

CHAPTER 5

TOXICITY, ACUTE IRRITANT EFFECTS, AND CARCINOGENICITY OF ENVIRONMENTAL TOBACCO SMOKE

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Irritation: Acute Exposure

Irritants in Environmental Tobacco Smoke

Tobacco smoke is a complex aerosol that contains several thousand different constituents (Hoffmann, Haley, Brunnemann 1983). Little is known about the health effects of most of these compounds individually and even less is known about their interactions. Tobacco smoke contains compounds established as irritants, toxins, mutagens, and carcinogens. The main irritants identified in environmental tobacco smoke (ETS) to date are respirable particulates, certain aldehydes, phenol, ammonia, nitrogen oxides, sulfur dioxide, and toluene. The range of concentrations of these irritants measured in mainstream smoke, in sidestream smoke, and in smoky air under "realistic" and "natural" conditions or as results of field studies is summarized in Table 1.

The levels of irritants in air contaminated with ETS vary considerably (Table 1). Some of this variation is due to differences in the number of cigarettes smoked, the amount of ventilation, the adsorptive properties of the surroundings, and measurement methodology. Triebig and Zober (1984) compared the measured concentrations of these irritants with the maximum permissible concentration (MAK) values for working areas and the maximum emission concentration (MIK) values for outdoor air pollution in the Federal Republic of Germany. They concluded that concentrations approximating or in excess of the MIK values can be found for respirable particulates, nitrogen dioxide, and acrolein. The other irritants generally do not reach the existing threshold limit values under realistic conditions. For phenol there is no MIK value. An evaluation of the hygienic and medical importance of the compounds in ETS based on threshold limit values is problematic for two reasons: first, MAK values for industries are established for healthy adults with an 8-hour exposure per day; MIK values are for the outdoor environment, and no indoor limit values exist for "everyday life." Second, the threshold limit values are valid only for single compounds; ETS contains many different irritants, which might interact to produce more toxicity than anticipated from the concentrations of individual compounds.

Many of the constituents of tobacco smoke are also produced by other sources that contribute contaminants to the indoor or outdoor environment. For example, sources unrelated to smoking such as urea formaldehyde foam insulation or certain wood materials can emit formaldehyde and may give rise to mean air concentrations as high as 100 to 400 ppb (Triebig and Zober 1984). In measuring the contribution of tobacco smoke to the levels of these constituents, some researchers (Weber et al. 1979a; Weber and Fischer 1980) have subtracted the measured indoor concentrations from the levels

TABLE 1.—Major irritants in environmental tobacco smoke (ETS), their concentrations in mainstream smoke (MS), sidestream smoke (SS) to mainstream smoke (MS) ratios, and levels in smoky air under realistic and natural conditions

Irritant	MS (per cigarette)	SS/MS (ratio)	Smoky air (range)
Acrolein	10-140 µg	10-20	6-120 ppb
Formaldehyde	20-90 µg	≈ 50	30-60 ppb ^a (CO: 1-43 ppb)
Ammonia	10-500 µg	44-100	1000-4580 ppb ^b
Nitrogen oxides	16-600 µg	4.7-50	1-370 ppb NO ^c 0-50 ppb NO ₂ ^c
Pyridine	32 µg	10	NA ^d
Sulfur dioxide	1-75 ppb	NA	1-69 ppb ^c
Phenol	20-150 µg	2.6	7.4-115 µg/m ³
Toluene	108 µg	5.6	0.04-1.04 mg/m ³
Respirable particulates	0.1-40 mg	1.3-1.9	55-962 mg/m ³

^a Measured under experimental conditions only.

^b Fischer (1979).

^c Difference: indoor concentration minus control value (unoccupied room or outdoors).

^d NA = not available.

SOURCE: Data from Collishaw et al. (1984), Remmer (1985), Triebig and Zober (1984), US DHHS (1984), except where noted.

measured either in the unoccupied room or in the outdoor environment near the room.

The measured concentrations of irritants listed in Table 1 are primarily the mean values in air samples collected over intervals of one-half hour to several hours. Substantial variation in levels can occur, depending on the proximity to a smoker and the air-mixing conditions in the room. Weber and Fischer (1983) measured peak concentrations of 3,330 to 99,680 ng/m³ for the particulates and 41 to 750 ppb for nitrogen oxide in the "blowing cloud" 1 meter from the smoker immediately after smoke exhalation. These high concentrations decreased very rapidly with time (half-life between 2 and 20 seconds) and distance from the smoker. Ayer and Yeager (1982) measured formaldehyde and acrolein concentrations in the side-stream smoke plume rising from a cigarette between puffs and obtained concentrations of some constituents up to three orders of magnitude above the occupational limits established for more extended exposures. e

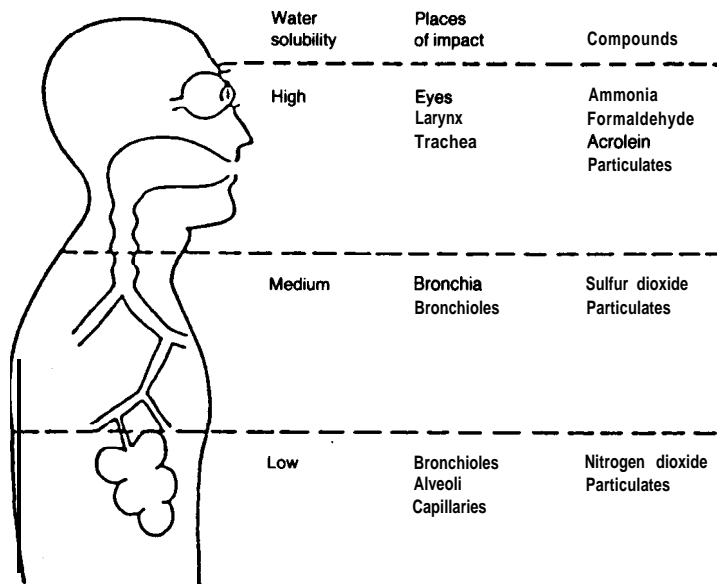


FIGURE 1.--Places of impact, and irritants in the eyes and respiratory tract in relation to water solubility

SOURCE: Valentin (1985).

Irritating and Annoying Effects of Environmental Tobacco Smoke

The main effects of the irritants present in ETS occur in the conjunctiva of the eyes and in the mucous membranes of the nose, throat, and lower respiratory tract. The main ocular symptoms are reddening, itching, and increased lachrymation; the main respiratory tract symptoms are itching, cough, and sore throat. The relationship of the site of the effect of some irritants in the eyes and in the respiratory tract to their water solubility is illustrated in Figure 1. The penetration of the particulates into the lung depends on their size; because most of the particulates in tobacco smoke are smaller than 1 μm , they can penetrate to the smallest airways.

Studies of Healthy Individuals

Field Studies

Several studies have shown that annoyance and irritation are the most common acute effects of ETS exposure. Shephard and Labarre (1978) surveyed more than 1,000 Canadian citizens aged 10 to 80 years. The interviewed population was representative of southern

Ontario with respect to both income and profession but underrepresentative of the elderly. Seventy-three percent of the nonsmokers were disturbed by tobacco smoke in restaurants and 53 percent by tobacco smoke in offices. The most frequently reported symptom was eye irritation. Complaints of nausea, dizziness, and wheezing as well as rhinorrhea were also reported, although much less frequently than stinging eyes.

Similar results were obtained in a survey conducted in three restaurants in Switzerland (Weber et al. 1979a). A multiple-choice questionnaire was administered to 220 guests. One-third to two-thirds of the respondents complained about air quality, and up to 12 percent reported eye irritation. In another survey of more than 2,100 white-collar employees, Barad (1979) found that nearly one-fourth of the nonsmokers reacted to smoke exposure with frustration and hostility.

Weber and Fischer (1980) surveyed employees in 44 worksite workrooms, located in seven different companies, that included offices, rooms for design and technical and clerical work, and conference rooms. The choice of companies and worksites was based on availability and therefore was not a random sample. In all workrooms, the concentrations of carbon monoxide (CO), nitrogen oxide (NO), acrolein, particulate matter (PM), and nicotine were measured in the air. The contribution of tobacco smoke to these levels was obtained by subtracting background levels obtained before working hours from the concentrations during working hours. These differences from the background levels were called Δ CO, Δ NO, and so on. Measurements were conducted in each room on 2 successive days (12 1-hour mean values per workroom), and 472 employees were questioned about irritation and annoyance as well as about their opinions on involuntary smoking.

Some of the exposure results are summarized in Table 2. The comparison of these Δ values with the measured absolute indoor concentrations revealed that 30 to 70 percent of the measured indoor concentrations of carbon monoxide, nitrogen oxide, and particulate matter were due to tobacco smoke. The correlations between the gas phase components Δ CO and Δ NO were relatively high (Pearson correlation coefficient $r = 0.73$). However, the correlations of Δ CO with Δ nicotine and Δ PM were low. Nicotine values were generally in the range of the lower detection limit of the method of measurement used (gas chromatography). The low correlation of the gaseous components with the particulate matter is probably due to the different physical properties (sedimentation, adsorption, and desorption of the particulates) and to the fact that the Δ PM values include particulates from sources other than tobacco smoke.

Approximately one-third of the employees described the quality of air at work as "bad" with regard to tobacco smoke. Forty percent

TABLE 2.—Air pollution due to tobacco smoke in 44 workrooms

Component	Number of samples	Mean values	Standard deviation	Maximum
δCarbon monoxide (ppm)	353	1.1	1.3	6.5
δNitrogen oxide (ppb)	348	32	60	280
δParticulate matter (μg/m ³)	429	133	130	962
δNicotine (μg/m ³)	140	0.9	1.9	13.8

NOTE: δ value = "indoor concentration during work" minus "indoor concentration before work."
SOURCE: Weber and Fischer (1980).

were disturbed by smoke. One-fourth reported eye irritation at work. Seventy-two percent of the interviewed nonsmokers and 67 percent of the smokers were in favor of a separation of the workrooms into smoking and nonsmoking sections; 49 percent supported a partial or total prohibition of smoking at work.

Contradictory results were reported by Sterling and Sterling (1984), who found no relationship between smoking conditions in offices and comfort complaints. A self-administered work environment questionnaire was given to approximately 1,100 employees working in nine buildings. Data were analyzed according to the smoking habits of the respondents and the office rules regulating smoking. The distribution of the responses to questions assessing the presence of symptoms (headache; fatigue; nose, throat, and eye irritations; sore throat and cold symptoms) were similar in environments with and without smoking. The researchers concluded that “smoking is not a pivotal source of indoor pollution of health-related building complaints.” No objective measurements of air pollution were carried out, however, and there were no descriptions of building ventilation. The researchers used a “building illness index” that included several different symptoms in addition to irritation (e.g., headache, fatigue), and the irritating effects on the most sensitive organ—the eyes—may have been masked by this use of an overall symptom index.

Experimental Studies

Harke and Bleichert (1972) examined the acute physiological response to ETS in a 170 m³ room. The electrocardiogram, blood pressure, heart rate, and skin temperature showed no change with exposure to ETS, even at extremely high exposure levels (150 cigarettes smoked in 30 minutes, corresponding to a carbon monoxide concentration of 60 ppm at the end of the exposure).

The influence of the temperature and humidity of room air on odor perception and irritation was investigated by Kerka and Humphrey (1956). They found that odor intensity was somewhat reduced by increasing the temperature at a constant humidity. Both odor and irritation intensity were reduced by increasing the humidity. Johansson and Ronge (1966) also observed that acute irritation is increased in warm and dry air. Johansson (1976) exposed 12 subjects in a 6.7 m³ climatic chamber for 29 minutes to the ETS produced by the smoking of 10 cigarettes. The air in the chamber was cold (18° or 19° C) or warm (25° or 26° C), and at each temperature, the relative humidity was evaluated at three levels from 30 to 80 percent. Under all conditions, subjective irritation, assessed by a questionnaire, increased during exposure; eye irritation increased more than nose irritation. No marked effect of temperature on the degree of irritation was observed, probably owing to the limited temperature range studied (18° to 26° C). Kerka and Humphrey (1956) demonstrated a thermal effect when the temperature range was greater than 8° C. The low relative humidity (7 to 20 percent) in aircraft may be responsible for the substantial level of perceived irritation due to ETS among passengers, despite the low levels of pollutants measured in aircraft (WHO 1984).

Basu and colleagues (1978) studied the effects of ETS on human tear film and observed a reduction in the stability of the precorneal tear film in subjects exposed to a smoke concentration corresponding to approximately 20 ppm CO. In the presence of ETS, the tear film breakup time was significantly reduced by 35 to 40 percent compared with baseline measurements without smoke. The researchers suggested that this reflects an alteration in the relative proportions of the constituents of tear film.

In these studies, the quantitative exposures to ETS either were not measured or were determined in a relatively imprecise way. More systematic studies, including measurements of several compounds of ETS, were carried out by Weber and collaborators (Weber et al. 1976, 1979a,b; Weber, Fischer, Grandjean 1977; Weber, Fischer, Gierer et al. 1977; Weber and Fischer 1983) and Muramatsu, Weber, and colleagues (1983). These experiments were carried out in a climatic chamber of 30 m³, with an air temperature of 20° to 24° C and a relative humidity between 40 and 60 percent. The ventilation rate could be varied between 0.1 and 16 air changes per hour. The smoke was produced by a Borgwald smoking machine under standardized conditions, and only the sidestream smoke of cigarettes was used. Healthy students were exposed to the sidestream smoke of cigarettes in groups of two or three in the climatic chamber. They all also participated in a control exposure with identical conditions, but without sidestream smoke in the air. The concentrations of the following compounds were continuously recorded: carbon monoxide,

nitrogen oxide, formaldehyde, acrolein, and particulate matter. The background levels before smoke production were subtracted from the measured concentrations during smoking; the resulting values were called Δ CO, Δ NO, and so forth. The degree of irritating and annoying effects of the exposed subjects was determined every 10 minutes by means of questionnaires and by measuring the eye blink rate, considered an objective measure for eye irritation.

In the first study, 33 subjects were exposed to continuously increasing smoke concentrations (Weber et al. 1976). The main results are summarized in Figure 2. The concentrations of CO, NO, formaldehyde (HCHO), and acrolein increased with the number of cigarettes smoked. Both mean subjective eye irritation and mean eye blink rate increased with increasing smoke concentration. Subjective nose and throat irritation was also evaluated. Nasal symptoms were less pronounced than eye symptoms, and the throat was the least affected.

In a second series of studies, acute effects were analyzed in relation to smoke concentration and duration of exposure (Weber et al. 1979; Muramatsu, Weber et al. 1983). The tobacco smoke concentrations corresponded to 1.3, 2.5, 5, and 10 ppm CO (Δ CO). Subjects were exposed to these smoke concentrations for 1 hour, each smoke concentration increasing linearly during the first 5 to 10 minutes and then remaining constant at the desired level for the rest of the hour. Because very high correlations ($r > 0.9$) were obtained in the first experimental series between Δ CO and each of the other compounds, only Δ CO was used to quantify the level of exposure to ETS.

The results obtained for subjective eye irritation and eye blink rate are shown in Figures 3 and 4. The mean reported level of eye irritation as well as the eye blink rate increased with increasing smoke concentration. Both irritation parameters also increased with the duration of exposure under conditions of constant smoke concentration. The same, but less pronounced, results were observed for nose and throat irritation.

Annoyance increased rapidly as soon as smoke production began and increased with increasing smoke concentration, but after 10 to 15 minutes the level of annoyance remained approximately constant during the rest of the exposure. Thus, the intensity of exposure was important in determining the degree of annoyance and the duration of exposure was less important.

These experiments demonstrated an objective irritant response in healthy adult subjects at levels of smoke exposure substantially lower than the levels at which an airway response has been demonstrated. Whether this difference represents a difference in threshold for irritation in the eye and airway or a limitation in the ability to measure subtle changes in the airway is uncertain.

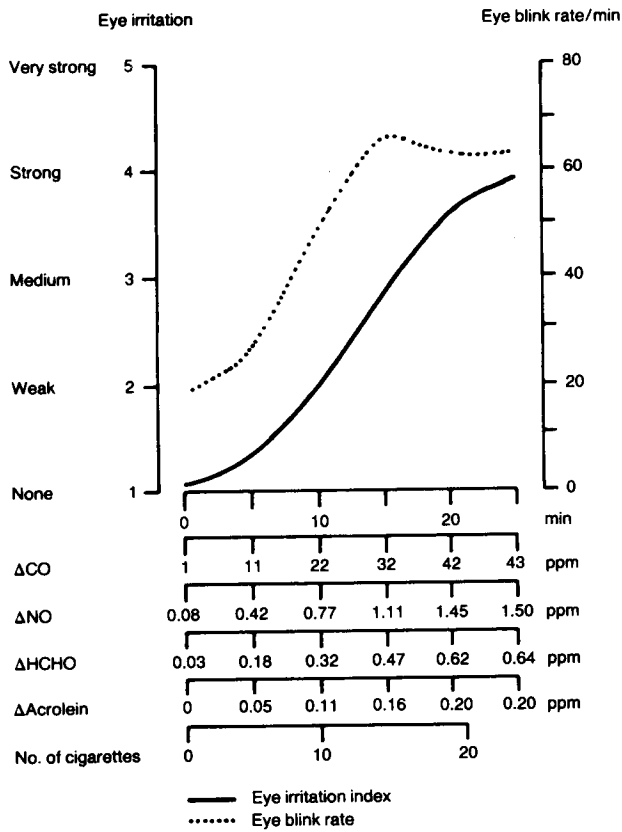


FIGURE 2.—Mean subjective eye irritation, mean eye blink rate, and concentrations of some pollutants during continuous smoke protection in an unventilated climatic chamber

NOTE: 33 subjects; 0 min: measurement before smoke production.
 SOURCE: Weber et al. (1976).

Hugod and colleagues (1978) and Weber and colleagues (Weber, Fischer, Grandjean 1977; Weber, Fischer, Gierer et al. 1977; Weber et al. 1979b) carried out several experiments in order to determine which compounds in ETS are responsible for irritation and annoyance. The results of the two studies were somewhat conflicting. Hugod and colleagues exposed 10 subjects in an unventilated 68 m³ room to high concentrations of sidestream smoke (concentrations corresponding to 20 ppm CO), to the gas phase of sidestream smoke alone, and to acrolein alone at concentrations three times those found in sidestream smoke alone. Irritation was assessed via a

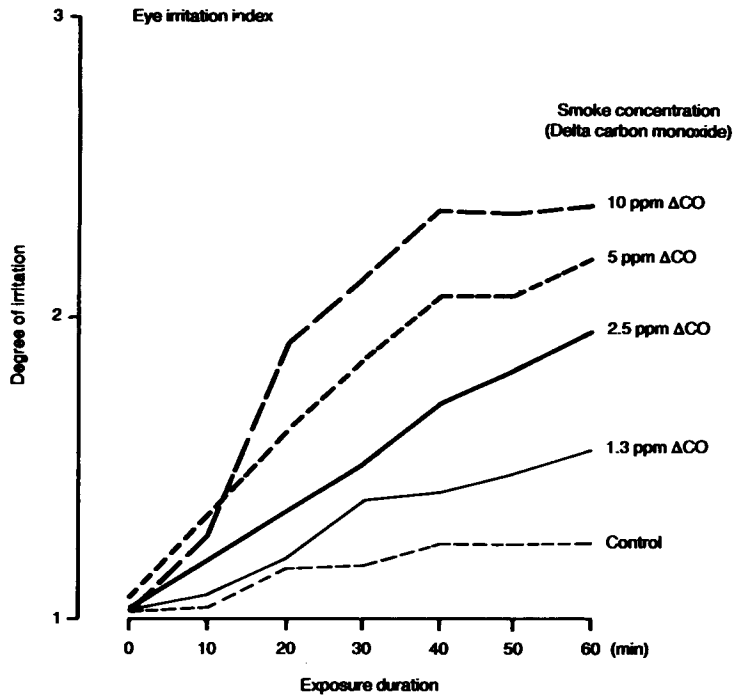


FIGURE 3.—Mean subjective eye irritation related to smoke concentrations (ppm delta CO) and duration of exposure

NOTE: 32 to 43 subjects; 0 min: measurement before smoke production; 0 to 5 min: increasing smoke concentration; 6 to 60 min: constant smoke production.

SOURCE: Muramatsu, Weber et al. (1963).

questionnaire. Both annoyance and irritation were reported at similar levels in the subjects exposed to the whole sidestream smoke or to the gas phase only. Exposure to acrolein caused only slight discomfort.

Weber and colleagues (Weber, Fisher, Grandjean 1977; Weber, Fischer, Gierer et al. 1977; Weber et al. 1979b) exposed students in groups of two or three in a 30 m³ climatic chamber to whole sidestream smoke, to acrolein alone, to formaldehyde alone, or to the gas phase of smoke. Subjective irritation and annoyance as well as eye blink rate were measured. The results indicated that acrolein and formaldehyde did not produce substantial irritation or annoyance at the levels used. The gas phase exposure resulted in high levels of reported annoyance, but was less important as a determinant of irritation. The objectively measured eye blink rate, as well as subjective eye irritation, was much lower with the gas phase alone

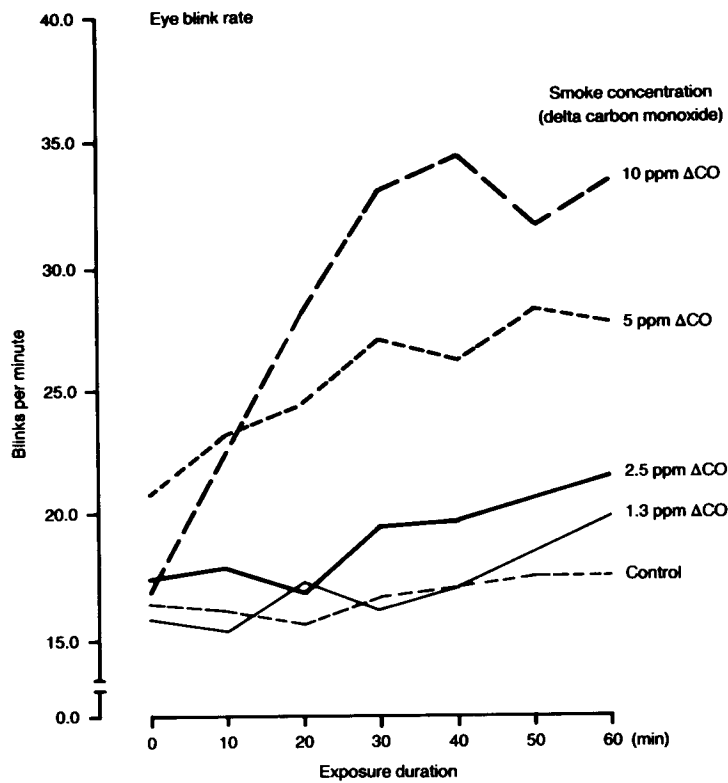


FIGURE 4.—Mean effects of environmental tobacco smoke on eye blink rate

NOTE: 32 to 43 subjects; 0 min: measurement before smoke production; 0 to 5 min: increasing smoke concentration; 6 to 60 min: constant smoke production.
 SOURCE: Muramatsu, Weber et al. (1983).

than with the total sidestream smoke, suggesting that the particulate phase is the major determinant of irritation. The researchers postulated that the irritating effects of the particulate phase are due to the semivolatile irritant compounds. These compounds, which volatilize rapidly during the process of combustion, recondense on the particulates with cooling and may deposit irritants in relatively high concentrations on the mucous membranes.

Studies of Sensitive Individuals

Children

Several investigators have used questionnaires to examine the subjective symptoms of children and young people with FTS exposure (Cameron 1972; Muramatsu 1977; Muramatsu, Muramatsu et al. 1983). The last group found that 81 percent of 13-year-old children disliked involuntary smoking and 82 percent complained of one or more kinds of irritation, the most common being eye irritation. Several epidemiological studies have shown that children with parents who smoke have an increased risk for respiratory illness (see Chapter 2).

Allergic Individuals

A few studies have assessed the effects of ETS on allergic individuals. Speer (1963) found that allergic individuals report irritation more frequently than healthy individuals. Weber and Fischer (1980) observed that employees suffering from hay fever reported significantly more eye irritation at work than those without hay fever.

Effects on the Lung

Cigarette smoking is associated with prominent changes in the numbers, types, and functions of respiratory epithelial and inflammatory cells. These alterations have been implicated in the development of pulmonary emphysema, chronic bronchitis, and respiratory tract cancers and in an increased susceptibility to infections. Chronic exposure to environmental tobacco smoke might cause similar changes. Because studies that directly address the effect of chronic exposure to environmental tobacco smoke on lung structure and biochemistry have not been conducted, this section reviews those studies in humans and animals that provide evidence on smoke exposures that may be relevant to ETS exposure.

Effects of Cigarette Smoking on Respiratory Epithelium: Studies in Humans

Extensive evidence shows that exposure to cigarette smoke has adverse effects on respiratory epithelial cells, and dose-response relationships have been established from these changes (Auerbach et al. 1961; Auerbach, Hammond, Garfinkel 1970). Studies involving the systematic examination of the bronchial mucosa from large numbers of human smokers have recorded three principal types of epithelial changes: epithelial hyperplasia, loss of cilia, and nuclear atypia. In an autopsy study of 402 adult male subjects (Auerbach et al. 1961), 98 percent of the sections of the tracheal and bronchial

**TABLE 3.--Sections with one or more epithelial changes,
by packs of cigarettes per day**

Group	Number of subjects	Number of sections	Total with one or more changes	
			Number	Percentage
Subjects without lung cancer				
Never smoked regularly	65	3,324	559	16.8
Smoked <1/2 pack/day	36	1,824	1,683	92.3
Smoked 1/2-1 pack/day	59	3,016	2,938	97.4
smoked 1-2 pack/day	143	7,062	7,021	99.4
Smoked \geq 2 pack/day	36	1,787	1,780	99.6
Subjects with lung cancer	63	2,784	2,778	99.8
Totals	402	19,797	16,759	
Average				84.7

SOURCES:Auerbach et al. (1961).

epithelium of the men who had smoked had epithelial changes. The most common abnormality observed was atypical nuclei, and a large proportion of sections had hyperplasia. Denudation of the ciliated epithelium was also present in most of those who had smoked. Other studies have observed that goblet cells were frequently increased in the airways of cigarette smokers (Regland et al. 1976; Jones 1961). The extent and severity of the abnormalities have been closely related to the intensity of smoking. A similar relationship of smoking habits to laryngeal lesions has been observed (Auerbach, Hammond, Garfinkel 1970), although the laryngeal lesions were less frequent and less advanced than those in the bronchi for a given smoking history.

The frequency and severity of epithelial lesions observed in smokers contrasts sharply with those in individuals who do not smoke regularly. In the study by Auerbach and colleagues (1961) (Table 3), 98 percent of the sections from the tracheobronchial tree from smokers contained abnormal epithelial changes; however, similar changes were observed in only 16.8 percent of the sections from nonsmokers. The most common lesion in nonsmokers was epithelial hyperplasia (9.4 percent); atypical cells were seen in only 4.8 percent of the sections from nonsmokers.

If it is assumed that the nonsmoking group included a subgroup of individuals who were chronically exposed to environmental tobacco smoke, an assumption that seems reasonable in light of the largely U.S. veteran population under consideration in the Auerbach group's study, then some information on the effect of chronic exposure to environmental tobacco smoke on the respiratory epithe-

lium can be inferred. Epithelial hyperplasia or nuclear atypia due to chronic exposure to environmental tobacco smoke may occur in some nonsmokers, but these findings are not common in the majority of nonsmokers.

Cigarette smoking also has adverse effects on the bronchial wall beneath the epithelium. Submucosal gland hypertrophy has been observed frequently (Auerbach et al. 1961; Regland et al. 1976; Jones 1981). The prevalence is related to the intensity of cigarette smoking. Mucous gland hypertrophy is seen in nonsmokers, but is not prevalent and is usually not extensive (Auerbach et al. 1961).

The loss of ciliary epithelium, the increased numbers of goblet cells, and the mucous gland hypertrophy frequently observed in cigarette smokers would predict mucociliary dysfunction. Indeed, available evidence indicates that long-term cigarette smoking impairs mucociliary transport (Wanner 1977). Once a cigarette smoker develops chronic bronchitis, mucus transport appears to be irreversibly damaged. Impairment persists even in patients who have abstained from cigarette smoking for many years (Santa Cruz et al. 1974). prior to the development of chronic bronchitis, however, partial recovery of function has been observed (Camner et al. 1973). Studies examining mucociliary dysfunction in humans due solely to chronic environmental smoke exposure have not been reported.

Effect of Cigarette Smoking on Lung Inflammatory cells

Studies in Humans

One of the earliest pathologic lesions found in the lungs of young smokers is a respiratory bronchiolitis (Anderson and Foraker 1961; McLaughlin and Tueller 1971; Niewoehner et al. 1974). Clusters of pigment-laden phagocytes, predominantly alveolar macrophages (AM), lodge in the respiratory bronchioles of cigarette smokers precisely at the sites of the earliest lung injury. The infiltration by AM precedes the development of emphysema and focal fibrosis (Cosio et al. 1978). Analyses of cells harvested by bronchoalveolar lavage complement the morphologic studies. Lavage fluid yields five to seven times more AM from the lungs of cigarette smokers than from nonsmokers' lungs (Harris et al. 1970; Reynolds and Newball 1974; Warr et al. 1976; Hunninghake et al. 1979; Hoidal et al. 1981). The alveolar macrophages from smokers appear to be activated morphologically and metabolically. The AM from smokers have increased size, endoplasmic reticulum, Golgi apparatus, glucose metabolism, hydrolytic and proteolytic enzyme activities (Pratt et al. 1971; Cohen and Cline 1971; Harris et al. 1970; Rodriguez et al. 1977; Hinman et al. 1980, Martin 1973; Cantrell et al. 1973), and increased rates of oxidative metabolism resulting in increased production of reactive oxygen species (superoxide radical, hydrogen peroxide, and hydroxyl radical) (Hoidal et al. 1981; Hoidal and Niewoehner 1982).

The strategic location of the alveolar macrophages and their altered function have led to the hypothesis that they may contribute to the alteration of the protease-antiprotease balance of the lower respiratory tract and thus foster the development of emphysema in smokers. Two plausible mechanisms have been identified by which AM may influence the proteases-antiprotease balance in cigarette smokers. The first is by directly increasing the lung protease burden. Human AM release enzymes with elastolytic activity *in vitro*, whereas those from nonsmokers do not (Rodriguez et al. 1977). The activity may originate from endogenous or exogenous sources. A metalloenzyme with activity against synthetic amide substrates, which have specificity for elastase, was detected in the bronchoalveolar washings of cigarette smokers (Janoff et al. 1983; Niederman et al. 1984) and was also found in the cell culture fluid of smokers' AM (Hinman et al. 1980). Alveolar macrophages can synthesize a metalloprotease capable of solubilizing elastin, they also contain a thiolprotease with such activity (Chapman and Stone 1984). The metalloprotease, if analogous to that of murine macrophage elastase, would be resistant to inactivation by alpha₁-protease inhibitor (α_1 PI) (Banda et al. 1980). These enzymes have not been demonstrated to cause emphysema. The content of elastolytic activity in AM at a given time is less than that of equal numbers of polymorphonuclear leukocytes (PMN); thus, AM may be only a minor source of enzymes capable of lung parenchymal destruction. However, their potential importance must be considered in light of their demonstrated ability to degrade elastin in the presence of serum protease inhibitors (Chapman and Stone 1984) and their capability of ongoing synthesis of elastolytic enzymes. Cell matrix contact may be critical for their matrix-degrading action, since the AM-derived enzymes are likely to be membrane bound.

Human AM also acquire elastolytic activity from exogenous sources. AM can bind and internalize neutrophil elastase by virtue of possessing a specific membrane receptor for this and other neutrophil glycoproteins (Campbell et al. 1979; Campbell 1982; McGowan et al. 1983). Studies to date suggest that the scavenged elastase accounts for much of the elastolytic activity in AM lysates. Sequestered PMN elastase may subsequently be released by AM over an extended period of time.

The second mechanism by which AM may influence the protease-antiprotease balance in cigarette smokers is by inactivating α_1 PI, a major antiprotease of the lower respiratory tract in humans (Gadek et al. 1981). Smokers' AM can inactivate α_1 PI through oxidant mechanisms *in vitro* (Carp and Janoff 1980). Studies on bronchoalveolar lavage fluids have identified oxidatively inactivated α_1 PI in some human smokers (Gadek et al. 1979; Carp et al. 1982), but this has not been a consistent finding (Stone et al. 1986; Boudier et al.

1983). studies that directly assess the status of α_1 PI activity in the alveolar space and interstitium of cigarette smokers are needed to clarify this issue.

The phagocytic capabilities of AM from cigarette smokers and nonsmokers are similar in most studies (Harris et al. 1970; Cohen and Cline 1971; Reynolds et al. 1975; Territo and Golde 1979), although a few studies (Martin and Warr 1977; Fisher et al. 1982) have suggested a modest decrease in the phagocytic abilities of AM from smokers. The experimental design of these studies has differed considerably, and technical factors may be responsible for the variable results. In particular, there are differences in cellular culture conditions. In view of the increased number of AM in cigarette smokers, it seems unlikely that a primary phagocytic defect of AM would account for the bacterial colonization observed in some cigarette smokers.

The possibility that increased numbers of PMN may be present in the lungs of cigarette smokers has been examined primarily because of the attention given these cells in the study of the pathogenesis of emphysema. PMN elastase is the only purified human enzyme with ready access to the lung parenchyma that has been demonstrated to cause emphysema when administered to animals. The number of PMN is increased in the distal airways and lung parenchyma of cigarette smokers. Bronchoalveolar lavage from some smokers yields increased PMN (Reynolds and Newball 1974; Hunninghake et al. 1979). More compelling evidence for increased PMN in the lungs of smokers comes from the morphologic evaluation and direct cellular analysis of the lung parenchyma. A fourfold increase in PMN infiltration has been observed in the lungs of cigarette smokers compared with the lungs of nonsmokers, using morphometric techniques (Ludwig et al. 1985). Analysis of cell suspensions from lung biopsies has also demonstrated increased PMN in the lung parenchyma of smokers (Hunninghake and Crystal 1983). The alveolar septa are the primary site of the PMN accumulation. Increased PMN are present in the alveolar walls of smokers both with and without emphysema, which suggests that other factors must also be involved in the development of the destructive lesion.

Factors that might influence the destruction of lung parenchyma by PMN elastase include the intensity of PMN influx, the amount of elastase per cell, the quantity and site of elastase released, and local factors that enhance or inhibit the elastolytic activity. Investigations of the relation of PMN elastase levels and the development of emphysema have provided discrepant results. Some studies have shown elevated levels of PMN elastase in patients with chronic obstructive pulmonary disease (Galdston et al. 1977; Rodriguez et al. 1979; Kramps et al. 1980), but others have not (Taylor and Keuppers 1977; Abboud et al. 1979). Other alterations in the PMN function of

cigarette smokers include the enhanced generation of reactive oxygen species in certain smokers (Ludwig and Hoidal 1932). After stimulation, the release of superoxide anion by PMN was 50 percent greater from smokers with peripheral white blood counts (WBC) greater than 9,000 per mm³ than from nonsmokers with similar WBC or from smokers or nonsmokers with WBC less than 9,000 per mm³. (Cigarette smokers have increased peripheral WBC counts compared with nonsmokers.)

The influence of cigarette smoking on many aspects of the immune system has been examined. Immunoglobulin (Ig) levels in the peripheral blood of smokers have been reported to be decreased (Gerrard et al. 1980, Ferson et al. 1979), but similar results have not been observed in all studies (Bell et al. 1931; Merrill et al. 1985). In contrast to the decrease of IgG in peripheral blood, cigarette smokers appear to have increased IgG levels in bronchoalveolar lavage fluid (Bell et al. 1981), primarily owing to an increase in IgG₁ (Merrill et al. 1985). Cell-mediated immunity may also be affected by cigarette smoking, but again, the results are somewhat conflicting. Peripheral blood T-lymphocytes and mitogen responsiveness have been reported to be increased (Silverman et al. 1975), unchanged (Daniele et al. 1977), or decreased (Petersen et al. 1983). Natural killer-cell activity in the peripheral blood of cigarette smokers appears decreased (Ginns et al. 1985, Ferson et al. 1979). Analysis of peripheral blood lymphocyte populations by monoclonal antibodies has demonstrated increased T-lymphocytes (OKT3+), with a decreased proportion of OKT4 + (helper/inducer), and an increased proportion of OKT8₊ (suppressor/cytotoxic) subsets in smokers with greater than 50 pack-years of smoking (Miller et al. 1982). Analysis of bronchoalveolar lavage fluid from cigarette smokers with a mean smoking history of 14 - 9 pack-years demonstrated a decreased proportion of OKT4₊ lymphocytes and an increased proportion of OKT8₊ lymphocytes (Costabel et al. 1986). In the latter study, the alterations in T-lymphocyte subsets observed in bronchoalveolar lavage were not present in peripheral blood. This finding and the increase in IgG in bronchoalveolar lavage fluid, but not in serum, raise the possibility of regional effects of cigarette smoking on the immune system.

The extent to which the alterations of inflammatory cell numbers and functions observed in smokers are also present in individuals who are chronically exposed to environmental tobacco smoke remains unknown. Studies in humans have not directly addressed this issue. Studies of dose-response relationships are absent, except for those cited that document a relationship of peripheral white blood cell count and lymphocyte T-cell subsets. If it is assumed that a subgroup of nonsmokers is composed of individuals who are chronically exposed to environmental tobacco smoke, then some inferences

are possible. As has been stated, the most common pathologic feature in the lungs of young cigarette smokers is an accumulation of pigment-laden macrophages in the respiratory bronchioles. In the study by Niewoehner and colleagues (1974), all 19 male cigarette smokers who died suddenly elsewhere than in a hospital had such lesions, which were present in all sections studied in 16 of the 19 subjects. In contrast, only 5 of 20 nonsmokers had similar lesions, and they were minimal in all but 2. One of the two individuals was a stoker in a foundry and the other was undergoing desensitization for severe hay fever. Although the inflammatory cell accumulation cannot be absolutely attributed to these extenuating circumstances, it is clear that the respiratory bronchiolitis is not common in young, healthy individuals who do not smoke regularly. In contrast, autopsy studies have observed focal inflammatory changes quite frequently in older subjects who had not smoked, but the lesions were of much less severity than in age-matched subjects who had smoked (Cosio et al. 1978). Similar changes have not been observed in studies on bronchoalveolar lavage fluids. The metabolic activation of the AM from younger and older nonsmokers is similar (Hoidal and Niewoehner 1982). These findings suggest that the characteristic inflammatory lesions seen in the lungs of smokers are usually absent or are modest in those individuals who do not smoke cigarettes and who are not exposed to an alternative inciting agent.

Experimental Models

The effect of cigarette smoke inhalation on lung inflammation and inflammatory cell function has been extensively studied in experimental animal models; however, studies have not investigated inflammatory cell alterations in models intended to simulate chronic environmental tobacco smoke exposure. Several studies have demonstrated that chronic cigarette smoke exposure produces an accumulation of AM within the respiratory bronchioles of many animal species, including dogs (Hernandez et al. 1966, Frasca et al. 1971, 1983; Park et al. 1977), rats (Kendrick et al. 1976; Coggins et al. 1980; Huber et al. 1981), hamsters (Bernfeld et al. 1979; Hoidal and Niewoehner 1982), and mice (Matulionis and Traurig 1977), that is strikingly similar to that seen in human smokers. In most studies, the accumulation of AM has been dependent on the duration and intensity of the smoke exposure (Hoidal and Niewoehner 1982; Huber et al. 1981). Increases in lysosomal enzyme activities have been observed in rats (Etherton et al. 1979) and mice (Matulionis and Traurig 1977) following tobacco smoke exposure. Increased elastase secretion by alveolar macrophages from mice chronically exposed to cigarette smoke has also been observed (White et al. 1979). Oxygen consumption, superoxide anion release, hydrogen peroxide production, and hexose monophosphate shunt activity were reported to be

increased in AM harvested by bronchoalveolar lavage from hamsters (Hoidal and Niewoehner 1982) and rats (Drath et al. 1978; Huber et al. 1981) chronically exposed to tobacco smoke. Accumulation of PMN in the alveolar septa of cigarette smoke-exposed hamsters, strikingly similar to that observed in human smokers, has also been reported (Ludwig et al. 1985). In contrast to the focal nature of the AM accumulation, the accumulation of PMN was diffuse. Studies of PMN function have not been systematically evaluated in smoke-exposed animals. One distinctive feature in rats has been a lymphocytic periairway infiltration (Innes et al. 1956, Huber et al. 1981). Similar alterations are not seen in humans. The lymphocytic infiltration may be due to complicating respiratory infections with mycoplasma or a respiratory virus, which have been common in rats.

Effects of Cigarette Smoking on Lung Parenchyma: Studies in Humans

The most striking alteration of the lung parenchyma associated with cigarette smoking is centrilobular emphysema. The relationships between smoking history, age, and the degree of emphysema have been examined. The effect of smoking on the development of emphysema is believed to be cumulative (Anderson et al. 1972; Auerbach et al. 1974). In a study of 1,824 autopsies from individuals who had died in the hospital, Auerbach and associates, using a semiquantitative scoring system, detected emphysematous lesions in all individuals who had smoked two or more packs of cigarettes per day, including 111 who had been under 60 years of age at the time of death. The extent of emphysema strongly correlated with the number of cigarettes smoked per day. However, some emphysematous changes, usually of a mild degree, were noted in 94 percent of the individuals who had regularly smoked less than one-half pack per day. In contrast, no emphysema was detected in 95 percent of the 175 individuals who had not smoked regularly, and only one case of emphysema of moderate severity had occurred in a person who had not smoked. These findings suggest that emphysema is rare in individuals who do not smoke regularly and do not have a genetic predisposition for the disease.

Summary of Lung Effects

Substantial evidence documents that active cigarette smoking produces adverse effects on respiratory epithelial cells and causes lung inflammation and alveolar septal disruption. Whether these effects occur following chronic exposure to environmental tobacco smoke cannot be definitively answered by the fragmentary data now available. It is possible that clinically significant pulmonary consequences of chronic exposure to environmental tobacco smoke in adults might occur only when this exposure interacts with other

factors in particularly susceptible individuals. In this regard, future studies directed at selected high-risk populations or animal models incorporating exposure to environmental tobacco smoke along with other exposures might be the most fruitful areas of investigation into the effects of chronic exposure to environmental tobacco smoke.

Carcinogenicity of Environmental Tobacco Smoke

This section reviews some of the more widely employed methods of evaluation of the carcinogenicity of mainstream smoke that may also be extended to the evaluation of ETS. The similarities, differences, and technical difficulties in employing these various bioassays with MS, smoke condensate, and ETS are discussed.

Inhalation Experiments

Because inhalation is the primary mode of exposure for both active and involuntary smoking, animal inhalational assays would appear to be the ideal approach to developing an animal system for carcinogenicity testing. However, the acute toxicity (mainly due to carbon monoxide and nicotine) have limited the exposures to whole smoke that can be tolerated by laboratory animals.

Two types of passive exposure systems offer the primary approaches to inhalation studies with small laboratory animals. These systems provide either the forced exposure of the whole body to tobacco smoke or exposure of the head only. The amount of smoke that is retained in the lower respiratory tract of the animals is the dosage variable of interest in assessing these studies. The particulate matter content of whole smoke is probably of greater importance than the vapor phase content (Wynder and Hoffmann 1967; Davis et al. 1975) for studies of carcinogenesis. Labeled particulate phase components have been used for determining the deposition of the particulate phase in the respiratory tract in smoke inhalation studies (Mohr and Reznik 1978). However, since such markers are applied to the tobacco column, they may be partially volatilized during smoking. Thus, some of the values reported in deposition studies of inhaled smoke aerosols in mice, rats, and hamsters reflect the deposition of the trapped particulate phase plus the gas phase of cigarette smoke in the respiratory tract. A less ambiguous tracer is decachlorobiphenyl (DCBP). It is added to the tobacco column of cigarettes, and after exposure of the animals to the smoke of the treated cigarettes, this tracer can be determined in extracts of various segments of the respiratory tract by gas chromatography with an electron capture detector (GC-ECD). The detection limit of DCBP is $< 5 \times 10^{-11}$ g (Lewis et al. 1973; Hoffmann et al. 1979). Using these techniques, only a small percentage of the smoke particulates of cigarette mainstream smoke can be shown to reach regions in the

lower respiratory tract of small laboratory animals. This may explain, at least in part, why the lifetime inhalation exposures of small animals to tobacco smoke have led only to limited numbers of lung tumors.

In mice, inhalation assays with cigarette smoke have generally led to hyperplasia and metaplasia in the trachea and bronchi of the animals (Wynder and Hoffmann 1967; Mohr and Reznik 1978). In one of the most extensive studies, the Leuchtenbergers (1970) induced pulmonary adenoma and adenocarcinoma in Snell's mice. However, only the gas phase, not the total smoke, induced a statistically significant number of lung tumors.

In another inhalation bioassay, male and female C57Bl mice (100 in each group) were exposed, nose only, to fresh mainstream smoke diluted with air (1:39) for 12 minutes every other day for the duration of their lives. Four lung tumors were detected in both the treated male mice and the treated female mice. No lung tumors were found among controls. A similar experimental design was used to examine the possible differences between the smoke of flu-cured Bright tobacco cigarettes and the smoke of air-cured Bright tobacco cigarette (Harris et al. 1974). Female Wistar rats (408 animals) were exposed, nose only, to a 1:5 smoke-to-air mixture for 15 seconds of every minute during an 11-minute exposure twice a day, 5 days per week, for the lifespan of the animals. Three of the rats exposed to cigarette smoke developed pulmonary squamous neoplasms of uncertain malignancy and one animal had an invasive squamous-cell carcinoma of the lung. No tumors were found in the 104 sham-control animals or in the 104 untreated female rats (Davis et al. 1975).

Fischer-344 rats (80 animals) were exposed, nose only, to a 1:10 smoke-to-air mixture for approximately 30 seconds of every minute that a cigarette was being smoked (Dalbey et al. 1980). In this manner, the animals were exposed to the smoke of one cigarette per hour, 7 hours per day, 5 days per week, for 128 weeks. The mean pulmonary particulate deposition during the smoke-aerosol exposure was 0.25 mg per cigarette, or 1.75 mg per rat per day. Ten respiratory tumors were observed in seven smoke-exposed rats. One alveologenic carcinoma and two adenomatoid lesions were observed in 3 of the 93 control rats employed in this study. A similar protocol was used to evaluate the effects of the inhalation of the smoke of cigarettes with varying tar deliveries. In this study (Wehner et al. 1981), squamous metaplasia of the laryngeal and tracheal epithelium was significantly increased in the smoke-exposed Fischer-344 rats.

Syrian golden hamsters (80 males and 80 females) were exposed, nose only, to a 1:7 smoke-to-air mixture for 10 to 30 minutes, 5 days per week, for a period no longer than 52 weeks. The incidence of

laryngeal leukoplakias ranged from 11.3 percent for the animal receiving the low dose to 30.6 percent for those animals receiving the highest dose of cigarette smoke. Such changes were not observed in the controls or in the hamsters exposed to the gas phase only (Dontenwill 1974). Exposing 102 male BIO 87.20 and BIO 15.16 hamsters nose only, twice a day, 5 days a week, for up to 100 weeks, resulted in almost 90 percent of the animals having hyperplastic or neoplastic changes in the larynx (Bernfeld et al. 1974). Laryngeal cancer was five times more frequent in the BIO 15.16 strain. Two animals in this strain also developed nasopharyngeal tumors. Another study using nose-only exposures and similar extents of exposure reported similar changes in the larynx of the smoke-exposed animals (Wehner et al. 1974). Increasing the exposure duration to the lifespan of the animals resulted in the development of squamous papilloma of the larynx.

Thirty rabbits in an inhalation chamber were exposed to the smoke generated from 20 cigarettes for up to 5 1/2 years. Thirty-one animals were used as controls. No tumors were found among the treated animals that could be related to the exposure to cigarette smoke (Holland et al. 1963).

Eighty-six beagle dogs, trained to inhale cigarette smoke through tracheostomata, were actively exposed to smoke from either filter or nonfilter cigarettes (Auerbach, Hammond, Kirman et al. 1970). Tumors of the lung were reported in 23 of the 62 dogs exposed to smoke from the nonfilter cigarettes. Two of the dogs in this group had small bronchial carcinomas. Noninvasive bronchioalveolar tumors were reported in 4 of the 12 dogs exposed to the smoke of filter cigarettes and in 2 of the 8 control dogs. The bronchioalveolar tumors tended to be multiple, with as many as 20 per lung, and were reported in 40 of the 203 lung lobes in the 29 dogs with such tumors.

Inhalation studies with SS or ETS have not been reported thus far with any of the laboratory animal inhalational assays. This lack of experiments has in large part been due to the absence of exposure devices that allow the appropriate delivery of the inhalant without incurring the loss of the test animals due to the toxicity of carbon monoxide and nicotine.

Other In Vivo Bioassays

Among alternative methods used to assess the relative carcinogenicity of mainstream cigarette smoke, the most widely utilized test is to collect the cigarette smoke condensate (CSC) and to bioassay this material for carcinogenicity. In the process of preparing CSC, many of the volatile and semivolatile components are lost. Furthermore, there are serious concerns regarding the influence of aging of the CSC, which can affect both the chemical composition and the biological activity. Despite these shortcomings, bioassays using CSC

have provided insight into mechanisms by which tumor induction in animal tissues is likely to occur. The application of CSC to mouse skin has helped to identify those agents that are active as tumor initiators and has shown that within the CSC subfractions are components that can act as tumor promoters or cocarcinogens, respectively. Thus, this approach allows the comparison of various condensates, especially when large groups of animals are used (>50 per group).

The application of CSC to mouse skin is the most widely employed assay for the evaluation of its carcinogenic potential. The mouse skin bioassays in tobacco carcinogenesis have been reviewed (Hoffmann, Wynder et al. 1983). A typical experiment uses two to three dose levels of condensate, generally 25, 50, and 75 mg of CSC, which are administered topically to the shaved backs of mice three to six times weekly for approximately 78 weeks. The CSC is most frequently applied as an acetone suspension (25, 33, or 50 percent). At the conclusion of such a study, skin tumors, some of which are malignant, generally are observed among the treated animals in a dose-related fashion. Such studies have shown that the carcinogenic activity of CSC is also a function of tobacco variety, is influenced by replacement materials such as tobacco sheet or semisynthetics, and may be influenced by the use of additives. Although such bioassays have been extensively performed for the tars from mainstream cigarette smoke, only one study has examined the carcinogenic potential of the condensate of sidestream cigarette smoke.

Cigarette tar from the sidestream smoke of nonfilter cigarettes that had settled on the funnel covering a multiple-unit smoking machine was suspended in acetone and applied to mouse skin for 15 months (Wynder and Hoffmann 1967). Out of a group of 30 Swiss-ICR mice, 14 animals developed benign skin tumors and 3 animals had carcinomas. In a parallel assay of MS from the same cigarettes, a 50 percent CSC:acetone suspension applied to deliver a comparable dose of CSC to 100 Swiss-ICR female mice led to benign skin tumors in 24 mice and to malignant skin tumors in 6 mice. This indicates that this smoke condensate of SS had greater tumorigenicity on mouse skin than MS tar ($p > 0.05$).

In Vitro Assays

Several short-term bioassays have been performed to evaluate the genotoxicity of the MS of cigarettes. These studies have been the subject of two reviews (DeMarini 1983; Obe et al. 1984). Although most of these studies have evaluated the effects of CSC, some investigations were focused on either the gas phase or the whole smoke. In recent years, there has been increased use of short-term assays to attempt to evaluate the relative genotoxic potential of environmental tobacco smoke.

The most commonly employed assay for mutagenic activity is done with various strains of *Salmonella typhimurium*. Whole smoke as well as CSC from four types of tobacco were found to be mutagenic in *S. typhimurium* TA1538 (Basrur et al. 1978). Sidestream smoke was also found to be mutagenic in a system where the smoke was tested directly on the bacterial plates (Ong et al. 1984). These studies lend support to the extensive assays performed with CSC that establish that tobacco smoke has significant mutagenic potential.

Several of the studies with CSC from mainstream smoke have been aimed at comparing the effects of various tobaccos, various tester strains, and various systems selected for metabolic activation. Most of the mutagenic activity was associated with the basic fraction of CSC (DeMarini 1983). For the CSC from mainstream smoke, mutagenic activity was primarily detected with the strains TA1538 and TA98, thus indicating the presence of the frame-shift type of mutagens. Except for studies on the effects of nitrate-treated cigarettes, metabolic activation was required to demonstrate mutagenic activity for most of the CSC studied.

Several short-term tests have been performed in eukaryotic systems. A solution of the gas phase of mainstream cigarette smoke dissolved in a phosphate buffer induced reciprocal mitotic recombination in *Saccharomyces cerevisiae* D3 and petite mutants in an isolate of strain D3 (Ixard et al. 1980). Whole mainstream cigarette smoke induced mitotic gene conversion, reverse mutation, and reciprocal mitotic recombination in strain D7 of *S. cerevisiae* (Gairola 1982).

Transformation of mammalian cells was also induced in several cell systems using the CSC from mainstream cigarette smoke (Lasnitzki 1968, Inui and Takayama 1971; Rhim and Huebner 1973; Benedict et al. 1975; Takayama et al. 1978; Rivedal and Sanner 1980). Transplacental exposure to mainstream CSC was reported to transform Syrian hamster foetal cells (Rasmussen et al. 1981). Transforming activity was reported in the acidic and basic fractions as well as the neutral fractions of CSC. Studies on subfractions of CSC have shown that the basic fraction and some of the acidic fractions are the most active in cell transformation (Benedict et al. 1975). The neutral fraction of CSC was also reported to inhibit DNA repair in normal human lymphocytes (Gaudin et al. 1972). Transformation of mammalian cells with SS or ETS has not been reported.

Summary of Carcinogenicity

At present, the scientific literature offers some information on the physicochemical nature of the sidestream smoke from tobacco products and of environmental tobacco smoke. Chemical analytical studies have already demonstrated that SS and ETS contain a wide spectrum of carcinogens such as polynuclear aromatic hydrocarbons,

volatile and tobacco-specific N-nitrosamines, and polonium-210. To date, only one study has demonstrated the carcinogenic activity of the particulate matter of sidestream smoke and a few isolated reports have dealt with the genotoxicity of SS and ETS. Therefore, bioassay studies with the mainstream smoke and the environmental tobacco smoke of cigarettes are needed. Although the resulting bioassay data will derive from tests of concentrations of environmental smoke that do not realistically occur in the human setting, these results will provide information about the relative carcinogenic potential of sidestream smoke in comparison with the mainstream smoke of the same cigarettes. In a comprehensive analytical approach, data should be generated to systematically determine the concentrations of toxic and tumorigenic agents in the ETS samples and to simultaneously measure the uptake of tobacco-specific agents by the body fluids of nonsmokers exposed to ETS.

Conclusions

1. The main effects of the irritants present in occur in the conjunctiva of the eyes and the mucous membranes of the nose, throat, and lower respiratory tract. These irritant effects are a frequent cause of complaints about poor air quality due to environmental tobacco smoke.
2. Active cigarette smoking is associated with prominent changes in the number, type, and function of respiratory epithelial and inflammatory cells; the potential for environmental tobacco smoke exposure to produce similar changes should be investigated.
3. Animal models have demonstrated the carcinogenicity of cigarette smoke, and the limited data that exist suggest that more carcinogenic activity per milligram of cigarette smoke concentrate may be contained in sidestream smoke than in mainstream cigarette smoke.

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