obtained from 12 unexposed children had CCRs ≤ 30 ng/mg (first column, figure 3). Urine specimens from 12 of 15 exposed children consistently (63 of 67; 94%) contained greater than 30 ng cotinine/mg creatinine (second column, figure 3). However, three exposed children consistently had urine CCRs that clustered near the 30 ng/mg breakpoint (13 of 18 = 72% of urine specimens tested had CCR < 30 ng/mg) (third column, figure 3). The sensitivity of urine CCR was 80%; 12 of 15 exposed children were consistently designated as exposed to ETS using urine CCR at the 30 ng/mg criterion level. Thus, for 24 children (12 exposed and 12 unexposed), accuracy of exposure categorization using urine cotinine was excellent at the 30 ng/mg criterion level. Only seven of 136 (5%) urine specimens tested from these children resulted in misclassification of exposure status. The remaining three exposed children were misclassified as not-exposed by 13 of 18 (72%) urine cotinine determinations using the 30 ng/mg CCR criterion. Two-day average home air nicotine levels in the homes of these three children were 1.45, 3.85, and 3.40 μg/m³. Home air nicotine averaged 2.90 μg/m³ for the three children not classified as exposed by urine cotinine compared to 3.95 μg/m³ for the 12 children whose exposure status was consistently correctly identified by urine cotinine determinations.

The analyses that follow were performed with and without the data from the three exposed children with consistently low urine CCRs. Exclusion of data of exposed children with low CCRs did not alter the pattern of any analytic result; data shown are for all exposed children.

Correlation of Home Air Nicotine and Urine Cotinine Levels

Among ETS-exposed children, there was a significant positive correlation between the average concentration of nicotine in home air over the 2 days of monitoring and the average logarithm of urine CCR on the mornings after the 2 evenings of home air monitoring. In figure 4, average CCRs are plotted in relation to average home air nicotine levels for each of the 15 exposed children. The Pearson correlation for this set of data was r = 0.68; p = 0.006. The regression equation for the linear correlation is given in the figure legend.

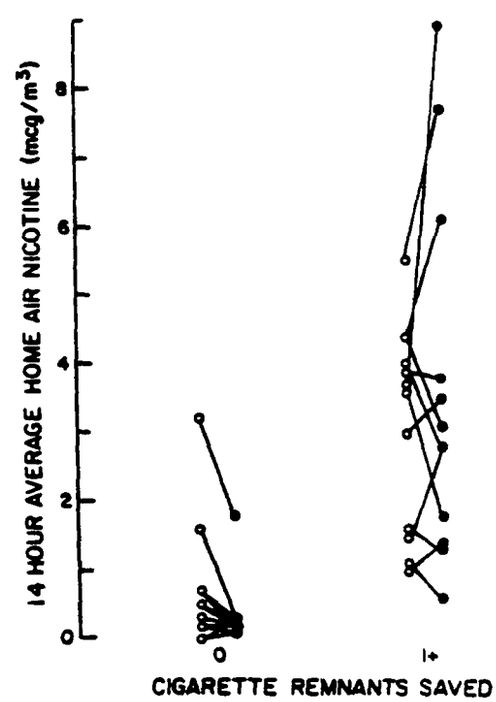
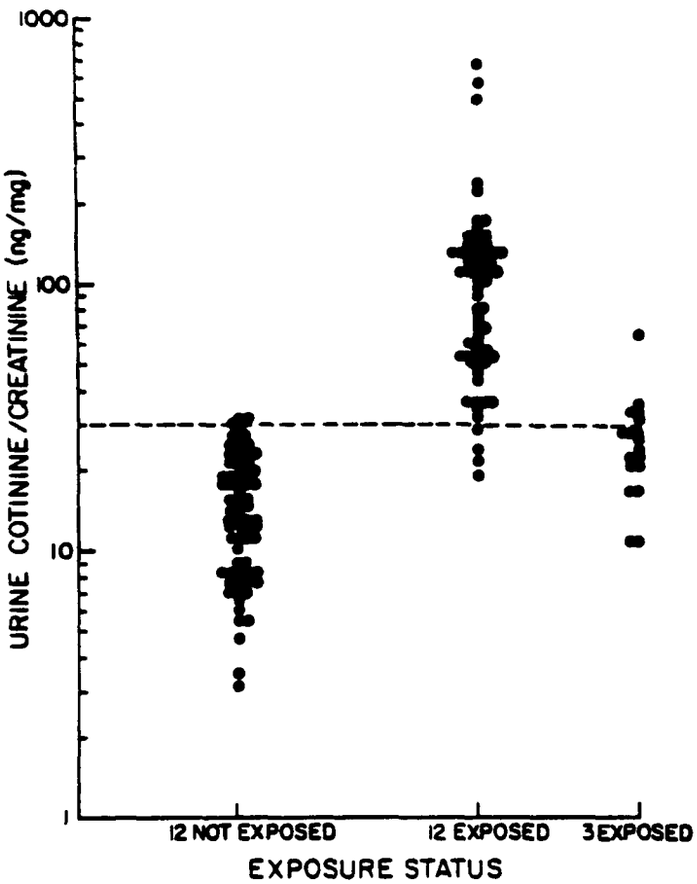


Fig. 2. Distribution of average home air nicotine concentrations for homes where cigarette remnants were and were not saved; measurements obtained on two consecutive days per home.



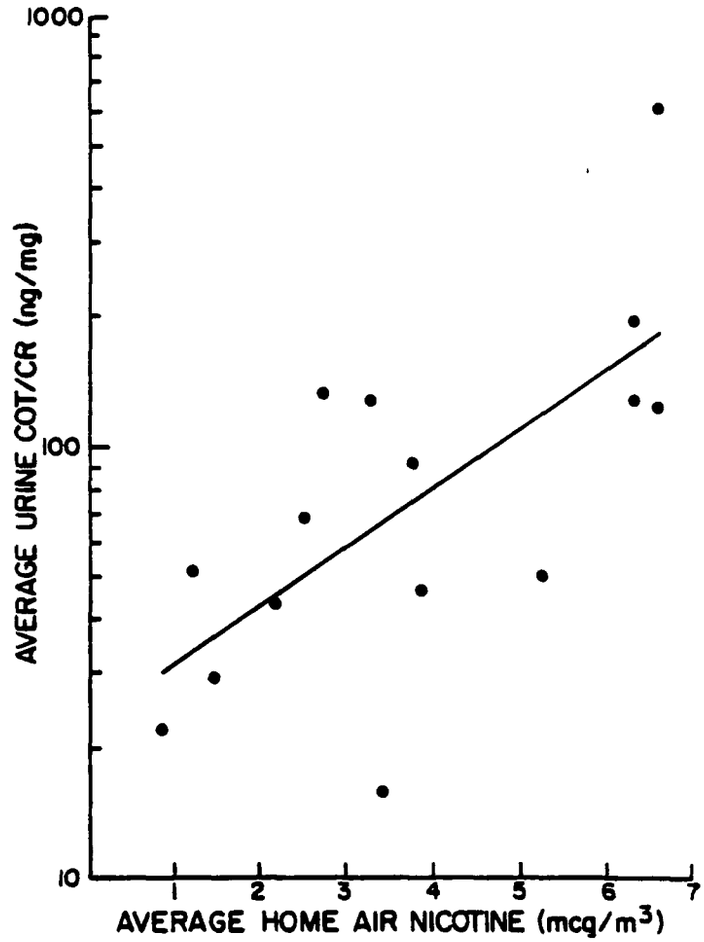


Fig. 3 (left). Distribution of all midmorning urine cotinine determinations from: column 1, 12 unexposed children (geometric mean CCR, 13.7 ng/mg; SEM, 2.3 ng/mg); column 2, 12 exposed children consistently classified as exposed with the CCR (geometric mean CCR, 86.5 ng/mg; SEM, 7.8 ng/mg); and column 3, three exposed children consistently classified as unexposed by urine CCR (geometric mean CCR, 24.5 ng/mg; SEM, 2.5 ng/mg).

Fig. 4 (right). Correlation between average home air nicotine concentrations for two monitoring days and average natural logarithm of urine CCR on the mornings after the two monitoring days for 15 ETS-exposed children. The equation for the regression line is: Ln CCR = 3.13 (SEM, 0.40) + 0.315 (SEM, 0.10) · average home air nicotine. ($F = 11.0$; $p = 0.006$.)

Observations Relevant to the Use of Urine Cotinine Determinations in Epidemiologic Studies

The urine collection protocol allowed us to examine whether time of urine collection during the day influenced urine cotinine levels. The means of $\log_e$ CCR did not differ significantly by sampling time. Furthermore, individual children maintained their absolute and relative levels of cotinine excretion throughout the two 24-h study intervals. In exposed children, rank correlations of sequential measurements ranged from $0.68 < r < 0.93$; $0.008 < p < 0.03$. There was no evidence that routine evening ETS exposures in children exposed chronically to ETS had acute effects on urine cotinine levels. For unexposed children, rank correlations of sequential urine CCRs were more variable, but as indicated previously the great majority of urine specimens (48 of 51 = 94%) had CCRs less than 30 ng/mg.

We then examined the stability of repeated urine cotinine/creatinine determinations over a 1-month time period. Urine specimens were collected while the children were at the day care center on each of four consecutive Thursdays. The data of 14 exposed children are shown in figure 5; one exposed child was absent frequently from the Center during the 1-month observation period. In exposed children, absolute and relative levels of urine cotinine were very stable over time. Rank correlations for sequential urine CCRs were all $r > 0.88$; $p = 0.0001$.

Discussion

Measurement of concentrations of cotinine in body fluids has been established as a useful method for identifying active smokers, persons exposed passively to environmental tobacco smoke, and neonates exposed to nicotine and cotinine in utero (1–13). Although active smokers can be distinguished readily from passive smokers with cotinine assays, these tests appear less accurate in distinguishing persons who are routinely exposed passively to ETS from those who are sporadically exposed. Furthermore, our knowledge of the usefulness of cotinine determinations for ranking the intensity of passive smoke exposure in populations of individuals is fragmentary. Most investigators who have attempted to examine this question have relied on questionnaire data to provide estimates of the intensity of ETS exposure in study populations. In the present work, we employed direct assays of home air nicotine concentrations to estimate the intensity of home nicotine exposure for study children.

The results of this study provide a firmer foundation for use of the CCR in urine as a biologic marker of ETS exposure in epidemiologic studies involving young children. Twelve of 15 preschool children routinely exposed passively to tobacco in the home had urine CCRs that were consistently > 30 ng/mg, whereas 12 of 12 children without home ETS exposure consistently had CCRs < 30 ng/mg. Three exposed children (20%) had urine

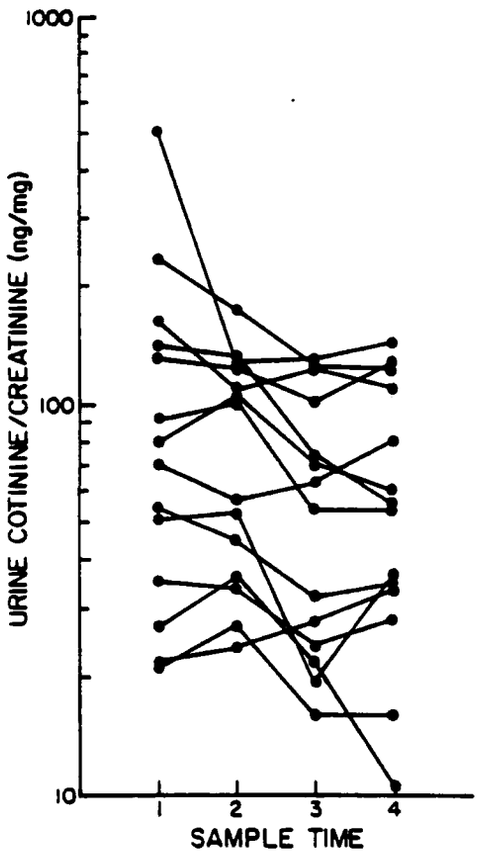


Fig. 5. Distribution of four consecutive weekly urine CCRs obtained from 14 ETS-exposed children. Rank correlations for sequential measurements are all greater than $r = 0.88$; $p < 0.0001$.

CCRs that were consistently in the upper range of values observed in unexposed children. Average home air nicotine levels were lower for these children than for exposed children with higher urine CCRs, but the difference was not statistically significant.

The correlation between average home air nicotine concentrations and the average $\log_e$ urine CCR among exposed children was $r = 0.68$, a significant association ($p = 0.006$). It is within the exposed group that a correlation must exist for cotinine to be a useful tool for assessing intensity of ETS exposure. The magnitude of correlation between air nicotine levels and urine CCR is dependent on the extent to which air sampling for nicotine in the home environment accurately reflected personal nicotine exposure and on the magnitude of individual differences in nicotine/cotinine metabolism for children with similar levels of environmental nicotine exposure. The degree of correlation between average home air nicotine concentrations and urine CCR observed in this study suggests that relative intensity of nicotine, and thus ETS, exposure can be estimated with a reasonable degree of accuracy using urine CCR.

Urine CCRs did not differ significantly by time of collection during the day. There was no evidence that usual evening ETS exposures altered urinary cotinine levels acutely. Furthermore, there was no evidence that short-term, high-level exposures, such as those occurring in closed automobiles, had a measurable impact on urine cotinine levels. Thus, for an individual child, any urine cotinine measured during a 24-h period was representative of the three others examined. Similarly, urinary CCRs were remarkably stable over a 1-month observation interval. Again, any single urine cotinine level was representative of the three others measured during the month. Relative urine CCR would have been assigned correctly using a single determination.

This study was conducted in a group of children who attended day care regularly. Urine cotinine levels might have been higher in young children who were exposed throughout the day to a smoking caregiver. The degree of overlap in urine CCR between some exposed and unexposed children might have been reduced in those circumstances. On the other hand, the study was conducted in the winter, when home ventilation rates are low compared to warm weather months. Performance of the study during the summer months could have reduced the accuracy of urine CCR as an exposure indicator.

These findings can be compared with those of other workers who have used salivary, serum, and urine cotinine determinations as indicators of passive smoking in children. Jarvis and coworkers (6) studied salivary cotinine levels in 569 nonsmoking English school children between 11 and 16 yr of age. Mean salivary cotinine concentrations were significantly higher in children with smoking parents than in children with nonsmoking parents. When both parents smoked, mean salivary cotinine levels were higher than when a single parent smoked. However, approximately 20% of children without smoking parents had salivary cotinine levels of 0.6 ng/ml or higher, whereas 20 to 30% of children with one smoking parent had levels below 0.6 ng/ml, indicating the potential for misclassification of exposure status. Coultas and colleagues (13) studied salivary cotinine levels in a population-based sample of adults and children in New Mexico. Salivary cotinine was detected in 38% of children less than 5 yr of age who lived in homes where no cigarettes were smoked. Conversely, 26% of children who lived in homes with smokers had no detectable salivary cotinine. In-home ETS exposure status of 33% of study subjects was misclassified using salivary cotinine determinations. These findings are comparable to those of Jarvis.

Pattishall and associates (9) studied serum cotinine levels in infants who attended the Frank Porter Graham Center, the site of the present study. Sera were obtained from 38 children at approximately 9 months of age; 20 children were identified by questionnaire as living in homes with smokers. Overall, there was a significant difference in the prevalence and average concentrations of cotinine in sera from exposed and unexposed children. However, cotinine was not detected in sera of 20% of children living in homes with smokers, while cotinine was demonstrated in sera of 26% of children living in homes without smokers. The accuracy of exposure assessment using serum cotinine was similar to that obtained by investigators studying salivary cotinine as a marker of ETS exposure in children.

Greenberg and colleagues (4) examined urinary and salivary cotinine levels in 46 infants: 28 with a history of passive tobacco smoke exposure and 18 with no exposure history. Urinary cotinine excretion distinguished exposed and unexposed children more reliably than did salivary cotinine determinations. Urine CCRs were significantly correlated with exposure dose estimated by questionnaire, when exposed and unexposed children were included in the analysis. When the correlation analysis was restricted to exposed children, no evidence of a dose-response relationship between reported maternal smoking intensity and infant cotinine excretion was observed (personal communication).

In a pilot study for the work presented here, we compared the sensitivity and specificity of serum cotinine concentrations and urine CCRs as correlates of home ETS exposure that had been documented by cigarette butt collections and home air nicotine monitoring. Cotinine was detected in sera of 16 of 26 (62%) children with home ETS exposure and in the sera of three of 20 (15%) children without home ETS exposure. Urine CCRs were more sensitive (21 of 26 = 81%) and more specific (18 of 20 = 90%) than serum cotinine levels for determining exposure status. For this reason, we focused our efforts on urine CCRs in the present study.

Determination of CCRs in urine samples is a reasonably accurate method for

identifying passive tobacco smoke exposure in young children. Because some exposed children have relatively low levels of cotinine in urine, cotinine determinations should probably be used in conjunction with questionnaire data regarding ETS exposure. Data presented here indicate that urinary CCRs are related quantitatively to the intensity of home nicotine exposure among ETS-exposed children. This and the relative stability of urine CCR over time support use of the assay in epidemiologic studies of ETS exposure in young children.

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Passive Smoking on Commercial Airline Flights

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In-flight exposure to nicotine, urinary cotinine levels, and symptom self-reports were assessed in a study of nine subjects (five passengers and four attendants) on four routine commercial flights each of approximately four hours' duration. Urine samples were collected for 72 hours following each flight. Exposures to nicotine measured during the flights using personal exposure monitors were found to be variable, with some nonsmoking areas attaining levels comparable to those in smoking sections. Attendants assigned to work in nonsmoking areas were not protected from smoke exposure. The type of aircraft ventilation was important in determining the levels of in-flight nicotine exposure. The environmental tobacco smoke levels that occurred produced measurable levels of cotinine (a major metabolite of nicotine) in the urine of passengers and attendants. Passengers who experienced the greatest smoke exposure had the highest levels of urinary cotinine. Changes in eye and nose symptoms between the beginning and end of the flights were significantly related both to nicotine exposure during the flight and to the subsequent urinary excretion of cotinine. In addition, subjects' perceptions of annoyance and smokiness in the airplane cabin were also related to in-flight nicotine exposure and urinary excretion measures.

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THE ADVERSE health effects on nonsmokers of passive, or involuntary, smoking include lung cancer and respiratory disease, the latter especially among children, as well as acute irritant effects. The scientific evidence for these effects has been reviewed and codified in the Surgeon General's *Report on the Health Consequences of Involuntary Smoking* published in 1986.[1]

Attention recently has been focused on the extent of exposure to passive smoking experienced in various indoor environments where smoking is allowed. The National Academy of Sciences reviewed the data on passive smoking in relation to the quality of indoor air environments in two separate reports also published in 1986. The first is a more general statement, *Environmental Tobacco Smoke: Measuring Exposures and Assessing Health Effects*,[2] and the second, specifically addressing the environment of airline flights, is *The Airliner Cabin Environment: Air Quality and Safety*.[3] The latter recommended a ban on smoking in all domestic commercial flights for four major reasons: to minimize irritation, to reduce health risks, to reduce fire hazards, and to bring air cabin quality into line with standards for other closed environments.

See also p 898.

Public opinion, by smokers and nonsmokers alike, is increasingly in favor of restrictions on smoking in public areas and in the workplace.[4,5] However, there are presently no regulatory standards that specify limits on pollutants affecting air quality in airplanes. Recent legislation in the United States, enacted in April 1988 on a trial basis, has banned smoking on all US carrier domestic flights of two hours or less. The ban is due to terminate in April 1990 unless additional congressional action occurs.[6] In Canada, smoking on flights of two hours or less was banned by the government in December of 1987. Subsequently, both major Canadian airlines voluntarily went beyond the two-hour ruling and banned smoking on all North American flights. One US airline has voluntarily banned smoking on flights of any length within the United States, except flights to and from Hawaii.[6] On Jan 1, 1988, the California legislature enacted a law that banned smoking on all forms of public transportation, including all intrastate airline flights, and limited smoking in transportation waiting areas. Reactions to the ban obtained in a March 1988 survey of 677 passengers and crew showed strong support among nonsmokers (95%) and smokers (57%) (University of San Francisco news release, April 6, 1988).

The Surgeon General of the Public Health Service requested that the Smoking, Tobacco, and Cancer Program of the National Cancer Institute conduct a research study to measure environmental tobacco smoke exposure on typical commercial flights. This study was undertaken (1) to measure nicotine levels in ambient air during flights of approximately four hours' duration and urinary cotinine levels at various points during the three days after the flights, and (2) to determine if these exposure and excretion measurements correlate with each other and with acute symptoms experienced during the flights.

METHODS

Subjects and Procedures

Nine subjects (four attendants and five passengers) participated on each of four flights. Based on smoking chamber data,[7] we determined that this study design would have 77% power for finding a difference in urinary cotinine excretion between subjects moderately exposed to environmental tobacco smoke and unexposed subjects. All subjects were nonsmokers; were not regularly exposed to smoke; were free of chronic respiratory disorders such as asthma, bronchitis, or emphysema;

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Table 1.—Inflight Nicotine Exposure*

Variable	Flight 1	Flight 2	Flight 3	Flight 4
Attendants				
1	3.0 (S†)	6.8 (S)	0.7 (NS)	7.7 (NS)
2	0.1 (NS)	9.4 (NS)	0.5 (S)	9.5 (S)
3	9.8 (NS)	0.2 (NS)	1.5 (S)	7.5 (S)
4	0.9 (S)	10.5 (S)	1.4 (NS)	5.4 (NS)
Passengers				
5	16.6 (S)	0.8 (NS-B)	5.5 (NS-B)	12.9 (S)
6	58.6 (S)	0.1 (NS-B)	32.1 (S)	27.3 (NS-B)
7	0.1 (NS-B)	3.5 (S)	2.5 (NS-B)	8.4 (S)
8	0.6 (NS-B)	0.3 (S)	8.3 (S)	71.0 (NS-B)
9	0.2 (NS-B)	12.6 (S)	27.5 (NS-B)	11.4 (S)
Average No. of smokers per count (No. of observations)	4.2 (9)	3.9 (7)	4.4 (7)	4.4 (5)

*Nicotine values from personal monitoring pump (assigned work or seating area). Values were adjusted for sampling time during which smoking was allowed. Units are micrograms per cubic meter (μg/m³).

†S indicates smoking section; NS, nonsmoking section; and NS-B, borderline between smoking and nonsmoking sections.

were willing to be assigned to the smoking section of aircraft; and volunteered to participate in the study. The protocol was approved by the human subjects institutional review board of the National Institutes of Health and by the participating airline. Eight of the nine subjects were recruited from Air Canada employees.

Data collection for the entire study took place over a 19-day period in May of 1988. The flights' schedules were as follows: flight 1, Toronto to San Francisco; flight 2, San Francisco to Toronto; flight 3, Toronto to Vancouver; and flight 4, Vancouver to Toronto. Seventy-two hours elapsed between all flights except flights 2 and 3, which were separated by four days. Flights 1 and 2 were on B727 jets with 100% fresh air. Flights 3 and 4 were on B767 jets with 50% of the air recirculated.

The same subjects participated in all four flights. The five passenger subjects (three men and two women) were seated in the smoking section or in the nonsmoking section bordering the smoking section. The four attendant subjects (three men and one woman) were assigned to work in smoking or nonsmoking sections of the cabin. All subjects rotated exposure conditions over the four flights, shown in Table 1.

Each flight had four smoking section rows, consisting of 24 seats in flights 1 and 2 and 28 seats in flights 3 and 4. There were 78 seats in nonsmoking in flights 1 and 2 and 93 in 3 and 4. Although the smoking section was fully occupied on the first three flights and there were only two empty seats on the fourth flight, some passengers in the smoking section did not smoke, eg, study subjects and children. Logistical considerations (ie, the airline's need for a rapid turnaround of aircraft) prevented a direct count of all cigarette butts produced by smokers on the flight. Smoking activity was estimated by the field coordinator, who observed the smoking section at intervals throughout the flight and counted the number of persons smoking each time.

The concentration of nicotine in the cabin air was used as a marker or surrogate for the exposure to environmental tobacco smoke. During the flights, nicotine was collected from the air by active sampling as described elsewhere.[8] A personal exposure monitor consisting of a pump sampling at 3 L/min through a cassette containing two filters was used. The first filter collected particles for a separate study of mutagenicity of extractable organics to be reported elsewhere. The second filter was treated with sodium bisulfate to collect the nicotine. The nicotine on the treated filters was desorbed in solvents and analyzed by gas chromatography with nitrogen-selective detection. The sensitivity was 0.07 μg of nicotine per cubic meter. Each subject wore an active sampling system during each flight to measure his or her actual nicotine exposure ("in-flight exposure").

Exposure to cigarette smoke between flights was monitored with both a diary, in which subjects recorded the extent and duration of exposure outside of the flights, and with a passive monitor. For 72 hours before and after each flight, each subject wore a small, lightweight passive monitor that contained a bisulfate-treated filter that collected nicotine by diffusion. These filters were also analyzed by gas chromatography with nitrogen-selective detection.[9,10]

In addition to a preboarding baseline urine sample, subjects collected all their urine for each of 12 six-hour periods after the flight. They recorded the total volume collected for each six-hour period and took a sample from the pooled specimen. All samples were shipped in dry ice to the testing laboratory and analyzed for cotinine and creatinine. The method of cotinine analysis was radioimmunoassay, as described by Haley et al.[11,12] All cotinine values were normalized by creatinine excretion.

Before each flight, all subjects were asked to complete a simple questionnaire about the following symptoms: eyes (itching, burning, dryness, teariness, or increased blinking); nose (dryness, itching, discharge, obstruction, or stuffiness); dry mouth; coughing; sneezing; scratchy or sore throat; and headache. These same questions were asked at the completion of each flight along with additional questions concerning annoyance from cigarette smoking during the flight ("During this flight, were you annoyed or irritated by cigarette smoke?") and an estimate of how smoky the flight appeared to be ("How smoky was the area of the plane in which you spent most of your time?").

Data Analysis

Air nicotine concentrations (in micrograms of nicotine per cubic meter of air) were corrected for the sampling time during which smoking was permitted and the pumps activated. The lengths of time the air sampling equipment was on were as follows: flight 1, 4.8 hours; flight 2, 4.5 hours; flight 3, 4.0 hours; flight 4, 3.8 hours. Since all flights had minimal time during which smoking was curtailed, these corrections were minor. Levels of air nicotine were classified as "high" (>12 μg/m³), "moderate" (1 to 12 μg/m³), or "low" (<1 μg/m³). Statistical significance was assessed by the Wilcoxon-Mann-Whitney rank test[13] and the Mantel linear trends test.[14] Both one- and two-tailed P values have been used, depending on whether the direction of the statistical comparison could be anticipated from prior knowledge.

Two types of analyses were done with the urinary data. In the first analysis, the relationship between air nicotine exposure during the flight ("in-flight exposure") and cotinine excretion over the 72-hour period after the flight was examined. Twenty-four–hour moving averages, ie, an average of the four cotinine values for a consecutive 24-hour period, were created to smooth out variability.[15] The cotinine moving average (MA) was computed for the mean cotinine values, normalized for creatinine in units of nanograms of cotinine per milligram of creatinine as follows (where UCP indicates urine collection period

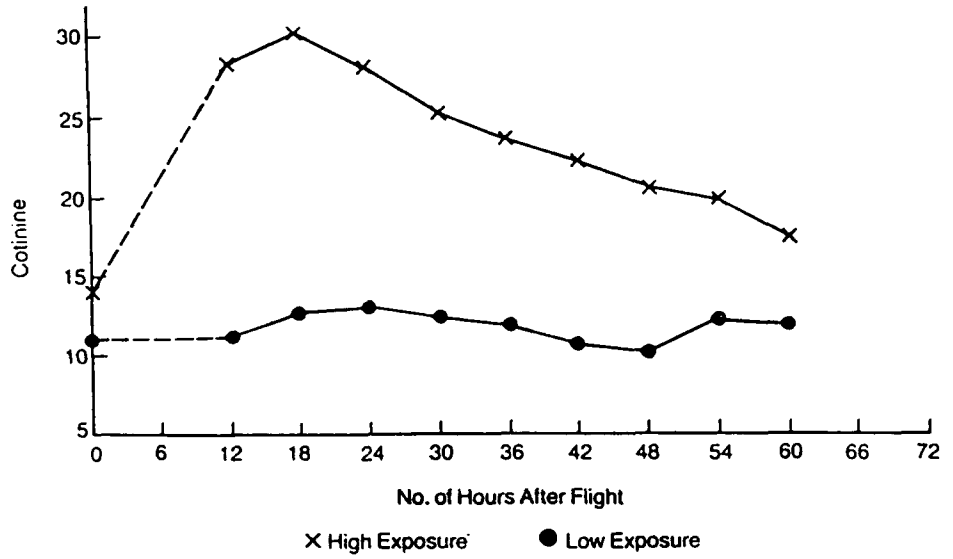

Fig 1.—Cotinine excretion over time. Twenty-four–hour moving average of cotinine excreted after flight. Value at $t = 0$ is average urinary cotinine before boarding flight. High in-flight exposure is defined as nicotine exposure greater than median value. Units are nanograms of cotinine per milligram of creatinine. See text for description of methods.

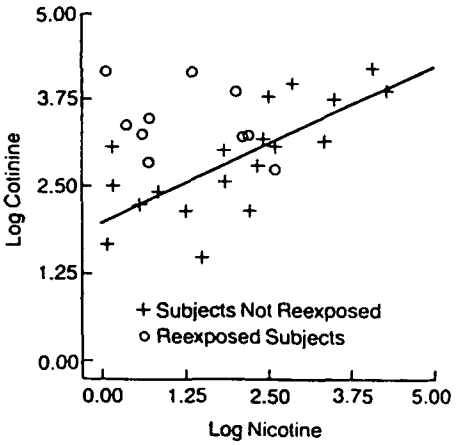

Fig 2.—In-flight nicotine exposure and cotinine excretion at 12 hours after flight. Values are natural logarithms of air nicotine (Ln [nicotine + 1]) and creatinine-normalized cotinine (Ln [cotinine + 1]). Regression line through data points for subjects not reexposed between flights, correlation coefficient = .74, $P = .0003$.

for the stated interval): MA (at 12 hours) = UCP (0 to 6 hours) + UCP (6 to 12 hours) + UCP (12 to 18 hours) + UCP (18 to 24 hours) divided by four, plotted at $t = 12$, the midpoint of that 24-hour interval. MA (at 18 hours) = UCP (6 to 12 hours) + UCP (12 to 18 hours) + UCP (18 to 24 hours) + UCP (24 to 30 hours) divided by four, and similarly for the remaining moving averages. Computed in this way, the 12 postflight urine collection periods yielded a single moving average curve consisting of nine points. Measured levels of in-flight air nicotine were partitioned into those above and below the median value for all flights. Twenty-four–hour moving averages of cotinine levels were plotted against time separately for subjects receiving high (above median) and low (below median) in-flight exposure.

In the second analysis, the dose-response relationship between nicotine exposure and cotinine excretion at 12 hours was examined by scatter plots of in-flight exposure vs cotinine excretion using log-transformed values for all nicotine and cotinine measures. The total number of urinary points available for $t = 12$ hours was 29 (instead of 36), due to missing data.

Linear plots of both raw and creatinine-normalized measures of urinary cotinine and plots of the log-transformed urinary cotinine data (original units were nanograms per milligram of creatinine) done early in the analysis revealed marked variation for some individuals. Some began a flight with unexpectedly high baseline values. For some subjects, peak excretion of cotinine occurred at irregular intervals, with some subjects peaking early, others late, and some showing multiple peaks. The most likely explanation for the patterns observed is that some subjects were reexposed while not on a flight.

The possibility of reexposure was analyzed by examination of the interflight nicotine badge worn between flights and the exposure diaries. Subjects whose passive nicotine badge values indicated exposure of 0.13 μg of nicotine or greater during the 72-hour between-flight interval were considered to have received at least moderately high reexposure. Data from the diaries documenting the day and time of exposures were used in combination with known half-life values for cotinine to determine which collection intervals were affected. The mean baseline cotinine level (nanograms of cotinine per milligram of creatinine) of subjects with interflight badge values of 0.13 μg of nicotine or greater was 34.3, and 12.1 for those with badge values of less than 0.13 μg of nicotine.

Urine samples collected after the following flights were considered unsuitable for analysis due to occurrence of interflight exposures to tobacco smoke: all data for attendant subjects 1 and 2, flights 3 and 4 for attendant subject 3, flight 1 for attendant subject 4, flight 4 for passenger subject 5, and flight 3 for passenger subject 8. These reexposed subjects were excluded from some analyses (Fig 1).

Both questions about symptoms and the questions concerning annoyance and smoke levels experienced during the flight were recorded on a six-point scale from zero to five. Differences in the symptom scores before and after the flight were calculated and categorized into three groups of roughly equal size, coded as 0, 1, and 2. For eye and nose score changes, −2 to 0 was classified as none or mild, 1 to 2 as moderate, and 3 to 4 as marked. For "annoyed" and "smoky," the corresponding categories were 0 to 2, 3, and 4 to 5. A logarithmic transform for both the nicotine values from the personal sampling pumps and the urinary cotinine values was used.

These coded symptom score changes were related to the nicotine and cotinine values by linear least squares regression with the continuous measurements (air nicotine or urinary cotinine) treated as the response variables. The analysis for the self-reported symptoms used *all* subjects and was based on those urinary cotinine values obtained at 12 hours after the flight, not moving averages. Twelve hours was chosen as an appropriate point where there was minimal contamination from reexposure to smoke experienced later during the data collection period.

RESULTS

In-flight Nicotine Exposure

Subject placement and exposure conditions for the four flights along with in-

flight nicotine measurements of air concentrations from the active personal exposure monitors worn in-flight are shown in Table 1. Measurable exposure to environmental tobacco smoke occurred for all subjects on all four flights. Review of the in-flight logs kept by the study field coordinator of counts of smokers done at least hourly indicates a maximum of eight smokers at any one time during the flight, with the average number of smokers per count ranging from 3.9 to 4.4 (Table 1). Table 2 also shows the frequency distribution of the in-flight nicotine readings and levels of statistical significance for various group comparisons.

The personal exposure monitors of subjects on flights with 100% fresh air (1 and 2) recorded less exposure than those on the two flights with 50% fresh air and 50% recirculating air (3 and 4), a statistically significant difference by both tests, with two-tailed *P* values. On flights 1 and 2, all passengers seated in the nonsmoking section (five samples) were exposed to less than 1 μg/m³ of nicotine with a median exposure of 0.2 μg/m³, while all passengers seated in the nonsmoking section of flights 3 and 4 (five samples) were exposed to more than 2.5 μg/m³ of nicotine, with a median exposure of 27 μg/m³. Flight 4 from Vancouver to Toronto had the highest levels of air nicotine, with all exposed to greater than 5 μg/m³.

Passengers in the nonsmoking border section experienced variability in their exposure, with some attaining exposures comparable to or higher than those in the smoking section. The three highest nonsmoking border readings (27.3, 27.5, and 71 μg/m³) were obtained on the flights with 50% recirculating and 50% fresh air. There was also a difference in spatial configuration of the seating between the two types of aircraft. *All* nonsmoking border readings on flights with recirculated air were greater than *all* nonsmoking border values on flights with 100% fresh air. The differences between exposures in the nonsmoking border and smoking sections were not statistically significant. No passenger subjects in the study were placed in the center of the nonsmoking section far from the border with smoking because of the expectation that exposures there would be very low.

Exposure among attendants was not statistically different from that of passengers, although none of the eight high exposures observed on the flights occurred among attendants (Table 2). Exposure among attendants assigned to work in the smoking section was not different from that among those working in the nonsmoking section.

Table 2.—Frequency Distribution of Air Nicotine Readings and Tests of Significance*

Pairs Being Compared	High	Moderate	Low	Significantly Different?	P† WMW	P† MLT
Flights 1 and 2	3	6	9	Yes	.054	.040‡
Flights 3 and 4	5	11	2			
Attendants	0	11	5	No	.15	.093‡
Passengers	8	6	6			
Passengers in smoking section	5	4	1	No	.075	.058§
Passengers in nonsmoking border	3	2	5			
Attendants in smoking section	0	6	2	No	.26	.30§
Attendants in nonsmoking section	0	5	3			

*All readings are from personal pump monitors worn by all subjects during the flights. Units are micrograms per cubic meter (μg/m³). High = >12 μg/m³; moderate = 1-12 μg/m³; and low = <1 μg/m³.

†*P* values are from the comparison between the members of each of the pairs indicated. Listed are the *P* values for the Wilcoxon-Mann-Whitney test (WMW) and Mantel linear trends test (MLT).

‡Two-tailed *P* value.

§One-tailed *P* value.

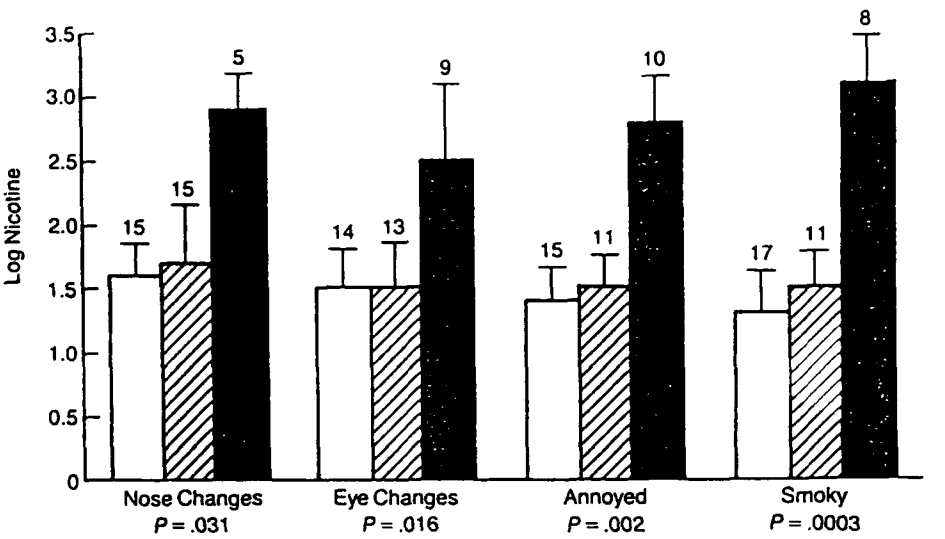

Fig 3.—Relationship of in-flight levels of air nicotine measured by personal monitoring pump to symptoms and perceptions during flight. Units are micrograms of nicotine per cubic meter (log transformed: Ln [nicotine + 1]). Numbers of subjects in each category are shown above bars representing 1 SE. Black bars indicate marked changes; striped bars, moderate changes; and white bars, no or mild changes. *P* values are one-tailed. See text for description of scoring system.

Urinary Cotinine Excretion

Postflight urinary cotinine excretion over time in subjects without appreciable reexposure between flights is shown in Fig 1. A median split was performed on the in-flight nicotine exposure values to divide these subjects into "high" and "low" nicotine exposure groups. The median nicotine exposure value was 5.5 μg/m³. The urinary cotinine level for each exposure group was plotted against time using a moving 24-hour average. In the high-exposure group, cotinine excretion increased quickly from preflight levels, rose to a peak value, and monotonically decayed back to a value slightly above the preflight levels by 72 hours. Similar plots of cotinine excretion over time for only the reexposed subjects showed highly elevated baselines, irregular excretion time courses, and no relationship to in-flight nicotine exposure.

Scatter plots of all data using different symbols to distinguish reexposed subjects were done to investigate dose-response relationships (Fig 2). Urinary cotinine excretion (creatinine corrected) at 12 hours after the flight for subjects not reexposed showed a clear correlation with nicotine exposure received during the flight. The correlation coefficient was .74 with $P = .0003$. There was no significant correlation between the cotinine and nicotine values for the reexposed subjects.

Self-reported Symptoms

Dry mouth, coughing, sneezing, scratchy or sore throat, and headache were not significantly related to nicotine exposure. On the other hand,

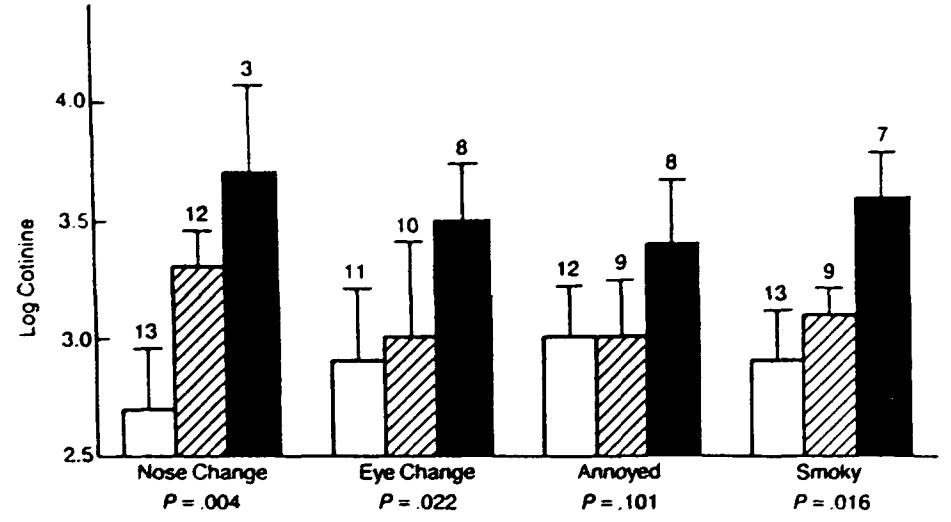

Fig 4.—Relationship of urinary cotinine excretion at 12 hours after flight to symptoms and perceptions during flight. Units are nanograms of cotinine per milligram of creatinine (log transformed: Ln [cotinine + 1]). Numbers of subjects in each category are shown above bars representing 1 SE. Black bars indicate marked changes; striped bars, moderate changes; and white bars, no or mild changes. *P* values are one-tailed. See text for description of scoring system.

Table 3.—Air Nicotine Levels (μg/m³) in Various Indoor Environments*

Environment		Mean	Range
Airplanes†	(NS)	14 (median = 2)	0.1–71
	(NSa)	4 (median = 3)	0.1–10
	(S)	17 (median = 12)	0.3–59
	(Sa)	5 (median = 5)	0.7–11
Airplanes‡	(NS)	7 (geom = 3)	ND–24
	(NS)	13 (geom = 8)	ND–40
	(NS)	8 (geom = 4)	ND–17
	(S)	11 (geom = 7)	0.4–42
	(S)	30 (geom = 7)	ND–112
	(S)	26 (geom = 22)	ND–77
Airplanes§		15	6–29
Trains‖		...	0.7–50
Offices‖		0.9	14 (peak)
Offices§		19	9–31
Offices¶		...	3–28
Pubs, coffee		..	25–52
shops‖		5	...
		10	...
Cafeterias§		26	12–42
Lobbies,		37	...
waiting		7	...
rooms‖		1	...
		3	...
Automobiles‖			
Open ventilation		65	..
Closed ventilation		1010	..
Rooms‖		500	...
Submarines‖		15–35	...

*NS indicates nonsmoking section; S, smoking section; NSa, nonsmoking section (attendants); Sa, smoking section (attendants); and ND, not detectable.

†This report.

‡Oldaker nonsmoking values were measured at or near the border with smoking in three different types of planes. Arithmetic means were presented originally[14]; geometric means (geom) were subsequently published.[19]

§Muramatsu et al.[20]

‖Summary across studies taken from National Academy of Sciences review of studies reported in the literature between 1957 and 1980.[7] The number of studies cited in the report are as follows: trains, three; offices, one; pubs etc, three; lobbies etc, four; automobiles, one; rooms, one; and submarines, one.

¶Hammond et al.[8]

changes in eye symptoms, nose symptoms, annoyance with smoking, and perception of a smoky environment were all related significantly to the nicotine exposures. These relationships are illustrated in Fig 3.

As with the nicotine analysis, no significant relationships were seen between the 12-hour log-transformed urinary cotinine values and dry mouth, coughing, sneezing, scratchy or sore throat, or headache. Like the nicotine data just described, these urinary cotinine data also showed significant relationships to eye and nose symptom changes as well as to the perception of the smokiness in the aircraft cabin (Fig 4). The relationship for the annoyance index was not statistically significant, but the observed changes were in the expected direction. Some evidence suggests that changes in eye symptoms may have been more marked in the two subjects wearing contact lenses.

COMMENT

Several other studies have measured air nicotine levels in indoor environments and report that a wide variety of factors act in combination to produce a particular "microenvironment." Differences in methodology, such as type of monitoring devices and assays, ventilation, selection of sampling times and number of smokers present, and their location relative to sampling devices, all limit precise comparisons across these studies. However, the levels of nicotine found in this study are comparable to the measurements reported in other studies shown in Table 3 and support the conclusion that air nicotine levels in the nonsmoking areas that border the smoking area may be at least as high as in similar indoor environments frequented by smokers.

A small number of studies have been published in the scientific literature that assess environmental tobacco smoke specifically on board aircraft. These studies have assessed exposure by measuring concentrations of carbon monoxide,[16,17] particulates,[16] and nicotine,[18-20] with one also assessing physiological absorption (ie, blood nicotine) resulting from exposure.[17] The results from the two studies employing in-flight nicotine measurements are summarized in Table 3.

In this study, air levels of nicotine were highly variable, with some nonsmoking areas attaining levels greater than those in some smoking sections. Seating section was a less important predictor of actual nicotine exposure. This bears out travelers' anecdotal observations that the section in which one sits is often not as important in determining exposure to smoke as is the environment generated by one's neighbors. This environment is determined by several factors, including the number of cigarettes smoked by neighbors, seating configuration, air flow patterns, and the percentage of recirculated air. In these flights, the average number smoking at any one time was only about four and was never observed to be greater than eight. This may represent relatively low exposure compared with flights with many more smokers.

The type of ventilation appeared to be an important factor in the levels of air nicotine attained. Planes with 100% fresh air (flights 1 and 2) had significantly less ambient nicotine than those with 50% fresh air and 50% recirculating (flights 3 and 4). Recirculation systems significantly increase the fuel economy and so are an integral part of the design of some newer aircraft. Passengers and attendants may be exposed to higher levels of environmental tobacco smoke in the next decade as the percentage of seat-hours on airplanes with recirculation systems increases from 30% in 1985 to an estimated 40% in 1990.[3]

Attendants are not confined to the section in which they are assigned to work and move through all areas of the plane. Although the attendants were assigned either to the smoking or nonsmoking sections, in fact there were only about four rows of smokers on each of the four flights and the attendants worked in both smoking and nonsmoking areas when they were assigned to the smoking area. Attendants assigned to nonsmoking areas may have received exposure from the first-class smoking section and from passing through the

smoking section in coach. This may explain why there was no significant difference in exposure between attendants assigned to the two sections.

Although the levels of exposure of attendants measured by the personal exposure pumps were less than those of passengers (although not statistically significant), the amount of nicotine and other cigarette smoke products actually inhaled and ingested may have been greater due to the greater physical activity and increased respiratory rate of the attendants. If true, this, together with the cumulative exposure attained from long periods of flight duty, could result in greater total exposure over the course of an attendant's career.

The levels of environmental tobacco smoke that occurred during the four-hour flights led to increased levels of cotinine (a major metabolite of nicotine) in the urine of both passengers and attendants. Subjects who experienced the greatest in-flight nicotine exposure generally had the highest levels of urinary cotinine and continued to excrete cotinine for 72 hours after the flight. The shape and time course of the decay pattern are consistent with a first-order pharmacokinetic decay process following an initial exposure to nicotine. The peak level of cotinine excreted is related to the dosage of nicotine received over the range of exposures encountered.

Reports on dose-response data under conditions of environmental tobacco smoke exposure are sparse, especially for the nicotine concentration range typically encountered by nonsmokers under free-living conditions. This analysis provides estimates of the response to a bolus of environmental tobacco smoke, delivered over a four-hour period, shown by a subsequent increase in urinary cotinine excretion over time synchronized across subjects. This study expands upon previous studies employing single-point estimates of cotinine or self-reported smoke exposure levels[21-25] and provides information on the shape of the excretion curve, delay to peak, amplitude to the peak, approximate functional form, and decay time of cotinine excretion after environmental tobacco smoke exposure.

Changes between the beginning and end of the flights in eye and nose symptoms indicative of acute irritation are related both to a measure of in-flight nicotine exposure and to the later urinary excretion of cotinine. In addition, perceptions of annoyance and smokiness in the airplane cabin were likewise related to the in-flight nicotine exposure and urinary cotinine excretion measures. Experimental studies under controlled conditions indicate that in smoky environments, eye, nose, and throat symptoms gradually increase over time with the duration of exposure even when smoke concentrations remain constant. Annoyance tends to rise quickly as soon as exposure begins and then remain constant over time.[26] The irritant effects of cigarette smoke are reflected in the numerous complaints about smoky conditions by attendants and passengers alike in records compiled by the Association of Flight Attendants and in government and industry surveys.[3,14,27-29] In these surveys, 60% of nonsmoking passengers and 15% of smokers reported being annoyed by in-flight tobacco smoke.[16] Ninety-five percent of cabin attendants reported irritation and annoyance,[27,28] with 69% in one study[29] perceiving smoky air to be a more serious concern than other work environment conditions such as temperature, odors, dust levels, and noise.

Taken together, data from this study on in-flight nicotine exposure, subsequent cotinine excretion, and acute symptoms demonstrate that total separation of smoking and nonsmoking sections was not achieved on the flights studied. The exposures experienced by passengers and attendants are comparable to those in other closed environments where smoking is allowed and represent another contributor to the cumulative health risk, acute irritation, and annoyance that nonsmoking individuals receive from passive smoking.

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