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TOXICOLOGY OF COMPLEX MIXTURES OF INDOOR AIR POLLUTANTS[1]

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INDOOR AIR POLLUTION

Indoor air pollution is now recognized as a potentially signficant public health problem. This recognition comes from evidence that human exposure to indoor air pollutants is often substantially greater than to outdoor air pollutants (1). The greater time period individuals spend indoors and the higher concentration and frequency of exposures to indoor pollutants account for this exposure. Studies of time activity patterns of individuals show that typically 22 hr out of 24 per day are spent in an indoor environment (2, 3). Although there are many different indoor microenvironments (e.g. offices, schools, public buildings, vehicles), approximately 73% of the time spent indoors is spent in the home. The much smaller dilution volume of indoor compared to the outdoor air results in higher concentrations of pollutants when the pollutant source is inside the home. As a consequence attention is now drawn to the toxicology and potential public health risk from indoor air pollutants.

Indoor pollutants originate either directly from indoor emission sources or from outdoor sources by infiltration. If the pollutant source is the outdoor air, the resulting concentrations indoors will usually be equal to or less than the concentrations found outdoors. Radon is an exception in that although it originates outdoors, primarily from soil gas, it operates like an indoor source in the basement, slab, or crawl space under the house. Many individual pollutants (e.g. carbon monoxide, nitrogen dioxide, formaldehyde, pesticides) found in indoor air are also found outdoors. The indoor air pollutants of most concern, however, are those that originate indoors. In these cases, since

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the indoor dilution air is small and ventilation in homes is generally less than one air change per hour (ACH) (4), the concentrations are higher than would be found outdoors. Toxicology studies of indoor air pollution are therefore focused on indoor sources.

Human exposure to complex mixtures of pollutants from indoor sources presents a potential public health problem for which we have the least knowledge. Although indoor air pollution began when man first built fires in caves, little is known about the toxicology of complex mixtures of indoor pollutants. International conferences on indoor air quality began with experts in air monitoring, odor, ventilation, and epidemiology (5). Later conferences included clinical researchers (e.g. allergists) (6). Recently, the presence of toxicologists marked a transition in the approach to assessing the potential hazard of indoor air pollutants(7). Very few chemical characterization data are available on indoor air pollution that indentify the most hazardous pollutants present. Chemical, physical, and toxicological interactions among the various chemical and biological pollutants in indoor air are also not characterized. Toxicology and clinical studies of these complex mixtures provide the most direct approach to elucidating any potentially adverse health effects. Increasingly, the importance is recognized of studying these pollutants as complex mixtures, rather than approaching each component as a separate toxicology problem. This review focuses initially on strategies for assessing the toxicology of indoor air pollutant mixtures. These strategies are illustrated by reviewing the current problems and approaches to the toxicology of indoor air pollutants from three indoor source categories which make a major contribution to human exposure: (*a*) environmental tobacco smoke, (*b*) combustion appliances, and (*c*) materials and products.

TOXICOLOGY STRATEGIES FOR COMPLEX MIXTURES: APPLICATION TO INDOOR AIR POLLUTANTS

Toxicology has traditionally involved the study of individual chemical compounds, even though most human exposures in environmental and occupational settings are to mixtures of chemicals. Complex mixtures present special problems for the toxicologist in exposure assessment, study design, pharmacokinetics, and data interpretation. The National Research Council recently published the evaluating and recommendations from the Committee on Methods for the In Vivo Toxicity Testing of Complex Mixtures (8). Although the committee was originally charged with evaluating and recommending new toxicology methods for complex mixtures, the committee concluded that: ". . . . a new approach, rather than new methods, is the primary need." Toxicologic test methods are developed to detect a biological change or

effect, rather than a specific agent, although specific effects may be diagnostic for a class of agents. The toxicology methods applicable to complex mixtures are, therefore, the same as those developed and validated using single agents. While new or improved toxicologic methods and models are needed to detect or better assess certain effects and diseases, such needs are not generally driven by complex mixture problems.

The focus of the NRC report on complex mixtures (8) was on the development of strategies and experimental approaches for evaluating the toxicity of mixtures. The strategies described in the NRC report include strategies for determining: (*a*) effects of mixtures, (*b*) causative agents in mixtures, and (*c*) predictability of mixtures toxicology. This review addresses the application of these strategies to the evaluation of complex mixtures of indoor air pollutants.

Exposure and Dosimetry

Characterization of potential exposures is a critical first step in any study design, including the selection of pollutant mixtures and exposure scenarios. Total human exposure assessment methods, developed to measure exposure to pollutants by all routes (9), show that for many pollutants, the air pathway is the most important. Sexton & Ryan (10), in a recent review of human exposure to air pollution, demonstrate the major contribution of indoor exposures to the total exposure to respirable particulate matter (RSP) for an individual (Figure 1). This approach requires characterizing the exposure in specific microenvironments as well as the fraction of time spent in each microenvironment. Two other approaches have been used: personal exposure monitoring (11) and biological monitoring using body fluids, or tissues (12).

A recent assessment of various indoor air sources was reported by the US EPA (13) with respect to the number of individuals exposed, pollutants emitted, and duration of exposure. Three of the most important sources identified were: (*a*) environmental tobacco smoke (ETS), (*b*) combustion appliances, and (*c*) materials and products that release mixtures of volatile organic compounds (VOC mixtures). Other important indoor air pollutants not discussed here, which are reviewed elsewhere (13, 14), include radon, biological contaminants, asbestos, pesticides, and building ventilation systems. The three indoor source categories discussed here (ETS, combustion appliances, and VOC mixtures) have been only generally characterized with respect to the magnitude of human exposure. The exact character and uniformity of the exposure to these three types of mixtures vary from relatively uniform and reproducible exposures for ETS to extremely variable for the VOC mixtures, depending on the materials and products. Selection of exposure conditions and materials for toxicology studies of these mixtures is a critical step in the toxicology strategy and study design.

Exposure to indoor air pollutant mixtures can be accomplished through one

Microenvironment Type	RSP Concentration (C)(μg/m³)	Time Fraction (t)	C×t (μg/m³)	Microenvironment Contribution (%)
Indoors at Home	15	0.75	11.25	47
Indoors at Work	50	0.15	7.50	31
Indoors, Other	25	0.04	1.00	4
In Transit (vehicle)	90	0.04	3.60	15
Outdoors	40	0.02	0.80	3

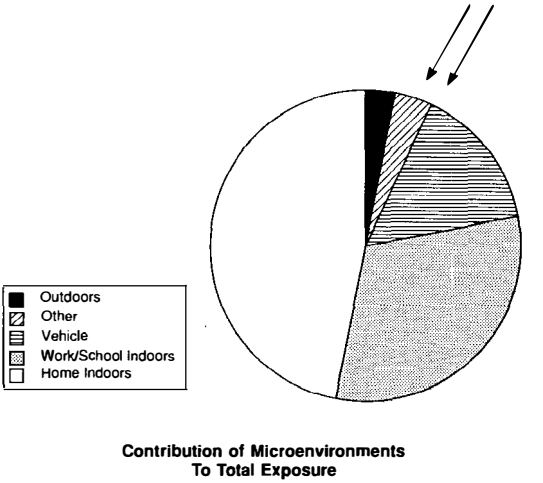

Figure 1 Example of the relative contributions of various microenvironments to total human exposure to respirable particles (RSP). Adapted from Sexton & Ryan (10).

of several approaches: (*a*) direct exposure to emissions as they occur, in either controlled laboratory conditions or in field studies, (*b*) exposure to mixtures collected or prepared from collected emissions (e.g., particle extracts from air filtration), and (*c*) exposure to constructed synthetic mixtures of individual chemicals. The NRC Commitee on Complex Mixtures (8) emphasized the importance of two key steps in designing a toxicology program on complex mixtures: (*a*) characterization of potential exposures and (*b*) definition of the problem so that specific questions may be addressed. The approach taken to select exposure materials and methods depends on both of these steps.

Dosimetry studies with complex mixtures present a challenge beyond the problems normally encountered with pure chemical exposures. Dose is considered to be the biologically effective quantity that interacts with the receptor (e.g. DNA) responsible for a particular effect (e.g. mutation). This implies that you need to know which agents in the mixture will interact with which receptors through understanding the mechanisms of action. Generally neither the active agents nor the receptors are known for complex mixtures of either indoor or outdoor air pollutants. Recent advances in the use of biological

markers for exposure, dosimetry, and effects have been reviewed (12–14) elsewhere. These advances have made several new methods available to estimate the biologically effective dose for complex mixtures as described here for one indoor air pollutant mixture (ETS).

Toxic Effects

In the initial stages of defining a complex mixture problem, what is known about the mixture itself and potential exposures should be assessed. Knowledge about the mixture includes information on both composition and effects. Although effects on that specific mixture may not be known, the composition of these emissions suggests which effects would be of concern. Before any toxicology studies were conducted on kerosene heater emissions, for example, the composition of the emissions suggested that genetic toxicity, cancer, and respiratory effects would be of concern. The fact that kerosene heater emissions contain soot and other incomplete combustion organics is sufficient to suggest evaluation of the genetic toxicity and carcinogenicity of the emissions. Respiratory effects would be suggested by the emission of carbon monoxide (CO), nitrogen oxides, sulfur oxides, and particulate matter.

The methods and strategies available for determining toxic effects of mixtures in cellular, organ, animal, and human systems are the same as those available for evaluating single chemicals (8, 15). If a mixture contains a large number of potentially toxic chemicals and there is no specific information to formulate an hypothesis regarding the expected effects, then a stepwise toxicological strategy should bc used to determine which target organs or systems may be affected (15). In the application of this strategy, the mixture is first evaluated in toxicology screening studies or first-tier tests using in vitro and acute in vivo exposures. As toxicity is identified in specific systems (e.g. genetic, systemic, reproduction) or organs (e.g. liver, kidney) then more definitive toxicology studies are conducted using repeated low-dose exposures and more extensive toxicological measurements.

Questions related to the effects of complex mixtures are of two types: (*a*) nature of the toxicity (e.g. What are the important
discussed above or (*b*) magnitude of the toxicity (e.g. How does the toxicity of one complex mixture compare to another?). Because of the potential diversity of individual complex mixtures, even within one category (e.g. combustion appliances), comparison of the potency (magnitude of effect per unit exposure) of the toxicity is a major focus of complex mixture research (8). Special considerations are required when designing comparative potency studies with complex mixtures, so that the results may be extrapolated to other similar mixtures (8, 16–18). The NRC highlights two related approaches, matrix testing and comparative potency approach, which have been used to

evaluate the toxicology of a category of related mixtures (e.g. fuels, diesel emissions) by evaluating the toxicology of a few members representing the range of possible mixtures. An example of how these two approaches have been combined in a study of the mutagenicity and composition of ETS as a function of two variables, nicotine and tar content (19) is shown in Figure 2.

Causative Agents

When complex mixtures are demonstrated to induce a specific toxic effect, questions are then raised as to which chemicals in that mixture are causing the toxicity (causative agents). In order to understand the mechanism of toxicity or the dosimetry of the active agents in the mixture, these chemical agents have to be identified. From a public health perspective, identifying the active agents provides critical information needed to develop methods to monitor either emissions, ambient concentrations or personal exposures. The design of pollution control devices or other methods to reduce human exposure to toxic agents in mixtures is facilitated by any information on the chemical or physical properties of those agents.

The NRC (8) reviewed and expanded on the strategies described by Claxton

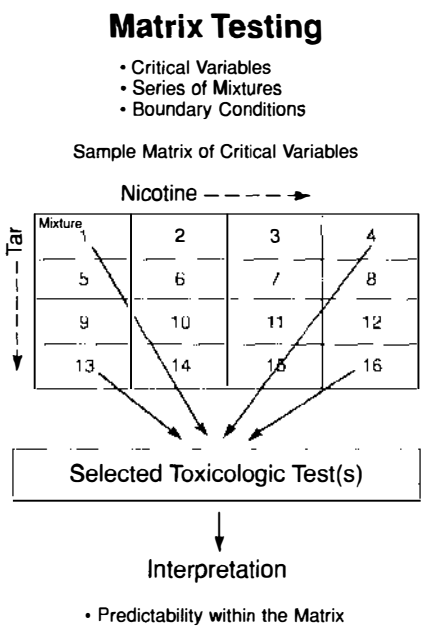

Figure 2 Example of matrix testing where a sample matrix is developed in which critical variables for a series of mixtures selected to define boundary conditions such that toxicologic testing of selected variables will result in predictability of effects within the matrix. Adapted from NRC (8) and Lewtas et al (19).

(20) for identifying biologically active agents in complex mixtures. Bioassay-directed fractionation combined with chemical characterization as illustrated in Figure 3 is the most efficient method for readily identifying biologically active fractions and components in very complex mixtures (20, 21).

The earliest application of this approach was a series of studies conducted in the 1920s that resulted in the identification of polycyclic aromatic hydrocarbons (PAH) in general and benzo(a)pyrene (BaP), specifically, as causative agents in cancers observed in animals treated with coal tar fractions (22). Later applications of this strategy have led to the identification of mutagens, carcinogens, and tumor promoters in cigarette smoke condensate (22), urban air particles (18), and diesel particulate emissions (21). Although this approach has been widely used in mutagenesis and carcinogenesis, in principle, it is applicable to identification of any causative agent that produces a measurable effect in the whole mixture (8) or a fraction of the mixture (e.g. particulate fraction of a combustion emission).

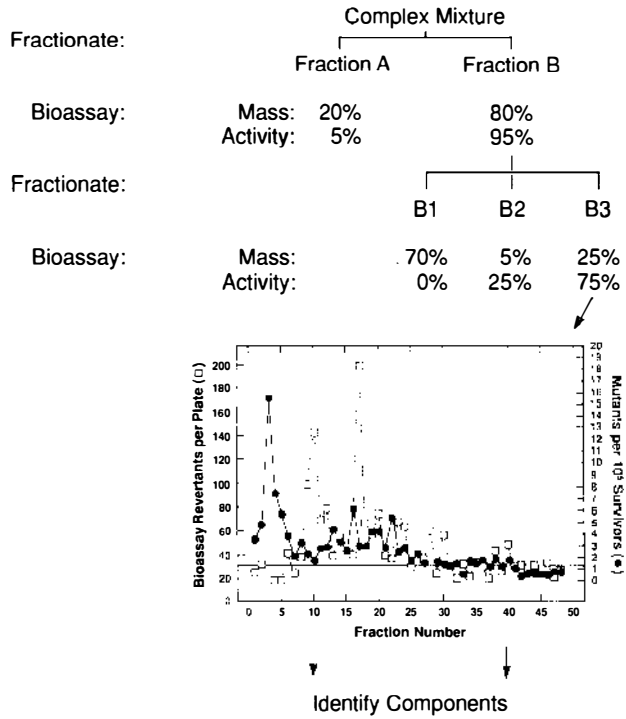

Figure 3 Diagramatic example of bioassay-directed fractionation designed to separate biologically active components from nonactive fractions conserving total mass and biological activity. Separation is sequentially performed until a fraction is obtained that is amenable to chemical characterization. Adapted from Lewtas (18).

Predictability

One of the most difficult problems to address in toxicology studies of complex environmental mixtures is the question of predictability of effects or agents from one mixture to another or from knowledge of the chemical constituents in the mixture (8). Toxicologic studies to examine the mechanisms of interaction between chemicals in a mixture have almost entirely been limited to two or three component mixtures as reviewed elsewhere (23, 24). The principles elucidated from these studies have little utility in predicting the toxicity of complex mixtures containing thousands of components of unknown composition.

Matrix toxicological testing is an approach that may provide predictability of toxic effects within certain boundaries. It involves the systematic manipulation of variables within a mixture to result in a matrix of mixtures for toxicology studies (8). Scala (25) has reviewed the application of this approach to the evaluation of the toxicity of different gasolines where the variables included aromaticity and boiling range. The principles of this approach have potential application to many complex mixture problems, including indooor air pollution. Studies in progress to evaluate the effect of tobacco product variables (e.g. tar and nicotine content) on the genotoxicity of ETS is one example shown in Figure 2.

Integration of Strategies of Indoor Air Pollutants

What is known about the exposure, composition, and toxicology of mixtures of indoor air pollutants varies from source to source. The three examples reviewed here (ETS, combustion appliances, and VOC mixtures) were chosen to illustrate the current status of the toxicology of indoor air pollutants and application of the strategies discussed above. These examples range from one where the toxic effects are not well established (VOC mixtures from materials and products) to an example where several effects and causative agents are known (ETS). Although any one of these problems can be approached from several different perspectives or hypotheses, the general principles identified by the NRC Complex Mixture Committee (8) suggest that a stepwise sequence in the application of these strategies will more effectively increase knowledge of the toxic effects, agents, and predictability. Figure 4 illustrates this stepwise sequence.

ENVIRONMENTAL TOBACCO SMOKE: A PROBLEM IN EXPOSURE AND DOSIMETRY

A large proportion of the US population is exposed to ETS. Forty (40%) percent of homes in the United States have one or more smokers and 50–65% of children have been exposed to ETS in their home over the past 20 years

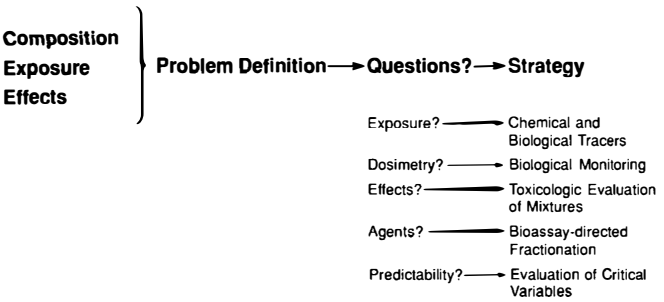

Figure 4 Diagramatic representation of complex mixture problem definition, stepwise selection, and integration of testing strategies as described by NRC (8).

(27). The exposures and health risks associated with ETS have been extensively reviewed by the International Agency for Research on Cancer (IARC) (28), the National Research Council (NRC) (27) and the US Public Health Service (Report of the Surgeon General) (29). ETS originates primarily from sidestream smoke (SS) emitted from the burning end of a cigarette or other tobacco product. Exhaled mainstream smoke (MS) makes a minor contribution to ETS. Most of the toxicological data on tobacco smoke in humans is from smokers who are exposed to both MS and SS; however, increasing numbers of epidemiological studies have been reported in passive smokers exposed to ETS (27, 29).

Although there are qualitiative similarities between SS and MS tobacco smoke, there are significant quantitative differences in the concentrations of constituents due to the lower temperature in the burning cone between puffs (when SS is generated). ETS also differs from both MS and SS in that oxidation, nitrosation, and other chemical and physical changes take place as SS is released to and diluted in the environment (28). SS, for example, contains 6–100 times higher concentrations of highly carcinogenic volatile N-nitrosamines than MS (30) and contains higher concentrations of aromatic amines, aldehydes, PAH, CO, ammonia, nitrogen oxides, and many other nitrogen-containing aromatics. The IARC working group concluded that . . . "it is unlikely that any effects will be produced in passive smokers that are not produced to a greater extent in smokers and that effects not seen in smokers will not be seen in passive smokers" (28). There is increasing evidence, however, that there are significant qualitative and quantitative differences in exposure, uptake, metabolism, and toxicity between exposure to ETS and active smoking. One animal study indicates that SS is more tumorigenic than MS (31). In this study, Swiss-ICR female mice were treated by skin application with a comparable dose of tar from SS and cigarette smoke condensate (CSC) similar to MS. Twice as many mice treated with SS developed benign

tumors (47% for SS compared to 24% for CSC) and 1.7× as many mice developed carcinomas (10% for SS compared to 6% for CSC). An inhalation study with Syrian golden hamsters is currently in progress to compare the carcinogenicity of SS to MS smoke from the same cigarettes (32). A report on the survival of animals after 15–18 months of the bioassay indicates a higher mortality for the SS-exposed hamsters compared to the MS-exposed (32). Although we know more about the potential toxicology of ETS than other indoor air pollutants, based on studies of MS and CSC, clearly more toxicology data are needed to understand the toxic effects of ETS itself, and dose-response relationships for these effects. The highest priority research recommended by both the NRC (27) and Surgeon General's report (29) is in the area of improving our understanding of ETS exposure and dosimetry.

The method most frequently used to assess human exposure to ETS has been a simple questionnaire to ask if individuals live with, work with, or have regular contact with persons who are smokers. Although this method has provided a simple means of classifying individuals into broad categories, it does not provide a quantitative measure of exposure and has problems associated with misclassification of exposures (27). The ideal method for assessing exposure to ETS, or other indoor air pollutants, would be to characterize and monitor exposure using a combination of physical/chemical and biological monitoring.

One approach to assessing exposure to a complex mixture such as ETS, is to monitor one of the emission products as a tracer. Of the emission products that have been measured as surrogate indicators of ETS exposure, nicotine and respirable particulate matter (RSP) are the most useful (27). Table 1 summarizes typical ETS exposure concentrations. An ideal tracer for ETS would be unique to tobacco smoke (e.g. nicotine, tobacco-specific nitrosamines, solanesol), be present in adequate concentrations to measure at low exposures, and would exhibit similar emission rates for all tobacco products. Nicotine meets these three requirements, while RSP is not unique since it is emitted from all combustion sources. An additional important factor is that the tracer should be present in a constant ratio to the toxic or carcinogenic components (causative agents) regardless of the environmental conditions that could effect physical changes (e.g. gas to particle phase distribution), surface deposition, and chemical transformation (27). Nicotine has the limitation that it is found primarily in the gas phase in ETS (33) and, because it is a strong base and potentially reactive with acidic materials, may be removed from the environment at a faster rate than particle-associated tars (34). These features may make gaseous nicotine a good tracer for volatile carcinogenic nitrosamines in ETS but may underestimate exposure to the particle-associated carcinogens and to the nonreactive alkenes and other organic gases (e.g. benzene, ethene, 1, 3 butadiene). Eatough (34) has

Table 1 Environmental tobacco smoke (ETS): Exposure characterization using chemical and biological assays

Measurement	Chamber Concentration[a]		Indoor Environments Range[b]	
Carbon monoxide	2.48	mg/m^3	<1–17	mg/m^3
Nitric oxide	68.0	μg/m^3		
Nitrogen oxides	72.8	μg/m^3		
Ethene	98	μg/m^3		
Ethane	138	μg/m^3		
Propene	105	μg/m^3		
1,3-Butadiene	19	μg/m^3		
Isoprene	225	μg/m^3		
Benzene	37	μg/m^3	20–317	μg/m^3
Formaldehyde	68	μg/m^3		
Acetaldehyde	77	μg/m^3		
Acrolein	19	μg/m^3	20–120	μg/m^3
Particulate matter (TSP)	349	μg/m^3	10–1900	μg/m^3
Mutagenicity	628	rev/m^3	<100–3500	rev/m^3
Nicotine	29	μg/m^3	<1–1010	μg/m^3
Human dose nicotine (4 hr)[c]	23.6	μg	2.4–120	μg
Human urinary cotinine[c] (ng/mg creatinine)				
Baseline before exposure (not exposed to ETS)	37	ng/mg	0–145	ng/mg
Peak (ETS exposed)	394	ng/mg	41–1885	ng/mg

[a] Average concentrations in 13.6 m^3 chamber used for human exposure studies where 1 cigarette is smoked every 30 min for a 4 hr period (43, 45).

[b] The range of indoor concentrations is taken from several reviews (27, 29) except for the mutagenicity taken from Lofroth et al (47).

[c] Nicotine dose and urinary cotinine were determined in children with smoking parents under controlled chamber exposure conditions (43, 45).

proposed that the much lower quantities of nicotine measurable in the particles could serve as a tracer for the particles. Although research is clearly needed to develop additional tracers for ETS, nicotine is the best currently available tracer of ETS exposure. Recent data suggest that environmental conditions indoors (humidity, surface areas, and other particle sources) may alter the phase distribution of nicotine in ETS; this would certainly alter the human dosimetry (unpublished data).

Personal exposure and dosimetry of ETS is dependent upon many factors, including uptake and metabolism. Nicotine and its metabolite, cotinine, measured in saliva, blood, or urine, are the most useful biological markers of ETS exposure since they are derived exclusively from tobacco (27–29). Nicotine uptake, metabolism, and clearance have been better characterized for smokers than nonsmokers (35–37), however there is some evidence that nicotine is metabolized slower by nonsmokers (38). Cotinine, the major

metabolite of nicotine, is readily detected by chemical or immunoassay methods (39) in saliva, serum, or urine of individuals exposed to nicotine in ETS (40, 41). The relatively long half-life of cotinine, 10–37 hr (35), results in relatively stable concentrations of urinary cotinine in individuals intermittently exposed to ETS. In a recent study of preschool children who are exposed to ETS in their homes before and after day care, urinary cotinine concentrations were remarkably stable regardless of sampling time during the day and over a one month observation period (42). This study also observed a significant correlation between the nicotine concentrations in the home and the children's urinary cotinine concentrations (42). These observations are consistent with our recent findings that the half-life of cotinine in preschool children (29 hr) is similar to that of adults (20 hr) (35) and ranges from 15 to 55 hr (43). The relationship between nicotine exposure concentrations (29 $\mu y/m^3$) and urinary cotinine concentrations for this study are shown in Table 1.

Microsuspension mutagenesis assays in *Salmonella typhimurium* have recently been used to measure exposure to mutagens from ETS (44–47). The application of bioassays to personal exposure or microenvironmental samples provides an integrated measure of biological response to many chemicals in the mixture. Mutagenesis studies on the extractable organic (tar) from ETS particles have been used to compare ETS to other combustion sources (18) and to compare the effect of tar and nicotine content of the tobacco to mutagenic emission rates for a series of different cigarettes (19). The concentration of airborne mutagens in indoor environments containing ETS is summarized in Table 1.

Urinary mutagenicity has been suggested as a biological marker of exposure to ETS. Smokers consistently exhibit increased concentrations of urinary mutagenicity that has been found to be related to the number of cigarettes smoked (48), even though the half-life of urinary mutagenicity is relatively short (49). Investigations of the use of urinary mutagenicity as a biological marker of ETS exposure in nonsmokers (46), even under controlled exposure conditions, have not resulted in reproducible increases in urinary mutagenicity related to the exposure (L. Claxton, and J. Lewtas, unpublished results).

Highly sensitive methods are now being used to measure protein and DNA adducts that may result from exposure to environmental pollutants. Several ETS constituents including benzo(a)pyrene, tobacco specific nitrosamines, and 4-aminobiphyenyl can be detected either as protein or DNA adducts (50–52). It is not yet known whether these methods are sufficiently sensitive to detect exposure to ETS in nonsmokers. The postlabeling assay for DNA adducts is particularly applicable to complex mixtures, since it does not depend upon prior identification of the specific chemical in the mixture that may form adducts (53). Randerath et al (54, 55) have shown tobacco-smoking

related DNA adducts in human placenta, bronchus, and larynx tissue. We have recently detected adducts using this method in alveolar macrophages obtained from smokers by bronchio-alveolar lavage (Gallagher et al, unpublished results). Although the postlabeling method can detect 1 adduct in 10^9-10^{10} nucleotides, no direct evidence of adducts resulting from exposure to ETS has been reported.

COMBUSTION SOURCES INDOORS: FINDING THE CAUSATIVE AGENTS

All types of combustion sources burning either fossil fuels (e.g. kerosene, oil, coal) or vegetative sources (e.g. wood, plants, food, paper, tobacco) result in the production and usually the emission of a very complex mixture of organic gaseous and particulate pollutants as well as some inorganic materials. The complex emission products of incomplete combustion are mutagenic and carcinogenic (56). The major gaseous pollutants CO, NO_x, and SO_x are known to produce a variety of acute and chronic noncarcinogenic health effects associated with the respiratory system. These and other gaseous emissions such as nitrous acid (HONO) are known to react with many of the organic emissions to form oxidized and nitrated compounds such as epoxides, lactones, and nitroarenes (57). Combustion also results in the emission of volatile organic compounds (VOC) that have been shown to induce tumors in animals or have been implicated as carcinogens in human epidemiological studies, including aldehydes (e.g. formaldehyde, acetaldehyde), aromatic hydrocarbons (e.g. benzene), alkenes (e.g. ethylene, 1,3-butadiene) (13).

Virtually every combustion process in which carbonaceous fuels are burned results in the production of soot particles. These fine (<2.5 microns) soot particles contain an organic fraction (solvent-extractable) and an elemental carbon fraction. The combustion of coal, wood, gasoline, and No. 2 diesel and residential fuel oil have all been shown to produce soot particles with extractable organic matter that is mutagenic in short-term bioassays and tumorigenic in animals (56). Chemical characterization of these organics shows that they contain carcinogenic PAHs as well as mutagenic nitrated PAHs (e.g. nitropyrene), oxidized PAHs (e.g. cyclopenta(c,d)pyrene dicarboxylic acid anhydride), and a variety of other oxygenated and nitrated polycyclic organic compounds.

Bioassy-directed fractionation closely coupled to chemical characterization has been shown to be the most efficient and effective approach to identifying the biologically active compounds in a complex mixture (18, 20, 21). This approach has been used to identify tumor initiators and tumor promoters in cigarette-smoke condensates (58), automotive-exhaust emissions (59), and urban-air particles (60). More recently, this approach has been coupled with

short-term genetic bioassays, including microbial mutagenesis assays, to identify mutagens and potential carcinogens in complex mixtures (21). We first employed this method to identify the chemical classes and specific components associated with diesel particulate emissions that were mutagenic in the Ames *Salmonella typhimurium* mutagenesis assay (61). Bioassay-directed fractionation of diesel emissions showed that very little of the mutagenic activity was in the the aromatic fraction that contains PAH. Most of the mutagenic activity was in moderately and highly polar neutral fractions. Conventional gas chromatography/mass spectroscopy identified many non-mutagenic fluorenones and methylated fluorenones as major constituents of these fractions. None of these or other identified constituents accounted for the direct-acting frameshift mutagenic activity observed. Studies with nitroreductase-deficient strains of *Salmonella typhimurium* showed a reduction in the mutagenicity of these organics, which suggested that nitrated compounds were present (62). Nitrated polycyclic aromatic hydrocarbons (NO_2-PAHs) are potent direct-acting frameshift mutagens originally detected in xerographic toners (63). A series of NO_2-PAHs in diesel extracts were then identified and quantitated in order to estimate their contribution to the mutagenic activity of diesel particulate emissions (21). These studies show that, although present in low concentrations, NO_2-PAHs, di-NO_2-PAHs, and hydroxy-NO_2-PAHs together account for much of the mutagenicity observed in *Salmonella typhimurium* (21).

Unvented space heaters and other indoor combustion appliances (e.g. cooking stoves) are a major pollution source of organic and inorganic gases, semivolatile organics, and soot particles (13). Kerosene heaters, which are one of the most widely used unvented combustion sources, have been studied in laboratory chamber and field studies. Ohnishi et al (64) reported that the initial start-up and burning of a radiant kerosene heater resulted in very high concentrations of mutagens indoors. Several other studies confirmed that several types of kerosene heaters under certain operating conditions result in a high emission of mutagens (65–67). These studies all show that kerosene-heater emissions are mutagenic in the *Salmonella typhimurium* TA98 reversion assay in the absence of metabolic activation and that the mutagenicity in nitroreductase-deficient strains is significantly reduced. This evidence together with chemical analysis suggests that nitrated polycyclic aromatic hydrocarbons and other nitroarenes may be responsible for a significant portion of the mutagenicity emitted from kerosene heaters. Recent studies of the comparative mutagenic emission rates showed that older and maltuned kerosene heaters have much higher mutagenic emission rates (Mumford et al, personal communication). We have also observed significant mutagenicity in the semivolatile organics collected on XAD-2 as well as the soot particle extracts (67).

Bioassay-directed fractionation and chemical analysis of the extractable organics from soot-particle emissions from a radiant kerosene heater has been recently reported by Kinouchi et al (68). These samples were fractionated into neutral, acidic, and basic fractions and the mutagenicity was measured with strains TA98, TA98NR, and TA98/1,8-DNP_6. The nitroreductase deficient strains (TA98NR and TA98/1,8-DNP_6) lack enzymes required to metabolize nitropyrenes (NR), dinitropyrenes (1,8-DNP_6) and other nitrated PAH (69). Most of the mutagenicity was recovered in the neutral fraction, as shown in Figure 5. Furthermore, the mutagenicity of the neutral fraction decreased in the nitroreductase-deficient studies in the order TA98>TA98NR>TA98/1,8-DNP_6, which suggested that this fraction contained nitropyrenes, especially dinitropyrenes. Chemical analysis confirmed the presence of both 1-nitropyrene and 1,6-dinitropyrene that can account for approximately 1% and 20%, respectively, of the mutagenicity (67). Mutagenicity assays on HPLC fractions showed that most of the mutagenicity eluted in the same fractions that contain dinitropyrenes (Figure 5). Dinitropyrenes are highly mutagenic in *Salmonella typhimurium* TA98 (63, 69) and are carcinogenic in animals (70).

VOLATILE ORGANIC COMPOUND (VOC) MIXTURES FROM MATERIALS AND PRODUCTS: WHAT ARE THE TOXIC EFFECTS?

Building materials and other products (e.g. adhesives, carpet, wall coverings, etc.) emit volatile organic chemicals (VOC) by outgassing. It has been postulated that these mixtures of VOC cause the acute, nonspecific sensory irritation and other sensory effect referred to as sick building syndrome (SBS). The World Health Organization (71) had described the SBS symptoms as generally including: (*a*) eye, nose, and throat irritation; (*b*) sensation of dry mucous membranes and skin; (*c*) erythema (skin irritation, redness); (*d*) mental fatigue and headaches; (*e*) high frequency of airway infections and cough; (*f*) hoarseness and wheezing; (*g*) itching and unspecific hypersensitivity; and (*h*) nausea and dizziness. These effects are based on a large number of case reports describing similar symptoms. Since the frequency of such nonspecific complaints is high in any population, it is difficult to establish a cause-effect relationship with one specific aspect of indoor air pollution. SBS has been costly in terms of lost work time and productivity and, in severe cases, abandonment of buildings (72, 73). Investigations of these "sick" buildings have not readily, or often, found a single cause (74, 75), due possibly to the psychological factors that may be involved (71) and the multiple factors, including ventilation, materials, products, and other sources, that add complexity to the problem.

One category of sick buildings is described (71) as temporarily "sick"

Fraction	Revertants/Plate/m³ (% of TA98)					
	TA98		TA98NR		TA98/1,8-DNP$_6$	
Crude	237	(100)	133	(56.7)	17.8	(7.5)
Neutral	206	(100)	46.4	(22.5)	12.9	(6.3
Acidic	1.7		0.2		0.8	
Basic	5.3		0.9		3.8	

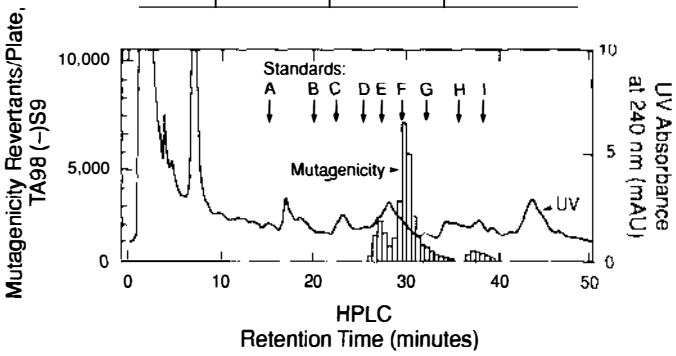

Figure 5 Bioassay-directed fractionation and identification of mutagens in kerosene heater emissions adapted from data reported by Kinouchi et al (68).

because the SBS symptoms occur when the building is either newly constructed or remodeled, and decrease with time, nearly disappearing after 6 months. A recent study in both a new office building and a new nursing home showed that VOC concentrations dramatically decreased (e.g. as much as 10-fold) between completion of construction and 5–7 months later (76). In a review of VOC concentrations in new buildings (0.5–19 mg/cubic meter), Molhave (77) found the total concentration to be about 10 times higher than the range found in old buildings (0.01–1.7 mg/cubic meter), and that 82% of these compounds are either known or suspected mucous membrane irritants, 25% were known or suspected carcinogens, and 30% have known or suspected odor thresholds below the average concentrations measured indoors (78).

Molhave et al developed a working hypothesis that these VOCs may be an important factor in causing the SBS symptoms related to mucous membrane irritation (79). To investigate this hypothesis, they conducted a controlled exposure to 22 VOCs of 62 healthy individuals (including 40% smokers) who had previously complained about typical SBS symptoms and who suffered from dry mucous membranes (79, 80). The mixture of 22 chemicals, shown in Table 2, was selected based on their frequent occurrence indoors, and that they were known or suspected mucous membrane irritants (79). Compounds known or suspected to be carcinogens were not included in the mixture. The concentrations were selected to represent clear air (zero mg/cubic meter), the

Table 2 Composition of VOC mixtures for human and animal toxicology

Compounds	Indoor Air		Water
	Human Chamber[a] $\mu g/m^3$	Animal Inhalation[b] $\mu g/m^3$	Animal Oral[c] ppm
ALIPHATIC HYDROCARBONS			
Alkanes			
n-Hexane	825	590	
n-Decane	825	1100	
n-Undecane	75	950	
n-Nonane	825		
Alkenes & Cycloalkanes			
1-Octene	8		
1-Decene	825		
Cyclohexane	75		
Terpenes			
α-Pinene	825	605	
Limonene		480	
AROMATIC HYDROCARBONS			
Benzene		204	5000
Toluene		378	5180
1,3-Xylene	8250		
Xylenes (1,3+1,4)		390	4070
Ethylbenzene	825	109	650
1.2,4-Trimethylbenzene	75	150	
n-Propylbenzene	75		
Naphthalene		70	
OXYGENATED HYDROCARBONS			
Alcohols			
Iso-propanol	75		
n-Butanol	825		
Phenol			3000
Ketones			
Acetone		157	6900
2-Butanone (MEK)	75		
3-Methyl-3-Butanone	75		
4-Methyl-2-Pentanone	75		
Aldehydes			
Formaldehyde		87	
Acetaldehyde		48	
n-Pentanal	75		
n-Hexanal	825	20	
Acid Derivatives			
n-Butylacetate	8250		
Ethoxyethylacetate	825		
HALOGENATES			
Chlorinated Aliphatic			
Chloroform (trichloromethane)			1460
Dichloromethane		5000	1670

Table 2 *(continued)*

Compounds	Indoor Air: Human Chamber[a] $\mu g/m^3$	Indoor Air: Animal Inhalation[b] $\mu g/m^3$	Water: Animal Oral[c] ppm
1,1-Dichloroethane	825		310
1,2-Dichloroethane	(825)[a]		6330
1,1-Dichloroethylene			240
1,2-trans-Dichloroethylene			730
Trichloroethylene			3820
1,1,1 Trichloroethane		60	1250
Carbon tetrachloride			540
Tetrachloroethylene			9680
CHLORINATED AROMATICS			
Chlorobenzene			100
1,4-Dichlorobenzene		230	

[a] Chamber exposure concentrations for the 25 mg/m^3 total mixture concentration. Molhave et al (80) used 1,2-dichloroethane shown in parenthesis whereas Otto et al (84) are using 1,1-dichloroethane.

[b] Maximum concentrations found indoors by DeBortoli et al (86) and mixed to produce 8.5 mg/m^3 total mixture for animal exposures by Glaser et al (85).

[c] Highest concentration of VOCs (1000x) in drinking water for NTP subchronic toxicity studies in rats and mice (87). Other organics and inorganics included in this mixture are: Arochlor 1260 (210 ppm), arsenic trioxide (200 ppm), cadmium chloride (880 ppm), chromium trioxide (900 ppm), di(2-ethylhexyl)phthalate (130 ppm), lead acetate (1200 ppm), mercuric chloride (10 ppm), nickel sulfate (210 ppm). The total concentration of all chemicals at the highest dose was 54,670 ppm. Lower doses include 0.1x, 1x, 10x, and 100x.

average concentration for new houses (5 mg/cubic meter), and the highest concentration measured in new Danish houses (25 mg/cubic meter) (81). After 2.75 hr exposure in a climate chamber a wide variety of variables were measured, including sensory, behavioral, physiological, affective, and cognitive motor performance. The principal findings reported by Molhave (79, 80) were that the VOC mixture caused an increased perception of sensory irritation and discomfort and affected performance on a digit-span test suggesting impairment of short-term memory. The results of this study have received considerable attention because the concentrations of each individual chemical in the mixture were well below the threshold limit value (TLV) for adverse health effects for 8 hr of continuous occupational exposure, as defined by OSHA (82). Molhave has proposed that the responses observed are to the total chemical mixture rather than to any single constituent and that the effects are mediated by trigeminal nerve function (83).

The importance of these findings and questions raised by the experimental design of the first study (79, 80) led the US Environmental Protection Agency to conduct a second human exposure study using the same mixture of 22 chemicals, with one chemical that has become a suspect carcinogen (1,2-

dichloroethane) replaced by 1,1-dichloroethane (84). There are several important differences in the EPA study design: (*a*) normal healthy, nonsmoking adults will be tested rather than subjects self-selected as sensitive to indoor air quality; (*b*) exposure and control conditions will be separated by a one week interval, rather than performing them on the same day, to avoid possible confounding effects; and (*c*) only one exposure concentration (25 mg/cubic meter) will be used. Its purpose is to confirm the major findings in the original Molhave study (79) of increased sensory irritation and possible neurobehavioral impairment (short-term memory) and to use a battery of tests to clarify the nature of the neurobehavioral response (84).

Controlled animal exposures to mixtures of 20 VOCs plus other indoor air pollutants were recently reported by Glaser et al (85). The 20 VOCs shown in Table 2 were selected to be those typically found indoors and were mixed proportional to the concentrations found as maximum values in homes (86) to give a final total exposure concentration of 8.5 mg/cubic meter for an initial 28 day continuous exposure. Although 7 of the chemicals were the same as used in the human exposure studies, the other 13 included a number of known or suspected carcinogens. These studies reported several significant biochemical effects and suggest that these mixtures caused hepatotoxicity as well as genotoxicity in lung cells.

These two cases both used a synthetic VOC mixture to provide a controlled exposure to simulate the indoor environment. In the case of human exposures, carcinogens that may produce irreversible damage were deleted from the mixture and emphasis was placed on selecting components that may be irritating. In the case of the animal study, the exposures included known and suspected carcinogens and hepatotoxins at maximal concentrations found indoor. In the past, complex mixture exposures have been generated using the actual source that generates the mixture in the environment, such as a diesel engine (16). VOC mixtures from building materials and products used in the homes (e.g. cleaning products) present a more heterogeneous and unpredictable mixture. Evaluation of exposures from hazardous waste sites has presented a similar problem and current toxicological studies of mixtures of the most prevalent contaminants in drinking water are being conducted using an approach similar to that described here, where a synthetic mixture is used to conduct animal toxicology studies (87) (Table 2).

SUMMARY AND FUTURE DIRECTIONS

Indoor air pollution is now recognized as an important public health problem. Pollutant concentrations indoors can exceed both US EPA's Ambient Air Quality Standards for outdoor air and the TLVs defined for occupational exposure limits by OSHA for healthy workers. In addition to long exposure

times and high concentrations, indoor air pollutants present a potential public health problem to sensitive populations such as infants, the elderly, and populations susceptible due to preexisting illnesses. These factors taken together have resulted in increased funding and interest in toxicology research on indoor air pollutants (13).

Human exposure to complex mixtures of indoor air pollutants presents a challenge to toxicologists to develop testable hypotheses based on clearly defined problems and objectives. The NRC (8) has recommended a series of strategies that may be applied in a stepwise approach to toxicological studies of complex mixtures. This review has focused on the application of these strategies to several indoor air pollution problems. After defining the problem and potential human exposures, the first step is to determine if there are any toxic effects from exposure to the mixture. In the case of VOC mixtures emitted from materials and products, controlled chamber exposure studies are being conducted with both humans and animals to test for neurotoxic, respiratory, and genotoxic effects. When an effect is defined, as in the case of mutagenic and carcinogenic effects from soot emitted from unvented combustion appliances, research is focusing on determining the causative agents. In the case of kerosene heaters, for example, it appears that the nitrated polycyclic aromatic hydrocarbons may account for a major portion of the mutagenic and potentially carcinogenic activity emitted indoors. Environmental tobacco smoke (ETS) is one of the major indoor air pollutants where there is a substantial data base on the human and animal toxicology of a related, but not identical, mixture—mainstream tobacco smoke. ETS research is focusing on developing chemical and biological markers of human exposure and biologically effective dose. New molecular methods for measuring protein and DNA adducts are being developed and applied in toxicological research on ETS.

The major emphasis in indoor air pollution research has been on chemical characterization and quantitation of indoor exposures as well as characterization of source emissions and the influence of ventilation systems (13, 87). Chemists and engineers conducting these studies are using a combination of studies in controlled laboratory chambers, test homes, and indoor field studies (13). Rarely do toxicologists participate in the design or implementation of these studies. New bioassay methods applied to indoor air exposures to measure the magnitude of biological response to a complex mixture of indoor air pollutants will facilitate understanding the potential health effects of indoor air pollution (89, 90). New molecular toxicology methods to detect and quantitate human exposure, dose, or effects from indoor air pollutants should be combined, wherever possible, in both controlled laboratory or field studies where microenvironmental and personal exposure measurements are being made. Major advances in our understanding of the toxicological effects of complex mixtures of indoor air pollutants will be brought about by inte-

grated multidisciplinary studies involving chemists, engineers, and toxicologists. Indoor air pollution presents a challenge to scientists to work toward initiating and fostering such interdisciplinary collaborations that will result in a better understanding of both human exposure and health effects from complex mixtures of these pollutants.

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