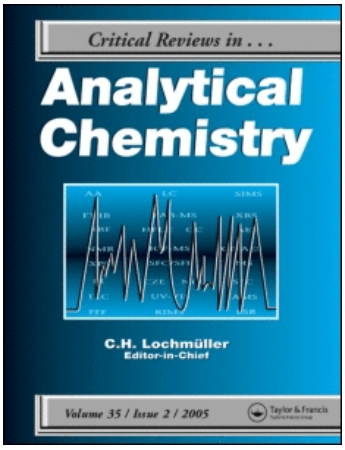

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ANALYSIS FOR AEROTOXICANTS

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I. INTRODUCTION

Increased knowledge of the cellular, viral, and chemical composition of outdoor, enclosed, and "concentrated" atmospheres in urban, farm, industrial, and wilderness areas in terms of aerotoxicants is necessary for a better understanding and control of the irritant and toxic effects of air pollutants on human beings and other life forms. We will discuss these various pollutants briefly (see Table 1 and 2) but will concern ourselves mainly with the chemical aerotoxicants. We will consider five conglomerate classes of chemical aerotoxicants, e.g., carcinogens, allergens, alkylating agents, lachrymators, and phytotoxicants. Each class has air pollutant satellites which have some form of sensitizing, enhancing, or antagonizing effect on the physiological properties of the main family of toxicants or have some analytical usefulness in furthering an understanding of the physiological effect of the toxicants.

With the aid of good analytical methods knowledge can be assembled on the synergistic effects of environmental (water, food, and contact) pollutants with "nature-derived" and combustion-derived air pollutants. We define "nature-derived" pollutants as those pollutants stemming from the undisturbed natural wilderness and from the ecologically disturbed outdoor nonurban and wilderness areas. We consider indoor air pollution to be a very important type of pollution since the average human being in the American industrialized society spends more time indoors than out. The composition of this relatively uninvestigated atmosphere should be investigated. Only in this fashion will we be able to understand the interplay of the various biological effects of combustion products, biological residue dusts, and airborne cellular and viral entities on the human being in his home, work, and recreation areas. It is in the enclosed atmospheres that bacterial, fungal, and viral aerotoxicants are usually found. In this type of atmosphere higher concentrations of house-dust allergens and other types of aerotoxicants create their havoc.

Some phytotoxicants are briefly described in Table 2. Their assay will be considered in a later section of the paper.

I would also like to mention briefly the diseases of commercial and domestic animals which can be attributed to airborne aerosols.[69a] These include

TABLE 1

Potential Human Aerotoxicants

Aerotoxicant	Physiological Effect
Bacteria[a] [1-4]	
Bacillus anthracis	Pulmonary anthrax
Bordetella pertussis	Whooping cough
Corynebacterium diphtheriae	Diphtheria
Diplococcus pneumoniae	Pneumococcal pneumonia
Klebsiella pneumoniae	Klebsiella pulmonary infection
Mycobacterium tuberculosis	Pulmonary tuberculosis
Neisseria meningitidis	Meningococcal infection
Pasteurella pestis	Pneumonic plague
Staphylococcus aureus	Staphylococcal wound and respiratory infections
Streptococcus pyogenes	Streptococcal respiratory infection
Fungi[a] [1-4]	
Aspergillosis fumigatus	Aspergillosis
Blastomyces dermatitidis	Blastomycosis
Coccidioides immitis	Coccidioidomycosis
Cryptococcus neoformans	Cryptococcosis
Histoplasma capsulatum	Histoplasmosis
Nocardia asteroides	Nocardiosis
Sporotichum schenckii	Sporotrichosis
Aerotoxicant	**Physiological Effect**
Virus[a, b] [1-7]	
Adenoviruses	b, bl, cc, fst, p.[c]
Chlamydozoaceceae[d] – psittacosis	p, ps
Herpesviruses – herpes	pharyngitis (adults)
Mycoplasmataceae – mycoplasma pneumoniae	b, bl, mrs, p
Myxoviruses – Influenza A, B, and C	b, bl, c, cc, i, p, t
Respiratory syncytial (RS)	b, bl (infants), c, cc, p
Parainfluenza	b, bl, c (infants), cc, p
Picornaviruses – Coxsackie A	cc, fst
Coxsackie B	cc, fst, pl
Rhinoviruses	b, cc, p
ECHO	c, cc, fst
Reoviruses – ECHO-10	d, mrs (children)
Rickettsiae – Coxiella burneti (Q fever)	p
Carcinogen Family[8-12]	
Benzene	Leukemia[13]
Methylpyrenes	Cancer in animals
Methylchrysenes	Cancer in animals
Benz[a]anthracene	Cancer in animals
Methylbenz[a]acridines	Cancer in animals
Methylbenz[c]acridines	Cancer in animals

TABLE 1 (Continued)

Aerotoxicant	Physiological Effect
Carcinogen Family[8-12]	
Benzo[b]fluoranthene	Cancer in animals
Benzo[j]fluoranthene	Cancer in animals
Benzo[a]pyrene	Cancer in animals, human cancer?[14]
Dibenz[a,h]acridine	Cancer in animals
Dibenz[a,j]acridine	Cancer in animals
Arsenic	Human cancer?[8,15]

a Pathogens recognized as causing human respiratory diseases; they are essentially limited to indoor spaces or too closely confined outdoor spaces.

b Airborne transmission is partially involved in chickenpox, German measles, measles, mumps, shingles, and smallpox.

c b=bronchitis, bl=bronchiolitis, c=croup, cc=common cold, d=diarrhea, fst=febrile sore throat, i=influenza, mrs=minor respiratory symptoms, p=pneumonia, pl=pleurodynia, ps=psittacosis, and t=tonsillitis.

d Nucleic acid core contains both RNA and DNA.

e Two types of promotion may be involved here. In the case of asbestos evidence is presented in support of the hypothesis that, in the induction of asbestos cancer, trace metals play the active role of increasing the residence time of the hydrocarbon in the lung; asbestos, a passive role as a metal carrier; and BaP (or related polycyclic arenes) derived from the environment, a critical mediating role. The initiation-promotion type may be involved in the experiments where *n*-dodecane causes a thousandfold increase in the enhancement of potency of low concentrations of BaP and benz(a)anthracene.[22b]

f Increased particle retention in respiratory tissues due to interference with ciliary activity and the flow of the mucous stream.

g There is some question about the allergic reaction of some of these dusts, although there is no doubt about their adverse effect on human beings.

h A billion tons released per year by vegetation over the surface of the earth. Present in air at concentrations of 2 to 20 ppb. Terpenes postulated as having something to do with asthmatic attacks.

i Belief that essential difference in mucosa of atopic individuals is the increased permeability to inhaled allergens.[41]

j Asthmatic attack rate varied with level of SO_2.

k Asthmatic attacks increased in Los Angeles area when oxidant level greater than 0.25 ppm.

l Many of these compounds are carcinogenic.

m 1 ppm produces eye irritation.[54]

n Two hundred times as potent as formaldehyde.[55]

Aerotoxicant	Physiological Effect
Carcinogen Family[8-12]	
Chromium	Human cancer?[8,15]
Beryllium	Cancer in animals[8,15]
Nickel	Human cancer?[8,15]
Cadmium	Human prostatic carcinoma?[16]
Selenium	Cancer in animals[17]
Pesticides	Animal tumors[8]
Asbestos and trace metals	Animal tumor-promoter[18,18a,18b]
Sulfur dioxide	Animal tumor-promoter (BAP)[19]
Phenols	Animal tumor-promoter[20-22]
Polyphenols	Animal tumor-promoter[20-23,23a]
Phorbol esters	Animal tumor-promoter[20,20a]
Long chain alkanes	Animal tumor-promoter[22,22a,22b,e]
Tobacco smoke	Animal tumor-promoter[22c]
Acetylperoxide, benzene, formaldehyde, formic acid, 2-methyl-2-butene, 2-methyl-pentane, peracetic acid, propylene oxide, etc.	Animal respiratory irritants[f 24,25]
Chrysene, dibenz[a,c]anthracene benz[a]anthracene + 6-methyl-anthanthrene	Animal tumor-initiators[20,20a]
Closely related compounds	Anticarcinogens[25a]
Quinaldine and isoquinoline	Synergistic effect on BAP[25b]
Ozone	Pulmonary adenomas increased in sensitive mice[25c]

Allergen Family[26]

Hayfever-causing Pollens	Relative Importance
Short ragweed (Ambrosia elatior)	+4
Giant ragweed (Ambrosia trifida)	+4
Western ragweed (Ambrosia psilostachya)	+2
Southern ragweed (Ambrosia bidentata)	+2
False ragweed (Franseria acanthicarpa)	+1
Perennial ragweed (Ambrosia coronopifolia)	+1
Perennial slender ragweed (Franseria confertiflora)	+1
Cocklebur (Xanthium commune et sp)	+2
Marsh elder (Iva ciliata)	+1
Burweed marsh elder (Iva xanthifolia)	+2
Annual sage (Artemisia annua)	+1
Biennial sage (Artemisia biennis)	+1
Prairie sage (Artemisia ludoviciana)	+1
Sagebrush (Artemisia tridentata)	+1
Pigweed (redroot) (Amarantnus retroflexus)	2
Spiny amaranth (Amaranthus spinosus)	1
Western water hemp (Acnida tamariscina)	2
Lamb's quarters (Chenopodium album)	2
Firebush (Kochia scoparia)	2
Russian thistle (Salsola pestifer)	2
Bermuda grass (Capriola dactylon)	3
Bluegrass (Poa pratensis)	4
Orchard grass (Dactylis glomerata)	4
Redtop (Agrostis palustris)	3
Timothy (Phleum pratense)	4
English plantain (Plantago lanceolata)	2
Hemp (Cannabis sativa)	1
Red sorrel (Rumex acetosella)	2

TABLE 1 (Continued)

Fungi[27-30]

Alternaria	Penicillium
Aspergillus	Phoma
Asterosporium	Scopulariopsis
Chaetomium	Stemphyllium
Cladosporium	Trichoderma
Curvularia	Trichophyton
Cytospora	Tricothecium
Epicoccum	
Fusarium	
Helicoma	
Helminthosporium	
Hormodendrum	
Libertella	
Monilia	
Mucor	

Algae[30a,b,c,d]

Hormidium
Bracteacoccus
Tetracystis A
Tetracystis 1

Vegetable Dusts[g 26,31-34]

	Disease
Acacia	Allergic
Karaya	Allergic
Tragacanth	Allergic
Cotton seed	Allergic
Flaxseed	Allergic
Castor bean	Allergic
Organic	Mill fever
Cotton	Bysinosis
Moldy sugar cane	Bagassosis
Grain	Grain asthma
Tarimand seed	Tarimand asthma
Moldy cotton yarn	Weaver's cough
Moldy hay, silage	Farmer's lung
Hemp dust	Cannabosis

Miscellaneous Allergens[26,35-37]

House dust, old feathers and old cotton fibers
Animal danders, insect debris,[38] kapok

Coallergens
Enhancers and Sensitizers

Terpenes[h 39]
Permeability enhancing factor[i] from ragweed and rye pollen[40]
Sulfur dioxide[42,43 j]
Nitrogen oxides, particulates, temperature, humidity[44]
Oxidants[k 44,45]
High barometric pressure[43]
Ozone[44]

Pre-allergens

Polyhydroxyaldehydes?
Polypeptides or proteins containing the free ϵ-amino group of the lysine moieties

Mutagens

Atmospheric alkylating agents?[46,47]
Epoxides, lactones, and peroxy compounds?[48-501]

Lachrymators[51-53]

Formaldehyde
Acrolein
Peroxyacetyl nitrate[m]
Peroxypropionyl nitrate
Peroxybenzoyl nitrate[n]

TABLE 2

Phytotoxicants[56,57]

Ethylene[58]
Fluoride[59,60]
Nitrogen dioxide[61]
Ozone[a] [62-64]
Peroxyacetyl nitrate[65,66b,67b]
Particulates
Sulfur dioxide[68,69]
Sulfuric acid[69]

a Chlorotic needle mottle has been produced in ponderosa pines after exposure to synthetic ozone at 0.3 ppm for 8 hr/day over 2 to 3 weeks.

b Arguments for and against the phytotoxicity of PAN.

the bacterial diseases, tuberculosis (Mycobacterium bovis) and glanders (Actinobacillus (Malleomyces)Mallei), fungal diseases such as Aspergillosis, Crytococcosis, and Coccidordomycosis, and viral diseases, such as hog cholera, equine influenza, swine influenza, feline distemper, canine distemper, New Castle disease, and infectious bronchitis.

When we think of chemical air pollution we think of the nitrogen oxides, sulfur oxides, ozone, benzo[a]pyrene, auto exhaust, smog, and cigarette smoke. There is much controversy about the toxicities of these various pollutants. However, there is one other type of air pollution that unequivocally does cause definite physiological effects on human beings and that does cause misery and suffering to millions of human beings, and this is pollution by aeroallergens. There is no such uncertainty about their toxic effects on humans, such as we have with the usual atmospheric concentrations of nitrogen oxides, sulfur oxides, ozone, benzo[a]pyrene, auto exhaust, and smog. Aeroallergens do cause misery and suffering, and it is possible that one of the main toxic effects of sulfur dioxide, ozone, etc. on humans is to enhance the adverse effects of allergens.

One other type of ubiquitous pollution should be considered. This is the pollution in the microatmosphere that encompasses the individual like a thick gaseous skin. The human skin, which is boundary layer between man and his environment, is separated from the ambient air by the microatmosphere. This layer of convecting air can contain a thick pall of fumes of cigarette or cigar smoke, pollens, fungi, miscellaneous emitted chemicals, and microorganisms. Under crowded or other adverse conditions even this type of pollution could become a problem, especially if there is a synergistic effect of the various types of pollutants on the skin, derived from the microatmosphere and from the external environment.

The complexity of the situation and the vital necessity for adequate analytical techniques may be emphasized by saying that a member of one class of chemical toxicants can have some form of synergistic or antagonistic effect on a member of another class. In the same way it is possible for the cellular, viral, and molecular aerotoxicants to have synergistic and antagonistic effects on each other.

As flu epidemics increase in frequency and severity and as ragweed and other harmful plants spread throughout the suburbs and farmlands, one wonders if these changes are accelerated by increasing amounts of some particular chemicals in the atmospheric miasma. Because of this complexity and the necessity of understanding it I shall discuss not only the analytical techniques for aerotoxicants but also the methods of analysis for the satellite pollutants. Stress will be placed on the necessity for identifying the many unknown aerotoxicants present in our indoor and outdoor atmospheric environments, on the lack of good analytical techniques for many aerotoxicants, on the shortcomings of many of the present methods, and on a critical discussion of the best available methods.

Due to space and time considerations a cutoff point had to be established. Analytical methodology for some of the possibly toxic air pollutants has been omitted. Some of these are the salts of metals, such as arsenic, beryllium, cadmium, chromium, mercury, nickel, selenium, and vanadium, and organometallic derivatives of lead and mercury. Other compounds which are not discussed include the nitrogen oxides and carbon monoxide, although the last compound can be deadly in high concentrations in an enclosed atmosphere.

II. CARCINOGENS AND ALLIED COMPOUNDS

Families of compounds of importance in this section include carcinogens, cocarcinogens, anticarcinogens, and irritants.

A. Carcinogens

The variety and concentrations of the common air pollutants have been reported.[12,71] A discussion of the larger organic air pollutants with stress on the variety and concentrations of the airborne carcinogens is available.[12] The families of aerocarcinogens include arenes, aza arenes, and imino arenes, many of which are enumerated in Table 1.

1. Arenes

Methods for the separation and determination of polynuclear aromatic hydrocarbons present in the human environment have been reviewed through 1962.[74] Of the general tests available for these compounds the piperonal test is the best known.[75,76] The reagent reacts readily with all aromatic molecules that have their highest electron density on a conjugated carbon atom and that are more basic than benzene. The procedure has been correlated with the atmospheric concentration of benzo[a]pyrene.[77] Polynuclear aromatic hydrocarbons react very well in the procedure. Highly colored diarylcarbonium cations are formed. Wavelengths of maximum absorption for the hydrocarbon-derived products range from 522 to 768 nm. Other sensitive aromatic aldehyde reagents that have been recommended for the determination of polynuclear aromatic hydrocarbons include furfural, 2-thenaldehyde, indole-3-aldehyde, 9-anthraldehyde and 3-nitro-4-dimethylaminobenzaldehyde.[78] These yield reaction products having molar absorptivities that range from 7500 to 75000. With 9-anthraldehyde polynuclear arenes gave chromogens absorbing between 790 and 965 nm; the type of chromogen formed is shown in the reaction with fluoranthene (Figure 1).

However, most analytical methods for the hydrocarbons involve separation followed by fluorescence or ultraviolet absorption methods of analysis. The former method has the advantage of sensitivity and the latter of selectivity due to the fine structure of the absorption bands.

Another general test which has been thought to give some measure of pollution by arenes is the evaluation of the "anthracene pollution index."[79] This is done by passing 100 l. of polluted air through 50 ml of cyclohexane, then diluting to 100 ml with cyclohexane, adding 10 ml of water, centrifuging to eliminate insoluble dust, and taking a reading of the cyclohexane layer at F 386.5/480.

A somewhat similar general method involves the measurement of the fluorescence intensity of the cyclohexane extract of airborne particulates at F 365/480. This index of fluorescence has been related to the concentrations of BaP (benzo[a] pyrene) and polynuclear aromatic hydrocarbons.[80]

A variety of chromatographic methods has been used in the analysis of the polynuclear aromatic hydrocarbons present in the atmosphere and in air pollution source effluents. These are summarized in Table 3, which also lists some of the air and air pollution source samples which have been analyzed for polynuclear arenes. The most popular solvents for the extraction of polynuclear arenes

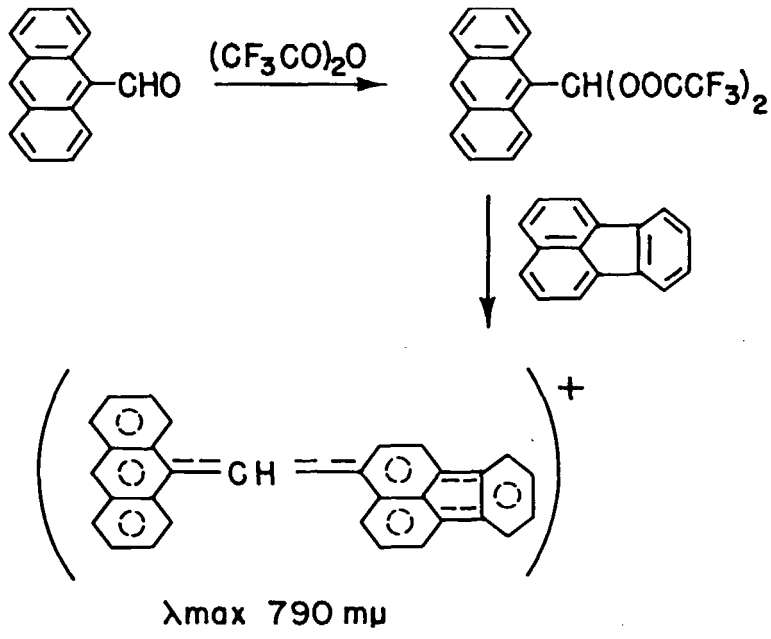


FIGURE 1. Colorimetric determination of fluoranthene with 9-anthraldehyde.

from airborne particulates have been benzene and cyclohexane. Although benzene is the more efficient extraction solvent, it is more toxic. Fairly high concentrations of benzene in an enclosed atmosphere can cause headaches and other symptoms; prolonged exposure can cause more severe damage.[134] Although cyclohexane is a much less efficient solvent it is reputed to extract most of the polynuclear arenes from air particulate mixtures.[86, 97] The various extraction solvents have been compared.[74, 135] Since cyclohexane extracts less colored material than benzene, the problem of interference by many of the polar components of the mixture is eliminated. On the other hand, care must be taken to ensure that a major portion of the polynuclear arenes is extracted when cyclohexane is used, and it must be remembered that if more polar components are to be assayed, a solvent more polar than cyclohexane is necessary.

The method of analysis used routinely for polynuclear aromatic hydrocarbons involves column chromatography on alumina followed by ultraviolet-visible absorptiometry. The disadvantages of this method are that approximately 100 mg of organic airborne particulates (representing 10,000 m^3 of air) are necessary for analysis, the column separation takes about half a day, and the total of two to three days is necessary to complete the assay of one sample, dependent on the efficiency of the analyst. This type of method does not lend itself to extensive routine assays for atmospheric polycyclic arenes by the smaller state, county, and city air-pollution laboratories. It is possible that the method can be automated with the help of recently developed liquid chromatographic instrumentation.

The most popular eluting mixture has been cyclohexane containing increasing amounts of ether.[81,83-87,92-94] The advantages of cyclohexane are that it can be obtained pure and it does not evaporate readily at room temperature. Occasionally, batches are obtained with benzene as an impurity; in this case analytical work cannot be done in the ultraviolet. A good technical grade of cyclohexane can be freed from aromatics by passage through activated carbon. The disadvantage is that evaporation of cyclohexane is necessary in the procedure and this involves time and the possibility of product decomposition by heat.

Pentane with increasing amounts of ether has also been used as an eluent.[88-91,127,132,133] A separation by this system is shown in Figure 2. The main disadvantage of pentane is that it evaporates readily at room temperature so cuvettes have to be tightly stoppered. The advantages are

TABLE 3

Determination of Polynuclear Arenes Present in Airborne Particulates

Extraction solvent	Analytical procedure[a]	Ref.
Chloroform	CC(Al_2O_3, cy) → SP(cy)	(81)
Benzene	CC(Al_2O_3, pet ether → pet ether + ether) → SP(pet ether)	(82)
Cy	CC(Al_2O_3, cy) → SP(cy)	(83–87)
Benzene	CC(Al_2O_3, pentane → pentane + ether) → SP(pentane)	(88–91)
Acetone	CC(Al_2O_3, cy → cy + ether) → SP(cy)	(92, 93)
Cy	CC(Al_2O_3, cy → cy + ether) → SPF(cy)	(94)
Benzene	CC(Si gel, i-octane → benzene) → CC(Al_2O_3, cy → cy + ether) → SP	(95)
Cy	CC(Si gel. i-octane → benzene → CC(Al_2O_3, cy → cy + ether) → SP	(96)
Cy	CC(Si gel, cy → benzene) → CC(Al_2O_3, cy → cy + ether) → SPF	(97)
Benzene	TLC(Si gel + caffeine, light petroleum + 4% pyridine) → SP(ethanol)	(98)
Benzene	TLC(Al_2O_3 – Cellulose acetate (1:1), hexane → methanol:ethyl ether: water(4:4:1) – SPF(cy) or LTF(heptane)	(99)
Benzene→ Methanol	LL(90% Methanol → cy) → CC(silica gel, cy) → PC + DMF(decalin – half-sat with DMF) → CC(Al_2O_3, cy) → SP + SPF(cy)	(100)
Cy; acetone; Benzene→Methanol	LL(90% Methanol → cy) → CC(silicic acid, iso-octane → benzene) → TLC[Cellulose acetate, ethanol:toluene:water(17:4:4)] → CC(Al_2O_3, cy → cy + ether) → SP(cy)	(101)
Benzene;benzene-methanol	LL[Methanol–water (4:1) → cy → nitromethane] → Methanol (4:1) → CC(Al_2O_3, hexane → hexane – benzene → benzene) → PC[acetylated paper, methanol:ether:water (4:4:1)] → PC[acetylated paper, methanol:ether:water (4:4:1)] → SP(cy)	(102, 103)
Vac. sublimation	GC(FID + ECD, 1% SE – 30 on Diasolid H, 60–80 mesh, stainless steel)	(104)
Benzene	CC(Al_2O_3 or Si gel) → GC(FID, 10% SE 30 on Chromosorb W, 60–80 mesh) → SP[b]	(105)
Chloroform	CC(Si gel, benzene) → CC(Al_2O_3, cy → cy + ether) → GC(FID, 10% SE-52 on 60–80 mesh Chromosorb W)[c]	(106)
Cy	LL[Methanol–water (4:1) → cy → nitromethane] → GC(FID, glass capillary column coated with SE 30 silicone rubber)[d]	(107, 108)
Benzene	GC(FID, 2% Apiezon L on 60–80 mesh Diatoport S)[e]	(109, 110)

[a] Al_2O_3 = alumina, CC = column chromatography, cy = cyclohexane, DMF = dimethylformamide, ECD = electron capture detector, FID = flame ionization detector, GC = gas chromatography, LL = liquid-liquid extraction, LTF = low temperature fluorescence at liquid-nitrogen temperatures, PC = paper chromatography, SP = ultraviolet and visible absorption spectrophotometry, SPF = spectrophotofluorimetry, TLC = thin-layer chromatography

[b] Not applied

[c] For characterization → SP(cy). Soots from ethylene and ethane diffusion flames analyzer

[d] Benzo[a]pyrene and benzo[e]pyrene not separated

[e] Benzo[a]pyrene, benzo[e]pyrene and perylene not separated. No cleanup used in this method.

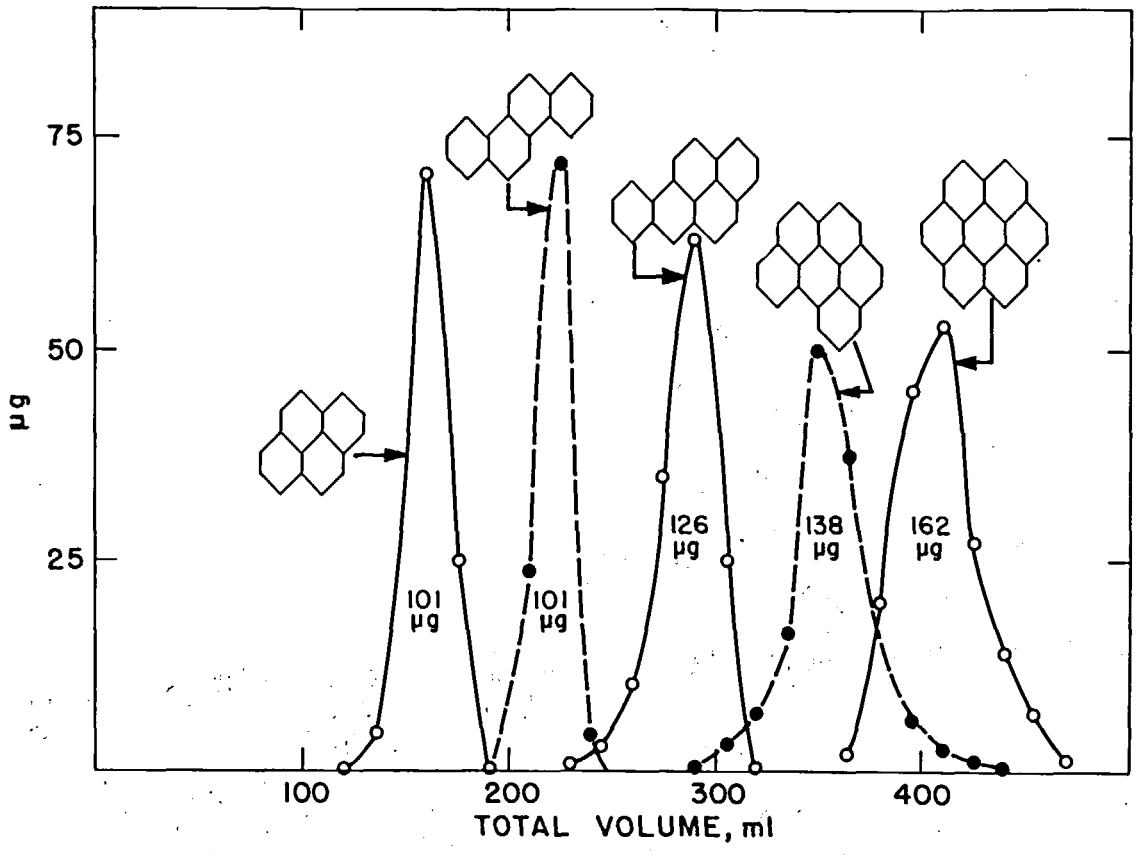


FIGURE 2. Column chromatographic separation on alumina of pyrene, chrysene, benzo[a]pyrene, benzo[g,h,i]perylene and coronene with pentane containing increasing amounts of ether. Reprinted from *Anal. Chem.*, 32, 810(1960). With permission of the American Chemical Society.

that it can be purified easily by distillation or evaporated quickly at room or lower temperature. It readily dissolves tri-, tetra-, penta-, and even some hexacyclic arenes. It has a low blank in fluorimetric analysis. Absorption spectra can be determined to 220 nm. Of the known hydrocarbons only benzo[a]pyrene has a triplet at 377, 379, and 382 nm in pentane; in cyclohexane it shows only one band and a shoulder in this region. Arenes are more easily characterized and assayed in pentane because their bands are sharper and narrower than in cyclohexane or other more polar solvents.

The following arenes can be readily characterized in airborne particulates of various types by the column chromatographic-absorptiometric procedure: anthracene, phenanthrene, pyrene, fluoranthene, chrysene, benz[a]anthracene, benzo[a]pyrene, benzo[e]pyrene, perylene, benzo[k]fluoranthene, benzo[g,h,i]perylene, anthanthrene, and coronene.[91, 136, 137] The spectral bands useful in characterization and assay are shown in Figure 3.[91] The tricyclic hydrocarbons are too volatile to be assayed quantitatively by the procedure. The tetracyclic hydrocarbons are somewhat volatile, so that reasonable results can be obtained only if due care is taken. The penta- and hexacyclic arenes are much less volatile

and so can be assayed more readily. In the same fashion urban particulates stored in an envelope in the dark for one year showed the following losses: pyrene 88%, benzo[a]pyrene 32%, benzo[g,h,i]perylene 10%, and coronene 1%.[111] On the other hand, the residue obtained by evaporating the benzene from the benzene-soluble fraction of a composite urban airborne particulate sample showed no losses after four years' storage in a closed bottle in a refrigerator.

Analysis for benzo[a]pyrene has usually been accomplished at the 382-nm absorption maximum with the base-line method.[84, 90, 138] At this wavelength the millimolar absorptivity of BaP is 30.5 in pentane while that of benzo[k]fluoranthene is 5.6.[136] So, obviously, there is some interference from the latter compound in the assay for BaP. Subtracting this interference can give corrected values.[113]

Other analysts prefer to use the 401-nm band

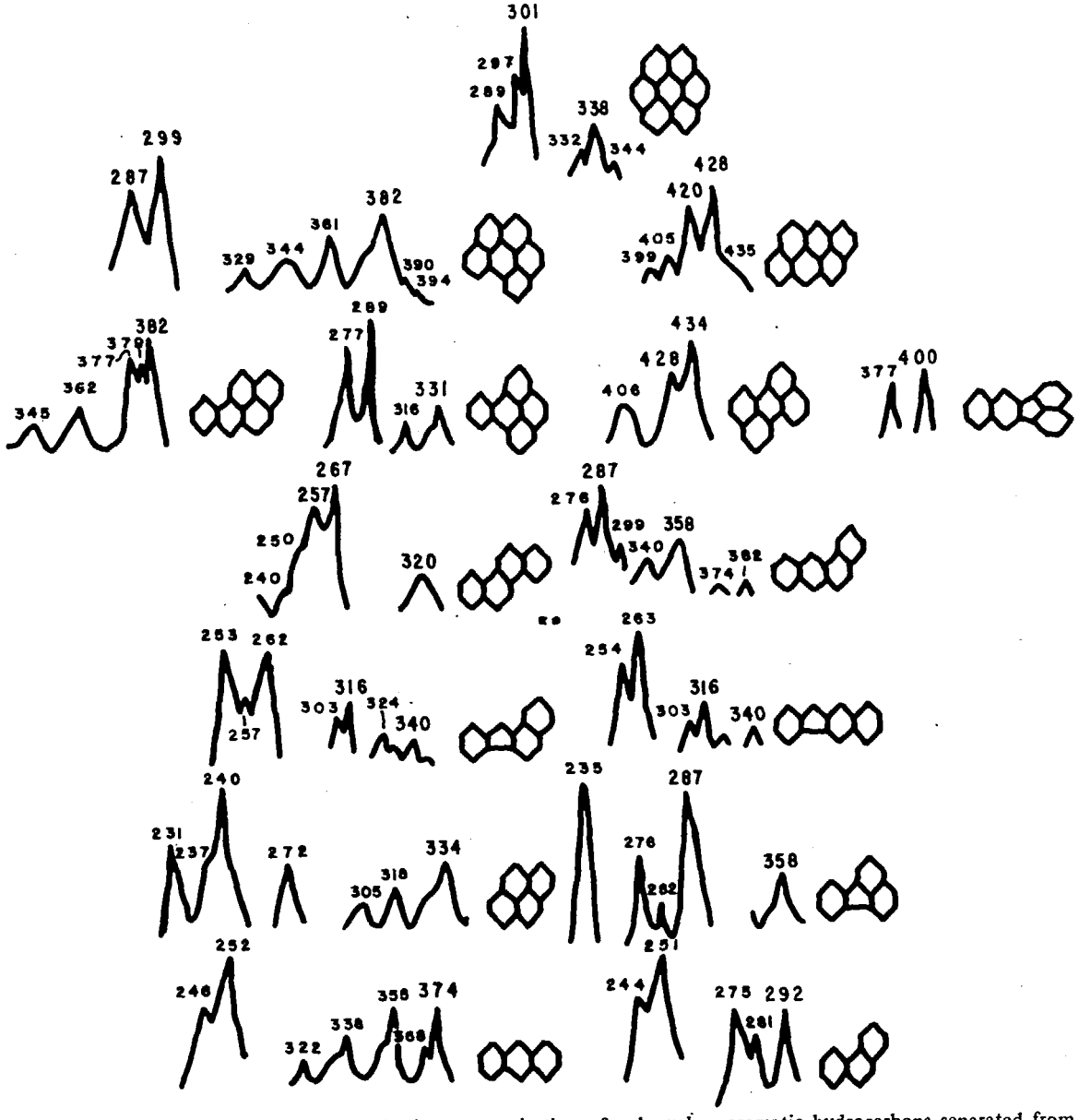


FIGURE 3. Ultraviolet-visible bands useful in characterization of polynuclear aromatic hydrocarbons separated from airborne particulates with column chromatography (alumina plus pentane containing increasing amount of ether). Reprinted from *Anal. Chem.*, 32, 810 (1960). With permission of the American Chemical Society.

of BaP.[92,93,139] One analyst found it necessary to multiply his BaP values by 0.33 to obtain the corrected results.[139] Since at 401 nm the millimolar absorptivity of BaP is 4.2, while the corresponding values for benzo[k]fluoranthene and perylene are 21.9 and ca. 20, respectively, considerable interference is present at this band. Measurement at this wavelength is reported both to be unreliable[97] and to be more reliable than other methods.[139]

Another method is to determine both BaP and benzo[k]fluoranthene fluorimetrically at two different excitation wavelengths and then subtract one value from the other to obtain the amount of BaP.[140]

All of these column chromatographic methods are believed to be estimations. This is primarily due to the minute amounts of hydrocarbons measured, but other contributing factors are particulate collection problems, the presence of benzo[k]fluoranthene in the BaP fraction and fluorescence quenching problems in the fluorimetric methods.

The variety of particulate mixtures which has been analyzed for polynuclear arenes by column chromatographic techniques is listed in Table 4.

A somewhat less popular method of assay for the polynuclear arenes includes column chromatography followed by fluorimetric assay, Table 3. The advantages are a much greater sensitivity and a usually greater selectivity. The disadvantages are poorer reproducibility due to instrumental, quenching, and fluorescence-enhancement problems, the last of which is derived from energy-transfer phenomena. Base-line methods in fluorimetry are highly questionable with present instrumentation. Spectral bands are not as sharp, distinct, and reproducible as they are with the ultraviolet spectral method. In mixtures a sharp intense absorption band can stick out from the background after some separation, but a fluorescent band could be partially or completely quenched by impurities, as has been shown in the determination of benzo[e]pyrene in the presence of BaP.[136]

Low temperatures, and especially the temperature obtained with liquid nitrogen, can be used to cause the normally broad fluorescence peaks of hydrocarbons to sharpen into fine-structure lines. A non-polar solvent, such as heptane, is used as the solvent. These quasilinear fluorescence spectra have proven useful in the determination of the polynuclear arenes.[141,149] Most of this work has dealt with the determination of BaP. The possible interference of benzo[k]fluoranthene needs study. Fluorimetric and phosphorimetric measurements of hydrocarbon carcinogens at -196° following thin-layer chromatography permit determinations of most of the carcinogens down to 0.1 μg.[150] The sensitivity for the quasilinear fluorescence spectral method is 0.1 to 10 ng/ml.[141] Nanogram amounts of some of the polynuclear arenes can be and have been characterized on paper and thin-layer chromatograms.[151-153] Correlation spectroscopy may also prove useful in air-pollution analysis based on quasilinear fluorescence spectra obtained at low temperatures.[153a] These various low-temperature luminescent methods are definitely of potential value in the determinations of carcinogens and analogous compounds.

Thin-layer chromatographic analysis of the family of atmospheric polynuclear arenes would have to involve two-dimensional TLC followed by spectrophotofluorimetry. This type of analysis has been used in the characterization of the arenes[154] and in the assay of BaP.[155] For potential assay by this technique a mixed adsorbent would be necessary. Cellulose-alumina and cellulose acetate-alumina have been used; the latter is preferred because of the superior separation of many of the arenes, as shown in Figure 4.[154] Two methods of assay could be used: direct fluorimetric assay of the spots on the plate, or fluorimetric or microabsorptiometric analysis after elution. If the spots

TABLE 4

Airborne Particulate Mixtures Analyzed for Polynuclear Arenes

MIXTURE
Urban atmospheres[82,84-86,88,90,93,94,96,97,99,100,104,106-112]
Non-urban atmospheres[113,114]
Combustion products from fuels[115]
Soot from ethylene and ethane diffusion flames[106]
Coal tar pitch[88,89,116-124]
Automotive exhaust[102,103,125-127]
Diesel engine exhaust[128-131]
Coal combustion effluent[88]
Wood smoke[101]
Industrial and residential heat-generation effluents[132]
Incinerator effluents[132]
Asphalt air-blowing emissions[133]
Industrial effluents[88,133]

cannot be obtained in a regular shape, the elution method would be preferable. The advantages and disadvantages of alumina, cellulose, and cellulose acetate for such separations have been discussed.[156,157]

It is even conceivable that a group of the polycyclic arenes could be assayed following one-dimensional thin-layer chromatography on alumina,[156] or possibly on polytetrafluoroethylene,[158] followed by microspectral analysis of the eluted spots. Two-dimensional separation on alumina[159,160] followed by microspectral analysis of the eluted spots presents another possibility in arene assay.

However, in all these TLC assays photodecomposition can take place if the material is left on the plate for several hours after separation.[160-164] Caffeine on a plate is reported to stabilize the polycyclic arenes toward air and daylight so that there is no change after four days.[160,161] The hydrocarbons are also much more stable on cellulose acetate than on alumina or silica gel.

Gas chromatography has been used to analyze for polycyclic arenes in coal tar pitch[116,118] and in air samples (Table 3). Gas chromatography has not been a popular method for determining atmospheric polycyclic arenes. Lack of sensitivity, the inability to completely separate the polycyclic arenes from each other and from other background material, and product breakdown have been the main problems in developing a reliable routine method. At the present stage of development it would appear that cleanup is necessary before gas chromatography followed by ultraviolet absorptiometric analysis of the GC fractions can be used successfully. For greater sensitivity (sometimes up to a thousand times greater), fluorescence analysis can be employed after chromatographic separation.

Methods for the determination of individual

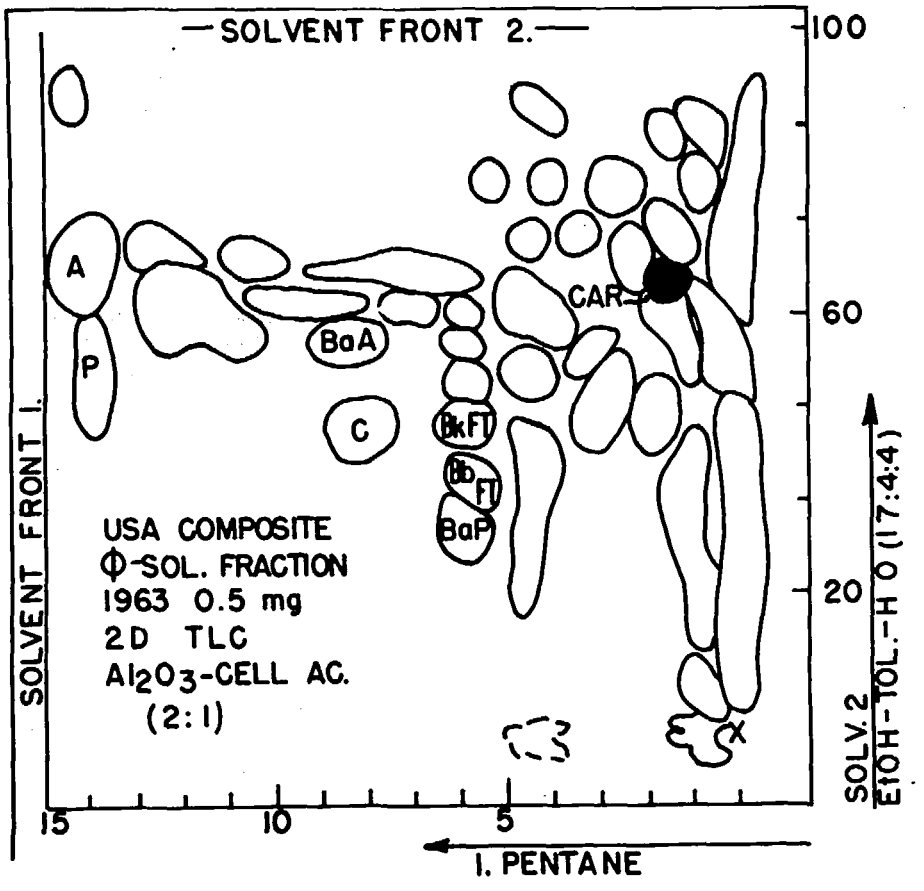


FIGURE 4. Two-dimensional thin layer separation of a benzene extract of composite sample of airborne particulates collected from 200 American communities With permission.

carcinogens of the arene type are also available. The criteria for interest in such compounds are that they are potent animal carcinogens (or can cause human cancer) and can be present in the atmosphere.

Benzene is highly toxic to blood-forming tissue[165] and is mildly leukemogenic.[166] It can be found in high concentrations in enclosed atmospheres. It is present in the atmosphere at ~0.02 ppm. At these atmospheric concentrations it probably does not present a problem. The best method of assay for benzene utilizes gas chromatography with flame ionization detectors.[167] The hydrogen flame ionization detector has also been used in the gas chromatographic analysis of C_6 to C_{10} aromatic hydrocarbons in automobile exhaust.[168] Gas chromatographic analyses have been reported for six- to ten-carbon aromatic hydrocarbons in various types of samples of air pollution interest.[169,170]

Benzene and other simple aromatics have been identified and estimated by mass spectroscopy of atmospheric[171, 172] and automobile exhaust[173] samples. In the latter case only benzene was estimated.

Alternative methods of analysis for atmospheric benzene include absorption of benzene onto silica gel followed by elution, fractionation, and finally either spectrophotometric analysis in the lower boiling component of the solvent system in the 253 to 280-nm range[174] or collection in methanol cooled with dry ice followed by measurement at 245.5, 268.5, and 272.0 nm.[175]

Methods are also available for the determination of benzene in the air in the presence of toluene and xylene by nitration to *m*-dinitrobenzene followed by coupling to acetone or 2-butanone in alkaline solution.[176-182] The absorbance of the anionic resonating chromogen is then measured, employing its absorption band in the long-wavelength portion of the visible spectrum.

Since benzo[a]pyrene is the most prominent atmospheric carcinogen, methods of analysis for this compound have received much attention, Table 5. Methods 8 and 9, which are very closely

TABLE 5

Determination of Atmospheric Benzo[a]pyrene

	Method[a]	Detm. Limit, μg[b]	(Ref.)
1.	Extn(b) → CC(Al_2O_3, p → p–e) → SP(p, λ375, 382, 390)[c]	10	(90,113)
2.	Extn(cy) → CC(Al_2O_3, cy) → SP(cy, λ399, 402, 405)		(84,93,139)
3.	Extn(cy) → CC(Si gel, b) → SPF(F308/400[d], F385/403[e])		(97,140)
4.	Extn(a) → CC(Al_2O_3, pet → pet – 30% b) → SPF(pet) F365/408		(183)
5.	Extn(a) → CC(Al_2O_3) → PC(cell. acetate) → SP[f]		(184–186)
6.	Extn(mc) → TLC(Al_2O_3 + cell. acetate, al–t–w, 17:4:4) → d SPF(F300/430)	0.003	(155)
7.	Extn(mc) → TLC$_2$(Al_2O_3 + cell. acetate, p → al–t–w, 17:4:4) → d SPF(F300/430)	0.003	(155)
8.	Extn(b or mc) → TLC(Al_2O_3, p–e, 19:1) → Elution(e) → SPF(H_2SO_4, F470/540)	0.003	(155,156)
9.	Extn(mc) → TLC(Al_2O_3, p–e, 19:1) → Elution(e) → FF(H_2SO_4)	0.01	(155)
10.	Subl. → TLC(Si gel, hex–dc–py, 10:1:0.5) → Elution(b) → SPF(b,F365/402, 405, 408)		(187–188)
11.	Extn → TLC(Al_2O_3) → LTF(hep, F365/403 at–197°C)		(144)
12.	Extn(b) → TLC(Al_2O_3, p–e, 19:1) → Elution(e) → SP(p, λ372, 382, 390)		(135,156)
13.	Extn(b) → CC(Al_2O_3, p → p–e, 4:1) → SPF(H_2SO_4, F470/540)	0.5	(155)
14.	Extn(b or mc) → LL(w:m, 1:4 → cy → H_2SO_4) → SPF(F470/540)	0.12	(155)
15.	Extn(mc) → Evapn → LL(hex → H_2SO_4) → SPF(F470/540)	0.01	(155)
16.	Extn(mc) → TLC(Al_2O_3, p–e, 19:1) → Elution(e) → GC	5	(155)
17.	Subl. → SPF(cy, F307/403[d], F382/403[e])		(189)
18.	Extn(cy) → SP(cy, λ300, 302, 304 and 381.5, 384.5, 387.5)		(190)
19.	Extn(b) → GC(ec, NaCl–Chromosorb G containing 2% SE 30; 2:3)		(191)

[a] a = acetone, al = alcohol, b = benzene, cy = cyclohexane, e = ether, hep = heptane, hex = hexane, mc = methylene chloride, p = pentane, pet = petroleum ether, pyr = pyridine, t = toluene, w = water,

Al_2SO_3 = alumina, CC = column chromatography, ec = electron capture, Extn = extraction, F308/400 = analysis with instrument set at excitation wavelength 308 nm and emission wavelength 400 nm, FF = filter fluorimetry, GC = gas chromatography, LTF = low temperature fluorimetry, PC = paper chromatography, SP = absorption spectrophotometry, SPF = spectrophotofluorimetry, d SPF = direct spectrophotofluorimetric examination of a separated spot on a chromatogram, Subl. = sublimation, TLC = thin layer chromatography, and TLC_2 = 2 dimensional chromatography.

Value X 2 = lower limit in mg of benzene-soluble fraction necessary to determine BaP in a sample containing 500 μg BaP/gram benzene-soluble fraction.
Value X 24 = lower limit in mg of airborne particulate sample necessary to determine BaP in a sample containing 50 μg BaP/g sample.
Value X 200 = lower limit in m^3 of air necessary to determine BaP.

Values corrected for presence of benzo[k]fluoranthene.
λ375, 382, 390 signifies base-line determination at wavelengths 375, 382, and 390 nm.

For benzo[k]fluoranthene.

For benzo[k]fluoranthene and benzo[a]pyrene. BaP then obtained by subtraction.

Indoor samples at iron and steel works.

related, are probably the most popular in the United States. They are very selective and sensitive, so sensitive indeed that a contaminated room or cigarette smoke can affect the results adversely. Where a less sensitive method is desirable, number 12 or some version of it can be used. In Canada method number 3, or some variation of it, has proven more popular. Since benzo[k]fluoranthene and benzo[a]pyrene have almost identical fluorescence emission spectra, variations of number 3 attempt to correct for the presence of benzo[k]fluoranthene.

BaP can also be estimated in 20 to 30 min once a particulate sample is obtained.[190] One to ten mg of particulate containing approximately 1 μg of BaP can be assayed. The method involves extraction with cyclohexane of the residue remaining after evaporation of chloroform from the chloroform extract, followed by spectral determination in two wavelength regions. Calibration is necessary against a reliable standard method for the determination of BaP.

In the gas chromatographic method, number 19, BaP has been separated from its usual contaminants, BeP (benzo[e]pyrene), benzo[k]fluoranthene, and perylene.[191] Unfortunately, a benzene-soluble extract was used in the analysis. The polar and aliphatic material in this mixture would probably clog the column and interfere in the analysis. A cyclohexane extract should be tried in this method. It certainly should be investigated further, simplified, and applied to routine assay if it proves worthwhile.

For automatic routine assays some methods which could have potential include (a) thin-layer chromatography on cellulose acetate of a cyclohexane extract of air particles, followed by direct fluorimetric assay, and (b) selective sublimation of BaP from glass-fiber paper into some appropriate spot, followed by direct fluorimetric assay or fluorimetric assay of a sulfuric acid solution of the sublimate. Simplified gas or liquid chromatographic procedures need to be investigated for eventual application to the routine analysis of BaP and other compounds.

The analytical procedure needs to be checked out at the beginning. This can be done by recovery experiments or by use of an internal standard not present in the original sample. Internal standards can be 1,3,5-triphenylbenzene, naphtho[a]pyrene, or a ^{14}C-labeled hydrocarbon. In addition, standards and blanks need to be run with every determination.

Large errors can result in the routine collection of air particles, in the extraction of organic material from these samples, and in the evaporation of these extracts. The errors are increased in the collection of BaP and other arenes from automobile exhaust. The thermal and oxidative instability of BaP makes it difficult to determine this component reproducibly in auto exhaust.[192, 193] Since the BaP content varies with sampling temperature and the exhaust tar is difficult to collect reproducibly, reliable and meaningful data cannot be developed until the BaP can be quantitatively obtained from the auto exhaust and a sampling procedure developed which is representative of the emission of BaP into the atmosphere.

In addition to these errors photodegradation of BaP can occur, especially on a thin-layer plate.[194] This type of decomposition can be prevented by taking care to perform the analysis without delay and to protect the sample or spot from the light. This can be readily done by installing yellow lights in the laboratory[126] or through the use of yellow Kodagraph filters between the sample and the light. These filters filter out light below 467 nm.

Benzo[a]pyrene has also been determined in automobile exhaust gas[102,103,126,193] through a carbon-14 technique, in wood smoke (101 and refs. therein), in coke oven effluents by GC→SP (195), and in various other sources.[12,74,197]

2. Aza Arenes

The basic fraction is only a small portion of the benzene extracts of urban airborne particulates[112,197] and other air-pollution source particles.[88,112,198-200] However, the basic fraction of urban[112] and air pollution source[199] samples contains several hundred conjugated basic compounds, some of which could be carcinogenic. Most of the compounds isolated from the spots separated by column chromatography followed by thin-layer chromatography have distinctive absorption and fluorescence spectra, and in many cases phosphorescence spectra as well. Of this large number of basic compounds only about 25 aza arenes have been characterized, either unequivocally or partially. The difficulty lies in characterizing alkyl derivatives and other unknowns for which standards are not available. Many national and international organizations are initiating projects involving the preparation, purification, and collection of pure standards. The World Health Organization has started some work in this field.

The importance of the composition of the basic fraction stems from the fact that a large number of basic organic compounds are carcinogenic to animals.[8-12] In addition, the necessity of knowing the composition of the basic, neutral, and other fractions stems from the possibility that some of the non-carcinogenic compounds of these fractions could have cocarcinogenic, synergistic, anticarcinogenic, or irritant effects in the cancer process; see Table 1. In this respect Spear, as cited by Cook,[25b] has shown that quinaldine and isoquinoline hasten the carcinogenic action of BaP. So the synergistic effect would be of some importance

Characterization is of importance in the analysis of the atmospheric aza arenes. Some of the methods found useful in the characterization include paper chromatography,[198,201] thin-layer chromatography,[112,198,199,201-203] paper electrophoresis,[204] thin layer electrophoresis,[204] column chromatography,[88,112,198,199,205,206] ultraviolet absorption spectrophotometry,[88,112,198,204-206] spectrophotofluorimetry,[112,135,198,199,202-204,206] fluorescence scanning,[204,206a] fluorescence tests,[198,199,202,204,206] quenchofluorimetry,[199,207-209] and spectrophotophosphorimetry,[201] Figure 5.

The complexity of the basic fraction is shown by the large number of fluorescent spots separated by thin-layer chromatography and the nineteen identified aza arenes found in the sample, Figures 6a and 6b, Table 6.[112] The following percentages of aza arenes were identified in the basic fractions: Atlanta, 1; Cincinnati, 1; Los Angeles, 0.06; Nashville, 2; New Orleans, 0.1; and Philadelphia, 0.2.[112] The carcinogen dibenz[a,j]acridine was found in the half dozen cities studied.

Techniques involving column chromatography followed by ultraviolet absorption spectrophotometry in pentane have been used to determine about eight aza arenes in urban atmospheres[112] and in effluents from the stack of a coal-heated residence,[88] several industrial sources,[88] air contaminated with coal-tar pitch fumes,[88] and automobile exhaust.[200] The column chromatographic

TABLE 6

List of Aza Heterocyclic Compounds Present in Nashville Urban Airborne Particulates[112]

1. Benzo(h)quinoline
2. Ra Benzo(h)quinoline
3. Rb Benzo(h)quinoline
4. Benz(c)acridine
5. Ra Benz(c)acridine
6. Rb Benz(c)acridine
7. Dibenz(a,h)acridine
8. Indeno(1,2,3-ij)isoquinoline
9. Phenanthridine
10. 11H-Indeno (1,2-b)quinoline
11. Acridine
12. Ra Benzo(f)quinoline
13. Benzo(f)quinoline
14. Rb Benzo(f)quinoline
15. Benz(a)acridine
16. Ra Benz(a)acridine
17. Rb Benz(a)acridine
18. Dibenz(a,j)acridine
19. Ra Dibenz(a,j)acridine

step is much too long, and it is probable that high-pressure liquid chromatography with the proper detector and column could simplify and speed up these analyses.

Values that could represent the possible cancer threat of the basic fraction could be the weight of basic fraction per cubic meter of air and per gram of air particulates, the concentrations of the various aza arenes in air and particulates, and the concentration of a representative readily determinable compound such as benz[c]acridine.

Benz[c]acridine was assayed by the following procedure. The particulate sample was extracted with benzene-diethylamine (4:1). After evaporation of solvent, the residue was dissolved in methylene chloride and separated by thin-layer chromatography on alumina, eluted, and then assayed in acid solution fluorimetrically at F 290/470.[135,203,210] Benz[c]acridine can also be determined directly on the plate at F 288/478 after two-dimensional separation on an alumina-cellulose plate.[203] In the filter fluorimetric method, two-dimensional separation is followed by elution, evaporation, solution in nitromethane-trifluoroacetic acid, and assay with a primary and secondary filter peaking at 405 and 490 nm, respectively. Thus, the greater interference of background in filter fluorimetry method is overcome with a solvent that quenches the fluorescence of the background material much more than it does that of the benz[c]acridine. Benzo[h]quinoline can also be determined by these various two-dimensional thin-layer chromatographic procedures. Acridine, a highly active mitotic poison (against the fertilized egg of sea urchin),[211] can be determined in airborne and other particulates through paper electrophoretic separation followed by direct fluorimetric scanning at F 345/475.[212]

3. *Imino Arenes and Benzanthrone*

Imino arenes have also been found in polluted

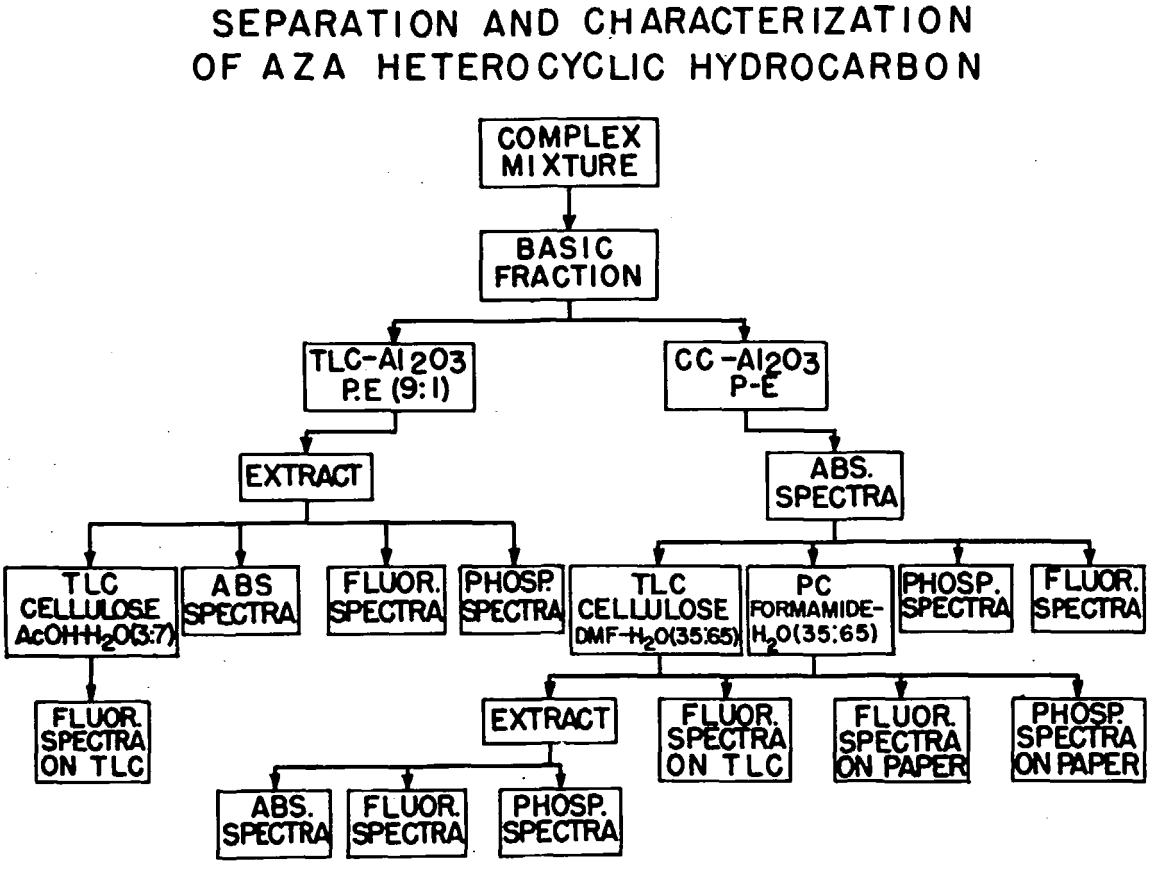
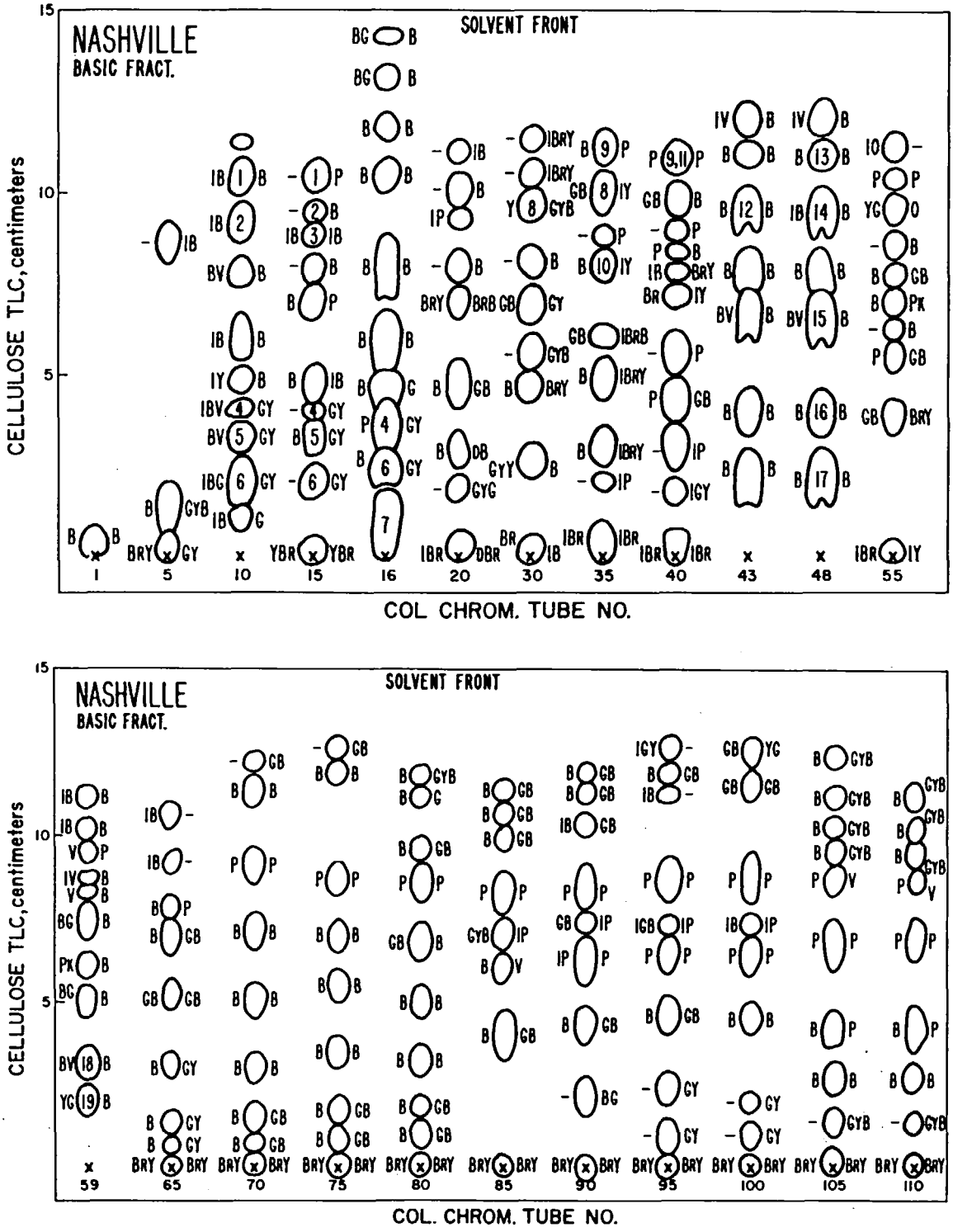


FIGURE 5. Scheme for separation and characterization of atmospheric aza arenes. With permission.



FIGURES 6a and b. Thin-layer chromatograms (cellulose-dimethylformamide: water, 35:65) of the alumina column chromatographic fractions of the basic fraction of a one-year composite airborne particulate sample from downtown Nashville. The aza heterocyclic compounds numbered from 1 through 19 are identified in Table 6. The letter(s) at the left of a spot represents the fluorescence color of the spot wet with solvent; the letter(s) at the right, the fluorescence color of the spot treated with trifluoroacetic acid fumes. B = blue, Br = brown, d = dull, G = green, Gy = gray, l = light, O = orange, P = purple, Pk = pink, V = violet, Y = yellow, and – = no fluorescence. With permission.

atmospheres. Carbazole has been identified in urban atmospheres and in coal-tar pitch-polluted atmospheres.[89, 154, 213] Benzocarbazoles have been identified in coal-tar pitch-polluted atmospheres.[154, 214] Analysis for carbazole involves alumina-column chromatography followed by absorptiometric assay. Analysis for the benzocarbazoles involves thin-layer chromatography followed by spectrophotofluorimetric examination of the separated spots.

The physiological activities of the weakly basic polynuclear ring-carbonyl compounds have been essentially uninvestigated, although 7H-benz(d,e) anthracen-7-one (benzanthrone) has been reported to cause a few tumors in white mice.[215] However, all the evidence available would seem to indicate that the carcinogenic activity of this compound is highly questionable.[215-217]

Thin-layer chromatographic characterization tests are available for these compounds,[218] as are thin-layer and column chromatographic methods of separation.[219] Phenalen-1-one and benzanthrone have been estimated in urban atmospheres and air pollution source effluents by two-dimensional TLC followed by either direct fluorimetric scanning[159,220] or elution and fluorimetric assay.[220] One-dimensional TLC followed by elution and spectrophotofluorimetric[210, 220] or filter fluorimetric[220] assay has been used.[210,220] Some of these techniques involve quenchofluorimetric methods. A rapid method involving instant thin-layer chromatography on glass-fiber paper impregnated with silica gel, followed by elution and spectrophotofluorimetric assay in sulfuric acid solution, has been developed for atmospheric benzanthrone and phenalenone.[221] Estimation of these compounds on the plate by eye is also possible.

B. Cocarcinogens

Possible atmospheric cocarcinogens or promoters include alkanes, monocyclic phenols, and polyphenols, Table 1. An initiator or initiating agent is one which, when applied on mouse skin in a single dose followed by repeated applications of a promoting agent, results in induction of benign and malignant tumors.[20a]

A promoting agent can be defined as a chemical, mixture, or some entity which, following initiating action by a carcinogen, applied over a period of time will produce tumors that the carcinogen in small initiating amounts would not produce. Somewhat analogous to this are chemicals which hasten the process of carcinogenesis. Thus, quinaldine and isoquinoline accelerate the carcinogenic action of benzo[a]pyrene.[72]

Cocarcinogenic action is a general term which refers to all forms of augmentation of tumor induction, usually brought about by concurrent administration of the carcinogen and the added factor although, in some cases, the added factor operates before or after the carcinogen.

1. Alkanes

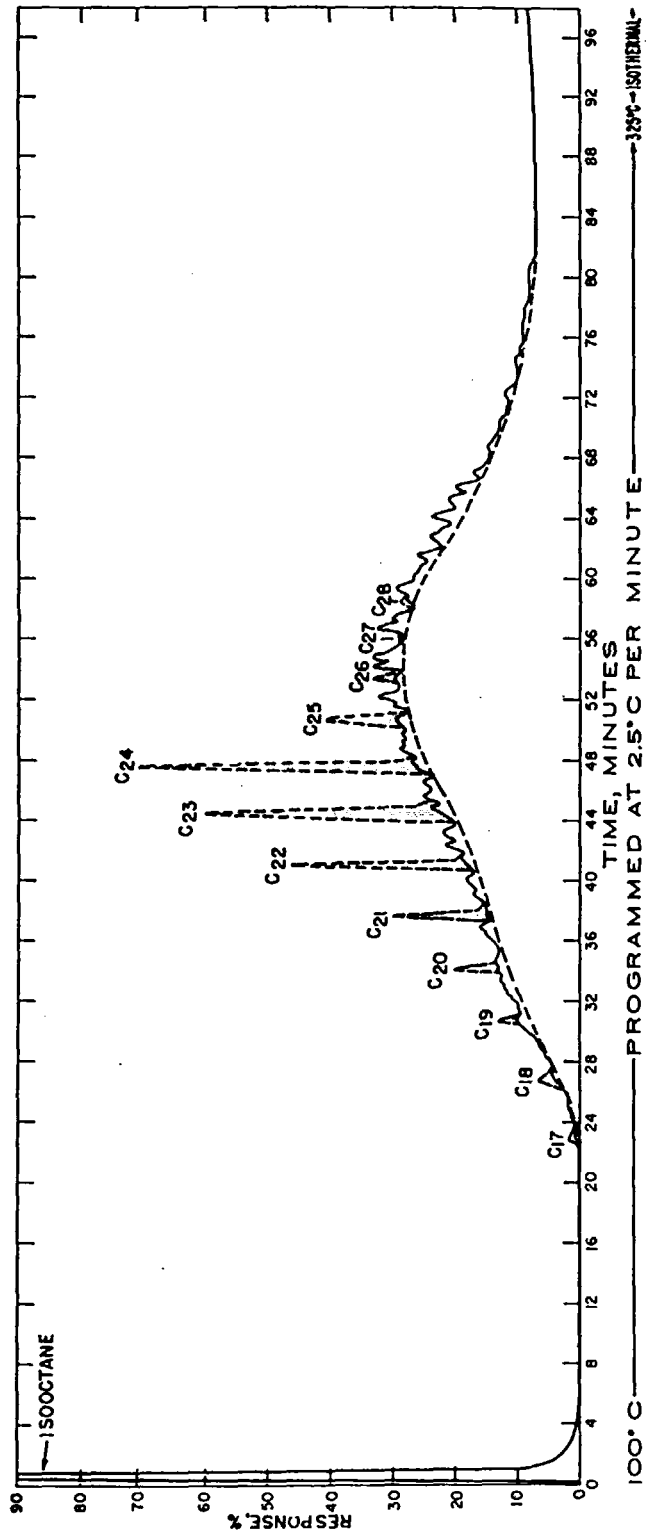
Since the higher molecular weight hydrocarbons of this type enhance the carcinogenicity of benzo[a]pyrene and analogous arenes, their presence in the atmosphere needs to be monitored. At the present time there are two ways of doing this. One is by subjecting about 100 mg of organic airborne particulates (e.g., benzene, cyclohexane, or another appropriate extract) to chromatography through an alumina column with pentane, hexane, or cyclohexane as the eluent. The first two tubes would contain the aliphatic fraction, which could be weighed after evaporation of the solvent. From this weight the amount of aliphatic fraction in $\mu g/m^3$ air and mg/g airborne particulates can be determined. The relative amount of this material would give some idea of the possible danger of the cocarcinogenic effect.

An additional, but more laborious, method would be to analyze for the individual hydrocarbons in the aliphatic fraction of airborne particulates. Gas chromatography with a differential hydrogen flame ionization detector has been used to determine atmospheric *n*-alkanes (*n*-heptadecane through *n*-octacosane).[112, 222, 223] The analysis was performed after removal of unsaturates and adsorption by a molecular sieve, Figure 7.[222]

2. Phenols

The most popular general methods of analysis for phenols are the ultraviolet and colorimetric methods. Colorimetric methods have been used most often in the analysis of atmospheric phenols. Thus, N,N-dimethyl-*p*-phenylenediamine,[224] diazotized *p*-nitroaniline,[225, 226] and 4-aminoantipyrine[227, 228] have been recommended as reagents for the analysis of atmospheric phenols.

The burning of domestic fuels was thought to be the major source of phenols in the air until it was found that greater quantities of phenols were present in automobile exhaust fumes than in



CHROMATOGRAM OF ALIPHATIC FRACTION AFTER REMOVAL OF UNSATURATES AND ADSORPTION BY MOLECULAR SIEVE

FIGURE 7. Gas chromatogram of aliphatic fraction of airborne particulates after removal of unsaturates and adsorption by molecular sieve. With permission.

emissions from domestic furnaces.[228] The analysis of automobile exhaust fumes for phenols then becomes of some importance. 4-Aminoantipyrine has been used.[229] A variety of general methods is compared in Table 7,[230] and many of the strong and weak points of the various methods are discussed in Table 7. The simplest procedure is the absorptimetric determination of the phenols in alkaline solution; however, in an efficient collection of phenols into one impinger, the large amount of acetophenone also collected interferes with the determination. This method could probably be improved. The methods of choice where simplicity and routine usability are desirable are the 4-aminoantipyrine B and *p*-nitroaniline procedures. The most sensitive method is the 4-aminoantipyrine procedure A; it is ideal for minute amounts of phenols where large amounts of test solutions are available. The piperonal and *p*-nitroaniline procedures yield reactions with a greater variety of phenols than do the other methods. These two methods would be the best for analysis of small volumes containing minute amounts of phenols. These methods provide the lowest detection limits. With the piperonal chloride procedure, *para*-substituted phenols can be readily distinguished from the other phenols and the conjugation-complexity of the phenol can be ascertained. This latter procedure could be simplified, and its sensitivity could be improved considerably by further research.

Methods of analysis for the individual members of the phenol family are available. The methods of separation include paper or thin-layer chromatography of azo dyes formed by reaction of the phenols with diazotized 2-nitroaniline or 4-nitroaniline, and gas chromatography of the unreacted phenols.

Analysis of automobile exhaust fumes is accomplished through paper chromatography of the

TABLE 7

Comparison of Methods for the Determination of Phenol[230]

	Wavelength of Maximum Absorption nm	Molar Absorptivity ($\epsilon \times 10^{-3}$)	Standard Deviation[a]	Dilution Factor	Sensitivity[b]	Detection Limit μg[c]	Color Stability, hours	Time Required minutes
Piperonal Chloride	522	46	1.6	10	4.6	2.0	>1	22
p-Nitroaniline	485	27	5.4	4	6.7	1.4	>1	12
4-Aminoantipyrine-A	455	17	10	0.2	86.0	5.5	>16	47
4-Aminoantipyrine-B	507	14	2.1	10	1.4	33.5	2.5	12
Nitrous Acid	412	5.1	0.6	10	0.5	18.4	>24	105
Sodium Hydroxide	287	2.6	1.0	1	2.7	11.0	>24	2

[a] Relative standard deviation based on 10 determinations.

[b] Sensitivity = $\epsilon \cdot 10^{3}$/dilution factor.

[c] Total micrograms of phenol in total test solution giving an absorbance of 0.1 in a 1-cm cell.

azophenols obtained by reaction of the phenols with diazotized 2-nitroaniline followed by elution with 95% ethanol and absorptimetric assay.[231]

Another method of assay involves the gas chromatographic determination of phenols with a flame ionization detector. The phenols can be determined in the free state[231-233] or as their methylated derivatives.[234] Free phenols have been determined in gas condensates[234] and automobile exhaust[231,232] by gas chromatography.

Polynuclear phenols have also been found in the atmosphere.[235, 236] These compounds have not yet been assayed quantitatively. The method of characterization consisted of thin-layer chromatography followed by spectrophotofluorimetry.

C. Anticarcinogens

Some polynuclear arenes have anticarcinogenic properties.[73] For this reason it is customary to determine as many atmospheric arenes as possible. In addition many of these compounds have synergistic effects, and therefore, the compositions of the atmosphere and of air-pollution source effluents have been investigated.

D. Irritants

Another class of compounds of importance in the lung cancer process are the irritants whose presence in the respiratory environment can interfere with ciliary activity and the flow of the mucous stream.[73] As a result, particulate matter accumulates on the underlying cells and the carcinogens present in the particulates have a longer opportunity to be leached out and trigger physiological processes which might result in the formation of malignant tumors or a greater susceptibility of the system to the effects of carcinogens. Air pollutants classified as irritants include acetaldehyde, acetyl peroxide, acrolein, benzene, formaldehyde, formic acid, 2-methylbutene-2, 2-methylpentane, peracetic acid, and propylene oxide. Pollutants showing cilia-movement inhibition include aldehydes, monocyclic arenes, carboxylic acids, epoxides, olefins, paraffins, peroxides, and phenols.

Selective methods of analysis for atmospheric acids, epoxides, and peroxides are not available. The methods for phenols have been discussed, while methods for aldehydes will be discussed in Section V on lachrymators. Total atmospheric hydrocarbons can be determined by gas chromatography using the flame ionization detector.[237] The FID is essentially a carbon-atom counter, but since its response to carbon atoms in different compounds is nonlinear, FID data are usually expressed in terms of a calibration gas. Since methane is so abundant in the atmosphere, a methane-saturated column can be used to absorb nonmethane hydrocarbons before analysis for methane by a flame ionization analyzer.[238] Thus, the nonmethane hydrocarbons can be determined by difference.[239] With gas chromatography most of the hydrocarbons up to the hexanes and hexenes can be individually separated and analyzed.

There is much controversy on the possible toxicity of atmospheric sulfur dioxide, especially since the report that laboratory animals live longer breathing air with 5 ppm sulfur dioxide.[240] However, the effect of SO_2 on hayfever and asthma is another story. In addition, atmospheric SO_2 is reported as increasing the susceptibility of mice to benzo[a]pyrene.[241]

In conclusion, it would appear that for a thorough study of the atmospheric carcinogenesis process the following state-of-the-art assays would be necessary: benzo[a]pyrene by thin-layer chromatography followed by spectrophotofluorimetry, polynuclear arenes by column chromatography followed by ultraviolet-visible spectrophotometry, organic fraction of the airborne particulates by weight, basic fraction of the airborne particulates by weight, benz[c]acridine either by column chromatography followed by ultraviolet spectrophotometry or by thin-layer chromatography followed by spectrophotofluorimetry, total phenols by colorimetry, the family of phenols by gas chromatography, particulate aliphatic fraction by weight, *n*-alkanes by gas chromatography, total atmospheric hydrocarbons less methane by flame ionization analyzer, total aldehydes by colorimetry, and sulfur dioxide by colorimetry or other appropriate methods.

III. ALLERGENS AND ALLIED COMPOUNDS

Although much time, money and effort have been spent on air-pollution studies, the aeromisergen conglomerate has been relatively unstudied. The following studies need to be performed in this field:

1. Collection, separation, and characterization of the individual members of the aeroallergen conglomerate.

2. Development of selective methods of assay for the various types of allergens present in airborne particulates collected indoors and outdoors.

3. Isolation and purification followed by determination of the chemical composition and overall composition of the various allergens.

4. Elucidation of the active sites in allergens.

5. Development of an automated biological particle collector to differentially count the various families of particles.

6. Creation of reliable fast analytical methods for the determination of the allergenicity of air pollutants and air particulate fractions.

7. Investigation of simple assay methods for allergen indicators, e.g., the non-allergenic air pollutants whose atmospheric concentrations are proportional to the aeroallergen concentration.

8. Creation and application of methods of characterization and assay for the various members of the aeroallergen conglomerate such as haptens, pre-allergens, co-allergens, anti-allergens, enhancers, and sensitizers. Essentially, coallergens are allergens that have synergistic effects, antiallergens are substances that decrease the activity of allergens, enhancers are non-allergens which enhance the symptoms of an allergic attack, sensitizers are non-allergens that make an individual more susceptible to the aero-allergens around him, and pre-allergens are non-allergens in the environment which become allergenic following chemical reaction.

9. Development of methods of continuous assay for the important members of the aeroallergen conglomerate so as to study more thoroughly co-allergenic, anti-allergenic, enhancing, senzitizing, and priming effects on the allergic individual. The priming effect results when contact with a small concentration of an aeroallergen sensitizes the individual to further contacts with allergen.

10. Increased study of the composition of airborne particulates and of the aeroallergen conglomerate in terms of their effect on asthma.

11. Understanding of the mechanism of the interaction of a solid particle, such as a pollen grain, with human internal tissue.

12. The structure and mode of activity of the active water-insoluble components of the allergenic bioparticles.

13. Identification of the pre-allergens in our environment, the mechanism of the toxic synthesis, and the structures of the final allergens.

A. Allergens

Aeroallergens are ubiquitous pollutants found in residences, work areas, and in the open air. Some are seasonal; others are present all year round.

Many types of airborne biological particles contain allergens. Some of the common particles of this type include pollen, fungi, house dust (a miscellaneous assortment of biological products and other types of particles), animal dander, bird feather dust, insect debris, vegetable dusts, algae, bacteria, viruses, and protozoa. Some of the most common aeroallergens are the pollens of wind-pollinated plants, and especially ragweed pollen, which is one of the main causes of hay fever. Aeroallergens are also associated with bronchial asthma.

Many people are extremely allergic (and sometimes show a strong asthmatic reaction) to the hair and dandruff of cats and dogs. In a personal communication Dr. Lloyd Monkman of Canada has pointed out that members of his laboratory staff are allergic to solvents, such as acetic acid, *n*-pentane, and benzene, in each case a different individual being involved.

The importance of this problem is demonstrated by the more than ten million people in the United States who suffer from seasonal allergic rhinitis (hay fever) and the large number who exhibit the more severe syndrome of bronchial asthma. The potency of the pollen is shown by the report that under conditions of natural exposure the inhalation of only about twenty ragweed pollen grains results in adverse symptoms in many patients.[242]

The analysis of aeroallergen pollution is based primarily on microscopic counts of collected samples from the air. In spite of its deficiencies, the "gravity slide" method for pollen sampling[243] has been accepted as the standard procedure by the Pollen Survey Committee of the American Academy of Allergy.[244] It is possible that in the near future automatic instrumentation could be developed that could collect air particles, separate them according to size, differentially stain them, and then count the particles of interest. It is also possible that the natural fluorescence of some of these particles could be used in their analysis.

A survey of the airborne biological particles containing allergens reviews the types, distribution, effect on humans, and counting techniques.[245] Emphasis is on ragweed pollen in this study.

The studies of the last 50 years to isolate and characterize the allergens in the pollen of short and giant ragweed and other plants and in other airborne biological residues have been contradictory. Many types of compounds have been "isolated" from ragweed pollen and stated to be allergens. These include proteins, glycoproteins, polypeptides, carbohydrates, lipid pigments, and combinations of these various biochemicals.[246] Since it has been extremely difficult to obtain the allergens in pure form the controversy continues, although modern research indicates the aeroallergens are probably protein, glycoprotein, and/or polypeptide in structure.

1. Proteins

Since foreign proteins can cause allergic reactions, the protein contents of the atmosphere and of particles suspended in the atmosphere are of prime interest. Atmospheric protein could be estimated through automated methods in the following fashion. The particulates would be collected, the organic nitrogen would be converted into nitrogen or ammonia, and this would then be determined by gas chromatography, colorimetry, or fluorimetry.

Another general method for determining atmospheric proteins[247] is through extraction of airborne particulates with water, evaporation, hydrolysis, and assay for the amino acids with the ninhydrin colorimetric method.[248]

The micro-Kjeldahl procedure, utilizing colorimetry with the Nessler reagent, was also used to determine atmospheric protein. From 5 to 66 μg of protein per m^3 of air was found in a grain mill area.[247] Airborne proteins have also been estimated by pyrolysis to hydrogen cyanide followed by electrochemical analysis.[249] Fluorimetric methods could also be used. The protein hydrolysate could be reacted with 2,4-pentanedione and then assayed.[250]

A little work has been done with atmospheric allergens. Two allergenic "polyglycoside peptides" have been isolated from airborne particles[251-253] and vegetable dusts and pollens[254] through the use of paper and thin-layer chromatography and electrophoresis.

The protein compositions of some airborne biological particles of hay fever and asthma interest have been reported. Extraction is a problem. The proteins of giant ragweed pollens have been extracted with aqueous dipolar aprotic solvents, Table 8.[255] An extraction-dialysis procedure was used to obtain these proteins. Aqueous pyridine extractions have also been used.[256] The proteinaceous material obtained in the latter extraction was analyzed by the standard Dumas combustion procedure[257] wherein the organic nitrogen is oxidized to nitrogen oxides which are then reduced to nitrogen and then measured in a azotometer. Alternatively, the relative amount of protein in a sample was determined after alkaline hydrolysis by the modified ninhydrin method of Moore and Stein.[258] The protein content of pollens may be as low as 11% and as high as 35%.[259]

Pollens of timothy, corn, birch, English plantain, broadleaved cottontail, hazelnut, orchard grass, sheep sorrel, sour dock, and giant ragweed have also been analyzed for protein.[260] Biuret reagent was used with readings taken at 450 nm. Algae have also been analyzed for protein with alkaline copper and phosphotungstic plus phosphomolybdic acid reagents and readings taken at 750 nm.[261]

The two most active allergens in ragweed pollen have been isolated and studied.[262-265] They are globular proteins with molecular weights about 28000. Their amino acid composition has been determined.[262] The main allergen, antigen E, comprises 6% of the soluble proteins; the other allergen, antigen K, comprises 3% of the proteins. These proteins contain less than 1% carbohydrate. Antigen E contains 90% of the activity; one picogram of this protein gives a positive skin test. A water-insoluble glycoprotein has also been isolated.[266] It represents 2% by weight of the pollen

TABLE 8

Average Amount of Protein Extracted from Giant Ragweed (*A. trifida*) (g protein/100 g protein)[255]

Solvent	Water Sol.	Ins.	Total
Ethylene carbonate-water (1:1)	22	3	25
t-Butylformamide-water (3:1)	27	5	32
Dimethylsulfoxide-water (3:1)	22	3	25
Acetonitrile-water (1:1)	12	4	16

and appears to be an extract of cell wall material somewhat reminiscent of the polysaccharide antigens isolated from pneumococcal and streptococcal cell walls. This identification of some of the allergens in ragweed pollen as proteins confirms previous work.[267-269]

2. Glycoproteins

A group of isoallergens has been isolated from rye grass pollen.[270, 271] They appear to be glycoproteins containing pentose, hexose, and heptose moieties. The heptose, found only in the allergenic fractions, was determined by reaction with cysteine in sulfuric acid and measurement of the absorbance at the 510-nm absorption peak. Starch-gel electrophoresis was used with protein staining by amido black 10B. It is believed that virtually all the allergenic activity in grass pollens may be recovered by aqueous extraction prior to, or after, defatting with ether.[270]

Airborne castor bean powder can cause allergic reactions. Its composition has been investigated. Four antigens were found in the CB-1A complex mixture of low-molecular-weight proteins and polysaccharidic proteins considered to be the principal allergens of castor benas.[272] The CB-1A mixture was analyzed colorimetrically through its Ponceau S complex. The principal antigen and a principal minor antigen were separated by cellulose acetate electrophoresis and shown to be allergenic.

A large number of atopic allergens are known which are components of biological particles found in indoor atmospheres.[273-276] Examples of atopens are house dust, trichophytin, human dandruff, succus liquiritiae, radix ipecacuanhae, and horse dandruff.[273] Atopic allergens elicit the clinical symptoms of asthma, hay fever, or atopic dermatitis in predisposed and sensitized human allergic individuals. Many of these allergens (not completely purified) contain 1-deoxy-2-ketoses conjugated in position 1 to the ϵ-amino group of lysine residues in the molecular framework of protein or glycoprotein carriers. These particular lysyl-sugar residues appear to participate in the elicitation of positive (wheal and flare) reactions during skin tests in atopic patients.[277] Berrens claims that two essential requirements are necessary to the activity of atopic allergens, e.g., incorporation of 1-amino-1-deoxy-2-ketoses in the molecular structure and a molecular weight of about 30000 to 40000.[274]

In these compounds two main types of absorption peaks are present: the peak at 278 nm derived from the tyrosine moiety, and the peak at 305 nm derived from the NH-CH=C(OH) chain involving the ϵ-amino group of lysine linked to carbon atom one of a 1-deoxy-2-ketose sugar in the 1,2-enol form. Thus, the Berrens hypothesis states that atopic allergens are formed from decomposing cellular material by Maillard reactions between peptides and aldehydosugars followed by an Amadori rearrangement of the Schiff base. In this respect, the atopic allergens show much higher A_{305}/A_{280} values than do the proteins.

Few if any of the allergens thus far isolated are completely pure. One explanation for some of the difficulty in isolation of a pure allergen is that many of these allergens may actually be intimate mixtures of physicochemically and structurally similar glycopeptides.[278]

Since the free ϵ-amino group of combined lysine could be of prime importance as a necessary structure for one type of pre-allergen, the determination of this group in our environmental mixtures could be of some importance. In this respect the 2,4-dinitrofluorobenzene procedure of Carpenter[279] has been used in a modified form to determmine "available" lysine in allergens[280] and could be used to determine this grouping in pre-allergens.

A reagent that could be used for a more sensitive determination of the free ϵ-amino group of combined lysine is 2,4-pentanedione.[250] Fluorescence analysis would then take place at F 405/470.

Since the 1-amino-1-deoxy-2-ketose moiety is claimed to be present in many types of atopic allergens, methods of analysis for this grouping could be of considerable importance. One method of analysis which has been used by Berrens is shown in Figure 8. Mild hydrolysis and dehydration are accomplished with oxalic acid at 100°. The 5-hydroxy-methylfurfural formed in the reaction is reacted with thiobarbituric acid and determined colorimetrically at 443 nm, the wavelength of maximum absorption of the reaction product.

Of great importance in the study of a chemical disease caused or aggravated through contact with an aerotoxicant is the development of a method of determining this type of biological activity in the chemicals and mixtures surrounding the human

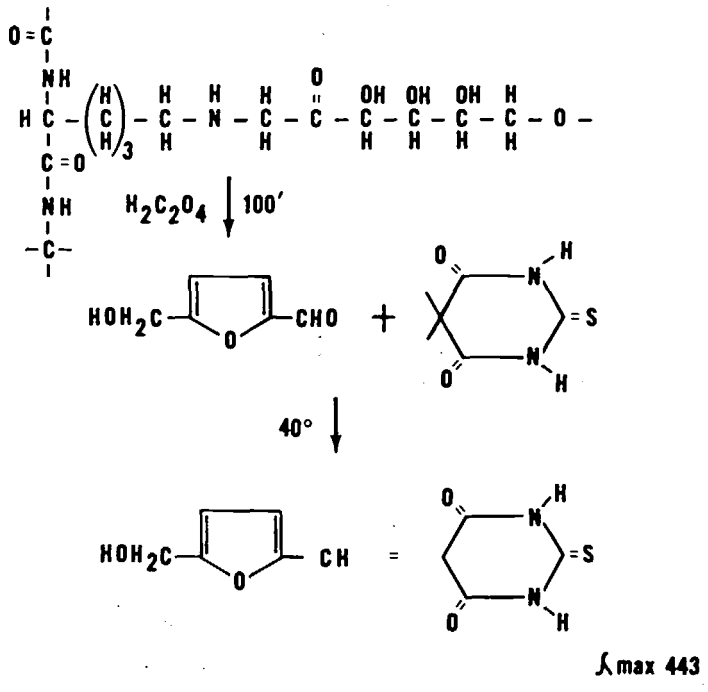
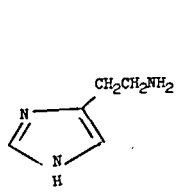
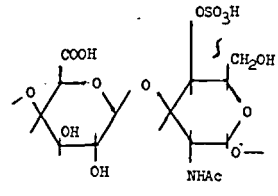


FIGURE 8. Thiobarbituric acid test for 1-amino-1-deoxy-2-ketoses in glycoprotein allergens.

being. For this purpose an appropriate living entity is necessary, preferably and if possible the human or some easily available part of him. Thus, live mammals (usually mice) are used in the determination of the carcinogenic activity of air pollutants, such as organic airborne particulates, auto exhaust particulates, benzo[a]pyrene, etc. Humans cannot be used because of the long duration of the experiment (10-60 years) and the immorality of such a test. However, animal experiments, although useful, involve a skeptical leap of faith.



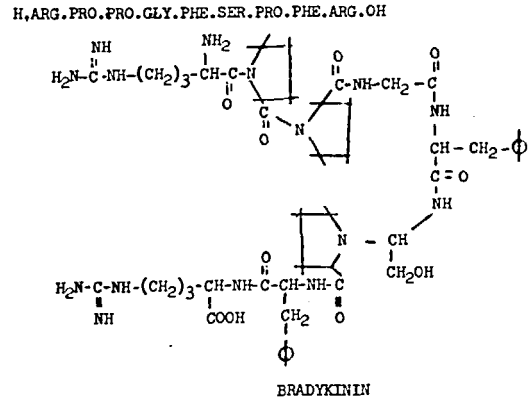
HISTAMINE
I



MUCOPOLYSACCHARIDE
II

3. Bradykinin, etc.

For allergic reactions an appropriate chemical test for biological activity of an air pollutant would be to determine the "caustic" chemical produced during the allergic reaction in a human being. Some of the "caustic" chemicals which could be released during such an attack would include histamine, I, a powerful vasodilator which is released in anaphylactic shock and can occur in blood urine and tissues; the highly acidic mucopolysaccharides, II, which cause edema, fibrosis, etc.; and the highly basic polypeptides, such as bradykinin, III, which causes vasodilation, itching, and increased capillary permeability in tissues.



BRADYKININ
III

Some of the available methods of in vitro allergen analysis include antibody precipitation, electrophoretic methods, column chromatography and dialysis, histamine release from human leukocytes, immunodiffusion, and radioimmune diffusion.

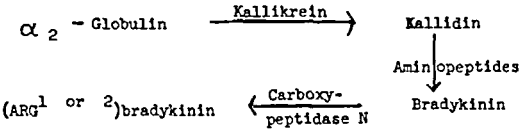
With a greater knowledge of the physiological properties of aeroallergens more knowledge can be derived about the allergenic and asthmatic reactions and better methods of assay can be developed for the aeroallergens.

Thus, trypsin cleaves proteins at the carboxy end of lysine or arginine. With the increase in the lysine ϵ-amino group there is greater cleavage.[280] With an increase in the combined lysine, LYS-NH-CH=COH-, there is an increase in the A_{305}/A_{280} ratio and a decreased cleavage of the peptide portions of these types of allergens according to Berrens.

Berrens has also claimed that atopic allergens can inhibit lysine and arginine peptide hydrolysis.[281,282] Thus, the hydrolysis of substrates such as N-lysyl- or N-arginyl-2-naphthylamine by serum naphthylamidases is inhibited by atopic allergens or compounds containing the protein - LYS-NH-CH=C(OH)-sugar structure. It is possible that colorimetric or fluorimetric methods for 2-naphthylamine could be used in the assay for such allergens.

The release and/or blockade of enzymatic activity is believed to be one of the essential features of the allergic reaction.[283] Enzymes cleaving N terminal lysine residues appear to be of particular interest here because this type of enzyme is involved in the physiological breakdown of the vasodilating and smooth-muscle-stimulating decapeptide kallidin (lysyl-bradykinin), which has been implicated in the response to the initial trigger of the allergen-antibody ("reagin") interaction in epithelial tissues.[281] It has been reported that activated plasma or tissue kallikreins act upon an α_2-globulin substrate to release kallidin first, and that this must be converted into bradykinin before the pharmacological activity can be completely abolished.[284] It is suggested that the inhibitory action of atopic allergens may, as a side effect, impair the ability of allergic individuals to inactivate kallidin generated in response to the initial allergen-reagin interaction.[281]

Thus, the following tentative first step mechanism is suggested[281] wherein the presence of atopic allergens inhibits the formation of the physiologically inactive des (arg) bradykinins and the "caustic" bradykinin accumulates.



Obviously, chemical or physical methods of assay for these peptides and enzymes would be of importance, and methods for bradykinin would be especially valuable. Kallikrein has been assayed spectrophotometrically by means of its ability to catalyze the hydrolysis of α-N-benzoyl-L-arginine ethyl ester.[285] Unfortunately, analysis is at 253 nm, at which wavelength both the ester and benzoly-L-arginine absorb with millimolar absorptivities of 2.3 and 3.45, respectively. Obviously a better method is needed.

4. Histamine Release

The assay of aeroallergens and the determination of the allergenicity of air pollutants could be accomplished through the assay for histamine released from human (or animal) leukocytes in contact with the allergen. The release of histamine from sensitized human leukocytes reacting in vitro with specific allergens was first described by Katz and Cohen in 1941.[286] Since then many other studies on histamine release have been published.[287-294]

Histamine is virtually absent from human plasma, platelets, erythrocytes, or lymphocytes.[295] Approximately half the total amount is found in the basophils, the remainder being distributed among the eosinophils and neutrophils. Ninety to 100% of the total cellular histamine can be released in an assay. Five to 10^4 pg of allergen can release 50% of the cellular histamine; 10^4 to 10^6 moles of histamine are released per mole of allergen. The substitution of rabbit blood for human blood in histamine release studies has the advantage of the "enormous amounts of histamine in rabbit blood."[295]

Extracts of some textile dusts are capable of producing histamine release from lung tissue in vitro.[296] Histamine release by other types of air pollutants has also been studied.[297]

In most of the procedures[288,295] the released histamine is usually assayed by the spectrophotofluorimetric *o*-phthalaldehyde technique of Shore et al.[298] as modified by Kremzner and Wilson.[299]

Many other methods are available for histamine assay, some of which have been used. These methods are compared in Table 9.[300]

Of the colorimetric methods the 4-dimethylaminocinnamaldehyde and the azobenzenediazonium fluoborate methods, which are shown in Figure 9, are the most sensitive and are highly reproducible with good color stability. Under present conditions only the *o*-phthaldehyde method has the requisite sensitivity for use in histamine-release studies of aeroallergens. However, the applied procedure is too long and complicated and wasteful of material. As the basic procedure is improved, analysts should be able to apply some of the other methods in the assay of aeroallergens, coallergens, antiallergens, enhancers, and sensitizers by the histamine-release procedure.[300]

5. Coallergens and Antiallergens

Practically no work has been reported on the analysis of coallergens and antiallergens. As far as other types of allergy effects are concerned, it would appear that the main effect of some of the common air pollutant gases on human health is in their effect on the allergic or asthmatic individual. Thus, higher concentrations of ozone, as found in some confined atmospheres, could sensitize the allergic individual to the aeroallergens in his environment. Atmospheric SO_2 probably enhances the allergic or asthmatic symptoms of many individuals suffering these symptoms. Methods of assay for these pollutants will be discussed in a later section.

6. Indicators

Compounds present with allergens in biological particles could be used as indicators of the allergens. Thus, rapid, simple, sensitive and selective methods of assay for families of compounds, or for prominent individuals, present in the important atmospheric bioparticles are needed. Wet and, eventually in some cases, automated methods need to be developed for the analysis of appropri-

TABLE 9

Comparison of Methods for Determination of Histamine[300]

	Reagent	λ max.(mε) or F exc/emis	Sens.[a]	Detn. limit,[b] μg	Color Stability min.	Beers law range, μg	Rel. Std. Dev., %	Anal. time, min.
1.	Sulfanilic acid	488(8.6)	3.4	17	<60	25–110[c]	± 4	6
2.	p-Nitrobenzenediaz. fluoborate	a460(15.4) b386(13.8)	5.1 4.3	2 3	20d	2–72.4	± 1.1	5 8
3.	4-Azobenzenediaz. fluoborate	a562(52.0) b418(55.0)	36 38	0.9 0.9	30	0.9–23	± 2	10
4.	2,4-Dinitrofluoro-benzene	357(16.1) 420s(5.6)[e]		6 18	<120			21
5.	4-Dimethylamino-benzaldehyde	560(25.8)	10.3	1.8	f	1.8–24	± 2.2	8
6.	4-Dimethylamino-cinnamaldehyde	639(62.5)	25	0.8	15	0.8–21	± 1.2	8
7.	o-Phthalaldehyde	F350/445		0.007	30	0.007–0.15	± 2.6	10
8.	Acetoacetaldehyde dimethylacetal	F405/485		0.6	15	0.6–16	± 4.5	25

[a] Sensitivity = mε/Dilution factor where dilution factor equals final vol/test solution volume

[b] At absorbance = 0.1

[c] Linear over this range. Does not obey Beers law.

[d] Fades 6% in 1 hour.

[e] Read at this wavelength because of interference of excess reagent at wavelength maximum.

[f] Read immediately because color fades 6 to 8% per minute.

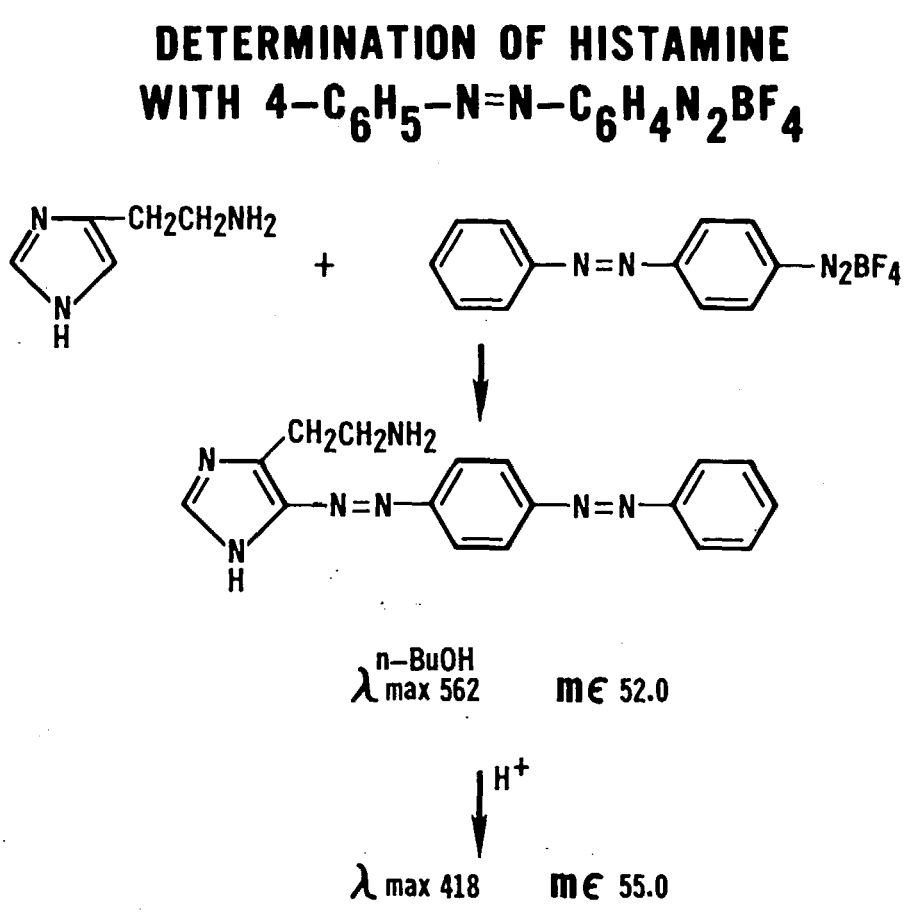


FIGURE 9. Determination of histamine with 4-azobenzenediazonium fluoborate. With permission.

ate groups of hydrocarbons, flavonones, carbohydrates, proteins, amino acids, nucleic acids terpenes, steroids, fats, etc., present in the bioparticles of interest.

Obviously, much work needs to be done in the field of aeroallergens. It is surprising that so little has been done considering the extensive ramifications of the allergy problem as it affects human health.

IV. ALKYLATING AGENTS, MUTAGENS, PESTICIDES AND ALLIED COMPOUNDS

A. Pesticides

Many varied methods of analysis are available for pesticides found in agricultural products, in soil, and in water.[301,302] Infrared, colorimetric, fluorimetric, and gas chromatographic methods have been used with the greatest reliance placed on the last of these. Except in unusual circumstances, the concentrations of pesticides in the atmosphere are much lower than those found in water, food, and soil. Analysis of atmospheres in communities where fogging operations took place have been reported.[303] Benzene extracts of air particulates were analyzed by gas chromatography with electron capture and sodium thermionic detectors. Obviously, some pesticides could not be collected quantitatively on glass-fiber papers by the high-volume technique.

As with too many other compounds gas chromatographic characterization and assay of sulfur, phosphorus, and chlorinated insecticides has depended on the belief that elution time unequivocally identifies the test compound. Unfortunately, this has too often been untrue. Consequently, artifacts have been assayed and quite a good many of the gas chromatographic literature data are in error. It is absolutely necessary in many of these

assays to make use of mass spectrometry to ensure accuracy.

B. Alkylating Agents

A large number of alkylating agents, many of which are mutagens and/or carcinogens, are known.[10, 304, 306] Individual alkylating agents have not as yet been identified as atmospheric components. However, qualititive tests with 4-(4-nitrobenzyl)pyridine and 4-acetylpyridine-4-nitrophenylhydrazone showed the presence of alkylating agents in organic airborne particulates.[46, 47] A large number of colorimetric and fluorimetric methods of analysis are available and have been used for the analysis of alkylating agents.[46, 47, 307] Gas chromatography can also be used, as it has been for the nitrosamines.[308]

As has been shown, two pollutants can be taken into the body from at least two different sources and cause a synergistic or enhancing effect of the physiological activity of one of the pollutants. It should also be possible for two pollutants taken into the body from two different sources to interact to form a much more physiologically active compound.

Secondary amines do react with nitrite to form nitrosamines [which are carcinogenic in a wide range of organs of various species[309]] in conditions similar to those in the mammalian stomach[310] and in gastric juice.[311, 312] It has been suggested that such in vitro and in vivo interactions might be significant to man.[311, 313] It is possible that individuals taking in nitrite precursors, such as atmospheric nitrogen dioxide and other nitrogen oxides and nitrite as well as secondary amine precursors and secondary amines, stand a risk of continual contact with these types of lethally-synthesized alkylating agents and eventual cancer. The entire environment can contribute to these types of risks since pollutants in air, water, food, medicine, etc. could play a role here.

V. LACHRYMATORS

The most bothersome manifestation of photochemical smog is eye irritation. The components of polluted air that cause eye irritation have not been entirely identified but, on the basis of laboratory experiments, are believed to be formaldehyde, acrolein, peroxyacyl nitrates, and other products of the oxidation of unsaturated hydrocarbons.[314-318] Some of the most important precursors of eye irritants are aromatic hydrocarbons with olefinic or paraffinic side chains.[55] All produce formaldehyde to a varying degree, and some also produce peroxyacetyl nitrate (PAN). It is possible that peroxybenzoyl nitrate could also be produced. This compound has not been demonstrated in the atmosphere but is two hundred times as potent a lachrymator as formaldehyde.[55] Other lachrymators which could be produced in sunny areas heavily polluted by automotive sources are the α-halo aralkyl ketones.[319] These compounds are alkylating agents and could be analyzed on the basis of that property.

A. Total Aldehydes

A few general methods are available for the determination of "total aldehydes" in the atmosphere. Titrimetric methods have been used but are not very sensitive or highly selective. Three types of titrimetric procedures have been used for auto exhaust samples. These include oxidation by alkaline hydrogen peroxide to carboxylic acids which are then titrated, and also reduction of silver oxide to silver followed solution of the silver and titration with thiocyanate.[320] The bisulfite procedure has been widely applied in air pollution and industrial hygiene. Ketones can also react to some extent. An addition compound is formed with bisulfite, excess bisulfite is destroyed, the combined bisulfite is liberated, and the bisulfite ion is titrated with standard iodine solution. The method has been used for atmospheric samples,[321-323] automobile exhaust,[324, 325] Diesel exhaust,[326, 327] and backyard incinerators.[328]

The *p*-rosaniline-sulfur dioxide procedure has been applied to the continuous monitoring of ambient atmospheres for aldehydes.[329] This method is more sensitive for formaldehyde than for the other aldehydes.

Probably the best colorimetric method available for total aldehydes utilizes 3-methyl-2-benzothiazolinone hydrazone (MBTH) as the reagent.[330] The three main aldehydes present in the atmosphere give the following millimolar absorptivities at a wavelength of 670 nm: formaldehyde, 65; acetaldehyde, 51; and acrolein, 23. With further modification, the second and third of these values could be increased. However, these differences are not too serious since total atmospheric aldehydes are composed of at least 50% formaldehyde and

about 10 to 15% acrolein.[331] The MBTH method has been modified so as to increase its sensitivity[332] and has been applied to the analysis of the atmosphere [333-340] and of automotive emissions.[341] A simplified and improved modification of the MBTH test has been used to determine water-soluble aliphatic aldehydes in atmospheric dusts.[342]

B. Formaldehyde

A large number of colorimetric and fluorimetric methods are available for the determination of formaldehyde. Some of the colorimetric reagents which can be used include chromotropic acid,[327, 343-357] J-acid,[356] phenyl J-acid,[356] 2-hydrazinobenzothiazole,[358] 2,4-pentanedione,[359] *o*-aminobenzaldehyde,[360] 1-ethylquinaldinium iodide,[361] *p*-rosaniline,[329, 362] and phenylhydrazine.[320, 363-368] Some of the fluorophor-forming reagents for formaldehyde which can be used include 2,4-pentanedione,[369] dimedone,[370] 1,3-cyclohexanedione,[370] and J-acid.[361]

Formaldehyde has been determined in auto exhaust with the chromatropic acid[357] and phenylhydrazine[320, 368] methods, in Diesel exhaust with the chromotropic acid[327,349-351] and phenylhydrazine[369] methods, in incinerator effluents with chromotropic acid,[353,354] and in the urban atmosphere with *p*-rosaniline[329, 362] and chromotropic acid.[321,344,347,348]

Of the sulfuric acid methods (chromotropic acid, J-acid, and phenyl J-acid), chromotropic acid is the one that is the least sensitive and that has seen the most use. The chromotropic acid procedure has been recommended as a tentative method for atmospheric formaldehyde and, except for precursor interferences, has been discussed thoroughly.[371] The chemistry of the color reaction as given is in error.[372] The reagents are highly selective for formaldehyde, but under the conditions of the test a large number of compounds can form formaldehyde and thus react. It could be that the atmospheric formaldehyde determined by this method could include combined formaldehyde. This seems reasonable since, in the analysis of diluted and diluted irradiated automobile exhaust, formaldehyde concentrations determined by the chromotropic acid method were 10 to 20% higher than those obtained by the 2-hydrazinobenzothiazole method.[357]

Of the hydrazine methods 2-hydrazinobenzothiazole is the most sensitive. Other aldehydes react to some extent. However, in the *p*-rosaniline methods acetaldehyde and propionaldehyde also react.

Although it is highly sensitive, the 1-ethylquinaldinium iodide method has never been tried in air-pollution studies.

Of the diketo reagents 2,4-pentanedione is the most highly selective. In fact, it is probably the most highly selective of all the reagents used in the determination of formaldehyde. For this reason the highly sensitive fluorimetric procedure should be applied to air pollution analyses.

C. Acrolein

Fairly complicated paper chromatographic,[373] gas chromatographic,[325, 374] and polarographic[375] methods have been used in the analysis for acrolein in automobile exhaust and in the atmospheres of paint and varnish plants.[376]

Colorimetric methods using phloroglucinol[377] and tryptophan[378, 379] lack sufficient selectivity and sensitivity to be useful in air analysis.

Other reagents which have been used in the colorimetric determination of acrolein include 4-hexylresorcinol,[380, 381] and anthrone;[382] fluorimetric reagents include J-acid[361] and anthrone.[383] The J-acid method is the most sensitive; 10 ng of acrolein can be determined. However, equal or greater amounts of formaldehyde interfere. In the anthrone methods crotonaldehyde and methacrolein interfere.

The most highly selective method for acrolein is the 4-hexylresorcinol procedure[381] as applied to air analysis.[380] A blue trimethine cation is formed absorbing at 605 nm, mϵ 20.0. Sulfur dioxide, nitrogen dioxide, ozone, ketones, olefins, and many types of aldehydes do not interfere.[340,380] Slight interferences are found with some dienes.[382] Acrolein and malonaldehyde precursors could interfere here.[384] This method has been used in the analysis of automobile exhaust,[357, 380] Diesel exhaust,[327,349,351] and atmospheric samples.[317,334]

The C_2-C_5 aldehydes have been determined in industrial emissions by gas chromatography using hydrogen flame detection and dinonyl phthalate on fire brick in the column.[385]

D. Peroxyacyl Nitrates

These compounds were originally called compound X,[386, 387] then peroxyacyl nitrites,[388]

and finally peroxyacyl nitrates.[389] They are believed to be both lachrymators and phytotoxicants. They were detected in the atmosphere and partially characterized with long-path infrared spectrometry.[386-388] The infrared bands at 8.6 and 12.6 μm are used in this type of analysis.[388] In recent years electron-capture gas chromatography has permitted measurements of these compounds in ambient polluted air at concentrations below 0.01 ppm.[389-391] Two- to three-milliliter samples of air containing 5 $\mu g/m^3$ (0.001 ppm) PAN can be analyzed. An automated gas chromatograph has been used.[391] The principal members present in atmospheric samples are peroxyacetyl nitrate and peroxypropionyl nitrate, the latter present at about one eighth the concentration of the former.

VI. PHYTOTOXICANTS

A. Ethylene

Samples of this gas can be collected before assay by grab sampling into an evacuated container,[392, 393] in freeze traps,[394] in mercuric solution,[395] on silica gel,[396] or can be analyzed directly.[167,397,398]

Detector tubes have been used in the estimation of atmospheric olefins.[396, 399, 400] Ten-thousand-and three-thousand-cubic-centimeter air samples can be analyzed for minimum amounts of 23000 and 10 $\mu g/m^3$ of ethylene.

A portable instrument is available which is based on the reaction of ethylene with mercuric oxide at high temperature to give mercury vapor which is then passed over selenocyanate paper to give a black coloration.[401]

Mass spectrometry has been used to determine ethylene in automobile exhaust[402,403] and in the atmosphere.[394]

Ethylene can also be determined by infrared spectroscopy in air,[387] Diesel exhausts,[392] automobile exhausts,[404, 405] and in incinerator effluents.[328, 406] The method does not have adequate sensitivity except when the long-path infrared cell is used.[387] The absorption peak at 10.5 μm is normally used, and a sensitivity better than 0.1 ppm is obtained.

The most popular method of assay is through gas chromatography.[407-409] A silica-gel-packed column at or near room temperature with a flame ionization detector gives rapid efficient separation with a sensitivity in the ppb range.[167, 393, 410-413] Other columns that are used include alumina,[414] dimethyl sulfolane,[349] hexadecane,[392] polypak-2,[398] multicolumn techniques,[415, 416] and open tubular columns.[397, 417] Gas chromatography is currently used to determine ethylene as, for example, in air,[51, 167, 411] auto exhausts,[397, 411, 413, 415] municipal wastes,[398] agricultural wastes,[414] and incinerator effluents.[392]

A review on ethylene as an air pollutant is available.[418]

B. Sulfur Dioxide

The properties of this air pollutant as a phytoxicant, an allergy enhancer, and a possible killer in a few intense air-pollution episodes make knowledge of its concentrations in the atmosphere a necessity.[419, 420] A bewildering number of publications are available on numerous modifications of a large number of methods for the determination of the common inorganic atmospheric air pollutants. Sulfur dioxide definitely falls in this class. The methods capable of measuring ambient concentrations of this aerotoxicant include conductometry;[421-424] coulometry;[425-427] titrimetry,[428,429] colorimetry with *p*-rosaniline,[430-437] fuchsin,[438-441] molybdate,[442] barium chloranilate,[443, 444] ferric iron and 1,10-phenanthroline,[445] iodine-starch,[446-450] and 4-aminoazobenzene;[451] turbidimetry;[452-454] polarography,[455] sulfation of lead dioxide (not an air-concentration measurement);[456-461] infrared interference spectrometry;[462, 463] correlation spectrometry at 300 nm;[464, 465] flame photometric detection[466] alone or after gas chromatography;[467] fluorescence decrease with 5-aminofluorescein;[468] and quadrupole mass spectrometry. Many of these wet and dry methods for measuring and monitoring atmospheric and effluent sulfur dioxide have been discussed.[469-472] Correlation studies between many of the more popular methods badly need to be done. Unfortunately, the correlation among the few of these methods which have been studied is not too good.[467,473,474]

Conductometric and colorimetric monitoring instruments have been the most popular in the continuous measurement of atmospheric sulfur dioxide. Among the non-automated methods the lead dioxide candle and the *p*-rosaniline colorimetric methods have seen extensive use.

In the conductometric methods the sulfur dioxide is oxidized by hydrogen peroxide to sulfuric acid; the increase in conductivity is then proportional to the amount of sulfur dioxide absorbed by the scrubbing solution. Some of the continuous monitoring instruments using this technique include the Thomas Autometer, the Davis Emergency Equipment Co. Monitor, and instruments manufactured by the Beckman Instrument Development Co., Research Appliance Co., Scientific Instrument Co., Industrial Scientific Co., and Scientific Industries, Inc. The specificities and accuracies of these instruments leave much to be desired.

In the coulometric method the sulfite ion is oxidized by bromine to sulfate and bromide ions, bromine is regenerated by electrolysis at the generator electrode, and the current required to regenerate the bromine is directly proportional to the amount of absorbed sulfur dioxide. Some of the continuous monitoring instruments using a similar principle include the Beckman, the Titrilog, and the Philips coulometric analyzers. The latter has been recommended as the most trouble-free and drift-free wet chemical method experience by the investigators.[471] Dr. Lloyd Monkman, in a personal communication, has stated that he has had very good results with an Atlas coulometric instrument using a reagent system based on triply distilled water.

In the titrimetric method sulfur dioxide is oxidized by hydrogen peroxide to sulfuric acid which is then titrated by alkali.

Of the colorimetric methods the *p*-rosaniline method[430,475] is the most popular. The method consists of the addition of the $-CH_2SO_3H$ group (from CH_2O and SO_2) to one, two, or three amino groups of the heavily protonated decolorized dye to form a highly colored dye (λ_{max}=575 nm, mϵ=37.0) containing two amino groups with basicity decreased enough so that they can act as resonance terminals in the highly acid solution. Interferences in the procedure have been neutralized in the following ways. The gas is collected in sodium tetrachloromercurate (II) which combines with sulfur dioxide to give a complex stable toward the oxidizing agents in the solution, ethylenediamine tetraacetate is added to complex the oxidizing heavy metals, enough time is allowed to elapse to ensure decay of ozone, and sulfamic acid is added to destroy the nitrite. Temperature, pH, and purity of the reagent have to be controlled. The dye is difficult to purify.[435,475,476] In addition, there is the problem of the nonstoichiometry on three active sites of the *p*-rosaniline molecule.[477] It would be no difficult matter to replace the *p*-rosaniline with a more readily purifiable reagent, such as a highly colored conjugated amine or a cationic resonance dye with two amino groups as resonance terminals. With such a reagent greater sensitivity and reproducibility could be obtained.

Some of the continuous monitoring equipment using colorimetry include the Technicon, the WACO, Precision Scientific Development Co., and the Kimoto Electric Co. instruments. The continuous determination of atmospheric sulfur dioxide by the *p*-rosaniline method has been discussed.[476a]

In the molybdate procedure, sulfur dioxide is reduced to hydrogen sulfide, which reacts with molybdate to form a blue-violet complex. In the phenanthroline procedure, sulfur dioxide reduces ferric iron to ferrous iron, which combines with the phenanthroline to give an orange complex. In the iodometric procedures, the reduction in the color of the iodine-starch complex is measured. The *p*-aminoazobenzene procedure is similar to the *p*-rosaniline procedure, except that the reagent is easily purified and the stoichiometry of its reaction with sulfur dioxide is theoretically 1:1. The turbidimetric method involves oxidation of sulfur dioxide to sulfate, reaction of sulfate with a barium salt, and measurement of the turbidity. The lead peroxide candle method involves oxidation of sulfur dioxide to lead sulfate and the gravimetric or colorimetric determination of this material.

Dry automated methods of analysis for sulfur dioxide will receive greater study in the future. Thus, a commercial model of a multiple scan infrared interference spectrometer has been developed by Block Engineering Corp. and has been utilized in the remote detection of sulfur dioxide in stack effluents of power plants. An open-path instrument based on correlation spectrometry using the fine-structure bands in the 300-nm region can determine low concentrations of sulfur dioxide over long path lengths. The use of gas chromatography and the flame photometric detector needs to be evaluated for the determination of sulfur dioxide as does that of quadrupole mass spectrometry (e.g., the commercially available EIC

Pollution Analyzer) not only for sulfur dioxide but also for other gaseous air pollutants.

The potential value of the attenuation of infrared laser lines for the determination of sulfur dioxide and other gaseous pollutants is derived from the narrow band width of the infrared laser lines and the fine structures in the infrared spectra of the gaseous pollutants.[478,479] The gas-filter cross-correlation method of detection also appears to have promise in this field.[480] These potential methods for the dry determination of sulfur dioxide need further development and thorough investigation of their strengths and shortcomings.

As yet highly sensitive fluorescent methods for the determination of sulfur dioxide have not been developed. The only method available in this field is the 5-aminofluorescein method, which involves the difficult measurement of a decrease in fluorescence. The development of highly sensitive fluorimetric methods of determining sulfur dioxide and the other gaseous air pollutants should not be too difficult a matter and this goal is well worth pursuing.

C. Ozone and Oxidants

This important pollutant is a definite aerotoxicant. It is a well known phytoxicant, probably a powerful allergen sensitizer, causes headaches and nausea in chemists using instrumentation that produces this gas, causes eye irritation, and aggravates respiratory diseases such as asthma. Many of these problems and the data on atmospheric oxidants have been discussed.[481]

Most methods for the analysis of ozone are based on its oxidizing properties. The simplest and least reliable involves cracking and deterioration of rubber by atmospheric ozone.[482,483] In spite of its many shortcomings the potassium iodide method has been the most popular method for the determination of atmospheric oxidants. Acidic,[484] alkaline,[485] and neutral buffered potassium iodide [486-492] have been used. The neutral potassium iodide method has been preferred on the basis of a claim that it is more stable, precise, and sensitive.[454,487,492] Titration[492] coulometry,[489,494-500] and colorimetry[484-491] have been used in this method.

Since ozone analysis suffers from the need of a reliable primary standard, the manual neutral potassium iodide method, in spite of its shortcomings, has become the unofficial reference method for calibrating ozone sources and oxidant methods. The stoichiometry of this reaction has been investigated;[493] in a neutral solution 1.54 mole of iodine is liberated per mole of ozone absorbed. The stoichiometry of this reaction needs further investigation.

In the titration method sodium thiosulfate is added to the triiodide solution and the excess thiosulfate is titrated. The coulometric methods are based on electrolytic conversion of triiodide to iodide ion by cathode reduction utilizing either an electrolytic or a galvanic cell.[495] There are two versions of commercial coulometers based on the type of cell used. Commercial versions of the Brewer cell are available from the Atlas and Mast Instrument Companies; one of the Hersch cell is available from Beckman. Most of the colorimetric potassium iodide methods involve the determination of the triiodide ion at about 352 nm.[487-490] Commercial analyzers using this technique include the Litton Industries, Beckman, and Technicon instruments. Alternatively, the iodine can be complexed with starch and measured colorimetrically.[491]

Atmospheric oxidants and reductants can be serious interferences in the determination of ozone by the iodide methods. Interference due to sulfur dioxide can be eliminated by prior treatment with permanganate[494] or hydrogen peroxide.[485] Alternatively, a good blank can be obtained by removal of ozone from half the sample; permanganate,[501] 2,3-dimethyl-2-butene,[502] and treated cotton wool[491] have been used for this purpose.

The sensitivity and reproducibility of the iodide methods could be improved considerably by reacting the iodine with an appropriate reagent to give a chromogen (or fluorogen) absorbing (or emitting) with greater intensity at longer wavelengths. For example, the large number of reagents developed for the determination of nitrite[503,504] could be modified for the determination of iodine. In this sense 4,4'-bis-(dimethylamino)thiobenzophenone has been used for the determination of iodine; it gives a band at 648 nm with $m\epsilon$=45.[503] This could be improved. Another possibility is the formation of highly colored free radicals.[504] Analysis could be by colorimetry or paramagnetic resonance spectroscopy. Reagents such as N,N,N',N'- tetramethyl-*p*-phenylenediamine, etc. could be used here. Alternatively, ozone could be measured directly with these free-radical precursors. Reagents such as bis-(1-methyl-2-quinolone)azine and bis-(3-methyl-2-benzothi-

azolinone)azine could be used in this fashion. Reagents which give stable free radicals could be used; these include octachlorophenothiazine[504a] and dinaphtho[2,3-c:2',3'-h]phenothiazine.[504b]

Other methods of assay for ozone include the NO_2-equivalent method, in which NO is oxidized by ozone to NO_2 and the latter is measured colorimetrically.[505] The blank could be a serious problem here. The ferrous thiocyanate procedure involves oxidation by ozone to the colored ferric thiocyanate.[506-509] The molar absorptivity changes drastically with the concentration of ozone. Anything that oxidizes or reduces the ferrous ion would interfere. The estimation has been done in this method with impregnated paper.[507] Alternatively, the ferric ion formed in the oxidation could be determined with some of the recently developed reagents which combine with this ion to form brilliant chromogens, or the ferric ion could be determined by oxidation of a free-radical precursor.

Other methods of determination are based on the oxidation by ozone of leuco or dihydro forms of chromogens such as phenolphthalein, methylene blue, and indigosulfonic acid;[510] leuco forms of fluorogens such as fluorescein[511] and acridine;[512] and diarylamines such as 2-anilinonaphthalene[513] and sodium *p*-diphenylaminesulfonate.[514] All of these methods suffer from interference from oxidizing and reducing agents.

A somewhat similar method involves the oxidation of 3,5-diacetyl-1,4-dihydrolutidine.[515] A solution of this compound has an affinity for ozone five hundred times as large as one of iodide. Peroxides and nitrogen and sulfur dioxides interfere little. Ozone is determined by measuring the decrease of absorbance at 412 nm. However, since the reagent is fluorescent, the decrease of fluorescence could be measured.

Another group of methods used in ozone analysis depends on the oxidation by ozone of compounds containing a R-CH=CH-R' grouping, thereby producing the aldehydes RCHO and R'CHO. The first of this series uses 4,4'-dimethoxystilbene as the substrate[516] and determines the resultant p-anisaldehyde by the fluoranthene test for aromatic aldehydes.[517] Since some of the reagents are caustic and sensitive to water, a more practical procedure was developed using 1,2-di-(4-pyridyl)ethylene as the substrate and 3-methyl-2-benzothiazolinone hydrazone (MBTH) as the reagent for the determination of the resultant 4-pyridinealdehyde.[518] Further investigations indicated that there were no interferences during a 30-min sampling time, and that only 1-hexene, hydrogen peroxide, and peracetic acid could interfere during a 24-hr sampling period.[519] This procedure has been further improved by substituting propionic acid for acetic acid.[492] Alternatively, the 4-pyridinealdehyde formed after oxidation could be determined with 2-diphenylacetyl-1,3-indandione-1-hydrazone.[520] The fluorescent azine could then be determined at F 470/530. Since the reagent also fluoresces (F 430/520), this interference would have to be overcome. Obviously, ozone could be determined through the oxidation of a reactive alkene (such as *trans*-2-butene[502]) to aliphatic aldehydes. The aldehyde(s) could then be determined by one of the sensitive colorimetric or fluorimetric methods described in this review. These methods are highly selective for atmospheric ozone. The MBTH method has been the most thoroughly evaluated and certainly needs to be investigated by a variety of laboratories.

A group of methods (mainly colorimetric) for the analysis of oxidants has been evaluated,[508] as have seven methods for the determination of ozone at low concentrations.[521] The wavelengths of maximum absorption and millimolar absorptivities of some of the colorimetric methods are given in Table 10.

TABLE 10

Spectral Properties of Reagents Used in the Colorimetric Determination of Ozone

Reagent	λ max, nm	mε	Ref.
Sodium *p*-diphenylaminesulfonate	593	2.5	(514)
3,5-Diacetyl-1,4-dihydrolutidine	412	7.7	(515)
Neutral KI	352	24.2	(521)
1,2-Di-(4-pyridyl)-ethylene	442	26.2	(518)
Phenolphthalin	545	26.9	(521)
Ferrous + thiocyanate	481	~30.0[a]	(509)
4,4'-Dimethoxystilbene	610	35.0	(516)
NO_2-equivalent method	540	38.2	(505)

[a] At ozone concentrations below 0.1 ppm; mε = 15 at 3 ppm ozone.

A variety of methods is available for the chemiluminescent determination of ozone. Thus, measurement is made of the chemiluminescence produced by the reaction of ozone (a) with luminol in the presence of hematin,[522] (b) with rhodamine B in the presence of gallic acid,[523] (c) with rhodamine B adsorbed on a surface, [524,525] and (d) with nitric oxide at low pressures.[526] The luminol method is sensitive to 5 ng of ozone and permits the determination of ozone at concentrations of 0.3 μg/liter of air. The gallic acid method involves the oxidation of gallic acid; the breakdown product(s) transfer their absorbed energy to rhodamine B which then emits it, as shown in the equation.

$$\text{Gallic acid} + O_3 \longrightarrow A^* + O_2$$

$$\text{Rhodamine B} + A^* \longrightarrow \text{rhodamine B}^* + B$$

$$\text{Rhodamine B}^* \longrightarrow h\nu + \text{rhodamine B}$$

The adsorbed rhodamine B method is a gas-phase reaction in which the intensity of the light emitted is measured with a phototube. The nitric oxide method is also a gas phase reaction. It is believed that nitrogen dioxide and sulfur dioxide do not interfere in the chemiluminescent methods. These methods, and especially the dry procedures, certainly need to be thoroughly studied since they could be more readily monitored. However, the strengths, and especially the shortcomings, of the chemiluminescence methods need more thorough investigation.

Long-path infrared spectrometry has been recommended as a specific method for determining atmospheric ozone.[527] Its sensitivity is poor, but with the help of laser techniques use could be made of the distinctive principal band near 9.6 μ which is quite free from interference by the bands of other atmospheric constituents. Thus, ozone may be an excellent candidate for the laser method of analysis since many strong CO_2 bands fall within the ozone band.[528]

A long-path ultraviolet spectrophotometer has also been used to determine ozone[501,529] which absorbs at 250 to 260 nm.[530] With a double-beam instrument use could be made of ozone-eliminating procedures so as to get a better blank and make the method more selective.[501]

An ozone analyzer has also been developed which measures ozone by running a gas stream over a Kr^{85}-containing quinol clathrate and observing the amount of radioactivity released, Figure 10.[531] The method is stated to be a hundred to a thousand times as sensitive as the iodide methods. However, nitrogen dioxide also reacts.

A quadrupole mass spectrometer can be used to measure atmospheric ozone, as well as other air pollutant gases. A commercial instrument, the Environmental Instruments Co. Pollution Analyzer, Model A-10, makes use of this principle. This instrument is said to be capable of being programmed for rapid repetitive monitoring and to be readily interfaceable with telemetry and computers for remote operation. Ambient air containing 1 ppm or less of ozone (and many other gaseous pollutants) can be assayed continuously. The evaluation of this type of instrumentation and the comparison with other methods of assay certainly need study.

Other complementary reviews on the determination of ozone and oxidants treated from entirely different viewpoints are available in the literature.[525,532,533]

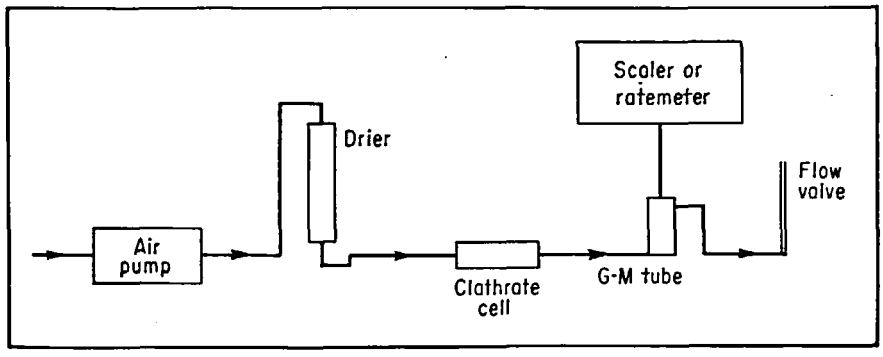


FIGURE 10. Principle of Hommel, Chleck, and Brousaides Ozone Analyzer. Air is dried and passed over Kr^{85}-containing clathrate. Ozone attacks the clathrate, releasing Kr^{85} to produce signal in G-M tube. With permission.

D. Fluorides

As an atmospheric pollutant fluorine presents special problems in analysis. It can be present in the gaseous phase as hydrogen fluoride, silicon tetrafluoride, carbon tetrafluoride and other fluorocarbon gases, or in the particulate phase as water-insoluble and -soluble inorganic and organic compounds. Some of these compounds may be extremely toxic while others may be relatively non-toxic. Fluorides can be measured as gaseous, particulate, or total fluorides. Obviously, methods of analysis for these components are imperfect since the analyst is not able to analyze for the specific fluoride compounds of known toxicity. Gaseous fluorides can be collected on filter paper or in an impinger. Collection, separation, and determination of fluoride have been discussed.[534-538] Of the fluoride separation methods the distillation procedure[539] is preferred and is usually used as the "standard" by which newer methods are evaluated. Ion-exchange and diffusion are also used,[539] and some workers believe them to be superior to the distillation method.

The fluoride methods may be categorized as depending on titrimetry,[538-540] fluoride-ion-selective electrodes,[534, 541] radiometry with I^{131},[542] colorimetry involving a bleaching effect by fluoride ion,[543-545] direct colorimetry,[537, 546-550] and fluorimetry involving the quenching effect of fluoride ion.[551] The fluoride electrode deserves more thorough investigation.

The colorimetric methods depend on the reaction of fluoride with a metal-dye complex to yield an insoluble or slightly ionized metal fluoride. Suitable metals include aluminum, bismuth, cerium, iron, thorium, yttrium, and zirconium. Some of the commonly used dyes include Alizarin Red S, Chrome Arurol-S, Ferron, Hematoxylin, PAN, SPADNS, Thorin, and Xylenol orange. Phosphate interferes by forming insoluble phosphates with the metals. The best of the lot uses the lanthanum alizarine complexone method.[537, 538, 546-550] This direct colorimetric method covers the range from 0.01 to 0.4 μg fluoride/ml.

Visual titrimetric methods usually use the thorium-Alizarin Red S-fluoride system. With the use of instrumental end-point detectors visual errors have been eliminated.

Silica-gel tubes[552] and monitoring[553, 554] have been used for the determination of hydrogen fluoride.

Surprisingly, there is no good fluorescence method for the determination of atmospheric fluoride. Some fluorescent methods are available for the determination of fluoride. The reagent mixture usually contains an aluminum salt and dyes such as Eriochrome Red B,[555] Superchrome Garnet-Y,[555] morin,[556] or quercitin.[556] A method has been reported which depends on the quenching by fluoride ion of the fluorescence of the zirconium 3-hydroxyflavone chelate.[551] A fluorimetric fluoride analyzer originally designed by Chaikin has been improved and modified recently.[557] This analyzer is also based on a quenchofluorimetric principle. It samples the atmosphere through parallel warmed glass tubes, one of which absorbs hydrogen fluoride in a thin coating of $NaHCO_3$. The two airstreams are then drawn through adjacent portions of a paper tape impregnated with the highly fluorescent magnesium salt of 8-hydroxyquinoline. The fluorescence of the ultraviolet-excited salt is quenched by the gaseous hydrogen fluoride, and the difference of emitted light is then measured on a recorder. A direct fluorescence method wherein the HF would induce fluorescence in a non-fluorescent molecule would work better here.

SUMMARY

For lack of space, methods for the determination of other types of aerotoxicants have not been discussed. For example, carbon monoxide-which has killed many individuals in confined areas and which is said to affect the central nervous system even at relatively low levels[558] -has been reviewed elsewhere.[559] Others like sulfur trioxide and nitrogen dioxide could be considered. Actually, long, thorough, and separate reviews on the analysis of each of the common air-pollutant gases would be invaluable.

The ideal situation in the analysis of these simple unique chemicals would be a relatively inexpensive little black box which would quantitatively measure the aerotoxicant in the airstream being pulled through the box. An alternative dry method would measure the aerotoxicant directly in the air over the distance covered by some laser beam. With the development of better monitoring techniques, improved manual techniques would also be necessary for calibration purposes.

Surprisingly, highly sensitive direct fluorimetric methods of analysis for most of the common

gaseous pollutants are unavailable, and yet they could be readily developed. Some possibilities have been discussed in the text. Others are readily apparent. Thus, nitrogen dioxide could be determined fluorimetrically with 2,3-diaminonaphthalene[560] or with some appropriate member of the large assortment of readily available *o*-diamines.

A bewildering number of analytical methods for the common atmospheric gases are available or are being developed. Methods of analysis are needed for the higher boiling and more toxic components of the air, many of which are of unknown composition. In addition, methods of analysis are unavailable and are badly needed for the huge class of "in-between" compounds present in the atmosphere. These are compounds which are neither common gases nor high-boiling solids.

The four main types of aerotoxicants which can affect human, animal, or plant life can be classified as bacterial, fungal, viral, and molecular. It is the analysis of the last of these that has been reviewed here.

The types of chemicals affecting human beings whose analysis has been reviewed include the aeromiserogens of the allergen family with their chronic and periodic effect on the individual's well-being; the aerocarcinogen family found in cigarette smoke, polluted air, and in the environment around us; the possible mutagens; and the lachrymators. Accumulation of some of these air pollutants has caused murderous air-pollution episodes[561] in the Meuse Valley of Belgium in December, 1930; in Donora, Pennsylvania, in October, 1948; in the hydrogen sulfide episode in Poza Rica, Mexico, in November, 1950; and in London in December, 1952. Air pollutants probably also play an important role in the alarming increase in emphysema.

The analysis of phytotoxicants has also been reviewed in this paper.

We must consider the total pollutant assault on human beings and other organisms if we are to properly evaluate the impact of any pollutant. Factors such as synergism, long-term effects of a pollutant, sudden aggravations of existing physiological problems, and the minor aggravations of lachrymators, sternutators, and headache-causers have to be considered.

As we obtain more and more information on the individual aerotoxicants we must turn our attention more and more to the mixtures around us. A reduction in the amount of one type of air pollutant or aerial organism may result in an overwhelming increase of a more toxic competitor. On the other hand, an increase in some air pollutant could have a synergistic effect on the physiological reaction of some other type of pollutant - chemical, bacterial, fungal or viral. Thus, it has been shown that during Asian flu epidemics polluted cities experienced a 200% increase in illness while the relatively unpolluted cities had only a 20% increase.[562]

Viruses and viral fragments have been postulated as playing a role in carcinogenesis. One suggestion is that independent fragments of virus-like genetic material, perhaps assisted at certain stages in their existence by viruses proper, are the key to many naturally occurring tumors.[563] In addition, there is the possibility that chemical carcinogens may have their effect mediated through viral agents or may react in combination with viruses.[564]

Another type of phenomena is the lethal or toxic synthesis. Thus, ozone, PAN, lachrymators, etc. can be synthesized in the polluted atmosphere while some allergens can be synthesized in airborne dusts from pre-allergens and some highly toxic chemicals can be synthesized in plants in contact with air pollutants. Thus, some forage crops in contact with high atmospheric concentrations of fluoride synthesize and accumulate the highly toxic fluoroacetate and fluorocitrate.[565]

On the basis of all these data I believe it is obvious that the total environment needs to be examined more thoroughly. I believe that analysis for just the primary aerotoxicants and the main atmospheric gases is not enough. Methods are needed for cofactors, irritants, antagonists, enhancers, sensitizers, pre-toxicants, etc. Analytical methods for many of these seemingly innocuous pollutants will be necessary because in the right mixture at the right moment these "harmless" chemicals may contribute to enhance, accelerate, or increase the duration of the physiological effect of the primary aerotoxicant.

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