Assessing Human Exposure to Power-Frequency Electric and Magnetic Fields

W.T. Kaune

EM Factors, 530 Lee Boulevard, Richland, WA 99352

This paper reviews published literature and current problems relating to the assessment of occupational and residential human exposures to power-frequency electric and magnetic fields. Available occupational exposure data suggest that the class of job titles known as electrical workers may be an effective surrogate for time-weighted-average (TWA) magnetic-field (but not electric-field) exposure. Current research in occupational-exposure assessment is directed to the construction of job-exposure matrices based on electric- and magnetic-field measurements and estimates of worker exposures to chemicals and other factors of interest. Recent work has identified five principal sources of residential magnetic fields: electric power transmission lines, electric power distribution lines, ground currents, home wiring, and home appliances. Existing residential-exposure assessments have used one or more of the following techniques: questionnaires, wiring configuration coding, theoretical field calculations, spot electric- and magnetic-field measurements, fixed-site magnetic-field recordings, personal- exposure measurements, and geomagnetic-field measurements. Available normal-power magnetic-field data for residences differ substantially between studies. It is not known if these differences are due to geographical differences, differences in measurement protocols, or instrumentation differences. Wiring codes and measured magnetic fields (but not electric fields) are associated weakly. Available data suggest, but are far from proving, that spot measurements may be more effective than wire codes as predictors of long-term historical magnetic-field exposure. Two studies find that away-from-home TWA magnetic-field exposures are less variable than at-home exposures. The importance of home appliances as contributors to total residential magnetic-field exposure is not known at this time. It also is not known what characteristics (if any) of residential electric and magnetic fields are determinants of human health effects. — Environ Health Perspect 101(Suppl 4):121–133 (1993).

Key Words: 60–Hz, health effects, epidemiology, tutorial

Introduction

This paper first discusses methods and data that pertain to occupational exposures. It then reviews the literature on residential exposures and exposure assessment, describing known sources of residential power-frequency fields and the methods that have been used to assess residential exposures. This paper also identifies and discusses current problems in occupational and residential exposure assessment, with the twin goals of drawing conclusions where possible and developing working hypotheses for future study. Finally, this paper proposes areas where future research might prove of value.

Methods for Occupational Exposure Assessment

Job Titles

All occupational epidemiology studies to date have assessed exposure by using job titles or categories of job titles. Wertheimer and Leeper (*1*) mentioned in the very first epidemiology paper concerned with magnetic fields that they had examined published data on occupations and cause of death and had found an elevation in the cancer rate of electrical workers relative to the general population. This category of workers included job titles such as power station operators, linemen and servicemen, electricians, and welders.

The first major study was reported by Milham (*2*), who stratified deaths by occupation in the state of Washington for the period 1950 through 1979 and found that electrical workers tended to have higher than expected mortality from leukemia. His classification of electrical workers was similar to that used by Wertheimer and Leeper.

Perhaps because occupational studies like the two described above require little field work and are, therefore, relatively inexpensive to perform, a substantial number have been reported in the literature. Several reviews of these studies have been published (*3–6*). Many of these studies found elevated rates of certain cancers among individuals holding electrical-worker job titles.

Because none of these studies reported exposure measurements, the connection between electrical-worker job titles and elevated exposures to electric and/or magnetic fields, while plausible, was unproven. Two occupational exposure studies have been performed that deal with this question (*7,8*).

Occupational Exposure Measurements

Deadman et al. (*7*) had 20 workers, with six electric utility jobs that were deemed to involve elevated exposure to power-frequency electric and magnetic fields, wear personal-exposure meters for periods of 1 week. This group consisted of 10 distribution linemen, three transmission substation electricians, two transmission linemen, two cable splicers, two apparatus mechanics, and one power plant worker. In addition, the authors had 16 electric-utility office workers from two different buildings wear meters for 1-week periods. The resulting data were divided into work,

Table 1. Measured work, nonwork, and sleep exposures of electric utility workers whose jobs involve, or do not involve, work near facilities used to generate, transmit, and distribute bulk electric power (*7*).

	Geometric mean electric field, V/m			Geometric mean magnetic field, μT		
Group	Work	Nonwork	Sleep	Work	Nonwork	Sleep
Exposed utility workers	48*	11	11	1.7*	0.31	0.16
Office workers	5	11	19	0.16	0.19	0.14

*Exposed group significantly higher than office workers

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Table 2. Occupation and residential exposures to ELF electric fields (*8*).

Job class	Environments	*N*	Magnetic field, μT	
			Geometric mean	Range
Electricians	Industrial power supply	1	4	—
Power line workers	Underground lines	2	0.8	0.5–1.2
	Overhead lines	2	158	120–206
	Home hook-ups	13	4	0–71
Welders/flame cutters	TIG	1	2	—
Power station operators	Transmission stations	3	290	165–621
	Distribution substation	3	72	22–222
	Generating station	7	0.4	0–4
	Control rooms	4	1	0.3–24
Electronic assemblers	Sputtering	1	6	—
	Soldering	2	8	8–9
	Microelectronics	2	2	0.8–3
Projectionists	Xenon arc	4	1	0–2
Forklift operators	Battery powered	1	0.2	—
Electronics engineers and technicians	Laser lab	4	2	0.6–8
	Calibration lab	4	2	0.5–4
	Office	1	1	—
Radio and TV repairers	Repair shops	11	45	4–110
Radio operators	Dispatchers	1	1	—
Electrical workers	All	67	5	0–620
Residential	In homes	178	2.5	0–79

Table 3. Occupation and residential exposures to ELF magnetic fields (*8*).

Job class	Environments	*N*	Magnetic field, μT	
			Geometric mean	Range
Electricians	Industrial power supply	1	10	—
Power line workers	Underground lines	3	5.7	3.8–9.1
	Overhead lines	2	4.2	3.2–5.7
	Home hook-ups	14	0.11	0.004–1.2
Welders and flame cutters	AC	4	4.1	2.4–9.0
	DC	4	0.65	0.4–1.6
Power station operators	Transmission stations	3	3.9	1.6–7.2
	Distribution substation	3	2.9	0.7–5.4
	Generating station	12	0.60	0.01–12
	Control rooms	8	0.21	0.1–0.4
Electronic assemblers	Sputtering	2	2.4	1.4–4.3
	Soldering	2	0.13	0.13–0.16
	Microelectronics	3	0.003	0.001–0.006
Projectionists	Xenon arc	7	1.4	0.1–4.5
Forklift operators	Battery powered	9	1.2	0.09–125
Electronics engineers and technicians	Laser lab	9	1.1	0.2–20
	Calibration lab	4	0.06	0.05–0.07
	Office	1	0.02	—
Radio and TV repairers	Repair shops	11	0.63	0.1–2.6
Radio operators	Dispatchers	3	0.03	0.02–0.04
Electrical workers	All	105	0.50	0.001–125
Residential	In homes	181	0.06	0.005–1.1

nonwork, and sleep periods. (During sleeping, the meter was not worn but was placed near the bed.) Time-weighted-average (TWA) exposures were calculated for each subject for these three periods.

The 20 electric utility workers studied by Deadman et al. would, in all likelihood, be included in anyone's definition of electrical workers. Consistent with this assignment, Deadman et al. found that these workers were exposed more highly while at work (Table 1). However, the nonwork and sleep exposures of the utility and office workers were the same (Table 1).

The results of Deadman et al. suggest that job titles might be a good surrogate for electric and magnetic field exposures. However, these data cover only a few highly exposed job titles within the much larger cohort of electrical workers and, therefore, do not provide a very strong test of this hypothesis.

Bowman et al. (*8*) measured spot electric and magnetic fields at 105 electric utility, aerospace, municipal government, motion picture theater, and television repair work sites. Their survey included at least one worker from every job title in Milham's 1982 electrical-worker category except for aluminum workers and conductors and motormen on urban rail systems. To provide a basis for comparison, electric and magnetic fields also were measured at 181 sites in 18 residences. The electric- and magnetic-field data are summarized in Tables 2 and 3, respectively.

The geometric mean electric and magnetic fields measured in the job sites of electrical workers were 5 V/m (Table 2) and 0.5 μT (5 mG) (Table 3), respectively. The comparable numbers for the residential measurements were 2.5 V/m and 0.06 μT (0.6 mG). The difference in electric-field exposures was due entirely to utility jobs that involved work around high voltages (overhead line and transmission and distribution substation workers). Apparently, the job-title class electrical worker is not an effective surrogate for electric-field exposure.

The difference between the occupational and residential magnetic fields in Table 3 was reliable statistically. All of the electrical workers had higher measured fields at their work sites except for electrical engineers and technicians working in offices and calibration laboratories, radio dispatchers, and microelectronic assemblers. Apparently, electrical workers, as a group, are exposed somewhat consistently to elevated magnetic fields.

There are several large projects currently examining exposures that occur in the telephone and electric utility industries. Consequently, it should be possible in a few years to discuss much more intensively occupational exposures to power-frequency electric and magnetic fields.

Sources of Residential Fields

The Electric Power Research Institute (EPRI) is executing a program to identify and characterize residential and nonresidential sources of power-frequency magnetic fields. This program started with a pilot study (*9*), and it is continuing with the characterization of the fields in 1000 residences selected randomly from a clustered sample of EPRI-member utilities. The pilot study identified the following five classes of residential fields sources: electric power transmission lines, electric power distribution lines, ground currents, home wiring, and household appliances. These are discussed in the next five sections.

Electric Power Transmission Lines

Electric power transmission lines operate at very high voltages (usually ≥50,000 volts, abbreviated 50 kilovolts or 50 kV) and may carry currents of many hundreds of amperes. Thus, these lines can produce relatively strong electric and magnetic fields. The exterior walls and roofs of most homes are fairly effective shields for electric fields (*10*), but they have little, if any, effect on the magnetic fields produced by power lines.

The magnetic field produced by a three-phase transmission line outside its right-of-way, where most human exposure occurs, usually can be calculated satisfactorily using the following formula (*11*):

$$B = \frac{I}{5R^2}\sqrt{\frac{S_{12}^2 + S_{13}^2 + S_{23}^2}{2}}, \qquad [1]$$

where B is the field's resultant flux density in μT, I is the current in amperes carried by each of the three phase conductors (these currents almost are equal for transmission lines), R is the distance in meters from the line to the point where the field is being calculated, and S_{ij} is the transverse distance in meters between the i^{th} and j^{th} conductors. This formula is valid when R is substantially larger than any of the S_{ij}.

The most common transmission line configuration has all three conductors arrayed in either a horizontal or a vertical plane. Equation 1 then simplifies to

$$B = \sqrt{3}sI/(5R^2) \qquad [2]$$

where s is the distance between adjacent conductors.

Figure 1 shows the fields produced 1 m above ground level by typical 115 kV (lower voltage) and 345 kV (higher voltage) transmission lines carrying currents of 300 A. Magnetic flux densities are shown for various horizontal distances from the lines. Note that fields ≥0.1 μT (≥1 mG) are produced up to about 70 m and 100 m from the 115 kV and 345 kV lines, respectively.

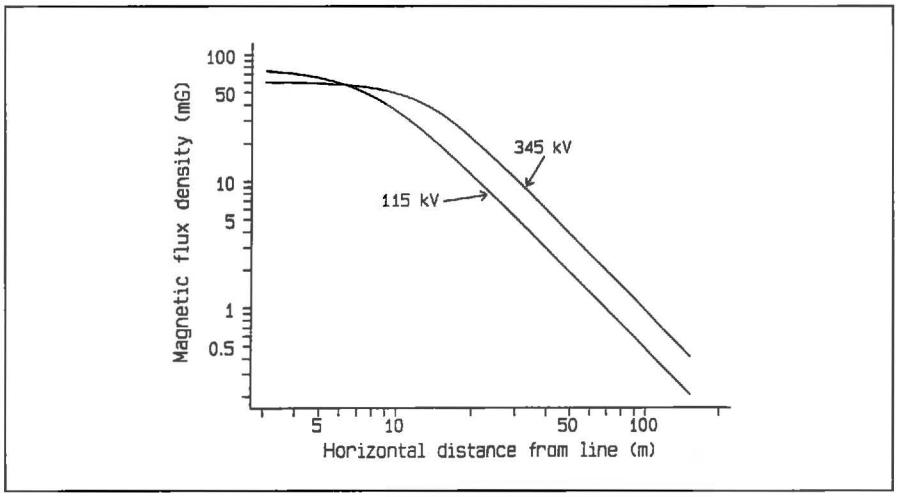

Figure 1. Magnetic fields produced by typical 115-kV and 345-kV transmission lines carrying

Electric Power Distribution Lines

Electric power generally is carried by electric power transmission lines to receiving substations located within a few kilometers of the ultimate consumers. At these substations, the voltage is reduced from transmission to distribution levels (4–34 kV), and the power is distributed on primary distribution lines to the immediate vicinity of the consumers. At this point, distribution transformers further reduce the voltage to the level of ultimate consumption (110–220 V for residential customers, 110–480 V for most commercial customers). Power is carried from distribution transformers on secondary distribution lines. Service drops to each customer are connected normally to the secondary distribution lines. Some may originate directly from the distribution transformer. While most primary and secondary distribution in the United States is by overhead lines, it is common for new installations to be underground.

Primary distribution lines can be either three-phase, two-phase, or single-phase. The first two of these categories are subdivided further into those lines with and without associated neutral conductors. Neutral conductors are operated at zero voltage (but not zero current) by connecting them to the earth (usually at many points) using ground rods or equivalent structures.

Because of their lower voltages, the conductors of distribution lines are placed much closer together than the conductors of transmission lines. Also, it is usual for distribution currents to be considerably less than transmission-line currents. Consequently, Equation 1 predicts that distribution lines will not, in most cases, produce magnetic fields much above ambient levels in areas that normally would be occupied by people. However, in practice, this is not always true because of the existence of net currents on some distribution lines.

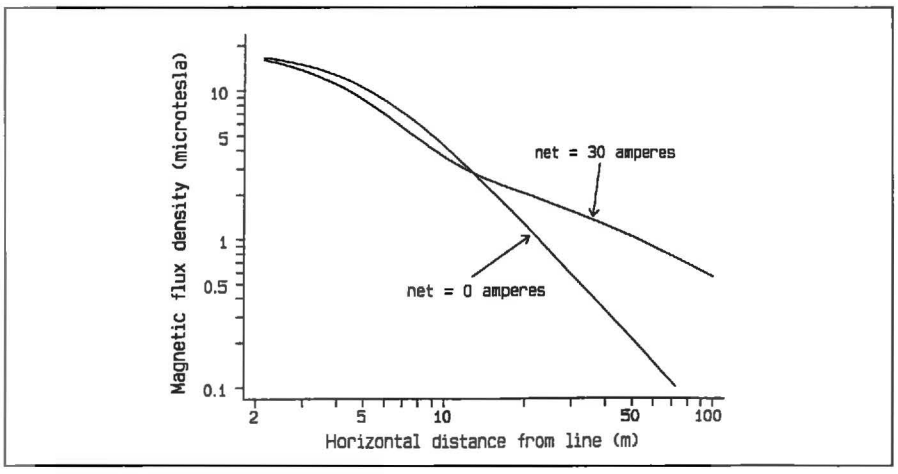

Figure 2. Magnetic field produced by typical primary distribution line carrying 100 A of load current in each phase conductor and net currents of either 0 or 30 A.

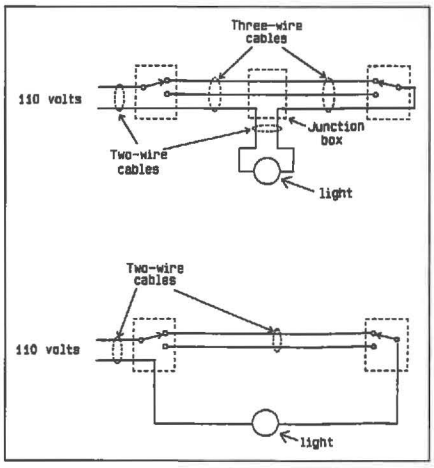

Figure 3. Alternative methods to control an electric light with two different (3-way) switches. The bottom installation could be a significant residential source of magnetic fields.

The net current, I_{net}, being carried by a power line is defined to be the algebraic sum of all the conductor currents. (This sum must be calculated taking into account both the magnitudes and phases of the individual currents.) In principle, I_{net}, = 0 for transmission and distribution lines. While this is nearly true for most transmission lines, it can be far from correct for primary and secondary distribution lines because of ground currents.

Net current is important because the magnetic field, B_{net}, produced by it depends on distance, R, from the line as $1/R$ and is, therefore, spatially more persistent than the magnetic fields produced by normal power-line currents. This is illustrated in Figure 2, which shows the fields produced by a distribution line carrying a load current 100 A and a net current of either 0 or 30 A.

Ground Currents

Ground and net currents produce spatially persistent fields. A point where current frequently enters the ground is at the service entrance of a residence because safety codes require that the neutral conductor be grounded at this point. This ground may be to a rod driven into the earth, but it is often to a metal water pipe. Often, the electrical service entrance is at the rear of a home, and the water main is in front of the home, so ground current in the water system must pass under the home. This current is not compensated by any return current in the vicinity, so its magnetic field is proportional to $1/R$. Individuals in a home may be exposed to magnetic fields from this source.

Wiring in a Home

Home wiring is not usually a significant source of magnetic-field exposure because the two wires connecting to a household load (e.g., a light or appliance) are located very close together and carry equal and opposite currents. However, there are unusual wiring configurations where this is not true. Of those known to the author, the most common are some three-way switch installations and homes having two or more separated circuit breaker panels.

Three-way switches are used where it is desired to control a load from multiple points. The most common application is probably lights that can be turned on or off from either end of a hall or stairway. Figure 3 shows two alternative ways that an installation could be made to control a light from two different switches. In the upper diagram, the various wires are routed in multiwire cables so that the net current in any of the cables is zero. Consequently, the magnetic fields from the conductors in any cable largely cancel, with the result that this installation would not be a significant source of residential magnetic fields. A different installation—one requiring less total wire—is shown in the lower panel of Figure 3. Here, a separate wire is routed from each switch directly to the light, and the direct connections between switches are made with a two-wire cable. However, this cable, and the wires connecting to the light, will carry a net current—the entire current required to energize the light. If these two elements are separated significantly, the magnetic field in their vicinity could be significant. (The author has studied a home where turning on a hall light raised the field from about 0.01 μT to 0.5 μT.)

As mentioned earlier, U.S. building codes require that the neutral bus in the main circuit-breaker (or fuse) panel protecting a home's electrical system be grounded. Some homes have multiple panels, usually because an addition to the home required more electric power than could be supplied by the original panel. Many electricians automatically will ground the neutral bus in these subpanels, creating two routes for current flow between the main and grounded subpanel, one through the neutral conductor connecting the two panels, the other through the ground. In this way, local net currents can be formed with the production of spatially more persistent fields, as explained above.

Home Appliances

The magnetic fields produced by many home appliances can be quite strong in their immediate vicinity, but these fields also are localized in space. Figure 4 shows magnetic-field data from Gauger (*12*) for five electric ranges (left graph) and three hand-held electric hair dryers. Note that the fields produced by these appliances were all less than 0.1 μT (1 mG) at distances from them exceeding 1 m. This is a characteristic of the fields from most household appliances (*12*) because of their small size, and because the magnetic fields produced by localized current sources decay as $1/R^3$ when R is large (*13*).

At this time, the relative importance of home appliances as sources of human exposure to magnetic fields is controversial. Some maintain that home appliances are important, if not the dominant, sources of exposure of humans to residential magnetic fields, while others argue that most appliance sources are unimportant. Although this controversy continues, there is general agreement that a few home appliances do contribute significantly to exposure. For example, most electric blankets clearly lead to significant whole—or near whole—body exposure because the distance between a

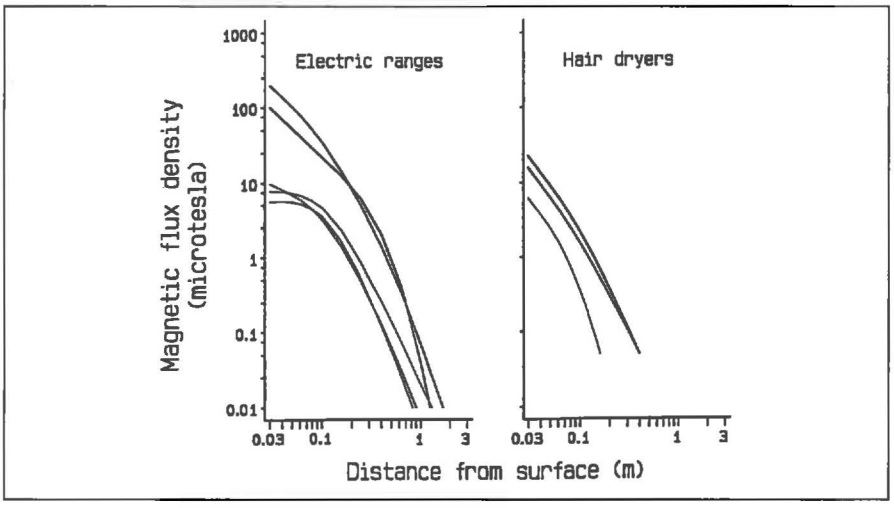

Figure 4. Magnetic fields produced by five electric ranges and three hand-held electric hair dryers. Fields are shown as function of distances from surfaces of appliances.

Table 4. Methods used to assess exposure to power-frequency electric and magnetic fields in published epidemiological studies.

Reference	Disease	Questionnaire	Wiring configuration	Theoretical estimates	Spot measurements	Fixed-site recordings	Geomagnetic field measurements
Wertheimer and Leeper (*1*)	Childhood cancer		yes[a]				
Fulton et al. (*18*)	Childhood leukemia			yes			
Wertheimer and Leeper (*14*)	Adult cancer		yes[b]				
McDowall (*33*)	Cancer			yes			
Tomenius (*19*)	Childhood cancer			yes	Outside front door		
Wertheimer and Leeper (*34*)	Fetal development	On electric blanket use					
Coleman et al. (*35*)	Adult leukemia			yes			
Severson et al. (*21*)	Adult leukemia	On appliance use	yes[b]		Inside home		
Savitz et al. (*17*)	Childhood cancer	On appliance use	yes[b]		Inside home		
Preston-Martin et al. (*36*)	Adult leukemia	On electric blanket use					
Myers et al. (*37,20*)	Childhood cancer			yes[c]			
Verreault et al. (*38*)	Testicular cancer	On electric blanket use					
London et al. (*25*)	Childhood leukemia	On appliance use	yes[b]		Inside and outside home	24-hr in bedroom	In child's bedroom

[a]Using the two-category Wertheimer-Leeper code

[b]Using the four-category Wertheimer-Leeper code

[c]Calculated magnetic field on basis of distance and line loading

Table 5. Published research studies on methods to assess human exposure to residential power frequency electric and magnetic fields.

Reference	Wiring configuration	Theoretical estimates	Spot measurements	Fixed-site recordings	Personal exposure measurements
Caola et al. (*39*)			yes		
Kaune et al. (*15*)	yes[a,b]		yes	In bedroom and family room	
Male et al. (*40*)			yes	In home	
Deadman et al. (*7*)					7-day at home and work
Bowman et al. (*8*)			yes		
Barnes et al. (*16*)	yes[a]	yes	yes		
Dlugosz et al. (*26*)	yes		yes		
Delpizzo (*28*)		yes			
Mader et al. (*41*)		yes			
Kaune et al. (*27*)	yes[a]		yes	yes	24-hr AMEX-3D at home and school
Kavet et al. (*22*)		yes	Inside and outside home	24-hr in bedroom	24-hr EMDEX at home and work

[a]Using the four-category Wertheimer-Leeper code

[b]Developed an alternative code

Table 6. Definition of Wertheimer-Leeper Wiring Code.[a]

Wiring structure	VHCC	OHCC	OLCC
Transmission line	≤50 ft	≤130 ft	
Thick 3-phase primary ≥ 6 primary phase wires	(15.2 m)	(39.6 m)	
Thin 3-phase primary	≤25 ft (7.6 m)	≤65 ft (19.8 m)	≤130 ft (39.6 m)
First-span secondary		≤50 ft (15.2 m)	≤130 ft (39.6 m)
Second-span secondary (not end pole)			≤130 ft (39.6 m)

[a]Houses not falling in VHCC, ONCC, or OLCC categories are in VLCC

user and an electric blanket is small relative to the blanket's dimensions (so the $1/R^3$ law does not apply) and because blankets are used by many for the entire nighttime period. (Recently, manufacturers have developed new blanket designs that greatly reduce their magnetic fields.)

Methods of Residential Exposure Assessment

Most research related to the assessment of residential exposures to power-frequency electric and magnetic fields has occurred in conjunction with on-going epidemiologic studies. Table 4 is a list of epidemiologic publications from these studies that present exposure-assessment data and techniques. Table 5 provides a list of publications whose primary purpose is to report results related to exposure assessment.

All residential assessments of exposure to power-frequency fields have used one or more of the following techniques: questionnaire, wiring configuration coding, theoretical estimation of fields produced by nearby electrical facilities, spot electric- and magnetic-field measurements, electric- and magnetic-field recordings at fixed locations covering periods of time from hours to days, personal-exposure measurements, and geomagnetic-field measurements. The exposure-assessment methods used by published residential studies are enumerated in Tables 4 and 5.

Questionnaires

Questionnaires have been used in residential studies to assess exposure to the power-frequency magnetic fields produced by electric blankets and other home appliances. Typically, a case or control subject (or a relative or care giver) would be questioned about their (or the subject's) pattern of use of these sources.

Wiring Configuration Coding

The first method developed for exposure assessment was the wiring configuration coding system of Wertheimer and Leeper (*1,14*). Originally criticized by many, this method has stood the test of time. Research has shown that wiring code is correlated with measured magnetic fields (but not electric fields) in residences (*15,16*). The code now normally in use was defined originally in Wertheimer and Leeper's 1982 paper (*14*). The types of overhead electrical wiring that enter into the code are transmission lines, three-phase primary distribution lines, and secondary distribution lines.

Primary distribution lines are divided into thick and thin lines according to whether or not their phase conductors are clearly larger in diameter than the standard secondary wire used in the Denver, Colorado, area (*14*). An alternative and more quantitative definition of thick and thin has been developed in terms of the ampacities (i.e., current-carrying capacities) of conductors used for primary distribution (*15*). This technique is appropriate when the wire materials and gauges can be determined. Visual discrimination of thick and thin conductors is the most subjective element in wire coding.

Sections of secondary distribution lines are further categorized as being first-spans or second-spans. A first-span secondary is that length of an overhead secondary distribution line extending from the pole on which the line's distribution transformer is located to an adjacent pole, which also is carrying electric power to more than two residential loads or one or more commercial loads. Secondaries not meeting this condition are called second-span secondaries. (Sometimes, the term short first-span secondary is used for a first-span not supplying sufficient load to be classed a first-span secondary.)

Wire coding consists of identifying transmission and distribution lines and measuring the distance of closest approach of each to the home being coded. Table 6, then, can be used to code each structure, and the final code for the home is taken as the highest of the codes for each of the lines. There are four possible codes: very high current configuration (VHCC), ordinary high current configuration (OHCC), ordinary low current configuration (OLCC), and very low current configuration (VLCC).

The process of wire coding is illustrated in Figure 5, which shows a schematic-plan view of a residence and the electrical wiring surrounding it. A transmission line passes within 145 ft (44.2 m) of the home. According to Table 6, this structure would be coded VLCC. The thin three-phase primary line passing 80 ft (24.4 m) north of the home is coded OLCC. The single-phase primary passing 40 ft south of the home is not coded at all in the Wertheimer-Leeper system (only three-phase primaries are coded). The pole southeast of the home has a transformer mounted on it that sup-

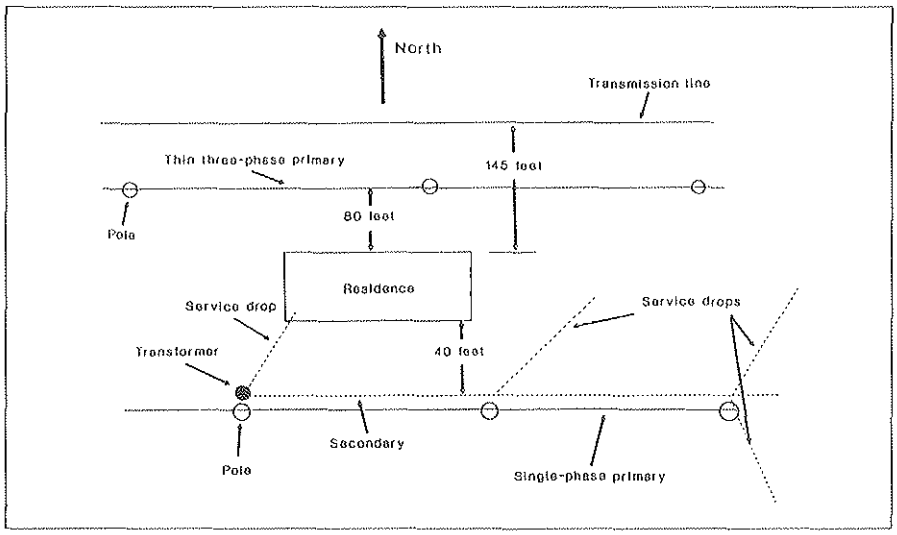

Figure 5. Schematic plan view of residence and electric power transmission and distribution wiring in its vicinity. Distances are not to scale.

plies a secondary line that passes by the home. The segment of this secondary passing by the house carries the power for three service drops. Consequently, this segment is a first-span secondary and is coded OHCC. Because the highest structure code is OHCC, the home is coded OHCC.

In practice, wire coding can sometimes be difficult. The most difficult and time-cosuming part of wire coding is the identification of first-span secondaries. The wires in a secondary often are bundled together, making it difficult to see details from the ground, and the coder's view of these wires often is obscured by trees. It also is sometimes difficult to determine where one secondary ends and another starts. Nevertheless, with all these difficulties, it is possible to train technicians to code reliably electrical wiring using the Wertheimer-Leeper method. For example, Savitz et al. (*17*) obtained 95% agreement between independent codings of homes made by trained technicians.

Houses served by underground primary wiring were placed in the VLCC category by Wertheimer and Leeper (*14*). Other researchers have chosen to treat houses with underground wiring as a fifth category (*17*).

Because magnetic fields are produced by electric currents, the overt purpose of wire coding is to discriminate between wiring configurations that carry, on the average, different levels of current. As described earlier, transmission lines are significant sources of magnetic fields, so their treatment in the Wertheimer-Leeper wiring code seems reasonable. This conclusion is not certain for primary and secondary distribution lines because, as noted earlier, net currents on these lines often are the primary sources of their magnetic fields, and net currents depend on the type of distribution line (whether or not it has a neutral) and local grounding practices. It may be that there is a statistical association between the total and net currents carried by distribution lines, which could explain the apparent ability of the Wertheimer-Leeper code to capture magnetic field levels produced by distribution lines.

Theoretical Estimation

The strength of the electric and magnetic fields produced by electrical facilities, such as power lines, transformers, and substations, depends in a known way on the system voltage, current, and geometry. Thus, assuming these parameters are known, one can calculate the electric and magnetic field produced at any distance from a source. Several studies have used this approach to assess magnetic-field exposure in residences located close to power lines.

Fulton et al. (*18*) used a combined theoretical and empirical method for their exposure assessment. They determined the closest distance, R, of approach of every power line passing within 45.7 m (150 ft) of a house under study. They placed the wires of each line into one of the following four classes: high tension (i.e., belonging to a transmission line), large-gauge (thick) primary, small-gauge (thin) primary, and secondary. They assigned to these classes nominal field values based on data published in Wertheimer and Leeper's original 1979 paper. They then weighted these nominal values by the quantities $1/R^2$ to allow for different distances between sources and the home under study, and they summed the weighted contributions from all sources.

Tomenius (*19*) simply noted in his study whether there was a visible electrical facility (6–200 kV high-voltage wires, substations, transformers, electric railroads, and subways) within 150 m of each home. (The actual epidemiological analysis performed by Tomenius defined exposure solely in terms of proximity to electric power transmission lines.)

Myers et al. (*20*) measured the distances between homes occupied by subjects of their study and all power lines (secondaries, primaries, and transmission lines) located in their immediate vicinities. In conjunction with the utilities operating these power lines, the authors estimated the load currents in each line, assumed these currents were balanced (i.e., equal currents in all phase conductors of a line), and calculated the resulting magnetic fields 1 m above ground at the center of each dwelling.

Spot Electric and Magnetic Field Measurements

A spot measurement is defined to be a measurement at a fixed location (usually inside a residence) that occurs over a period of time less than a few minutes. Survey meters customarily have been used for these measurements, but some studies now in progress are using personal exposure meters for this purpose. Savitz et al. (*17*) and Severson et al. (*21*) used identical plastic fixtures to position Model 113 survey meters (Electric Field Measurements Company, Lenox, MA) in three orthogonal directions to measure the three vector components of electric field and magnetic flux density.

Savitz et al. (*17*) and Severson et al. (*21*) jointly introduced the notion of low- and high-power spot measurements. Low-power spot measurements were made after electric power consumption in a home was reduced (by turning off lights and appliances) to as low a level as practical. These measurements were interpreted as being most reflective of magnetic fields produced by sources outside the residence under study. Similarly, high-power measurements were made after as many lights and appliances as possible were energized. These latter measurements were thought to contain maximal contributions from field sources inside the home.

Fixed-Site Magnetic-Field Recordings

Kaune et al. (*15*) made the first published fixed-site recordings in 43 Seattle residences using a data acquisition system constructed for this purpose. Three magnetic field sensors and one electric field sensor were located at fixed positions in each home, and data from these sensors were recorded on magnetic tape at 2-min intervals for a 24-hr period.

With the advent of small, battery-powered data acquisition systems, such as the EMDEX family of meters, longer term measurements have become much less intrusive and, thus, more practical. Tables 4 and 5 list studies that have reported fixed-site recordings.

Personal Exposure Measurements

Although EMDEX and IREQ/Positron personal exposure meters have been available for several years, only two studies that the author is aware of have published residential personal exposure data (Table 5). In both of these, subjects were asked to wear personal-exposure meters for periods from 24 hr (*22*) to 7 days (*7*). There are currently several studies underway that are collecting large amounts of personal exposure data.

Geomagnetic Field Measurements

Blackman et al. (*23*) published a paper reporting that a biological response elicited in the laboratory by exposure to extremely low frequency electric and magnetic fields was apparently also affected by the strength of the static geomagnetic field (i.e., earth's magnetic field). These authors found that the response occurred only when the frequency of the alternating exposure fields lay in certain bands, and they showed that the frequencies of these bands were dependent on the geomagnetic field (i.e., the static-magnetic field, usually due largely to the earth's magnetic field). Because this model has had considerable success in describing a variety of laboratory results [summarized by Liboff et al. (*24*)], some have decided to incorporate geomagnetic field measurements as part of their residential exposure assessment protocol (*25*).

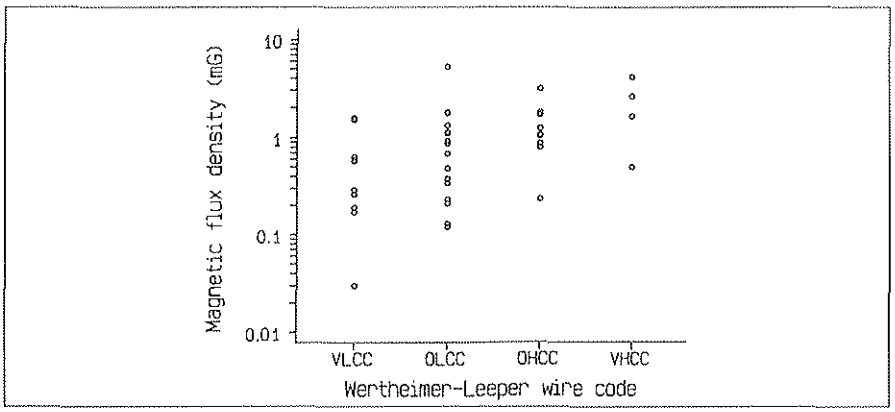

Figure 6. Scatter plot of 24-hr-average house magnetic flux densities and Wertheimer-Leeper wiring code. Data from Kaune et al. (*15*).

Current Issues in Exposure Assessment

This section discusses issues of current interest concerning the assessment of human exposure to power-frequency electric and magnetic fields.

Occupational Exposure Assessment

As related in the section "Job Titles," all published occupational studies have used job titles as surrogates for electric- and/or magnetic-field exposure. Separate research indicates that the exposures of electrical workers are, in fact, elevated relative to those received in most other occupations and at home (*7,8*). However, job titles, by themselves, must be regarded as a crude measure of exposure. There are certainly exposure differences within the general category of electrical workers or even within workers holding the same job title, differences that could perhaps be exploited to help detect the presence of confounders or dose–response effects.

What is needed in future occupational studies is a job-exposure matrix. In its simplest form, the rows of this matrix would be labeled by job titles, and a single column would contain exposure estimates for each job title. A more complex matrix could contain several columns, each for a different definition of exposure (i.e., a different exposure metric) or different latency periods for disease onset after exposure. A complete job-exposure matrix also should contain information about exposures unrelated to electric and magnetic fields. In particular, because many jobs that fall within the electrical workers category also involve the use of chemicals and possible exposures to fumes, it is important that data on these factors be included in the job-exposure matrix.

The construction of a complete job-exposure matrix is a daunting task. Electric- and magnetic-field measurements of current exposure in the job titles under study will be necessary in many, if not most, studies. In case–control studies, the exposure of interest occurred in the past. Consequently, historical changes in exposure patterns will have to be assessed during the construction of the job-exposure matrix. Such historical changes may be more pronounced for chemicals than for electric and magnetic fields.

Table 7. Summary of published arithmetic and geometric means of magnetic field measurementsin and near residences.

	Magnetic flux density, μT					
	Low Power		Normal power		High power	
Study	AM[a]	GM[b]	AM[a]	GM[b]	AM[a]	GM[b]
Savitz et al. (*16,17*)	0.08[c,d]	—	—	—	0.11[c,d]	—
Severson et al. (*21*)	0.09[c,d]	—	—	—	0.11[c,d]	—
Kaune et al. (*15*)	—	—	0.10[d,e]	0.05[d,e,f]	—	—
Deadman et al. (*7*)	—	—	—	0.15[g,h]	—	—
Bowman et al. (*8*)	—	—	—	0.06[c,d]	—	—
Dlugosz et al. (*26*)	—	—	0.53[c,i]	0.40[c,i]	—	—
London et al. (*25*)	0.06[e,g]	0.03[e,g]	0.11[e,g]	0.10[e,g]	—	—

[a]Arithmetic mean
[b]Geometric mean
[c]Spot measurement
[d]Home average
[e]From 24-hr recording
[f]Estimated using median field
[g]Measurement in bedroom
[h]Recorded during sleeping period
[i]Measurement on street corner

Between-Study Variation of Spot and Fixed-Site Measurements

Magnetic field data from spot measurements and fixed-site recordings are summarized in Table 7 for seven studies. The low-power and high-power data (all from spot measurements) are from the Denver, Seattle, and Los Angeles metropolitan areas in the United States and seem reasonably consistent. However, the normal power data, consisting of spot measurements (*8,26*) and fixed-site recordings (*7,15,25*) show considerable differences between studies. For example, the geometric means measured by Deadman et al. (*7*) are about three times larger than those measured by Kaune et al. (*15*) and Bowman et al. (*8*). It is unknown if this difference is attributable to geographical differences, measurement protocol differences, or instrumentation differences. The data from Dlugosz et al. (*26*) are much larger than the other normal power data. However, these measurements were taken on the sidewalks outside homes and may reflect more strongly sources under and above city streets.

Wiring Codes and Measured Electric and Magnetic Fields

The Wertheimer-Leeper wiring code was developed to provide a surrogate measure of long-term historical exposure to power-frequency magnetic fields that could be obtained without entry into residences (*1,14*). Three studies have now been performed that report a statistically elevated risk of cancer for children living in high-current-configuration homes. Two of these studies (*1,17*) were performed in the Denver, Colorado, area, with different groups of children. The third study was performed in Los Angeles County (*25*). These findings have stimulated a strong interest in wire codes and in various physical factors that might be associated with wire codes.

Several published studies have found that wiring configuration is associated statistically

with magnetic fields measured in homes. Wertheimer and Leeper, in both of their original studies (*1,14*), present magnetic-field data, measured outside homes, that show an association between wire code and magnetic-field levels. Kaune et al. (*15*) recorded electric- and magnetic-field data for 24-hr periods in the bedrooms and family rooms of 43 homes in Seattle, Washington. These authors observed no relationship between wire code and measured residential electric-field levels. However, there was an association between wiring code and residential magnetic fields (Fig. 6): Log-transformed averages of 24-hr mean magnetic fields were significantly different for different codes, with the largest differences being between the VLCC and OLCC taken as one group and the OHCC and VHCC as the other group. However, this model left unexplained 79% of the total variation between homes.

Barnes et al. (*16*) analyzed magnetic-field spot-measurement and wiring-configuration data from the Savitz et al. (*17*) study and reached a similar conclusion. These authors state:

> The proportion of variance in fields explained by the wire codes, however, is a rather modest 19%. In combination, these findings indicate that the relationship between fields and wire codes is well beyond chance but that the correlation is far from perfect.

In addition, London et al. (*25*) have reported recently that a relationship between the Wertheimer-Leeper wiring code, spot, and 24-hr magnetic-field measurements has been observed in Los Angeles County. This is interesting because utility distribution practices are different in many areas of Los Angeles County from those in the Seattle or Denver areas. In particular, the grounding system for a distribution line in Seattle and Denver is integrated along its entire length and typically might include 1000 to 2000 homes, whereas in Los Angeles, the grounding system for a secondary distribution line (typically serving 1–10 homes) may be electrically isolated. Thus, the Seattle, Denver, and Los Angeles results suggest that ground currents may not be an important source of residential magnetic fields or, at least, of that component of a residential field captured by the Wertheimer-Leeper code.

There is evidence that wiring codes only are associated weakly with spot measurements and fixed-site recordings of residential magnetic fields. It is tempting to conclude that, for epidemiological purposes, wiring code is a poor measure of magnetic-field exposure. While this conjecture ultimately may be proven true, its validity is not certain at this time. For epidemiological purposes, exposure generally is placed in categories (e.g., low and high) and the definitive test of wiring configuration (or any other surrogate measure of exposure) is its ability to place individual exposures in the correct category. It is important to realize that the ability of a measure to explain variability between homes is not the same as its ability to place homes correctly in exposure categories.

Relative Effectiveness of Wire Codes and Spot Magnetic Fields

Several authors have discussed the possibility that wire codes are better predictors of long-term historical exposure to magnetic fields than are spot or 24-hr measurements of the present magnetic fields in a residence (*4,1,14*). This notion is discussed in this section.

Wire codes seldom change over periods of months or years because utilities seldom change their transmission and distribution systems. In fact, the historical stability of wire codes is the reason most often advanced to explain their hypothetical superiority in assessing historical magnetic-field exposure. However, it seems that this property of wire codes will only be of virtue if long-term magnetic-field exposure is, itself, historically stable.

Assuming that long-term exposure is historically stable, we still need to explain why spot (or 24-hr) magnetic-field measurements are not historically stable indicators of human exposure to residential magnetic fields. There seem to be three possibilities: *a*) Spot measurements exhibit such large short-term variability that they are very poor indicators of mean magnetic-field levels, whether in the present or the past. *b*) The spatial variability of residential magnetic fields is so large that spot or 24-hr measurements, even if temporally stable, could not be used to assess present or past human exposure. *c*) Spot or 24-hr rmeasurements

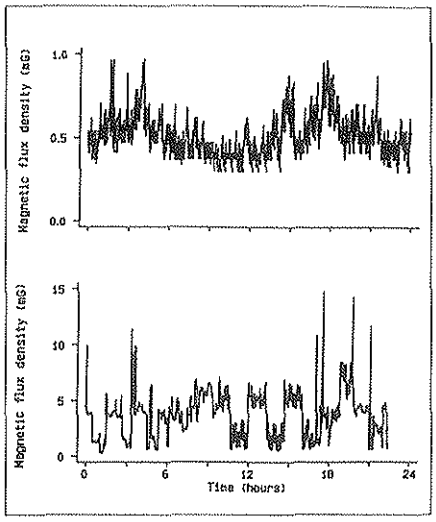

Figure 7. Magnetic fields measured in bedrooms of two homes during 24-hr periods.

exhibit much greater long-term variation than does personal magnetic-field exposure. These three possibilities are discussed in the following paragraphs.

Short-term Temporal Variability of Spot Measurements. Figure 7 shows magnetic field records, covering approximately 24 hr, taken in the bedrooms of two homes. These records, which consist of a large number of spot measurements taken one after another, clearly show short-term, apparently almost random, variation. Although the variability shown in this figure seems large, several groups have found that spot measurements taken at different times are strongly correlated.

Dlugosz et al. (*26*) made spot magnetic-field measurements during seven successive evenings on 33 street corners in Buffalo, New York. The intraclass correlation between these seven measurements was 0.94, indicating a high degree of stability

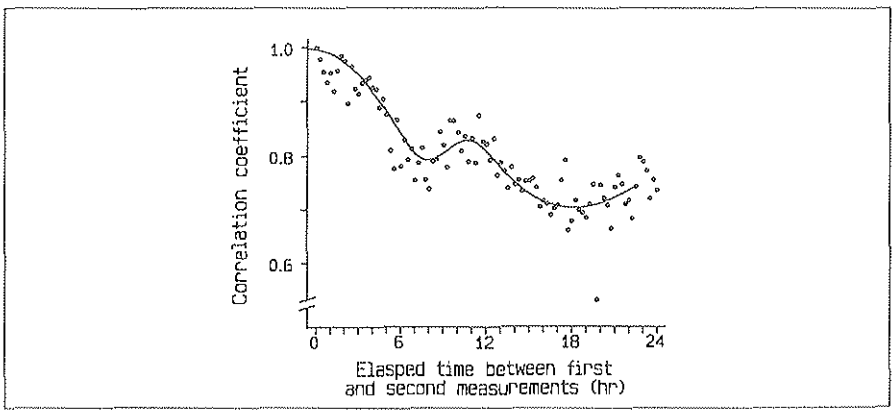

Figure 8. Correlation between spot measurements made at two different times in bedrooms of 29 homes.

Table 8. Geometric statistics for at-home and away-from-home personal exposures measured for young children by Kaune et al. (*27*) and Kavet et al. (*22*).

	Kaune et al.		Kavet et al.	
	Geometric mean, μT	Geometric, S.D.	Geometric mean, μT	Geometric, S.D.
At-home exposure	0.096	2.38	0.148	1.79
Away-from-home exposure	0.100	1.42	0.182	1.41

during the week. It should be noted that these data, alone, are far from conclusive because they were measurements taken outside homes where fields may be more stable.

Kaune et al. (*27*) recently obtained 24-hr EMDEX records in the bedrooms of young children living in 29 homes. These records were regarded as a series of spot measurements made every 15 min over 24-hr periods, and the correlations between two spot measurements separated in time by varying intervals were computed. Figure 8 shows the results of this analysis. Clearly, there is considerable stability between spot measurements made at two times separated by as little as 15 min or as much as 24 hr.

Delpizzo et al. (*28*) tested the ability of spot magnetic-field measurements to correctly classify exposure. In this case, exposure was defined in terms of the 24-hr average magnetic field measured in 40 homes. Exposure was termed either high or low, depending on whether it was greater than or less than 0.075 μT (0.75 mG). The authors then classified exposure using a single spot measurement and found that this technique had at least an 80% chance of classifying homes correctly. Furthermore, this probability was not significantly increased as the number of spot measurements used to estimate exposure was increased above one.

Thus, available data suggest that spot measurements may be rather stable over periods up to one week in length.

Correlation of Spot and Personal Exposure Measurements. The second possibility enumerated above to explain why wiring coding might be a better estimator of historical long-term exposure than a spot or 24-hr measurement is that the spatial variability of the magnetic fields in a residence might be so great as to render a spot value ineffective as a measure of residential human exposure. However, available data suggest that this might not be the case.

Kaune et al. (*27*) found that a time-weighted average of a bedroom spot (or 24-hr) measurement, a kitchen spot measurement, and a family-room spot measurement were well correlated with the measured personal exposures (measured using AMEX-3D meters) of 29 young children. (The correlation coefficient between the log-transformed measured and predicted exposures was 0.8.) Wiring code, on the other hand, was associated weakly with the measured exposures.

Kavet et al. (*22*) made the following measurements in 45 homes: spot measurements in at least three rooms of each home, 24-hr fixed-site bedroom measurements, and 24-hr personal exposure obtained by asking an adult resident to wear an EMDEX meter. Thirty of the 45 subjects lived close to transmission lines, so their data may not be representative. Limiting analysis to those 15 who lived away from transmission lines, the correlation between the measured at-home log-transformed exposures and the log-transformed averages of the spot measurement taken in each home was 0.77. (The comparable correlation for the entire sample of 45 homes was 0.76.)

Two exposure assessment studies, both with only small numbers of subjects, do not provide a substantial basis on which to make any firm conclusions. Nevertheless, if the trend continues—spot measurements predict contemporaneous exposures better than wiring code—it will become progressively more difficult to argue that wiring code is a better predictor of long-term magnetic-field exposure than spot measurements.

Long-term Variation of Spot Measurements. The third possibility introduced above to explain why wire codes might work better than spot measurements to assess long-term historical exposure of people to residential magnetic fields is that spot measurements might exhibit more long-term variability than does exposure. This issue has been examined experimentally for the first time by a recent study (*29*) in which a new set of measurements were made during 1990 in 80 Denver-area homes that were part of the original Savitz study (*17*). This study found correlations of 0.71 and 0.75 respectively, between their log-transformed low- and high-power spot measurements and those made by Savitz et al. in 1985. This level of correlation was present in both high-current configuration and low-current configuration strata. Linear regression analysis showed that the slopes of the relationships between the 1985 and 1990 low-power and high-power spot measurements were near 1.0. Apparently, spot measurements in Denver are remarkably stable over a 5-year period.

Let us now return to the original question: Are wiring codes or spot measurements a better method of assessing long-term historical exposure to power-frequency magnetic fields? First, evidence from three studies suggest that short-term variability in spot measurements is not large enough to render them ineffective estimators of TWA exposure. Second, evidence from two studies suggest that spot measurements are as, or more, effective than wire codes in assessing concurrent TWA exposure. Third, one study found that spot measurements made in 80 Denver homes about five years apart are correlated well. These results, while far from conclusive, seem to offer evidence suggesting that spot measurements may be at least equivalent to, if not superior to, wire codes as measures of historical TWA exposure to residential magnetic fields.

At-Home and Away-From-Home Exposures

Two new studies have measured separately the residential and nonresidential components of the total exposure of children and adults to power-frequency magnetic fields. Kaune et al. (*27*) had 29 young children (ages 4 months through 8 years) wear AMEX-3D meters for 24-hr periods. Each child was given two meters, one to be worn while at home, the other while away from home. The cumulative exposure measured by each meter was divided by the total time it was worn, yielding the TWA magnetic field to which it was exposed. Table 8 presents geometric means and standard deviations summarizing these two components of total exposure. Note that the geometric mean exposures at home and away from home were both about 0.1 μT (1 mG), but that the geometric standard deviations for these two exposures were very different, with the at-home component being much more variable than the away-from-home component. That is, most of the differences in exposure between subjects occur during their time at home rather than when away from home.

Kavet et al. (*22*) measured residential and nonresidential exposures for 45 adults. Geometric statistics summarizing the at-home and away-from-home exposure fields for the 15 subjects who did not live close to transmission lines also are given in Table 8. The same pattern is observed in these adult data: The at-home component of exposure is more variable than the away-from-home component.

The results discussed in the preceding two paragraphs were quite surprising. They suggest the possibility that total time-weighted exposures of children and adults can be categorized accurately by studying only their residences. If valid, this would be a very important result; but at this time, it should be regarded only as a working hypothesis. Considerable additional research is needed to test this result among other populations.

Table 9. Statistics for at-home personal exposure measurements and residential spot measurements made in 45 homes by Kavet et al. (*22*).

	Arithmetic statistics		Geometric statistics	
	Mean μT	S.D. μT	Mean μT	S.D.
At-home personal exposure	0.18	0.12	0.15	1.79
Spot measurements	0.13	0.13	0.08	2.95

Table 10. Correlation among selected magnetic field exposure indices during nonwork hours (*32*).

Exposure index	Correlation with TWA exposure
Geometric mean	0.74
Median	0.69
Peak (largest recorded field)	0.64
99th percentile	0.69
90th percentile	0.80
Percent above 0.78 μT	0.68
Percent below 0.20 μT	0.79
Percent in range 0.78–1.56 μT	0.57
Percent in range 0.20–0.39 μT	0.69

[a]Data are from 36 subjects

Contribution of Home Appliances to Residential Exposures

Questions about the importance of home appliances to residential exposures have been raised for years. It is well known that appliances such as hair dryers, curling irons, and electric razors can deliver substantial short-term partial-body exposures to their users. However, it is not clear that TWA exposure is affected substantially by these sources. Delpizzo (*30*) has performed a theoretical analysis of exposure to electric blankets, waterbed heaters, and concrete slab heaters, and concludes that these sources can make significant contributions to total exposure.

One way to examine this question is to compare magnetic fields measured with a personal-exposure meter (which presumably captures appliance contributions) to spot measurements (which are generally made to exclude appliance contributions). This comparison can be performed using the data (Table 9) of Kavet et al. (*22*). A *t*-test on log-transformed data confirms that the at-home exposure values are significantly larger ($p = 0.0004$) than the spot fields, suggesting the presence of significant appliance contributions to personal exposure. However, many additional data are needed to confirm this observation.

Exposure Metrics

An exposure metric is a function of an applied electric or magnetic field that yields a value useful for predicting or describing a biological response of interest. The simplest and most widely used metric is TWA exposure, in this case, the average electric or magnetic field during the period of exposure. But there are other possibilities. For example, exposure assessment for radio-frequency electromagnetic fields commonly uses the square of the electric- or, sometimes, of the magnetic-field strengths.

Past epidemiological studies, as well as most laboratory studies of electric and magnetic fields, have used TWA-field strength as their explicit or implicit measure of exposure. The validity of this approach is currently being questioned because of several recent developments: *a*) Some biological responses observed in the laboratory exhibit a complex dependence on intensity and frequency of the exposure field (e.g., intensity and frequency windows) as well as on the strength of the static magnetic field. *b*) Some biological systems may be sensitive to a power-frequency magnetic field only when its strength is abruptly changed (*31*). *c*) The use of TWA magnetic-field exposure to explain the relationship between wire code and childhood leukemia has not proven fruitful (*25*).

Because of the considerations listed in the preceding paragraph, some effort has been devoted to identifying characteristics of residential or occupational magnetic fields, other than TWA exposure, that might serve as alternative exposure metrics. Presumably it would be desirable to identify metrics that are not correlated strongly with TWA exposure, but this might be a difficult goal to achieve. Armstrong et al. (*32*) calculated correlations between a wide variety of electric- and magnetic-field exposure indices and found, for nonwork exposures, that many were well correlated with TWA exposure (Table 10).

One alternative that was not considered by Armstrong et al. (*32*), and is discussed frequently, is exposure to temporally fluctuating magnetic fields. This concept is illustrated in Figure 9, which shows actual 24-hr magnetic-field recordings taken in two homes (*27*). In both cases the TWA fields were about 0.36 μT (3.6 mG), but the short-term variability of the field in the upper chart was clearly much greater than that in the lower. It would not be difficult to invent a metric function to discriminate between these two exposures.

Research Recommendations

This section identifies research areas where progress can be made to improve and clarify exposures and exposure-assessment methods related to power-frequency electric and magnetic fields.

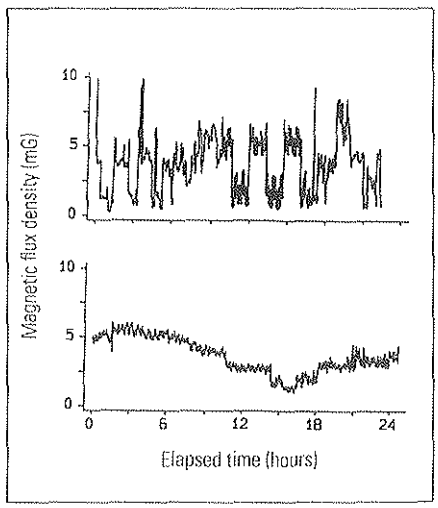

Figure 9. Twenty-four-hour records of magnetic fields in two homes. Time-weighted-average exposure was 0.36 μT for both homes.

Development of Job-Exposure Matrices for Electrical Workers

Because a number of studies have found elevations in the rate of mortality from various cancers in electrical workers, it would be appropriate to develop detailed job-exposure matrices for both electric-field, magnetic-field, and chemical exposures received by members of this group. With such a matrix, electrical-worker job titles that were exposed to fields could be separated from those that were not, and confounding exposures could be evaluated.

Prediction of Historical Exposure

The ability of wiring codes and spot measurements to predict long-term historical exposure needs to be thoroughly evaluated. In addition, techniques need to be investigated that possibly could utilize available historical information, such as residential billing records and utility loading data, to sharpen historical residential-exposure estimates.

Alternate Exposures Associated with Wiring Codes

Because of the possibility that the Wertheimer-Leeper Code is detecting some underlying factor that is unrelated to magnetic fields, an intensive and multidisciplinary search for environmental correlates of wire codes is needed.

Between-Studies Variability in Spot and Fixed-Site Magnetic Field Measurements

Spot measurements and fixed-site recordings of residential magnetic fields show considerable differences between studies, particularly for normal-power measurements (Table 7). Research is needed to determine if these differences are due to geographical, measurement protocol, or instrumentation differences. In this context, the latter two possibilities are of particular concern because they imply the existence of measurement errors that are not understood.

Residential and Nonresidential Exposures

As discussed earlier, there are data suggesting that the nonresidential exposures of children, and perhaps adults, are considerably less variable than residential exposures. This finding could be of great importance, but it needs to be confirmed in different geographical areas with a variety of different groups of adults and children.

Temporal Variability of Residential Exposure

No direct data exist on the variability or stability of residential exposure over time periods greater than 24 hr. It was inferred from spot measurements previously that exposure might be, in fact, stable over periods of years, but this hypothesis needs to be tested with direct measurements.

Alternate Exposure Metrics

Biological hypotheses that include specification of the appropriate exposure metrics need to be developed for testing in future epidemiological studies. Although much of the rationale for a particular model must come from laboratory research with in vivo and in vitro models, exposure-assessment research may contribute by identifying metrics that are associated with wiring codes.

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Problems and Priorities in Epidemiologic Research on Human Health Effects Related to Wiring Code and Electric and Magnetic Fields

Jack Siemiatycki

Epidemiology Research Unit, Institut Armand-Frappier, 531 boul. des Prairies, Laval-des-Rapides, Quebec H7V 1B7 Canada

Because of a reported excess of cancers among children living near power lines, there is some concern that electric and magnetic fields (EMFs) induced by electric power sources may affect human health, and this possibility has provoked considerable controversy. The scientific question of whether there are such health effects is far from resolved. Building upon a set of detailed reviews of the available evidence, this paper proposes research priorities and places particular emphasis on epidemiologic research. The most pressing need is to verify the validity of the claim that childhood cancer risk is affected by the type of wiring code in the vicinity of the household. More useful work can be done to verify this in the areas in which such studies have already been carried out, and additional studies should be done elsewhere. Methodological investigation of the interrelationships among different measures and proxies for EMF is needed, and this could feed back to influence the type of EMF measures used in epidemiologic studies. Studies of cancer among adults in relation to EMFs in the work place are needed. Of lower priority are studies of adverse reproductive outcomes in relation to parental EMF exposure and studies of the neurobehavioral impact of chronic EMF exposure. This article also discusses the structural impediments of conducting environmental epidemiology research and argues that bold, large-scale epidemiologic monitoring systems are needed. There is a discussion of the interface between epidemiology and public policy in a topic area as controversial as EMFs. —— Environ Health Perspect 101(Suppl 4):135–141 (1993).

Key Words: Electromagnetic fields, neoplasms, leukemia, review, environmental epidemiology

Few issues have excited as much public health concern and controversy in the past decade as the alleged harmful health effects of extremely low frequency electric and magnetic fields (EMFs). The controversy does not show any signs of abating. The set of papers in this volume provides not only a review of the scientific evidence concerning the possible human health effects of exposure to EMF, but more importantly, they provide a number of prescriptions for future research in this area (*1–6*). All of these authors has drawn their own conclusions about future research needs based on the evidence they presented. This paper serves to provide an evaluation of the research priorities across the various areas covered by the authors of the accompanying papers based on the background data they have assembled. Also, this paper will reflect on the reasons for controversy in this area and discuss the implications for both scientific research and public health practice.

By way of introduction, it is useful to summarize briefly, albeit rather simplistically, the current state of knowledge regarding health effects of EMFs (*2–7*). Based on retrospective case–control studies, associations have been reported between type of electrical wiring configuration in the vicinity of the household (referred to as wiring code) and risk of childhood cancers, notably leukemia and brain cancer. [See (*2*) for an explanation of wiring code.] Some studies have reported relative risk estimates in the range of 1.5 to 3.0 among subjects classified as very highly exposed, with lower 95% confidence limits near 1.0. Other studies have found no association. While the evidence is not strong, it is suggestive. There is some correlation between type of wiring code and levels of magnetic fields in the home, but the relationships still are poorly understood. While electric and magnetic fields can be measured with relative ease, it is not clear whether contemporary measurements in homes have much relevance to the estimation of past EMF levels. The few attempts to relate contemporary measured fields to cancer risk have produced equivocal or null findings. It is possible that a true association with EMFs has not been detected because the etiologically relevant EMF exposure variable has not been assessed. Based on the epidemiologic studies alone, the statistical evidence is stronger for an association with wire codes than it is for an association with measured fields.

Many studies, most of them based on death certificate notation of the decedent's occupation, have examined relationships between cancer risk among adults and so-called electrical occupations. The interpretation of these studies is complicated by countervailing biases. On one hand, there may have been biases in reporting results from such surveillance-type studies, namely, positive findings are more likely to be reported than null findings. Also, these studies generally involved posthoc delimitation of certain job titles as exposed (i.e., the investigators usually patched together a grouping of electrical occupations after examining the results for individual job titles). On the other hand, there were biases in the opposite direction, including the questionable validity of the job title and cause of death data and the crudeness of the job title designations as indicators of occupational exposure. Overall, there is a modest degree of consistency among these studies that shows a slight excess of leukemia and brain cancer in such workers.

There has been suggestive evidence of a link between long-term use of certain appliances in the household and childhood leukemia risk. There also is some concordance between the target organs apparently related to domestic wire code in children and to so-called electrical occupations in adults. The most obvious common factor,

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if both of these associations were confirmed, would be extremely low frequency electric and/or magnetic fields.

The biological plausibility of significant human health effects due to electric power sources remains controversial. Studies have identified various physical and biological mechanisms that might explain such effects, if real, but these are considered speculative. One of the possible mechanisms proposed, mediated by inhibition of pineal melatonin, would predict the greatest effect of EMFs on hormone-dependent tumors such as breast tumors. Research to test this prediction adequately has not been conducted yet, though there are some hints of excess male breast cancers in some electrical occupations.

Because of the frequently close relationship between carcinogenesis and teratogenesis, it is instructive and prudent to question the reproductive effects of EMF. While plausible mechanisms can be envisaged, the evidence is still too scant to provide the basis for any inference.

There has been concern that the central nervous system would be particularly susceptible to perturbation. Although there have been several studies purporting to show some effects of EMFs or their correlates on neurobehavioral outcomes, such as suicide, these have, for the most part, been flawed or inadequately reported studies.

Priorities

The listing of priorities is based on the following considerations: What issue is driving the scientific controversy and concern? What is the strength of evidence for different health effects? What types of studies would be needed to evaluate different associations? What methodologic advances would help most in resolving the uncertainties?

In the history of medicine, by far the most important basis for the discovery of true etiologic relations has been, and probably will continue to be, empiric evidence rather than deduction from biologic principles. As Doll (*8*) illustrated in the area of occupational carcinogenesis, most true associations were discovered first as a result of the chance observation of a cluster of like-exposed cases. If and when a human risk factor has been identified, basic research can be helpful in elucidating its mechanism of action. But, as exemplified by the procedures and experience of the International Agency for Research on Cancer (IARC) Monograph Program for Evaluating Human Carcinogens (*9*), epidemiologic evi-dence continues to be the cornerstone of the process for determining whether a given agent causes a given disease among humans.

The driving force in this whole controversy has been the observation initially made by Wertheimer and Leeper in Denver (*10*), then by Savitz et al. in Denver (*11*), and by London et al. in Los Angeles (*12*) of an association between childhood cancer, notably leukemia and brain cancer, and the type of wiring distribution in the vicinity of the home, referred to as wiring code. A fourth study, in Rhode Island, found no association (*13*), but it has been criticized as having used methods that biased the results towards the null (*14*). A fifth study, in Sweden, reported no association for leukemia but a positive association for central nervous system tumors (*15*). This was based on a simplified method for assessing wiring code. In aggregate, this body of evidence supports the hypothesis of an association between wiring code and childhood tumors. There are four possible interpretations: *a*) a positive association that reflects a true association between EMFs and cancer has been identified correctly, *b*) wiring code was confounded by a non-EMF risk factor for childhood cancer that was not adequately controlled, *c*) there was a bias generated by the study design or data collection method, or *d*) it was a statistical fluke.

While most attention has focused on the first interpretation, the others also merit consideration. Although there is no documentation currently available on the issue, it is unlikely that wire codes are randomly distributed through a city. There must be many social and geographic correlates of different wiring codes such as spacing of houses, distribution of single versus multidweller units, and age of the housing development. Perhaps the true risk factor is a characteristic of the neighborhood, such as air pollution, population density, or levels of local immunity to infectious agents; possibly it is a characteristic of the home related to its age or building materials, or a characteristic of the family, such as residential mobility. The complex pattern of leukemia risk as a function of crowding and mobility, possibly mediated by infectious agents, that was hypothesized by Kinlen (*16,17*) in Britain illustrates the kinds of complex and subtle factors that must be considered. While the reports by Savitz et al. and by London et al. made some efforts to take socioeconomic status into account, those attempts were far from comprehensively accounting for the range of possible social and geographic confounders.

Another objection to the Denver and Los Angeles studies that has been raised, and that has not been answered satisfactorially, has to do with the possibility of a biased population control group. Namely, it is alleged that nonresponse, including noneligibility and nonparticipation, may differ among cases and controls and may also be correlated with wire code exposure. For certain parameters, such as use of appliances, the results may also have been biased by differential quality of response from cases and controls.

Finally, the interpretation of the positive findings as a set of statistical flukes cannot be dismissed, because the handful of relative risk estimates has been of borderline statistical significance and have not shown clear dose–response patterns. Even if we could be satisfied that biases were not responsible for those findings, the strength of the accumulated body of evidence (in the sense of a metaanalysis) would not justify concluding that there is an association before additional studies were consistent in demonstrating an association.

Although the investigators used wiring code as a proxy for EMFs and subsequent work has shown that wiring code is correlated with EMFs, the attempts to relate childhood cancer to EMFs directly have not succeeded yet. This is not to say that the available evidence disproves an association with EMFs, but it does not support such an association even to the modest degree that it supports an association with wiring codes. The physical and biological mechanisms that have been postulated to explain the alleged harmful effects of EMFs on humans are largely speculative. The two legitimate reasons for according this issue a high level of attention are the epidemiologic evidence linking wiring code to childhood cancer and the fact that EMF exposure is so pervasive. Were it not for the empiric observations of an association between childhood cancer and wiring code, the issue of EMF and health would merit little more scientific attention than the potential harmful effects of many other common physical and chemical exposures. It might be argued that its apparent association with wiring code has served to open the issue of EMF exposure and cancer risk and that it is now appropriate to study more credible measures of EMF exposure. However, it is not opportune to put all the research eggs in the EMF basket, and it would be more cost-effective to give some priority to establishing the validity of the Wertheimer–Leeper observation. In part, this judgment is based on the fact that the Wertheimer–Leeper observation is a fairly straightforward hypothesis to evaluate; conversely, the investigation of one or another of the measured EMF metrics would be

much more expensive and time-consuming to evaluate, and any null finding in respect to a given measure of EMF exposure will be unconvincing, because it will be argued inevitably that the wrong metric of EMFs was studied. If the Wertheimer–Leeper observation were confirmed, it would reinforce the high priority of research in this general area and it would suggest two lines of research, the effects of EMFs on cancer risk and the significance of other correlates of wiring codes as possible risk factors. The combination of positive findings on the Wertheimer–Leeper observation and null results on attempts to correlate measured EMFs with childhood cancer would provide an extremely important lead in searching for the etiology of childhood cancer. If the association with wiring codes is not confirmed, then the general priority level for research in this area would be lowered, and the issue of exploring non-EMF correlates of wire codes would be eliminated.

Thus, if the problem is formulated as the search for the etiology of childhood cancer rather than the search for the health effects of EMF exposure, the top priority is to determine whether there is an association between childhood cancer, notably leukemia and brain cancer, and wiring code. Of slightly lower priority is the closely related question of whether other, more direct measures of EMFs are associated with cancer risk. In evaluating these related issues, there are several facets that require specification, including the types of cancer on which to focus, whether to assess an effect in children or in adults, and how to measure the exposure variable. Finally, the last priority would be to evaluate the effects of EMFs on other health outcomes. Methodologic developments would be needed at several steps.

Revisit the Available Case–Control Data Sets on Childhood Cancer and Wire Codes

It is important to try to address the confounding and selection bias issues in Denver and Los Angeles more thoroughly than previous studies. It should be possible to ascertain that the observed findings are not due to uncontrolled confounding by the types of neighborhood characteristics, dwelling characteristics, and family and/or social characteristics mentioned above. There may be relevant data already available to the investigators of the Denver and Los Angeles studies. If not, it would be desirable to conduct some additional data collection in these areas to detect and to deal with potential confounders. Ideally, this could involve visits to the homes of study subjects already interviewed. If this is not feasible, it could involve examinations of the social and geographic correlates of wiring codes in representative samples of households in these cities (i.e., to characterize the exposure–confounder arm of the conventional confounding triangle).

In the studies of Savitz et al. (*11*) and of London et al. (*12*), random digit dialing (RDD) was used to ascertain eligible controls. It would be informative to compare, on a sample basis, the kinds of households elicited by an RDD procedure as compared with those elicited by different procedures and then to estimate the prevalence of different wiring codes in these cities. Because of the possibly idiosyncratic nature of telephone coverage and social behavior, it would be preferable to carry out these methodologic studies in Denver and Los Angeles rather than trying to transfer inferences from another locale.

Studies to Replicate Cancer Found in Children

Even if the supplementary studies recommended above confirmed the associations with wire codes initially reported, these findings would have to be replicated elsewhere to provide some assurance that they were not statistical flukes or products of uncontrolled bias. Fortunately, wiring code is a relatively easy exposure variable to assess and it does not require access to households. It might be possible for investigators who have previously carried out leukemia or brain cancer case–control studies to piggyback a new evaluation of wire codes onto their previous studies.

Although the evaluation of the childhood cancer and wire code associations is a sufficient motivation for additional case–control studies by itself, if feasible, it would be important to use that opportunity to evaluate again the role of measured EMFs and appliance use. Because the nature of the risk factor is completely unknown, it would be prudent to include an exposure assessment protocol in as many different types of exposure metrics as possible, including various functions of spot measurements in subjects' places of residence, work, or school and continuous monitoring of personal exposure. It also would be useful to explore alternative exposure metrics such as the resonance model and exposure to transient fields, though methods for so doing in an epidemiologic study are not apparent (*2*).

Any new studies undertaken to tackle this issue ideally should be carried out in areas that have had relatively stable populations to minimize complication of the retrospective exposure assessment because of immigration, and in areas that contain an adequate proportion of high wire code homes. Also, they should involve larger numbers of study subjects than previous studies. Because of the rarity of childhood cancers and the desire to subdivide them by histological subtypes, it may be necessary to resort to multicenter studies. A population-based, case–control approach with data collected on social and geographic characteristics of neighborhoods, dwellings, and households (so that those factors can be incorporated into analyses) would be the design of choice.

The issue of control group selection is important not only in this area of EMF exposure and cancer but also in any case–control studies. Thus, a brief digression is in order. While convention holds that a set of population controls selected from the free-living general population represents the optimal choice in a population-based case–control study, the practical aspects of implementing this strategy may render it decidedly less attractive than alternatives. Obtaining a sampling frame is not straight forward, since in North America, at least, there are few, if any, continuously updated population registers. Random digit dialing has become a popular method for control selection, but there is little understanding of the biases that might ensue from noncoverage due to having no telephone service, being unavailable to answer an initial call, or being unwilling to respond honestly to the most elementary eligibility questions. Once subjects are eligible, their willingness to participate may differ between cases and controls, and once they are willing to participate, the quality of their participation may differ. Differential losses at any stage can result in bias, as can differential quality of response. Alternative methods of population control selection (e.g., birth certificates, immunization rosters, school lists, utility company lists, address directories) also may have problems with differential losses (selection bias) and differential quality information (information bias). A properly chosen disease control group (e.g., selected from the same hospitals as the cases and among diseases having similar referral patterns) may minimize selection bias and avoid information bias. Because it is impossible to be certain of the relative pros and cons of different potential control groups within a given study, it is prudent and efficient to use two control groups, one a so-called population control

group and the other a so-called hospital control group. This design is shunned sometimes for fear that it might produce conflicting, and therefore difficult to interpret, results. However, this argument is flawed; its logical conclusion is that there should not be more than one study on any issue because of the possibility of conflicting results. The use of multiple control groups can be seen as a way of carrying out multiple (albeit not independent) studies at a small additional cost. In any case, the verdict on these hypotheses regarding wire code or EMFs will not be based on any single study but on the body of studies, some already complete, some now in progress, and some perhaps coming later. It would be a pity if all of these studies used basically the same control group strategy and thus were open to the same set of criticisms. If an association were found using different types of control groups, this would be the most powerful way to disarm critics.

In this section on studies of childhood cancer and domestic exposure, case–control rather than cohort-type studies are recommended. It is unlikely that lists to constitute a retrospective cohort of exposed children for follow-up are available anywhere. The possibility of establishing a prospective cohort of children, with baseline information on exposure to wire codes and possibly other exposure variables, and follow-up through morbidity or mortality registers is a daunting prospect when the outcome of interest is as rare as childhood cancer. Furthermore, for a variable with long-term stability such as wiring code, it is likely that a properly conducted case–control study would provide results equivalent to those of a properly conducted cohort study. For a variable that is very unstable over time, as some of the EMF metrics may very well be, a measure taken at the outset of a prospective cohort study may be no more meaningful than a measure taken in a retrospective case–control study.

Exposure-Related Methodology

The development of general epidemiologic methodology, including insights into design, fieldwork methods, or analysis, may not be specific to this particular problem, but it would benefit research in this area. Of specific relevance to research in this area are methodologic studies focusing on the measurement and meaning of the exposure variables. There are many sources of EMF exposure and many approaches to measuring it. Among the most prominent approaches to measuring nonoccupational exposure are spot measurements in the home; personal, portable dosimeters; and wiring code. The relationships among these and their stability over time are crucial to planning and interpreting epidemiological studies but are understood poorly.

Given the current available epidemiologic literature, the top priority is to investigate the significance of wire codes. This should include some general description of the historic evolution and current urban geography of wire codes. Empirical studies should be undertaken to correlate spot measurements of electric and magnetic fields in houses with wire code. Various exposure metrics should be examined to determine which are best correlated with wire code.

Temporal stability of spot measurements, both short-term and long-term, requires further documentation. Ideally, there should be a representative panel of households monitored over a long period, perhaps 5 years, with spot measurements, personal dosimetry, and wire code data collected periodically. The relative importance of at home versus away from home EMF exposure should be evaluated in a general population, as should relative contribution of appliances in the home to the overall burden of domestic EMF exposure. Such panel studies should be conducted in at least two geographically separate locales so an estimate of the generalizability of these interrelationships may be determined.

Occupational Studies of Cancer Occurrence

Despite the problems with the occupational studies alluded to above, the relatively consistent evidence of slightly increased risk of brain tumors and leukemia in so-called electrical occupations deserves further evaluation. A possible advantage of occupational studies over residential studies is that they may provide clearer exposure differentials. However, there needs to be better characterization of the exposure factor than the job title. This could be accomplished by using some sort of mechanism that measures exposure levels either by means of a job-exposure matrix or by measurement protocols. Studies should be devised to obtain exposure information not only on EMF but also on occupational and nonoccupational exposures that may confound the association between EMF and cancer. Useful studies could be carried out in the context of cohort studies of workers known to be highly exposed (some major studies of utility workers are already in progress) or in the context of population-based case–control studies. While most interest should be in leukemia and brain tumors, there is sufficient uncertainty about other cancers to justify examining many cancer sites, especially skin melanoma, lymphoid tissue, breast, and prostate.

Databases for Attributing Occupational Exposure to EMF

It is possible for experts in hygiene to make useful estimates of past occupational exposures to chemicals (*18*). It has also been shown that experts can make useful estimates of EMF exposure ranking in a cohort of utility workers (*19*). But the validity of such expert judgments depends on the availability of some exposure measurements in various occupations. There is very little information available on the relative levels of EMF exposure in different occupations. Most measurements have been made among utility workers. There is only a scattering of data on other occupations (*20*), and no indication of the relative stability of occupational levels of EMFs over long time periods. Surveys should be carried out to document EMF levels in representative samples of many occupational groups. The development and availability of data bases on relative EMF exposure levels in different occupations and on the temporal stability of such levels would aid in the interpretation of past occupational studies and in the conduct of new ones.

Animal Carcinogenicity Studies

Although uncertainty about the ability to extrapolate evidence of carcinogenicity across species still exists, it is widely accepted that evidence of carcinogenicity in one species increases the plausibility of carcinogenicity in another. Thus, some evidence concerning the animal carcinogenic potential of these exposure variables would benefit the planning and interpretation of epidemiologic studies in this area. The wiring code variable, which may represent a complex of sociological as well as physical parameters in epidemiological studies, has no meaningful equivalent in animal experiments. If an animal model of EMF carcinogenesis can be developed, it would help greatly in elucidating which exposure metrics might be useful to assess in epidemiologic studies. Animal experiments to investigate various exposure metrics should be set up. The most interesting end points would be leukemia and brain tumors. If there is any further support for the hypothesis that melatonin levels are affected by EMFs, then mammary tumors also would be worth examining. Different types of experimental protocols would be needed to evaluate different possible mechanisms of action (e.g., complete carcinogen versus promoter).

National Survey of Domestic Exposure and Ecological Studies

Little information is available on how the exposure variables, wiring code, or the various EMF metrics may vary from region to region and from city to city. Such information would, at the very least, be useful in the selection of appropriate sites for carrying out the case–control studies mentioned above. But even further, such information may be useful in assessing the feasibility of, and eventually the implementation of, ecological studies. Although the limitations of ecologic studies are not to be minimized (*21*,*22*), these limitations should not prohibit the use of such studies where they may be useful. The opportunity for distortion in ecologic studies is minimized when the intercommunity variation in the exposure variable is relatively large compared to the intracommunity variation. Wiring characteristics and/or electric and magnetic fields in buildings may be factors that vary substantially from county to county and from city to city. If so, these would be beneficial variables to include in a geographic correlation study of childhood cancer risk, notably leukemia and brain cancer. Because mortality rates are readily accessible for all causes, many types of cancer and even noncancer death rates can be assessed. Also, it would be desirable to use incidence rates, but this would limit the outcomes and the possible geographic areas to those covered by tumor registries. Such a study would relate some aggregate measure of exposure to wire codes and/or EMFs to rates of any type of cancer or any other health outcome. In fact, for a couple of reasons, it would probably be more successful in detecting an association with a childhood tumor than with an adult tumor. First, the induction period probably would be shorter for childhood tumors; thus, the current aggregate exposure index would be more etiologically relevant for childhood tumors. Second, the opportunity for confounding by other factors is probably greater for adult tumors than for childhood tumors because the web of causation is probably more complex and drawn out in time, which would make it more liable to vary from place to place.

Because information on wiring characteristics and fields is not readily available at the aggregate (e.g., city, or county, or state) level, it would require some effort to carry out representative field surveys in selected areas. It would be desirable to collect information on potential confounding factors among the aggregate units. Some would be available from sources such as the census bureau. Some confounder data, notably social characteristics of the families residing in different homes, could be collected in conjunction with the field surveys of wiring codes and EMFs. The collection of such data would allow estimation of exposure-confounder associations at both the individual and aggregate levels.

Surveys of as few as 30 to 100 representative households per area may be enough to address the issue of interarea versus intraarea variability in wiring code distribution. If the interarea variability in wiring code is large compared to the intraarea variability, then an ecologic study with as few as 10 to 20 ecologic units may provide useful results. Such a study could be quite easy and not too expensive to mount. For relatively low marginal cost, it also would be possible to evaluate some simple measured EMF metrics.

Neurobehavioral Effects

The biologic plausibility for neurobehavioral effects is somewhat higher now than it is for other disease outcomes (*5*). However, despite a substantial amount of literature, the evidence for such effects is too flimsy, and the biologic rationale is insufficiently compelling to justify giving high priority to research in this area. Nevertheless, methodologically sound studies should be encouraged in this area, and the findings should be monitored. For short-term effects, it should be possible to generate fairly clear indications of whether there is an effect.

Reproductive Effects

The evidence concerning reproductive effects is inconclusive and inconsistent. Because of the public's concerns and because of the possible link between carcinogenicity and teratogenicity, it would be justifiable to pursue studies in this area. Because the induction period between exposure and disease may be shorter than the period for carcinogenic effects, it may be easier to relate exposure to reproductive effects than to cancer, if there are such effects. The disadvantage of studying reproductive outcomes as opposed to cancer is the notorious difficulty of ascertaining outcomes. The reproductive effects may be nonspecific; thus, it may be appropriate to focus on such things as birth weight, congenital malformations as a class, and perhaps even sperm quality. As the impetus for such studies would come from the analogy between carcinogenicity and teratogenicity, rather than from evidence about reproductive effects of EMFs, many of the arguments used to prioritize cancer studies would prevail here as well. Outcomes should be studied in relation to domestic wire code, measured EMFs, electrically related occupations, and appliance use.

Adult Cancer and Nonoccupational Exposure

There is weak evidence of a differential cancer risk in adults from residential wire codes. Additional case–control studies could be carried out along the lines of the childhood cancer studies. It would be worthwhile to examine risks of leukemia, brain cancer, and following Stevens' (*4*) conjecture, cancers of hormone-dependent tissue. However, such studies would be difficult to carry out properly since the etiologically relevant exposure period might be many decades before disease onset (e.g., in puberty for breast cancer), and it would be desirable to obtain at least wire code information on all homes inhabited since childhood—a daunting prospect. If an ecologic study in which information on wire codes and EMFs are collected from different areas is carried out and if the interarea variation is large, then it would be interesting to submit adult cancer rates to an ecologic correlation analysis. As indicated above, however, the interarea variation in other risk factors for adult cancers might be quite significant and would confound the observed associations. It appears that the investigation of adult cancers in relation to EMF exposure would be more effectively conducted in occupational studies, not nonoccupational studies, because there may be a greater likelihood of estimating past relative exposure levels.

General Comments

The above ranking is rather arbitrary. I would consider the top six items to be of high priority and the bottom four to be of low priority. There are studies in progress that correspond to several of the themes listed above. For instance, there are multicenter case–control studies of childhood leukemia in the United States, in Canada, and in New Zealand; there are cohort studies of utility workers in the United States, in Canada, and in France; there are long-term animal carcinogenicity studies in the United States and in Canada, and so on. As they become known, results of these studies may alter significantly our view of the research directions to be fostered. In the above listing, I have included epidemiologic, measurement, or toxicologic research whose results might have a direct impact on epidemiology. Research on basic biological effects of EMFs will continue undoubtedly at its own rhythm, and

the findings may influence the conduct of epidemiologic research in this area.

Problems in the Science and Public Health Aspects of EMF Research

Unique Aspects of EMF Epidemiology

In most respects, the problems of research design, delimitation of disease outcome categories, confounding, and defining appropriate comparison groups are similar in EMF studies to what they would be in other environmental epidemiology studies. However, while many exposure variables are difficult to measure and do not lend themselves to a self-evident exposure metric, EMFs pose particular challenges in this regard. First of all, it is uncertain if the etiologically relevant exposure variable is electric field, magnetic field, or some other correlate of wire code. Furthermore, there is no such thing as a truly unexposed group. Finally, if effective exposure depends on theories of resonance or on exposure to transients, then the notion of monotonic dose–response, which serves with chemical exposures as an additional means of juxtaposing exposed and unexposed, may not be operative. Many exposure circumstances in environmental epidemiology are very difficult to characterize or measure (e.g., air pollution, toxic waste sites), and many are so ubiquitous that it is difficult to identify a truly unexposed group (e.g., ultraviolet light, motor vehicle exhaust). But the peculiar hypothesized models of dose–response (e.g., the resonance model or the transients model) may be unique to the EMF issue. The rest of this paper does not concern specifically issues related to EMFs or wiring code, but rather, it concerns the social and scientific contexts in which the issue is being addressed

Structural Impediments to Environmental Epidemiology

EMFs from power sources have been part of the urban environment for most of this century, and over a decade has passed since the initial Wertheimer–Leeper report. The available epidemiologic evidence on this issue is still very thin. Our species will continue to live in an environment full of potential health hazards. The hypothetical matrix of all exposures by all diseases is virtually limitless. Our capacity to evaluate each possible association is very limited. These limitations affect our ability to detect and evaluate the effects of any environmental agent, including EMF. What measures, if any, can be taken to enhance the capacity of epidemiology to provide more rapidly better quality evidence for a larger part of the hypothetical matrix?

The number of epidemiologists, and particularly environmental epidemiologists, who cope with the multitude of potential environment–disease associations is small. This can be improved by increasing the training and job opportunities in environmental epidemiology. Although this would help, within the bounds of feasibility, it is unlikely to make a significant dent in the problem. Pouring more money into EMF-related research might help, but it would detract from other equally worthy issues. We need to develop and implement more efficient methods for studying environmental disease associations. The use of experimental *in vivo* and *in vitro* procedures for testing environmental agents is not capable of serving as an effective proxy for human evidence. Epidemiologic evidence is still essential.

The traditional biomedical research paradigm of studying a single hypothesis at a time does not serve well when addressing a problem of this magnitude. More efforts should be devoted to large-scale data collection endeavors. Population-based disease registries, such as tumor registries, represent one essential element of a useful intelligence system. Systems for routine registration of exposure are less common but also would be of use. The ideal might be a system that goes beyond the traditional passive disease registry system to something approaching an ongoing case–control study (*18,23*). As cases are ascertained in the system, a data collection procedure can be implemented to obtain different kinds of information such as occupational history, residential history, and dietary habits. As time goes on, the accumulated data can be analyzed to examine the relationships between the diseases covered by the registry and the various exposure variables routinely collected. A permanent infrastructure to run such a system would be an extremely cost-effective tool that could be mobilized to generate hypotheses in periodic analyses or to test hypotheses suggested by other evidence. The institution of such systems, which should combine the breadth of traditional vital statistics functions with the depth of so-called analytical epidemiology projects, would greatly increase our capacity to confront rationally the hypothetical matrix of all environmental exposures by all diseases. The main impediments to such development are in the anachronistic attitudes toward research that doesn't fit into the narrow hypothesis-testing mold of conventional biomedical research and in the structural difficulties of funding such endeavors.

Epidemiology, Controversy, and Public Policy

Since the 1960s, there has been a growing consciousness of the potential harm that environmental pollution can cause. It has become widely accepted that extrinsic factors (as opposed to genetic factors or pure chance) play a role in many, if not most, cases of disease. It has also become clear that the benefits of modern technology have been accompanied by significant degradation and pollution of the environment. In this context, it has been easy for the public and for scientists to entertain, if not readily accept, the hypothesis that pollutant *X* causes disease *Y*. The initial reports of carcinogenic effects of EMFs were greeted with skepticism by much of the scientific community. But there was sufficient scientific interest to foster concern in nonscientific circles. In such a context, it was natural for epidemiology to be called upon to carry out the studies to resolve the issue. Like other sciences, epidemiology operates iteratively between hypotheses and empirical evidence. Generally speaking, as valid scientific data accumulate, the underlying truths are elucidated, and at least a consensus about which hypotheses are untenable may develop among informed scientists. But there is no law of nature that determines how much data on a given issue are needed before a consensus develops. Unlike other sciences, epidemiology is frequently drawn into a public policy terrain with little tolerance for uncertainty or equivocation. Issues such as EMFs and human health force epidemiologists to draw inferences concerning causal relations before the data are developed enough to support conclusions. Furthermore, the issue may be so enmeshed in adversary or ideological interests that it becomes difficult to address it in the ideal scientific context of objective disinterest. Scientists are not unwilling victims of this process. Research careers and funding opportunities can be enhanced greatly by engaging in controversial and high-profile research.

The rules of the game differ when scientists move onto this adjoining turf. It is not necessarily the best science that prevails. Suboptimal science, whether motivated by altruistic or base motives, can thrive in such a context. Epidemiology is more susceptible to misuse than other disciplines, because it can be carried out by persons who have little or no specialized training in the area. Because of the

invisible and ostensibly mysterious nature of EMF, research in this area may be susceptible particularly to the social pressures that may detract from methodological rigor. It is impossible to establish hard and fast rules to regulate epidemiologic or scientific competence. Certainly, science must make room for mavericks who are often at the origin of important developments. However, even if mainstream science is sometimes slow to accept new ideas, it is supple enough that valid ideas would not be shunned indefinitely.

As with many environmental health issues, the scientific evidence concerning effects of EMF is weak. This may be because the relative risk is too low to be readily detected by the epidemiologic methods used or because there really is no effect. For public health purposes, this is a crucial distinction, because even a relative risk that is low by epidemiologic standards can produce a nontrivial burden of disease if it is due to a prevalent exposure. Perhaps this is the case with EMFs. What type of public policy should be recommended in such a situation? Should the standards for evaluating causality be relaxed in the case of a potentially significant pathogen? Should we be prepared to conclude that there is an association when the evidence is weak or even contradictory? No. This would not serve science, and in the long run, it would not serve public health. The temptation to cry wolf on the grounds of prudence will begin discrediting epidemiology and science in general. We may have seen some of this already. Is a scientifically conservative attitude tantamount to licensing pollution until the ephemeral scientific certainty is achieved? No. Public health decisions are made with a different set of rules than scientific ones. Scientific evidence is one of the parameters that enters the equation, but there are also issues of social values, economic costs, political will, technological alternatives or fixes, and so on. It is entirely defensible and even desirable to contemplate a policy of prudent avoidance of substances for which there is weak evidence of health effects. Within reasonable economic constraints, we should try to minimize pollution by any substance, irrespective of known toxic effects, because what we know of toxic effects of environmental agents is only the tip of an iceberg. However, we must resist the temptation to disguise a tough public policy decision in a cloak of ostensible scientific rigor and precision, whether this abuse of science is in defense of vested corporate interests or the preservation of public health.

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Introduction and Recommendations: Working Group on Indoor Air and Other Complex Mixtures

Jonathan M. Samet[1*] and Frank E. Speizer[2]

[1]Pulmonary and Critical Care Division, Department of Medicine; and the New Mexico Tumor Registry, Cancer Center, University of New Mexico, Albuquerque, NM 87131; [2]The Channing Laboratory, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA 02115

Air in indoor and outdoor environments typically contains many gaseous and particulate pollutants that may affect adversely any individual at sufficiently high concentrations and more sensitive individuals at lower concentrations. The public health relevance of addressing the effects of mixtures is becoming increasingly evident as we improve the concept of total personal exposure to pollution and obtain more data from personal monitoring. The papers within this volume represent the deliberations of a working group assembled with the goal of improving the epidemiologic approach to investigating the health effects of indoor air pollution and other complex mixtures. The group, composed of epidemiologists, human and animal toxicologists, and experts on biomarkers, comprehensively reviewed the methodologic issues involved in investigating complex mixtures. Members noted the deficiencies of current epidemiologic methodology for studying complex mixtures and called for broad-based advances in study design, exposure assessment, outcome assessment, and data analysis and interpretation. Understanding the health effects of complex mixtures will require multidisciplinary research using not only epidemiologic studies incorporating the new methods of exposure assessment but animal and clinical toxicology. — Environ Health Perspect 101(Suppl 4):143–147 (1993).

Key Words: Complex mixtures, air pollution, exposure assessment, health effects

Introduction

The gaseous and particulate pollutants that are typically present in the air of indoor and outdoor environments may have an adverse effect upon any individual at sufficiently high concentrations and upon more sensitive individuals at lower concentrations. The complexity and components of the pollutant mixture may vary as human activities influence the sources, as meteorology alters the distribution and dilution of the pollutants, and as components of the mixture undergo chemical transformation (*1*). For example, sources of indoor air pollution are diverse and include building occupants themselves and their activities, combustion, building materials and furnishings, biological agents, and entry of contaminated outdoor air and soil gas (*2,3*). The air of a home might contain nitrogen dioxide (NO_2) from the unvented emissions of a gas stove or space heater, respirable particles from cigarette smoking, cooking, occupant activities, and outdoor air, formaldehyde from furnishings and plywood, tetrachloroethylene from recently dry-cleaned clothes, and allergens from a family cat. The contaminant levels would vary with occupant activities, such as cigarette smoking and cooking. For example, concentrations of environmental tobacco smoke components would be greatest during the smoking of cigarettes and the characteristics of the environmental tobacco smoke would change as the mixture aged (*4,5*). The potential health effects of indoor air pollution are equally diverse, spanning from short-term annoyance and discomfort to permanent disability, cancer, and even death.

Similarly, pollutants in outdoor air are present in complex mixtures, although strategies for regulation and source control have tended to focus on single pollutants; adverse effects of concern span from short-term toxicity to chronic diseases reflecting long-term exposure. These mixtures of primary and secondary pollutants vary from urban to rural settings and across microenvironments.

Although the complex nature of air pollution is recognized, most epidemiologic studies of air pollution and health have focused on the effects of single pollutants or, at most, two specific pollutants such as total suspended particles and sulfur dioxide or on a single outcome measure in relation to several exposures such as respiratory symptoms in children, NO_2, and environmental tobacco smoke (*6,7*). Some pollutant mixtures, such as environmental tobacco smoke and photochemical pollution, have been investigated as though the mixture were a single agent, using a component of the mixture or indicators of source strength as indices of exposure in epidemiologic studies. The restricted focus undoubtedly reflects, in part, the difficulty of accurately estimating personal exposures to multiple pollutants and assessing multiple health outcomes. However, even studies directed at a single pollutant inherently examine the effect of that pollutant on a background of exposure to a complex mixture of other pollutants.

It should be noted that in the context of this collection of papers, the term *complex mixture* is used in several ways. Sometimes it is used to refer to binary mixtures of single compounds, sometimes to binary combinations of a complex mixture and a single compound such as environmental tobacco smoke and NO_2, and sometimes to mixtures of more than two compounds such as mixed volatile organic compounds. A more precise definition might well restrict the use of the term complex mixtures to mixtures of more than two constituents. Its broader use in this document is allowed on the grounds that in the context of epidemiologic research, a number of the problems encountered when trying to measure the effects of two factors are only compounded when the researcher is confronted with higher order mixtures (see Working Group, Recommendations, below).

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*Author to whom correspondence should be addressed.

The public health relevance of addressing the effects of mixtures is becoming increasingly evident as we refine the concept of total personal exposure to pollution and obtain more data from personal monitoring (*1*). Recognition of the complexity of pollutant mixtures in indoor and outdoor air has led to concern that synergism among the components of mixtures may produce adverse effects, even though effects would not be anticipated from the concentrations of individual components. For example, mixtures of volatile organic compounds, with individual compounds present below permissible exposure limits specific to the compounds, are a suspect cause of some outbreaks of sick-building syndrome (*3*). For protection of public health, identification of the specific components of mixtures that result in toxicity should lead to more specific and effective control strategies.

Difficult questions concerning the effects of mixtures, increasingly raised as we recognize the complexity of indoor and outdoor air pollution, pose new challenges to environmental epidemiology. The state of the art is largely reflective of study designs that have been tailored to studying single pollutants, although the data may be secondarily used to address other pollutants, sometimes to test hypotheses, but often only to control for a potentially confounding or modifying exposure. For example, the Harvard Six Cities Study was designed to assess the effects of sulfur oxide and particulate pollution; the original design assumed that a gradient of exposure to the same type of pollutants could be established across the six cities (*8*). Subsequently, the data were used to test hypotheses concerning indoor air pollution and additional outdoor pollutants (*9,10*). In a prospective cohort study in New Mexico of indoor nitrogen dioxide exposure and respiratory infections in infants, restriction has been used to remove the potential confounding or modifying effects of environmental tobacco smoke (*11*). By design, all subjects reside in homes having no adults who smoke.

In some investigations, data have been collected on indicators of exposures to multiple pollutants. Most of these studies have been cross-sectional in design and incorporated surrogates for indoor and outdoor exposures to complex mixtures. In those investigations that have attempted to address the effects of multiple pollutants, the most widely used approach for assessing joint effects has been multivariate regression analysis, incorporating variables for the main effects of the pollutants and often product terms for the interactive effects of the pollutants. Thus, for two exposures, an additive regression model would take the form:

$$Y = f(a + b_1x_1 + b_2x_2 + b_3x_1x_2), \quad [1]$$

where x_1 and x_2 represent the two pollutants, b_1 and b_2 describe their independent effects, and the coefficient b_3 describes their joint effect.

Such regression methods now are used routinely for assessing the joint effects of multiple pollutants. Software for these methods is available and applied readily. However, regression alone does not offer a solution to the problem of understanding complex mixtures. Measures of exposure are used generally with the assumption that the surrogate measures of particles or specific gases are similarly applicable in different environments. Statistical models inherently simplify complex biological phenomena, and the relations assumed among exposures included in a model may represent inappropriately the underlying disease mechanism. Often understanding of causal pathways is insufficient for assuring that the model correctly represents biological mechanisms, and statistical considerations alone may direct model development.

Improvement in the state of the art for studying complex mixtures will require broad-based advances in study design, exposure assessment, outcome assessment, and data analysis and interpretation. Epidemiologic studies of indoor and outdoor air pollution have been almost exclusively observational in design. Experimental approaches might be designed to control variation in exposure to a complex mixture; hybrid designs combining observational approaches with controlled exposures of certain subjects also might be informative. Methods for assessing exposures of individuals are evolving rapidly (*1*), but little consideration has been given yet to strategies that can be employed in epidemiologic studies of complex mixtures. Most outcome measures in studies of complex mixtures are nonspecific; newer approaches of assessing intermediate markers of outcome may augment sensitivity and possibly improve specificity. Epidemiologists use the term *interaction* in referring to interdependence of effect of multiple exposures (*12,13*). Approaches need to be designed for strengthening the links between toxicologic research and epidemiologic research to provide a common and biologically based framework for addressing interaction. The limitations of epidemiologic methods for addressing interaction also need further investigation, with emphasis on the consequences of the measurement error that inevitably affects studies of pollution.

This group was assembled with the goal of improving the epidemiologic approach to investigating the health effects of indoor air pollution and other complex mixtures. Achieving this goal will require multidisciplinary research using not only epidemiologic studies incorporating the new methods of exposure assessment but animal and clinical toxicology. Working group participants thus included an animal toxicologist (JL Mauderly), a human toxicologist (WF McDonnell), experts on exposure assessment (BP Leaderer, PJ Lioy, and JD Spengler), epidemiologists involved in air pollution research (DW Dockery, JM Samet, CM Shy, and FE Speizer), an expert on biomarkers (TC Wilcosky), and two epidemiologists with expertise in epidemiologic methods (S Greenland and NS Weiss). Similarly, broad expertise was provided by members of the Health Effects Institute Research Committee (C Harris, L Gordis, and M Utell). Additional observers included representatives of the sponsoring organizations (IH Billick, R Calderon, and RS Dyer). Working group participants were charged with considering the state of the art in their assigned areas, identifying barriers to research on complex mixtures, and proposing new research to reduce these barriers. Each member reviewed the status of his or her assigned area in a draft document that was circulated within the group. Subsequent discussion led to revision of these drafts, and the deliberations of the working group produced the overall recommendations of the participants.

The papers authored by the participants accompany this overview; they provide reviews and perspectives on various facets of the epidemiologic investigation of complex mixtures in inhaled air. Some of the authors provide useful research recommendations extending beyond those formally made by the whole group.

General epidemiologic concepts relevant to investigating complex mixtures are considered by Weiss (*14*). Weiss overviews circumstances under which observational studies are most informative and discusses threats to their validity, including selection bias and confounding. Investigation of the health effects of complex mixtures implies a research focus on the combined effects of the mixture's components. Greenland (*15*), in the Methodologic Issues document, reviews the general conceptual advances made in the epidemiologic literature in regard to distinguishing interaction among agents from the statistical, biological, and epidemiological perspectives. He illustrates

the problems of interaction assessment and points to evolving approaches for addressing these problems.

Two papers focus more specifically on research designs relevant to complex mixtures in inhaled air. Dockery (*16*) reviews the strengths and limitations of the conventional epidemiologic designs (cross-sectional surveys, cohort studies, and case–control studies) for investigating complex mixtures; he acknowledges that such research often is challenging because the agents of interest are ubiquitous and the anticipated levels of effect may be small. He suggests that no particular study design is optimal and calls for rigorous planning at the design stage. Outcomes other than adverse respiratory effects also may be associated with inhaled complex mixtures. Shy (*17*) addresses the investigation of neurotoxic, reproductive, and carcinogenic effects. He considers the data resources, such as registries, available for addressing these health outcomes and overviews research designs that might be used in investigating them.

In investigating the health effects of any environmental agent, exposure and outcome need to be accurately assessed if unbiased and informative results are to be obtained. Samet and Speizer (*18*) consider the approaches used to assess respiratory health effects; although standardized methods have been developed for measuring some of these health outcomes, nonspecificity limits interpretation of pollutant-outcome associations. Biological markers have been advanced as an approach for improving the sensitivity and specificity of outcome assessment. Wilcosky (*19*) reviews the biologic framework for applying biomarkers and specific markers that might be used for inhaled pollutants. As for the conventional outcome measures considered by Samet and Speizer (*18*), Wilcosky (*19*) points to lack of specificity as limiting current biomarkers of outcome.

Leaderer et al. (*20*) set out the concepts and methods of exposure assessment in relation to complex mixtures. They discuss the difficulties of measuring multiple contaminants for individual subjects in epidemiologic studies, in spite of the advances that have been made in personal monitoring techniques. Feasible approaches to assessing exposures to complex mixtures include selecting marker pollutants, employing passive personal samplers if available, collecting information by questionnaire on exposure to sources and time–activity patterns, and using nested designs that involve more intensive data collection for selected subjects.

In clinical studies, volunteer subjects are exposed to pollutants in the controlled circumstances of the laboratory. McDonnell (*21*) examines the potential uses of the clinical study approach for investigating complex mixtures. The clinical study design affords the opportunity of evaluating the effects of pollutants alone and in the form of a mixture. Animal studies also provide this same opportunity. Mauderly (*22*) comprehensively reviews toxicologic studies of complex mixtures. Surprisingly few studies have been directed at complex mixtures; barriers include the costs of such studies and the large numbers of experimental animals needed.

Several themes extend throughout these individual contributions. The authors emphasize the difficulties of approaching complex mixtures and the need for multidisciplinary investigative teams. None identified anticipated new techniques in methodology for exposure or outcome assessment that would rapidly advance our capabilities for investigating complex mixtures.

Working Group Recommendations

Introduction

The recommendations that follow are based on intensive discussions among the working group. Members were asked to consider investigative approaches to studying health effects of four complex mixtures of concern. The examples were intended to illustrate the range of challenges faced in testing hypotheses concerning the effects of complex mixtures. Subsequently, general recommendations were developed for new research methodology that would facilitate studies of complex mixtures.

General Considerations

For the purpose of the these proceedings, complex mixtures were considered to contain at least two pollutants potentially associated with the health effect of interest. While a mixture of only two pollutants might not be labeled as complex in other contexts, the methodologic issues raised in studying the joint effects of two pollutants merit this designation from the epidemiologist's perspective. Working group participants also acknowledged that some pollutants that might be treated as a single agent in an epidemiologic study are complex mixtures themselves, such as environmental tobacco smoke and diesel exhaust.

Working group members noted that many of the methodologic issues faced in conducting studies of complex mixtures in inhaled air were equally challenging in studying single pollutants and, in fact, were inherent throughout environmental epidemiology. The group suggested that concepts and methodology already available needed to be applied more generally in studying indoor air and other complex mixtures. Laxity in applying these concepts and methods potentially extends from the initial step of hypothesis formulation to the final step of data interpretation. In regard to complex mixtures, hypotheses need to be specified with a level of clarity that is often lacking. The effect measure of interest should be determined, and the anticipated pattern of joint effects should be described, both in terms of direction (synergism or antagonism) on the measurement scale selected and in terms of quantitative magnitude. Such specification of the hypothesis of interest is needed to guide study design and sample size estimation. If this level of specification is not met, the resulting vague hypotheses concerning interaction, synergism, or antagonism cannot be tested rigorously.

The conceptual framework for considering joint effects of two or more agents has been the subject of numerous publications in the epidemiologic literature. A consensus has been achieved for using departure from the additive scale as indicating interaction of public health significance (*12,13*). The pitfalls associated with using models that implicitly make assumptions concerning the underlying form of biologic interaction also have been well described. Working group members supported the development of biologically based analytic strategies, while recognizing that the needed understanding of pathogenetic mechanisms was lacking for many pollutants. The recommendation of the participants for interdisciplinary approaches to complex mixtures was prompted, in part, by the need for experimental data to support biologically driven data analysis.

Errors in estimating exposures and in assessing outcomes also limit epidemiologic studies of complex mixtures. The consequences of measurement error and strategies for adjusting effect measures for error have been considered extensively in recent publications. Techniques for staged sampling of exposures, moving from less intensive and costly to more valid and more costly, have been described (*1*). This emerging literature also needs specific extension to inhaled complex mixtures.

Specific Examples

To illustrate problems encountered in investigating complex mixtures, the working group considered approaches for four

scenarios of exposure to complex mixtures of current concern: the combined effect of exposure to environmental tobacco smoke and nitrogen dioxide on respiratory infection in infants, the combined effect of indoor radon and environmental tobacco smoke on lung cancer in never-smokers, the combined effect of ozone and acid aerosols on respiratory morbidity, and the consequences of exposure to multiple volatile organic compounds indoors.

The first example addressed by the group was the combined effect of nitrogen dioxide and environmental tobacco smoke (Table 1). Environmental tobacco smoke has been associated with increased lower respiratory infections during the first two years of life; nitrogen dioxide exposure is a suspect cause of respiratory infection as well, although the evidence presently is less consistent. Both agents may act by reducing the efficacy of host defenses against infectious organisms. Thus, because the two agents may share the same step in a causal pathway, the additive scale was considered biologically appropriate for assessing the combined effect.

The case–control design was eliminated because all children have multiple episodes of illness and selection of controls would therefore be problematic. The proposed cohort design incorporates staged determination with sampling for both outcome and exposure. The resulting data would make possible the estimation of the degree of error and permit correction for error in the data analysis. The proposed analytic strategy would test for departure from additivity and then employ modeling to describe the pattern of joint effect across the range of the two exposures.

The second example was the combined effect of radon and environmental tobacco smoke. Radon, an occupational carcinogen, is found in the air of all homes, reaching concentrations as high in some homes as that found in underground mines. Exposure to environmental tobacco smoke also is a cause of lung cancer in never-smokers. Investigation of the combined effects of the two exposures might be motivated by the large numbers of persons exposed to both agents in their homes. Biologic rationale for investigating the joint effect can be found in the altered dosimetry of radon progeny in the presence of environmental tobacco smoke and the potential actions of the two agents at different points in a multistage carcinogenic process.

A case–control study was considered the only feasible approach. Three distinct design objectives were identified that might guide study design: testing the hypothesis that the combined effect is the same as observed in underground miners who smoked, comparing the additive with the multiplicative models, and obtaining sufficient data to describe the combined effect with specified precision. Exposure assessment would be accomplished by placing radon detectors in living areas in the present residence and, where possible, in previous residences, and using a questionnaire to classify exposure to environmental tobacco smoke. The cases would include persons with histologically diagnosed lung cancer; to potentially improve specificity, histologic type of lung cancer would be determined.

The analysis potentially would be limited by measurement error and missing data for radon exposure and misclassification of environmental tobacco smoke exposure. Misclassification also would likely affect the diagnosis of lung cancer. In this example, sampling strategies that apply more in-depth measurement approaches for samples would not be possible. Thus, the analysis would explore the sensitivity of the findings to varying degrees of error.

In the third example, a substantial proportion of the population is exposed to both acid aerosols and photochemical oxidants. Historical data link secondary ambient pollutants (sulfates and acid aerosols, and photochemical oxidants) with health effects. The air pollution disasters earlier in the century, such as Donora in 1948 and London in 1952, showed that acid aerosols were associated with excess mortality. For photochemical pollution, the evidence from controlled human exposures and studies of lung function during outdoor activities in the so-called camp studies shows that oxidant pollution can have short-term adverse effects on lung function (*23*). Recently developed monitoring techniques for acid have shown that acid aerosols and oxidant pollution, as indexed by level of ozone, commonly occur together and that levels may be especially high during the summer. Thus, an assessment of the combined effects of these two mixtures is needed for public health protection.

Because these pollutants generally undergo long-range transport, the monitoring strategy for assessing exposure could be based regionally and study designs might be based on comparing health status across regions rather than attempting to establish exposure gradients within regions. For example, morbidity has been compared across regions using hospital and health practitioner contacts as outcome measures. Other outcomes to be considered in an epidemiologic investigation include emergency room visits for respiratory diagnoses or status of patients with pulmonary disease, as assessed by symptoms or lung function. For a study of acute effects, daily concentrations of ozone and acids in the study communities might be used.

The investigation of chronic effects requires the estimation of cumulative exposure; such exposure estimates may be problematic because of lack of historical data and uncertainty with regard to the biologically appropriate exposure window. Outcome measures in a study of chronic effects might be chronic symptoms and cross-sectional differences in lung function level. In adults, and to a lesser extent in children, confounding and modifying effects of other exposures would require consideration (e.g., cigarette smoking).

Finally, the need to study the effects of mixtures of volatile organic compounds is signaled by the occurrence of sick-building syndrome in the occupants of many buildings. The presence of many volatile organic compounds with irritant and neuropsychologic effects has led to the hypothesis that exposure to mixtures of volatile organic compounds may cause at least some outbreaks of sick-building syndrome. Barriers to planning

Table 1. Design features of a cohort study of the combined effect of environmental tobacco smoke (ETS) and nitrogen dioxide (NO_2) on respiratory infection in infants.

Study hypothesis	The incidence rate of respiratory infection in the jointly exposed subjects exceeds the value expected on the basis of additivity.
Outcome assessment	Prospective assessment of all subjects by periodic telephone follow-up. More detailed clinical evaluation for a sample of ill children.
Exposure assessment	Description of exposure sources for all children. More detailed assessment, possibly including monitoring of the homes for NO_2 and respirable particles, personal monitoring of the subjects for nicotine and NO_2, and use of biomarkers of ETS exposure.
Data analysis	Initial calculation of incidence rates and direct testing of departure from additivity. Subsequent modeling to describe the two-dimensional response surface of incidence rate versus measures of ETS and NO_2. Further modeling to take account of error in assessing outcome and exposure.

a study include the lack of standard methods for measuring both exposure and outcome. The components of the mixture potentially responsible are unknown, and the outcome measures of interest are both nonspecific and not readily validated.

Any study would need a multidisciplinary team equipped to measure exposure and to assess outcomes. Cross-sectional, cohort, and case–control designs might be used. Comparisons of affected and nonaffected individuals might incorporate biomarkers of exposure and of response; for example, nasal lavage might be used to assess irritation. Observational studies should be designed to take advantage of the natural experiments that occur when buildings are altered. In fact, intervention designs could be implemented feasibly and ethically. Thus, concentrations of volatile organic compounds could be reduced by increasing the rate of exchange of indoor with outdoor air.

Hybrid designs that combine observational approaches with controlled human exposures would permit further characterization of affected and nonaffected subjects in an epidemiologic investigation. Blinded challenges to suspect volatile organic compounds could be performed to validate questionnaire reports of symptoms and to assess the effects of individual components of the mixture.

General Recommendations

Based on the presentations of individual participants and discussions involving the entire group, the following recommendations were made: *a*) The investigation of the health effects of complex mixtures needs multidisciplinary approaches involving epidemiology, exposure assessment, and toxicology. Mechanisms for promoting regular and sustained interaction among researchers in epidemiology, exposure assessment, and toxicology need to be developed. *b*) Methods should be developed to link controlled human and animal exposure studies to complex mixtures. *c*) Methods should be developed to link controlled human exposure studies and epidemiologic studies. *d*) Statistical methods should be developed to combine human and animal toxicologic data with epidemiologic data to obtain overall estimates of risk. *e*) Methods should be developed to use activity pattern data to quantify cumulative exposures to complex mixtures. *f*) Many already available statistical and epidemiologic techniques relevant for studying complex mixtures have not been utilized appropriately. Demonstrations of these techniques in relation to complex mixtures are needed. The development of user-friendly software would facilitate their application. *g*) Approaches for estimating measurement error for both exposures and outcomes should be developed further. *h*) Meta-analysis may provide a more powerful assessment of complex mixtures than can be achieved by the findings of single studies. Data should be published in a form that will facilitate the conduct of meta-analysis.

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Assessment of Health Effects in Epidemiologic Studies of Air Pollution

Jonathan M. Samet[1*] and Frank E. Speizer[2]

[1]Pulmonary and Critical Care Division, Department of Medicine; and the New Mexico Tumor Registry, Cancer Center, University of New Mexico, Albuquerque, NM 87131; [2]The Channing Laboratory, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA 02115

As we increasingly recognize the complexity of the pollutants in indoor and outdoor microenvironments, a broad array of inhaled mixtures has assumed scientific, public health, and regulatory importance. Few adverse effects of environmental pollutants are specific, that is, uniquely associated with a single agent; the adverse effects that might be considered in an investigation of the consequences of exposure to an inhaled complex mixture are generally nonspecific. In the context of this paper, we will refer to binary mixtures as complex, though we realize that a more precise definition of complexity would restrict the term to mixtures of three or more constituents. Their causes potentially include not only pollutant exposures through the medium of inhaled air but other environmental agents, such as infectious organisms and radiation, and inherent characteristics of the exposed persons, such as atopy. We review the outcome measures that have been used in epidemiologic studies of the health effects of single pollutants and complex mixtures. Some of these outcome measures have been carefully standardized, whereas others need similar standardization and modification to improve sensitivity and specificity for investigating the health effects of air pollution. — Environ Health Perspect 101(Suppl 4): 149–154 (1993).

Key Words: Complex mixtures, health outcomes, lung function, respiratory symptoms, respiratory illness

Introduction

As we increasingly recognize the complexity of the pollutants in indoor and outdoor microenvironments, a broad array of inhaled mixtures has assumed scientific, public health, and regulatory importance. For many of these mixtures, the respiratory tract is the sole or predominant portal of entry and the principal locus of injury. Some agents, however, such as volatile organic compounds, may not damage the lungs but affect other target organs after uptake and distribution. Few adverse effects of environmental pollutants are specific, that is, uniquely associated with a single agent. The association of mesothelioma with asbestos exemplifies a highly specific link of a single agent to a single disease. By contrast, bronchogenic carcinoma has multiple causes, including cigarette smoking, occupational agents, and radiation, which may interact in a synergistic fashion.

The adverse effects that might be considered in an investigation of the consequences of exposure to an inhaled complex mixture are generally nonspecific (Table 1). Their causes potentially include not only pollutant exposures through the medium of inhaled air but other environmental agents, such as infectious organisms and radiation, and inherent characteristics of the exposed persons, such as atopy. It is unlikely that any new investigational techniques will soon become available that will provide more specific indicators of pollutant effect. Thus, investigative approaches should be developed with acknowledgment of the nonspecificity of the usual outcome measures.

Past investigations of outdoor and indoor air pollution incorporated the outcome measures listed in Table 1 (*1–3*). Descriptive studies of community morbidity and mortality used such routinely collected data as death counts or death rates, hospitalization or emergency room visit rates, and absenteeism rates. In some investigations, categories of respiratory diagnoses were selected as outcome measures. Community-based epidemiologic studies of both cross-sectional and longitudinal design typically included assessment of respiratory symptoms using standardized questionnaires and of lung function using spirometry or peak flow measurement. A few investigations added measurements of nonspecific airways responsiveness, using challenge with a pharmacologic agent or cold air.

The extensive experience gained with these approaches for outcome assessment clearly documents the lack of specificity of the measures used at both the community and individual levels. Cause-specific mortality rates, for example, vary with disease prevalence and severity, patterns of medical care usage, and death certificate coding. Respiratory symptoms have multiple determinants. For example, a mother's report that a child has a chronic cough might reflect the presence of underlying airways hyperresponsiveness, an effect of parental smoking, persistent symptoms after a recent

Table 1. Health outcome measures in studies of indoor air and other complex mixtures.

General	Respiratory	Neuropsychological
Overall mortality	Acute and chronic symptoms	Reduced performance on neurobehavioral testing
Morbidity index	Acute infections	Neuropsychological syndrome
	Chronic respiratory diseases	Neuropsychological disease
	Degree of nonspecific airways responsiveness	
	Reduced level of lung function	
	Increased rate of lung function decline	
	Decreased rate of lung function growth	
	Exacerbation of a chronic respiratory disease	
	Hospitalization for a chronic respiratory disease	
	Lung cancer	
	Death secondary tu a chronic respiratory disease	

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*Author to whom correspondence should be addressed.

lower respiratory tract infection, and bias because the mother has a cough. Cross-sectionally measured reduction of lung function might be produced by obesity, cigarette smoking, or past occupational exposures.

In this paper, we review the outcome measures used in epidemiologic studies of the health effects of single pollutants and complex mixtures. In the context of this paper, we will refer to binary mixtures as complex, though we realize that a more precise definition of complexity would restrict the term to mixtures of three or more constituents. The emerging research on use of biomarkers is discussed elsewhere in these proceedings.

Conventional Outcome Measures

Introduction

This section reviews the outcome measures that might be used in assessing the health effects of complex mixtures of current concern. For the principal outcome measures, we briefly consider pathophysiologic mechanisms, accuracy, and potential sources of bias.

Overall and Cause-Specific Mortality

From the 1930s through the 1950s, episodes of excess mortality at times of extremely high outdoor air pollution provided dramatic evidence that air pollution can cause excess deaths (*1*). While overall mortality rates increased during these episodes, the excess deaths tended to be placed into cause-of-death categories for cardiovascular and respiratory diseases. Although such dramatic air pollution episodes are now infrequent in most developed countries, research continues on the effects of outdoor pollutants on overall and cause-specific mortality.

In investigations of air pollution and mortality, routinely collected vital statistics data for specific geographic areas are used as the health outcome measures, while air pollution exposure of the areas' residents is estimated from outdoor monitoring sites assumed to be representative for the populations. Association between mortality rates and pollutant levels is generally assessed using regression methods. For example, Schwartz and Dockery (*4*) examined variation in daily mortality rates in Steubenville, Ohio, in relation to daily levels of total suspended particles and sulfur dioxide. After controlling for season and temperature, the level of particles was significantly associated with the daily mortality counts in a regression model.

All-cause mortality is not subject to error from assignment of cause of death. However, pathophysiologic considerations typically lead to research hypotheses focused on cause-specific mortality, such as ischemic heart disease or chronic obstructive lung disease. Exposure to pollutants might cause death in persons with underlying chronic obstructive lung disease by further incapacitating those with little functional reserve; for such patients, pollutant exposure, by diminishing the efficacy of host defenses, also might increase the incidence or severity of respiratory tract infections. Persons with ischemic heart disease are vulnerable to pollutants, such as carbon monoxide, that impair oxygen delivery to tissues (*5*).

Misclassification of the underlying cause of death by death certificate designation has been well documented (*6*); accuracy of cause-specific mortality data is influenced potentially by the extent of the population's contact with medical care, the diagnostic acumen of clinicians in the study areas, the accuracy of information on the death certificate, and the rate of error in coding the death certificate to a particular cause of death. Because of recent concern about increasing asthma mortality, the validity of death certificate designation of deaths as due to asthma has been examined in several countries (*7*). However, while the validity of death certificate data on respiratory cancer has been specifically evaluated (*8*), comprehensive assessments of the quality of death certificate data for other major chronic respiratory diseases and for acute respiratory infections have not been performed. Misclassification of the underlying cause of death in vital statistics data would be anticipated to occur randomly in relation to the level of pollutant exposure. Such random misclassification attenuates exposure–response relations and reduces the statistical power of an investigation to detect an effect of pollution.

All-cause and cause-specific mortality rates are also nonspecific outcome measures. Mortality rates vary with the background distribution of risk factors determining the incidence of disease and with the survival rate of those who have developed disease. Thus, assessments of the effects of air pollutants on mortality can be sharpened if these other factors can be considered in data analysis.

Indices of Morbidity

Epidemiologic studies of the health effects of air pollution have incorporated diverse indices of general morbidity, including absenteeism from school and work; days of restricted activity spent at home; and rates of utilization of outpatient medical facilities, of visits to emergency rooms, and of hospitalization (*1,9*). For example, in an investigation in Steubenville, Ohio, the relation between the numbers of visits made to the principal hospital's emergency room and daily air pollution levels was assessed (*10*). Like mortality rates, the general morbidity indices are nonspecific and subject to misclassification.

Respiratory Infections

Diverse microorganisms can cause respiratory tract infections, including mycoplasma, viruses, bacteria, and fungi (*11*). The spectrum of infecting organisms and of clinical manifestations vary from infants through the elderly (*12*). Research on air pollution and respiratory infection has focused largely on infants and younger children. Children, particularly infants, have been considered susceptible to inhaled pollutants because their lungs are maturing and rates of respiratory infection in this age group are the highest of any (*12,13*).

The occurrence of respiratory infections can be monitored using subject reports of symptoms or illnesses or by using inpatient and outpatient records of clinical facilities. The usual clinical respiratory illness syndromes include upper respiratory tract infections ("colds"), otitis media, and lower respiratory illnesses; the latter category includes croup, tracheobronchitis, bronchiolitis, and pneumonia (*11*). Standardized and uniformly accepted clinical definitions have not been developed for these illnesses, and health care practitioners apparently develop their own operational criteria. In fact, a single unimpeachable gold standard for establishing the presence of a respiratory infection is unavailable; a clinical diagnosis and a positive culture for a pathogenetic organism represent the most valid basis for documenting infection.

In some studies of children and of adults, illness histories have been obtained retrospectively by questionnaire. While such retrospective information can be collected readily, bias is likely, with subjects symptomatic or ill at the time of data collection more likely to report past illnesses (*14*). Prospective surveillance of illness avoids the potential problem of recall bias but requires a more elaborate system for ascertaining the occurrence of illness. Surveillance approaches using calendar diaries for recording of symptoms have been applied successfully in community-based studies on respiratory illnesses (*15–17*) but have been used in only a few studies of inhaled pollutants. For example, in a cohort study in progress in Albuquerque, New Mexico, on nitrogen

dioxide and respiratory infections, infants are enrolled shortly after birth and the occurrence of illness is ascertained by completion of a daily symptom diary and telephone contact every 2 weeks (*18*). To assess the validity of this system for illness ascertainment, a sample of ill children is evaluated by a nurse practitioner according to a standardized protocol.

The occurrence of illness also can be documented by using diagnoses made by clinicians at the time of outpatient visits or hospital discharge diagnoses. However, illness rates based on contact with health care providers have potential determinants other than incidence, including patterns of access to health care, the severity of the illnesses, and diagnostic practices of the clinicians. More severe illnesses are likely to prompt contact with a health care provider, and thus illness rates based on clinical diagnoses are lower than those obtained by community-based surveillance. Therefore, in the United States, community-based surveillance studies show that children have about two lower respiratory tract illnesses during the first year of life (*12*); by contrast, from 20 to 30% of children receive a physician's diagnosis as having a lower respiratory tract illness during this same age range (*19,20*). Nevertheless, studies of both indoor and outdoor air pollution have used indices of respiratory infection derived from clinical encounters (*11,21*). However, confounding may be introduced into studies using such clinical indices, because both pollution exposure and patterns of health care utilization may be associated with demographic and socioeconomic factors that also determine illness rates (*11*).

Respiratory Symptoms

Standardized respiratory symptom questionnaires, initially developed during the 1950s, are widely used in epidemiologic research for assessing the occurrence of the cardinal respiratory symptoms: cough, sputum production, wheezing, and dyspnea (*22*). The presently used questionnaires have evolved from the questionnaire originally developed by the British Medical Research Council; like the first questionnaire, the presently available instruments emphasize chronic symptoms and are insensitive for detecting acute symptom responses. Limited data have been published on the validity and reliability of individual questions (*22,23*). In the United States, an American Thoracic Society committee initially adopted the Medical Research Council questionnaire for adults in 1969. In 1978, the American Thoracic Society's Epidemiology Standardization Project published a revised questionnaire for adults and a new questionnaire for children (*24*). Proper use of these questionnaires reduces the potential for interviewer bias and assures comparability with data from other populations collected with the same techniques.

For pollutants with quickly changing concentrations and mechanisms of action associated with acute symptom responses, short-term longitudinal studies, often called "panel studies," may be carried out to examine the relation between pollutant levels and symptom occurrence on the time scale of a day or less. Typically, symptom status is tracked by asking subjects to complete a diary that covers such items as the occurrence of cough, sputum production, wheezing, sore throat, hoarseness, and fever (*25*). In studies involving controlled laboratory exposures, asthmatics are more susceptible to a number of inhaled pollutants than nonasthmatics (*26*). The diary approach has been applied to investigate the health effects of pollutant exposure on asthmatics and also on patients with chronic obstructive pulmonary disease in the community setting (*27,28*). In studies of asthmatics, medication pattern and use of health care services may be tracked in addition to symptom status. Standardized instruments for diary studies have not been published.

Pulmonary Function

Toxicologic considerations suggest that complex mixtures of current concern might have either irreversible or reversible effects on lung function. Permanent loss of function could reflect the development of emphysema, airways fibrosis, and interstitial fibrosis. Acute, reversible loss of function could be secondary to airways inflammation, bronchoconstriction, or other mechanisms. In a cross-sectional study, an irreversible loss of function would be reflected as a lower level of function in comparison with an unexposed "normal" population. In a longitudinal study, irreversible loss of function during childhood would be manifest as a reduced rate of lung growth, whereas during adulthood, accelerated decline of function would be expected. Acute adverse effects of pollutant exposure on lung function can be detected by longitudinal monitoring of function with comparison of preexposure to postexposure measurements.

Spirometry, involving the timed collection of exhaled air during the forced vital capacity maneuver, has been the most widely used technique for measuring lung function in epidemiologic studies of air pollution. Spirometers are available for field use and are inexpensive, portable, and durable. Standardization of spirometry has long been advocated and recommendations are available from the American Thoracic Society (*24,29*) and a Working Party of the European Coal and Steel Community (*30*). These recommendations cover specifications for spirometers, testing protocols, and test interpretation. Data collected following these recommendations and using proper equipment have small within-subject variability (*23,31*). In a few studies, other types of measurements have been made, including the single breath nitrogen test (*31*). However, these tests, as well as other types of testing used in clinical pulmonary function laboratories, have greater variability than spirometric measures of lung function, and the equipment is more complex and expensive than a simple spirometer.

Spirometry provides measurements of the forced vital capacity (FVC), the total amount of exhaled air, as well as the volume of air exhaled in the first second (FVC_1) or at other time points. A spirometer integrated with a microprocessor can measure flow rates at various lung volumes. These spirometric measures are sensitive to processes impairing ventilatory function of the lung, but injury cannot be inferred at specific anatomic loci because of particular patterns of abnormality of spirometric parameters (*32*). However, abnormalities of flow rates at lower lung volumes are associated with adverse effects on the small airways of the lung (*31*).

Although spirometry has proven effective for community-based studies, it cannot be used readily in large numbers of subjects to track function on a day-to-day basis. In many studies investigating the relation between short-term variation in lung function and pollution exposure, peak expiratory flow rate (PEFR) has been measured using portable and inexpensive instruments that can be used by subjects themselves. Peak expiratory flow rate measurement takes only a few minutes and can be performed at multiple times throughout the day; measurements can be made before and after episodes of exposure. Accurate measurement of PEFR requires calibration of the peak flow meters and standardized protocols for subject training and data collection (*33,34*).

Nonspecific Airways Responsiveness

Nonspecific airways responsiveness refers to the extent of bronchoconstriction evoked by a nonantigenic stimulus (*35*). The pharmacologic agents most widely applied to assess nonspecific airways responsiveness are methacholine and histamine; other

alternatives, including hypoactive and hyperactive aerosols, exercise, and hyperventilation with cold air, also have been used. Asthmatics, by definition, have airways hyperresponsiveness. In populations, the distribution of nonspecific airways responsiveness appears to be unimodal, with skewing towards hyperresponsiveness (*35*). In controlled exposure studies of asthmatics and healthy nonasthmatic subjects, nonspecific airways responsiveness often has been one of the monitored outcome measures (*36*). In the community setting, assessment of nonspecific airways responsiveness might provide a sensitive indicator of the effect of exposure to a complex mixture. The protocols for measuring nonspecific airways responsiveness are time consuming, however, and the possibility of adverse consequences of testing necessitates the presence of a physician. Thus, nonspecific airways responsiveness has not been used yet in large-scale epidemiologic research on the health effects of air pollutants.

Neuropsychological Responses

Exposure to mixtures of volatile organic compounds in indoor air can be postulated to have neurobehavioral consequences. In fact, volatile organic compounds have been postulated to be etiologic factors in the nonspecific sick-building syndrome. A variety of tests of neurobehavioral outcomes are available (*37*), and such tests have been applied in epidemiologic investigations (*38,39*). However, standardized approaches for assessing neurobehavioral outcomes have not been developed (*37*). Molhave (*40*) recently summarized symptomatology and commonly used tests for behavioral effects caused by volatile organic compounds. The tests are designed to assess sensory, cognitive, affective, and motor disorders. Although most of the tests have been used in the neurobehavioral field for a number of years, their applicability to field studies of indoor air health effects has been demonstrated only recently and standardization in such studies has not been achieved.

"Objective measures" of health impacts of indoor exposures to volatile organic compounds should relate to the patterns of reaction that can be anticipated (*41*). Acutely perceived reactions include odor, irritation of the skin, and the sensation of reduced air quality or the need for more ventilation. Subacute reactions manifest the beginning of the development of an inflammatory reaction with dilation of peripheral vessels: stinging, itching, or pain in the skin, and changes in skin temperature. Finally, subacute or chronic effects relate to discomfort, and complaints of headache, drowsiness, and changes in eye and nose liquids, odor threshold, performance, and mood. These latter signs can be assessed objectively with a variety of diagnostic techniques. For example, eye dryness can be assessed by the time required to clear a fluorescein dye placed in the eye (*42*). Change in pulmonary function over the course of the day, a commonly used measure in assessing occupationally related respiratory diseases, also has been used to assess the more toxic irritations seen in indoor air exposures.

Specific Examples

Environmental Tobacco Smoke and Nitrogen Dioxide

Environmental tobacco smoke and nitrogen dioxide (NO_2) are highly prevalent indoor exposures; slightly less than half of U.S. homes have gas cooking ranges and ovens, the principal sources of NO_2 indoors, and about 40% of U.S. homes have at least one adult smoker (*2*). Environmental tobacco smoke itself is a complex mixture, representing the combination of sidestream smoke with exhaled mainstream smoke (*21,43*). Its components include irritants, inflammatory agents, and carcinogens. Exposure to environmental tobacco smoke has been associated with increased lower respiratory tract infections in young children, increased respiratory symptoms and reduced lung growth in children, and lung cancer in adults who have never smoked (*21,43*). Nitrogen dioxide, an oxidant gas, also might increase rates of respiratory infection through adverse effects on respiratory defense mechanisms and, by causing airways inflammation, produce respiratory symptoms and reduce lung function (*44*). Thus, exposure to the combination of environmental tobacco smoke and NO_2 can be hypothesized to increase rates of respiratory infection and respiratory symptoms and to reduce lung function. Additive effects might be postulated because the effects might be mediated through similar pathways for the two agents.

Respiratory infections are extremely common during childhood; active surveillance methods show that children have two or more episodes of lower respiratory tract infection during the first year of life and about twice as many upper respiratory tract infections (*11*). By contrast, only about 20% of children visit a physician for a lower respiratory tract infection during the first year of life, and hospitalization for such an illness is rare. Selection of an outcome measure for a study of environmental tobacco smoke and NO_2 needs to be made in light of the underlying hypothesis. If joint exposure is postulated to increase severity of infections, then physician visit or hospitalization should be selected. Alternatively, if joint exposure is postulated to increase incidence, then an active surveillance method for illness is appropriate. Recall of illnesses by a parent may not be adequate for describing incidence but may suffice for characterizing more severe illness occurrence.

To address the joint effect of exposure on respiratory symptoms and lung function in older children, conventional methods would include spirometry and completion of a standardized symptom and illness questionnaire by a parent. Symptoms and lung function level have multiple determinants, and the effects of the pollutant mixture cannot be assessed without controlling for these other factors, such as age, sex, and presence of asthma. The specificity of analysis might be improved by a priori identification of those symptoms and lung function measures of particular interest. Thus, for environmental tobacco smoke and NO_2, the symptom of chronic cough may be of greatest interest because of the temporal pattern of chronic and sustained exposure to the two agents; spirometric flows at low lung volumes might be selected for investigation because the dose of NO_2 may be greatest for the small airways, as suggested by the results of dosimetric analyses (*45*).

Environmental Tobacco Smoke and Radon

Environmental tobacco smoke is causally associated with lung cancer in never-smokers; radon exposure in underground mines causes lung cancer in both smokers and never-smokers, and active smoking and radon exposure interact in a synergistic manner (*23,46*). Thus, synergism between environmental tobacco smoke and radon exposure may be postulated. Environmental tobacco smoke potentially affects the dosimetry of radon progeny within the respiratory tract; tobacco smoking is a strong source of aerosol, and the presence of smoking may reduce the unattached fraction of radon progeny, thereby retarding removal through plateout and reducing the dose of alpha energy delivered to target cells (*47*). Points of interaction between the two agents that might affect lung cancer risk include altered exposure to radon progeny in the presence of environmental tobacco smoke, the effect on lung dosimetry of the inhaled progeny, and joint effects in the multistage process of carcinogenesis.

The outcome of concern, lung cancer, comprises a heterogeneous group of malignancies from the histologic perspective; four major types account for the majority of cases: squamous carcinoma (30%), adenocarcinoma (25%), small-cell carcinoma (20%), and large-cell carcinoma (15%) (*48,49*). In never-smokers, adenocarcinoma is the most common histologic type, but all types may occur. Radon-exposed underground miners have an increased frequency of small-cell, but this proportion declines as the interval since the start of exposure lengthens (*46*). Newer techniques of cellular and molecular biology have not provided more sensitive techniques for linking specific exposures to specific types of lung cancer yet.

A case–control study could be designed to address interaction between environmental tobacco smoke and radon. Interpretation might be clouded, however, by the present impossibility of assuring that some degree of disease misclassification is not present.

Acid Aerosols and Ozone Outdoors

Both acute and chronic effects of mixtures of acid aerosols and ozone can be anticipated from the known chemistry of these agents. Clinical chamber exposure studies suggest that physiologic changes suggestive of inflammation of the airway can occur after acute exposure to ozone (*50*). Animal studies of aerosols of H_2SO_4 suggest changes in clearance of particles, which increase as exposure increases (*51*). Most of the efforts to assess the combined exposure to ozone and H_2SO_4 have not shown synergistic effects; however, in some studies, a combined effect of the two agents is apparent (*52*).

To address these two agents in acute studies requires the use of panels of subjects exposed over time, with repeated studies of conventional outcomes, including symptoms and lung function, in conjunction with monitoring of exposure. In particular, potentially sensitive subgroups of subjects, as well as normal persons, need to be evaluated. Ideally, some measure of average minute ventilation during periods of exposure would be useful to assess delivered dose more quantitatively. For chronic exposure, prevalence of symptoms and level of pulmonary function, particularly in well-characterized groups of children, can be used as a measure of cumulative lifetime effects and compared among exposed and unexposed groups.

Conclusions

Epidemiologic studies of the health effects of air pollution have used an array of nonspecific outcome measures. The effects postulated to be associated with pollution exposure also are caused by other factors such as cigarette smoking, occupation, and subject characteristics. In interpreting effects attributed to pollutant exposure, careful control of confounding and assessment of joint effects is warranted by the nonspecificity of outcome measures. Moreover, the effects of pollution may vary with the background of other exposures.

Some of the outcome measures used in epidemiologic studies of air pollution, such as respiratory symptom questionnaires and spirometry, have been carefully standardized, and extensive data are available from pollutant-exposed and unexposed populations. Other outcome measures need similar standardization and modification to improve sensitivity and specificity for investigating the health effects of air pollution.

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Toxicological Approaches to Complex Mixtures

Joe L. Mauderly

Inhalation Toxicology Research Institute, Lovelace Biomedical and Environmental Research Institute, P.O. Box 5890, Albuquerque, NM 87185

This paper reviews the role of toxicological studies in understanding the health effects of environmental exposures to mixtures. The approach taken is to review mixtures that have received the greatest emphasis from toxicology; major mixtures research programs; the toxicologist's view of mixtures and approaches to their study; and the complementary roles of toxicological, clinical, and epidemiological studies. Studies of tobacco smoke, engine exhaust, combustion products, and air pollutants comprise most of the past research on mixtures. Because of their great experimental control over subjects, exposures, and endpoints, toxicologists tend to consider a wider range of toxic interactions among mixture components and sequential exposures than is practical for human studies. The three fundamental experimental approaches used by toxicologists are integrative (studying the mixture as a whole), dissective (dissecting a mixture to determine causative constituents), and synthetic (studying interactions between agents in simple combinations). Toxicology provides information on potential hazards, mechanisms by which mixture constituents interact to cause effects, and exposure dose–effect relationships; but extrapolation from laboratory data to quantitative human health risks is problematic. Toxicological, clinical, and epidemiological approaches are complementary but are seldom coordinated. Fostering synergistic interactions among the disciplines in studying the risks from mixtures could be advantageous. — Environ Health Perspect 101(Suppl 4):155–165 (1993).

Key Words: Toxicology, chemicals, mixtures, bioassay, interactions, synergism, pollutants, animals, tobacco smoke, exhaust, radon

Introduction

There is a considerable body of literature on the toxicology of mixtures; however, our understanding of the significance of exposures to compounds in mixtures, in contrast to single exposures, is sparse. To date, toxicology has remained primarily the science of individual poisons, even though people are rarely, if ever, affected by single agents in isolation from other agents that might influence risk. Toxicological studies of mixtures inherently are difficult, and the science of studying mixtures is not refined or codified highly. Understanding risks from simultaneous or sequential exposures to multiple agents, particularly at low levels of exposure, is the single greatest challenge to toxicologists today. This paper is perspective in nature and constitutes a summary review of the role of toxicology in understanding the health effects of human exposures to mixtures of toxic chemical and physical agents. As used here, the term toxicology refers to laboratory investigations that do not involve human subjects but involve *in vitro* and *in vivo* experimental systems encompassing molecules, cells, tissues, and laboratory animals. Although not emphasized in this review, analytical chemistry is often incorporated into toxicological studies of mixtures.

This review summarizes the background and current status of toxicological studies of mixtures but is not intended to be exhaustive. The purpose is to present the scope of efforts in mixture toxicology, the manner in which toxicologists approach the issue, and the role of toxicological studies in relation to epidemiological and clinical studies. A more detailed treatise on issues surrounding the study of mixtures was developed by the National Research Council (NRC) Committee on Methods for the *In Vivo* Toxicity Testing of Complex Mixtures and was published as the text *Complex Mixtures* (*1*). The reader is encouraged to refer to that text; the present review does not attempt to reiterate or abstract its contents but presents some additional perspectives.

The approach taken in this review is first to summarize past, major efforts in the toxicological study of mixtures, because this background provides several examples that are used later to illustrate different issues and research approaches. A latter portion of this review describes in general conceptual terms how toxicologists view and approach the problem of mixtures. In doing so, this section also describes the role of toxicology in our understanding of the health risks from mixtures and contrasts the strengths of toxicology with those of epidemiology and clinical studies. The last section briefly discusses approaches to toxicological studies of specific mixture-related problems.

Past Toxicological Research on Mixtures

One can gain a view of how toxicologists envision the problem of mixtures and how they have approached that problem experimentally by examining the major research efforts of the past. The following summary does not recount all past work but indicates the principal areas of focus on mixtures to date. Studies of specific mixtures representing major thrusts are described, followed by mention of integrated programs that have been funded to address the issue of mixtures more broadly.

Research on Specific Mixtures

Tobacco Smoke. Tobacco (almost entirely cigarette) smoke is a complex mixture long studied by toxicologists and remains one of the most important mixtures affecting human health today. Early studies were summarized in 1967 by Wynder and Hoffmann (*2*), and research through the mid-1980s was summarized in detail in a 1986 International Agency for Research on Cancer (IARC) monograph (*3*). Research on cigarette smoke continues at present, with recent efforts directed toward comparisons of effects of conventional and alternate types of cigarettes (*4*), comparisons of alternate methods for animal exposures (*5*), and molecular mechanisms of smoke-induced mutagenesis (*6*). The full range of *in vitro* and *in vivo* toxicological assays has been used to study tobacco smoke, including aerosol characterization, analytical chemistry, dosimetry, metabolism, mutagenesis, teratogenesis, lung defenses, physiology, and long-term and acute inhalation bioassays. Nearly all past research on tobacco smoke was done using mainstream smoke; relatively little information has been published on sidestream or environmental tobacco smoke (ETS).

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It is instructional to consider the history of cigarette smoke toxicology in view of the present thrust to examine the health effects of mixtures, particularly ETS. That history will not be recounted in detail; suffice it to state that despite the hundreds of studies over five decades and the clear epidemiological signal for a range of adverse effects, the specific components of smoke causing the effects, the precise mechanism of the effects, and the reason for marked individual differences in susceptibility to the effects remain only partially understood. Toxicology has shown that cigarette smoke is cytotoxic, mutagenic, and carcinogenic, and that chronic exposure can cause chronic respiratory disease and impairment of lung defenses in animals. However, most of this information also is evident from observations of humans. The fact that toxicology has refined our understanding of the toxicity of tobacco smoke but has not yet resolved many key issues suggests caution against undue optimism for the study of ETS and other mixtures.

Toxicology has been hampered particularly by the lack of a good animal model for the pulmonary carcinogenicity of cigarette smoke as it is observed in man. Repeated heavy inhalation exposures of substantial numbers of subjects for the majority of a life span have been attempted only with rodents. It has been speculated that the failure of these studies to clearly demonstrate lung cancer induction resulted from the stressful, labor intensive, nose-only exposure methods used for exposures, which coupled with the avoidance responses of rodents exposed "puff-by-puff" have resulted in inadequate dosing. Indeed, compared to human heavy smokers, this is a somewhat unique example of underdosing in animal toxicological studies. In addition, studies to date have lacked statistical power by including small numbers of subjects living sufficiently long for cancer to be expressed. There is a current effort to use whole-body exposures in an attempt to overcome these difficulties (*5*), but it remains to be seen if the approach will succeed. Whole-body exposures also are being used in newly initiated studies of ETS sponsored by the Center for Indoor Air Research. Although *in vitro* and short-term animal studies suggest that nitrosoamino compounds may play a substantial role in the carcinogenicity of cigarette smoke, the relative contributions of the many potential carcinogens in smoke to pulmonary carcinogenicity of inhaled smoke remain unknown.

Combustion Products. There is a body of literature, summarized in the NRC book (*1*), on the acute effects of the products of flame combustion or heat pyrolysis of wood, plastics, and other materials. Studies, as exemplified by those of Alarie and Anderson (*7*), typically have used single, short exposures and have characterized effects by mortality, respiratory responses, and respiratory tract histopathology. These studies have been empirical and generally have treated each exposure atmosphere as a whole. Little information has been generated on chronic or repeated exposures, the mechanisms of response, or the components of the exposure atmospheres responsible for the effects.

Engine Exhaust. There is a body of literature (although perhaps surprisingly small) on the toxicology of gasoline engine exhaust. The earliest major studies were those begun in the early 1960s by the U.S. Public Health Service in Cincinnati starting with short- and long-term exposures of rodents (*8*) and followed in 1965 by the initiation of long-term exposures of dogs (*9*). The dog studies also included groups exposed to raw, irradiated exhaust and mixtures of sulfur dioxide and sulfuric acid. Mild changes in respiratory function were observed in physiological parameters during the 5-year exposures (*9*), and both physiological and histopathological changes indicative of chronic, terminal airway and centriacinar disease were observed after the exposures (*10,11*). This study is unique in that it is the only substantive long-term study of dogs exposed to inhaled nonradioactive toxicants, and it demonstrated the persistence of exhaust-induced lesions at 3 years after exposures ended.

More recently, studies at two laboratories of rats and hamsters exposed chronically to diesel engine exhaust also included groups exposed to gasoline engine exhaust. Neither laboratory published the results from gasoline exhaust exposures in detail. Results from a study of mice, hamsters, and rats exposed for 2 years to gasoline exhaust at the Fraunhofer Institute were presented in part in 1985 at an annual meeting of the American Association for Aerosol Research. Results from a study of rats and hamsters exposed for 2 years to gasoline exhaust at the Battelle–Geneva laboratory were described partially in two publications (*12,13*). The detailed results were submitted as a final report to the sponsors (Committee of Common Market Automobile Constructors [CCMC], a consortium of European automobile manufacturers), but the report has not been made public. Both the Fraunhofer and Battelle studies produced subtle physiological changes but little evidence of chronic lung disease and no carcinogenesis resulting from gasoline engine exhaust exposure. In another study at the Fraunhofer Institute (*14*), rats were treated with dipentylnitrosamine to induce a high background incidence of respiratory tract tumors and also were exposed chronically to gasoline exhaust. Exhaust exposure reduced the nitrosamine-induced lung tumor incidence but increased the incidence of nasal tumors.

Diesel exhaust is undoubtedly the complex mixture receiving the most intense toxicological study, surpassing even tobacco smoke in the number of funding sources and research institutions committing major efforts to the evaluation of potential health effects of occupational and environmental exposures (*15–17*). With major efforts beginning in the late 1970s, these studies continue at present in the United States and abroad. The first major commitment was that of the U.S. Environmental Protection Agency (EPA), which launched large-scale, multifaceted studies during the late 1970s based on the finding that diesel soot-associated organic compounds were mutagenic and the prediction that the use of diesel engines in the United States automobile fleet would increase to bolster fuel economy. This was followed by the initiation of research by automobile manufacturers (General Motors, Ford, Volkswagen, and a consortium of European automobile manufacturers) and government agencies (Department of Energy [DOE] and National Institute of Occupational Safty and Health [NIOSH] in the United States, the Japan Automobile Research Institute, and the German environmental agency), numerous university laboratories, and the Health Effects Institute. This research is probably the best example of an approach combining dose–effect studies of a "real life" complex mixture with dissecting studies to determine the most hazardous components of the mixture.

The studies of diesel exhaust have encompassed nearly the entire range of toxicological, epidemiological, and risk assessment approaches (*17*). The composition of exhaust from different engines and under different operating conditions has been analyzed extensively. Animals have been exposed acutely and chronically to whole exhaust and to filtered exhaust gases and vapors. *In vitro* mutagenicity and cytotoxicity assays have been used in the biodirected analytical chemistry of soot-associated organic compounds. Animals and cells have been exposed to individual compounds found in exhaust and to representative compounds attached to model carrier particles simulating diesel soot. Health effects have been evaluated at the molecular, cellular, tissue, organ, animal, and human levels. The effects of engine type, operating conditions,

and exhaust clean-up devices on the toxicity of emissions have been studied. Risk factors derived from animal studies and epidemiology have been compared.

It is useful to reflect on the extent of our understanding of potential health effects that has developed from over a decade of research on diesel exhaust. We know that inhaled diesel exhaust is toxic and has a potential for causing cancer; however, this was known in general terms before recent efforts began. We now know with great certainty that chronic inhalation exposure of rats to high concentrations causes lung cancer in a dose-related manner and that this effect is associated with the carbonaceous core of the soot fraction of exhaust. We now know with some confidence the approximate upper bound of risk for lung cancer among exposed humans; our confidence is bolstered because we have substantive epidemiological information to complement the large base of toxicological data. We do not yet know, however, if the dose–response data from rats can be used with much accuracy to estimate unit risks for humans. This is because the mechanism of cancer induction in rats remains uncertain, and it is possible that the mechanism might not be relevant to human risk. We know that soot exposure increases DNA adduct levels in the lung cells of animals, but we do not know if this is related to mutagenic, soot-associated compounds. There is good evidence that nitroaromatic compounds in soot extract are responsible for much of the bacterial mutagenicity of the extract, but we do not know the relative contributions of these and other carcinogenic compounds to the pulmonary carcinogenicity observed in animals and thought to occur in man.

It is worth noting that the diesel exhaust research effort also has included the only major studies of the toxicology of combined exposures to mineral dusts and exhaust, perhaps the most complex mixtures studied to date. The NIOSH study (*18*) included rats exposed for 24 months to atmospheres containing diesel exhaust at 2 mg soot/m^3, coal dust at the same concentration, and a combination of diesel exhaust and coal dust at 1 mg/m^3 each. Among the several health-effects endpoints evaluated, the effects of diesel exhaust and coal dust were similar, with coal dust being slightly less toxic. No synergistic interactions between the exposure materials were noted. The series of studies of diesel exhaust at the Inhalation Toxicology Research Institute included one (*19*) in which rats were exposed for their life span to diesel exhaust at 3.5 mg soot/m^3, to either raw or retorted oil shale dust at 5 mg/m^3, or to combinations of diesel exhaust and the shale dusts at total particle concentrations of 8.5 mg/m^3. Although the results have not been reported fully, the effects of diesel exhaust and shale dusts generally were less than additive for delay of particle clearance; additive for respiratory function impairment; and greater than additive for lung collagen, airway fluid indicators of inflammation, and lung tumors.

Air Pollution. Outdoor air pollutants present the classic problem of understanding the toxicology of mixtures of agents that are physically and chemically complex. Most research on air pollutants has focused on individual chemicals and compounds rather than on mixtures, although the engine exhaust studies described above certainly address a complex component of outdoor air pollution. There has been little research on the toxicology of actual ambient air pollutant mixtures largely because of the complexity and variability of outdoor air contaminants. One of the few examples is Bils' 1966 study (*20*) in which mice of different ages were exposed to ambient Los Angeles air during pollution alerts.

One step removed from studying actual ambient air pollution is the study of synthetic pollutant mixtures generated by reacting selected compounds. An early example of this approach is Bils' 1967 study (*21*) in which he exposed mice to synthetic smog produced by reacting propylene, nitric oxide, and carbon monoxide under ultraviolet radiation. A more recent example is the effort at the University of California, Irvine Air Pollution Health Effects Laboratory, involving exposures of animals at rest and during exercise to mixtures generated by reacting various combinations of ozone, sulfur dioxide, nitrogen dioxide, sulfuric acid, ammonium bisulfate, and ferric oxide (*22,23*).

The third approach used to study air pollution mixtures is the study of the effects of two representative air pollutant constituents administered alone and in combination. The most extensive database is comprised of studies addressing interactions between oxidant gases and acid aerosols. As reviewed in 1990 by Last (*24*), synergy was reported between the oxidants ozone and nitrogen dioxide and between acid aerosols in collagen synthesis and inflammation in rats exposed to the agents singly or in combination. Kleinman et al. (*22*) exposed rats to combinations of ozone and/or nitrogen dioxide with acid sulfates. Those investigators observed synergy between ozone and sulfuric acid in the development of lung lesions. Schlesinger et al. (*25,26*) studied the effects of nitrogen dioxide and sulfuric acid aerosol, alone and in combination, on particle clearance mechanisms. The effects of mixtures differed from those of the single agents, although a consistent pattern of synergy was not observed.

Major Programs Funded to Study Mixtures

DOE Complex Mixtures Program. During the mid-1970s, the Energy Research and Development Administration (ERDA), the predecessor of DOE, initiated a major research effort on the toxicology of complex chemical mixtures. This program was predicated on the emergence of alternate fossil fuel technologies that involved process streams, emissions, or products consisting of chemical mixtures. This was the first major, agency-sponsored, multilaboratory effort to understand and estimate the health risks of toxic mixtures. Continued by the DOE and administered by the Office of Health and Environmental Research (OHER) within the Office of Energy Research, the program reached a peak annual funding level of approximately $57 million in fiscal year 1981. The majority of this research was conducted at five DOE laboratories: Argonne National Laboratory, Inhalation Toxicology Research Institute, Los Alamos National Laboratory, Oak Ridge National Laboratory, and Pacific Northwest Laboratory (although researchers at several universities also were involved).

During the early 1980s, therefore, the emphasis of the DOE program shifted away from assessing technology-specific mixtures toward gaining a more fundamental knowledge of how complex chemical mixtures interact with people and the environment. The complex mixtures program diminished during the mid-1980s concurrent with the shift of OHER's health-effects research emphasis toward the more basic mechanisms of disease induction by energy-related materials and the emergence of the human genome program. The Complex Chemical Mixtures Program ceased to exist as a formal entity during the late 1980s, although OHER continues to sponsor some chemical toxicology research.

Many of our current approaches to studying mixtures have roots in the DOE program. It spurred advances in analytical chemistry and methods for sampling complex atmospheres and process streams. It fostered the development of bioassay-directed chemical analysis, which remains a mainstay of the field. It demonstrated the usefulness of modifying complex mixtures

to reduce toxicity (i.e., the reduction of the mutagenic potential of hydrocarbon mixtures by hydrogenation). The DOE program also fostered the development of air, aqueous, and solid media transport models for chemicals and mixtures of chemicals. It contributed substantial literature on the tissue, cellular, and molecular dosimetry of chemicals. The DOE program generated risk-assessment paradigms for dealing with complex exposure issues.

NIEHS Hazardous Chemical Mixtures Program. Beginning in approximately 1983, The National Institute of Environmental Health Sciences (NIEHS) and the NIEHS National Toxicology Program (NTP) began an initiative to study the toxicity of chemical mixtures, which continues at present (*27*). A major thrust began in 1985 to study mixtures of chemicals identified as groundwater contaminants (*28*). This program began as an interagency agreement with the Agency for Toxic Substances and Disease Registry (ATSDR) in response to the Comprehensive Environmental Response, Compensation and Liability (Superfund) Act.

This program took the approach of developing a standard mixture of compounds identified as groundwater contaminants and studying the toxicity of the mixture as a whole in a variety of test systems. Nineteen organic and six inorganic chemicals were selected from those identified in groundwater in the vicinity of hazardous waste disposal sites by two surveys sponsored by the EPA. The initial study involved rats and mice exposed for three months via drinking water to the mixture at concentrations ranging from 0.1 to 1000 times a baseline concentration of each chemical, which was the average concentration of each chemical near the waste sites. A variety of mortality, morbidity, histopathological, clinical chemistry, cytogenetic, and neurobehavioral endpoints were evaluated during exposures and up to 3 months after exposure. The number of chemicals and their proportional concentrations remained constant; there was no adding, subtracting, or manipulation of the ratios of individual chemicals to examine synergy or cause–effect relationships. Although its scope has broadened since these first studies, this program remains one of the best examples of an approach that uses synthetic mixtures containing large numbers of chemicals.

This program continued with studies funded from NIEHS/NTP and studies done in collaboration with the EPA's Health Effects Research Laboratory (HERL), and Brookhaven National Laboratory complemented the Superfund studies. In fiscal year1989, studies of pesticide and fertilizer mixtures and of the effect of chemical mixtures on bone marrow cell proliferation after irradiation were added to the program. Collaborative studies with EPA/HERL focused on neurobehavioral toxicity and hepatotoxicity. The fiscal year 1990 funding level for this effort was approximately $3.5 million. Publications resulting from the toxicological studies began to appear in 1989 (*29–31*), and several reports are in the publication process.

Research on Sequential Exposures

The issue of sequential exposures to single agents or mixtures is linked inextricably to the issue of simultaneous exposures to multiple agents as mixtures. Although sequential exposures have been studied, the present information base is smaller than that for mixtures. The most extensive example of research on sequential exposures is the study of initiation–promotion phenomena in carcinogenesis (*32*). Another example, closely related to concerns for environmental exposures to mixtures, is the work of Chameaud and French co-workers (*33*) on interactions between cigarette smoke and radon in causing lung cancer. Although numerous issues of interpretation remain, Chameaud and colleagues reported that exposure of rats to smoke before exposure to radon caused no more tumors than exposure to radon alone, but exposure to smoke after radon appeared to act synergistically to increase the lung tumor incidence.

The largest current singly funded program on sequential exposures is the DOE-sponsored research at the Inhalation Toxicology Research Institute on combined and sequential exposures to radiation and chemicals in the nuclear workplace. This program includes both *in vitro* and *in vivo* studies. An example of the former is the finding of Brooks et al. (*34*) that beryllium impairs the ability of cultured cells to repair DNA damage caused by radiation. Two long-term carcinogenesis studies are under way, one examining interactions between inhaled and retained plutonium dioxide particles and subsequent multiple exposures to whole-body x-irradiation (*35*), and the other examining interactions between inhaled plutonium dioxide and subsequent inhalation of beryllium (*36*). The latter study already has shown that small amounts of beryllium in the lung can retard the clearance of plutonium particles markedly, thus increasing the radiation dose. A third major study was initiated during 1991 to examine interactions between single exposures to radionuclides and chronic inhalation of cigarette smoke.

As is true for studies of mixtures, toxicologists are typically more able than epidemiologists to view the issues of combined or sequential exposures with a real hope of dissecting interactions in a definitive manner. Although precautions might be necessary, such as consideration of agents contained in feed, water, or culture medium, the exposures of animals or cells to multiple agents can be controlled in a manner not possible in studies of humans.

Toxicologists' Approaches to Studying Mixtures

This section describes, in general terms, the perspective of toxicologists toward the issue of mixtures. It describes the views that toxicologists likely have regarding the issues, the types of experimental designs typically used, and how their views and approaches might differ from those of epidemiologists and investigators conducting clinical studies (experimental exposures of humans).

Toxicologists have distinct advantages over epidemiologists in their greater ability to exert three general types of control over their studies of mixtures: *a*) control of the population (e.g., a selection of systems ranging from DNA to intact animals; a selection of species, strain, age, gender; and previous exposure history); *b*) control of exposures (e.g., precise knowledge of the type and concentrations of atmospheric constituents, and control of the timing of exposures); and *c*) control of endpoints (e.g., nearly unlimited selection of sampling time and frequency, use of invasive and destructive tests, consistency and completeness of health status evaluations). Toxicologists also have a much greater experimental latitude than those conducting clinical studies regarding the range of exposures and response endpoints. These advantages cause toxicologists to readily envision a broader range of experiments than can be envisioned by investigators studying humans.

Toxicologists also can link more directly exposures to effects than typically is possible in studies of humans. The fact that both the subject's preexposure history and exposures are known and controlled makes cause–effect linkage easier. In addition, the toxicologist often can determine that an exposure actually results in a dose to the tissue manifesting an effect. Their greater potential for linking exposure to effect and controlling extraneous influences leads toxicologists to consider studying a wider range of interactions (as described below) than is typical for investigators studying humans.

Of course, toxicologists have the major disadvantage that their data are not derived from humans but must be extrapolated to humans with varying (and sometimes huge) degrees of uncertainty. The results

of many toxicological studies are difficult to extrapolate directly to man because of the uncertainty that effects, resulting from high-level exposures, would occur at the lower levels of human exposure. This is largely a problem of not knowing the shape of the exposure–response relationship at low exposure levels. Moreover, it is often uncertain whether or not the mechanisms resulting in effects from high-level exposures are even operative at lower exposure levels. Building extrapolation bridges from *in vitro* test systems and animal responses to human responses continues to be a weakness of both toxicologists and investigators of humans. Despite the extrapolation problem, their experimental advantages engender in toxicologists an optimism about their ability to address the issue of mixtures.

Types of Toxic Interactions Envisioned by Toxicologists

Interactions among Components of Mixtures. Toxicologists generally use the term interactions when speaking of the combined effects of two or more agents. This use of the term is questionable; it might better be reserved for the physical–chemical interactions that occur between agents in a mixture, and the term combined effects used for responses. Regardless, interactions will be used in deference to common usage. The three most commonly considered types of interactions between two agents are additivity, synergism, and antagonism, with respect to each measurable effect of exposure. Different possible manifestations of these three types of interactions are described below and illustrated by equations. Two points must be made. First, the interactions described include both those for which examples exist and those for which examples are not known currently but must be considered as potentially operable in exposures to mixtures. The presentation is conceptual; no attempt is made to give specific examples. Second, there is presently no codified method used by toxicologists for expressing the following interactions in equation form. The symbology used herein is illustrative but not standardized and probably not optimal.

Additivity occurs when the combined effect of two or more agents (or components of mixtures) equals the sum of the individual effects. This can be represented by the following expression for the case in which two agents (1 and 2), each having effect A, have effect $A + A$ when administered together:

$$1 = A,\ 2 = A : 1 + 2 = A + A. \quad [1]$$

Synergism is said to occur when the combined effect of two or more agents given together exceeds the sum of the effects of the agents given singly, as in

$$1 = A,\ 2 = A : 1 + 2 = A + A + A. \quad [2]$$

In this case, a single effect, caused by both agents alone, is amplified or expressed in a more than additive manner when the two agents are given together.

An important caveat is necessary here. Toxicologists are likely to call the above response synergism even if only one dose of each agent is used in a three-group study (exposure groups 1, 2, and 1 + 2). However, the outcome, $A + A + A$, might not represent strictly synergism. The outcome simply might have reflected a nonlinear dose–response curve, particularly if agents 1 and 2 caused effect A by the same mechanism. That is, it also might be true that increasing the dose of either agent caused the apparently synergistic response, as in

$$1 = A : 1 + 1 = A + A + A, \quad [3]$$

or

$$2 = A : 2 + 2 = A + A + A. \quad [4]$$

This raises a critical point that is often overlooked by toxicologists—the need for understanding the dose–response relationship for both agents if interactions between them are to be studied. Studies using single doses of multiple agents are useful as an initial exploration of the potential for a nonadditive interaction; however, they are seldom definitive. Ideally, studies of interactions would include enough groups to examine the dose–response surface encompassing the entire range of exposure concentrations of concern for each agent involved in the combined exposures. This approach is not always possible, however. The dose–response caveat applies to the cases that follow; however, it will be assumed for simplicity that the dose–response relationship for each agent is known as linear through the range of interest.

A different case likely to be termed synergism by toxicologists is one in which an agent has an effect and another has none (or at least none measurable) but interacts with the first agent to yield an effect greater than that of the first agent given singly, as in

$$1 = A,\ 2 = 0 : 1 + 2 = A + A. \quad [5]$$

Yet another possible interaction that would be termed synergism could arise when the effects of two agents given singly differ, but the combined effects are greater than the single effect of one or both agents, as in

$$1 = A,\ 2 = B : 1 + 2 = A + A,\ A + A + B,\ B + B,\ A + B + B, \quad [6]$$

or

$$A + A + B + B. \quad [7]$$

Antagonism occurs when the effect of two or more agents given together is less than the sum of the effects of the agents given singly, as in

$$1 = A,\ 2 = A : 1 + 2 = A,\ a,\ 0, \quad [8]$$

in which the lowercase letter represents an effect qualitatively identical to but of a lesser magnitude than the effect represented by the uppercase letter (i.e., $a < A < A + A$), and 0 represents no effect. In parallel to the different cases of synergism shown above, antagonism also could occur if one agent has no effect when given alone, or if the two agents have different effects when given alone, as in

$$1 = A,\ 2 = 0 : 1 + 2 = a,\ or\ 0, \quad [9]$$

$$1 = A,\ 2 = B : 1 + 2 = a + b,\ a,\ b,\ or\ 0. \quad [10]$$

Under the controlled conditions of toxicological studies, it is theoretically possible (and often practical) to dissect additional types of interactions in which the combined effects of multiple agents do not fit the above paradigms; that is, they are not simply equal to, greater than, or less than the sum of individual effects. Interactions between two agents could involve an additional, or third effect, not known to be caused by either of the agents given alone. Although this type of interaction has received little attention, there should be an awareness that these possibilities exist when studying mixtures. The epidemiologist has little potential for dissecting such an interaction from the complex interplay possible among the undocumented (and sometimes unknown) exposures that all of their subjects incur during life. Toxicologists have greater potential for discovering interactions because of their control of confounding factors, which gives confidence that unanticipated effects actually resulted from the experimental exposures and not from unknown exposures, and because of their ability to determine that exposures and effects are mechanistically linked and not just coincidental.

Cases of interactions involving an unexpected effect could arise either when the

two or more agents have different effects given alone or when one or more of the agents has no known effect when given alone. Examples of these interactions are illustrated by the following expressions:

$$1 = A, 2 = B: 1 + 2 = C, A + C, B + C,$$

or

$$A + B + C, \quad [11]$$

$$1 = A, 2 = 0: 1 + 2 = C$$

or

$$A + C. \quad [12]$$

An increased focus on mixtures is certain to bring with it an increased awareness of the potential for various complex interactions, particularly in view of our parallel increasing awareness of the complexity of biological mechanisms producing some effects. The developing awareness of the multistep process of carcinogenesis is an example. A mixture could contain agents affecting different steps (e.g., adduction of DNA and growth factor secretion) or alternate pathways contributing to the same steps (e.g., different growth factors). It will be left largely to the toxicologists to address this issue. As a simplistic example, it might be difficult for the epidemiologist to differentiate between the following cases, particularly if exposure to agent 2 was unsuspected:

$$1 = C; \quad [13]$$

$$1 = 0, 2 = B: 1 + 2 = C; \quad [14]$$

and

$$1 = 0, 2 = 0: 1 + 2 = C. \quad [15]$$

Interactions Resulting from Sequential Exposures. When considering toxic interactions among components of mixtures, it is important to remember that adverse effects also can result from interactions among agents encountered as a result of sequential exposures. Although this is not the same issue as simultaneous exposures to multiple agents in mixtures, it is related closely and of considerable importance. The potential for exposures to multiple agents, and thus for adverse responses due to toxic interactions among agents, is greater for sequential than for simultaneous exposures because the potential for different types of serial exposures is nearly unlimited. Both environmental and occupational exposures present numerous opportunities for the expression of combined effects due to sequential exposures. As a common example, interactions between cigarette smoking and occupational exposures to inhaled agents typically involve alternating rather than simultaneous exposures.

In epidemiological studies of the effects of mixtures, it must be remembered that effects that result from interactions among components of a mixture might alternately have resulted from, or been influenced by, previous exposures. Although the initial uptake of multiple agents might have been separated in time, the actual exposure of a critical cellular or molecular target site to either the parent agents or active metabolites still could have been simultaneous. One can envision the simultaneous exposure of a target site to two agents taken in at different times due to the retention time of the first agent or to differences in the rates of metabolism or excretion of the agents. When simultaneous exposure occurs, the effect manifested at the target site could be the same whether the subject was exposed to the agents as a mixture or sequentially. Toxicologists are expected to resolve these issues.

Toxicologists' Experimental Approaches to the Study of Mixtures

Test Systems. The toxicologist's toolbox contains a wide range of biological test systems from subcellular units to intact animals. Cell systems include cultured mammalian cells and bacteria, such as the Salmonella system for assessing bacterial mutagenicity (Ames test). Numerous species and strains of animals can be exposed to mixtures by all imaginable routes, although the majority of exposures are done by the oral, dermal, inhalation, intravascular, and intraperitoneal routes. There are few limitations on the range of health-effects end points that can be evaluated, although many of the more clinical types of assays, such as physiological measurements, are done more readily using the larger species. Most assays, such as histopathology, serum chemistry and urinalysis, can be applied to animals exactly as they are to humans; however, the assays requiring subject interaction, such as respiratory function or neurobehavioral function, are modified to differing degrees from those applied to man.

Toxicologists frequently incorporate analytical chemistry into their studies to characterize both exposure materials and biological samples. Extensive analytical capability also is used in concert with biological response systems in an interactive, decision-making mode to determine the constituent of a mixture responsible for an effect. The terms biodirected fractionation or bioassay-directed fractionation often are used for this approach. Biodirected fractionation has been used to determine the active agents in several mixtures, an early example being the use of the mouse skin carcinogenesis assay to determine the tumor-initiating fractions of cigarette smoke condensate (*37*). More recent examples are the use of short-term mutagenicity and cellular transformation assays to determine the mutagenic constituents of cigarette smoke (*38*) and diesel soot extract (*39*).

Basic Experimental Designs. There are three fundamental approaches to the toxicological study of mixtures. Although several terms are used for these approaches, the terms integrative, dissection, and synthetic will be used here. Toxicological studies of diesel exhaust will be used to illustrate the differences among these approaches and how they are interrelated.

The Integrative Approach. The integrative approach involves exposure of test systems to the intact mixture and conducting exposure-response studies to evaluate the nature and magnitude of the hazard associated with exposure. This is often the initial experimental approach to the study of mixtures of a generic nature, such as the real-life mixtures tobacco smoke and diesel exhaust, or representative mixtures, such as the 25-compound mixture of water contaminants studied in the NIEHS program. This is the type of toxicological study most related to epidemiology, and it is often used in clinical studies as well. The exposure regimen and biological end points used by toxicologists might be generalized and exploratory in nature if there is little advance knowledge of the hazard, or they might be narrowly targeted if particular hazards are recognized or suspected in advance. These studies are often observational or phenomenological in nature, but they also can be carefully targeted to test specific hypotheses. In addition, the observational studies are not superficial; they can provide a great deal of detailed information if properly designed. Good examples of integrative studies are the several long-term studies of the carcinogenicity of diesel exhaust [recently reviewed by Mauderly (*18*)]. Some of these studies also provided detailed information on a range of noncancer effects, such as that conducted at the Inhalation Toxicology Research Institute, which provided detailed information on dosimetry and particle clearance (*40*), inflammatory responses (*41*), effects on immune responses in lymph nodes (*42*), lung structure–respiratory function correlates (*43*), and adduction of lung DNA (*44*), as well as carcinogenesis (*45*).

The Dissection Approach. The dissection approach seeks to understand the contributions of individual constituents or families of constituents to the toxicity of the mixture. Studies of this type often follow

Table 1. Example of a matrix study of two agents (*A* and *B*).

		Doses of agent A		
		A_0	A_1	A_2
Doses of agent B	B_0	A_0, B_0	A_1, B_0	A_2, B_0
	B_1	A_0, B_1	A_1, B_1	A_2, B_1
	B_2	A_0, B_2	A_1, B_2	A_2, B_2

the demonstration of an adverse effect by integrative studies. The ultimate goal of dissection studies usually is to identify the active agent in order to *a*) determine the causal mechanism of the effect, *b*) develop more accurate risk estimates by using a dose term that is better focused than using the entire mixture as the dose term, or *c*) reduce exposures by reducing the amount of the agent in the mixture.

In the dissection approach, the mixture is separated into individual constituents or families of constituents, which are then tested for biological activity. Biodirected fractionation is a case of the dissection approach in which a mixture is separated progressively into fractions containing fewer and fewer constituents, and each fraction is tested in a biological response system in an iterative manner (*1*). The fractionation is biodirected in the sense that the biological response indicates which fraction to pursue in subsequent iterations. Although the implementation is not always easy, the approach is a conceptually straightforward method for identifying the cause of the biological activity.

In the case of diesel exhaust, studies employing the dissection approach actually preceded and led to the flurry of recent integrative studies. The dissection studies began with the finding of Kotin et al. in 1955 (*46*) that solvent extracts of diesel soot were carcinogenic to mouse skin. Two decades later, EPA investigators found that the extracts were mutagenic to bacteria (*47*). Biodirected fractionation was used extensively to locate the primary source of mutagenic activity in diesel exhaust in the aromatic hydrocarbon fraction of the soot-associated organic compounds (*48*) and resulted in a focusing of attention on the nitropolycyclic aromatic compounds (*49*). Biodirected fractionation does not always involve *in vitro* test systems and short-term assays. On a larger scale, but identical in philosophy, biodirected fractionation using long-term animal exposures was employed by the Fraunhofer Institute (*50*) to determine that the pulmonary carcinogenicity of diesel exhaust was associated with the soot fraction rather than the gas-vapor fraction. Similarly, long-term animal exposures were used recently at the Inhalation Toxicology Research Institute to determine that the organic fraction of diesel soot is not required for the effect (*51*).

The Synthetic Approach. In the synthetic approach, the toxicologist begins with simple, laboratory-synthesized mixtures of compounds or agents, and usually compares the effects of the mixture to the effects of the individual constituents. This approach is used to study interactions between specific agents, to study combined effects using simple systems, and to identify constituents responsible for effects by studying them in a sequential, additive manner. The goal is to gain an understanding of causal interactions among agents by studying a small number of constituents in a stepwise manner. These studies usually begin with two agents and sometimes use increasingly complex combinations of agents to work toward an understanding of the causative agents, or mechanisms, of the effects of the complete mixture to which humans are exposed.

The synthetic approach sometimes takes the form of a matrix study, in which the combined effects of two agents in a range of concentrations are explored in a series of experimental cells. An exposure–matrix approach to studying interactions between two agents, *A* and *B*, each at two concentrations, or doses, is shown in Table 1. In the matrix shown, the A_0, B_0 cell is the control group, and the top row and left column of cells represent graded treatments with single agents. The matrix, therefore, contains four cells in which interactions between the two agents can be observed. This approach has practical limitations; a very large, three-dimensional experimental matrix would be required to fully examine interactions among three agents. For this reason, the matrix approach is used typically to examine interactions between only two agents, as in the case cited earlier of the plutonium–beryllium study at the Inhalation Toxicology Research Institute (*36*). The matrix approach can be simplified by using single-dose levels or by studying only a few of the cells of a matrix involving more than two agents, as exemplified respectively by the studies of oxidants and aerosols by Last and Warren (*52*) and by Kleinman et al. (*22*).

Studies of diesel exhaust have involved different types of synthetic experiments. One type is represented by the work of Wolff et al., who examined the lung retention in rats of a model organic carcinogen, nitropyrene, inhaled either alone or adsorbed on a model particle, carbon black (*53*). Wolff et al. also compared effects on particle clearance and inflammation of multiple models of diesel exhaust constituents, nitropyrene, benzo[a]pyrene, sulfur dioxide, and particles, when administered alone and in combination (*54*). Quite a different type of synthetic experiment is represented by the work of Henderson et al. (*55*), who examined the mutagenicity of solvent extracts of diesel soot from an engine burning simple, laboratory-synthesized fuels containing single aromatic hydrocarbon compounds.

Examples of Toxicological Approaches to Specific Mixture Problems

Three current issues involving exposures to mixtures are used below as examples of problems and potential approaches involved in the toxicological study of mixtures. These examples were selected because they also are used in other papers discussing epidemiological and clinical study problems and approaches.

Environmental Tobacco Smoke and Nitrogen Dioxide: Effects on Lung Growth and Susceptibility to Infection

One difficulty in the study of ETS is defining and generating a representative exposure material. Environmental tobacco smoke is a mixture of exhaled mainstream smoke, sidestream smoke, and reaction products of the constituents of smoke and of smoke with other agents in the environment. Sidestream smoke, diluted and aged, is probably a useful simulant, and the simulation can be improved by the admixture of some mainstream smoke generated by a puffing device. Cigarette smoking devices practical for creating such a mixture are available commercially. Simulating ETS for toxicological studies is not practical when using actual exhaled smoke and a room environment. These factors may not influence the toxicity of ETS strongly; regardless, they are too variable to simulate well. Systems for exposing cells or animals to various dilutions of simulated ETS are readily fabricated, and a range of well-defined experimental cigarettes is available from the University of Kentucky Tobacco

Health Research Institute. Nitrogen dioxide (NO_2) is available in compressed gas cylinders or can be generated by vaporization of the N_2O_2 dimer (*56*).

Another difficulty in addressing this issue is the choice of an experimental model for lung development. Until more is known about the mechanisms controlling cell division and differentiation during growth, studies of lung development will continue to use developing animal lungs rather than cells in culture. A difficult choice is incurred by the differences among species in the maturity of the lung at birth and its postnatal development (*57*). The lungs of laboratory animals and man go through similar stages of development, however, and animals can be used successfully in developmental studies if care is taken that the stage of development, rather than age, is the basis of comparison. Most studies of perturbations of lung development have used rats, and this model is being used presently by Pinkerton and colleagues (unpublished data) at the University of California, Davis, to study the impact of ETS on lung development.

Based on the above considerations, a useful approach for studying the effects of ETS and/or NO_2 on lung growth would be to expose rats to various concentrations of these agents between 1 week and 5 months of age. These represent the ages at which rapid alveolarization of the lung begins and the number of alveoli is complete, respectively, and would approximate exposure of humans between birth and approximately 8 years of age. The primary end points would be morphometric, to examine structural effects, and biochemical, to examine connective tissue effects. Respiratory function measurements would be a useful adjunct, but might not be as sensitive as detailed morphometry. Exposure of rats to 10 ppm NO_2 from birth to 6 months of age was not shown to affect respiratory function evaluated by state-of-the-art functional assays (*58*).

The issue of susceptibility to infection can be addressed by both *in vivo* animal studies and *in vitro* cellular studies, such as the phagocytosis and killing of microorganisms by pulmonary macrophages. There is substantial literature on the effects of inhaled pollutants on the susceptibility of animals, particularly rodents, to infection with bacteria, but there have been fewer studies using viruses. The effects of oxidants in these models were reviewed in 1989 by Frampton and Roberts (*59*). Because viral infections of children are the concern, infectivity models using viruses would be more relevant than those using bacteria. Influenza viruses have been used most frequently as models for studying pulmonary defenses against viruses (*59,60*). Animal models have included rodents, rabbits, monkeys, and dogs.

Laboratory studies addressing the influence of NO_2 and ETS on viral infections in children should not just focus on susceptibility to infection by examining morbidity or mortality but also should address the ability to develop immune responses to relevant types of viruses. Furthermore, studies are needed that examine the interplay among susceptibility to viral infection; development and severity of acute parenchymal and airway inflammation; lung growth; and subsequent development of airway hyperresponsiveness, sensitivity to allergens, and asthma. This is clearly a tall order for the infectivity models of the past. An example of an approach showing promise for studying these interrelated phenomena is the canine adenovirus model being developed and used at the University of Arizona (*61*). This model has potential applicability for incorporating pollutant mixtures into studies of growth and airway responsiveness.

Acid Aerosols and Oxidants: Effect on Respiratory Morbidity

As described earlier, there have been toxicological studies of the combined effects of inhaled acids and oxidants. Schlesinger et al. at New York University (*25,26*) have examined interactions between NO_2 and acid sulfates in affecting alveolar macrophage function and mucociliary clearance of rabbits. Kleinman et al. (*22*) and Mautz et al. (*23*) at the University of California, Irvine, have studied the interactive effects of ozone and NO_2 with acid sulfates on respiration, lung surfactant, pulmonary histopathology, and cell proliferation in the respiratory tracts of rats. Last (*24*), at the University of California, Davis, has studied interactions between ozone and NO_2 and acid sulfates in changing connective tissue synthesis in airway tissues.

Although the particle clearance studies of Schlesinger et al. approach indirectly the issue of morbidity, the primary concern for human exposures to atmospheric acids and oxidants has been for morbidity in the form of airway responses and aggravation of asthma. These issues have not received much attention from toxicologists, partly because of the limitations of animal models for hyperreactive airways and responses during exercise. The guinea pig has been used for years as a model for airway constrictive responses to acute inhalation exposures to acids and oxidants. The airway responsiveness of the guinea pig appears more comparable to man than small laboratory animals. However, most studies have used gross changes in breathing pattern or breathing mechanics as the response end point. It is not clear how closely these end points are related to the responses measured in man by forced expiratory tests.

In a program at the University of Cincinnati, Leikauf and colleagues (unpublished data) are using the guinea pig model and both physiological and molecular end points to explore interactions between acid aerosols and ozone. This and similar approaches should provide information that is applicable to the issue of respiratory morbidity in humans, even though the experimental end points might be different from those applied to humans. Leikauf and colleagues are exploring the use of cDNA probes for mRNA expression of phospholipase A_2, endothelin, and fibronectin as markers of hyperreactivity, comparing these markers to results of physiological measures of airway constriction. A similar approach is being used for mucin and transforming growth factor beta (TGF-β), possible markers for hypersecretion. These approaches have potential for examining oxidant–acid interactions at exposure levels below those for which the physiological studies of the past have demonstrated effects.

Environmental Tobacco Smoke and Radon-Induced Carcinogenesis

There are no reports of toxicological studies of the potential carcinogenic interactions between ETS and radon, but the issue of tobacco smoking (mainstream smoke) and radon has been given some attention. As reviewed recently by Guilmette et al. (*62*), exposure to radon (progeny) has been linked to increased risk for lung tumors in rats in laboratories in the United States and France and in dogs in one study in the United States. Although attempts have been made to study interactions between radon exposure and smoking in animal studies, the results have an uncertain interpretive value. One significant problem is the lack of a good animal model for smoking-induced respiratory carcinogenesis, as described earlier. Without a reliable model for smoking-induced cancer, the ability to explore carcinogenic interactions between tobacco smoke and other agents is hampered.

Chameaud et al. (*33,63*) exposed rats to simulated mainstream cigarette smoke either before or after exposure to radon. The animals were placed in a dome into which mainstream smoke was drawn from several cigarettes that burned simultaneously,

rapidly, and continuously by drawing a vacuum through a cigarette manifold. The rats remained in a static atmosphere of the resulting high concentration (uncharacterized) of smoke for 15 min, and then the dome was flushed with clean air. Although the rats were exposed to smoke in ten 15-min sessions four times weekly for a year, the relevance of the exposure method and pattern to human smoking is uncertain. Regardless, these investigators found that, while exposure to smoke before exposure to radon had no effect on radon-induced tumor incidence, exposure to smoke after radon increased the tumor incidence 2- to 3-fold. Exposure to smoke alone did not induce significant incidences of lung tumors.

Cross et al. (*64*) exposed a small number of dogs via mask to smoke from 10 cigarettes/day, with smoke inhaled during every tenth breath for 4 to 5 years, and also exposed the dogs to radon. Although the study was not statistically robust, the incidence of lung tumors was lower in the smoke + radon group than in dogs exposed to radon alone.

The above results do little to resolve the issue of potential carcinogenic interactions between ETS and radon. The rat study suggested that smoke acted as a promoter of radon-induced carcinogenesis. If the current attempt to establish a model of tobacco smoke-induced carcinogenesis in rats using whole-body exposures (*5*) is successful, this model could be applied readily to studying interactions between smoking and radon. In the absence of an animal model demonstrating smoking-induced carcinogenesis, animal studies of ETS-radon interactions are of questionable value.

In vitro or a combination of *in vivo* and *in vitro* approaches might be used to determine if radon and tobacco smoke are synergistic in causing preneoplastic changes. Cultured cells can be exposed to radon, or the alpha particle irradiation from radon progeny can be simulated by irradiation from other sources. As an example, Thomassen et al. (*65*) used electroplated sources of plutonium-238 to irradiate primary cultures of tracheal epithelial cells and to compare the preneoplastic transformation (induction of growth variants) of tracheal cells by α-irradiation and direct-acting chemical carcinogens. This transformation assay could be used similarly to study interactions between α-irradiation and cigarette smoke or smoke condensate *in vitro*. An alternate approach would be to expose animals to radon and cigarette smoke and to determine preneoplastic transformation in primary cultures taken from the exposed animals. Neither of these approaches has been used to date.

Summary: The Role of Toxicology in the Study of Mixtures

Overall, toxicology provides a degree of experimental selection and control that has potential for providing more detailed information about hazards from mixtures than is possible with epidemiology or clinical studies. First, toxicology provides a means of evaluating whether or not exposure to a mixture poses a health hazard without relying on human experience. This is important for exploring risks from a new mixture or combination of exposures for which human experience has not yet been accumulated or identified. This also is important, however, for exploring risks from mixtures to which humans have been exposed but for which the effects cannot be strictly identified as having resulted from the particular exposure of concern.

Second, toxicology provides a means of determining the causal constituent among components of a mixture shown to cause an adverse effect. In some cases, this also might be possible in clinical studies but is never possible in epidemiological studies.

Third, toxicology provides a means of determining the mechanism by which an effect occurs. This includes determining interactions among mixture constituents that are responsible for the effect, the toxicokinetics resulting in the dose of the critical agent to the critical biological site, and the mechanism by which the adverse effect results from the critical exposure. In rare situations, the mechanism of response might be determined in clinical studies, but is beyond the capabilities of epidemiology.

Fourth, toxicology provides a means of exploring, in a precise, stepwise manner, the existence and nature of adverse effects resulting from exposures to multiple agents, ranging from simple combinations of two agents to chemically and physically complex mixtures. Again, this might be done to some extent in clinical studies but not by epidemiology.

On the other hand, using its nonhuman test systems, toxicology alone can seldom provide accurate estimates of human health risk from exposure to mixtures. The qualitative extrapolation from nonhuman test systems to man is often satisfactory and is strengthened by developing an understanding that the same basic biological mechanisms are operative in the test systems and humans. However, the quantitative extrapolation of exposure–effects data from nonhuman test systems to man is difficult typically and often impossible to accomplish with a high degree of confidence. Not only are humans a different species than those used in laboratory studies, but they also live in an environment that is much more complex than the experimental setting. For some nondestructive, readily measured end points, clinical studies can serve as an extrapolation bridge between toxicology and epidemiology.

Toxicology is an important predictive and dissective science that complements the observational science of epidemiology. It is just as impossible to fully duplicate the human environment in the laboratory as it is to exert experimental control over human exposures in the environment. There is a greater potential for using experimental exposures of humans as an extrapolation bridge between toxicology and epidemiology than has been exploited in the past. Animals certainly can be exposed in any pattern to any experimental atmosphere to which human subjects can be exposed, and there is good potential for simultaneous exposures of humans and animals in the same chambers. This constitutes an area for exploration.

Coordination of complementary research using toxicological, clinical study, and epidemiological approaches is a worthwhile and reasonably achievable goal that has received little attention. Research sponsors could give emphasis to fostering such coordination, and researchers in the three disciplines should make greater efforts to reach out toward each other. Many research sponsors fund work in two or all three disciplines, but there has been little effort to actually coordinate how issues are addressed by the disciplines. Few scientific societies or journals consciously integrate the disciplines, a noteworthy exception being the American Thoracic Society, whose meetings and publications serve as an intersection for the three areas of interest. For the most part, the three disciplines remain separate sciences with only superficial contacts among them.

Advantage could be gained by fostering collaborations among toxicology, clinical studies, and epidemiology in addressing health risks from environmental mixtures. The goal of this closer collaboration would be to identify the next step for each discipline, to identify opportunities for directly comparative studies among disciplines, and to identify points at which research could be handed off from one discipline to another. As an example, epidemiology (including environmental sampling) could provide toxicology with information on the composition of mixtures to which humans

are exposed, patterns of exposure, populations of concern, health outcomes of concern, and the level of effects observed (or observable). Clinical studies could provide information on short-term responses and dose–response relationships, biomarkers revealing short-term exposures and effects, and the likelihood of sensitive subpopulations. Toxicology, in turn, could provide feedback on the biological plausibility of the suspected exposure–response relationship, the potential for chronic disease resulting from repeated exposures, causal and predictive relationships between acute and chronic effects, finer definition of dose–response relationships, active constituents of mixtures, and the effect of exposure pattern. Coordinated clinical and toxicological studies could, in some cases, provide direct human–animal comparisons that bolster confidence in the relevance of animal responses and provide quantitative extrapolation bridges. Other forms of complementary cross-feed among the disciplines are envisioned; the preceding examples are only illustrative. In general, the goal appears worthwhile.

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Assessing Exposures to Inhaled Complex Mixtures

Brian P. Leaderer[1*], Paul J. Lioy[2], and John D. Spengler[3]

[1]John B. Pierce Laboratory and Division of Environmental Health Sciences, Department of Epidemiology and Public Health, Yale University School of Medicine, 290 Congress Avenue, New Haven, CT 06519; [2]Exposure Measurement and Assessment Division, UMDNJ-Robert Wood Johnson Medical School, 675 Hoes Lane, Piscataway, NJ 08854; [3]Department of Environmental Health, Harvard University School of Public Health, 665 Huntington Avenue, Boston, MA 02115

In the course of daily activities, individuals spend varying amounts of time in different spaces where they are exposed to a complex mixture of gas, vapor, and particulate contaminants. The term complex is used in this paper to refer to binary mixtures as well as truly complex mixtures of three or more constituents. The diversity of the environments where pollution may occur, the number of pollutants that may be present, and the nature of the activity in the environment combine to pose a challenge to investigators of the health effects of air pollutants. This article discusses several methods of measuring or assessing exposure to complex mixture air contaminants that include time–activity assessments, personal monitoring, biomarkers of exposure, and microenvironmental models that can be employed singly or in combination in a protocol for exposure assessment. The use of nested designs, involving more intensive data collection from samples or subjects, is also considered. — Environ Health Perspect 101(Suppl 4):167–177 (1993).

Key Words: Pollutant, complex mixture, exposure assessment, microenvironmental model, nested designs, time–activity assessment, biomarkers, monitoring

Introduction

Human activities routinely involve exposure to the complex mixtures of gases, vapors, and particulate matter that contaminate the air in most indoor and outdoor environments. The diversity of environments, where exposure may occur, and the of number of pollutants that may be present pose a challenge in investigating the health effects of air pollutants. For example, in the course of a typical day, individuals spend time in a variety of both indoor and outdoor environments, such as residences, industrial and nonindustrial work places, automobiles, public buildings, and urban or rural outdoor locations. The many different activities of work and leisure time also affect the personal exposures. Although this paper focuses on inhaled pollutants, it is important to recognize that exposures also take place through media other than air and by routes of entry other than the respiratory tract.

The lack of information on the characteristics of the complex mixtures found in most environments makes investigation of the health effects difficult. Concentrations of the key compounds of many mixtures considered relevant to public health have not been quantified well, and even the identities of many mixture components are unknown.

Complex mixtures can be classified into three groups that *a*) originate from single sources (e.g., environmental tobacco smoke from active smoking), *b*) result from physical mixing of primary emissions from multiple sources (e.g., a range of volatile organic compounds [VOCs] emitted from building furnishings), or *c*) result from physical mixing of emissions from multiple primary sources with agents created by chemical transformations of those emissions (e.g., precursors of smog [like nitrogen oxides, hydrocarbons, and sulfur oxides] reacting to form ozone and acid particles mixed with other oxidants and metals). The term *complex* is used in the context of this paper to refer to binary mixtures as well as to truly complex mixtures of three or more constituents.

The methodologic challenge faced in assessment of exposure to each of these types of mixtures is evident. The components of mixtures, which are relevant to the health outcomes of interest, may not be known; and, therefore, the measurement of all components of mixtures in the context of an epidemiologic investigation is not possible for most mixtures of concern. In any case, such detailed information might not be readily interpretable without an adequate biologic framework.

As an alternative to full characterization of mixtures, marker components (also referred to as tracers, proxies, or surrogates) have been used to represent exposures to the mixtures. Markers or indicators may be speciational elements, chemical compounds, size-fractionated airborne particles, metabolites in biologic specimens, variables derived from questionnaire responses, or model estimates. Ideally, a marker of exposure to a complex mixture should be unique to the mixture's source, readily detectable in air at low concentrations, present in air in a consistent ratio to other components, and measured easily and accurately at an affordable cost (*1*). Unfortunately, exposure measures of a single marker for a complex mixture may not reflect toxicity from synergistic interactions among the components fully.

Tobacco combustion illustrates a single-source complex mixture found in indoor environments. Environmental tobacco smoke (ETS) is comprised of hundreds of different compounds in the particle and vapor phases (*2*). Many toxic and carcinogenic agents have been identified in ETS, and ETS has been linked to a wide range of acute and chronic health effects and to loss of comfort (*2*). Although it is not possible to measure all components of ETS, several specific air contaminants and categories of contaminants (nicotine, carbon monoxide, pyridine, aldehydes, and respirable particles) have been identified as markers for ETS (*1–4*). These markers have proved to be useful in studies of health

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*Author to whom correspondence should be addressed.

effects, for validation of questionnaires, and the development of exposure models (*5*).

Questionnaires have also been used to assess exposure to ETS by characterizing smoking in the environments where subjects spend time. Typical questions are directed to the smoking habits, especially locale and intensity, of family members or coworkers (*4,6,7*). Biologic markers of ETS exposure, including carbon monoxide level in exhaled air, carboxyhemoglobin level, and concentrations of nicotine and its metabolite cotinine in body fluids, have also been used to assess exposure (*2*).

Volatile organic compounds are a complex mix of contaminants resulting from multiple sources that exemplify the second class of mixtures. Hundreds of VOCs can be found in indoor and outdoor air. The many sources of indoor VOCs include industrial processes, consumer products, and home and office furnishings. Volatile organic compounds are suspected to be the cause of a wide range of adverse health and comfort effects (*8*). A particular VOC may be singled out as relevant to a specific health effect, for example, benzene and leukemia. However, no single compound has been identified as the causative agent of irritant and neuropsychologic effects. For example, no single marker compound has been identified for investigation of the hypothesized association of building-related symptoms with VOCs. For experimental human exposures, one investigator (*9*) has designed a mixture of 22 VOCs typically found in offices, while others use the total mass of VOCs as an exposure indicator.

The third group of complex mixtures is illustrated by photochemical smog, which includes the primary pollutants sulfur dioxide, nitrogen oxides, and hydrocarbons and diverse reactive species produced by atmospheric chemical reactions. Mixtures that concern health include photochemical oxidants and acidic gases and particles. Because identification and measurement of all the reaction by-products of this group are not possible, markers such as ozone, formaldehyde, and acid sulfates are used to assess exposure. These individual pollutants have also demonstrated adverse effects; for example, ozone is a criterion pollutant that causes transitory, and possibly long-term, effects at concentrations at or below the current standard of 0.12 ppm.

The challenge faced in assessing exposures in order to investigate the health effects of each of the three types of complex mixtures is evident. This article reviews the methods presently available for assessment and covers concepts of personal exposure, time–activity assessment, methods for measurement of contaminants, and the use of questionnaires and biomarkers. The article ends with a discussion of integrated approaches for exposure assessment and suggestions for further research.

Concepts of Exposure and Exposure Assessment

Concepts of exposure and exposure assessment evolved and matured during the 1970s and 1980s from studies involving large-scale measurements of the exposures of individuals and sample populations. The 1991 report of the Committee on Advances in Assessing Human Exposure to Airborne Pollutants of the National Research Council sets out these concepts and details approaches for using them in the context of epidemiologic studies (*10*). The committee defined exposure as "an event consisting of contact at a boundary between a human and the environment at a specific environmental contaminant concentration for a specified interval of time; the units to express exposure are concentration multiplied by time." Dose is defined as the amount of the pollutant absorbed or deposited in the exposed person over a particular period of time. These definitions must be considered in the context of the averaging time relevant to the biological response of concern. In an environmental epidemiology study, exposure assessment approaches should be based on an understanding of the biologically relevant time frame for the exposure–effect association under study. For example, exposures to radon in a study of lung cancer need to be assessed over periods of years rather than days or weeks. The time frame for assessing exposures to some single agents with well-characterized adverse effects may be evident; but for complex mixtures, however, it may be difficult to specify the biologically relevant time frame because the principal active components of the mix may be uncertain and the time course of the independent and combined effects unknown. The study of VOCs as a potential cause of sick-building syndrome exemplifies these problems. The mixture of VOCs indoors is diverse and variable over time, the health effects examined are varied, and exposures may take place in a number of different microenvironments over varying intervals.

The National Research Council committee provided a general framework for relating sources, media of exposure, exposure, dose, and health effects (Fig. 1). For air pollutants, a less complex microenvironmental model has been used to guide the development of exposure assessment approaches. This model is appropriate for pollutants for which air is the sole medium of exposure (e.g., carbon monoxide and ozone). A microenvironment is generally defined as a location where the concentration of a pollutant is considered to be spatially uniform during the time that individuals are exposed in that location. Integrated individual exposure from a medium or media to a pollutant can be estimated as the weighted average of the concentrations in the relevant microenvironments by using the time spent in the microenvironments as the weights (*10–13*).

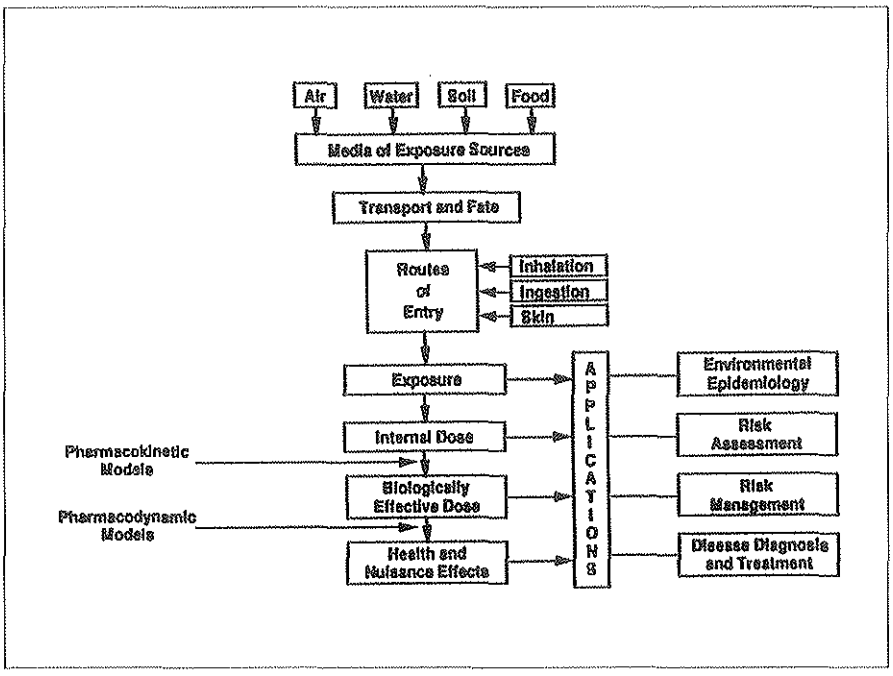

Figure 1. Contaminant sources and effects continuum (*10*).

Table 1. Analytical method selection.[a]

Factor	Ideal condition
Sensitivity	Detects analytes at levels below those causing adverse health effects; sensitivity 0.1X level of interest; range 0.1X–10X level of interest; precision and accuracy +/ –5% easy and accurate calibration
Selectivity	No response to similar compounds that might be present simultaneously with the analyte of interest
Rapidity	Short sampling and analysis times compared with biological response time or with significant changes in contaminant concentration; response time 90% in less than 30 sec; RS232 or equivalent output
Comprehensiveness	Sensitive to all contaminants that could result in adverse health effects
Portability	Sampling and analysis device is rugged and can be worn without modifying the normal behavior of individual; low power consumption; battery operated; stabilization time less than 15 min; temperature range –20 to 40° C; humidity range 0 to 100%
Cost	Cost of sampling and analysis is not prohibitive; inexpensive, readily available components; few consumables; low maintenance

[a]Data from the National Research Council (*10*)

Table 2. Status of personal monitor development.

Pollutants	Monitor needed		Monitor under development		Prototype under development		Tested and evaluated		Used in pilot studies		Used in large field studies		Ready for routine use	
	D	I	D	I	D	I	D	I	D	I	D	I	D	I
CO	√	√	√	√	√	√	√		√		√		√	
NO_2	√	√	√	√	√	√	√	√		√		√		√
Vapor phase nicotine for ETS	√	√		√		√		√		√		√		√
Inhalable particles (<10 μm diameter)	√	√	√	√	√	√		√		√		√		√
Formaldehyde	√	√		√		√		√		√		√		√
VOCs	√	√		√		√		√		√		√		
Polar VOCs	√	√				√								
Pesticides	NA	√		√		√		√		√		√		
Radon	√	√	√	√	√	√								
PAH	NA	√		√		√								
Biological aerosols	NA	√		√		√		√		√		√		√
House dust	NA	√		√		√		√		√		√		√
O_3	√	√		√		√								

Abbreviations: D, direct readout; I, integrating collection of samples; NA, not applicable

[a]Data from the U.S. Environmental Protection Agency (*17*)

Approaches to measuring personal exposures to air pollutants can be classified as direct or indirect (*10*). In the direct approaches for inhalation exposures in the microenvironments of interest, measurements of exposure are obtained by direct personal monitoring in the breathing zone or by the use of appropriate biological markers. Indirect approaches are based on the microenvironmental model. Measurements of a pollutant are made in the relevant microenvironments, and information is gathered on the human time and activity patterns and the weights for the concentrations in the exposure model. The measurements and the time and activity information are used to calculate integrated exposure. With this model, it is possible to apportion the contribution from various sources and locations.

Personal Monitoring

Of the approaches currently available for exposure assessment, personal monitors offer the most promise for minimizing uncertainty about the degree of contact with a contaminant (*10*). However, there are inherent difficulties involved in personal monitoring, including determination of the appropriate duration of monitoring, obtaining valid samples without altering the subject's behavior or activities, and the development of small and reliable devices. The Committee on Advances in Assessing Human Exposure to Airborne Pollutants of the National Research Council has enumerated the factors to be considered in assessing monitoring devices (Table 1). Personal monitors appropriate for epidemiologic research should provide adequate instrumental specificity and a wide detection range for the biologically active or surrogate compounds of concern.

The use of a personal monitoring technique should be accompanied by simultaneous data collection on locations where time is spent and on activities in the locations (see below) (*10,12,14–18*). Data from a study that integrates assessment of sources and their locations with personal monitoring can be analyzed for the contribution of specific sources to exposures and can also provide a basis for developing exposure models (*19,20*).

In 1988, the U.S. Environmental Protection Agency (EPA) summarized the status of personal monitors (Table 2) (*17*). The status of the monitors varies widely among the pollutants. For some pollutants, the personal monitoring technology is available, but the devices still require improvement. The VOCs are particularly problematic, due in large part to the myriad of compounds that may be found indoors and outdoors. For some compounds, the time needed to collect an adequate personal sample may be too long to link exposure with an acute biologic response.

Of the personal monitors now available, several are relevant to the example mixtures

considered by the working group. Passive badges have been developed for exposures to NO_2 generated by combustion and for nicotine in tobacco smoke (*10*). Nicotine samples have been collected for periods as short as one day (*10,21*). Techniques for personal monitoring of radon, ozone, and the VOCs are not as well developed (*10*). Personal VOC samplers that use Tenax or a series of sorbents for passive and/or active collection are available, but these accurately detect only a limited number of compounds (*10*). The current efforts to employ multiple sorbents in a sampler have increased versatility by enabling the sampler to collect more compounds.

A personal dosimeter recently developed for radon was used in a personal exposure study conducted in New Jersey (*22*). Most epidemiological studies of radon have used microenvironmental measurements; however, these measurements do not capture the exposures that occur in microenvironments other than the home (*23*). The new personal radon dosimeter is an example of a long-term personal monitor that should decrease uncertainty in individual exposure estimates in environmental epidemiological studies of lung cancer.

Personal monitors for ozone exposure are being developed (*17,24*). However, these monitors provide integrated exposure estimates over periods of several hours or days, depending on the concentration of ozone, and most of them do not measure biologically relevant short-term exposures between 1 and 4 hr.

Techniques for measuring reactive acidic particles and gases using a system of annular denuders and filter packs have been developed (*25*). The system has been employed as an indoor and outdoor monitor especially for acid sulfate and nitrate species. Some effort also has been made to develop a proto type personal monitor that measures a limited number of ionic species. All monitors, however, measure only total particle acidity, as hydrogen ion (H+), and do not speciate H_2SO_4 from NH_4HSO_4, which may be of greater biologic relevance (*26,27*).

In contrast to the estimation of other example pollutant exposures, estimation of exposures to ozone and acidic sulfate particles may not require personal monitoring because they are regional pollutants, and outdoor monitors may be sufficient for estimating the personal exposures sustained by a population (*28,29*). However, dose estimation of these compounds for individuals also requires valid questionnaire information on the amount of time the individuals spent outdoors and their level of participation in athletic activities and other activities that increase pulmonary ventilation (*10*).

For the mixtures addressed in these papers, representative personal and microenvironmental monitors are shown in Table 3 (*30*). Although these monitors hold promise, their sensitivity and time resolution may not be compatible with current research needs. In addition, the fixed samplers are not always available in a form that can be used in all microenvironments. An exception is the recent particle size selective samplers, which are integrating devices designed for operation within residential settings, outdoors, or in work place settings (*10,26,31,32*). Real-time, continuous NO_2 and O_3 monitors are still unavailable for convenient operation in residential settings. The fixed-site outdoor sampler typically used in a trailer or similar location is still the only accurate instrument available for monitoring NO_2 and O_3, and these instruments are not incorporated readily into studies of the indoor residential environment.

Time–Activity Assessment

Time–activity patterns determine the duration of exposure to complex mixtures in relevant microenvironments. Three general categories of microenvironments can be used to describe most exposures to complex mixtures: *a*) environments where exposures to complex mixtures result from multiple sources emitting a class of contaminants (e.g., VOCs from building materials and furnishings); *b*) environments where exposures to complex mixtures occur because the source emits contaminants with dissimilar properties (e.g., gas and kerosene combustion can produce inorganic reactive gases, organic and inorganic particulate matter, organic vapors, and nonreactive inorganic compounds); and *c*) environments where exposures to complex mixtures occur because dissimilar source types are present (e.g., soil gas containing radon and cigarette smoke producing ETS).

The collection of time–activity data may be essential for estimating exposures to complex mixtures in each of these three types of environments. People integrate exposures to single-compound and complex mixtures through a range of common activities. These activities place people in specific microenvironments and determine their proximity to sources. In addition, activities relevant to the estimation of exposure may be diverse among individuals and highly variable for each individual. Activities vary among individuals because of age, ethnicity and race, socioeconomic status, health status, weather, and other factors. The variation in types and levels of activity probably is a strong determinant of variation in exposures to complex mixtures.

The application of time–activity information to estimation of exposures in an epidemiologic study requires in-depth consideration of the biologically relevant exposure measures. For short-term responses, it may be necessary to assess time–activity with a degree of temporal resolution that is not appropriate for long-term responses. The relevant microenvironments should also be determined in the analyses. As approaches to data collection are developed, emphasis should be placed on accurately measuring time in the microenvironments where subjects are exposed to the mixture being studied and on describing activities that may lead to contact with one or more components of the mixture.

Diary, recall, and observational approaches have been used to assess time and activity patterns (*10,18,20*). In the diary approaches, subjects are asked to complete a log of their activities that typically captures sequential information on each activity, its location, and its duration. An alternative approach asks subjects to account for each time interval of a given period in regard to activities and locations. Subjects may also be asked to supply information on time and activity patterns by recall. Direct observation of subjects has received little application in studies of human exposure.

Techniques for applying time–activity data to studies of total human exposure and the data sets that are available have been reviewed by Ott (*20*) and Robinson (*18*). Specifically, time–activity data from both the time budget and national travel surveys have been used to describe time spent in pollutant-relevant microenvironments for selected groups in the population (*33,34*). As new time–activity data have become available, researchers have updated and validated exposure models (*35,36*). Higher resolution time–activity data should improve predictions in the absence of personal exposure data.

Data from a recent statewide study of time–activity patterns of Californians over 11 years of age are used in Figure 2 to illustrate the activities and percent of time spent in a few generalized locations (*37*). A finer resolution of these patterns is required to quantify health effects, identify populations at risk, and formulate effective management strategies. For example, Jenkins et al. (*37*) were interested in identifying the duration, frequency, and location of exposures to specific indoor sources. Table 4, reprinted from Jenkins et al. (*37*), describes the frequency and duration of adult activities associated with exposures to

Table 3. Monitoring equipment for particulate matter for indoor air quality studies.[a,b]

Pollutant sampler	Manufacturing company	Sensitivity and Integrating time	Approximate cost
Radon: track etch	Terradex Corporation 460 N. Wiget Lane Walnut Creek, CA 94598 (415) 938-2545	1 to 3 month exposure 1 to 4 pCi/L	$20 to $60 depending on sensitivity desired
Radon: charcoal canister detector	RTCA 12 West Main Street Elmsford, NY 10523 (914) 347-5010	4 days 0.1 pCi/L	$35/canister includes shipment and analysis costs
Organic vapors	Industrial Scientific Corporation 355 Steubenville Pike Oakdale, PA 15071 (412) 758-4353		
Organic vapors: hydrocarbon chemical reaction tubes	National Draeger Inc. P.O. Box 120 Pittsburgh, PA 15230 (412) 787-8383	100 to 3000 ppm for 4 to 8 hr	$3/tube, $900 for pump and accessories
Organic vapors: charcoal badges	3M Corporation Technical Service Department 3M Center St. Paul, MN 55144 (612) 733-1110	Depends on vapors and sampling times; minimum level, 10/mg	$10/badge; $50 to $300 analysis by GC or GC/MS
Formaldehyde: diffusion tube	Air Quality Research, Inc. 901 Grayson Street Berkeley, CA 94710 (415) 644-2097	5 to 7 days	$48 /kit, includes 2 monitors, analysis and report
Formaldehyde: pro-tek adsorption badge	E.I. Dupont Company Applied Technical Division P.O. Box 110 Kennett Square, PA 19348 1 (800) 344-4900	1.6 to 54 ppm/hr up to 7 days or 0.2 to 6.75 ppm/8 hr TWA	$20/badge; $25 to $80 for analysis
Formaldehyde: diffusion monitor	3M Corporation Technical Service Dept. Building 260-3-2 3M Center St. Paul, MN 55144 (612) 733-1110	0.1 ppm for 8 hr	$37/monitor and analysis
NO_2: personal and alarm	MDA Scientific 405 Barclay Boulevard Lincolnshire, IL 60069 1 (800) 323-2000	2 to 3 ppm; 1/3 TLV electrochemical cell based 15 min to 8 hr TWA	$800/detector; $100/output; $2075/dosimeter; $1045/read-out unit
NO_2: diffusion tubes	Environmental Sciences and Physiology Harvard School of Public Health 665 Huntington Avenue Boston, MA 02115 (617) 432-1000	500 ppb/hr integrated	$10/tube, research only
NO_2: diffusion badge	Environmental Sciences and Physiology Harvard School of Public Health 655 Huntington Avenue Boston, MA 02115 (617) 432-1000	50 ppb/hr	$15/badge, research only
CO: passive badge	Lab Safety Supply Co. P. O. Box 1368 Janesville, WI 53547 (608) 754-2345	50 ppm for 8 hr produces color change	$3/holder; $12.75/10 indicating papers

(Continued)

Table 3. Monitoring equipment for particulate matter for indoor air quality studies [a,b] **(continued)**.

Pollutant sampler	Manufacturing company	Sensitivity and integrating time	Approximate cost
CO: detector tube integrated	National Draeger Inc. P.O. Box 120 Pittsburgh, PA 15230 (412) 787-8383	2.5 ppm for 8 hr	$255 pump and accessories; $3/tube
CO: detector tube grab	Sensidyne Inc. 12345 Sparkey Road Suite E Largo, FL 33543 (813) 530-3602	5 ppm/min	$130 pump; $2/tube
Nicotine for ETS diffusion monitor	John B. Pierce Laboratory 290 Congress Ave. New Haven, CT 06519 (203) 562-9901	0.01 µg sampling rate of 24 mL/min	$55/sample
Integrated gravimetric; particles <3.5 µm diameter	Cyclone separators with filter. Several manufacturers of cyclones, filters, and pumps	1.7 L/m	Pumps $200 to $700; filters $2; cyclones $20 to $100
Integrated gravimetric; particles between 10 and 3 µm and less than 3 µm diameters	National Bureau of Standards Under EPA Contract U.S. EPA Research Triangle Park, NC 27711 (919) 541-2350	6 L/m Separates using filters in series; batteries	Unknown
Instantaneous (2/10 sec); TSP or RSP; 0.1 to 10 µ forward light-scattering	GCA-Mini-RAM (personal aerosol monitor) GCA Corporation 213 Burlington Road Bedford, MA 01730 (617) 275-5444		$2500
Continuous; RSP submicron light-scattering multi-sensor monitor	Handheld Aerosol Monitor (HAM) PPM Inc. 11428 Kingston Pike Knoxville, TN 37922 (615) 966-8796	>10 µg/m^3 mass concentrations; 1.5 L/sec	$3000 to $10,000

[a]Particles can be measured using a variety of techniques. Using cyclone or impactor separators, smaller size fractions can be collected on filters. Mass can also be measured using the optical properties of particles. Measuring particles usually requires equipment costing several hundred to a few thousand dollars. Equipment using filters requires that they be preweighted and postweighted in a temperature- and humidity-controlled room.

[b]Data from Samet et al. (*30*)

Table 4. Adult diary activity episodes with smokers present in locations with high frequencies or exposure times.

Location of activities	Total number of activity episodes	Number of episodes with smoker present	Percent of episodes with smoker present	Average minutes per episode with smoker present
Own living room or family room	4653	442	9	88
Restaurants	778	327	42	68
Car	5420	323	6	33
Own kitchen	4045	215	5	44
Office building/bank	841	188	22	153
Industrial plants/factories	340	125	37	173
Shopping malls	755	120	16	86
Bars/nightclubs	133	104	78	99
Other public buildings	245	62	25	135
Playgrounds/parks	248	51	21	120
Hospitals/doctors' offices	284	37	13	133
Others' homes	155	9	6	11
Beauty parlors/barber shops	49	9	18	138

[a]Data from Jenkins et al. (*37*)

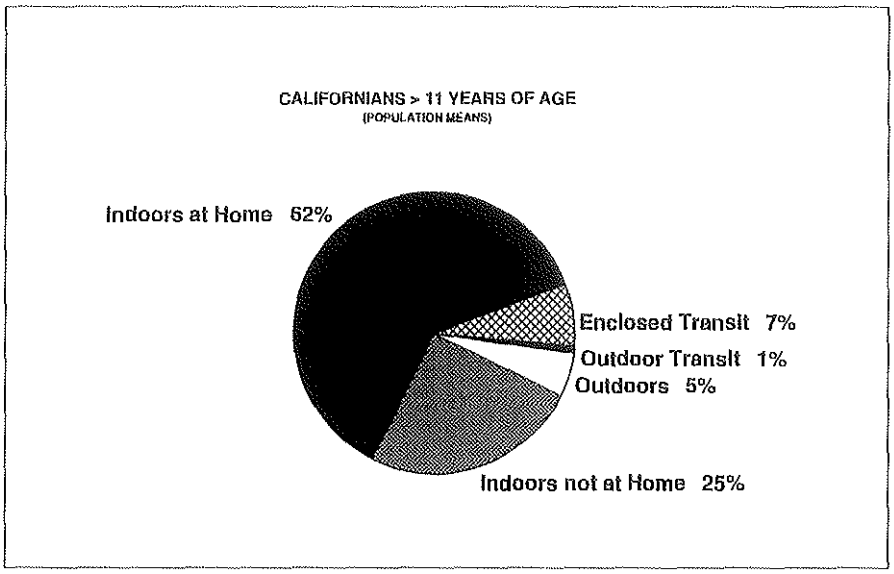

Figure 2. Percent of day spent in different locations. From Jenkins et al. (*37*).

environmental tobacco smoke. Overall, the largest number of contacts with smokers occur inside the home environment, but the percentage of home activities associated with smokers is actually small. On the other hand, visits to restaurants, bars, and nightclubs are frequently associated with the presence of smokers. With a knowledge of the concentrations encountered in these microenvironments and of the potency of the complex mixture, the health risk to the population or to specific subgroups could be assessed.

Questionnaires

Questionnaires are the least expensive method for obtaining either retrospective or prospective information on the exposures of large populations, and they have been the method most commonly used for exposure assessment in epidemiologic studies. Questionnaires can be used to categorize exposures to sources and to describe the environmental characteristics that affect concentrations and activities, which, in turn, affect exposures and doses of inhaled pollutants. Questionnaires have been used extensively to provide a classification of potential exposures to sources and to obtain information on potential confounding and modifying factors. Questionnaires frequently ask simple questions such as "Do you live with a smoker?" or "Do you have a gas stove?" Others may be more specific, aimed at a particular source or pollutant.

In the indirect method of exposure assessment based on the microenvironmental model, the approach is to model the factors that govern the generation, dispersal, and removal of the air contaminant mix. Inputs to the models may include information collected by questionnaire, such as time–activity information, a source inventory, and patterns of source use. For example, in modeling NO_2 levels in a residential environment, questionnaires might ask for information on the sources (presence of a gas range and number of pilot lights, presence of a gas water heater, and presence of a gas dryer), source condition (age of the range), source use (number of burners used, length of time used, flame setting, and use of oven to heat the house), and the removal and dispersal of contaminants (use of outside-vented range hood and volume of home).

Questionnaires can play a major role in assessing exposure to complex mixtures. Exposures to ETS and NO_2, which are ubiquitous in indoor environments, can be assessed with sufficient accuracy in large-scale studies by using several different questionnaire approaches. For example, an initial screening instrument for sources might determine the smoking status of the household members and the presence of gas appliances in the home. The resulting source-based categories could be refined by questionnaires that assess the time spent in other environments, such as in day care, work, and outdoors, where exposures also may occur. The results could be further refined by questionnaire information related to the characteristics of the sources and the patterns of their use. For example, estimates of ETS exposure could incorporate the location of smoking in relation to the subject and the number of cigarettes smoked in the home. The questionnaire approach should be designed to assess exposures on a relevant time basis.

Questionnaire-based exposure measures can be made more accurate if supplemented by air monitoring—for example, passive monitoring for nicotine and NO_2 indoors and both passive and active monitoring for NO_2 outdoors—and by use of biomarkers such as urine cotinine. Leaderer et al. (*38*) have described nested approaches for applying the more costly and intensive techniques of air monitoring and measurement of biomarkers within an epidemiologic study population. The more accurate information obtained in the nested study can be used to estimate the error associated with the questionnaire method applied to the entire study population.

In the assessment of exposures to acidic aerosols and photochemical oxidants, another mixture considered by workshop participants, questionnaires would be of little value for estimating the concentrations in microenvironments. However, questionnaires would be useful for determining the time–activity patterns, the time spent outdoors, and the level of physical activity (*29*). Although acid aerosol and photochemical oxidants are primarily outdoor contaminants, there are indoor sources such as kerosene heaters, which may produce acid species, and malfunctioning air cleaners, which may emit ozone. The importance of these sources could be assessed by questionnaires. In addition, contaminants from outside can penetrate the building at a rate determined largely by the type of building and the air treatment equipment. Questionnaires can provide some information on these factors.

Questionnaires also have been used widely to provide retrospective assessment of exposures in the examination of the relationship between lung cancer and ETS and residential radon exposures in case–control studies. The questionnaires are used to assess ETS exposures in several microenvironments, to obtain residential histories, to estimate the time spent in each residence, and to determine other sources of exposure. This kind of questionnaire data is subject to both random and nonrandom sources of bias (*39*).

Exposures to VOCs, another example mixture, cannot be assessed readily by questionnaire. Volatile organic compounds are emitted from a large number of sources, and exposures occur in nearly all microenvironments. However, questionnaires can be used to assess the presence, absence, or use of potential sources such as cleaners, paints, new carpets, or dry cleaning, and indicate potential exposure. In studies of sick-building syndrome, such questionnaires form a major part of the exposure assessment component of the study (*40*).

Biomarkers of Exposure

Biomarkers show promise in epidemiologic studies as indicators of internal dose, biologically effective dose, early biological effects, altered function, and clinical disease (*41,42*). Within the context of assessment of exposure to air contaminants, biomarkers of exposure refer to cellular, biochemical, or molecular measures that are obtained from biological media such as human tissues, cells, or fluids and are indicative of human exposure to air contaminants (*10*). The markers are indicators of changes or events in human biological systems (*10*) and include indicators of both the internal dose and the biologically effective dose. A measure of internal dose indicates the amount of the contaminant absorbed into the body over a period of time. It is also a measure of the contaminant itself or its metabolites—for example, lead level in blood; cotinine or nicotine levels in urine, blood, or saliva; and concentrations of VOCs in exhaled air. The biologically effective dose refers to the amount of the contaminant or active metabolites delivered over a period of time to the target site. Some markers of biologically effective dose include protein adducts, DNA adducts, and sister chromatid exchange.

Recently, the use of biomarkers of exposure in epidemiologic studies has been discussed as offering methodology that may be useful in *a*) assessing the integrated exposure from all routes of entry (total exposure); *b*) reconstructing exposures; *c*) reducing error in respondent-provided exposure information resulting from biased recall, deliberate misinformation, inability to remember, and lack of knowledge; *d*) reducing exposure-associated misclassification and thereby enhancing study power; *e*) describing exposure–dose–response relationships, particularly when the target contaminant and its metabolic by-products can be identified and measured, for example, as with carbon monoxide or lead; *f*) identifying individuals or populations at risk through high exposure; and *g*) providing an independent measure of exposure for validating other measures (such as questionnaires or models).

The relationship between the biomarker and exposure may be complex. It might vary with other environmental factors such as sources and activities and with the uptake, distribution, metabolism, location, and mode of action of the compound or compounds of interest. Biomarkers of exposure are indicators of dose and do not directly represent actual environmental exposures. Although external measurements and pharmacokinetic or pharmacodynamic models are needed to estimate quantitative exposure from measured biomarkers, biomarkers do provide an indication that exposure has occurred.

To be useful, a biomarker of exposure for an air contaminant should be chemically specific; detectable in trace quantities; measurable in samples obtainable by noninvasive techniques; inexpensive to collect, handle, and assay; and quantitatively associated with exposures encountered in the community setting (*10*). The utility of a biological marker in an epidemiologic study also depends on its biological relevance, the level of understanding of its pharmacokinetics and pharmacodynamics, the temporal relevance of the marker to the exposure of interest, its background levels, and the feasibility of its application. Additional considerations for using biomarkers for complex mixtures include the uniqueness of the marker for the mixture; the relation of the marker to concentrations of other components; and the relation of the marker to the uptake, distribution, metabolism, location, and mode of action of the other compounds.

Assessment of exposure to environmental tobacco smoke demonstrates the advantages and disadvantages of using biomarkers as indicators of exposure to complex mixtures. Many biomarkers have been proposed as indicators for ETS (*2,3*), including thiocyanate, carboxyhemoglobin, nicotine, cotinine, *N*-nitrosoproline, aromatic amines, and protein or DNA adducts. Although these biomarkers indicate that exposure has taken place, they may not indicate the contaminants in the mixture that cause the adverse effect under study. Available biomarkers for ETS also show considerable variability between individuals, and most of them capture only short-term exposures. These markers have not been evaluated adequately in controlled conditions for sensitivity, specificity, reproducibility, and relation to air exposures at realistic environmental levels. Some of the markers, such as carboxyhemoglobin, thiocyanate, and DNA adducts, are not specific to ETS exposure, while others, such as thiocyanate and carboxyhemoglobin, are not sufficiently sensitive for the concentrations of ETS typically encountered. Nicotine and its metabolites, principally cotinine, are used widely as specific biomarkers of exposure to ETS. However, we lack data that relate the levels of these biomarkers to air exposures in different environments, such as the work place and the home, or to long-term exposures. Cotinine, however, has been useful for validating questionnaires and identifying high- and low-exposure groups.

Exhaled levels of specific VOCs recently have been explored for use as biomarkers of exposure to complex mixtures of VOCs (*10*). Measurements of exhaled VOCs can indicate that exposure to specific compounds has taken place. However, the relationships between levels of individual VOCs and the complex mixtures present in different microenvironments are undoubtedly complex and variable and depend on uptake, metabolism, and excretion of the compounds.

At present, biomarkers cannot be used alone as indicators of exposure to complex air contaminant mixtures in epidemiologic studies because they do not provide sufficient information on the frequency, duration, and magnitude of exposure. However, they may provide insights into dose–response relations in the population under study; and they can reduce misclassification of exposure to specific compounds. Biomarkers only provide an indirect measure of exposure and should be used in combination with direct measures, such as air sampling, questionnaires, and models.

Integration of Exposure Methods

The selection of one or more methods of assessing exposure for an epidemiologic study should consider the specificity of the stated hypothesis, identification of the complex mix of contaminants or sources, and the existing state of knowledge. When designing an exposure assessment protocol, it is important to consider many issues like the available resources (such as finances, work force, air sampling equipment, and laboratory analytical support), the size of the study population, the willingness of the subjects to participate, the time frame for completing the study, the suitability of the exposure methods available (biological markers, air monitors), and the acceptable level of uncertainty in the assessment of exposure.

No single method of assessing exposure to complex mixtures is without drawbacks. Personal monitors can provide only a measure of individual compounds; they cannot determine the microenvironments in which the exposures take place or provide information on the factors controlling the concentration. They also represent a respondent burden. Biomarkers of exposure, although provided an indication of dose, may not be related readily to exposures, particularly exposures to complex mixtures. Biomarkers may also be limited by inadequate sensitivity and specificity, and they require costly and invasive approaches for specimen collection. Air monitoring of different microenvironments tends to be compound-specific and needs to be combined with time–activity

information to generate estimates of personal exposures. Questionnaires, as we have discussed, are subject to error and can introduce misclassification of exposure status.

The goal of any exposure assessment effort made in support of epidemiologic studies of the effects of complex mixtures is to provide sufficiently accurate and precise measures of exposure and dose in a cost-effective manner. Strategies are needed to integrate and utilize the strengths of the various exposure assessment methods. One such approach, the nested exposure assessment strategy (*1,38*), utilizes questionnaires to acquire an easily measured indicator of exposure on the whole population under study, while simultaneously obtaining more detailed exposure information by using more sophisticated and expensive measurement techniques such as personal monitoring and biomarkers, on ever-decreasing numbers of subjects (Fig. 3). In this strategy, the questionnaires provide a measure of exposure with a higher level of uncertainty; the more intensive measures provide a lower level of uncertainty. The more intensively monitored groups could be randomly selected or purposefully sampled to address specific exposure issues. Measures of exposure in the intensively monitored subgroups could then be used to model the exposure to the full population and to provide an estimate of the magnitude and direction of the uncertainty associated with exposures estimated from the questionnaires. Additional exposure issues which could be addressed in different nested studies include the generation, transport, and fate of compounds; the development and validation of predictive models; the evaluation of monitoring techniques; the relation between average and peak concentrations; and the evaluation of, or relation between, air exposures and biomarkers of exposure.

The nested exposure assessment strategy could, for example, be utilized in assessing exposures to ETS and NO_2 in a study of the effect of these pollutants on respiratory infections in children. At the first level of exposure assessment, biweekly telephone questionnaires could be used for the whole study population to acquire respondent-estimated exposure to passive smoke and NO_2 in different environments. This would be accomplished by asking questions about the number of cigarettes smoked in the home, the location in the home where they are smoked, gas stove and kerosene heater use, and the time spent in the environments (outdoors, day care, school, and home). During one 2-wk period, a sample of respondents (second level of monitoring) could receive, in addition to the questionnaire, passive monitoring for vapor phase nicotine (an ETS marker) and NO_2 in one or several locations in the home. At this level, sampling might be conducted several times during the course of the study to as certain temporal variations. The third level of exposure assessment, conducted on a smaller sample, could include, in addition to the measures employed in the first and second levels, more detailed assessments such as personal passive monitors for nicotine and NO_2, passive monitoring in environments other than the home, and time and activity diaries. A fourth level of assessment, conducted on a still smaller sample, could employ yet a greater level of exposure assessment detail by acquiring urine samples for cotinine analysis, continuously monitoring NO_2 and respirable particles, collecting source–use diaries, counting or collecting cigarette butts, and measuring ventilation rates, all in addition to the measures obtained in levels one through three.

The example mixtures considered in these papers (VOCs, radon and ETS, ETS and NO_2, and photochemical smog and acidic aerosols) exemplify the types of mixtures that are of present public health concern as well as the difficulties of characterizing population exposures to complex mixtures. Exposure characterization studies have been conducted for individual components of complex mixtures, such as radon, ETS, NO_2, O_3 and a limited number of VOCs (*3,10,23,29,30,43,44*). The extent of the information is limited, and not all relevant microenvironments have been adequately assessed.

Population-based data are not available for any of the four mixtures. Further, few epidemiologic studies that involved comprehensive multiple-contaminant monitoring, particularly with personal monitoring, have been performed. The most comprehensive investigation to date is the Harvard Six Cities Study, and even in that study, selected participants wore monitors for only brief durations during the studies of short-term health responses. The exposure estimates in the Six Cities Study were based primarily on microenvironmental studies of the outdoor air (*45*).

Summary

During the last decade, substantial progress has been made in developing methodologies for assessing exposures to specific environmental contaminants in inhaled air. The newer techniques include personal monitoring, microenvironmental models, and biomarkers of exposure. We also have recognized that measurement error is inherent in most exposure measures used in epidemiologic research; approaches have been developed for minimizing this error and for evaluating its consequences.

While these advances have been incorporated effectively into studies of the health effects of single contaminants, the assessment of exposures to complex mixtures of air contaminants continues to present a formidable challenge. At present, no immediate and major advance in methodology that will offer resolution to the problems of estimating exposure to complex mixtures can be anticipated. We suggest that progress can be made through more effective application and continued evolution of already available methods, for example, *a*) development and validation of standardized questionnaires on

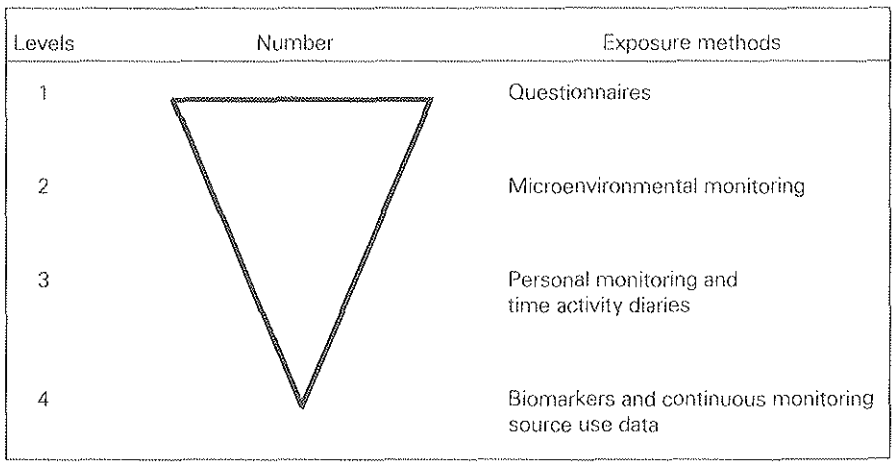

Figure 3. Representation of "nested" exposure assessment strategy that utilizes questionnaires to acquire an easily acquired measure of exposure in the whole study population, while simutaneously obtaining more detailed exposure information by using more sophistticated techniques on ever-decreasing numbers of subjects.

sources, source use, building characteristics, and interactions of subjects with indoor environments; *b*) development and validation of prediction models for concentrations in the most frequently encountered microenvironments and for personal exposure estimates; *c*) continued development and critical evaluation of personal monitors and biomarkers for complex mixtures; and *d*) development of efficient statistical designs for nested assessment of exposures using the more intensive and accurate techniques. The needed advances will be best achieved by interdisciplinary teams that include epidemiologists, statisticians, and persons with expertise in exposure assessment and monitoring.

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Complex Mixtures and Indoor Air Pollution: Overview of Epidemiologic Methods

Noel S. Weiss

Department of Epidemiology, School of Public Health and Community Medicine, University of Washington, Seattle, WA 98195, and Division of Public Health Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA 98104

The likelihood of an epidemiologic study correctly identifying an adverse health outcome associated with exposure to indoor air pollutants is increased if *a*) substantial variation exists in the frequency or level of exposure among study subjects otherwise at similar risk of the health outcome; *b*) the number of study subjects or study communities is large; *c*) the health outcome can be assessed with accuracy; *d*) relevant exposure levels can be measured with accuracy; *e*) an unbiased sample of exposed and nonexposed subjects is selected for study; and *f*) other determinants of the adverse health outcome can be measured. Nonetheless, given a strong enough impact of exposure to one pollutant or a mixture of pollutants on the risk of illness, it is possible for epidemiologic studies to discern a relation even if only some of the above circumstances are present. — Environ Health Perspect 101(Suppl 4):179–181 (1993).

Key Words: Air pollution, epidemiology

Epidemiology can be thought of as the study of the variation in disease occurrence and of the reasons for that variation. Operationally, it involves making observations in individuals or groups of individuals on the rates of disease associated with different levels of an exposure or characteristic, followed by inferences concerning the basis for any differences in rates seen. At its simplest, epidemiology can involve nothing more than seeking to correlate published rates of illness in various population groups with levels of present or past exposure in such groups. However, it generally is true that stronger inferences can be based on studies of the occurrence of illness in exposed and nonexposed individuals. Such studies occasionally involve randomization of individuals to differing environmental exposures to determine if the subsequent rate of illness (or marker of illness) differs among exposure groups. More commonly, no randomization is done, but investigators simply observe rates of illness in persons who happen to have differing levels of exposure (cohort studies). Also, especially for health outcomes that are uncommon, it is possible to identify persons with and without a disease and attempt to retrospectively resurrect exposures that persons in each group had sustained (case–control studies).

For an epidemiologic study to provide useful information regarding causes of the disease, several circumstances need to be met. These circumstances are discussed below:

A. *Among individuals otherwise at similar risk of the disease, there exists substantial variation in the frequency or level of exposure.* From an epidemiologist's point of view, this circumstance is best met when the variation occurs within members of a community (e.g., the presence of both cigarette smokers and nonsmokers in a given population). However, when dealing with certain exposures (e.g., outdoor pollution from acid aerosols and oxidants or from arsenic), the exposure may be communitywide, with little variation among individuals within that community. In this situation, it becomes particularly necessary to make comparisons among populations of differing exposure status (e.g., air pollution levels) rather than among individuals within the same population. In many instances, comparisons among populations are facilitated by the fact that routine data are available for a variety of health outcomes (e.g., mortality, cancer incidence) on a large number of populations over a long period of time. Nonetheless, studies that compare populations rarely can be used for anything but the generation of hypotheses regarding disease etiology, because a substantial degree of movement of individuals between communities occurs in most parts of the world in which these studies are likely to be conducted. This would generally be expected to dilute any true association between communitywide exposures and disease occurrence. Also, other bases for a difference in rates among populations are often quite hard to measure and therefore cannot be taken into account when looking at the exposure of interest.

For these reasons, some investigators have attempted to study communitywide exposures on health outcomes by returning to the study of individual persons within a community, exploiting the substantial degree of migration that would have occurred in years past. For example, in a study of cancer in relation to ingestion of asbestos in drinking water, Polissar et al. (*1*) compared persons with and without cancer who resided in one western Washington county. These cases and controls were contrasted with respect to the amount of time they had lived in those particular areas of western Washington in which there had been an extraordinarily high concentration of asbestos in the water supply. Clearly, this approach can be successful only if the induction period of the disease from the exposure in question is reasonably long.

For residential exposures that truly do vary within a community, this tendency of persons to change households frequently will act to minimize variation among individuals in that community. For example, Lubin et al. (*2*) note that among Americans in the 1980s there had been a change of household on the average of every 5 years. If one were attempting to study cancer in relation to household radon exposure, for example, movement between households of differing radon levels would tend to neutralize the more extreme differences that might be present if individuals had resided in a single household for a longer period of time.

Occasionally, there will not only be interindividual quantitative differences in exposure (e.g., levels of intensity or duration

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of exposure) but qualitative differences as well. Studies of individuals (or groups of individuals) who vary with regard to type of exposure can suggest what aspect of exposure might be important in disease etiology. For example, the observation that occupational exposure to amphibole, more than chrysotile, asbestos is associated with a particularly high risk of mesothelioma and lung cancer (*3*) has *a*) provided hypotheses regarding the pathogenesis of asbestos carcinogenicity and *b*) served, in some countries, as the basis for different standards for permissible workplace air levels of amphibole versus chrysotile asbestos.

B. ***Whether of individuals or communities, the number of units being compared need be large enough to reliably identify an adverse health effect of the exposure if one is present.*** If one or more indoor air pollutants have a substantial relative impact on the occurrence of a disease, it generally is possible to identify this in a study of but modest size. For example, once mesothelioma was identified as such, a study of only a small number of individuals with and without this condition was needed to determine that inhalation of asbestos fibers was associated strongly with its occurrence. However, for many indoor air pollutants, there are reasons to believe that the true impact on disease occurrence, if any, would be small in magnitude given the relatively low levels of exposure to these pollutants and the limited variation in exposure to them in members of the population. The detection of small relative increases in disease incidence can require a study that includes a very large number of subjects, even if exposure status can be measured accurately and possible confounding factors can be taken into account. Some strategies for achieving a large number of subjects have included combining in a single study exposed groups that are scattered over a wide geographic range. For example, in attempting to evaluate the influence of occupational inhalation of formaldehyde on the occurrence of lung and other forms of cancer, individuals exposed to formaldehyde in a number of different work settings and industrial processes in a variety of locations in the United States were enrolled in a collaborative study (*4*). By means of meta-analysis (*5*), one can formally aggregate the results of multiple studies that pertain to the health impact of a particular exposure.

C. ***The health outcome can be assessed with accuracy and in an unbiased way.*** Obviously, the inability to recognize distinctive pathologic process as such will impair our ability to recognize the determinants of that process. It was not until the last half of this century that mesothelioma was identified regularly as being present in patients who truly had this malignancy. Had mesothelioma been routinely diagnosed in earlier years, undoubtedly our understanding of the carcinogenic potential of asbestos fiber inhalation would have been achieved earlier as well.

Inaccurate assessment of health outcomes also can give rise to false positive associations with respiratory exposures. This is particularly true when the outcome is defined solely on the basis of symptoms. When knowledge of a person's exposure status could influence his or her reporting of these symptoms, great care has to be taken to standardize assessment between exposed and unexposed subjects. Occasionally, it will be necessary to focus the analysis on the occurrence of symptoms of great severity. For example, in their study of possible neurologic sequelae of swine flu vaccination, Marks and Halpin (*6*) labeled only patients with bilateral lower motor neuron weakness of acute onset as having Guillain-Barré syndrome. They feared that, because of the concern that many patients and their physicians had regarding this vaccine, less specific neurologic illnesses would be identified more completely in vaccinated than in unvaccinated persons.

D. ***Exposure levels can be (or have been) measured accurately and at the appropriate time relative to the induction period of the disease under study.*** In many studies, whether cohort or case–control in type, the cases of disease have occurred already by the time of the study. Exposures that have occurred earlier in time need to be assessed. One way of doing this is to ask subjects, both those with and without disease, about their prior exposures. An advantage of this approach is that information can be sought about several different time periods. The primary disadvantage of the approach, however, is the relative imprecision with which the information generally can be provided. While persons might know they have been exposed to some extent to environmental tobacco smoke, for example, they would find it difficult to quantify this exposure in an accurate way. For other types of exposure (e.g., radon), no subjective assessment is possible. Direct measurements of present exposures can be made, but responsibility falls on the investigator to take steps to assess their comparability to exposures that the subject sustained in the past. For some (e.g., residential radon), this is more feasible than for others, because prior radon exposures can be estimated from present ones given the known decay of this element combined with additional information on structural and other alterations to the residence.

At first glance, it would seem that studies in which measurements are made at the time the study begins, with subsequent monitoring of the occurrence of illness, would have substantial advantages over those that try to ascertain exposures in a retrospective way. However, there are at least two important limitations of these prospective studies: *a*) Unless the follow-up period is very long, the study population very large, or the disease under study very common, the number of health outcomes that occur may be small and may yield highly tentative results. *b*) Depending on the length of the induction period for the disease, single measurements made at the start of the study may not be relevant for long to disease occurrence. For example, in their prospective study of environmental tobacco smoke in relation to the occurrence of fatal coronary heart disease, Garland et al. (*7*) assessed exposure to spouse's smoking via an interview. Among members of this cohort, the occurrence of fatal heart disease was then monitored during the next decade but with no additional information regarding continued exposure to spouse's smoking. If exposure to environmental tobacco smoke predisposes to the occurrence of fatal heart disease through a relatively short-term mechanism (perhaps via acute toxicity of elevated levels of carboxyhemoglobin), this research approach would be a relatively insensitive means of addressing the hypothesis, given the occurrence of changes in the exposure to spouse's smoking during the extended follow-up.

E. ***An unbiased sample of exposed and nonexposed individuals has been selected for study.*** While this is a concern in any study, it is a particular problem for those that are cross-sectional in nature. In such a study, exposed and nonexposed individuals are contrasted for their prevalence of disease. A seriously biased underestimate of the health impact of the exposure will be obtained if persons who have suffered disease because of the exposure are no longer present at the time of sampling (e.g., through premature retirement from a hazardous occupation or due to death). For example, in the 1940s, Fleischer et al. (*8*) noted only a low prevalence of asbestosis among men who had been employed as pipe coverers in a shipyard and who, through this employment, had been exposed to asbestos. Undoubtedly, the selective removal from employment of those who already had been affected by asbestos led to

the overly optimistic conclusion by the authors that there was little to be feared in terms of levels of asbestos exposure present in that occupation at that time.

F. *Other factors besides the exposure in question that relate to the occurrence of disease have been (or can be) measured as well.* Measurement of such factors will enable, first, the control of potential confounding effects of these other variables (and thus the prevention of the distortion of the true association between the exposure and disease). For example, in a study of respiratory infection during childhood in relation to exposure to environmental tobacco smoke and nitrogen dioxide, it would be important to ascertain such things as exposure to infected individuals, household crowding, etc. Second, the characterization of other exposures can enhance the power of the analysis by allowing an examination of the effect of the exposure in question according to the presence or absence (or level of) other risk factors for disease. If, for example, domestic exposure to radon were a cause of lung cancer only in the presence of active cigarette smoking, an analysis that failed to examine the association separately in cigarette smokers and nonsmokers would provide a blurred result. On the other hand, if domestic radon exposure and cigarette smoking acted via separate causal pathways to produce the disease (as appears at least in part to be the case for occupational radon exposure and cigarette smoking) (*9,10*), then the relative impact of exposure to domestic radon would be far more discernible in nonsmokers with their low background rate of lung cancer than among cigarette smokers in whom there is a high background rate (*11*).

Conclusions

The foregoing has indicated some of the major threats to the sensitivity and validity of epidemiologic studies of the health consequences of indoor air pollution. While these threats are real, it would not be prudent to allow their specter to paralyze prospective investigators and discourage them from performing research in this area. Not all of the above criteria need be met in order for a study to produce some useful information. For example, the hypothesis that military service during the Vietnam war era predisposed people to the subsequent occurrence of suicide received strong support from a study (*12*) that found an increased rate of suicide among men whose birthdates made them eligible to be drafted during that time. Despite the great imprecision with which actual military service in Vietnam was assessed (it is estimated that only 25% of individuals with draft eligible birthdates even entered the armed forces) and the modest size of the association (the study observed a relative risk of 1.13), the randomized nature of the investigation and its ability to neutralize the effect of potential confounding variables made for convincing results.

Imprecise exposure assessment also was a problem in a cancer registry-based study of the hypothesis that homosexual men are at increased risk for the occurrence of anal cancer (*13*). Registry data do not provide information on sexual preference, but they do contain data regarding marital status. The investigators found that the percentage of men with anal cancer who had never been married was more than three times that of demographically comparable men with colon or rectal cancer. Of course, being a single male is hardly an accurate predictor of homosexual preference. Nonetheless, given the exceedingly strong association between a history of anal intercourse and anal cancer (found subsequently in response to the registry-based study), even a study that measured exposure status as imprecisely as this study was able to make a contribution.

The important findings in these last two studies, studies that had serious flaws as measured by the criteria that have been put forth here, should serve to dispel the notion that only perfect studies will permit progress toward understanding the harmful effects of indoor air pollution on health. Imperfect studies, properly interpreted, are far better than none at all.

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Epidemiological Studies of Neurotoxic, Reproductive, and Carcinogenic Effects of Complex Mixtures

Carl M. Shy

Department of Epidemiology, School of Public Health CB7400, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599-7400

Neurotoxic, reproductive, and carcinogenic effects are potentially important health end points in epidemiological studies of complex mixtures, particularly when such mixtures contain volatile organic compounds or trace metals. Epidemiological studies of neurotoxicity often will require direct clinical, behavioral, and/or physiological testing of study subjects, because these effects are likely to be subtle and not identifiable as clearly defined diseases. Peripheral nervous system toxicity can be assessed by clinical neurologic examinations, by electrophysiological tests of nerve conduction, and by physiological tests of thresholds for neurosensory perception, though these tests require considerable standardization for use outside the clinical setting, and most of the available tests have not been assessed for their utility in detecting effects of neurotoxic exposures. Neurobehavioral effects of exposures to solvents, as examples of complex mixtures, have been studied widely; but batteries of tests are often used, and these have not been well standardized and are generally unfamiliar to most research investigators in this area. Recently standardized neurobehavioral test systems developed by the World Health Organization and by a U.S. group for use in field studies, show promise in detecting neurobehavioral effects at relatively low environmental exposures. Similarly, new and sensitive measures of disturbed reproductive function, such as time-to-conception and biochemical indices of early pregnancy loss, are affected by some low-concentration environmental agents; but those measures have not yet been applied to studies of complex mixtures. Because of the long latency problem and small expected relative risks, population- or community-based studies of the carcinogenic effects of complex mixtures are unlikely to yield data of adequate quality to justify more than exploratory studies of carcinogens in ambient air. — Environ Health Perspect 101(Suppl 4):183–186 (1993).

Key Words: Neurotoxicity, reproductive effects, cancer, indoor air, air pollution

Introduction

The objective here is to consider the feasibility of performing and using epidemiological studies of neurotoxic, reproductive, and carcinogenic effects in populations to assess the human health consequences of exposure to indoor air and other complex mixtures of air pollutants. The rationale for considering effects other than nonmalignant respiratory effects of air pollutants may be quite obvious but worth expressing: The primary effect of the most commonly studied air pollutants, such as fine particulates and ozone, is directly on respiratory tissue. However, carbon monoxide and lead are two primary air quality pollutants that affect other organ systems. Mixtures of air pollutants containing these compounds or other trace metals or volatile organic compounds also are likely to induce nonmalignant effects in organ systems other than the respiratory. Epidemiological tools are now reasonably available to study neurotoxic, reproductive, and carcinogenic effects. Effects on other tissues or organ systems such as the liver, kidney, skin, endocrine, and immune systems also are possible, but considerable methods development is necessary before these categories of effects can be studied systematically in populations.

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Neurotoxic Effects

Sources of Data

Unlike cancer and birth defects, there are no regional or national registries for neurological diseases. Medical records from hospital admissions or medical insurance claims may be acceptable sources for studies of environmental factors in the etiology of defined diseases such as Parkinson's or Alzheimer's, but the major problem in epidemiological studies of these chronic neurological disorders is the probable latency between etiological exposures and disease manifestation. Some neurotoxic effects, such as disturbance in cognition or in nerve conduction, may be linked closely in time to environmental exposures, but these effects are likely to be subtle and insidiously manifested, and they are not identifiable as clearly defined diseases. Acute pesticide poisonings, usually due to organophosphate toxicity, are reported in some states, but reported episodes are thought to underestimate greatly the true incidence of such events. In general, in contrast to cancer and some reproductive effects, epidemiological studies of neurotoxicity induced by complex mixtures will require direct clinical, behavioral, and/or physiological testing of study subjects by the investigator. Exceptions to this general statement are potential case–control studies of defined clinical neurological diseases such as Alzheimer's, Parkinson's, and other entities, though there is little likelihood that retrospective estimates of determined exposures to complex mixtures could be satisfactorily for studies of such diseases.

Range of Neurotoxic Effects and their Measurement

For this discussion, it is assumed that typical ambient and indoor concentrations of complex mixtures containing neurotoxic components will not cause acute toxicity, but rather that the investigator will attempt to evaluate the effect of low concentrations of agents such as solvents, agricultural chemicals, other volatile organics, or trace metals in the indoor or ambient environment. Two primary classes of neurotoxic effects can be studied in population groups: peripheral nervous system toxicity, and neurobehavioral impairment reflecting central nervous system toxicity.

Peripheral nervous system toxicity can be assessed by clinical neurological examination, by electrophysiological tests of nerve conduction velocity, and by tests of

thresholds for neurosensory perception (e.g., visual, hearing, odor, and cutaneous vibration thresholds). The clinical neurological examination has the disadvantage of requiring highly skilled clinicians who are notoriously difficult to standardize in their procedures, and the results are only semiquantitative at best. There is no evidence that clinical examinations have detected early effects of neurotoxic exposures. Tests of nerve conduction velocities are fully quantitative and can be performed by trained technicians using standardized techniques. Such tests have been used to detect early effects of increased lead exposures among workers (*1,2*). A distinct disadvantage of nerve conduction tests is that they can be uncomfortable and therefore poorly received by test subjects. Tests for changes in hearing thresholds are very common and well standardized but have not been used often in epidemiological investigations of potential neurotoxic exposures. Tests of thresholds for visual, odor, and cutaneous vibration thresholds (*3*) are used less frequently, but the potential for standardization and use by trained technicians is clearly present. There is as yet little data for assessing the utility of neurosensory threshold tests for detecting the effects of environmental neurotoxin exposures.

The neurobehavioral effects of exposure to solvents (*4–6*) and to lead (*7,8*) have been evaluated in a considerable number of epidemiological studies. A wide range of impairments can be addressed, such as alterations in memory, learning, cognition, mood, attention, and neuromuscular performance (e.g., eye–hand coordination). Neurobehavioral function can be studied by means of questionnaires and by objective, physiological, and psychological tests. A major problem with their use in epidemiological studies has been a lack of standardization of test batteries and a lack of familiarity with the use of these tests for field studies. These problems are being addressed, however, at the national and international level. The World Health Organization (WHO) (*9*) has developed a well-validated neurobehavioral test battery, the Neurobehavioral Core Test Battery, comprising measures of auditory memory, affect, manual dexterity, visual perception and memory, attention and response speed, and perceptional motor speed. The disadvantage of the test battery is that it requires 50 min per subject and a highly trained test administrator. A second test battery has been developed in the United States (the Neurobehavioral Evaluation System [NES]) by Baker et al. (*10*). This battery tests the same functional domains as the WHO Core Battery, requires the same length of time, but is computerized and thus does not require the presence or administration of a highly trained test administrator. The NES holds great appeal because multiple subjects can be tested simultaneously and results are scored and tabulated immediately by computer linkage.

Epidemiological Applications

Cross-sectional studies of lead-exposed workers have shown a variety of neuropsychological effects quantitatively related to blood lead levels (*11–15*). Needleman et al. (*16*) and Bellinger et al. (*17*) performed longitudinal studies on the growth in cognitive function of children exposed to relatively low to moderate ambient lead concentrations; these studies provide evidence for small but important decrements in the development of cognitive and neurobehavioral function in children who had normal to high-normal blood leads in the first years of life. Relatively few longitudinal studies of lead-exposed adults have been reported. One example is a study by Baker et al. (*7*) suggesting an improvement in neurobehavioral function in a cohort of workers exposed to lead at a foundry in which hygienic conditions were significantly improved.

Several reports from Scandinavia (*18*) illustrate epidemiological studies designed to assess the long-term neurobehavioral effects of complex mixtures, including carbon disulfide in textile plants and solvents in paints. In several studies, psychomotor and intellectual functions of exposed workers were affected, and on the whole, higher intellectual functions seemed to be more affected by exposure than psychomotor performance. Gamberale (*18*) notes that while some aspect of neurobehavioral performance was found to be impaired in the great majority of studies, differences in test results between exposed and nonexposed groups of workers sometimes disappeared when the groups were matched on intellectual level. Differences still persisted in tests of reaction time, which are not correlated with intellectual level.

Because our focus of concern here is on low-level indoor and ambient exposures, it is more likely that serial tests of the same persons will be necessary to detect neurotoxic effects. Investigators will have to identify population groups, particularly workers, in whom preexposure and postexposure test results can be compared. Alternatively, results obtained during and after termination of exposure may be compared. This strategy avoids some of the serious selection biases encountered in cross-sectional studies of populations, in which exposure can induce selective loss of susceptible persons. A distinct advantage of neurotoxic studies is that the tests may be sensitive enough to detect effects with a minimal latency, thus allowing estimates of exposure to be made from concurrent measurements. Like cross-sectional studies, case–control strategies have the inherent problem of addressing past exposures; and for many neurological impairments, cases are difficult to identify from medical records.

Recommendation

Because volatile organic compounds and trace metals may often appear as components of complex mixtures and because these substances include known or potential neurotoxins, neurotoxic effects should be evaluated intensively in exposed populations. Standardized test batteries are now available for such studies, but they have not been used widely; and although standardized, their efficacy for evaluating low-level neurotoxic exposures has not been validated. The topic is broad and important enough to warrant its own workshop. Multiple issues need to be addressed, such as training of neuroepidemiologists, dissemination of information on the availability and applications of neurotoxic testing, methodological studies to determine whether some subsets of the lengthy test battery are appropriate particularly for low-level combined mixtures, and requests for applications (RFAs) to foster interdisciplinary epidemiological studies of neurotoxic exposures. Neuroepidemiology is not a developed subspecialty of epidemiology compared with levels of activity in the developing areas of biochemical, reproductive, and pharmacoepidemiology, to say nothing of the well-developed fields of cardiovascular, cancer, and infectious disease epidemiology. Several federal agencies have a legitimate reason for promoting the development of neuroepidemiological research; these include the National Institute of Environmental Health Sciences (NIEHS), National Institute of Neurological Disorders and Stroke (NINDS), United States Environmental Protection Agency (EPA), National Institute of Occupational Saftey and Health (NIOSH), and Agency for Toxic Substances and Disease Registry (ATSDR). The Health Effects Institute could bring together representatives from these agencies, along with appropriate academic disciplines, in a workshop on the development and use of neuroepidemiological studies of environmental exposures.

Reproductive Effects

Source of Data

Several states now have birth defects registries. Some reproductive outcomes, such as birth weight and gestational age, are listed on birth certificates. Hospital and clinic records are sources of information on clinical spontaneous abortions, complications of pregnancy, (preeclampsia, *abruptio placenta*, etc.), length of gestation, APGAR scores at birth and overt congenital defects at birth. An increasing number of ultrasound tests and amniocenteses are being performed *in utero*, and these tests provide additional data on fetal development.

Range of Reproductive Effects for Use in Epidemiological Studies

Although birth defects, birth weight, and clinical spontaneous abortions are the most frequent reproductive outcomes considered in epidemiological studies, they may not be sensitive to low-level exposures. Detecting changes in birth defect rates requires very large study populations, and thus studies of environmental exposures and birth defects are often infeasible. Birth weight is a quantitative outcome and, as such, provides adequate sample sizes for detecting small effects. However, racial and socioeconomic factors are strong epidemiological determinants of birth weight, and there is little evidence that environmental chemicals contribute to variations in the population distribution of birth weights. Clinical spontaneous abortions probably reflect less than half of all spontaneously terminated pregnancies; epidemiological methods now exist to detect very early pregnancy losses but have not been applied to study environmental exposures. Several studies have used time-to-conception as a quantitative measure of subfecundity and have demonstrated an effect of cigarette smoking (*19*) and of the anesthetic gas, nitrous oxide (*20*). Disturbances of menstrual cycles and of sperm counts and sperm mobility are known to be induced by environmental factors, but these effect measures also are relatively new and are not validated. Overall, rapid developments are taking place in the reproductive biology disciplines, and these are providing new tools for epidemiological assessment of reproductive effects. Several of these tools are now ready for application to populations exposed to potential reproductive toxins in the environment, and the number of qualified investigators and of studies in progress indicates that this will be a fruitful area of research throughout this decade. The entire spectrum of reproductive effects from preconception events through conception, pregnancy, and early childhood development is essentially unexplored in terms of sensitivity to environmental exposures.

Recommendation

Epidemiological measures of reproductive effects are available, and their utility in epidemiological studies has been well demonstrated (*21*). On a national scale, more effort is being put into reproductive epidemiology than into neuroepidemiology. However, the newer and potentially more sensitive measures of reproductive function, such as biochemical indices of early pregnancy loss and menstrual cycle disturbances, have not been applied to environmental exposures. Some distinct advantages of these end points are that latency is short, contemporaneous exposures are relevant, and they are common events. Although laboratory research on specific exposures and reproductive outcomes is clearly desirable, human studies need to proceed simultaneously because environmental complex mixtures cannot be readily reproduced in the laboratory, and variations in human versus animal susceptibility are not understood well enough to make confident extrapolations from animal models. Therefore, applications of the newer and potentially more sensitive measures of reproductive outcomes to selected population exposures should be encouraged. Methodological and applied epidemiological research is needed across a broad range of exposures.

Cancer

Sources of Data on Cancer Incidence and Mortality

The majority of states now have statewide cancer registries, though many of these are in the first years of implementation. Tumor registries at teaching hospitals and medical records at all hospitals are important data sources for case–control studies of cancer risks. However, the major obstacle to effective use of these registries and data bases is the time lag between first diagnosis and entry of cases into the registry. To obtain useful information on environmental and occupational exposures of cases, often it is necessary to interview cases directly and to do so while they are still able to be interviewed. Thus, the time lag between diagnosis and interview is critical, and requires case identification more rapidly than most cancer registries function. Ideally, cases would be interviewed while they are still in the hospital for their first evaluation. Methods have been used at some research centers to administer standardized questionnaires to all patients admitted for a cancer work-up, and these data have been fruitful for exploring various environmental risk factors. However, the most abundant source of evidence on specific environmental risk factors has been industrial cohort studies in which exposure estimates are based on the work history of cases and controls as recorded in the historical personnel record of employees.

Source of Data on Exposure to Carcinogens in a Complex Air Environment

With a few exceptions, population or community-based studies in contrast to occupational cohort studies are unlikely to yield exposure data of adequate detail or quality to justify more than exploratory studies of carcinogens in ambient air. The major obstacles to definitive studies in the general community environment are long latencies; small expected relative risks; a low proportion of the population exposed to specific and clearly defined complex mixtures; difficulty in estimating these exposures for past decades with any degree of specificity; and high mobility of populations between various home, work, and geographical environments. A few reasonably common and measurable exposures to environmental carcinogens (proven or potential) have been assessed in community studies (e.g., indoor radon, electromagnetic fields, and environmental tobacco smoke). Most complex mixtures in indoor or ambient air are unlikely to be as common as these three risk factors or as amenable to drawing distinctions between exposed and nonexposed persons in the community environment. The most feasible epidemiological investigations of complex mixtures containing carcinogens are likely to be studies of defined occupational cohorts, among whom at least qualitatively distinct exposure groups can be identified and for whom records of cumulative exposure are available. A relatively recent example is the NIOSH study of laryngeal cancer and acid mist exposures of steel workers involved in pickling operations (*22*).

While occupational cohort and case–control studies offer the greatest promise for analytical epidemiological studies of complex mixtures, there is a need to develop and apply to exposed community-populations studies of the occurrence of biological markers of carcinogenic exposure. Typically, communities adjacent to toxic waste sites or to point sources that process toxic wastes, such as incinerators and chemical treatment facilities, are concerned

about cancer risks. Epidemiological studies of cancer risks per se are practically infeasible in these environments due to inadequate populations at risk, undefined exposures, and short time since first exposure. However, it is feasible to study the biological uptake of some of the associated compounds in these populations. Thus a battery of biological markers might be a useful epidemiological tool to evaluate the potential for human exposures at these sites.

Recommendations

Occupational cohorts should be characterized with respect to exposures to carcinogenic complex mixtures that occur in indoor or ambient air environments. These cohorts should be the first choice for epidemiological studies of the potential carcinogenic effect of these mixtures.

Batteries of biological markers of carcinogenic exposures should be developed for use in epidemiological evaluations of populations exposed to point sources of complex mixtures containing suspected human carcinogens.

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Epidemiologic Study Design for Investigating Respiratory Health Effects of Complex Air Pollution Mixtures

Douglas W. Dockery

Environmental Epidemiology Program, Harvard School of Public Health, 665 Huntington Avenue, Bldg. 1-1414, Boston, MA 02115

Epidemiologic studies of the respiratory health effects of air pollution are intrinsically difficult because exposure is common, expected effects at concentrations found in developed countries are weak, random misclassification of exposure is common, and the respiratory health indicators have multiple etiologies. Exposures to air pollutants also are multidimensional, generally consisting of a mixture of gases and particles. In this paper, epidemiologic study designs are described, and their potential for evaluating effects of complex pollutant mixtures are discussed. Power to detect the independent effects of individual pollutants in a complex pollutant mixture or to measure their interactions is in general very weak unless the study is specifically designed to test such hypotheses. However, with innovative and creative design, the independent and joint effects of multiple pollutants should be estimable in epidemiologic studies. — Environ Health Perspect 101(Suppl 4):187–191 (1993).

Key Words: Epidemiology, study design, air pollution, mixtures, respiratory health effects

Introduction

From its earlier roots, epidemiologists have recognized air pollution as a potentially important determinant of increased morbidity and mortality. In the classic analysis of the Bills of Mortality in 1662 (*1*), Graunt attributed the high week-to-week variability in mortality to changes in the "airs" of London. Modern air pollution epidemiologists have attempted to attribute health effects to specific constituents of these "airs." However, it has become clear that these airs are in fact a complex mixture of contaminant gases and particles.

Methods for epidemiologic studies of the health effects of air pollution have been reviewed comprehensively by the National Research Council Committee on the Epidemiology of Air Pollutants (*2*). This paper builds on that state-of-the-art report, plus discussions by Samet and Lambert (*3*), to consider epidemiologic study designs for assessing health effects of complex air pollution mixtures.

Difficulties in Air Pollution Epidemiology

Epidemiologic studies of air pollution are particularly challenging. Air pollution exposures are universal and, as Rose (*4*) has pointed out:

> the more widespread is a particular environmental hazard, the less it explains the distribution of cases. The cause that is universally present has no influence at all on the distribution of disease, and it may be quite unfindable by the traditional methods of clinical impression and case–control and cohort studies, for all of these depend on heterogeneity of exposure.

The challenge, therefore, is to develop study designs that provide contrasting exposures in natural settings. Given that environmental exposures are generally to multiple pollutants, studies that differentiate response to the air pollution mixture will require careful and innovative designs.

A second problem is that while exposures are common, the risks tend to be low. Environmental controls that have been put in place in the United States have reduced exposures generally to levels below the National Ambient Air Quality Standards. The standards, established by the EPA, and based on the best available scientific data, were set to prevent any adverse health effects, even among the most sensitive members of the general population. Thus, expected health effects of air pollution at concentrations currently observed in the United States should be expected to be weak, that is, with relative risks less than 2 and often less than 1.5 for typical exposures.

At the present time, it is not sufficient to demonstrate that a certain air pollutant, or mix of air pollutants, is associated with an adverse health effect. Adequate information is available to demonstrate adverse health effects at high concentration. Regulators now require quantitative estimates of the exposure–response associations at concentrations below the National Ambient Air Quality Standards to evaluate adequacy of the standards and for risk and cost-benefit analyses as required under the most recent amendments to the Clean Air Act (*5*).

Misclassification of exposure is a particular problem in air pollution studies. Personal exposures to air pollution may differ substantially from ambient air data. Innovative methods have been developed for measuring personal exposures, but these methods are labor intensive and often very intrusive on the participants. Thus, the investigator should expect substantial random misclassification of exposure in designing an epidemiologic study. This means that statistical associations will be weakened and larger sample sizes required. Particular attention should be given to the potential for information bias associated with exposure misclassification.

Adverse health effects of environmental pollutants, and air pollution in particular, are generally nonspecific. For example, the development of chronic-obstructive pulmonary disease is a cumulative process in which air pollution is only one of many factors that produce irreversible loss of lung function. Likewise, reversible changes in lung function, as in asthma, may be triggered by many environmental exposures, including allergens (e.g., house dust mites, pollens, mold spores, fungi, and animals),

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infections, medication, exercise, heat, cold, and air pollution (SO_2 and O_3). This implies that respiratory health end points are often common in the study populations. However, this also implies that studies to evaluate the health effects of air pollution must carefully consider such covariates in the design.

Just as the cause of the respiratory health end points is likely to be multifactorial, exposures to air pollution are, in general, multidimensional. It is the purpose of this paper to address methods of designing and analyzing epidemiologic data to evaluate the health effects of such complex air pollution mixtures.

As an example, consider the association between environmental tobacco smoke (ETS) and lung cancer. It is clear that there is a strong association between active smoking and lung cancer. If these risks are extrapolated down to the exposures expected for a never-smoker exposed to environmental tobacco smoke (ETS), the estimated risk ratios would be of the order 1.4 for men and somewhat lower for women (*6*). Estimates combining results from case-control and cohort studies of lung cancers among nonsmoking women married to smokers in the United States (*6*) produce a summary relative-risk estimate of 1.14. At such low relative risks, alternative environmental causes, such as indoor radon, must be considered. Estimates from population-based studies may be biased toward the null because exposure to ETS is so common that it is impossible to identify a truly nonexposed control population. Thus, risk estimates in ETS epidemiologic studies are based on comparisons to controls with low, rather than no, exposure.

Respiratory Health Effects of Concern

For most air pollutants, indoor or outdoor, singly or in complex mixtures, the respiratory system is the sole or predominant portal of entry into the body and the principal locus of injury. The definition of what constitutes an adverse health effect has been addressed by a committee of the American Thoracic Society (*7*). Health effects generally are divided into acute and chronic effects. Acute effects are characterized by sudden onset; are usually short-lived, that is, lasting minutes to days; and may be reversible. Chronic effects are characterized by conditions that persist over extended periods of time, possibly years. Although there may be recovery from chronic effects, they may be irreversible and may lead to early mortality.

Examples of acute respiratory effects of air pollution include triggering or aggravation of asthmatic attacks, exacerbation of symptoms of chronic obstructive disease, increased upper or lower respiratory infections, transient changes in pulmonary function, increased respiratory symptom reporting, increased respiratory hospital admissions or doctor visits, and increased daily mortality.

Examples of chronic respiratory effects of air pollution include promotion of the development of asthma, increase in nonspecific airway responsiveness, reduced level of lung function, increased rate of lung-function decline, decreased rate of lung growth, development of chronic-obstructive pulmonary disease, increased reporting of persistent respiratory symptoms, lung cancer, and increased mortality.

Epidemiologic Study Designs

Epidemiologic methods applied in air pollution research can be described by a small number of study designs. Some study designs are not appropriate or have not been applied to air pollution. Discussing these designs provides a structure for evaluating the potential for investigating the health effects of complex air pollution mixtures.

Cross-Sectional Studies

In cross-sectional studies, health and exposure information are determined at a single point in time. These studies are often described as surveys. This approach is most appropriate for acute rather than chronic effects, that is, health effects that are temporally close to the exposures. They also are appropriate for exposures that have been stable over time. Cross-sectional studies are readily feasible with manageable costs. In such study designs, it is possible to perform intensive monitoring of exposures to complex mixtures.

Cross-sectional studies are not appropriate for studying the effects of exposures (or mixtures) that are changing over time or health effects that occur only after a long latency period. In particular, cross-sectional data cannot describe the longitudinal relation between exposure and the health end point. The potential for selection and information bias in such studies must be considered carefully.

Ecologic studies are a class of cross-sectional studies in which a group rather than an individual is the unit of comparison. Aggregate information rather than individual information is used to describe both exposure and effect. Ecologic studies are straight-forward, easily undertaken, and low in cost. However, confounding can be a severe problem in these studies. In air pollution epidemiology in particular, semi-ecologic studies are common in which individual health-status data is collected but exposure is determined from a single ambient-air pollution monitor.

In designing cross-sectional studies, it is often possible to select study populations such that exposures are limited to only one pollutant, or the range of exposures to one pollutant is very limited. For example, exposure to ETS could be limited in a study of NO_2 or radon by restricting the population to households with no smokers, as in the Albuquerque study of respiratory illness and NO_2 exposures in infants (*8*). In studies of oxidants, exposures to acid aerosols could be limited by considering only communities with low sulfur emissions (e.g., west coast communities). By such restrictions, the effects of individual pollutants that usually are found in mixtures can be assessed.

Alternatively, a factorial design can be implemented in which groups of participants having similar proportions of exposure are chosen based on prior knowledge of exposure or some marker of exposure. A factorial design allows estimation of the separate effects of each pollutant, as well as estimation of the effect of interaction.

In the Six Cities Study of indoor ETS and NO_2 (*9*), participating households were selected randomly from strata defined by previously obtained reports of smoking in the home and the presence of an unvented combustion appliance. The correlation between annual mean concentration of respirable particles ($PM_{2.5}$) and NO_2 measured in these homes was only 0.1, so that the effect of $PM_{2.5}$ and of NO_2 could each be estimated without strong confounding by the other pollutant. In the Harvard 24 Cities Study of the health effects of acid aerosols and ozone, study communities were selected to provide a contrast in the two pollutants (*10*). Existing ozone measurements for each community were examined along with measured sulfate and other indicators of the potential for acid-aerosol exposure. The purpose of this design was to optimize the power of this study to estimate the separate effects of acid aerosols and ozone. Similar selection criteria could be used in selecting households for inclusion in a study of ETS and radon.

Populations also can be studied cross-sectionally in time. For example, rates of diseases can be compared temporally within a community with time-varying air pollution. Chronic effects can be estimated by

comparison of annual disease rates with changing concentrations of air pollution. For example, can communities be identified in which sulfates concentrations, a marker of maximum aerosol acidity, have dropped while ozone concentration has risen?

Acute effects, such as daily mortality or hospital admissions, can be compared with daily air pollution measurements. These acute health-effects studies are usually described as time-series analyses. For a complex mixture, if the pollutants are not correlated perfectly, it is possible that the separate and joint effects can be estimated. In studies of ozone and acid aerosols, there is generally high correlation between the two exposures. An alternative strategy might be to perform a time-series study in separate communities with contrasting mixtures of these pollutants. For example, a community with both ozone and acid aerosols versus a community with ozone alone might be studied. Optimally, we would want to study a community with acid but no ozone.

In this sense, point sources of pollution may offer unique opportunities to investigate individual effects of pollutants that are usually found in complex mixtures. For example, NO_2 usually is found in photochemical smog along with CO and O_3. Shy et al. (*11*) examined the effects of NO_2 produced by a TNT plant in Chattanooga, Tennessee. Similarly, a study of a community adjacent to a sulfuric acid plant could provide unique information on the health effects of acid aerosols in the absence of oxidants.

Populations in developing countries are exposed routinely to air pollution concentrations and mixtures that are no longer seen in the United States or elsewhere in the developed world. Unique opportunities exist in such communities for studying mixtures of air pollution at extreme concentrations or in mixtures of pollutants not generally observed in the United States.

Cohort Studies

In cohort studies, subjects are selected based on exposure status and are followed to monitor the development of a specific health end point. Cohort studies can be conducted prospectively or retrospectively. In a prospective cohort study, exposure status is determined from current or historical records and the subjects are followed to monitor the development of disease. This design is not appropriate for rare diseases but works well for common end points. Many disease end points can be considered simultaneously with little increase in cost. For prospective cohort studies, extensive exposure assessment can be undertaken. Prospective cohort studies are especially efficient for assessing acute associations of air pollution exposures and respiratory health end points that vary over time.

The disadvantages of this design are the potential difficulty and high cost of implementation. The follow-up of study populations over extended periods of time is difficult. Large numbers of subjects are required if rare diseases are to be considered. This study design generally has weak power to measure interactions.

As in the cross-sectional study, interaction between pollutants in a complex mixture can be limited by restriction criteria on the sample cohort such that one pollutant is missing or its range is limited. Factorial designs also can be implemented to insure adequate sample sizes for each pollutant individually and for the joint distribution.

For a two-pollutant mixture, a factorial design allows the separate and joint effects of each pollutant to be estimated. In such a design, study subjects are selected such that there are equal numbers (or constant proportions) in each of the four cells defined by dichotomized exposure (high versus low) to one pollutant crossed with dichotomized exposure to the second pollutant. As an example, in a study of indoor radon and ETS exposures, never-smoking subjects could be selected based on radon levels in their homes (e.g., above or below 4 picocuries/m^3) and having a spouse who is a smoker (yes or no). A cohort with equal numbers of subjects in each of the four exposure groups would allow estimation of separate effects of radon and smoking, as well as their interaction. However, as has been noted earlier, for a rare event or an end point with a long latency, such as cancer, such a factorial cohort study would require extremely large sample sizes.

Prospective cohort studies have been used successfully to evaluate the acute effects of time-varying exposures to single air pollutants on daily reports of symptoms and changes in pulmonary function. For example, Pope et al. (*12*) studied a panel of school children and asthma patients in a location with pollution from particles only. Symptom reporting, peak flows, and medication for asthma were each associated with PM_{10}. Clinical studies have suggested that exposure to one pollutant may potentiate the subsequent effect of exposure to a second pollutant. For example, Koenig et al. (*13*) found that exposure to ozone potentiates the subsequent response to sulfur dioxide among adolescent asthmatics. In the ambient environment, however, exposures to complex mixtures usually are highly correlated temporally such that differentiating associations may be impossible. Study populations with unique characteristics may allow the investigation of serial exposure to multiple pollutants. For example, the acute effects of ETS and NO_2 may be different among subjects exposed to both pollutants simultaneously, as opposed to subjects exposed only to ETS at work and NO_2 at home.

Case–Control Studies

In a case–control study, subjects with a specific outcome of interest, the cases, are identified. A control series also is identified consisting of persons without the disease who potentially would be selected as cases if they were to develop the disease. Exposure histories of both cases and controls are determined and compared to estimate the risk of disease associated with exposure.

Case–control studies are efficient particularly for assessing risks associated with infrequent diseases and diseases with long latency periods. Generally, only one health end point can be considered, but multiple exposures can be evaluated with little additional cost.

Exposure is ascertained retrospectively or estimated from current measurements. Thus, there is potential for substantial random misclassification of exposure. Information bias is possible if there is not careful blinding of disease status of the participants. Selection bias is possible if cases and controls are not drawn from comparable populations. Case–control studies of the effects of air pollution have been infrequent perhaps because of the difficulty of reconstructing past exposures with acceptable precision (*1*).

Nested case–control studies are a hybrid design in which cases and controls are selected from within a larger cohort of subjects being followed historically or prospectively. The disease outcome is determined for all subjects in the cohort, but exposure information is determined only for the subset of subjects who develop the disease, that is, all cases, and a subset of subjects selected as controls. Nested case–control studies have been efficient particularly in cohort studies in which blood or other biological samples have been obtained and stored as part of regular evaluations of the study cohort. This approach makes efficient use of the measurement of biomarkers when the costs of the measurement are high. If biologic indicators of exposures to air pollutants can be identified, this design could be especially efficient.

The case–control design has been used widely to investigate the associations of lung cancer with exposure to ETS and to indoor radon. However, because exposures are estimated retrospectively, it is not clear that such a study can be designed to assess interaction of pollutants. Lubin et al. (*14*) have shown that testing for the interaction of active smoking and indoor radon exposure will require substantial numbers of subjects, possibly more than would be feasible in a single study. Evaluating interactions of indoor radon with ETS will be even more difficult. The off-diagonal exposures, that is, subjects with exposure to one but not both pollutants, can be enriched by selecting cases from populations with limited exposure to one of the pollutants. Restriction can improve the power of the study to estimate separate effects of pollutant mixtures. For example, cases and controls could be identified in areas with low smoking rates to reduce exposure to ETS but with high potential for radon exposures, or in areas with low radon potential to investigate the univariate associations with ETS.

Intervention Studies

In intervention studies, the investigator adds or reduces exposures to a cohort and then follows the cohort, assessing the impact of the intervention. In medical interventions, this approach, in which patients are assigned randomly to a treatment regimen (the randomized clinical trial), is considered the standard for inference and tests of causality. Studies in which air pollution is increased for specific subjects may be unethical. However, studies of subjects with reduced exposures would be acceptable.

In particular, Goldstein et al. (*15*) have described a cohort study of the acute effects of NO_2 in which lung function of women was measured before and after cooking a meal on a gas range. Lung function was also measured before and after cooking an equivalent meal with a portable electric stove replacing the gas stove. A larger scale intervention could be considered in which a cohort of subjects were evaluated for acute effects before and after changing their stove from gas to electric or electric to gas. A less intrusive intervention might be possible through the installation of an air-cleaning device specifically to remove ETS or to vent the exhaust of the cooking stove.

Special opportunities can sometimes be found in which specific pollutants are controlled unexpectedly. For example, Pope (*16*) performed an elegant analysis of the effects of particulate air pollution on hospital admissions based on a strike at a steel mill in Utah Valley. The steel mill was the primary source of particulate pollution in the valley. During the winter, when inversions develop, particulate levels build up to concentrations above the standards. Concentrations of other pollutants usually associated with particulates, that is, SO_2, NO_2, and O_3, were low. During the winter of 1986–1987, the steel mill was closed because of a strike, and particulate concentrations were reduced substantially. Comparison of respiratory hospital admissions in the strike year compared to the years before and after showed a 2-fold decrease in admissions among children. This study of opportunity has provided the clearest information yet on the effects of particulates alone, a pollutant usually observed in a mixture with other pollutants.

Occupational Studies

Occupational cohorts are valuable resources for epidemiologic studies of environmental risks. Cohorts are assembled easily and exposure estimation methods are well developed. The range of exposures can be large, facilitating the detection of associations. On the other hand, exposures often are much greater than those relevant for air pollution studies.

Occupational studies may provide opportunities to study exposures to single pollutants that are not possible in the ambient environment. For example, ozone exposures can be found in occupational settings without acid aerosols or nitrogen oxides.

Occupational studies also have furnished information on interactions that provide guidance for environmental studies. The interaction of active smoking and radon exposures has been demonstrated in uranium miners. Direct tests of interaction may be possible only at such extremes of exposure.

Migrant Studies

Epidemiologic studies of migrant workers have been useful particularly in disentangling the effects of heredity and environment. It is possible that studies of families moving into or out of areas of high pollution could provide insights into the relative contribution of individual components of multipollutant mixtures. For example, families moving from southern California, where oxidant concentrations are high but where acid aerosol concentrations are very low, to the Northeast, where both oxidants and acid aerosols can be elevated, could provide information on the modification of the ozone effect by acid aerosols. However, there are many other environmental changes that would be associated with such a move that also must be considered. Selection bias is also possible if the families have moved, at least in part, for health reasons.

Summary

Epidemiologic studies of the respiratory health effects of air pollution are difficult for the following reasons: *a*) Exposures are common, so developing contrasts is challenging. Maximum exposures have been reduced in the United States by control strategies. Populations free of exposure to air pollution cannot be found. *b*) There may be substantial misclassification of exposure. Ambient monitors do not reflect the range of exposures experienced by individuals. Personal monitors provide only a short sample of an individual's time-varying exposure. *c*) Exposures are multifactorial. Air pollution exposures are universally to multiple pollutants. In addition, other environmental insults, such as temperature and aero-allergens, may be correlated with air pollution exposures. *d*) Respiratory health end points are multifactorial, with air pollution being only one, and possibly only a minor, etiologic factor. *e*) Effects are weak and, therefore, difficult to detect. Nevertheless, information is needed to quantify health effects to the lowest observed concentrations.

If the investigation of mixtures of pollutants is not considered in the design of an epidemiologic study, then it is unlikely that the study will have sufficient power to detect interactions or even the separate effects of the individual pollutants in the analysis. Nevertheless, with innovative and well-thought-out study designs, it should be possible to measure the separate and joint effects of multiple pollutants in a mixture. No particular study design stands out as offering the most potential for disentangling the separate and joint effects. Creative epidemiologic designs and studies of opportunity can provide insights into these issues. If epidemiology were simply a matter of analyzing health and exposure data, we could set a computer to work regressing the vast stores of national health data against the immense amount of air pollution data that has been gathered. Fortunately for the epidemiologist, an elegant study design is more compelling than an elegant analysis.

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Biological Markers of Intermediate Outcomes in Studies of Indoor Air and Other Complex Mixtures

Timothy C. Wilcosky

Center for Epidemiologic and Medical Studies, Research Triangle Institute, P.O. Box 12194, Research Triangle Park, NC 27709-2194

Biological markers of intermediate health outcomes sometimes provide a superior alternative to traditional measures of pollutant-related disease. Some opportunities and methodologic issues associated with using markers are discussed in the context of exposures to four complex mixtures: environmental tobacco smoke and nitrogen dioxide, acid aerosols and oxidant outdoor pollution, environmental tobacco smoke and radon, and volatile organic compounds. For markers of intermediate health outcomes, the most important property is the positive predictive value for clinical outcomes of interest. Unless the marker has a known relationship with disease, a marker response conveys no information about disease risk. Most markers are nonspecific in that various exposures cause the same marker response. Although nonspecificity can be an asset in studies of complex mixtures, it leads to problems with confounding and dilution of exposure–response associations in the presence of other exposures. The timing of a marker's measurement in relation to the occurrence of exposure influences the ability to detect a response; measurements made too early or too late may underestimate the response's magnitude. Noninvasive markers, such as those measured in urine, blood, or nasal lavage fluid, are generally more useful for field studies than are invasive markers. However, invasive markers, such as those measured in bronchoalveolar lavage fluid or lung specimens from autopsies, provide the most direct evidence of pulmonary damage from exposure to air pollutants. Unfortunately, the lack of basic information about marker properties (e.g., sensitivity, variability, statistical link with disease) currently precludes the effective use of most markers in studies of complex mixtures. — Environ Health Perspect 101 (Suppl 4):193–197 (1993).

Key Words: Biological markers, assays, environmental exposure, air pollution, complex mixtures, pulmonary disease, health outcomes

Introduction

Most studies of health effects in humans exposed to complex air-pollutant mixtures have used such outcome measures as hospital admission rates during air pollution episodes, symptom reports from questionnaires or diaries, and disease prevalence or mortality rates in communities with different levels of air pollutants. A few studies in controlled exposure settings have attempted to assess the combined as well as separate effects of mixture components. Such approaches provide useful information; but studies of overt disease often are insensitive to low-dose exposure effects, and they focus on the extreme end of the disease spectrum, where only a small proportion of the exposure-related disease burden occurs.

Biological markers of intermediate health outcomes (i.e., early pathologic changes or events predictive of disease) could provide a superior alternative to traditional measures of pollutant-related disease. Markers can have greater sensitivity to exposure effects, they may appear sooner after exposure and at younger ages, and they may detect a greater proportion of the exposure-related disease burden compared to measures used in the past. Because some early pathologic changes detected by markers do not progress to symptomatic conditions, more people will show positive marker responses than overt disease; so studies using markers can potentially have increased statistical power due to the more numerous outcomes. However, the markers are useful only to the extent that they have a known relationship to clinical diseases of interest.

In general, biological markers are indicators of events occurring in the body that are difficult to measure directly. Markers can indicate that an exposure, a response to exposure, or an early pathologic change has occurred; other markers, often enzyme phenotypes, indicate an individual's increased susceptibility to disease from a particular exposure. Such markers differ from genetic markers, which are usually defined in the current genetic literature as discrete phenotypes controlled by genes that occur in close proximity on chromosomes to other genes of interest. A good genetic marker will be correlated highly with the presence of the gene of interest, which may be undetectable.

The following presentation discusses some methodologic issues associated with the use of biological markers of intermediate health outcomes arising from exposures to complex pollutant mixtures. In most instances, this article focuses on outcomes related to four examples of complex mixtures: *a*) environmental tobacco smoke (ETS) and nitrogen dioxide (NO_2), *b*) acid aerosols and oxidant outdoor pollution, *c*) ETS and radon, and *d*) volatile organic compounds (VOCs). No standard definition exists for the term biological marker. For example, the National Research Council's (NRC) 1989 book *Biologic Markers in Pulmonary Toxicology* (*1*) defines biological markers as "indicators of events in biologic systems or samples," while others [e.g., Hulka et al. (*2*)] limit the definition to indicators measured in biological specimens obtained from a person. The NRC definition includes, for example, spirometry, which involves no assays of biological samples. This discussion uses the more restrictive definition of Hulka et al. (*2*). These two references provide additional information about markers in general and about the specific markers discussed here.

Methodologic Issues

Link with Disease

The most important property of any biological marker of effect is its link with the health outcome of interest. Although markers in a target tissue (i.e., tissues that give rise to the disease of interest) usually provide the best early indicator of an adverse event, markers

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outside of the pathogenic pathway can be superior for a variety of reasons. For example, target tissues such as the lung are relatively inaccessible, markers in the target tissue may require higher exposures to show a response than do markers in a nontarget tissue, and markers in a target tissue may have less persistence due to rapid cell turnover or other mechanisms of marker loss.

A study of micronuclei measured in exfoliated epithelial cells in sputum of uranium workers exposed to radon and tobacco smoke illustrates how markers outside of the pathogenic pathway can show a stronger statistical association with the disease under consideration. Micronuclei, which are caused by agents that damage DNA, are small secondary nuclei formed during mitosis when whole chromosomes or chromosome fragments fail to become incorporated into daughter nuclei. As summarized by Loomis et al. (*3*), the micronucleus assay in exfoliated buccal cells is sensitive to ionizing radiation as well as tobacco smoke; uranium miners show a clear radon-related excess of lung cancer, but neither radon exposure nor cigarette smoking was associated with a higher prevalence of micronuclei in Loomis's study of sputum cells from the miners. In contrast, other studies of uranium miners (*4*) and persons with residential radon exposure (*5,6*) show increased levels of structural chromosome aberrations in blood lymphocytes. Although chromosome aberrations in lymphocytes have no direct role in lung cancer, their association with radon exposure suggests that this marker, compared to micronuclei in sputum cells, has a stronger statistical link with lung cancer arising from radon exposure.

Other markers of premalignant changes with potential interest for studies of carcinogenic exposures (e.g., VOCs or radon and ETS) include sputum cytology, the hypoxanthine-guanine phosphoribosyl transferase (HGPRT) assay for *in vivo* mutations, and assays of oncogene activation. To date, human studies using the HGPRT assay primarily have examined peripheral lymphocytes, but the assay could be adapted for pulmonary macrophages obtained through bronchoalveolar lavage (BAL) (*1*). By using macrophages, the marker would detect mutations occurring in the lung, although lung tumors do not arise from macrophages themselves. Theoretically, activated oncogenes could be detected in exfoliated cells in lavage fluid to characterize developing lung tumors (*1*). The use of almost any marker of intermediate outcomes would increase the number of exposure-related outcomes (compared to the number of cancers) in the study while reducing the necessary time interval between exposure and measurement of the outcome. At this point, however, most markers of intermediate outcomes have an unknown relationship to clinical disease; so their value is limited.

The rationale for using nontarget tissues and cells for assessing evidence of early disease is that pathologic changes observed in nontarget tissues often occur in the target tissues. For example, exposure to acid aerosols and oxidants can exacerbate airway hyperreactivity in asthmatics (*1*), with a resulting increase in pulmonary and blood eosinophils. Chronic deposition of eosinophils in the lung may cause airway inflammation, but eosinophils are much easier to measure in blood than in the lung. Even if markers in target tissues compared to nontarget tissues do show a stronger association with the ultimate outcome of interest, their inaccessibility may preclude their use in observational studies.

In general, the selection of markers involves a tradeoff between a marker's positive predictive value for the disease and such practical issues as specimen availability, marker sensitivity, and assay cost. Unfortunately, many potentially useful markers have an unknown relationship to lung disease. For example, the relationship between changes in constituents of BAL fluid (a potential source of myriad markers of intermediate outcomes) and pollutant-induced injury requires further study (*1*). Markers of events that occur further down the disease pathway, such as fibrosis, moderate airspace enlargement, or mutations, have clearer biological links with clinically apparent disease. In the absence of important advantages associated with other markers, biologically plausible markers having obvious biological links with disease are preferable to markers without such links. The validity of markers that occur in nontarget tissues or early in the pathogenic process would be assessed ideally in prospective studies, where their positive predictive value for subsequent overt disease can be estimated. Such studies would be difficult, especially when cancer is the outcome of interest, because of the need for large study populations and long follow-up periods.

Studies of associations that can be measured cross-sectionally are more feasible. One could, for example, ascertain whether a marker measured in blood has a high positive predictive value for inflammation in the lung. A marker with a high predictive value for inflammation could be used as an outcome variable in a study to determine whether exposure to a complex mixture causes inflammation.

Specificity and Confounding

Some markers respond to specific environmental exposures, while others respond to a wide variety of agents. For studies of complex mixtures, nonspecificity can be an asset, because the marker response will reflect the combined effects of multiple, sometimes unidentified, agents. Furthermore, the airways can react to inhaled toxic materials in a limited number of ways, so a wide variety of exposures lead to a small number of health effects. For example, many inhaled toxicants, such as acid aerosols and oxidant gases like NO_2 and ozone, cause inflammation in the respiratory tract. Exposure to ETS and NO_2 in children increases the risk of respiratory infections, which also cause inflammation. Chronic or repeated inflammation may in turn lead to irreversible lung injury and, eventually, clinically apparent diseases such as emphysema. Therefore, indicators of inflammation or early loss of elasticity can serve as markers of intermediate outcomes from numerous exposures, the effects of which converge on a common pathologic pathway.

The convenience of using a nonspecific marker that responds to a variety of complex mixtures is offset by the possibility of dilution and confounding from exposures other than those of interest. As discussed by Weiss and Liff (*7*), the problem of dilution, where an exposure–response association is obscured by other associations, arises when different causal pathways lead to the same end point, as is the case with nonspecific markers of intermediate outcomes. If two different exposures (or sets of exposures), E_1 and E_2, cause the same marker response through independent pathways, they increase the overall marker response rate in an additive manner; but relative measures of association (e.g., relative risk, odds ratio, etc.) are based on the assumption of a multiplicative model of association. As a result, the relative risk of the response due to E_1 will be influenced by the background incidence of the response due to E_2. In this situation, use of the risk difference rather than relative risk to compare marker responses in persons exposed and unexposed to E_1 helps avoid the problem of dilution from a high background incidence from E_2.

Another strategy for mitigating the problem of dilution is to stratify an overall group of end points into its more homogeneous components (*7*). Inflammation from different exposures, for example, may have slightly different manifestations detectable by different markers. Each marker would have greater specificity for a given exposure than would a marker that detected overall

inflammation. The feasibility of this approach for studies of complex mixtures is unclear until additional basic information on properties of markers of intermediate outcomes becomes available.

Weiss and Liff (*7*) point out that studies of intermediate outcomes sometimes facilitate the identification of a particular causal pathway. For example, if exposure to one complex mixture leads to pulmonary disease through inflammation, while another exposure causes the same disease through a noninflammatory process, the complex mixture would show a stronger association with inflammation than with the pulmonary disease. However, this approach is feasible only when the intermediate outcome has a known relationship to the clinical outcome of interest—a rare situation.

Confounding could arise in studies of inflammation due to exposure to acid aerosols and oxidants, for example, if exposed persons tend to be heavy smokers or have occupational exposures that also cause inflammation. Problems with confounding are essentially the same whether one uses nonspecific markers of intermediate outcomes or actual diseases as study end points. The usual epidemiologic approaches for controlling confounders (i.e., stratified analysis, matching, or restriction) can remove the effects of extraneous variables. Exposure-specific markers would be less prone to confounding than would nonspecific markers, but outcome markers that arise only from single agents would have limited value for studies of complex mixtures.

Sensitivity

In many instances, different markers can be used to detect the same outcome. Inflammation, for example, involves numerous physiological changes that can be used as markers of the inflammatory response. For a given degree of inflammation, however, some markers will be easier to detect than will others. Markers that detect the mildest inflammation (i.e., those that are positive with the lowest exposures) would have the greatest sensitivity.

An animal study (*8*) illustrates several issues associated with marker sensitivity for intermediate outcomes. The investigators evaluated different markers of connective tissue metabolism (a response to injury in the lung) in urine or BAL fluid. In one exposure protocol using 0.5-ppm NO_2 exposure for 4 weeks, hydroxylysine urinary excretion increased significantly, but levels of hydroxylysine and angiotensin-converting enzyme activity in lavage fluid remained normal and lung histology showed no damage. Compared to other markers of effects on connective tissue, urinary hydroxylysine apparently has greater sensitivity.

Although this controlled study of NO_2 exposure in rats only has indirect relevance to free-living human populations exposed to complex mixtures, it does illustrate that different markers vary in sensitivity, and that the same marker measured in different biological materials also can have different sensitivities. For reasonably benign exposures, such as many commonly occurring complex mixtures, exposure chamber studies can characterize a promising marker's properties (e.g., sensitivity, dose–response, and interindividual and intraindividual variability) in humans under controlled conditions. These studies can evaluate markers of acute outcomes but precise estimates of such properties will rarely, if ever, be available for marker responses from chronic exposures.

In general, a marker's sensitivity and positive predictive value can be increased by studying susceptible populations. For example, exposure to acid aerosols–oxidants can exacerbate symptoms of asthma. Sensitized asthmatics compared to nonsensitized asthmatics and nonasthmatics are likely to show an inflammatory response at lower exposure levels, so markers of inflammation will have greater sensitivity in studies of sensitized asthmatics. Similarly, ETS–NO_2 exposure may increase the risk of respiratory infections in children more than in adults, possibly through alterations in immune function; theoretically, markers of such alterations may have a greater sensitivity (i.e., occur at relatively low exposure levels) in children given their apparent increased susceptibility to infections compared to adults. Given the known susceptibility of such groups as asthmatics and children to some mixtures, they also may be susceptible to other pollutant mixtures, so that adverse health outcomes and associated markers could be detected at relatively low exposure levels.

Sensitivity also can be increased for a given ambient concentration by studying people with a relatively high internal dose of a pollutant mixture, such as those having a high rate of ventilation due to physical activity. Persons who spend a large proportion of time outdoors also will have relatively high doses of ambient outdoor air pollutants. Thus, for a given exposure level, marker responses would probably be more pronounced in persons with biological susceptibility and in those with behaviors that increase either their internal dose or their contact with ambient pollutants.

Temporal Aspects

Markers can appear hours, days, or years after exposure. For example, nasal irritation is commonly associated with indoor air pollution (e.g., VOCs and other complex mixtures). Markers of cell and mediator changes in nasal lavage fluid could be useful for studies of such pollutants (*1*), and the markers would probably appear within hours of exposure. In contrast, several months or years of exposure to acid aerosols and oxidants may be necessary to detect airspace enlargement using morphometry, while changes in alveolar cell populations may appear after days or weeks of exposure.

For transient markers, the timing of measurements is especially crucial. The influx of neutrophils and eosinophils into the respiratory tract, for example, usually occurs during the first 3 to 7 days of an inflammatory response (*1*). Measurements of these markers of inflammation in BAL fluid immediately after exposure would underestimate the inflammatory response, as would measurements taken after the response subsided. Protein influx reflecting pulmonary epithelial damage, however, should be measured relatively soon after exposure. For sustained ongoing exposures, such as occurs with VOCs or residential radon exposure and ETS, transient markers will be replenished, and measurements can be made any time during seasons when buildings are likely to be poorly ventilated.

The timing of measurements is less important for markers of chronic exposure-related changes. Irreversible airspace enlargement, for example, can be measured long after exposure ends, and it will reflect cumulative exposure effects. Altered populations of alveolar epithelial cells due to oxidant air pollution exposure eventually revert to normal proportions, but these markers can probably be detected for at least several weeks after the end of exposure. Timing is still important in the sense that the exposure must be sufficiently long for the marker response to occur. Note that for some markers of chronic pathogenic processes, such as the markers of connective tissue degradation in the study by Evans et al. (*8*), the marker response diminishes after the exposure stops ,even though the associated damage may be irreversible.

Approaches for Using Markers

The effective use of a marker in epidemiologic studies of complex mixtures depends not only on the marker's properties but also on the availability of suitable biological ma-

terials and moderately priced assays. Numerous markers of intermediate outcomes are inappropriate for field studies because of their invasive nature. The following section mentions some noninvasive markers with potential usefulness for studying complex mixtures, and it discusses strategies for using invasive markers.

Noninvasive Markers

In general, markers measured in such materials as urine, sputum, blood, and nasal lavage fluid are well suited for field studies because specimen collection involves relatively little inconvenience or risk for study participants. Urine could be valuable especially for studies of ETS and NO_2. ETS exposure can be estimated from urine samples, as can some markers of connective tissue metabolism associated with NO_2 exposure. Sputum cytology, a marker of disease that is nonspecific with regard to exposure, may reveal early evidence of carcinogenic changes from such exposure as VOCs or ETS and radon gas. Standardization of sputum collection and preparation might alleviate the problems encountered by Loomis et al. (*3*) and allow detection of increased micronuclei from these exposures. Loomis et al., who used archived specimens, could not control the source of sputum and its cellular content; and laboratory manipulation of the old samples may have caused a loss of some cell structures.

Blood is a source of numerous and diverse markers. As noted earlier, radon exposure at levels that increase the risk of lung cancer are associated with chromosome abnormalities in blood lymphocytes. Markers of altered immune function, which increases the risk of respiratory infections, also can be measured in blood. Some studies suggest that markers of pulmonary hypertension, which apparently is caused by several toxic chemicals, also may be present in blood (*1*); possible markers include elevated plasma copper levels and ristocetin cofactor activity relative to plasma von Willebrand factor.

Many changes in constituents of blood and nasal lavage fluid reflect the changes that occur in less accessible BAL fluid. For example, the distribution of lymphocyte subpopulations, a marker of air pollution effects, is similar in blood and BAL fluid (*1*). Nasal lavage fluid, which contains several markers that respond to a variety of constituents found in complex mixtures, may be especially useful in studies of indoor air pollutants that cause nasal irritation (*1*). Comparisons between markers in nasal and BAL fluid are necessary to ascertain the usefulness of nasal lavage markers as indicators of events in the lower respiratory tract.

Invasive Markers

Some of the most informative markers of intermediate outcomes occur in relatively inaccessible biological materials such as the lung. One approach to obtaining lung specimens is BAL, which uses a modified bronchoscope for collecting pulmonary cells and fluids. Lavage fluid contains a variety of biological materials in which to measure markers of intermediate outcomes. The method is used primarily for diagnostic purposes and in controlled-exposure chamber studies. Although its invasive nature precludes the routine use of BAL in field studies, small studies of individuals with exposures to naturally occurring complex mixtures could detect numerous potential exposure-related effects. The use of personal monitors in conjunction with lavage measurements would provide a high degree of precision in estimates of exposure-outcome associations. Retrospective collaborative studies using archived lavage specimens also may be feasible if unexposed controls from chamber studies in cities having different levels of air pollution could be identified. Differences in specimen collection and storage among research centers, however, may preclude such retrospective studies.

Much of the recent animal research and some human studies of air pollution have used morphometric measurements for assessing pulmonary damage. The morphometric approach uses an overlay of points or lines that is placed over an electron micrograph or other two-dimensional image of a lung section. By estimating the proportion of points or numbers of lines that fall on cell or airway structures, one can use a set of formulas to estimate various cell and tissue parameters, such as cell size and structure, proportions of cell types, or airway diameters (*9*).

Morphometric studies of lung tissue are limited to specimens obtained from surgery or autopsy. This severe constraint raises a host of methodologic problems, but the potential value of morphometric measurements argues in favor of further exploring this promising technique. For example, morphometry has been used to study postnatal lung growth (*1*), an outcome relevant to ETS–NO_2 exposur; and numerous morphometric studies have found pulmonary changes in animals exposed to single pollutants and pollutant mixtures (*10*). Limited studies of air pollution using morphometry with human autopsy specimens point to the feasibility of moving from animal to human tissues. The application of morphometric techniques to human lung specimens may eventually provide the most direct evidence of pulmonary damage from chronic low exposures to complex mixtures. However, basic work remains to be done. Studies are necessary to describe lung lesions in persons of different ages exposed to ubiquitous background pollutants (ETS, automobile exhaust, etc.) and to investigate the effects on lesion measurement of different protocols for collecting, handling, and storing lung specimens in autopsy settings.

Conclusion

Indoor and outdoor air pollutants potentially can affect the health of virtually everyone in the United States. Animal studies using controlled chronic exposures are important for identifying the pollutants responsible for adverse health effects, but epidemiologic studies address the effects of complex pollutant mixtures that actually occur in exposed humans. Biological markers of intermediate outcomes offer new opportunities for advancing the study of these pollutants.

Unfortunately, many research opportunities associated with markers remain theoretical. Much basic information critical for valid application of markers is lacking. For example, few markers have been characterized regarding their statistical properties, such as assay variability and variability in samples collected from the same individual at different times. Furthermore, protocols for collecting, storing, and analyzing biological specimens have not been standardized. More important, the relationship between markers of intermediate outcome and clinical disease remains a matter of speculation. Investigators studying exposures to complex mixtures therefore cannot interpret the health implications of a positive marker response, nor can they confidently attribute a lack of an exposure–response association to a true absence of a biological effect when a marker's sensitivity and variability are unknown. Also, the nonspecific nature of currently used markers of intermediate outcomes leads to potential problems with dilution and confounding of exposure-outcome associations. A combination of controlled exposure studies and systematically conducted epidemiologic studies could readily address the validity issues, but the high cost of many marker assays discourages their use in large-scale epidemiologic studies.

Progress in applying markers to studies of human exposures and diseases requires considerable effort from both toxicologists and epidemiologists. However, neither group has great incentive to undertake the mundane systematic studies necessary to characterize marker properties in a statistically valid manner: bench scientists usually prefer to investigate promising new techniques while epidemiologists are primarily interested in etiologic associations. Progress in the use of markers is likely to occur slowly until answers to basic questions become available.

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Utility of Controlled Human Exposure Studies for Assessing the Health Effects of Complex Mixtures and Indoor Air Pollutants

William F. McDonnell *

Clinical Research Branch, Health Effects Research Laboratory, U.S. Environmental Protection Agency, Research Triangle Park, NC 27711

The study of health effects induced by exposure to mixtures of pollutants is a complex task. The purpose of this paper is to identify areas of research in which the conduct of human controlled exposure (clinical) studies may contribute to better understanding health effects of exposure to indoor air and other mixtures. The strengths and weaknesses of clinical studies in general are reviewed, as well as examples from the literature of approaches that have been used. Human chamber studies play an important role alongside epidemiologic and animal toxicologic studies in such research. Human chamber studies are limited with regard to assessing chronic effects, rare effects, or effects from long-duration exposures but are powerful in assessing acute, reversible effects from short-duration exposures in humans. The areas in which human chamber studies are most likely to contribute include identification of effects or markers of effects for exposure to a given pollutant or mix of pollutants; direct dose–response assessment of effects for individual compounds and mixtures of set composition; identification of individual compounds responsible for the effects of a mixture; study of the joint effects of a binary mixture; development of markers of acute exposure for particular compounds; development of outcome measurements to be used in the field; and identification, characterization, and testing of sensitive subpopulations. — Environ Health Perspect 101(Suppl 4):199–203 (1993)

Key Words: Clinical studies, complex mixtures, indoor air pollution, air pollution, exposure chambers

Introduction

Inferences about the health effects of exposure to mixtures of air pollutants are based generally upon data from some combination of clinical studies, epidemiologic studies, and animal toxicologic studies. The relative contribution of information from each of these study types is dependent on the exposure of interest, the nature of the health outcome, the relationship between exposure and outcome, the existence of natural experiments, and the availability of suitable animal models, among others. In many circumstances, the data generated are complementary, and simultaneous assessment of information from those sources allows gaps in knowledge to be filled and allows the consistency of findings among the different disciplines to be examined. In other circumstances, hypotheses may be generated in one type of study with subsequent testing in another type.

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*Address reprint requests to W. F. McDonnell, Med. Res. Bldg. C (CB 7315), Mason Farm Road, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599-7315.

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The purpose of this paper is to identify ways in which controlled human-exposure studies can be used for direct measurement of exposures and effects, and can be integrated into a program of epidemiologic research to enhance our understanding of the health consequences of exposure to indoor air pollution and other complex mixtures.

Clinical studies are most useful in situations in which the mixtures of interest are well defined and easily produced and measured, and the outcomes of interest occur acutely, are reversible, and are measured easily with little error. At the other extreme are mixtures that are difficult to characterize and generate, and outcomes that occur only after a long period of exposure, are chronic in nature, and are rare and difficult to assess at an early stage. In the former case, effects of exposure can be assessed directly in human chamber studies. In the latter case, clinical studies can provide at best some information that may result in a more efficient epidemiologic study design or may provide information that lends plausibility to observed results.

Strengths and Weaknesses of Controlled Human Exposure Studies

A consideration of the utility of clinical studies must begin with a discussion of the strengths and weakness of such studies. A major advantage includes the control that one exerts over the conditions of exposure. For example, the effects of either the individual components of a complex mixture or the mixture itself can be studied without having to identify locations with appropriate ambient conditions. This is important particularly when one is interested in studying the individual and joint effects of pollutants, such as ozone and acidic aerosols, which commonly occur together. Control of the exposure also allows one to concentrate on the conditions of most interest. Studies primarily interested in worst-case scenarios may include exposures with high pollutant concentrations and levels of ventilation and long duration, while other studies may focus on lower level exposures similar to those that occur for large segments of the population. Dose–response information can be generated for individual compounds or a specific mixture, and interaction between two compounds theoretically can be studied by varing the relative concentrations of each component. Because controlled human exposure studies are conducted generally in a permanent facility with resident staff, the availability of sophisticated equipment and expertise allows measurement of a greater variety of end points than may be feasible in field studies.

Another strong point of clinical studies is reduction of bias, leading to greater internal validity. Foremost among the strengths of clinical studies is the random

assignment of subjects to treatment groups, which reduces both confounding and selection bias. Elements of exposure, such as concentration, ventilation, and duration, typically can be measured more precisely than under conditions of ambient exposure. Similarly, depending on the end point, health outcome usually can be measured more precisely. Such reduction in measurement error of both exposure and effect reduces misclassification bias experienced in epidemiologic studies. A further advantage of clinical studies is the obvious temporal order in which exposure and effects occur. The ability to manipulate effects by varying exposure greatly increases the confidence that a given exposure causes a particular effect.

While clinical studies are powerful in the assessment of many effects of interest, there are some relevant exposures and outcomes that cannot be studied experimentally in humans for ethical or practical reasons. Obviously, one cannot conduct ethically a study in which permanent effects are induced in subjects. This appropriate ethical concern excludes direct study of the induction of all chronic diseases that generally have major impact on the affected individuals. Furthermore, the study of reversible effects that require prolonged exposure of subjects is not practical. For example, the effect of a season of exposure to ozone and acid aerosols on bronchoalveolar inflammation cannot be studied in an experimental setting.

Another potential limitation of clinical studies is the relatively small number of individuals that can be studied. Exposure chambers usually can accommodate only one to four individuals at a time. Given the duration of exposure, the need for multiple exposures, the amount of time required for measurement of outcomes and for maintenance and auditing of chamber equipment, one realizes quickly the constraints on the clinical study of large numbers of individuals. The statistical limitations imposed by restricted numbers of subjects makes it difficult to study exposures that produce small or imprecisely measured effects. In these cases, it is hard to detect changes that are small due either to the nature of the effect or to bias toward the null. Small numbers also make selection among competing dose–response models for a single compound and assessment of interactions between mixtures of pollutants difficult. Human chamber studies also are inefficient for the direct study of rare events. This deficiency can be overcome somewhat by measuring markers of the outcome of interest that may occur more frequently and that can be measured with more precision. An example of this would be the production of asthma attacks by mixtures of ozone and acid aerosols. Actual attacks are the outcome of direct interest, but they occur rarely after a single chamber exposure. An increase in airway hyperreactivity, however, which may be associated with increased asthma attacks, can be measured easily in each exposed subject. Other potential approaches to the limitation of small numbers of subjects is aggregation of data from several studies and identification and study of particularly sensitive individuals for whom frequency and magnitude of effect is larger than for the population as a whole.

Experimental study of some complex mixtures is not realistic, either because mixtures are characterized poorly or are heterogeneous or because artificially generated mixtures are not comparable to those experienced in ambient air. An example of the former is the wide variety of volatile organic compounds (VOC) that are found in indoor air and that are suspected of causing sick-building syndrome. Neither one substance, nor a small number of compounds, nor a characteristic mix of compounds has been identified as most likely responsible for the syndrome. Rather different mixtures of compounds have been identified with a variety of sources (*1*). One approach to the study of poorly defined, variable mixtures has been the exposure of volunteers to a mixture containing 22 volatile organic compounds that are produced from a variety of sources (*2,3*). An example in which it is difficult to produce the complex mixture found in ambient air involves the study of the effects of acidic aerosols combined with photochemical oxidants. Differences in chemical composition and deposition characteristics may exist between naturally occurring aerosols and those generated artificially, and the mix of photochemical oxidants that occurs in ambient air is difficult to replicate because of the aging of the mixture that normally occurs. As a result, ozone, a chemically active and representative oxidant, has been used in such studies without inclusion of other oxidant species, while the aerosols usually have been limited to one-chemical species. While considerable information can be gained about some mixtures or about representative compounds in this manner, the true impact of ambient exposure to many complex mixtures can only be approximated in chamber studies.

A further concern about human experimental exposure studies as they have been traditionally carried out is that the population represented by the samples studied has not been consistently well defined. While the subjects often are well characterized, they are generally volunteers recruited through advertising and, in many cases, from university campuses. Furthermore, in the interest of decreasing heterogeneity of responses and increasing internal validity, very homogeneous groups are usually studied, such as very healthy, never-smoking, white, male individuals, or mild asthmatics not on medication. This process for subject selection raises questions about the ability to generalize findings to other segments of the population not represented by these samples. In many instances, this may not be a major concern, and in cases where it is, different methods of recruitment and subject selection can be used to improve the external validity of a given study.

Historical Use of Human Exposure Chambers for Study of Mixtures

Historically, chamber studies of air pollutants have been conducted once an exposure of interest has been identified. For a number of individual pollutants (e.g., ozone and sulfur dioxide), the purposes accomplished include identification and description of health effects, exposure–response characterization, assessment of individual variability in response, identification of sensitive or susceptible populations, quantification of retained dose, and some insights into mechanisms of action. Study of more than one pollutant generally has been limited to comparisons between clean air and complex mixtures or to assessment of the individual and joint effects of a single concentration of each of two substances during simultaneous or sequential exposure. As has been pointed out by Greenland (*4*), unless the dose–response characteristics of each of the individual pollutants are known, this latter study design is inadequate for completely assessing the nature of the interaction between effects of more than one compound.

Mixtures that have been studied in chambers include, among others, ozone and a variety of acidic aerosols, ozone and sulfur dioxide, ozone and nitrogen dioxide, sulfur dioxide and acidic aerosols, ozone and peroxyacetyl nitrate (PAN), a complex mixture of 22 volatile organic compounds, and environmental tobacco smoke. Stacy et al. (*5*) exposed individuals to a mixture consisting of one gaseous pollutant (air, ozone, nitrogen dioxide, or sulfur dioxide) and one aerosol pollutant (air, sulfuric acid, ammonium sulfate, ammonium bisulfate, or ammonium nitrate). They observed no

joint effects that were different statistically from those produced by ozone alone, although the mixture of ozone and sulfuric acid produced effects that suggested some additional effect. A number of other investigators have also studied the effects of ozone combined with a variety of aerosolized acidic substances. While one recent abstract suggested that responses to mixtures of ozone and either sulfuric or nitric acid are somewhat larger than the sum of effects of the individual compounds (*6*), most studies have found no such evidence (*7–12*). For simultaneous exposures to a mixture of ozone and sulfur dioxide that also may result in production of sulfuric acid particles, some investigators have observed evidence of a joint effect slightly larger than that due to the sum of the effects of the individual pollutants (*13,14*), while the majority of investigators have not observed such an effect (*15–17*). For exposure to mixtures of ozone and nitrogen dioxide (*18–22*) and ozone and carbon monoxide (*23*), there is little convincing evidence that exposure to any of the mixtures has much effect beyond that attributable to ozone.

Dreschler-Parks et al. (*24*) reported that a mixture of ozone and peroxyacetyl nitrate causes lung function decrements larger than those due to exposure to individual pollutants. Avol et al. (*25*) used a slightly different approach in assessing the joint effects of exposure to the mixture of pollutants common to the Los Angeles basin. They exposed volunteers to purified air that contained 0.16-ppm ozone on one occasion, and on another occasion, they exposed the same individuals to ambient air that contained a similar concentration of ozone in addition to the other pollutants commonly found in the Los Angeles basin. Avol et al. observed no differences in the magnitude of acute respiratory responses of the ambient air compared to the purified air with ozone alone. They concluded that the acute respiratory effects of exposure to the complex mixture making up ambient air in Los Angeles could be attributed to ozone.

No single chemical or mixture of chemicals has been observed to be responsible for the variety of complaints that are associated with the sick-building syndrome. Rather, a variety of mixtures of diverse chemicals has been identified in buildings in which the number of complaints seem to be elevated. Molhave et al. (*2*) and Otto et al. (*3*) measured responses to a mixture of 22 volatile organic compounds that seem to occur often in buildings in which complaints are recorded. Sensory irritation was observed in both studies, and memory deficits were observed in one study but not the other. Evidence indicates that this mixture also may result in an influx of inflammatory cells in the nose (*26*). Using a similar approach of measuring response to an entire mixture, Willes et al. (*27*) observed that exposure to environmental tobacco smoke (ETS) results in upper-respiratory symptoms and increased nasal resistance.

Use of Human Experimental Exposure Studies in Future Investigation

Having considered the strengths and weaknesses of clinical studies and the type of information that has been collected in the past both for exposure to individual pollutants and to mixtures of pollutants, one can better evaluate the possible contributions that clinical studies can make to direct assessment of effects of indoor air and other complex mixtures and to providing ancillary information that may enhance the design and interpretation of epidemiologic studies. Two approaches described by the U. S. Environmental Protection Agency (EPA) (*28*) include the "top down" and "bottom up" research strategies. The top down approach involves study of the mixture as a whole, with further study of fractions of the mixture to identify the causative agents and interactions among them. The bottom up approach involves study of the individual compounds as a first step followed by examination of the joint effects of mixtures of these individual compounds. Mauderly (*29*), in this supplement, refers to similar approaches used in toxicological assessment of mixtures: an integrative approach and a dissective approach (both top down) and a synthetic approach (bottom up). These paradigms also are useful for identifying areas of clinical research that may prove fruitful.

The integrative approach, as part of a top down strategy, concerns itself with assessment of the mixture as it exists in the ambient environment. This generally requires that the mixture, or a reasonable approximation, can be generated in a chamber setting. Two situations that appear worthy of study include the effects of environmental tobacco smoke and the effects of the mixture of 22 volatile organic compounds used to simulate an indoor environment in new buildings. Areas of research that seem most promising include generation of empirical evidence that either of these particular mixtures causes a given effect or a marker of a given effect; direct dose–response assessment of acute, reversible outcomes for the mixtures; development of markers of acute exposure for particular compounds representative of the mixture; development of outcome measurements that also could be used in the field; and identification or characterization of sensitive subpopulations. Environmental tobacco smoke has been documented to produce symptoms and physiological effects in the nose. The VOC mixture produces nasal inflammation and symptoms and may produce neurobehavioral effects. Both of these mixtures can be produced and controlled during chamber studies: ETS by "smoking machines" that generate sidestream smoke and VOC by evaporation of the mixture of interest. Further elucidation of the spectrum of effects for each of these mixtures and dose–response characterization of these effects seem to be worthwhile pursuits. This may include assessment of nasal and ocular inflammation, stimulation of neural elements in the nasal cavity, alterations in breathing pattern or airway reactivity, and behavioral effects. Because many of the complaints about ETS or sick-building syndrome are subjective, identification of physiological outcomes may help elucidate the mechanisms underlying the symptoms. Many of the outcome measurements developed for chamber studies also could be modified for use in the field to assess effects of exposure in epidemiologic studies. Promising techniques include nasal washes, sampling of tears, and neurobehavioral testing. Questionnaires could be developed and standardized for use in both clinical and epidemiologic studies to facilitate comparison between studies.

Cotinine often is used as a marker of exposure to ETS. Because metabolism of nicotine may vary among individuals and among groups of different age or gender, and because many different exposure scenarios can result in a given cotinine level at one point in time, further work in developing cotinine as a marker of exposure can be carried out during periods of exposure or nonexposure to ETS in chamber studies. Similar pharmacokinetic studies could be carried out for individual VOCs contained in indoor mixtures. Relationships could be established between inhaled dose and concentrations in blood, urine, or exhaled air. Such information potentially could be useful for assessing exposure in free-living individuals participating in epidemiologic studies.

Another promising use of chamber exposures to ETS or VOC is as part of a hybrid epidemiologic–clinical study. In a questionnaire survey of an exposed population, one might identify individuals who

are unusually sensitive and others who are nonsensitive. These groups could be exposed under controlled conditions and examined both for concordance with reported symptoms during ambient exposure and for physiological differences in response that could account for symptom differences. Furthermore, depending upon the question to be addressed, the a priori ability to identify responsive individuals can increase the study efficiency through proper selection of subjects. Alternately, one could document in the chamber the responses of a group of individuals who were to move into a new building. Concordance between responses measured in the chamber and those in the new ambient environment may provide insight into the host factors responsible for differences in response and into the underlying basis for reported symptoms. Such information could be useful for study design and control of confounding in future epidemiologic studies.

Another area that should be explored for feasibility is the use of environments other than existing chambers for quasi-controlled human exposures. For example, many model houses used for air monitoring information exist (*30*). The pollutants in these structures represent exposures of interest and are well measured. The feasibility of exposing individuals to these mixtures in these facilities and measuring responses should be explored. Similarly, facilities in which the atmospheric chemistry of photochemical oxidants is studied could provide an opportunity to assess the effects of exposure of individuals to a number of representative mixtures, including ozone and acid aerosols. A third approach involves the use of mobile chambers, which would allow the hybrid epidemiologic–clinical studies discussed above to be conducted at many more locations. Atmospheres could be generated for study of individual responses to single compounds or to specific mixtures of pollutants at the site of an epidemiologic investigation. Alternately, ambient air from various locations at an epidemiologic study site could be drawn into the mobile chamber for measurement of individual responses and inhaled doses. Such an approach would allow the random assignment of individuals to environmental conditions.

The dissective component of the top down strategy begins with understanding the effects of exposure to the mixture and then involves further work to identify the individual pollutants responsible for the observed effects. Willes et al. (*27*) have done some preliminary work in this area by measuring the responses of sensitive individuals to different components of ETS. Such an approach also could be undertaken with VOC, and the approach of Avol et al. (*25*) with the mix of photochemical chemicals could be refined and expanded. The use in knowing the compound of greatest interest is that exposure assessment in epidemiologic studies could be directed to that individual compound, and reduction of exposure to a single noxious agent may be a more efficient method of reducing effects than reduction of exposure to the entire mixture.

The bottom up or synthetic approach involves understanding the effects of exposure to individual pollutants (e.g., ozone and one acid-aerosol species) and then assessment of the joint effects of exposure to mixtures of these individual pollutants. This has been the method used most often in human chamber studies. This approach can also be extended to study the joint effects of two complex mixtures, such as VOC and ETS, or one complex mixture with one pure compound, such as ETS and nitrogen dioxide or ozone, and a mix of acid-aerosol species. As mentioned, the chamber study is a powerful tool in establishing causality between a given exposure and effect. From a theoretical perspective, it is very attractive for quantifying the individual and joint effects of two or more substances. Because of practical limitations on the amount of resources that can be devoted to a particular question, however, the actual utility is restricted. This is reflected in the number of subjects that can be studied.

Studies in which maximal utility can be made of this method include the effects of ozone and sulfuric acid aerosol upon respiratory symptoms and changes in lung function, or the effects of ETS and VOC exposure on symptoms and number of leukocytes in nasal lavage. Other studies in which some contribution could be made might include the effects of nitrogen dioxide and ETS on incidence of respiratory infection or the effects of ozone and sulfuric acid aerosol on frequency of asthma attacks. In the former case, some of the outcomes of interest can be measured directly; in the latter case, the incidence of respiratory infection and asthma attacks following a single exposure is likely to be too low to study efficiently. One might use an attenuated virus to study directly the effects of pollutant exposure on infection rates. Alternately, one may choose a surrogate measure for likelihood of infection, such as a decrease in phagocytosis of virus by alveolar macrophage harvested by bronchoalveolar lavage, or a surrogate measure for asthma attacks, such as an increase in responses to methacholine, cold air, exercise, or, more invasively, antigen. Similarly, identification of outcomes that occur following acute exposure and are in the pathogenetic pathway for a given chronic disease might allow inferences to be drawn from acute responses about the effects of chronic exposure to a given mixture.

In order to make maximal use of this method for assessing risk from exposure to varying levels of two compounds, one must be able to define the response surface for all possible combinations of the two substances. Assuming that response to each substance is nonlinear, one needs at least four concentrations for each pollutant for a total of 16 cells. Depending upon the precision of the measurements of interest and the variability in responsiveness to each pollutant, one needs a minimum of 10 to 20 individuals per cell. Such a study allows description of the entire response surface for the given exposure protocol and might allow one to distinguish between competing statistical models of interaction. Achievement of this latter goal requires substantial a priori knowledge of individual dose–response characteristics so that the optimal concentrations and conditions for study can be chosen and the number of models tested can be kept to a minimum. Definition of the response surface for exposure to two substances is further complicated by adding the multiple dimensions of time. Issues such as duration of exposure and latency period for effect development for each pollutant are critical for definition of response and add tremendously to the complexity and expense of this approach.

A simpler approach, which gives limited information but requires far fewer resources, involves study of fewer combinations of exposure and a selected duration of exposure and times of measurement. Because one often has some information about dose–response characteristics for each component of the mixture, one can usually choose a concentration for each substance that gives reproducible responses and that is either near the threshold of response or on a linear portion of a dose–response curve. Using clean air and a single concentration of each pollutant, one can measure the effect of each pollutant compared to a clean-air exposure and the joint effect of exposure to both compared to the effects of exposure to each and to clean air. While one cannot choose between different models of statistical interaction with this study design, depending upon how exposure concentrations are chosen, one can decide whether the joint effect compared to air exposure is large enough to justify concern, whether addition of a noneffect level of one pollutant to another pollutant produces

increases in response, or whether the combination of two pollutants with small, individual effects results in a much larger effect or in a reduction of effect.

Another use of the synthetic, or bottom up, approach is in identification of sensitive individuals for epidemiologic study. As mentioned for the top down approach, identification and study of individuals with optimal rates of the outcome of interest due to either exposure alone or to the joint exposure can result in more efficient epidemiologic studies. Furthermore, if other risk factors for the response of interest can be identified in chamber studies, control of potential confounding by these factors can be controlled in subsequent epidemiologic studies.

One can conclude that human experimental exposure studies play an important role alongside epidemiologic and animal toxicologic studies in the investigation of health effects that are the result of exposure to complex mixtures. The human chamber studies are limited with regard to assessing chronic effects, rare effects, or effects from long duration exposure but are powerful in assessing acute, reversible effects from short-duration exposures in the species of interest. The areas in which chamber studies are most likely to contribute include identification of effects or markers of effects for exposure to a given pollutant or mix of pollutants; direct dose–response assessment of effects for individual compounds and mixtures of set composition; identification of individual compounds responsible for the effects of a mixture; study of the joint effects of a binary mixture; development of markers of acute exposure for particular compounds; development of outcome measurements that can be used in the field; and identification, characterization, and testing of sensitive subpopulations. ϕ_p^h

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Introduction to Working Group on Tropospheric Ozone, Health Effects Institute Environmental Epidemiology Planning Project

Ira B. Tager*

Department of Medicine, University of California, San Francisco, Department of Veterans Affairs Medical Center, 4150 Clement Street, San Francisco, CA 94121

The working group on tropospheric ozone of the Health Effects Institute has evaluated the need for epidemiologic studies on the health effects of ozone (O_3) exposure. This paper summarizes current data and identifies possible research questions. The extent to which ozone exposure results in chronic health effects is largely undefined and is the central issue for epidemiologic studies. Most current data focus on transient endpoints; the link between acute changes in symptoms and/or lung function and possible chronic efects has not been established. Concepts of ozone-induced health effects have been extended to include processes of chronic disease (e.g., markers of ongoing inflammation and repair, markers of accelerated lung aging). Traditional epidemiologic studies performed have focused only on accelerated lung aging and are limited by a number of methodologic problems. Recent, very preliminary, studies suggest new opportunities for the use of human lung tissue and a variety biological response markers as part of epidemiologic studies. The identification of sensitive subpopulations with regard to ozone-induced health effects has been studied incompletely and is important both in terms of study efficiency and mechanistic insight. Methodologic advances in the reconstruction of past ozone exposure are seen as essential, as is the incorporation of emerging markers of biologic response to ozone into traditional epidemiologic study designs. Finally, more data on the joint and independent contribution of other ambient air pollutants to putative ozone-induced health effects is warranted. — Environ Health Perspect 101(Suppl 4):205–207 (1993).

Key Words: Ozone, health effects

Background

An extensive body of data has been developed on the biologic, physiologic, and health effects of ozone. Two recent comprehensive reviews (*1,2*) summarize this information. From these reviews, it is clear that epidemiologic studies represent only a small part of the current knowledge base on possible health effects of ozone and that considerable opportunities exist for epidemiologic studies to fill in many of the gaps in the current state of knowledge about these effects.

The working group on tropospheric ozone has addressed Health Effects Institute's (HEI's) objectives with regard to needed epidemiologic research through the following steps: identification of general research questions with regard to ozone-induced health effects that the committee felt most needed to be addressed and formulation of these research questions in terms suitable for epidemiologic study designs. At each stage of this process, consideration was given to the theoretical and practical advantages of available study design alternatives and the need to consider new design approaches.

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*Present address: 140 Earl Warren Hall, University of California, Berkeley, School of Public Health, Berkeley, CA 94720

The remainder of this introduction is devoted to a brief summary of the working group discussions on the general research needs that guided the development of the papers that follow.

The extent to which ozone results in chronic health effects in humans remains largely undefined and is a central issue for epidemiologic studies. Data from primate exposure studies suggest that permanent or poorly reversible changes in the distal airways and proximal alveolar regions of the lung might be important consequences of prolonged ozone exposure (*3,4*). Recent chamber studies of the acute effects of ozone exposure on the dispersion of inhaled aerosol boluses support the possibility that similar small airway alterations occur in otherwise healthy humans (*5*) and could be precursor lesions to a more chronic process.

To date, most studies of ozone-induced health effects in humans have focused on specific transient endpoints (e.g., symptoms, change in lung function after acute exposure, one-time assessment of cellular and/or biochemical markers of inflammation and repair). Moreover, the link between current measures of acute symptomatic and/or functional responses to ozone (*6,7*) and the occurrence of chronic effects has not been established in humans, nor has it been established that the mechanisms that underlie acute effects [e.g., airways hyperreactivity (*8*), reflex neural alterations of measured vital capacity (*9*), inflammatory changes (*10*)] are the same as those that underlie chronic effects (e.g., loss of lung elastic recoil, deposition of excess collagen).

Analogous to studies of the natural history of the effects of cigarette smoke on lung function and the subsequent occurrence of chronic pulmonary disease (*11*), concepts of ozone-induced health effects have to be extended beyond the evaluation of specific endpoints to include processes indicative of chronic disease (e.g., markers of ongoing inflammation and repair, biochemical and/or physiological markers of accelerated lung aging, etc.). To date, traditional, population-based epidemiologic studies that have tried to address process (e.g., accelerated decline in lung function) have been restricted to the evaluation of a limited range of lung function measures. They also have been handicapped by a number of problems: *a*) large losses to follow-up (*12,13*), which on the surface do not appear to have affected validity; *b*) inadequate characterization of individual and group exposure to ozone prior to study onset; *c*) difficulties in obtaining detailed data on concurrent ozone exposure for large numbers of subjects; *d*) lack of data on modifying and/or confounding factors; and *e*) the relatively short duration of follow-up (*12,13*) or the purely cross-sectional nature of their evaluation (*14*). These

studies have derived their epidemiologic appeal from a perceived need to have study samples from whom generalizations can be made to some population at large. These studies were considered to be of continued relevance for comprehensive policy analysis into the health and economic impact of exposure to ozone, but they were thought to be of less importance in terms of efficiency for studies aimed at the identification of acute and chronic health effects and chronic alterations of biological processes (e.g., premature aging of the lung). Epidemiologic studies in targeted populations (e.g., persons moving between localities with different ozone exposure characteristics, asthmatics) were considered as alternatives to provide valid data with greater efficiency for the determination of the extent to which acute and chronic health effects occur and for the characterization of such effects.

To address the issue of pathophysiologic process, innovative study designs that use new sources of data on the response of humans to ozone were considered in detail. Despite a number of significant limitations, the working group felt that the recent autopsy study of Sherwin and Richters (*15*), which reported an excess of severe respiratory bronchiolitis in the lungs of young accident victims who lived in Los Angeles, suggested important new opportunities for epidemiologic studies of chronic ozone-induced health effects. The development of well-designed autopsy studies and living population studies of inflammatory markers might provide a means to identify early pathologic changes that, by analogy to data for cigarette smoke (*16,17*), represent precursor lesions for more serious chronic effects and/or relate to acute and subacute functional changes in the lung. Study designs that utilize individuals who move, either temporarily or permanently, to and from areas with major differences in ambient ozone concentrations were seen as creative means to test hypotheses about functional changes in the lung and health effects in general (*18*).

The identification of sensitive subpopulations with regard to ozone-induced health effects (especially chronic effects) was considered to be important both for study design efficiency and for mechanistic (biologic) insight. Asthmatics, in particular, were singled out for consideration because of their increased reactivity to a variety of environmental stimuli. This approach was supported by population-based studies that demonstrated an association between acute asthma and other respiratory morbidity (emergency room visits) and other air pollutants (*19*). The failure of controlled exposure studies to identify, unequivocally, asthmatics as a sensitive subpopulation was not seen as limiting in this context, since it may have been the result of *a*) inadequate exposures used in the controlled exposure studies that have been conducted to date (*20*), *b*) the inclusion of only milder asthmatics, *c*) the exclusion of asthmatics with acute respiratory illness or its exacerbation, and *d*) failure to simulate the complex mix of air pollutants to which such individuals may be exposed (*13*). Epidemiologic studies, in conjunction with additional controlled exposure studies (*20,21*), were considered essential to address the above problems and to identify more clearly other sensitive subgroups and the factors that define sensitivity to ozone. A recent study that indicates that ozone exposure may enhance lung airway responsiveness to aeroallergens (*22*) adds further impetus to this focus.

Standardized questionnaire assessment and measurement of forced expiratory volumes and flows have been the primary outcomes that have been used in controlled exposure and epidemiologic studies to identify individuals who respond to ozone exposure (e.g., report of a symptom(s) or a change in level of volume or flow after exposure). On a more limited basis, markers of lung inflammation, as assessed through analysis of the fluid and cellular phases of bronchoalveolar lavage, have been employed to expand the concept of response to ozone (*23*). Given the importance attached to the ozone-sensitive population in the working group's formulations, it was imperative that the studies of ozone-induced health effects include more proximate markers of biologic effect (damage, repair, etc.) and measures of pulmonary function that may be more sensitive markers of effect or may reflect a broader range of functional alteration [e.g., changes in the distribution of inhaled aerosols (*5*)].

Although not unique to epidemiologic studies of ozone-induced acute and chronic health effects, methodologic advances were seen as necessary for the retrospective reconstruction of past ozone exposure status (e.g., identification of suitable, long-term, air pollutant monitoring databases, development of indices of relevant past exposure, time-activity indices, etc.). It was felt that the need for valid and reproducible methods that assess retrospective and concurrent exposure that are suitable for epidemiologic studies is of sufficient importance to merit independent study. Moreover, the suitability of existing (*24,25*) and evolving (*25*) models to estimate individual ozone exposure in epidemiologic studies was considered to be in need of further definition, as were models that have utility for a wide variety of epidemiologic study designs.

In this context, ozone exposure indices need to account for the independent and joint effects of other air pollutants. The relationship of the distribution of ozone to other pollutants (e.g., acid aerosols) was seen as playing an important role in the selection of study locations and the integration of results from studies from a number of differing geographical locations.

While issues of statistical analysis were not the principal purview of the working group, statistical issues figured prominently in the discussions. In particular, the working group felt that study designs of ozone-related health effects need to be able to incorporate new approaches to the analysis of within- and between-subject variability in response to given ozone exposures. Moreover, this variability, particularly within individuals, was considered an important endpoint for study in its own right, especially given the evolutionary adaptation to ozone that has been observed in some controlled exposure studies (*26,27*).

Research Questions for the Study of Ozone-Induced Health Effects

The working group established a series of discrete questions to which the members have addressed their presentations. Within the framework of a particular question, a brief background is presented that is intended to provide a context for the discussion rather than a comprehensive summary of available research data. Where appropriate, issues of exposure assessment and the specification of a range of study designs are discussed or very specific designs are suggested (*28,29*). In these latter cases, the designs that are presented are done so because the working group felt that the illustration of a specific design best captured the research needs in question. Finally, while several of the papers discuss issues of analysis, the overall views of the working group have been synthesized in a single, more statistically oriented presentation (*30*).

The following questions reflect the synthesis of the working group's deliberation and the guideline for the articles in this volume.

Chronic Effects and Processes

What study (studies) is (are) required to determine whether ozone causes chronic health effects? How can population studies be utilized to determine if respiratory bronchiolitis is caused by ozone? Is premature aging of the lung related to this process (as a consequence

of ozone exposure)? What is (are) the optimal methodologic approach(es) to determine if ozone leads to premature aging of the lung?

How can the direct analysis of lung tissue be used to evaluate the chronic effects of ozone exposure? What is (are) the optimal method(s) for retrospective exposure assessment that would be needed for such tissue analyses? What are the optimal methods for subject and tissue selection, analyses, and data management that would ensure valid comparisons over wide geographic areas?

Acute Effects

What is the full range of acute outcomes due to ozone exposure, and which of these outcomes relate to specific chronic disease outcomes and chronic changes in physiological processes?

Sensitive/Susceptible Populations with Regard to Ozone Exposure

Are there sensitive subpopulations in relation to ozone exposure and how can they be identified? What laboratory-based endpoints are almost ready for use in epidemiologic studies? What new laboratory-based endpoints need to be developed for the identification of susceptible individuals in epidemiologic studies? What is the relationship of the epidemiology of asthma to ozone exposure?

Analytical Issues

What are the important (and new) issues required in the design and/or the analysis of epidemiologic studies of the health effects of ozone? What study designs most appropriately reflect the need to study biological variability in response to acute and chronic exposure to ozone?

The paper of the working group address each of these issues separately. A concluding paper (e.g.) will pull together common threads that appear throughout each of the sections to highlight the central research issues that will form the basis for specific recommendations.

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Use of Human Lung Tissue for Studies of Structural Changes Associated with Chronic Ozone Exposure: Opportunities and Critical Issues

Morton Lippmann

Institute of Environmental Medicine, New York University Medical Center, Tuxedo, NY 10987

Definitive information on the chronic effects of exposure to ozone (O_3) in humans is not available. There is a strong concern that ozone could produce chronic lung damage in humans on the basis that exposures are ubiquitous at levels that produce transient symptoms, function deficits, and lung inflammation in humans and chronic lung damage in laboratory animals. Both prospective and national population surveys suggest an association between chronic O_3 exposure and reduced lung function, and a pilot investigation of autopsied lungs of accident victims in Los Angeles reported an unexpectedly high incidence of disease in the centriacinar region, the lung region known to receive the highest dose of inhaled O_3. This paper discusses the advantages and limitations of further studies of structural changes in human lung tissue in relation to chronic O_3 exposure. The major advantages of such studies are that *a*) measurable effects may be related to realistic chronic exposures, *b*) the effects may be described quantitatively and compared directly to those obtained in chronic animal inhalation exposures, and *c*) evidence for chronic effects may be obtained much more rapidly than in prospective studies. The major limitations are the difficulties in obtaining sufficient reliable information on residential history, physical activity out-of-doors, and smoking and other confounding exposures to lung irritants from next of kin, and limited availability of adequate air quality data for determining ambient concentrations at places of residence and/or outdoor exercise. The paper also discusses approaches to minimizing these limitations in the design of specific studies. — Environ Health Perspect 101(Suppl 4):209–212 (1993).

Key Words: Chronic ozone exposure, respiratory bronchiolitis, centriacinar region disease, human lung tissue, post-mortem analyses, retrospective exposure assessment

Introduction

While it is well established that short-term exposures of humans to ozone (O_3) produce a plethora of transient responses such as reduced ventilatory function; increased symptoms, permeability, and reactivity (*1*); and an influx of inflammatory cells and mediators (*2*), there is relatively little known about the roles of repetitive transient exposures and the responses they induce in the development of cumulative lung damage and/or disease. Many of the transient responses produced by exposures to O_3 are similar to those produced by cigarette smoke, a known causal factor for chronic lung disease. Since about half of the U.S. population lives in communities having O_3 concentrations that exceed the current National Ambient Air Quality Standard, there is an ample basis for research on the effects of chronic O_3 exposure.

While past research studies on the chronic effects of O_3 have not been definitive, there are some provocative indications that there may be substantial adverse effects. The indications include: greater rate of loss of lung function in nonsmoking men and women in both Glendora, California, (high oxidant) and Long Beach, California (moderate oxidant and moderate SO_x), than in Lancaster, California (moderate oxidant and low SO_x) (*3,4*); reduced baseline lung function when annual average O_3 concentration is greater than 40 ppb, based on a national population sample (*5*); and an unexpectedly high incidence of centriacinar region disease in the lungs of adolescents and young adults examined post-mortem in Los Angeles County (*6*).

There are a variety of ways in which epidemiologic research can provide evidence of adverse chronic health effects in humans resulting from long-term exposure to O_3 and/or the other ambient air pollutants that coexist with it. Prospective cohort studies in well-defined populations of interest could be performed with suitable and careful measurements of exposure, activity patterns, symptoms, lung function, etc. However, it may be hard to justify such a study at this time for several reasons, including lack of firmer evidence that chronic effects are occurring, the very high costs of properly performed prospective studies, and the long time frame for results (i.e., at least 7–10 years).

For the above reasons, retrospective human lung studies may be most appropriate at this stage of inquiry, despite the great difficulties in adequate characterization of past exposure to O_3 and copollutants and adequate evaluation of confounding and modifying factors. Such studies have their own inherent advantages (i.e., the existence and extent of early chronic lesions in the peripheral lung tissues can be quantified), and no other kind of human studies can provide such information. Thus, quantitative comparisons of the extent of lesions in the lungs of well-matched individual cases that have lived in areas with different pollutant exposures can indicate whether there is an association between pollution and chronic lung damage. Furthermore, if there are differences in lung structure associated with chronic exposure, the nature and extent of such differences would provide an extremely valuable resource for designing follow-up studies of function and symptoms in living populations and chronic inhalation exposure studies in laboratory animals. In other words, knowledge of the structural changes that occur in humans should guide the selection of end points and measurement methods that are

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likely to produce significant results in more conventional studies.

This brief paper outlines the rationale for a study of chronic effects of O_3 exposure based on postmortem lung tissue as well as the opportunities and problems that face an investigator in conducting such a study.

Rationale

There are a series of specific factors that help establish the appropriateness of a study of postmortem lung tissue of individuals having definable variations in chronic exposure to oxidant air pollutants. First, predictive lung uptake models (*7,8*) indicate that delivered ozone (O_3) dose is greatest in respiratory acinus of humans, rats, and other species. This region of the lung is inaccessible for studies based on direct in vivo examination, except that cells from this region can be recovered by bronchoalveolar lavage along with cells from adjacent regions. Second, chronic and subchronic exposures of rats (*9*) and monkeys (*10*) at near ambient levels of exposure produce changes in epithelial cell size and distribution in terminal bronchioles and immediately distal airways. These exposures also produce evidence of lung inflammation. All of these results are consistent with predicted uptake sites for O_3. Third, intermittent exposures of monkeys (4 weeks on, 4 weeks off) produce changes that are similar to or greater than those seen in monkeys exposed continuously (and, therefore, having twice the total exposure) (*10*). These results have implications for both seasonal and daily patterns of human exposure. Fourth, the structural changes seen in the chronic and subchronic exposures in rats and monkeys are associated with the functional changes consistent with emphysema and a stiffening of the lungs, both of which correspond to premature aging of the lungs (*11*). Fifth, an autopsy study of 107 lungs from 14- to 25-year-old fatal injury victims in Los Angeles County by Sherwin and Richters (*6*) showed that 27% had what the authors judged to be severe degrees of structural abnormalities and bronchiolitis not expected for such young subjects, and another 48% of them had similar, but less severe, abnormalities. In the absence of corresponding analyses of lungs of comparable subjects from communities having much lower levels of air pollution, the possible association of the observed abnormalities with chronic O_3 exposure remains speculative. Some of the abnormalities observed could have been due to smoking and/or drug abuse, although the authors noted that published work on the association between smoking and small airway effects showed lesser degrees of abnormality (*12*).

Hypothesis

The lung abnormalities produced by subchronic and chronic O_3 exposures in rats and monkeys at near peak ambient levels are sufficiently similar to those seen in 14- to 25-year-old residents of Los Angeles to suggest that long-term ambient exposures to O_3 contributed to these effects. Furthermore, the data suggest that such exposures, if continued over a greater proportion of normal life span, could lead to reduced ventilatory capacity later in life and perhaps to chronic lung diseases such as chronic obstructive lung disease and emphysema.

Discussion

The kinds and degrees of abnormalities seen in the studies involving analyses of animal and human lung tissues discussed above would be largely subclinical and poorly related to conventional lung function indices. Measurement of spirometry and pulmonary flow resistance are generally controlled by airway calibre in the large and midsized conductive airways, whereas the locus of damage associated with O_3 is in the small airways, which normally contribute little to overall flow restriction at early stages of disease progression.

The lungs of rats, monkeys, and humans were all examined at relatively young ages. Thus, there is concern that continued chronic O_3 exposure could lead to further progression of the structural and functional changes and thereby accelerate the normal rate of loss of lung function with age in a manner analogous to the accelerated loss of function associated with chronic cigarette smoke exposure.

Research Opportunities

To test the hypothesis that O_3 exposure can cause or facilitate an accelerated loss of lung function with age in human adults, it is necessary to show that there are significant differences in age-adjusted lung abnormalities in appropriately matched populations living in areas of relatively high and relatively low ambient O_3 concentrations.

Additional requirements, aside from appropriate matching or adjustment for smoking, age, gender, ethnicity, etc., would include climate and lifestyle. High oxidant, low acidic aerosol California communities would best be matched by other Pacific Coast communities that have relatively low levels of both types of secondary pollution, such as Santa Barbara, California; Portland, Oregon; Seattle, Washington; Victoria and Vancouver, British Columbia; etc. Cities in the midwest with moderately high oxidant and acid aerosol concentrations, such as Chicago, Illinois; Cleveland, Ohio; Detroit, Michigan; Buffalo, New York; and Toronto, Canada, might be matched with more westerly cities that have lower concentrations of such secondary pollutants such as Minneapolis, Minnesota; Milwaukee, Wisconsin; Kansas City, Missouri; and St. Louis, Missouri. For hot, humid cities, Houston, Texas, with high oxidant concentrations, could be matched with lower oxidant communities in Florida such as Tampa Bay, Orlando, Miami, and Fort Lauderdale to minimize possible confounding by differences in ambient temperature and humidity.

Specific Exposure-Related Research Needs

Of all the criteria pollutants, O_3 probably has the most extensive data base for ambient community levels. Quality-assured federal and state network data are readily available, and exposure modeling for locations within a monitored area is relatively straightforward. Temporal variations on a daily and seasonal level are largely predictable, and as a secondary pollutant, O_3 concentration variations within local regions are less extreme than those for primary pollutants such as carbon monoxide and lead. Some of the same considerations apply to acidic sulfate particles, another class of secondary pollutant that also deposits preferentially in small conducting airways.

The health effects associated with sulfates are most likely due to the associated hydrogen ion rather than the ammonium ion or sulfate itself (*13*). The H^+/SO_4^{-2} ratio is highly variable, and SO_4^{-2} concentration data are usually available only on the basis of 24-hr averages every sixth day. For chronic effects studies, the available data on SO_4^{-2}, SO_2, O_3, temperature, and humidity are thought to be sufficient to permit good estimates of long-term average exposure to SO_4^{-2} and H^+, at least for an examination of potential interaction of acidic sulfates and ozone in the production of accelerated aging of the human lung.

The development of protocols for obtaining residential and personal risk factors information on fatal injury victims whose lungs are analyzed is a specific research need. For those for whom such information can be obtained reliably and who have no complications of smoking or occupational exposures to lung irritants, cumulative O_3 exposure based on ambient concentrations at pollution

monitoring sites adjacent to or surrounding the residence or work sites can be calculated. To assess the cumulative exposures of individuals whose lungs are studied, these data should be obtained: *a*) residential histories—inclusive years at each address; *b*) distances from nearest continuous quality-assured monitoring sites for O_3 and other pollutants at each residential address; *c*) participation in outdoor activities, sports, and regular exercise (including intensity, duration, location, and time of day); *d*) history of acute or chronic lung diseases; *e*) cigarette smoking as well as occupational and hobby exposures; *f*) residential exposures to confounding factors environmental tobacco smoke (ETS), unvented gas and kerosene cookers or space heaters, wood smoke, molds, mildew, etc.); and *g*) commuting patterns resulting in different levels and types of air pollution exposures.

The development of alternate indices of cumulative pollutant exposure for correlation with activity patterns to yield individual exposure metrics is another research need. The exposure indices would then be correlated with the extent of observed lung abnormalities. Pollutant data resources of reasonably reliable quality include *a*) EPA and local monitoring data for O_3, NO_x, SO_2, SO_4^{2-}, and PM_{10}; *b*) weather bureau data such as temperature, humidity, wind speed and direction; and *c*) airport visibility data (which can serve as surrogates for fine particle concentrations).

With regard to research needed to develop methodologies for retrospective exposure assessment, some preliminary research of this type has been performed at New York University (*14*) and Harvard (*15*); further research in this area is continuing. It involves improving and validating predictive models by using available pollutant concentrations and meteorological data bases. While the preliminary work is encouraging, much more needs to be done.

Retrospective exposure assessment research needs for chronic O_3 epidemiology studies include delineation, investigation, and development. Delineation of the influence of various factors on local outdoor O_3 concentrations and indoor/outdoor (I/O) O_3 ratios is necessary for this type of research. Outdoor factors include sources of O_3 scavengers such as NO from motor vehicles, elevation, and local micrometerology. Factors affecting the I/O ratio include air exchange ratios and the nature of indoor surface sinks for O_3. Investigation of the reliability of models for estimating ambient concentrations of H^+ from intermittent (every sixth day) measurements of SO_4^{2-} or continuous measurements of fine particle mass or light-scatter coefficient is another research need. Also necessary is the delineation of the influence of various factors on local outdoor aerosol H^+ concentrations and I/O ratios. Major factors here are the strengths of the indoor and outdoor sources of ammonia (NH_3) and the rates of neutralization of H^+ by NH_3 from outdoor and indoor sources. A final retrospective exposure assessment research need is development and validation of exposure models that combine air concentration data and activity data to yield personal estimates of total hourly or daily inhalation rates.

The design and evaluation of a personal history questionnaire about residential and occupational histories and personal risk factors of the accident victims whose lungs are to be analyzed is also needed in exposure-related research. Among the data that should be acquired, as possible, for each individual are: *a*) analysis of blood for COHb and/or cotinine as well as for evidence of substance abuse that might produce lung abnormalities; *b*) residential history from next of kin, to be verified to the extent possible by information from driver's licenses, school records, etc.; *c*) occupational history, if any, from next of kin, to be supplemented by employers' records; *d*) patterns of outdoor activity from next of kin, supplemented by records of team sports, running clubs, etc.; and *e*) records on location of nearest air pollution monitoring site(s) for residences lived in for the past 10 years or longer, if appropriate, along with a listing of the pollutants monitored at each site.

A final research need is the identification of suitable cities and collaborating pathologists for maximizing the range of chronic ozone exposures and access to suitable lungs in those communities. Consider possibilities for matching for climate, socioeconomic levels, and other potentially confounding factors. In terms of the selection of the communities that may provide the best opportunities for the collection of lungs having the greatest range of air pollutant exposures of interest, it would be ideal to have *a*) high ozone with low H^+, *b*) high H^+ with low ozone, *c*) high ozone with high H^+, and *d*) low ozone with low H^+.

In reality, there are no large communities that meet any of these criteria, except that large portions of the greater Los Angeles area fall into the first category. Areas with complex terrain and variations in altitude, such as Los Angeles, can include people with highly variable pollutant mixtures. On the other hand, many large metropolitan areas in the midwest and along the Atlantic coast have relatively uniform concentrations of secondary pollutants such as O_3 and H^+. The tall stack SO_2 emissions that lead to H^+ formation in the areas several hundred to several thousand km downwind vary from west to east, and the most westerly of the large midwestern cities such as Minneapolis, Minnesota; Kansas City, Missouri; Dallas, Texas; and Fort Worth, Texas, should have much lower H^+ exposures than other large cities on the plains or Great Lakes such as Detroit, Michigan; Cleveland, Ohio; and Toronto, Canada. Ozone, formed closer to ground level, should have lesser east to west gradients. Major cities having low concentrations of both O_3 and H^+ include Seattle, Washington; Portland, Oregon; and Vancouver, British Columbia. Smaller communities include Santa Barbara and the Monterey Peninsula area in California.

The rate at which numbers of lungs that can be obtained from young accident victims and processed in a uniform manner sufficient to permit sensitive quantification of pathological abnormalities, localized morphometry, and cell type distributions will be quite limited, especially for the smaller cities or regions, it may not be feasible or desirable to look for differences in means of responses by city or region. An alternative is to treat the results on each individual case as an independent observation related to that individual's cumulative exposure scores for O_3. There should be enough O_3 monitoring data in the higher O_3 areas to devise a numerical value for each case with long residence in a high or moderate O_3 area. It may or may not be useful or desirable to semiquantitatively rank them by H^+ exposure based on measured sulfate levels and background knowledge of atmospheric chemistry (*14*).

Specific Health Effects Assay Needs

The Sherwin and Richters (*6*) study, while a pioneering effort of great interest, raised more critical issues than it settled. The distinctly abnormal centriacinar lesions they found could have been due less to air pollution than to cigarette smoking, drug abuse, or other stresses of the lower socioeconomic status (SES) that affected many of the individuals studied. Furthermore, the prospects of obtaining adequate background data on residence, occupation, and other critical variables on these and similar individuals are relatively poor. Another problem is that tissues were sampled for up to 48 hr postmortem. Information from animal exposure studies suggests that samples should be collected within one hour or less to obtain satisfactory results. The animal studies also indicate that some additional analytic protocols should be performed on

human lungs in future studies, to improve the prospects of seeing changes of interest in the tissues receiving the highest doses of oxidant, and to provide complementary analyses for interspecies comparisons.

It should be possible to overcome many of the limitations of the Sherwin and Richters protocols in designing future studies of human lung tissue from individuals chronically exposed to different levels of air pollution. For example, it may be possible to obtain fresher tissues from transplant donors. If such a source is used, the prospects of gathering adequate personal history data from next of kin should be better than for medical examiner cases. Selection of more optimal protocols for tissue analyses should be based on the recommendations of an expert panel convened to design such protocols.

The major developmental need for measuring the health impact of chronic ozone exposure is to refine and standardize the pathological protocols used in selecting target populations by age, location, background, etc. and used for selecting, storing, processing, inflating, fixating, preparing, devising analytical protocols for, managing data, etc., for human lungs. Consideration must be given to matching end points to those that have been or can be used in the chronic animal exposure studies. In terms of standardization of pathological analyses, there is a need to convene one or more expert panels of pulmonary pathologists with research backgrounds in irritant responses and let them establish suitable analytic criteria that would be used for all specimens.

Summary and Conclusions

Parallel studies on quantitative methods for retrospective exposure assessment need to be undertaken along with methods for quantitatively characterizing lung pathology, morphometry, and cell distributions. Neither aspect is trivial or easy, but the opportunities to do both are quite real and feasible.

Arrangements need to be made for coordination, standardization of protocols and procedures, and quality assurance for each collaborating investigator and group of investigators. Workshops should be conducted at the beginning and intermediate stages, and the results and experiences among the different groups should be used to develop and refine more optimal protocols. Furthermore, consideration should be given to the need for and benefits from continuing interchange of information with toxicologists performing chronic animal inhalation studies. ehp

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Examining Acute Health Outcomes Due to Ozone Exposure and Their Subsequent Relationship to Chronic Disease Outcomes

Bart D. Ostro

Air Pollution Epidemiology Unit, California Office of Environmental Health Hazard Assessment, Berkeley Way, Annex 11, Berkeley, CA 94704

Current evidence indicates that individuals exposed to short term elevations in ambient ozone may experience both upper and lower respiratory effects. Some respiratory symptoms and spirometric changes are mild and reversible in nature, while others involve more severe outcomes, including hospital admissions and emergency room visits. However, many questions remain about the effects of acute ozone exposure and the implications of this exposure for chronic disease outcomes. For example, the identification of sensitive subgroups, the delineation of the entire spectrum of health effects due to exposure to ozone, the potential synergy between viral infections and ozone exposure, and the nature of adaptation to ozone are not well characterized. In addition, studies that examine the association between acute responses to ozone and potential biological indicators of a chronic disease process would be desirable. This paper serves to provide an overview of the types of epidemiologic studies that may be appropriate and factors to consider in addressing these questions. — Environ Health Perspect 101(Suppl 4):213–216 (1993).

Key Words: Ozone, acute morbidity, panel study, epidemiology, respiratory

Introduction

Previous epidemiologic and field studies have examined the effects of ozone on several different acute health outcomes, including incidence of asthma attacks, hospital admissions, emergency room visits, cough and other respiratory symptoms, changes in lung function, and decreased exercise performance. Controlled chamber studies of exercising adults have recorded the occurrence of respiratory symptoms, spirometric changes, and effects on bronchial reactivity of 1, 2 or 7 hr ozone exposures. Taken together, such studies indicate that individuals experience both upper and lower respiratory symptoms, apparently of a mild and reversible nature, in response to current ambient levels of ozone. However, at this time, many questions remain about the health effects of acute ozone exposure. For example, the existence of a sensitive subpopulation, the role of respiratory infection prior to exposure, the effects of ozone on allergic response, the interactions between ozone and other pollutants or aeroallergens, the relevant averaging time for ozone exposure, the relationship between exposure and response, the lowest level at which effects are observed, and the role of averting behavior all are not well characterized at this time. In addressing these uncertainties, several factors need to considered, including: *a*) How representative are the groups that have been and are being studied in relation to the general population? *b*) What acute health outcomes should be examined? *c*) Which acute health outcomes, if any, indicate the existence of a chronic disease process? *d*) What is the nature of adaptation to ozone exposures over time? *e*) How should ozone dose be measured to better represent actual exposures? *f*) What confounders and effect modifiers need to be incorporated into any analysis of ozone effects? and *g*) What study design is most appropriate for examining the acute effect of ozone?

This paper provides an overview of the types of epidemiologic studies that may be useful and factors to consider when addressing these questions. It begins with a brief survey of previous epidemiologic studies of the acute effects of ozone.

Previous Epidemiologic Studies

Existing epidemiologic studies provide an incomplete picture of the acute health effects of ozone. To date, panel studies, which incorporate both cross-sectional and time-series components, have focused on selected groups that include asthmatics and female student nurses in Los Angeles. Studies of asthmatics (*1,2*) suggest that moderate levels of air pollution, including ozone, result in an increase in the exacerbation of asthma. However, these studies can examine the response for only a small subset of asthmatics. In addition, questions about the role of medication, exercise, averting behavior, indoor exposures, and viral infections remain. Also, in these studies, asthmatics who reported only a small number of symptoms during the survey period were dropped from the sample because of the statistical methods employed (i.e., individual-level logistics would not reach convergence). Studies of female nurses in Los Angeles (*3–5*) explored the relationship of daily oxidant (rather than ozone) concentrations to daily symptoms that included cough, chest discomfort, sore throat, and eye irritation. These studies suggest that eye irritation, cough, and chest discomfort are related to daily exposure to oxidants.

Evidence of morbidity from acute ozone exposure also is provided from studies of hospital admissions. For example, in southern Ontario, Canada, Bates and Sizto (*6*) reported a relationship between hospital admissions and higher concentrations of the existing "summer haze" consisting of ozone and sulfur compounds. Because of high covariation among the various pollutants, however, it is difficult to determine the extent of an independent effect of ozone. Wayne and Wehrle (*7*) showed that high oxidant (and particulate matter) levels were associated with decreased athletic performance among high school students in Los Angeles.

Analysis of large, cross-sectional data sets also provides some evidence of acute health effects of ozone exposure. For

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example, analyses of the National Health Interview Survey, an individual-level survey of 50,000 households conducted by the National Center for Health Statistics, suggest an association between ozone and respiratory symptoms causing minor restrictions in activity (*8,9*).

Several field studies have examined the impact on lung function of daily ozone exposure. Study groups have included children in summer camps in New Jersey and in the San Bernardino mountains of California (*10–12*), and healthy, exercising, nonsmoking adults in New York (*13*). These studies indicate a dose-dependent relationship between ozone and lung function parameters, including FEV_1 and peak flow. However, the implications of these small changes in lung function for either acute symptoms or chronic respiratory effects are uncertain. Of note, the measured changes in pulmonary function were greater than those predicted from comparable levels of ozone administered in controlled chamber studies, suggesting that chamber studies do not accurately represent effects of the mix of exposures experienced by the general public.

Representativeness of Previous Findings

It is uncertain whether the existence and magnitude of these same acute health effects related to air pollution can also be expected to occur for the population as a whole. For example, it is unclear whether the effects exist only for those people receiving a high effective dose of ozone (ozone concentration *x* duration of exposure *x* ventilation rate), such as children at play or adults vigorously exercising, or for people who may be particularly vigilant about reporting changes in health status (i.e., student nurses or asthmatics). To date, epidemiologic studies have used 1-hr maximum concentrations of ozone as the averaging time; no study has examined, for example, the impact of 7-hr daily averages of ozone. Mismeasurement of exposure would result in lower detection of an effect of air pollution. Thus, current evidence does not clearly indicate the entire range and severity of effects and whether they are related to exposure to ozone alone or to a more complex mix of pollutants. While setting ambient air quality standards requires only the determination of concentrations that cause effects in a subpopulation, comprehensive policy analysis necessitates an understanding of the true scope of the health and economic impacts of air pollution.

Measures of Acute Effects

Given the uncertainties cited above, additional studies of acute exposure experienced by the general population are warranted. The full spectrum of potential health outcomes should be explored, from relatively minor and reversible outcomes, such as respiratory irritation and pulmonary function changes, to exacerbations of existing chronic respiratory disease, incidence and duration of respiratory infections, physician and hospital visits, and mortality. If pulmonary function changes are utilized as an end point, their relevance to acute symptoms and chronic outcomes should be explored.

In examining the effects of ozone, it would be particularly useful to consider the coherence (i.e., the joint occurrence) of multiple health end points within or across sample sites. The observance of a continuum of effects would lend credibility to the epidemiologic approach and findings. Thus, if an association between ozone exposure and emergency room visits is found, one should also be able to detect, under similar ambient conditions, an association between ozone exposure and less severe outcomes. Such an examination would involve, for example, the collection of individual-level data on symptoms along with group-level data on hospital admissions and emergency room visits within a given catchment area. Another option would be to recruit individuals making respiratory-related emergency room visits for a subsequent study of acute respiratory symptoms.

Relating Acute and Chronic Effects

Because of the difficulties inherent in conducting long-term epidemiologic studies, few studies have attempted to relate chronic exposure to ozone to subsequent health effects. Some studies compare two or three different cities and statistically relate the differences in respiratory symptoms or pulmonary function to the general ambient air pollution levels observed in those cities (*14*). These cross-sectional studies typically suffer from several shortcomings, including imprecise or unmeasured pollution exposure during and prior to the study and the lack of information on commuting patterns, income and education, health habits and practices, and averting behavior. Nevertheless, the findings of these studies suggest that the development of chronic disease may be associated with long-term exposure to ozone.

An understanding of the implications of acute health outcomes and exposures for the development of chronic disease continues to be of particular importance. Animal studies indicate that ozone exposure may result in increased fibronectin or collagen in the lung, which may indicate a chronic disease process. Devlin (*15*) presents a more complete discussion of the status and use of various indicators of chronic disease. Study designs that examine the link between acute exposures and biological markers of chronic disease would be an important contribution to the understanding of the effects of ozone. Such a study would investigate the extent to which biochemical changes indicative of a chronic disease process are associated with changes in lung function, airway reactivity, or symptomatology in a cohort of individuals exposed to a mix of pollutants including ozone.

Research by Ostro et al. (*16*) demonstrated that a quantitative relationship exists between changes in FEV_1 and reports of both mild and moderate lower respiratory symptoms. Ideally, associations between these acute health end points and assays that indicate lung damage could also be examined with respect to the potential for chronic disease. This effort could be part of a general study linking ozone to acute respiratory effects among, for example, a panel of healthy individuals. Individual-level regression analysis could be used to differentiate levels of response to ozone. This would facilitate subsequent comparisons of those individuals who are most responsive to ozone with those who have little or no response. Between these two distinct groups, it may be easier to differentiate biochemical indicators that are believed to be indicators of a chronic disease process. Investigators could examine the length and level of ozone exposure necessary (if it relates at all) before biochemical signs of chronic disease occur. This may entail simultaneous long-term cohort studies in parts of the county experiencing different patterns of peak and long-term ozone concentrations.

Adaptation

Another issue that deserves consideration within the context of epidemiologic studies of acute health effects is that of adaptation. Some clinical studies indicate that repeated daily exposures to ozone cause reduced functional and symptomatic responses (*17*). However, this has not been examined epidemiologically, particularly among individuals with moderate or severe preexisting respiratory disease. Also, there is uncertainty about the duration of the attenuation after

exposure, the levels of exposure necessary to induce these attenuated responses, and whether the inflammatory process continues in human lungs even with adaptation.

From a study design perspective, the examination of adapration may necessitate repeated administrations of a survey instrument and physical measurements within and across seasons at the same and possibly other locations. Adaptation can also be explored by focusing on certain periods within the ozone season (e.g., during consecutive high ozone days, after a one day spike at the beginning and the end of the season, etc.) and examining the severity of response during these times. By considering different locations and seasons, researchers could begin to determine the differential impact of peak versus longer term cumulative ozone exposure on adaptation. It would also be of interest to test for a difference in acute response among a group that has lived in a polluted area over a long period of time and a group that has a long residential history in a relatively low pollution area.

Exposure

Readings from fixed-site monitors should be adjusted, to the best extent possible, to refine exposure estimates by including factors such as study participants' time spent outside; use of air conditioning; and the time, location, and intensity of exercise or other heavy exertion. Until now, few epidemiologic studies have collected or used such information to improve the measurement of exposure. It would be useful to know which, if any, of these factors actually make a difference in the estimated pollution effect. For example, it may be more effective to have broad indicators about the time, location, and level of exercise for a 3- to 6-month period than very detailed (e.g., every 15 min) time–activity diaries for only short periods of time. Likewise, it may be sufficient to have information on simply whether a gas stove or air conditioner was used on a given day (or even if one is in the house) rather than exactly when and how long these appliances were used and the precise location of the survey respondents. The less detailed questions will facilitate longer study periods and perhaps larger sample sizes. With this information, subsequent research efforts could make better use of survey resources, and could improve and streamline survey instruments.

An additional issue relating to ozone exposure is the appropriate length of the averaging time. Because the acute toxicity of ozone appears to be dose-related and because people spend more time outdoors on the sunny days that favor ozone formation, it has been proposed that the ambient air quality standard for ozone incorporate a longer averaging time (*18*). Several studies indicate that exposures of 7 hr at concentrations as low as 0.08 ppm ozone elicit respiratory symptoms and significant decrements in pulmonary function (*19,20*). Therefore, measurement of ozone concentrations as both 1-hr daily maximum and longer-term daily averages, especially in areas where these measures are not highly correlated (i.e., where there is a large peak to mean ratio), would be useful.

Confounders and Effect Modifiers

Survey research methods for collection of relevant data should be developed to account for such potential confounders or effect modifiers as temperature and humidity, active and passive smoking, and use of gas stoves and air conditioners. In evaluating ozone effects, it is important to collect and evaluate data on pollens and on other ambient pollutants including, but not limited to, fine and inhalable particulates, sulfates, nitrates, and acidic aerosols. Because of high correlation among these pollutants at certain locations during certain seasons, it may be necessary to examine multiple sites or conduct repeated sampling at a given site to ascertain the impacts of individual pollutants.

Recent clinical evidence indicates that prior exposure to ozone enhances the subsequent response to sulfur dioxide (*21*) and may increase sensitivity to aeroallergens (*22*). Therefore, exposure information on other ambient pollutants and aeroallergens on days prior to and subsequent to the ozone exposure should be explored and developed. Seasonal influences can be minimized by careful selection of criteria in the study design. This may necessitate either the completion of the study within a given season across many different sites or the continuation of a study into several different seasons within a given study site. For study samples with known disease and high health awareness (e.g., asthmatics), it also may be important to identify behavior that is adopted to avoid effects from exposure to pollution or other potential irritants (i.e., averting behavior). This may include data on medication, changes in exercise or activity, reduction in the amount of time spent outdoors, or the use of filters or air conditioning. Likewise, it would be useful to collect and use information on study participants' respiratory infections. This would facilitate a test of interactive effects with ozone, since various respiratory viruses cause prolonged bronchial hyperresponsiveness (*23*). Most chamber studies deliberately exclude individuals with respiratory infections. However, these individuals may be particularly vulnerable to the effects of air pollution.

Study Design

Research on acute effects should focus on study designs, such as the use of panel data, that minimize the potential for confounding and omitted variables. With panel data, the collection of health and exposure data for many individuals over time enables the use of analytical techniques where individuals serve as their own statistical controls. It would be useful to develop panels from one source when possible (e.g., one medical practice) to minimize reporting or demographic differences and differences in diagnostic and treatment patterns. In addition, the concurrent analysis of healthy individuals with those with chronic respiratory disease may be useful. Panel data can be used to explore changes on both an individual and group level. On an individual level, the panel can be used to examine the relationship between individual response rates to ozone (based on individual-level analysis) and other factors such as the existence and severity of disease, allergic status, the indoor environment, or health awareness and practices. It would be useful to use multiple sites for this study design. This would aid in determining factors unique to each study population or location (e.g., pollens, allergies, weather, and pollutant mixtures) that may affect the baseline rate of disease and the response to air pollution as well as the reproducibility of the effect.

Despite administrative and subject recruitment costs, there are several distinct advantages to large-scale studies. For example, these studies have greater ability to detect an effect (i.e., statistical power) among a population if one truly exists. Also, with a larger and more heterogeneous sample comes the ability to stratify the sample and thereby enhance the likelihood of identifying sensitive subgroups, differential responses to air pollution, and interactive effects between air pollution and other risk factors. It may be useful to obtain and use more detailed data for a subset of the entire group to improve exposure estimates and determine the existence and degree of the effect of exposure misclassification.

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Detection of Chronic Respiratory Bronchiolitis in Oxidant-Exposed Populations: Analogy to Tobacco Smoke Exposure

David V. Bates

Department of Health Care and Epidemiology, University of British Columbia, Mather Building, Vancouver V6T 1W5, British Columbia, Canada

Studies in nonhuman primates indicate that one pathophysiologic consequence of ozone exposure is chronic bronchiolitis in terminal bronchioles. Modeling dosimetry suggests that a similar phenomenon is possible in humans. These findings may constitute an important analogy to the respiratory bronchiolitis that is associated with tobacco smoking in young adults. This analogy could form the basis for future research related to chronic respiratory health effects of ozone. The smoking data are reviewed and several research strategies are proposed that will be developed more fully in subsequent articles in this volume. — Environ Health Perspect 101(Suppl 4):217–218 (1993).

Key Words: Ozone, oxidant pollution, air pollution, tobacco smoke, bronchiolitis

Laboratory studies in animals and controlled exposure studies in humans consistently have demonstrated an upper and lower respiratory tract inflammatory response to ozone exposure. The hallmark of this response is the polymorphonuclear leukocyte, but other markers of activated inflammatory and repair responses have been observed. These phenomena probably are accompanied by increased lung permeability. Animal studies of the effect of ozone exposure, including most particularly those on nonhuman primates (*1*), suggest that one pathophysiologic consequence of the inflammatory response to prolonged or repetitive ozone exposure is a chronic bronchiolitis in terminal bronchioles. This is consistent with modeling dosimetry calculations that indicate that this zone will be the location of maximal deposition in the human (*2*). The animal database has shown that primates are more sensitive to ozone exposure than rodents and that morphological changes induced with long-term or repetitive exposures are not reversible.

A report by Sherwin and Richters (*3*) of studies of the autopsy lungs of 107 youths between 14 and 25 years who died of nonrespiratory causes in Los Angeles indicates that a severe chronic bronchiolitis was present in the same region of the lung in one-third of the cases. Although quantitative morphometry on the specimens and control data from other, less polluted cities are not yet available, these preliminary observations offer impetus to the search for a relationship between respiratory bronchiolitis and ozone exposure.

The above observations suggest that there may be an important analogy to the respiratory bronchiolitis associated with tobacco smoking in young adults (*4*). Studies of the respiratory bronchiolitis that has been associated with cigarette smoking are reviewed briefly to illustrate the types and limitations of data that will be required to establish a similar link in relationship to chronic ozone exposure.

Respiratory bronchiolitis of variable sever-ity in smokers was first described by Niewohner and his colleagues (*4*). A recent comparison of small airway inflammation in 42 smokers and 13 nonsmokers in Vermont (*5*), in which the bronchiolar changes were evaluated by quantitative morphometry, indicated that in the 20- to 30-year-old subjects, the mean score for bronchiolar wall inflammation was about three times larger in smokers than nonsmokers. This confirms earlier work indicating the same phenomenon.

The physiological correlates [tests of small airway function) of these changes have been noted in many surveys (more than 70 in 1989 (*6,7*)] of smokers and nonsmokers. These findings can be summarized as tests of small airway function that indicate differences between smokers and nonsmokers (even in teenagers) in the following parameters (*1*): *a*) single breath nitrogen slope, *b*) closing volume, *c*) mid-expiratory flow rates, *d*) change of dynamic compliance with respiratory frequency, *e*) differences in regional ventilation in the lung measured with radioactive xenon, and *f*) changes in alveolar-arterial oxygen tension differences with posture. Of particular importance is the fact that all of these differences have been documented to occur when the FEV_1 is still normal or nearly so (95% of predicted).

Because it is not clear yet whether cigarette smoking induces a premature loss of lung recoil (*6*), the elevation of residual volume (that could produce the above pathophysiologic changes) found in 30-year-old smokers before FEV_1 has been reduced might be due to loss of recoil and/or to the small airway lesions noted above. It has been reported that in smokers small airway tests are not predictive of later FEV_1 decline (*8*). However, the observation that smoking cessation is followed by small, but significant, functional improvement (*9,10*) supports the view that reversible respiratory bronchiolitis may be present.

Given the acute inflammatory responses that have been observed with ozone exposure, the recent study by Richards et al. (*11*) provides an important bridge between the smoking data and possible effects of ozone exposure. These investigators observed that the fall in midexpiratory flow rates in smokers under the age of 30 was accompanied by increasing levels of neutrophil activation (measured by chemiluminescence). In 60 healthy young smokers (mean age 28 years), the flow rate tests of small airway function were performed in a laboratory setting. The standard error of the mean of the tests varied between 1.1 and 4.9. The mean FEF25-75, as a percent

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of predicted, was 121.9% in the group with the least affected function and 71.3% in the worst group. The mean FEV1 was 105.5% of predicted in the former and 94.9% of predicted in the latter group. Although the midexpiratory flow rate measurements have a higher coefficient of variation than the FEV_1, it is clear from this study that they not only possess much more discrimination than the FEV_1 in relation to the early changes in smokers (which morphological studies have indicated are changes of bronchiolitis), but they also have a sufficiently low coefficient of variation to be used for this purpose, at least in the laboratory setting.

There is no evidence that the early appearance of respiratory bronchiolitis in smokers is related to the later development of emphysema. Perhaps this is not surprising in view of the known complexity of the steps that lead to lung destruction. For this reason, some physicians have dismissed too readily the considerable body of evidence indicating that smoking induces respiratory bronchiolitis. There is subsequent evidence from studies of occupationally exposed cohorts and individual cases (*6*) that midflow rates predominately reflect small airway changes; this is true in allergic alveolitis, exposures to irritant gases, and dust exposures, including asbestos. Sarcoidosis and postviral bronchiolitis provide the best examples of nonoccupational small airway involvement (*6*).

Two approaches address the question of whether oxidant exposure is associated with respiratory bronchiolitis. The study of Sherwin and Richters (*3*) suggests that the use of autopsied lungs would be of value, and this approach is developed in detail by Lippmann in this volume (*12*).

The study of Richards et al. (*11*) suggests that biological markers of inflammation, in conjunction with the appropriate measures of small airways' functions, would offer another fruitful approach to this problem. Devlin's paper in this volume (*13*) provides details on markers of ozone exposure, and Hatch and Thomas' paper (*14*) provides general guidelines for the utility and application of biological markers in epidemiologic studies. Moreover, the issues of site selection for studies; the requirements for valid, retrospective exposure assessment; and the selection of metrics for ozone exposure are detailed by Lippmann. Studies that employ biological markers of response to ozone in conjunction with appropriate measures of lung function would be important adjuncts to data derived from autopsy studies and studies derived from controlled exposures to ozone (*13,15*) that seek to define more clearly the biochemical and cellular responses of the lung to ozone exposure.

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The Role of Ozone Exposure in the Epidemiology of Asthma

John R. Balmes

Lung Biology Center, Center for Occupational and Environmental Health, Department of Medicine, University of California, San Francisco, Box 0843, San Francisco, CA 94143

Asthma is a clinical condition characterized by intermittent respiratory symptoms, nonspecific airway hyperresponsiveness, and reversible airway obstruction. Although the pathogenesis of asthma is incompletely understood, it is clear that airway inflammation is a paramount feature of the condition. Because inhalation of ozone by normal, healthy subjects causes increased airway responsiveness and inflammation, it is somewhat surprising that most controlled human exposure studies that have involved asthmatic subjects have not shown them to be especially sensitive to ozone. The acute decrement in lung function that is the end point traditionally used to define sensitivity to ozone in these studies may be due more to neuromuscular mechanisms limiting deep inspiration than to bronchoconstriction. The frequency of asthma attacks following ozone exposures may be a more relevant end point. Epidemiologic studies, rather than controlled human exposure studies, are required to determine whether ozone pollution increases the risk of asthma exacerbations. Asthma affects approximately 10 million people in the United States and, thus, the answer to this question is of considerable public health importance. Both the prevalence and severity of asthma appear to be increasing in many countries. Although increased asthma morbidity and mortality are probably of multifactorial etiology, a contributory role of urban air pollution is plausible. The epidemiologic database to support an association between asthma and ozone exposure is limited, but the results of several studies suggest such an association. Some potential approaches to further investigation of the relationship between asthma and ozone, including those that would link controlled human exposures to population-based studies, are considered. — Environ Health Perspect 101(Suppl 4):219–224 (1993).

Key Words: Asthma, airway hyperresponsiveness, ozone, sensitive populations, epidemiology

Asthma and Ozone

Under the provisions of the Clean Air Act of 1970, the Environmental Protection Agency is required to set a standard for ambient air quality regarding ozone that will protect the health of the general population, including sensitive subgroups such as persons with asthma. Considerable research has been conducted on the health effects of ozone exposure over the past two decades in an effort to provide an adequate scientific foundation for regulation of ozone concentration in ambient air. Despite this research effort, there is still some uncertainty about whether persons with asthma are particularly sensitive to ozone.

Asthma is a clinical condition characterized by intermittent respiratory symptoms (e.g., dyspnea, chest tightness, wheezing, cough), airway hyperresponsiveness to a variety of nonspecific stimuli, and reversible or variable airway obstruction (*1*). Although the pathogenesis of asthma remains incompletely understood, it is clear that the condition is of multifactorial etiology. Currently, airway inflammation (i.e., edema; infiltration by leukocytes, especially eosinophils; epithelial injury) is considered to be the paramount feature of asthma (*2*). Several of the stimuli known to cause increased airway responsiveness, such as viral respiratory tract infections (*3*) and inhaled antigen (*4*), also cause airway inflammation. Recurrent exacerbations of asthma associated with increased airway inflammation may lead to the development of chronic airway obstruction (*5*), and recently published guidelines for the treatment of asthma stress the importance of preventing such exacerbations by the avoidance of exposure to inciting agents and the use of prophylactic antiinflammatory medications (*6–8*). It is clear that airway hyperresponsiveness alone does not define asthma since there are many persons with this physiologic characteristic who do not have symptomatic asthma. Whether such persons are at increased risk of developing asthma with viral respiratory tract infections or exposure to pollutants is not known.

Controlled Human Exposure Studies

The results of multiple controlled human exposure studies have documented that inhalation of ozone causes respiratory symptoms (*9*), acute decrements in pulmonary function (*10–12*), and increased airway responsiveness to nonspecific stimuli such as methacholine or histamine (*13,14*) in a dose-dependent manner in normal, healthy subjects. Most of these studies have focused on short-term exposures (i.e., $\leq$ 2 hr), perhaps because the National Ambient Air Quality Standard (NAAQS) is based on a 1-hr maximum concentration (0.12 ppm). With short-term exposures, concentrations of ozone > 0.12 ppm are usually required to cause significant mean decrements in forced expiratory volume in 1 sec (FEV_1). However, there is considerable interindividual variability in the magnitude of the response, with 10 to 25% of subjects tested at 0.12 ppm developing decrements in FEV_1 of $\geq$ 10% after 1 to 2 hr of exposure (*15*). Longer exposure duration and increased minute ventilation with exercise are required to see significant mean effects at ozone concentrations < 0.12 ppm (*16,17*).

Seltzer et al. found ozone-induced increases in methacholine responsiveness to be associated with the presence of excess polymorphonuclear cells (PMNs) in bronchoalveolar lavage (BAL) fluid that was collected 3 hr after intermittently exercising healthy subjects were exposed to 0.4 ppm for 2 hr (*14*). A subsequent study by EPA investigators using an identical exposure protocol demonstrated that the increase in PMNs was still present when they collected BAL fluid 18 hr after exposure (*18*). In addition, these investigators also reported significant mean increases in various biochemical end points indicative of an inflammatory response in BAL fluid collected

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after the 2-hr ozone exposure as compared to that collected after sham exposure. More recently, the same team of EPA investigators has reported their findings in BAL fluid obtained 16 hr after 6.6-hr exposure of healthy subjects to 0.1 and/or 0.08 ppm ozone during continuous moderate exercise (*19*). Similar to what has been noted with pulmonary function responses at lower concentrations of ozone, there was considerably greater intersubject variability in the degree of inflammatory response observed at 0.10 and 0.08 ppm as compared to the earlier study using 0.4 ppm. This series of BAL studies, in healthy human subjects coupled with studies using dogs (*20*) and guinea pigs (*21*), have clearly demonstrated the potential for inhaled ozone to cause airway inflammation.

Given the impressive data base on the responses of normal, healthy subjects to controlled exposures to ozone, in terms of both increased airway responsiveness and evidence of inflammation, one would expect asthmatic subjects to be particularly sensitive to ozone. Thus, it is somewhat surprising that most controlled exposure studies that have involved asthmatic subjects have not documented significantly greater mean responses for these subjects.

Several studies in the late 1970s looked at symptomatic and pulmonary function responses of asthmatic subjects to short-term exposures to ozone at concentrations in the range of 0.2 to 0.25 ppm. In a study of 22 asthmatic subjects exposed to 0.2 to 0.25 ppm ozone for 2 hr with intermittent light exercise, Linn et al. found no significant changes in either spirometric indices of pulmonary function or respiratory symptoms (*22*). A similar study by Silverman of 17 asthmatic subjects exposed to 0.25 ppm ozone while at rest also demonstrated no significant mean changes in symptoms and spirometry after exposure (*23*). However, in 6 of the 17 subjects, there was a >10% decrease in FEV_1 after ozone exposure, which suggests the possibility of a more sensitive subgroup. Another study by Linn et al. involved exposures to polluted ambient air in a mobile laboratory in which the mean (± SD) ozone concentration was 0.22 (± 0.09) ppm (*24*). Other pollutant concentrations were low except for total suspended particulate matter, which was 182 (± 42) $\mu g/m^3$. Thirty asthmatic and 34 normal subjects were tested using a protocol identical to that of the previous study by these investigators. The responses of the asthmatic and normal subjects were not generally different. Most subjects exhibited slight decrements in lung function and mild increases in respiratory symptoms. The investigators did note, however, that a possible explanation for their failure to find any difference in response between the groups was that many of their normal subjects had a history of respiratory allergy and "appeared atypically reactive to respiratory insults."

Two more recent studies by Koenig et al. involving exposure of adolescents to lower concentrations of ozone also failed to show a significant difference in response between asthmatic and healthy subjects. The first study compared the responses of 10 asthmatic and 10 healthy adolescent subjects to exposure to filtered air or 0.12 ppm ozone for 1 hr while at rest (*25*). There were no significant pulmonary function changes in either group and no measurable differences between the two groups. A follow-up study involved exposure of 10 asthmatic and 10 healthy adolescents to filtered air, 0.12 and/or 0.18 ppm ozone, for 30 min while at rest, followed by 10 min of moderate exercise (minute ventilation 30–40 $L/min/m^2$) (*26*). Decrements in FEV_1 were in the range of 3 to 6% for both groups and, again, there were no significant differences between the groups.

The latest study to examine the responses of asthmatic subjects to inhaled ozone, by Eschenbacher et al. (*27*), was designed to provide a greater exposure to ozone than was administered in the previous studies. A higher concentration was used (0.4 ppm), exposures were longer (2 hr), moderate exercise (minute ventilation 30 $L/min/m^2$) was performed for sufficiently long periods (15 min of exercise alternating with 15 min of rest), and bronchodilator medications were withheld. Under these conditions, a differential response between asthmatic and nonasthmatic subjects was demonstrated. Mean decrement in FEV_1 across the 2-hr exposure was 24% for asthmatic subjects and 13.2% for healthy controls. The results of this study, which have been criticized because a methacholine challenge test was performed immediately prior to each exposure and because most of the asthmatic subjects developed exercise-induced bronchospasm to the filtered air exposure, challenge the widely held position that persons with asthma are not more sensitive to ozone than normal, healthy persons.

Given the paucity of data on the response of asthmatic subjects from controlled studies that have been conducted with adequate exposure, it is relevant to review the limited data on the response of atopic subjects without clinical asthma. Holtzman et al. exposed nine atopic subjects (i.e., with a personal history of allergic rhinitis or childhood asthma and at least two positive responses to a battery of seven skin-prick tests with common aeroallergens) and seven nonatopic subjects to 0.6 ppm ozone or filtered air for 2 hr while at rest (*13*). Airway responsiveness to histamine was measured before and after each exposure and was increased in most of the subjects following ozone exposure. Although the authors concluded that the response to ozone was not affected by atopic status, the increase in histamine responsiveness was greater in the atopic subjects than the nonatopic subjects. This difference did not achieve statistical significance due to the small sample size and variability of response among the atopic subjects.

A similar study by McDonnell et al. at the EPA involving 26 nonasthmatic subjects with allergic rhinitis exposed to 0.18 ppm ozone or filtered air also failed to demonstrate a markedly different response to ozone as compared to previously tested nonatopic subjects (*28*). Because McDonnell and coworkers had anticipated that the magnitude of the response to ozone would be associated with baseline airway responsiveness, they speculated that both the size of their sample and the range of airway responsiveness in their subjects may have been too small to detect this relationship.

A recently completed study by Aris et al. (*29*) was designed to determine whether exposure to nitric acid fog would enhance the effects of ozone on pulmonary function in healthy subjects. It generated results contrary to those reported by McDonnell and coworkers. The Aris study protocol involved screening prospective subjects for ozone sensitivity based on a 10% decline in FEV_1 across a 3-hr exposure during moderate exercise (minute ventilation 40 L/min) to 0.2 ppm. Ten subjects selected in this manner demonstrated greater responsiveness to methacholine than 10 prospective subjects excluded from the full study protocol because of their lack of sensitivity to ozone. The study by Aris and coworkers provided a broader range of both baseline airway responsiveness and responses to ozone than did the McDonnell study.

The Aris study results are supported by a recent study by Linn et al. in which 8 of 12 subjects (responders), selected because of their sensitivity to ozone (as measured by decrements in FEV_1), demonstrated hyperresponsiveness to methacholine (*30*). These two recent studies, coupled with the Eschenbacher study involving subjects with asthma, suggest that persons with nonspecific airway hyperresponsiveness, whether clinically asthmatic or healthy, may be a subgroup that has an enhanced sensitivity to

ozone. Because asymptomatic, nonspecific airway hyperresponsiveness is common, the issue of whether such persons are at increased risk for ozone-induced pulmonary toxicity is of obvious importance to the design of adequately protective regulatory strategies.

The acute decrement in FEV_1 in response to ozone inhalation is thought to be due more to chest discomfort and/or neuromuscular mechanisms limiting deep inspiration than to bronchoconstriction (*31*), and it may be that asthmatic subjects are not especially sensitive to ozone for this end point. The frequency of asthma attacks following ozone exposure may be a more relevant end point to monitor. Epidemiologic studies, rather than controlled human exposure studies, are required to answer the question of whether ozone pollution increases the risk of asthma exacerbations.

Epidemiologic Studies

Asthma affects approximately 10 million people in the United States (*32*). Both the prevalence and severity of asthma appear to be increasing (*33*), despite improved understanding of the pathophysiology of the disease and the availability of effective drugs for its management.

Asthma mortality in the United States declined from 1968 to 1978; it has been steadily increasing since 1979 (*34*). The annual number of asthma deaths has increased over 30% since 1980 (*35*). The asthma mortality rate has increased more rapidly for females than for males and for older persons than for young. Because asthma hospitalization and mortality rates are considerably higher for African-Americans than for Whites, it has been argued that the urban poor's decreased access to and utilization of health care are the primary factors responsible for the increase in asthma mortality. A provocative study by Weiss and Wagener confirmed that the asthma mortality is higher in African Americans than Whites, that this gap is widening, and that New York City and Chicago had the highest rates of asthma deaths (*36*).

Decreased access to or availability of appropriate health care for asthma is not likely to be the sole explanation for increasing asthma morbidity and mortality in the United States, because these rates also are increasing in other Western countries, such as Canada, France, Denmark, and Germany, with more equitable health care delivery systems (*37*). In the United Kingdom, asthma mortality rates have risen more rapidly than in the United States (*38*). At the peak of an epidemic of asthma deaths in New Zealand in the 1970s, the mortality rate for asthma was 10 times higher than that in the United States (*33*).

Other proposed explanations for the observed increases in asthma morbidity and mortality include the following: *a*) the 1979 change in the *International Classification of Diseases* (ICD) coding of asthmatic bronchitis as asthma rather than bronchitis, *b*) a shift in physician diagnosis away from bronchitis to asthma, *c*) an improved ability of physicians to diagnose asthma through greater availability and use of pulmonary function tests, *d*) increased toxicity due to asthma medications, and *e*) a true increase in the prevalence and/or severity of asthma (*33*). Which of these explanations is playing an important role is unclear, but it is likely that the rise in asthma mortality is of multifactorial origin.

The results of the Weiss and Wagener study indicate that the change in ICD coding is not the only factor at work since the increase in asthma death started in 1978, before the latest version of the ICD was introduced, and continued unabated through 1987 (*36*). The observation by Weiss and Wagener that there were geographic areas with exceptionally high asthma mortality (New York City; Cook County, Illinois; Maricopa County, Arizona; Fresno County, California) also suggests that improved physician diagnosis of asthma is unlikely to have caused the increases in asthma morbidity and mortality. A companion study by Gergen and Weiss found that among children 0 to 17 years old, there was a 4.5% annual increase in asthma hospitalization from 1979 to 1987 (*39*). The authors found that while much of the increase in hospitalizations for asthma could be explained by a shift in diagnostic coding from bronchitis to asthma, other factors such as environmental pollution may be playing a role.

Although consideration must be given to other possible explanations for the increase in asthma mortality, the weight of the evidence favors a true increase in asthma prevalence. Asthma prevalence data from the United States (*40*), the United Kingdom (*41*), and New Zealand (*42*) are remarkably consistent in documenting an increase. For example, a study by Gergen and coworkers using serial National Health and Nutrition Examination Survey (NHANES) data showed that the prevalence of ever having asthma increased from 4.8 to 7.6% among children 6 to 11 years old from the early to the late 1970s (*40*). While it has been postulated that this rise in asthma prevalence is due to an increase in environmental allergens, an increase in nonallergenic environmental pollution is just as plausible. Weiss has reported a seasonal pattern of asthma mortality, with deaths in patients 5 to 35 years old peaking in June through August, that is consistent with an ozone effect (*43*).

The epidemiologic data base supporting the concept that air pollution can cause exacerbations of asthma is reasonably convincing, but the evidence linking ozone to asthma attacks is limited. In 1961, Schoettlin and Landau reported the results of a study of a panel of 137 asthmatic subjects in Pasadena, California, that showed there were significantly more attacks on days with a maximum 1-hr oxidant concentration greater than 0.2 ppm than on days with lower levels of oxidant pollution (*44*). A later study by Whittemore and Korn examined the relationship between daily asthma attack occurrence and 24-hr average pollutant concentrations and meteorologic conditions using 16 panels of asthmatic subjects residing in six Los Angeles communities (*45*). An innovative statistical approach involving a separate multiple logistic model for each subject's asthma attack probability was employed, and variables representing previous attack history, day of week, and time since the start of the study were included in the regressions. The dominant predictor of attacks was the presence of an attack on the preceding day; but oxidant concentration, particulate concentration, and cool temperature also were positively associated with asthma attacks. Based on their data, the authors calculated that a 0.1-ppm increase in the 24-hr average oxidant concentration would lead to an increase in asthma attack probability of approximately 15%. Potential problems with this study include covariation between air pollutants, high panelist dropout rate, and lack of generalizability to all persons with asthma because relatively severe asthmatic subjects were used on the panels.

Holguin et al. applied the approach of Whittemore and Korn to a study they conducted in Houston to estimate the probability of an asthma attack as a function of maximum hourly ozone concentration (*46*). The daily maximum ozone concentrations ranged from 0.02 to 0.27 ppm during the 6-month period of study from May to October. A greater effect of ozone on asthma attack probability was found in this study as compared to the previous Los Angeles-area study. Assuming the baseline probability of an attack was 10%, an increase in the 1-hr maximum ozone concentration of 0.1 ppm was calculated to

increase the attack probability to 16%, a 60% increase or four times the effect predicted by Whittemore and Korn.

An important study of the relationship between hospital admissions and levels of various pollutants (including ozone), the Ontario Air Pollution Study, has been reported in a series of publications by Bates and Sizto (*47–49*). The study involves all 79 hospitals in a region of southern Ontario that admit acute cases and pollutant data from 17 sampling stations in a monitoring network that covers the region. Hospital admissions for respiratory disease have consistently been associated with daily levels of ozone, sulfates, and temperature during summer months throughout a period of study more than 10 years. Admissions for a group of nonrespiratory conditions showed no such association. The major problem with this study is the inability to isolate the effect of a specific pollutant. Bates and Sizto have summarized the evidence for and against the role of ozone (*49*). It is the only one of the pollutants studied that has been shown to be an irritant at the ambient concentrations measured in southern Ontario. When the region was divided into subregions and each sampling station was associated with a group of adjacent hospitals, respiratory admissions on high ozone (0.08 to 0.2 ppm) days were approximately 7% greater than admissions on low ozone (0.01 to 0.06 ppm) days, if only the same days of the week in the same season in the same year are compared. Ozone concentration also showed a stronger association with admissions for asthma than did the levels of other pollutants and temperature. However, in June of 1983 when ozone levels were unusually high, there was no increase in hospital admissions for respiratory disease. Further, sulfate concentration had a higher correlation coefficient with admissions for all respiratory diseases than did ozone concentration. Because of the ambiguities in their data analysis, Bates and Sizto have concluded that neither ozone nor sulfate alone was responsible for the observed association with acute respiratory admissions. They have speculated that ozone may increase airway responsiveness and thus render individuals more susceptible to other pollutants and/or allergens. There are animal data to support such an effect (*50–52*).

The results of a recently reported controlled human exposure study supported the animal toxicologic evidence that ozone exposure may enhance sensitization to inhaled antigens (*53*). A group of investigators from Toronto exposed seven asthmatic subjects with specific sensitization to either ragweed or grass to 0.12 ppm ozone for 1 hr while at rest. As expected for these conditions of ozone exposure, there were no acute changes in FEV_1 or methacholine responsiveness. This level of exposure did cause, however, a significant shift to the left in the dose–response relationship during inhalation challenge testing with the specific antigens to which the subjects were sensitized. Although the Toronto investigators could only speculate about biological mechanisms responsible for this finding, it is possible that ozone-induced airway inflammation leading to increased epithelial permeability allows increased penetration of antigen to submucosal mast cells. The investigators did note that the public health implications of the study are great; thus, the current NAAQS for ozone is probably not adequately protective of the health of persons with asthma. A major caveat to such an interpretation of this study's results is that the number of subjects who completed the complicated protocol is small ($n = 7$).

Research Needs

The two major questions that need to be answered concerning the relationship of asthma and ozone are the following: Does chronic exposure to high ambient concentrations of ozone contribute to the development of new-onset asthma? Does ozone pollution contribute to exacerbation of preexisting asthma? These questions cannot be answered fully by further controlled human exposure studies alone. Rather, population-based approaches will be required.

The epidemiologic database to support an association between asthma and ozone exposure is limited, but the results of several studies suggest that high ambient concentrations can precipitate asthma attacks. As noted above, ozone is likely to be an inducer of increased nonspecific airway responsiveness and airway inflammation that renders persons with asthma more likely to develop bronchoconstriction upon subsequent exposure to substances such as allergens and sulfur dioxide. If ozone inhalation causes airway inflammation in persons susceptible to asthma (e.g., persons with asymptomatic airway hyperresponsiveness and/or atopy) that is sufficient to cause asthmatic symptoms, then ozone can cause new-onset asthma. Further controlled human exposure and epidemiologic studies are necessary to determine both the degree of ozone sensitivity of persons with asthma and the probability that exposure to ambient ozone is contributing significantly to the rise in asthma morbidity and mortality.

One way to link controlled human exposure studies with epidemiologic studies that plays on the strength of each type of study would be to characterize the acute responses of a panel of asthmatic subjects in the laboratory and then to follow this panel over time. The simplest method of characterizing asthmatic subjects' sensitivity to ozone would be to measure the decrement in FEV_1 over a 2- to 4-hr exposure to a sufficiently high concentration of ozone that would cause a considerable percentage of the subjects to have decrements $\geq$10% (e.g., 0.2–0.4 ppm). However, increased airway responsiveness to methacholine and/or evidence of inflammation on BAL after such an exposure could also be used as markers of ozone sensitivity. Whether acute responses to high ambient levels of ozone administered in a chamber have any predictive value with regard to real-life responses (both acute and chronic) is an important but unanswered question.

What outcome variables should be measured in this panel study? Both respiratory symptoms and serial peak expiratory flow rates can be recorded with relative ease in diaries. Periodic spirometry and methacholine responsiveness, while more difficult to measure, also could be obtained. Serial BAL would be the most direct method of assessing the degree of ozone-induced injury or inflammation in the panel members, but it also would be the most invasive and cumbersome to perform.

Following a panel of asthmatic subjects is fraught with methodologic difficulties. In addition to problems in exposure assessment due to varying patterns and intensity of activity, there will also be problems with exercise-induced bronchospasm independent of ozone exposure and variable medication use. Current progress in personal ozone dosimetry should continue to the point where individual doses can be measured rather than calculated. Time and activity monitoring techniques are improving to the point where level of exercise can be recorded with reasonable accuracy, and perhaps any independent effect of exercise can be controlled for during analysis. Confounding due to variable medication use among panel members perhaps can be minimized by measuring outcome variables, including medication use, during seasons with low ozone exposure and using each subject as his or her own control in the analysis. Although panel studies of the responses of asthmatic subjects to a pollutant may appear difficult to conduct, a

recent study of the effects of atmospheric acidity on a panel of subjects with asthma in Denver provides a successful model (*54*).

Another approach to the question of whether persons with asthma are more susceptible to adverse health effects from ozone exposure would be to follow the Bates and Sizto model of monitoring the rates of emergency room and hospital admissions for exacerbations of asthma in conjunction with regional air quality. Recently, this approach has been applied quite successfully to the study of the effect of particulate pollution on populations residing in several Utah valleys (*55,56*). Pope showed that hospital admissions for respiratory illnesses among both children and adults correlated with changes in PM_{10} concentrations (*55*). However, the success of the Pope study largely depended on the somewhat changeable air quality of the Utah Valley. Most of the particulate pollution came from one source, a steel mill that was shut down intermittently for economic reasons; and concentrations of other pollutants, including ozone, were generally low. The major limitation with further application of the hospital admission versus air quality study model to the epidemiology of ozone-related health effects is the identification of regions where ozone concentrations are high in the absence of elevated concentrations of other pollutants and in the absence of extremes of temperature and humidity.

Comparative studies of rates of hospital admissions or emergency room visits for asthma in different cities with different levels of ozone pollution may provide useful information on dose–response. Recent data indicate that while there is no association between asthma attacks and the relatively low ambient ozone concentrations in Vancouver (*57*), there is a positive association between emergency room visits for asthma and the higher summer ambient ozone concentrations in Atlanta (MC White, unpublished data). When planning studies linking ambient air monitoring data with rates of hospital admission or emergency room visits for asthma, it is important to consider that the ozone concentrations for the several days prior to the admission or visit may be more relevant than the concentration on the day of the attack. Another concern in this type of study is potential misclassification of asthma as acute bronchitis, especially when dealing with children.

It may not be necessary to collect data linking rates of asthma to ambient ozone concentrations in a prospective fashion. Considerable environmental monitoring data already exist; these measurements could be correlated with hospital records from multiple hospitals across a given geographic area, such as the Los Angeles basin, or with NHANES data across several geographic areas with varying levels of ozone pollution.

The issue of whether persons with asthma are more susceptible to ozone-induced respiratory tract injury is of epidemiologic interest because asthma is a common condition that appears to be increasing in terms of both prevalence and severity. Given that many of the over 10 million Americans with asthma live in ozone nonattainment areas, a well-defined association between ozone and either new-onset asthma or exacerbation of preexistent disease would be of great importance to public health.

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Identification of Subpopulations That Are Sensitive to Ozone Exposure: Use of End Points Currently Available and Potential Use of Laboratory-Based End Points under Development

Robert B. Devlin*

Health Effects Research Laboratory, U.S. Environmental Protection Agency, Research Triangle Park, NC 27711

A number of epidemiological studies have attempted to assess the effect of recurrent ozone exposure in humans. For the most part, they have failed to document convincingly an association between chronic ozone exposure and differences in lung function performance or respiratory symptoms. This is not surprising given the small respiratory effects observed in animals chronically exposed to ozone and assuming that people with abnormal respiratory function resulting from other occupational or environmental exposures, such as tobacco smoke, would make up a much larger percentage of the population than people with respiratory effects attributable to ozone. Therefore, either more sensitive end points must be developed to detect subtle changes due to chronic ozone exposure, or ways of selecting subpopulations that are especially sensitive to ozone must be devised. It has been well documented that there are large and reproducible differences in the acute response of individuals to ozone as measured by pulmonary function tests. Recently, it has also been shown that there are large differences in the acute response of individuals to ozone as measured by inflammatory and other biochemical parameters. This paper discusses the problems of selecting individuals who are sensitive to ozone depending on the end point chosen. It also describes potential new sensitive end points that might be available for ozone epidemiology studies in the near future. — Environ Health Perspect 101(Suppl 4):225–230 (1993).

Key Words: Ozone, sensitive subpopulations, epidemiology, humans, biomarkers

Introduction

It is widely accepted that humans exposed to known concentrations of ozone under controlled conditions exhibit reversible changes that affect the large and small airways as well as the alveolar region of the lung. These changes include reduction in pulmonary function performance, narrowing of small airways, increased nonspecific airway reactivity, alveolar inflammation, damage to pulmonary epithelial cells, and increased leakage of vascular components into the lung. In addition, acute reduction of pulmonary function performance following ozone exposure has been documented in epidemiological studies in which small groups were followed over a short period (*1–6*).

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*Address correspondence to R. B. Devlin, Medical Research Building B, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599.

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However, it is not known if recurrent exposure to ozone results in induction of chronic disease. Some studies of chronic animal exposure suggested that rats and nonhuman primates exposed to levels of ozone below 0.5 ppm for up to 2 years exhibit permanent or slowly reversible lesions in the distal airway and proximal alveolar region of the lung. An ongoing low-level inflammatory process with increased numbers of neutrophils was accompanied by increased numbers of type II alveolar cells, thickening of the basement membrane, and increased numbers of collagen fibers in the interstitium (*7–9*). Measurement of pulmonary function in these animals suggested very small changes in some variables, including total lung capacity and the diffusing capacity of carbon monoxide (*10,11*).

A number of epidemiological studies have attempted to assess the effect of recurrent ozone exposure in humans; they are summarized by Ostro in this volume. By and large, these studies have failed to document convincingly an association between chronic ozone exposure and differences in lung function performance or respiratory symptoms. In a discussion of why chronic effects have not been observed, McDonnell (*12*) noted that it is not surprising that epidemiologists have experienced difficulty in observing respiratory effects due to chronic ozone exposure, given that small respiratory function changes could be found only in chronically exposed animals that had developed advanced cellular and biochemical lesions. If similar lesions occur in humans, and only a small proportion of individuals with lesions go on to develop frank disease, it would be difficult to identify such individuals in an unselected population, particularly given the rate of frank disease and abnormal respiratory function resulting from other occupational or environmental exposures. Thus, it would seem that either more sensitive end points must be developed to detect chronic effects, or ways of selecting subpopulations that are sensitive to ozone must be devised.

There is some evidence that individuals sensitive to ozone can be identified. As mentioned above, acute decrements in forced expiratory volume in 1 sec (FEV_1) have been reported to occur in a concentration-dependent manner in both controlled exposure studies and some field studies. In these studies, the magnitude of response to

similar concentrations of ozone varies considerably among individuals. For example, decrements in FEV_1 that range from 2 to 48% have been reported in subjects exposed to levels of ozone between 0 and 0.4 ppm (*13–16*). The variation in magnitude of response is also reproducible when individuals are reexposed to the same level of ozone up to 13 months later (*17,18*). These findings suggest that differences in individual response to ozone are not due to random experimental error nor to different environmental conditions between exposures, but rather to factors that are inherent in individuals and that are stable over time. If this suggestion is true, it may be possible to screen large numbers of individuals in a controlled setting and to select those with the largest decrements in pulmonary function for inclusion in epidemiological studies of chronic health effects.

To identify a sensitive subpopulation, however, one must define the criteria used to select the population. This is not necessarily straightforward, since there are a number of parameters that have been shown to be altered in humans exposed to ozone. In addition to changes in lung function tests that primarily measure inability to take a deep breath (e.g., FVC), changes in airway constrictions, demonstrated by an increase in specific airway resistance (SR_{aw}), have been reported in animals and humans exposed to ozone. Increases in nonspecific airway reactivity have also been observed in humans exposed to levels of ozone as low as 0.08 ppm (*19,20*). Bronchoalveolar lavage (BAL) has been used to sample both cells and fluid removed primarily from the alveolar region of the lung of humans and animals exposed to ozone. An influx of neutrophils; increased permeability; decreased macrophage phagocytic ability; and increases in arachidonic acid metabolites, some cytokines, fibronectin, lactate dehydrogenase (LDH), coagulation factors, elastase, and plasminogen activator have been reported in humans exposed to levels of ozone as low as 0.08 ppm (*21–24*). As is the case in pulmonary function tests, there is a wide range of individual responses to ozone as measured by these assays, particularly to low levels of ozone (*21–23*). It is not known if the magnitude of change in these cellular and biochemical end points is reproducible over time for a given individual, as is the case with more traditional markers of lung function, such as FEV_1.

Preliminary analysis of some of the end points listed above suggests that none of them is strongly correlated with another; individuals who show large decrements in FEV_1 following ozone exposure do not necessarily have the largest neutrophil influx or the most nonspecific airway reactivity. Furthermore, drugs that block formation of cyclooxygenase products appear to ablate partially the pulmonary function decrements seen after human exposure to ozone (*25,26*), but they do not appear to affect neutrophil (PMN) influx, cell damage, leukotriene production, or increased permeability reported in humans exposed to ozone (*27*). Moreover, there does not appear to be a strong correlation between the cellular and biochemical assays when they are compared with each another. For instance, rats depleted of PMNs still show lung damage, production of inflammatory cytokines, and increased permeability of vascular components across the epithelial barrier after ozone exposure (*28*). Airway hyperresponsiveness may also occur prior to and in the absence of PMN influx in rats (*29*) and guinea pigs (*30,31*). More basic research into the underlying mechanism of ozone damage probably will be needed to understand fully the apparent lack of coherence of different end points.

The above discussion does not designate which end point(s) should be used to select sensitive individuals. Traditionally, lung function measurements derived from forced expiratory maneuvers have been used to define such people, probably because these tests are noninvasive, inexpensive, and performable on large numbers of individuals in the field. However, it might be that end points that measure airway hyperreactivity, inflammation, production of fibrogenic compounds, or some combination of these may be better suited for the selection of individuals who may be at high (or higher than average) risk for developing frank disease from chronic ozone exposure.

Additional studies are needed to define more precisely both the range and reproducibility (over the long and short term) of individual responses measured by the various end points discussed above. Such studies will need to combine a number of end points, including symptoms (e.g., cough, minor respiratory irritation), pulmonary function changes that reflect lavage and small airway function, and assays that measure cellular changes involved in inflammation and lung damage. Ozone concentration–response curves for each end point will also be needed to determine if different doses of ozone alter the response profile. In addition to defining the range and reproducing the response, it will be necessary to determine which, if any, of these end points are concordant (i.e. which ones are consistently elevated in responders but not in nonresponders). If both invasive and noninvasive concordant end points are identified, it will be important to know if noninvasive measures such as symptoms, standard lung function measurements, or measurements of airway resistance can be used as surrogates for more invasive procedures that measure inflammation, lung injury, reduced levels of antioxidants, or reduction in host defense capability. If concordant end points are not found, the relevance of pulmonary function changes to chronic outcomes should be more fully explored before using this end point to select responders for longitudinal epidemiology studies.

Under the assumption that suitable end points are identified, a combination of two approaches would be most useful for the identification of subpopulations sensitive to ozone. One approach would be to select subjects for an epidemiological study on the basis of their response to ozone in controlled chamber studies. This would allow the selection of individuals who respond to precisely known ozone concentrations and exposure regimens that could mimic those found in the geographical area in which the individual resides. One could also be certain that selected individuals truly are responsive to ozone rather than other occupational or environmental agents. The alternative approach would be to select responders from a cross-sectional study and attempt to duplicate their response in a controlled environment. A combination of both approaches would allow a better understanding of the relative contribution of ozone and other environmental agents to individual range of response.

One problem faced by epidemiologists attempting to carry out these studies is the selection of suitable end points. Traditionally, lung function assays have been used to assess pulmonary changes in humans exposed to ozone. This approach is suitable for detecting acute changes, but it is not likely to detect minute changes in small airways that have been demonstrated in animals chronically exposed to ozone. It is possible to monitor changes in inflammatory or fibrogenic compounds in the lungs of humans exposed to ozone with biochemical and molecular assays by using BAL to obtain cells and fluid lining the airways and alveolar region of the lung. However, the procedure is relatively invasive and probably not applicable for studies involving more than several dozen people. Techniques that measure changes in the lung not currently detected by standard lung function assays,

yet applicable to large-scale epidemiology studies are required. Summarized below are four promising approaches that are currently being developed in laboratories for potential use in epidemiology studies.

Tests that Measure Changes in Small Airways

There is much evidence that humans exposed to ozone develop reversible decrements in FEV_1 and FVC, which have been attributed to a reduction in total lung capacity (TLC) that is mediated by neural mechanisms. There also are obstructive changes in the large airways that are characterized by small increases in specific airways resistance (SR_{aw}) and airways reactivity (*32–35*). These measurements, however, do not provide convincing evidence of functional changes in the small airways of the lung. Yet, studies of animals acutely and chronically exposed to ozone show that tissue damage occurs first in the small airways, particularly in the bronchoalveolar duct region (*36–38*). It is likely that ozone perturbation of this region also would be reflected by measures that detect functional change in the airways. To detect small airways functional changes, tests are needed that do not rely on vital capacity maneuvers. This is because any acute small airways change from ozone that might be detectable from forced expiratory flows at low lung volumes will probably be obscured by the reduction in FVC.

Several potential tests of small airway function have been described. One such test involves the measurement of the dispersion of an inhaled bolus of a small (0.5–1 mm) aerosol. This assay has been used to demonstrate differences between a group of healthy, nonsmoking individuals and a group of asymptomatic smokers with otherwise normal lung function; it suggests the presence of small airways abnormalities in the latter group (*39*). Healthy, nonsmoking volunteers exposed to 0.4 ppm ozone also show differences in the dispersion of an aerosol bolus, which suggests that ozone exposure results in functional changes in the small airways (*40*). This bolus technique is relatively noninvasive, easy to perform, and can be done on large numbers of people. It has the potential to measure functional changes in the region of the lung known to be most sensitive to ozone inhalation, which may allow for detection of changes too small to be detected with traditional functional measurements derived from forced expiratory maneuvers. However, there needs to be more research comparing the aerosol bolus dispersion measurement with other putative small airways tests, such as multiple-breath nitrogen washout or radioactive gas boli, and examining functional tests of the large and small airways derived from forced expiratory maneuvers. Experiments also need to be designed to understand better the underlying structural and functional changes in the lung that can induce dispersive processes. Finally, the sensitivity and specificity of this test needs to be assessed in relation to other small airways tests.

Tests that Measure Changes in Nasal Passages

The nose is the primary portal of entry for inspired air in humans, and therefore it is the first region of the respiratory tract that is in contact with airborne pollutants such as ozone. If these pollutants are respiratory irritants capable of causing cellular damage, as ozone is, then effects should be detected in the nasal passages. Since many of the cell types found in the nasopharyngeal region are the same or similar to cells found in the trachea and bronchi, the responses of nasal cells to ozone may be similar to the response of airways and alveolar cells.

Nasal lavage (NL) is simple and economical to perform, relatively noninvasive, and allows multiple sequential sampling of both nasal secretions and cells from the same person. This procedure has been used to study nasal inflammation (as determined by an influx of PMNs) in humans exposed to rhinovirus (*41*) as well as to study mediators produced in human nasal fluid during allergic reactions (*42*). It has also been used to study changes in the cells and nasal fluid of humans exposed to tobacco smoke (*43*) and workers exposed to cotton dust (*44*). In a recent study, an increase in PMNs has been demonstrated in the NL fluid of humans exposed to volatile organic compounds (VOCs) in equivalent concentrations to those present in a new house (*45*). These studies clearly show the utility of using this technique in a variety of settings.

Work with animals has demonstrated that ozone causes damage to the epithelial cells of the nasal passages and ultimately results in an influx of PMNs (*46*). Two studies with humans have shown that an acute exposure to 0.5 or 0.4 ppm ozone results in a large increase in PMNs in the NL fluid (*47*), as well as increased levels of albumin and tryptase (a marker of mast cell degranulation). In the latter study, BAL was performed on the same individuals, and a qualitative correlation was found between the numbers of PMNs in the NL fluid and in the BAL fluid (*48*). Thus, there is a possibility that the noninvasive NL procedure can be used as a surrogate for the more costly and invasive BAL in epidemiological studies with some assurance that inflammation seen in the nasal passages is mirroring inflammation present in the alveolar region of the lung.

More work is needed to strengthen this assertion, particularly when considering low ozone concentrations. It is not known if NL is as sensitive as BAL in the detection of inflammation or production of other mediators. Work is also needed to extend the range of mediators that can be detected in the NL and to determine if a correlation exists between BAL and NL for them. This would include arachidonic acid metabolites, cytokines, and measurements of cell injury such as LDH, fibronectin, etc.

In addition to measuring NL fluid for the presence of mediators, it is also possible to perform nasal brush scrapings in which several thousand cells are removed. These cells can be analyzed by recently developed techniques such as RNA *in situ* hybridization and polymerase chain reaction (PCR), which are capable of analyzing mRNAs present in only a few hundred cells. Recently mRNAs coding for cytokines and other relevant mediators has been quantified in human upper airway cells removed by brush scraping (*49,50*). This approach may extend both the sensitivity and the range of end points that can be assayed. Clearly this newly emerging area needs more developmental work to validate the very preliminary results described to date.

Noninvasive Methods of Imaging the Lung

The 1973 discovery that nuclear magnetic resonance (NMR) signals could be spatially encoded through the use of magnetic gradients has spawned an entire field of research and application in magnetic resonance imaging (MRI). A subject is placed in a strong magnetic field that causes hydrogen protons to align synchronously about the direction of the applied field. Radio frequency pulses are then applied to stimulate the protons and to generate a radio frequency echo in the tissue. This signal is spatially encoded, which permits the construction of a two-dimensional image of the selected plane through the subject. Contrast is achieved by changes in the number of protons per cubic centimeter, the spin–lattice relaxation time, and the spin–spin relaxation time. These parameters reflect how much water is present in a tissue and how the water is bound to various other

molecules. The technique is completely noninvasive, involves no ionizing radiation, and can be performed repeatedly on the same individual over time. MRI has been used as a diagnostic tool for only the past 6 years, and its current applications include detection of pathologic changes in a number of soft tissues, including hepatitis and fatty liver, that are associated with chronic liver disease, infarction of skeletal muscle, renal lesions, soft tissue tumors, and joint lesions (*51–55*).

Recently a number of researchers have exploited the potential of this tool to develop MR microscopy, in which sections through soft tissues can be viewed at <50 mm resolution to permit noninvasive microscopy on live animals (*56–59*). This approach has been used to quantify edema and fibrosis in the lungs of animals exposed to paraquat (*60*), as well as edema in animals exposed to hyperoxia and ozone (J. Crapo, personal communication). This technique has the potential to become a powerful tool with the ability to detect noninvasively ozone-induced lesions in humans. MRI microscopy can focus on the whole lung or on a small portion such as the bronchoalveolar duct region, where ozone-induced lesions are first visualized by conventional microscopy.

A number of problems must be solved before MRI microscopy is ready to apply to humans in an epidemiological study. Resolution is partly dependent on the size of the magnet though which the subject must pass, and current magnets that are large enough to accommodate humans yield a resolution of only a few millimeters. It is particularly difficult to create an image of the normal lung because it has fewer protons than other tissues, and the microscopic structure of the air/water interface in the alveoli produces distortions resulting in very long scan times. In addition, distortion due to breathing can reduce resolution. Fortunately, research in this field is progressing very rapidly. In the past several months many of the problems associated with imaging the lung have been solved, and now images have greatly increased resolution and decreased scanning time. If the progress in magnet technology continues at the same pace it has had the past few years, it can be anticipated that magnets suitable for visualizing the human lung with high resolution will be available sometime in this decade. Developments are currently underway in which three-dimensional reconstruction of the lung will allow any plane to be viewed from any angle without loss of resolution.

It is tempting to overstate the importance MRI microscopy because of its potential to provide the epidemiologist with a powerful tool that can visualize the interior of a human lung with microscopic resolution. Clearly there is a considerable amount of developmental research which must be done, much of it with animals, before this technique can be applied to epidemiology studies. However, most of the current developmental effort is focused on disease models, and research funds must be provided to encourage development of models appropriate to environmental epidemiology studies. There are at least two general areas of research that should be encouraged in the near future. The technology necessary to measure inflammation and edema and to visualize lesions in animals chronically exposed to ozone exists. Studies are needed in which MRI imaging, conventional quantitative histology, and BAL (which can measure inflammation and lung damage from a cellular and biochemical perspective) are all applied to animals exposed to ozone for varying periods of time. This would allow an assessment of the ability of a noninvasive technique such as MR microscopy to detect acute and chronic changes in the lung that have already been described using histology and BAL. Studies are also needed to assess the ability of MR microscopy to detect edema and inflammation in the lung of humans exposed acutely to ozone. Even if current MR technology does not permit visualization of small structures in humans, MR is capable of detecting more pervasive changes such as inflammation and edema. These studies would allow an assessment of whether MRI could be used as a surrogate for BAL in epidemiology studies.

Development of Biomarkers of Exposure and Predictive Biomarkers

An important quality for any epidemiological study would be the ability to perform a simple test that could quantify the exposure of an individual to a pollutant or perhaps even detect individuals who are susceptible to exposure to a specific pollutant . The molecular technology necessary to identify specific macromolecules that could serve as dosimeters of exposure to specific pollutants or to identify components that could serve as predictive biomarkers of sensitivity exists today. Furthermore, the technology needed when analyzing changes in a tiny number of cells (100–1000), which greatly expands the range and type of tissues that potentially can be sampled in an epidemiology study, also exists. Nearly all of the developmental work and most of the current applications are focused on the identification and quantification of biomarkers related to disease. Very little effort has been made to develop or apply this technology to problems related to exposure of humans to environmental pollutants. Those operating the laboratories most suited to the development of the kinds of biomarkers needed for epidemiology studies are usually not aware of nor appreciative of the utility or value of developing these kinds of biomarkers. In addition, very little funding has been provided by the epidemiology community for long-term research dedicated to developing suitable biomarkers. However, if such biomarkers are ever to be developed, funding must be provided and lines of communication must be opened between epidemiologists and molecular biologists.

Research, preferably in easily accessible tissue such as nasal epithelium or blood, is needed to define the range of proteins and mRNAs that are induced by exposure of humans to ozone and other pollutants. It is likely that different sets of macromolecules are induced by different pollutants, similar to the induction of different rat liver proteins by different toxicants (*61*). If such sets of proteins or mRNAs are found in animals or humans exposed to ozone, studies will be needed to determine if a quantitative relationship exists between induction of any protein or mRNA and the level of ozone exposure. ELISA or RIA assays could then be developed to quantify specific proteins (and PCR to quantify specific mRNAs) whose level of induction is dependent on the concentration of ozone exposure. This would allow these macromolecules to serve as molecular dosimeters of ozone exposure. If suitable criteria for sensitivity can be determined, similar approaches can be used to identify proteins or mRNAs present in humans judged sensitive to ozone. It is likely to take at least several years to develop and validate biomarkers suitable for epidemiological studies of ozone, and the difficulties and pitfalls in attempting to identify such markers have been well documented by Hatch and Thomas (*62*). However, the potential power and utility of these biomarkers suggest that research in this area should be pursued.

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Design and Analysis of Studies of the Health Effects of Ozone

Alvaro Muñoz

Department of Epidemiology, The Johns Hopkins School of Public Health, 624 N. Broadway, Baltimore, MD 21205

The design and analysis of studies that investigate the effect of exposure to ozone on health outcomes need to define carefully the methods for the assessment of the exposure and to determine precisely which is the outcome of biological relevance. The estimation of sample size for longitudinal studies requires the expected rates of change among the exposed and unexposed, the variance of the outcome, and the correlation of measurements taken within an individual. Methods of analysis whose primary interest is in the combination of cross-sectional studies for the determination of the marginal distribution of the outcome are particularly appropriate for biological processes where the effect of exposure is acute. Conditional models are particularly useful for investigating the effect of changes in exposure on changes in outcome at the individual level. In addition, conditional models incorporate a dampening effect of exposure that may provide a reasonable agreement with several biological mechanisms. The identification of susceptible individuals and the description of the behavior of their outcomes over time may be better accomplished by using the within-individual variance as the outcome of interest. Discrepancies of the within- and between-individual regressions may be suggestive of chronic effects, and methodological research in this area is needed. Studies of the health effects of ozone exposure need to address the incorporation of missing data, measurement error, and the combination of complementary studies. — Environ Health Perspect 101(Suppl 4):231–235 (1993).

Key Words: Longitudinal studies, regression methods, missing data, environmental effects

Background

A central objective of studies on the health effects of ozone is to determine whether individuals who have been exposed to ozone have adverse health outcomes. The designer of a study needs to consider carefully the specific methods for measuring the exposure to ozone as well as to define precisely the outcome which will be used to identify adverse health effects. It is crucial to measure exposure accurately and to construct summaries that include duration and dose. In addition, it is important to study populations that are exposed to a range of exposures so that informative comparisons among groups can be made. There are three types of outcomes according to the number of possible values of the outcome. The simplest outcome is binary (i.e., yes/no) and typically refers to the presence of disease or a symptom. The occurrence of asthma is an example of a binary outcome that has received considerable attention in respiratory disease epidemiology. The next level of an outcome variable is the case of a categorical outcome including severity of disease. Typically this is the case of an outcome defining stages of disease as severe, moderate, mild, or absent. The third type of outcome is continuous (i.e., any value within the range of biologically possible values). This is the case of commonly used measures of pulmonary function, including the forced volume vital capacity and the forced expiratory volume after the first second in a spirometric maneuver.

Studies for epidemiological research are either cross-sectional or longitudinal in nature. Cross-sectional studies involve the assessment of exposure and outcome at a fixed point in time. Longitudinal studies can be viewed as a collection of cross-sectional studies performed in the same group of individuals, thus providing repeated measures of exposure and outcome for each individual at different points in time. The design of cross-sectional studies requires the estimation of the sample size needed to detect the expected health effect with a high probability. The design of a longitudinal study requires the determination of the appropriate sample size as well, but in addition, one needs to specify the frequency of visits and the lag between them. Differences between designs of longitudinal studies can be characterized by three variables, namely, the number of individuals (N), the number of visits (V), which is provided by each individual, and the time lag (T) between the baseline and the last visit (Table 1). Panel studies typically have V large, T small, and N moderate. An example of a panel study is the case of daily follow-up of 100 individuals for a year (i.e., $V = 365$, $T = 1$ year, and $N = 100$).

Longitudinal studies per se typically refer to the case of V small, T moderate or large, and N large. An example of a longitudinal study would be the yearly follow-up of 1000 school children from grade 4 to grade 12 (i.e., $V = 5$, $T = 8$ years, and $N = 1000$). Laboratory experiments on animal models or chamber studies typically have V small or moderate, T small, and N small. An example of a chamber study would be the observation of 30 individuals on a weekly basis for 3 months (i.e., $V = 12$, $T = 3$ months, and $N = 30$).

The analysis of data related to the effects of exposure to ozone on health outcomes requires the use of methods that allow for the incorporation of the simultaneous effect of different exposures or risk factors. The analysis of cross-sectional studies is considerably simpler than that of longitudinal studies. Methods for the analysis of longitudinal data need to incorporate the

Table 1. Types of longitudinal studies.

Type of study	Number of individuals (N)	Number of visits (V)	Time lag (T)
Panel	Moderate	Large	Small
Longitudinal	Large	Small	Moderate or large
Laboratory	Small	Small or moderate	Small

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intrinsic correlation of the repeated measurements within each individual. Since T ;(i.e., T/V) is typically 6 months or a year, the likelihood of incomplete profiles is high. Profiles are incomplete due to permanent or temporary dropouts. Permanent dropouts are those who have data missing after the last time they were seen in the study, and temporary dropouts are those with intermediate visits missing. The statistical methods for analysis need to include incomplete profiles in an efficient manner.

Longitudinal studies provide data to measure the changes of the outcome at the individual level and to relate those changes to the individual's exposure over time. Methods of analysis can be classified according to the extent of the parametrization of the functional form of the outcome over time. Fully parametric models use polynomials (of which linear regression is the simplest case) or exponential or logarithmic functions to model the growth curve of individuals over time. Time series analysis models (e.g., autoregressive processes) are not as restrictive, but they assume a specific form of the correlation structure. Fully nonparametric models include smoothing algorithms and graphical summaries of the outcome data over time.

The remainder of this paper is divided into three sections. The first and second sections discuss issues related to studies of acute and chronic effects, respectively. The third section discusses issues common to both studies.

Acute Effects Studies

Although there have been several studies documenting acute (i.e., transient) health effects of exposure to ozone O_3, there is a need to carry out further studies to determine the full range of acute outcomes. The outcome of interest could be either continuous (e.g., FEV_1) or dichotomous (e.g., symptoms), with the main interest being the investigation of the effects of O_3 concentrations on the levels and variability of the outcomes over time.

Exposure Measurement

The geographic extension of study populations of acute studies is usually of limited range, so that the ambient concentrations of O_3 to which the population is exposed at a given time, t, is relatively homogeneous. Let E_t denote the O_3 concentration at time t of the geographic area where the study population lives. The major contributor to the differential exposure for different individuals is the pattern of indoor/outdoor and exercise activities. Let P_{it} denote the pattern of indoor/outdoor and exercise activities of the i^{th} individual at time t. It is the combination of E_t and P_{it} that provides the basis for calculating the exposure of the i^{th} individual at time t (E_{it}). Under the current technological limitations for directly measuring E_{it}, studies of acute effects of O_3 need to devote special care in measuring P_{it}. Individuals whose P_{it} does depend on t (i.e., pattern of indoor/outdoor and exercise activities is not the same for all t) provide data on individual changes of exposure even if the regional exposure, E_t, is constant over time. Conversely, even if $P_{it} \equiv P_i$ (i.e., individual i has the same pattern of activities for all t), the changes in E_t will result in E_{it} depending on t. Studies where E_t and P_{it} are constant (i.e., do not depend on t) for all individuals, i, are of limited utility because they reduce to individuals with patterns of activities that make some of them exposed and others unexposed. Although, in principle, this difference in exposure provides the basis for testing its effect on health outcomes, the confounding between high activity and favorable outcome may intrinsically preclude the detection of the putative effect of O_3 exposure.

Besides the central issues related to the elements needed to determine exposure at the individual level, epidemiological studies attempting to assess the effect of ozone exposure on health need to collect extensive and detailed data on other variables that could positively or negatively confound or modify the exposure of interest.

Outcomes

The outcome (continuous or dichotomous) of acute effects studies is longitudinal in nature. The repeated measurements of the outcome provide the basis for assessing the changes on an individual over time. Correlation of these changes with the changes in the exposure at the individual level should be a central objective of studies designed to investigate the health effects of ozone exposure.

Design and Analytical Approaches

It is important that, at the planning stage of a longitudinal study, the investigators incorporate the correlation structure of the data for the estimation of the sample size needed to detect the differences of interest. It is not always appropriate to base the calculation of sample size on expected cross-sectional differences.

For the case of a continuous outcome, the simplest model incorporating the correlation of the outcome over time corresponds to the linear model with a random intercept. Specifically, if there are n_0 unexposed and n_1 exposed individuals, the outcome Y_{it} is modeled as $\alpha + \Delta_0 t + e_{it}$ for

$$(1 \le i \le n_0)$$

and

$$\alpha + \Delta_1 t + e_{it}$$

for

$$(n_0 + 1 \le i \le n_0 + n_1), \qquad [1]$$

where e_{it} are normally distributed with mean zero, variance σ^2, and the within-correlation of ρ. Thus, the variance of the mean of any indefinitely large number of observations for each individual (i.e., the between-individuals variance) will be $\rho\sigma^2$, while the variance of each observation about the population regression line for an individual (i.e., the within-individual variance) will be $(1 - \rho)\sigma^2$ and all individuals in a group will have the same slope. The main hypothesis of interest is whether $\Delta_0 = \Delta_1$ (e.g., decline of FEV_1 is the same on individuals unexposed and exposed to O_3). Standard procedures of generalized least squares methods show that the individual coefficients Δ_1 and Δ_0 have standard errors

$$SE(\Delta_1) = SE(\Delta_1) = \sigma(1-\rho)^{\frac{1}{2}} \left(\sum_{t=0}^{V} \left(t - \frac{V}{2} \right)^2 \right)^{-\frac{1}{2}} \qquad [2]$$

The asymptotic power to detect a difference $\Delta_1 - \Delta_0$ at 5% level having available n_0 unexposed and n_1 exposed individuals is given by

$$\Phi\left((\Delta_1 - \Delta_0) \left(n_1 n_0 \sum \left(t - \frac{V}{2} \right)^2 \right)^{\frac{1}{2}} \left(\sigma^2 (1-\rho)(n_0 + n_1) \right) - 1.96 \right), \qquad [3]$$

so the power is directly related to the magnitude of the difference $(\Delta_1 - \Delta_0)$, the number V of repeated observations on each individual, and the correlation ρ between the repeated measurements as well as the sample sizes of both groups. The power is inversely related to the variance σ^2.

Methods to compute the power of studies allowing for the slopes Δ_0 and Δ_1 to be different for different individuals are needed (*1*). Similarly, there is need to develop methods to calculate the power of models that directly relate the changes in exposure over time with changes in outcome.

Investigators carrying out longitudinal studies for the investigation of acute effects

of ozone exposure need to use analytical methods that focus on the quantification of the changes in outcome associated with changes in exposure. Under the assumption that the effects are acute and immediately disappear if the exposure is not present, marginal models treating the within-individual correlation as a nuisance are appropriate and particularly attractive since robust methods of estimation have been developed and are readily available (*2,3*). If the effects are purely acute, then the comparison of an exposed individual with another, unexposed individual does not need the incorporation of the previous history of the exposure of those individuals. In this case, a cross-sectional design is sufficient, and if longitudinal data are available, the task of the longitudinal analysis consists of combining the different cross-sections or visits into an overall estimate of the effect of the exposure. It is for this reason that the robust methods for combining correlated cross sections are particularly useful.

For many biological processes the effect of an exposure is not purely acute. An exposure needs to be present for a certain amount of time for the outcome of interest to change, and once the exposure is absent it takes a certain amount of time for the outcome to clear the past effects of the exposure. An acute effect can be thought of as one for which current exposure is more important than previous exposures. Statistical procedures allowing past exposure to have a dampening effect (i.e., the further away the exposure, the lower the effect on current outcome) have been proposed and should be explored when analyzing longitudinal data (*4*). Autoregressive models not only provide an approach to incorporate the correlation structure of the within-individual measurements, they also incorporate the effect of previous exposure on the outcome of interest. In particular, the simplest autoregressive model of the form

$$Y_{it} = \alpha + \gamma Y_{i,t-1} + \beta E_{it} + e_{it}, \qquad [4]$$

with e_{it} independent and identically distributed as normal with mean 0 and variance σ^2, yields Y_{it} in terms of the baseline Y_{i0} and the exposure history E_{is} for $1 \le s \le t$ as

$$Y_{it} = \alpha(1-\gamma^t)(1-\gamma)^{-1} + \gamma^t Y_{i0} + \beta \sum_{k=0}^{t-1} \gamma^k E_{i,t-k} + e^*_{it} \qquad [5]$$

where e_{it} are normally distributed with mean 0, variance $\sigma^2(1-\gamma^2)^{-1}$ and corr $(e^*_{it}, e^*_{it'} = \gamma^{|t-t'|}$. Since $0 < \gamma < 1$, then $\gamma^t < \gamma^{t-1}$ ... $< \gamma < 1$, and hence the coefficient of exposures at times prior to the current value decrease as the time lag increases. This may not necessarily be the case for certain outcome-exposure associations, and alternative analytical approaches should be employed. On the other hand, it offers a simple way to model a mechanism that is expected in many biological processes.

The above models are useful for the analysis of continuous outcome (e.g., forced expiratory volume after one second). If the outcome is binary (e.g., presence of asthma), appropriate methods using logistic regression models should be used (*5*). In this case, one models the log of the odds of having an asthma attack in the next examination on the basis of the presence of asthma in the current examination and the current environmental exposures. Another important aspect of acure studies of the health effects of ozone is the identification of susceptible subgroups. A susceptible individual is the one who changes more relative to another under the same change in exposure to ozone. In other words, a susceptible individual may tend to have higher within variance. Experimentation with high within variance as a selector of sensitivity may be well justified, but we cannot be certain that it will work. Longitudinal data provide the basis for the estimation of the within-individual variance, and analytical methods using the within variance as the outcome should be explored.

Chronic Effects Studies

Only a few epidemiological studies have been done on the chronic effects of ozone exposure. More studies are needed, and they should be designed to distinguish between acute/transient effects and those that have a long term effect on premature aging of the lung, symptoms, and mortality.

Exposure

In contrast to the acute effects studies, the studies to investigate chronic effects should pay special attention to cumulative exposures. Methods to summarize the duration and intensity of long-term exposure have been extensively studied in occupational epidemiology (*6*) and should be useful in studies designed to investigate the chronic health effects of ozone exposure.

Given the relatively homogeneous ozone concentration on a limited geographic area, it is important that locations with different histories of ozone concentrations be studied and compared. Obviously, valid inferences require the use of populations comparable to each other except for the exposure to ozone. Studies should be appropriately designed to provide comparable populations.

It is difficult to reconstruct the exposure E_{it} of the i^{th} individual at the time t in the past. It requires that the regional ozone concentration E_t at time t in the past be available and that the individual provide an accurate history of his or her pattern of activities P_{it} at time t in the past. In the absence of these data, it would be very useful to use markers M_i of the cumulative exposure to ozone on the i^{th} individual. The availability of these markers may provide the basis of simple cross-sectional comparisons.

Outcomes

Studies investigating the chronic effects of ozone exposure should compare cross-sectional means and patterns of change of the outcome over time. The simplest measure of change over time is the difference between two time points ($Y_{it} - Y_{i,t-1}$). Recent work by Ware et al. (*7*) discusses appropriate methods for statistical analysis and uses change as the outcome variable. Alternative methods (*4*) focus on the difference between the observed outcome at a given time t and the expected value of the outcome given the past values of the outcome

$$\left(Y_{it} - E\left[Y_{it} \mid Y_{i,t-1}\right]\right) \qquad [6]$$

Both methods attempt to identify the variables that explain the variability of the changes over time. Of primary importance is the test of the effect of the exposure to ozone after adjusting for confounders and other known explanatory variables.

An alternative outcome of interest may be the within variance of the outcome on individual i. It may be that the chronic effect of ozone is to make the outcome very susceptible (or volatile) to a given exposure. This approach is close to the challenging experiments (e.g., histamine) done in the area of respiratory disease epidemiology. In this case, the response to a challenge is used as an outcome as opposed to the usual setting of investigating its effect on pulmonary function. It could very well be that individuals with a long-term exposure to ozone are more reactive to a challenging exercise.

Design and Analytical Approaches

A central purpose of a study design to investigate the chronic effects of exposure to ozone is to obtain populations that are comparable to each other except for the exposure to ozone. One approach is to design a study where all individuals have been under the same regional exposure (i.e., close geographic location) but whose patterns of

indoor/outdoor and exercise activities are diverse. An advantage of this design is that the regional nature of the study population makes the individuals comparable in several respects, including coupling with exposures to other pollutants. The main difficulty is that very active individuals (i.e., more likely to be exposed to ozone) may have associated a favorable outcome. Another approach is to select different locations with different histories of ozone concentration and compare the outcome of groups of individuals from the different locations. The main difficulty here is that ozone elevations are typically coupled with other environmental exposures, making the effect nonidentifiable. Furthermore, individuals in different geographic locations intrinsically may have different patterns of activities, making it difficult to distinguish the independent effects of ozone exposure.

A design that may offer some advantage could involve the comparison of groups of individuals in different locations with different histories of ozone elevations but could match the individuals in different locations by their pattern of indoor/outdoor and exercise activities. The objective would be that individuals who have the same pattern of activities but are subject to different ozone concentrations may provide different outcomes.

A very important aspect of the analysis of data from studies investigating the long-term effects of ozone exposure is the identification of patterns of exposure with outcome. The main components for the determination of pattern of exposures are the duration and intensity of the exposure. Much effort has been dedicated to advantages and disadvantages of different summary measures, including the maximum concentration, the integral of the concentrations over time, the weighted mixture of different concentrations at different times, etc. Attention should also be given to patterns incorporating transitions of past exposures (*8*).

Analytical methods for longitudinal data have been the subject of active statistical research in the last decade. The emphasis has been on how to incorporate the correlation structure of the repeated observations on a given individual. Robust methods, random effects, and autoregressive models correspond to different ways of handling the within-individual correlation of the outcomes taken at different visits. An important result is that when modeling the cross-sectional (e.g., marginal) distribution of the outcome, the estimation of the regression coefficient should not be affected by the method of handling the correlation of the within-individual outcome measures. Discrepancies between estimates of the regression coefficients when using different methods for the incorporation of the correlation of the outcome over time could be used as a diagnostic regression measure of inconsistencies of the between- and within-individual regressions. If the effect of exposure using individual regressions (i.e., changes in exposure to changes in outcome) are of lesser magnitude than the effect of exposure using the between-individual regressions, a chronic (i.e., long-term) effect may be suggested. Therefore, a diagnostic regression procedure may be useful for the identification of chronic versus acute effects. Refinement and specificity of the diagnostic tool is needed.

Issues Common to Acute and Chronic Studies

The general issues of confounding and effect modification are present in both acute and chronic studies of the health effects of exposure to ozone. Since elevated ozone concentrations are correlated with elevations of other pollutants and environmental conditions, it is important to obtain complete information so that controlling for confounding can be done with the appropriate analytical procedures. The investigations of interactions between ozone and other pollutants that cause adverse health effects are of equal importance. It is possible that only situations where critical levels of other atmospheric pollutants occur are associated with a poor outcome.

Longitudinal studies are bound to have missing data on intermediate visits or on the last visit for those individuals who drop out of the studies. Analytical methods are available (*9*) to incorporate into the analysis visits that are not equidistant due to gaps caused by intermediate visits missing. The missing information on individuals who permanently drop out is of greater impact because they directly affect the ability to assess the long-term effect of the exposure. Investigators in other areas of research have used multiple imputation (*10*) for the handling of missing data. Use and applicability of these methods is an area of important research in the context of the health effects of ozone exposure. The methods of multiple imputation typically assume the missing data to be caused by a random mechanism. In studies of health effects of ozone, the dropouts may be related to disease progression and alternative methods for the incorporation of informative censoring will need to be developed.

Given the measurement errors to which both the exposure and the outcome are subject, it is essential that replicate measurements be taken if feasible. Regression methods incorporating the measurement error have been developed and should be used. These methods require data on duplicates to estimate the error variance. Failure to correct for the measurement error may increase the probability of not rejecting the null hypothesis when the alternative is true.

Both acute and chronic studies need to incorporate data on treatment of chronic respiratory illnesses (e.g., asthma) and interventions. Studies are needed on the effect of treatment under different exposures to ozone. As with exposure, the treatment may also be time dependent and, thus, the analytical issues are similar.

During the last decade, methods have been proposed to combine studies to provide an overview (i.e., metanalysis) or to combine studies with strengths in complementary aspects. In particular, the prevalent and incident subcohorts of a longitudinal study in infectious disease epidemiology describe the mature and early stages of the natural history. Several approaches (*11,12*) have been proposed to combine these components into a unified data set for the determination of the incubation period of AIDS. Although of a different nature, acute and chronic effects are complementary. The outcome of acute studies can be viewed as the exposure of chronic studies. Acute studies may establish that exposures to ozone are associated with acute/transient changes of outcome. Chronic studies may establish that individuals whose outcome has a high within variance are those who will prematurely age with respect to the outcome of interest. Methods to combine studies of this nature should be the subject of future research. The issues presented here have a role similar to that of surrogate markers for the evaluation of effective therapies in infectious disease epidemiology. Bridging the methodological issues will be an important contribution to scientific research.

In the context of the studies of the health effects of ozone, there are opportunities to combine laboratory experiments with longitudinal studies. Chamber studies provide a measure of the changes in outcomes due to a controlled exposure. Using multivariate methods (e.g., principal components), one can determine the individuals with the highest variability and enroll them in a follow-up study to assess the effects of environmental exposure. The analysis of these data will be more informative if the studies are formally combined into a comprehensive analysis.

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Summary of Papers and Research Recommendations of Working Group on Tropospheric Ozone, Health Effects Institute Environmental Epidemiology Planning Project

Ira B. Tager*

Department of Medicine, University of California, San Francisco, Department of Veterans Affairs Medical Center, 4150 Clement Street, San Francisco, CA 94121

This paper summarizes the themes and recommendations that emerge from the papers presented by the Working Group on Tropospheric Ozone. In terms of current knowledge, the following are considered of particular importance: *a*) lack of clear evidence for a human analogue of the terminal bronchiolar and proximal acinar changes observed in the lungs of ozone-exposed animals; *b*) lack of evidence for a connection between the acute respiratory effects of O_3 and possible chronic respiratory effects; *c*) need to better define the characteristics of O_3-susceptible individuals; *d*) the lack of adequate exposure assessment tools for reconstruction of lifetime O_3 exposure; and *e*) incomplete information on the role of other ambient environmental pollutants in the facilitation of O_3 effects or as a cause of effects attributed to O_3 in human populations. Based on the above, several recommendations for epidemiologic research on health effects of O_3 are offered. *a*) Studies to investigate the existence of chronic health effects of O_3 are essential, particularly those that include autopsied human lung tissue and biologic and physiologic response markers. *b*) Studies are needed to link acute responses with chronic effects and should include joint epidemiologic and controlled-exposure assessments. *c*) Studies are needed to identify susceptible subgroups. Such studies should include newly emerging biologic markers of O_3 exposure. *d*) Accurate and precise tools for chronic O_3 exposure assessment need to be developed for use in retrospective and prospective studies. *e*) Collaborative studies between epidemiologists and laboratory investigators are needed to develop and evaluate markers of O_3 exposure and to test O_3 exposure models. — Environ Health Perspect 101(Suppl 4):237–239 (1993).

Key Words: Ozone, health effects

This paper will summarize the themes and research recommendations that emerge from the papers presented by the Working Group on Tropospheric Ozone, of the Health Effects Institute Epidemiology Planning Project. The order of presentation of the themes and research recommendations is motivated by issues of coherence and is not intended to imply prioritization on the part of the working group. References to the individual papers that develop the themes are provided to assist readers who may choose to read this summary paper prior to the individual presentations.

Animal research has provided coherent evidence that the earliest effects of ozone (O_3) on the lung can be found in the terminal airways and acinus and are consistent with the changes (acute inflammation, fibrosis) and anatomic location that could be the antecedents for the occurrence of accelerated aging of the lung and chronic lung diseases.(*1–3*). Although human dosimetry calculations and data from bronchial lavage studies are compatible with a comparable process in humans, there is no direct evidence that the early lesions observed in animals occur in man and that chronic lung disease can be attributed to O_3 exposure (*1–4*). Therefore, epidemiologic studies need to make use of methods that permit a direct test of the hypothesis that O_3 does produce changes in the lung that are compatible with the occurrence of chronic lung disease. The use of human postmortem lung specimens (*1,2*) and the parallel use of markers of biologic response (e.g., inflammation) and physiologic response (e.g., pulmonary function at the level of small airways) are considered to be essential elements in future epidemiologic studies of the health effects of O_3 exposure (*1–5*).

Most epidemiologic studies and all controlled exposure studies on the health effects of O_3 have focused on acute responses of one kind or another. The implicit assumption of such studies has been that there is some relationship between these acute responses and the subsequent occurrence of chronic disease (*3–5*). Nonetheless, this relationship has not been established firmly (*4*). Moreover, the range of acute effects that can be attributed to O_3 exposure and the public health burden that they impose remain incompletely defined (*4,5*). The use of panel studies alone or nested within larger cross-sectional or longitudinal studies (*4,6*), the combining of traditional epidemiologic study designs with controlled exposure protocols, and the use of multiple biologic and physiologic response markers within a given study offer new possibilities to expand the current body of data with regard to the acute health effects of O_3 and their long-term consequences (*3,4*).

The need to define the diversity of individual and group susceptibility to the adverse health consequences of exposure to O_3 is a feature of each of the papers of the working group. The current database on the health effects due to O_3 exposure largely has ignored this issue, with the exception of the focus on asthmatic individuals and individuals with hyperactive airways as assessed by bronchoconstrictor challenge testing (*4,5*). The definition of the susceptible individual or group is complex: There is no uniform marker or set of markers that define(s) O_3-susceptibility either to acute or chronic effects (should they exist)

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*Present address: 140 Earl Warren Hall, UC Berkeley, School of Public Health, Berkeley, CA 94720.

(3), and susceptibility may be defined in terms of increased variance in response as well as in terms of the more traditional considerations of level of change (6). The later emphasis on individual variability in response imposes the need for the kind of repetitive measurements made in panel studies or more traditional longitudinal designs. Moreover, the issue of susceptibility is complicated by the fact that there may not be concordance between factors that define susceptibility to acute effects and those that define susceptibility to chronic effects (unpublished material) and, as noted above, there may not be a clear relationship between the occurrence of acute response to O_3 and the subsequent occurrence of chronic health effects. The identification of susceptible individuals and groups has implications for design choice, efficiency of epidemiologic studies, and analysis of data (3,4,6). To identify susceptible individuals, epidemiological studies will have to rely increasingly on biologic and physiologic response markers that more closely parallel the hypothesized effects of O_3 on the respiratory system (1–3). Currently, there are insufficient data on the validity, reproducibility (under conditions of known exposure), and specificity of the range of possible markers of O_3 susceptibility and exposure (3). Studies specifically designed to provide data on validity, reproducibility, and specificity should proceed in parallel with or, preferably, as an integral part of epidemiologic studies of the health effects of O_3 exposure.

Exposure assessment is an area of central concern for epidemiologic studies of O_3 effects (2,4). Epidemiologic studies that deal with chronic health effects must provide at least a semiquantitative estimate of O_3 exposure prior to the onset of the study (including longitudinal studies). To do so requires that available O_3 monitoring databases be used to estimate likely cumulative exposures that individuals have had prior to coming under direct observation (or prior to time of death in the case of autopsy studies). Such estimates depend upon detailed information on factors such as residential histories, typical activities (type, frequency, intensity), time spent indoors versus outdoors, etc. (2,4). Instruments need to be developed and validated for retrospective reconstruction of exposure history. Assessment of cigarette tobacco use and occupational exposures will have to be an integral part of such instruments. The use of such instruments also will be required for longitudinal studies, but the possibilities of conducting validation substudies make the task here less daunting than for studies that will have to depend solely on retrospective exposure assessment. In this context, there is an obvious need for continued development and validation of O_3 exposure models. As is the case for markers, such studies should proceed in parallel with or as part of longitudinal epidemiologic studies. The continued development and application of technologies that permit direct monitoring of individuals (personal dosimeters) should be encouraged as part of epidemiologic studies (4).

Closely related to the issue of exposure assessment for O_3 is the role of other ambient environmental pollutants in the facilitation of O_3 effects or as the cause of effects that have been attributed to O_3 (2,4). Of particular interest is the role of acid aerosols and particulates of the PM10 fraction. Careful selection of study sites will be essential to control for these effects and should figure prominently in the design of epidemiologic studies of whatever type (2,4). If epidemiologic studies take place in a single geographical location, the location should be chosen to minimize the possibilities that other ambient pollutants are either modifying or confounding any health effects that are attributed to O_3 exposure. The advent of personal O_3 dosimetry or the identification of specific molecular markers of O_3 exposure would lessen the need for such a restriction (3,4).

Bates, in his lecture entitled "Health Indices of the Adverse Effects of Air Pollution: The Question of Coherence" (7), developed the concept of coherence of data from epidemiologic studies of air pollution. Bates defined coherence, in the context of air pollution health effects research, as the interrelationships between different indices of health. Coherence, in this context, is a central issue for epidemiologic studies that attempt to relate acute responses to O_3 exposure and chronic health effects (4), but the concept is relevant to all aspects of epidemiologic studies of the health effects of air pollution. Implicit in the concept of coherence is the explicit linkage of epidemiologic studies that address differing elements in the chain of relationships between O_3 exposure and health effects. The choice of study design(s), the location(s) of the studies, and the specific health effects to be investigated should form a series of studies that are planned as a logical unit that is designed to produce a set of data that can be evaluated as a logical unit (4). The nested study designs, (8) are the simplest examples employed to date.

The above synthesis forms the overall framework from which the following set of specific research recommendations are put forth (9).

Recommendations for Specific Epidemiologic Research

Epidemiologic studies specifically targeted to investigate the occurrence of chronic health effects due to O_3 are neccesary. Innovative study designs that are based upon the use of autopsied human lungs and on the employment of combinations of biologic and physiologic response markers should be developed to test the hypothesis that chronic O_3 exposure can produce a respiratory bronchiolitis that is related to premature functional decline of human respiratory function. Studies that investigate the relationship between acute responses to short-term O_3 exposure and the occurrence of chronic respiratory tract symptoms and alterations of function should be undertaken. Such studies should employ traditional epidemiologic study designs in combination with panel studies and should provide samples that make population estimates feasible. Epidemiologic studies that investigate individuals who have recently moved (temporarily or permanently) from or to areas with markedly different O_3 and other pollutant profiles should be encouraged.

Epidemiologic studies of the full range of acute health effects that can be attributed to O_3 exposure are neccesary. Epidemiologic studies of acute health effects should investigate the phenomenon of adapration that has been observed in studies with controlled exposures and determine its role in the epidemiology of acute O_3-related health effects. Epidemiologic studies should be closely linked to controlled exposure studies in this regard.

Epidemiologic studies that investigate the role of other ambient air pollutants in the occurrence of O_3-related health effects are neccesary. Collaborative epidemiologic studies over several regions that are chosen on the basis of their patterns of O_3 and other specific pollutants (e.g., acid aerosols) should be encouraged.

Epidemiologic studies to identify O_3-susceptible subgroups and individuals are necessary. Epidemiologic studies of the role of O_3 exposure as an etiologic factor for asthma and as a factor in increases in the morbidity and mortality of asthma should be undertaken. Such studies should include a combination of traditional epidemiologic study designs and controlled

exposure protocols both for the selection of subjects and the investigation of outcomes. Epidemiologic studies should be undertaken to provide data for estimation of the validity, specificity, and predictive values of new O_3 susceptibility markers that are under development in the laboratory. Close collaboration between epidemiologists and laboratory-based investigators for the development of markers that can be applied in epidemiology study protocols of O_3 health effects is to be encouraged. Epidemiologic studies of O_3, regardless of their design, should attempt to include repetitive measures of effect for individual subjects whenever feasible.

Epidemiologic studies to develop accurate and reliable (precise) tools for O_3 exposure assessment are neccesary. The development of methods for retrospective reconstruction of cumulative O_3 exposure in epidemiologic studies is of particular importance. There should be close collaboration between epidemiologists and investigators who are involved in the development and testing of exposure models.

Recommendations for Needed Studies in Support of Epidemiologic Protocols

There were a number of research recommendations considered by the working group that do not translate strictly into epidemiologic studies. However, the working group felt that developments in these areas were of sufficient importance to epidemiologic studies of O_3-related health effects that research into them should be supported in the context of an overall "Epidemiology Planning Project."

Laboratory investigations to identify sensitive and specific molecular, cellular, and physiologic markers of O_3 health effects susceptibility are needed. Laboratory investigations to identify highly specific molecular and cellular markers of O_3 exposure also are desirable. Such efforts should occur in close collaboration with epidemiologic investigators, and studies to determine the validity, specificity, and predictive values of such markers in epidemiologic studies should be undertaken.

The further development and testing of models of O_3 exposure should be supported as a component of selected epidemiologic studies. Also, the further development, testing, and application of personal O_3 dosimeters should be supported as a component of selected epidemiologic studies, particularly those studies with an emphasis on the development of overall methods for exposure assessment. ehp

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Health Effects Institute
141 Portland Street
Cambridge, MA 02139