PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY A MATHEMATICAL, PHYSICAL & ENGINEERING SCIENCES

Photochemical and Biological Implications of the Atmospheric Reactions of Amines and Benzo(a)pyrene

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Photochemical and biological implications of the atmospheric reactions of amines and benzo(a)pyrene

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The discovery of direct mutagenic activity, as determined by the Ames *Salmonella typhimurium* reversion assay, in the organic fractions of ambient aerosols collected throughout Southern California, led us to investigate the reactions of benzo(a)pyrene (BaP) deposited on glass fibre filters with ambient photochemical smog, as well as with O_3, NO_2, and peroxyacetyl nitrate (PAN) in simulated atmospheres. A variety of products are readily formed, including phenols, diphenols, dihydrodiols, etc. Directly mutagenic nitroderivatives are formed upon exposure of BaP (a carcinogen and pro-mutagen) and of perylene and pyrene (non-mutagens) to 1 part/10^6 of NO_2 and a trace of HNO_3 in air. If such reactions occur in urban atmospheres they may account in part for the 'excess' carcinogenicity (over BaP and certain other polycyclics) observed in organic particulates collected from smog and exhausts of spark ignition and diesel engines. However, such gas–solid interface processes may also have occurred on the filters commonly used in particulate sampling. Thus the possibility of 'filter artefacts' must be recognized.

Carcinogenic *N*-nitrosamines have been detected in air at or near several industrial plants. Therefore, reactions of their possible precursors, e.g. secondary and tertiary methyl and ethylamines with NO_x–HONO were studied both at levels of less than 1 part/10^6 in air in a 50 m^3 outdoor chamber and at levels around 4 parts/10^6 in a long path (720 m) outdoor infrared cell with the use of Fourier transform spectroscopic techniques. At concentrations less than 1 part/10^6 diethyl and triethylamine readily form photochemical smog (O_3, PAN, aerosols, etc.). Additionally, small but significant amounts of diethylnitrosamine $(C_2H_5)_2NNO$ are formed in the dark from diethylamine (DEA) but destroyed in sunlight. In contrast, $(C_2H_5)_2NNO$ is initially formed on irradiation of triethylamine (TEA)-NO_x mixtures, then photodecomposed. Other significant nitrogenous compounds formed in sunlight include dialkylnitramines (R_2NNO_2, a major product) and substituted amides; small amounts of acetamide are present in the particulate phase from DEA and TEA. Both $(CH_3)_2NNO_2$ and CH_3CONH_2 are carcinogens in animals, though less potent than nitrosamines; the activity of $(C_2H_5)_2NNO_2$ is not known.

Environmental implications of both systems are discussed.

1. Introduction

Historically, researchers and control officials have focused their attention on the major air pollutants: sulphur dioxide, carbon monoxide, nitrogen dioxide, photochemical oxidant (including ozone) and total suspended particulates. In the United States these are often referred to as 'criteria pollutants'; thus, during the last decade the U.S. Environmental Agency (E.P.A.) has set Federal Ambient Air Quality Standards based to a large extent on the Air Quality Criteria documents developed for each pollutant, and has implemented strategies for their control.

Recently, however, considerable interest has developed in better understanding the sources, transport, and associated chemical and physical transformations, and sinks of several types of

'non-criteria' pollutants. Certain of these species, though present in urban atmospheres at much lower concentrations than the criteria pollutants, are known to produce disproportionately severe health effects in experimental animals – and possibly in man. A classic example is the polycyclic aromatic hydrocarbon (PAH) benzo(a)pyrene (BaP). It is formed during the combustion of fossil fuels and was identified by its fluorescence spectrum in domestic soot in 1949 (Goulden & Tipler 1949). In 1952 it was identified and measured in ambient soot particles collected at ten stations throughout Great Britain (Waller 1952). Today it is recognized as being distributed globally.

A second class of 'non-criteria' pollutants of current concern are the *N*-nitrosamines. Recently, they have been identified in a variety of systems including pesticides, synthetic cutting and grinding fluids, and drinking water (Fine 1978). However, in contrast to the ubiquitous nature of BaP, they have been identified as air pollutants only in a limited number of locations, including those in or near industrial plants in East Germany (Bretschneider & Matz 1973) and the U.S. (Fine, Rounbehler, Belcher & Epstein 1976; Fine *et al.* 1977; Pellizzari 1977; U.S. E.P.A. 1977). These industrial plants employed dimethylamine (DMA) or dimethylnitrosamine (DMN) respectively in organic syntheses.

The carcinogenic activity of soot particles has been of concern for over two centuries (Pott 1775) and that of *N*-nitrosamines since it was first shown in 1956 (Magee & Barnes 1956). Today the possibility that environmental contaminants may be responsible for a significant fraction of cancer in man is being widely discussed (Epstein 1974; Heidelberger 1975; Searle 1976; de Serres 1976*a*, *b*). Thus, while to date no direct link has been established unequivocally between urban air pollution and lung cancer there is substantial indirect evidence that a relation may exist (National Academy of Sciences 1972). Quantitative studies of the atmospheric chemistry of PAH and nitrosamines and their precursors therefore seem desirable.

Pronounced societal interest in these classes of compounds is illustrated by the amendments to the U.S. Clean Air Act passed by Congress and signed into law by President Carter in August 1977. Section 104b requires the Administrator of the Environmental Protection Agency (E.P.A.) to review and critique all available information on 'nitric and nitrous acids, nitrites, nitrates, nitrosamines and other carcinogenic and potentially carcinogenic derivatives of oxides of nitrogen'. Similarly in Section 122(a), the Administrator is required to 'determine whether or not emissions of polycyclic organic matter (POM) into the ambient air will cause, or contribute to air pollution which may reasonably be anticipated to endanger public health'.

The highly specific nature of these Congressional mandates is historically somewhat unusual in federal air pollution legislation. Clearly, they reflect growing concern over increasing emissions of oxides of nitrogen (NO_x) and possible undesirable consequences of proposed major shifts to a far greater use of coal, shale oil, diesel powered motor vehicles, etc., to relieve in part the energy crisis in the U.S.

This charge of Congress to the E.P.A. poses a variety of difficult scientific problems. For example, to establish the nature and magnitude of a health effect, one needs reliable dose–response curves for the air pollutant(s) in question. Elucidation of the 'dose', of course, requires detailed knowledge of the physical (i.e. gas, liquid, solid, adsorbed on solid, particle size, etc.) and chemical nature of the pollutant (and co-pollutants) at the site of its impact on the biological target. In addition, the actual 'dose' administered to man or animals may not necessarily be chemically the same as that determined by using current sampling and analytical techniques, a point illustrated in §2.6, where filter artefacts are discussed.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY A MATHEMATICAL, PHYSICAL & ENGINEERING SCIENCES

Central to the issue, as the title of this Discussion implies, is the fact that during transport in the atmosphere in the presence of sunlight, oxygen, water and a spectrum of co-pollutants, primary organic pollutants undergo a variety of chemical and physical transformations. Consequently, the mixtures of gaseous and particulate pollutants which impact urban man are complex, often containing hundreds of species. Furthermore, some pollutants of interest often occur at concentrations in the range of 1 part/10^9 or less. Thus detailed analysis for chemical carcinogens in ambient particulates, e.g. by gas chromatography – mass spectrometry (g.c.–m.s.) is complicated at best.

Compounding the problem is the fact that there not only is a dearth of reliable information in the literature but also a significant amount of misinformation on atmospheric transformations of 'non-criteria' pollutants. For example, clouding the issue with respect to the PAH is the statement that, as air pollutants, 'They are chemically inert and thus are removed from the air only by rain or the slow sedimentation of the particulate' (Berry & Lehman 1971; Fishbein 1976, p. 232). Actually, although the evidence to date is limited (see below) it is clear that many PAH, including BaP, are highly reactive compounds. These should undergo a variety of atmospheric reactions, thermal and photochemical, with a number of co-pollutants, both molecular and free radical species. The problem is that little is known about these processes (for detailed reviews see National Academy of Sciences (1972) and Hoffmann & Wynder (1977)).

With respect to nitrosamines, a recent E.P.A. document (1976) states that in solution 'Unlike the secondary amines which can form nitrosamines – only a few tertiary amines form nitrosamines.' This is clearly not the case; tertiary amines react readily in solution with aqueous nitrous acid to form secondary *N*-nitrosamines (Guether 1864; Smith & Loeppky 1967; Walters, 1977). The real question is whether or not tertiary, as well as secondary, amines can react with NO_x–HONO in the gas phase to form *N*-nitrosamines.

In this paper are presented current results from our laboratory dealing first with reactions in simulated and real urban atmospheres of the carcinogen and mutagen BaP and of its non-mutagenic (in the Ames reversion assay) isomer perylene, and second with reactions of mixtures of simple aliphatic amines and NO_x in air containing traces of nitrous acid (HONO). We shall also discuss the mutagenic activity of ambient urban particulates and show how the Ames test, which employs the bacterium *Salmonella typhimurium* to test for mutagens, can provide microbiological clues useful in establishing the chemical identity of the species responsible.

Although at first glance the two systems, particulate PAH and gaseous nitrosamines, may seem quite unrelated, they both participate in chemical transformations in which hydroxyl (OH) and hydroperoxyl (HO_2) radicals and NO_x play major rôles – just as they do in tropospheric photochemical smog and in determining the stratospheric ozone balance, the two general topics of this Discussion. For detailed considerations, the full papers should be consulted.

2. Atmospheric chemistry of PAH

2.1. *Carcinogenicity of organic particulates*

Domestic soot was shown to be carcinogenic over 50 years ago (Passey 1922), and it has been known for over three decades that extracts of the organic fraction of particulates collected in ambient urban air in the U.S. are carcinogenic when administered subcutaneously to mice (Leiter, Shimkin & Shear 1942; Leiter & Shear 1943). Subsequently, this effect was seen in experimental animals administered extracts from ambient particulates collected in 1954 from

Los Angeles photochemical smog (Kotin, Falk, Mader & Thomas 1953) and in seven U.S. cities (Hueper *et al.* 1962). Similar results have been found with ambient samples collected in major urban centres throughout the world.

The carcinogenicity of the neutral fraction of organic particulates has been mainly attributed to the presence of PAH such as BaP and benz(a)anthracene. Recently, by using fluorescence and/or combined g.c.–m.s. techniques, these compounds and a large number of other PAH have been identified in ambient particulates (Gordon & Bryan 1973; Lao, Thomas, Oja & Dubois 1973), motor exhaust (Boyer & Laitinen 1975), diesel engine exhaust (Spindt 1974) and in soil near mountain highways (Blumer, Blumer & Reich 1977). Indeed, the latter group suggested a correlation between the higher cancer death rates and the PAH emissions due to automobile traffic in the Swiss mountain area where their study was conducted. This has, however, been contested (Litman 1978).

In addition to PAH, other chemical carcinogens have been identified in ambient particulates, including aza-arenes such as benzocarbazoles in the neutral fraction, and benzacridines and dibenzacridines in the basic fraction (Sawicki *et al.* 1965; Sawicki 1967; Cautreels & Van Cauwenberghe 1976; Dong, Locke & Hoffman 1977; National Academy of Sciences 1972).

However, it is notable that the observed carcinogenicity in animals or transformation in cell cultures is significantly greater than can be accounted for by the amounts of polycyclics present in the samples administered (Kotin *et al.* 1954; Hueper *et al.* 1962; Epstein *et al.* 1966; Rigdon & Neal 1971; Freeman *et al.* 1971; Mohr *et al.* 1976; Grimmer 1977). Thus, Gordon and coworkers reported that in airborne particles collected in the Los Angeles area, the benzene extract had 100–1000 times the cell transformation activity attributable to its BaP content. Furthermore, the methanol extract, while containing only about one-thirtieth of the BaP in the total sample, had activity comparable to the benzene extract (Gordon *et al.* 1973).

With respect to auto exhaust, Mohr *et al.* (1976) studied the effects of the condensate on the Syrian golden hamster lung. They concluded not only that the condensate displayed a carcinogenic effect (100 % rate of multiple pulmonary tumours) but also 'Considering the relatively low total dose of BaP contained in the condensate, this pronounced neoplastic response cannot be explained alone by the effects of this well known carcinogenic hydrocarbon' (Mohr *et al.* 1976).

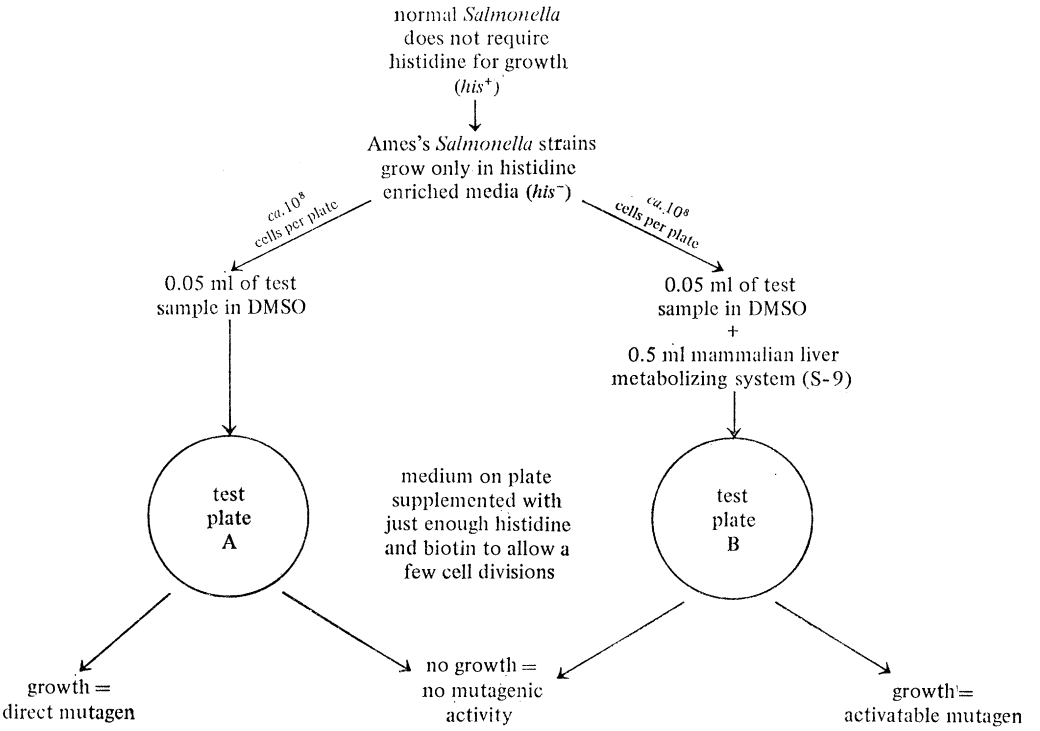
2.2. *The Ames test for mutagenic activity*

Because of the chemical complexity of ambient particulates and motor exhaust, and because animal tests for suspected carcinogens are time consuming and expensive, experiments directed to establishing the identity of the compounds responsible for the 'excess' carcinogenicity have been in general relatively limited. Recently, however, a rapid and relatively inexpensive microbiological assay for mutagenic activity has been developed by Ames and coworkers (Ames, Durston, Yamasaki & Lee 1973; Ames, McCann & Yamasaki 1975; Ames & McCann 1976). It is a reverse mutation system employing histidine-requiring mutants of the bacterium *Salmonella typhimurium*. In tests of some 300 compounds, about 85–90 % of the known carcinogens were also found to be mutagens, while the number of false positives and negatives ranged from 10 to 15 % (McCann, Choi, Yamasaki & Ames 1975). This observation has been confirmed by some investigators (Sugimura *et al.* 1976) but disputed by others (Ashby & Styles 1978). In any case, the Ames test is now generally recognized as a useful, *though by no means exclusive*, screening test for chemical mutagens in complex environmental samples (Bartsch

1976; Bochkov, Sram, Kuleshov & Zhurkov 1976; Commoner 1976; Dean 1976; de Serres 1976*a*, *b*; Sobels 1977).

We have been using the Ames test to screen for mutagenic activity in particulate samples collected from real and simulated atmospheres, and as a means of obtaining microbiological clues to the nature of the compounds responsible for the 'excess' carcinogenicity. Of course, we caution that the observation of mutagenic activity in bacterial assays conducted on environmental samples does not necessarily imply carcinogenic activity in man.

TABLE 1. DESCRIPTION OF AMES'S MICROBIOLOGICAL ASSAY FOR MUTAGENIC ACTIVITY



The experimental sequence used in the Ames test is outlined in table 1. In the test, one strain of *his*$^-$ *Salmonella*, TA1535, is reverted to the normal *his*$^+$ form with accompanying growth of colonies on the test plate by chemicals that are base pair substitution mutagens. Examples are *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine and alkylating agents such as β-propiolactone. Other strains, TA1537, TA1538 and TA98, respond to chemicals which produce frameshift mutations such as aromatic amines (e.g. 2-aminofluorene and 2-acetylaminofluorene) and PAH such as BaP. Strain TA100 detects both base-pair substitution and frameshift mutagens.

Experimentally, one first adds portions of the test sample to a series of tubes, each containing agar and one of the test strains. These are then poured onto plates filled with minimal salts agar. If no significant increase of colonies above background (the background is caused by spontaneous *his*$^-$ to *his*$^+$ reversions) is observed for TA1535 but a pronounced growth in colonies is seen for TA1538 (or another frameshift sensitive strain, e.g. TA98), one concludes first that the sample is *directly* mutagenic and second that only chemicals causing frameshift mutations were present. Thus at least some of the compounds in the sample must be directly active frameshift mutagens.

This behaviour is in contrast to species such as BaP or 2-acetylaminofluorene which are *promutagens* (Lu *et al.* 1976); they are inactive *per se* and must be metabolized to other species before they can cause mutations in this bacterial assay. Microsomal activation is achieved by adding to the test sample a small amount of an enzyme system (S-9 solution, table 1, test plate B) derived from the livers of rats injected with Aroclor (a polychlorobiphenyl) or other such inducing agents.

Now, if we add S-9 and do *not* observe on plate B a significant increase in the number of TA1538 colonies above that seen on plate A we can conclude that *only* directly mutagenic frameshift chemicals are present. If, however, the number of revertants per microgram sample on B is significantly larger than on A, we can conclude that *both* direct and activatable (e.g. BaP) chemical mutagens are present.

Finally, the test can be highly sensitive to relatively small changes in chemical structure. Thus, for example, 2-acetylaminofluorene is a strong frameshift mutagen [108 revertants per nanomole (108 rev./nmol)] while the 4-isomer is weak (0.3 rev./nmol, McCann 1976).

The phenomenon of strain specificity toward different frameshift mutagens underlines the desirability of employing each strain (i.e. TA1535, TA1537, TA1538, TA98, TA100) at least once during initial screening tests for mutagenicity in complex environmental samples. For example, of the 12 monohydroxy-isomers of BaP, only five phenols were found to be directly acting frameshift mutagens, primarily 6-OH and 12-OH but also 1-, 3- and 7-OH-BaP to a smaller extent. The remaining seven isomers were non-mutagenic. However, the activity of the 6-OH and 12-OH isometers was reported to be weak when strain TA1538 was used but moderate with strain TA98 (Jerina *et al.* 1976*a*, *b*; Wislocki *et al.* 1976).

It is also worth noting that seven of these phenols (4-, 5-, 6-, 7-, 8-, 9-, and 10-hydroxy-BaP) were also tested for carcinogenicity on mouse skin (Kapitulnik *et al.* 1976); all proved negative. Furthermore, although 6-OH BaP was moderately active in Chinese hamster V79 cells, 12-OH-BaP was inactive (Wislocki *et al.* 1976). Such findings illustrate the need for caution if one uses conclusions based on results obtained from one particular kind of mutagenicity assay system to predict the behaviour of another type.

Finally, in view of its increasingly widespread use, the need for standardization of the Ames test, including plate incubation temperature (*precise* control at 37 °C is important (Winer *et al.* 1978)) as well as the number of cells per plate, plate volume, concentrations of rat liver S-9 homogenate, etc., is becoming increasingly urgent (Pitts *et al.* 1978*a*). Collaborative programmes to achieve this objective are currently under way.

2.3. *Mutagenic activity of urban organic particulates*

In 1975 the mutagenicity of organic fractions from particulates collected at several sites in the Los Angeles Basin was demonstrated using Ames's assay system (Pitts 1975). This phenomenon has now been reported in studies at Ohmura and Fukuoka, Japan (Tokiwa *et al.* 1976), Kobe, Japan (Teranishi, Hamada & Watanabe 1978), Buffalo, New York and Berkeley, California (Talcott & Wei 1977), New York City, New York (Daisey, Hawryluk, Kneip & Mukai 1978) and Chicago, Illinois (Commoner, Madyastha, Bronsdon & Vithayathil 1978).

Recently, as a means of obtaining more information about the chemical nature of the organic particulates and hence about atmospheric transformations of PAH, we expanded our programme and collected 23 samples of airborne particulates at 11 urban sites in California's South Coast Air Basin (Pitts *et al.* 1978*a*, *e*). Results of the Ames tests can be summarized as follows:

(i) All 23 samples exhibited frameshift-type mutagenic activity without requiring metabolic activation with strains TA1537, TA1538 and TA98.

(ii) All solvent and filter blanks were inactive.

(iii) Typically, assay of 0.1–2.0 mg of airborne organic particulates resulted in a 5- to 20-fold increase in the number of histidine revertants per plate above the background of spontaneous revertants in strains TA1537, TA1538 and TA98, and in up to a twofold increase in strains TA100.

(iv) No activity was observed in any of the assays with strain TA1535, which is reverted by base-pair substitution mutations.

(v) Microsomal activation by S-9 solution did not greatly increase the activity of most of the samples tested.

(vi) Assay of extracts of a size-resolved sample collected in downtown Los Angeles revealed that all mutagenic activity was associated with organic species present in particles of diameter 1.1 μm or less. This is consistent with the well documented distribution of organic particulate pollutants such as BaP with respect to particle size (Kertesz-Saringer, Meszaros & Varkonyi 1971; Natusch & Wallace 1974; Pierce & Katz 1975; Friedlander & Miguel 1978).

As noted earlier, the carcinogenic activity of organic fractions extracted from ambient particulates taken from the Los Angeles atmosphere is well known, so that our finding frameshift-type mutagenic activity in all urban samples is not surprising. What is interesting chemically is that metabolic activation was *not* required for any sample. Thus these urban particulates must contain mutagens other than the carcinogenic PAH, such as BaP, which require activation. This is consistent with the numerous observations of 'excess' carcinogenicity in animals or in cell transformation activity discussed above, and the low average concentrations of BaP in the Los Angeles Basin (Gordon & Bryan 1973).

2.4. *Atmospheric reactions of benzo(a)pyrene and perylene*

We recently proposed that the presence of these direct mutagens in ambient particulates may be due in part to the reactions of BaP and other PAH with species present in photochemical smog such as ozone, nitrogen dioxide, peroxyacetyl nitrate (PAN), singlet molecular oxygen ($O_2{}^1\Delta$), and the free radicals OH and HO_2 (Pitts *et al.* 1977*a*, 1978*c*). Some of the products formed in these reactions are analogous to the metabolites of BaP and other PAH in mammalian cells (Jerina *et al.* 1976*a*, *b*; Fahmy & Fahmy 1976), so that reactions of PAH in exhaust effluents and in the atmosphere could account for the presence of directly active mutagens in urban particulates.

Furthermore, we recently observed significant differences in the mutagenic activity of polar neutral fractions of ambient particulates sampled during daytime and nighttime (Pitts *et al.* 1978*b*). Since the concentrations of O_3, NO_2 and PAN in urban air exhibit pronounced diurnal profiles, the observed differences in activity may reflect different exposures of PAH to these oxidizing species.

Also relevant to the postulated importance of oxidative processes during atmospheric transport of PAH are several reports of the carcinogenic activity of polar fractions of organic particulates (Hueper *et al.* 1962; Epstein, Mantel & Stanley 1968; Wynder & Hoffman 1965; Asashina *et al.* 1972; Gordon *et al.* 1973), products of ozonized gasolines (Kotin, Falk & McCammon 1958) and products of oxidation of aliphatic hydrocarbons (Kotin, Falk & Thomas 1956); and of the toxicity of the photooxidation products of a commercial fuel oil (Larson, Hunt & Blankenship 1977).

There are also several reports that BaP and other PAH readily undergo photochemical transformations when adsorbed on a variety of support materials such as filters, silica gel and carbon (soot) particles (Kotin *et al.* 1954; National Academy of Sciences 1972; Falk, Markul & Kotin 1956; Tebbens, Thomas & Mukai 1966; Tebbens, Mukai & Thomas 1971; Boyer & Laitinen 1975; Barofsky & Baum 1976).

Finally, it has been stated that polycyclic quinones are present in ambient particulates and that they may be formed by atmospheric photooxidation of PAH (Masuda & Kuratsune 1966; Fatiadi 1967; Jaeger 1971; Pierce & Katz 1976).

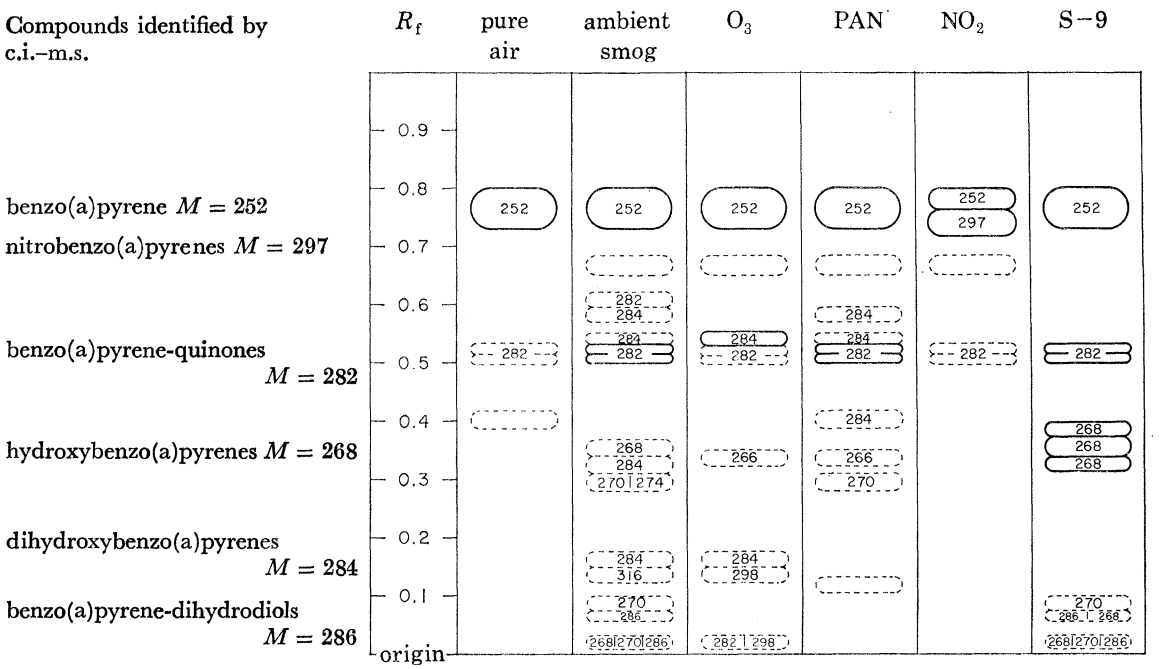


FIGURE 1. Thin layer chromatography separation of the reaction mixtures from BaP exposed to several gaseous pollutants, to ambient photochemical smog and to pure air. For reference, t.l.c. bands are also shown of metabolites formed from the action of liver homogenate (S-9) on BaP. The solvent was toluene:dichloromethane:methanol (25:10:1).

2.4.1. *Reactions of benzo(a)pyrene*

In order to test our hypothesis, a series of experiments was carried out in both real and simulated atmospheres. The first question to be answered was 'Can BaP deposited on a filter react with the gases present in ambient photochemical smog to form compounds which, unlike BaP, are direct mutagens?'

Experimentally, two washed and fired Gelman A/E glass fibre filters were mounted in series in a high-volume sampler capable of drawing 1130 l/min of ambient air through them. The upstream filter, a 'blank', simply collected all ambient particulates and allowed only the gaseous pollutants present in the photochemical smog to pass through to the second. This filter was coated with BaP (*ca.* 1 mg) that could interact with the gaseous pollutants. In these and all other runs, the filters coated with PAH were kept in the dark throughout the exposure. In a separate 'blank' run without BaP no transfer of mutagenic species from the first filter to the second was observed.

Four such runs were carried out, two in Los Angeles and two in Riverside. At each site one

test was conducted during the day and one at night; total exposures to ambient air were about 40 h per sample.

After exposure, the filter samples were extracted by ultrasonication and the concentrated organic extracts tested with *Salmonella* strains TA98 and TA100. All samples were directly mutagenic; typically with TA98, 10 µl of extract produced 150–250 revertants per plate *without* metabolic activation. Thus exposure of BaP in the dark to the gases in ambient smog forms compounds that are directly active mutagens.

In order to determine the chemical nature of these species, a similar experiment was performed in which ambient Riverside air was drawn through the pair of filters for 72 h. The average concentrations during this period for O_3, PAN, NO_2 and CO were 0.035, 0.0025, 0.050 and 1.10 parts/10^6 respectively.

The BaP-coated filter was then extracted and separated into fractions by thin-layer chromatography (t.l.c.) on silica gel plates. Each band was recovered in methanol and analysed by methane chemical ionization mass spectrometry. The t.l.c. bands and the molecular masses of the species formed are shown in the column headed 'ambient smog' in figure 1. Also shown at the left of the figure are the products identified by direct probe mass spectrometry and the t.l.c. R_f values (solvent toluene–dichloromethane–methanol, 25:10:1).

Clearly, not only does exposure of a sample of BaP to ambient smog cause it to become directly mutagenic, but contrary to some reports (Berry & Lehman 1971; Fishbein 1976), a variety of compounds are readily formed. Those tentatively identified by t.l.c. include, in order of decreasing polarity: BaP-dihydrodiol(s) (molec. mass 286), BaP-diphenol(s) (284), BaP-phenol(s) (268), BaP-quinones (282), and what appear to be dicarbonyl compounds (284) formed by ring-opening oxidations.

The recovery of pure polar compounds from t.l.c. plates is somewhat inefficient. Furthermore, the treatment of the BaP–smog products during extraction, separation and analysis was rather severe and time consuming so that some initial products may have reacted before their final identification. Thus, although no mass spectral evidence for BaP-epoxides was obtained, the presence of dihydrodiol(s) suggests epoxides may have been formed as initial, labile products. Detailed experiments using h.p.l.c. are currently under way to resolve this matter.

Based on elementary carbon analysis one obtains a rough estimate of the conversion of the BaP on the filter to products, which indicates that the surface area of the BaP directly exposed to the polluted air is the factor limiting the degree of oxidation in experiments of this type. This is consistent with the observations of Lane & Katz on the ozonization of BaP (Lane 1976; Lane & Katz 1977).

To obtain more specific information about the reactions of BaP with individual oxidants present in photochemical smog, another series of experiments was carried out under similar conditions. In these, glass-fibre filters coated as above with BaP were exposed in the dark to clean, particle-free air containing (1) 11 parts $O_3/10^6$ (exposure time 24 h at a flow rate of 85 l/min), (2) 1.3 parts $NO_2/10^6$ containing 10 parts nitric acid/10^9 (24 h, 28 l/min), or (3) 1.1 parts PAN/10^6 (16 h, 85 l/min). Control runs with BaP exposed to pure air (24 h at 85 l/min) and with blank filters exposed to NO_2, O_3, or PAN were also included. No mutagenicity was observed in any of the control runs but all the other exposures resulted in direct mutagenicity of the reaction mixtures.

After exposure, the products and unreacted BaP were separated by t.l.c., analysed by mass spectrometry and tested separately for mutagenic activity by the Ames test, as described earlier.

Microsomal activation was achieved by adding 0.5 ml of liver S-9 solution from Aroclor-induced rats. In a separate experiment, for product identification and comparison purposes, a sample of 9 mg of BaP was incubated for 30 min at 37 °C with the liver S-9 homogenate and the metabolites formed in this microsomal activation system analysed and tested for mutagenic activity.

It is evident from the results in figure 1 that BaP reacted with O_3 and PAN to give several oxidized products; with NO_2 and a trace of nitric acid it gave almost exclusively mononitrobenzo(a)pyrene(s). BaP reacted to only a slight degree with pure air, but the mixture was non-mutagenic without activation.

In recent experiments, the exposure levels of BaP on glass fibre filters were lowered to about 100 parts $O_3/10^9$ and 250 parts $NO_2/10^9$; similar results were observed. Conversion yields measured by fluorimetry were *ca.* 20 % for the nitration (8 h exposure) and *ca.* 47 % for the reaction with ozone (1 h exposure).

The nitration of BaP by levels of NO_2 of the order of 1 part/10^6 in air is catalysed by levels of nitric acid or sulphuric acid around 1 part/10^9. This species was shown by long-path (100 m) Fourier transform infrared spectroscopy to be present in our tanks containing known amounts of reportedly 'pure NO_2 in nitrogen.'

The t.l.c. bands containing the unreacted BaP (R_f = 0.77) were not directly active as expected and required metabolic activation to produce mutagenic effects. The bands containing the BaP-quinones (R_f = 0.54) were complex; they contained, in addition to quinones, trace amounts of an unidentified directly mutagenic compound. Authentic samples of the three purified quinones (BaP-6,12-quinone, BaP-1,3-quinone and BaP-3,6-quinone) were tested in our laboratory and were found to be non-mutagenic in the Ames assay system. However, caution should be expressed in the interpretation of t.l.c. separation patterns in terms of the formation of BaP-quinones in the exposures of BaP both to ambient air and to ozone. Indeed, recent separation of the reaction mixture of BaP with O_3 by h.p.l.c. techniques did not reveal the presence of any quinones in the reaction mixture. Obviously, at least some formation, and therefore detection, of quinones in the t.l.c. separation of BaP-containing mixtures may be attributed to photochemical transformation of the BaP adsorbed on the t.l.c. plate.

Treatment of BaP with liver S-9 mix resulted in the appearance of a complex t.l.c. band (figure 1) containing directly active mutagens. Its R_f (0.35) and the molecular mass (268) of its components are consistent with those of isomers of hydroxybenzo(a)pyrene.

Exposure of BaP to *ca.* 1 part $NO_2/10^6$ (and a trace of HNO_3) in air resulted in the appearance of only one major t.l.c. band; it was directly mutagenic. Its R_f (0.74) and molecular mass (297) are consistent with the structure mononitrobenzo(a)pyrene (nitro-BaP). By changing from a mixed t.l.c-solvent system to toluene alone, this band was resolved into two bands, one yellow and one orange. The latter had a lower R_f value. By comparison of ultraviolet-visible spectra shown in figure 2 and their mass spectra with those of authentic samples synthesized by the method of Dewar, Mole, Urch & Warford (1956*a*), we assigned the structure 6-nitro-BaP to the compound in the yellow t.l.c. band; the orange t.l.c. band consisted of a mixture of the 1-nitro and 3-nitro isomers.

Co-chromatography of the two t.l.c. bands with authentic samples further confirmed these assignments. The two isomers present in the orange t.l.c. band were then resolved by gas chromatography with the use of a glass capillary column. Interestingly, the relative concentrations of the 1-nitro and 3-nitro isomers were 1:1 in the mixture synthesized according to

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY A MATHEMATICAL, PHYSICAL & ENGINEERING SCIENCES

Dewar *et al.* (1956*a*) and 10:1 in the orange t.l.c. band formed upon exposure of BaP to NO_2 in air.

Both 6-nitro-BaP and the mixture of the 1-nitro and 3-nitro-BaP isomers proved to be direct frameshift mutagens when tested with strains TA1537, TA1538, TA98 and TA100. The 6-nitro-isomer was a moderate mutagen (32 rev./nmol for TA100) while the mixture of 1- and 3-isomers showed strong mutagenic activity (210 rev./nmol for TA98). Addition of liver S-9 homogenate significantly enhanced the mutagenic activity observed with strains TA1537, TA1538 and TA98. Thus, with S-9 the 6-nitro-BaP caused 418 rev./nmol (TA100) and the mixture of 1- and 3-nitro 5200 rev./nmol (TA98). However, the addition of S-9 actually reduced the activity of the mixture of 1-nitro and 3-nitro-BaP on TA100. Dose–response curves for the mutagenic activity of the authentic samples were in excellent agreement with those of the nitro-BaP isomers formed upon exposure of BaP to NO_2 in air. No base-pair substitution activity was found when the samples were tested with strain TA1535.

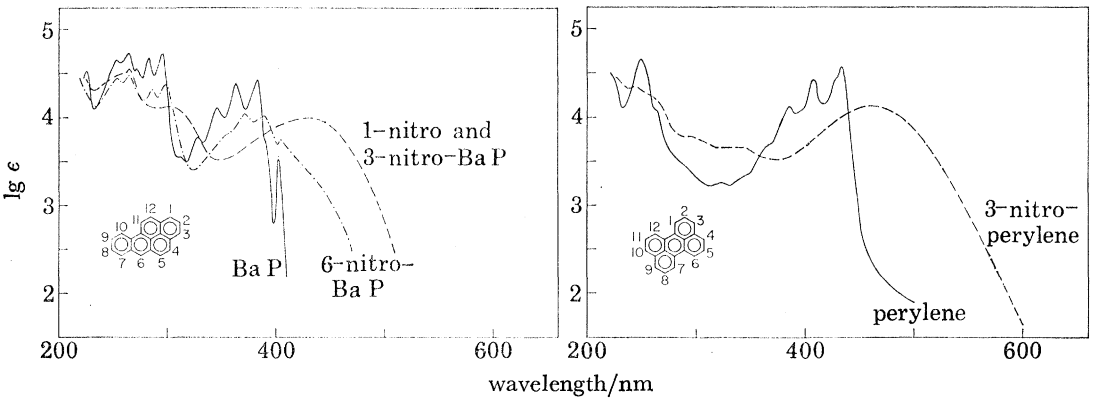


FIGURE 2. U.v. – visible spectra in methanol of the nitro derivatives of BaP and perylene formed upon exposure of the PAH deposited on a filter to 1 part $NO_2/10^6$ (and a trace of HNO_3) in air.

We can conclude from these observations that the pattern of compounds resulting from the exposure of BaP to ambient photochemical smog can be reconstituted in part by combining the individual effects of ozone and PAN on the BaP (figure 1). Furthermore, it is seen that metabolities formed by treating BaP with S-9 rat liver homogenate coincide both in molecular mass and mutagenicity, with several t.l.c. fractions observed when BaP was treated with ambient photochemical smog.

Several factors may explain the fact that nitro-BaP was not found in the experiment when BaP was exposed to ambient smog. Thus, NO_2 levels are customarily low in Riverside and for the 72 h exposure averaged only 0.050 part/10^6. It is also possible that the prolonged exposure of 72 h to other oxidizing species present in the smog resulted in the degradation of any nitro-BaP compounds that may have been formed initially. Recently, 6-nitro-BaP was detected in the air in Prague (Jäger 1978).

2.4.2. *Reactions of non-mutagens perylene and pyrene*

Since exposure of a known carcinogen and activatable mutagen BaP to only 1 part $NO_2/10^6$ in air produced directly mutagenic mononitro derivatives, we were interested to see if under similar conditions the same type of reaction could take place with a non-mutagenic (in the Ames reversion assay) PAH. To answer this question perylene, a closely related isomer of

BaP that is also found in organic particulates from petroleum and diesel engine exhaust, tobacco smoke, effluent from coal-fired furnaces, etc., was chosen. It was deposited on glass-fibre filters and, as with BaP, exposed 24 h in the dark to 1 part $NO_2/10^6$ (and a trace of HNO_3) in air at a flow rate of 28 l/min.

Separation of the exposed sample by t.l.c. yielded only one reaction product band. It was brick red, and from its mass spectrum and u.v. spectrum (figure 2), the compound responsible was identified as 3-nitroperylene. When tested with strains TA98 and TA1538, 3-nitroperylene was found to be a directly active, moderate mutagen (37 rev./nmol, TA1538) whose activity was significantly enhanced by addition of liver S-9 mix (94 rev./nmol, TA1538).

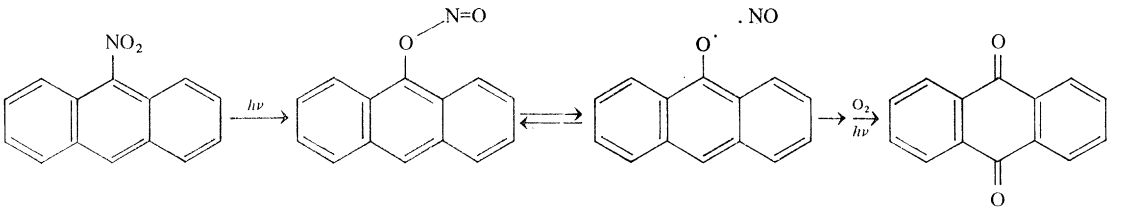
To check the generality of this transformation, i.e. conversion of a non-mutagenic (in the conventional Ames test), non-carcinogenic PAH to a directly active species, we recently deposited the quadricyclic PAH pyrene on a glass-fibre filter and exposed it to *ca.* 1 part $NO_2/10^6$ (and a trace of HNO_3) in air under similar conditions. Indeed, 1-nitropyrene was formed, though, as expected from theoretical considerations on aromatic nitrations in solution (Dewar, Mole & Warford 1956*b*), at a lower rate and lower yield than 6-nitro-BaP, 1- and 3-nitro-BaP, or 3-nitro-perylene. 1-Nitropyrene proved to be a direct mutagen (TA1538, *ca.* 100 rev./nmol) whose activity was in fact reduced when S-9 was added. Such S-9 suppression is quite common and the possibility must be kept in mind when carrying out metabolic activation.

In a similar experiment chrysene, an isomer of pyrene, was *not* nitrated. This seems reasonable since the relative rates for aromatic nitration in solution are: chrysene = 1, pyrene = 4.9, perylene = 22 and BaP (6-position) = 31 (Dewar *et al.* 1956*b*).

2.5. *Photochemical reactions*

The fact that upon photolysis 9-nitroanthracene forms 9,10-anthraquinone, both in solution (Chapman, Heckert, Reasoner & Thackaberry 1966) and in recent experiments on silica gel (Pitts *et al.* 1978*c*), suggests that similar reactions may represent, at least in part, the fate of nitro-BaP (and possibly other PAH) in ambient atmospheres. Thus, we feel that the photo-oxidation of PAH by sunlight in air (Falk *et al.* 1956; Tebbens *et al.* 1966; Tebbens *et al.* 1971; Boyer & Laitinen 1975), for the formation of polycyclic quinones found in polluted air, e.g. in Toronto, Canada (Pierce & Katz 1976). Indeed, recent experiments in our laboratory have shown that irradiation of 6-nitro-BaP deposited on silica gel yields the expected quinones.

At present one can only speculate whether or not the mechanism proposed by Chapman *et al.* (1966) for the formation of 9,10-anthraquinone as a major product of the irradiation of 9-nitroanthracene would also apply to 6-nitro-BaP.



Certainly, the electron impact mass spectra of the nitro derivatives of BaP and perylene are similar in some respects to that of 9-nitroanthracene. Thus loss of 30 u, corresponding to NO, is an important process in all cases. Presumably, on electron impact the nitro group rearranges

to a nitrite with subsequent loss of the NO. This process is analogous to the photorearrangement postulated by Chapman *et al.* (1966) for 9-nitroanthracene. Such parallels between intramolecular rearrangements observed photochemically and by electron impact in mass spectrometers are well known (Calvert & Pitts 1966) and support the hypothesis that 6-nitro-BaP may photooxidize in smog to form the quinones. Experiments to test this idea are under way in our laboratory.

2.6. *Environmental implications*

The formation of mutagenic nitro derivatives by exposure of non-carcinogenic as well as carcinogenic PAH to levels of NO_2 around 1 part/10^6 warrants further investigations of the modes of formation and atmospheric fate of these compounds, especially in situations where relatively high levels of PAH and oxides of nitrogen may coexist and direct mutagens may be formed. These could include spark ignition (Wang, Sawyer & Wei 1978) and diesel exhausts (Huisingh *et al.* 1978) as well as plumes from coal-fired power plants (Chrisp, Fisher & Lammert 1978).

The significant reactivity of BaP and perylene (and even pyrene), we observed with NO_2 in the dark, as well as BaP with O_3, and PAN and ambient photochemical smog, strongly suggests that similar processes could occur in ambient atmospheres – possibly to a major degree. Furthermore, these reactions with BaP do not constitute unique pathways for the formation of such oxidized species as the quinones. Thus at least two other mechanisms can be invoked for the atmospheric photodegradation of PAH.

One path would be electrophilic attack on the PAH by singlet molecular oxygen, $O_2(^1\Delta)$, presumably to produce in part hydroperoxides. These could then decompose to phenoxy-type radicals, which in the case of BaP would be converted to BaP-quinones (Inomata & Nagata 1970). Atmospheric sources of $O_2(^1\Delta)$ in sunlight include triplet energy transfer from photoexcited organic species such as PAH or benzaldehyde to ground state molecular oxygen (Pitts, Khan, Smith & Wayne 1969). Evidence for this mechanism has been obtained recently in experiments in which BaP coated on particles suspended in air was oxidized to quinones by $O_2(^1\Delta)$. The latter was produced by energy transfer from particles coated with rose bengal and illuminated at 365 nm (Grossman 1976; Grossman & Van Cauwenberghe 1976, private communication).

Other highly reactive species present in photochemical smog include hydroxyl and hydroperoxyl radicals, both of which would undoubtedly attack PAH. Not much is known about HO_2, but certainly the reactions of OH with gas phase aromatics are very fast, greater than 3×10^9 l mol^{-1} s^{-1} (Perry, Atkinson & Pitts 1977; Atkinson *et al.* 1978*a*), and would probably lead to BaP-phenols and ultimately BaP-quinones.

Finally, we should emphasize that our studies were conducted with PAH deposited on the surface of *glass fibre filters*. Whether PAH adsorbed on the surface of airborne particles (soot, fly ash, etc.) will react with co-pollutants in a similar fashion *in the atmosphere* is currently a matter of conjecture. Thus, the atmospheric reactions may be influenced not only by the levels of PAH and gaseous pollutants but also by many complex factors typical of surface chemistry and aerometry including particle size, sunlight intensity, relative humidity, atmospheric mixing and transport time, etc. Similarly, little is known about the extent of possible reactions of PAH on the filters employed to collect ambient particulates. However, our results suggest that they may indeed be significant.

Therefore, the elucidation of possible filter 'artefacts' is important because virtually all

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY A MATHEMATICAL, PHYSICAL & ENGINEERING SCIENCES

previous studies of the carcinogenic and mutagenic activity of organic particulates present in polluted air and motor exhaust have been based upon samples collected on various types of filters.

3. Atmospheric chemistry of amines

In the recent E.P.A. document ('S.T.A.R.') on nitrosamines in the environment (U.S. E.P.A. 1976) the following statement is made: 'In the face of these uncertainties, the existing information on the atmospheric chemistry of nitrosamines can have only vague implications regarding the control of atmospheric nitrosamines. Thus, to decide whether the nitrosamine problem is an ambient-atmosphere problem or an industrial-atmosphere problem requires that the nitrosamine to precursor dependencies be better known.'

We shall now discuss current results bearing on the problems cited above: the reactions of amines and NO_x in real and simulated urban atmospheres. In doing so, examples will be given of situations in which injudicious extrapolation of results on photochemical processes and reaction mechanisms obtained under 'laboratory' conditions (i.e. ranging from pressures around 1 Torr to concentrations around 1 part/10^6) to predict the behaviour of amines and NO_x in the parts/10^6 to parts/10^9 concentration range typical of actual ambient atmospheres can lead to erroneous conclusions.

The uncertainties inherent to such kinetic and mechanistic extrapolations – while far from the magnitude of those toxicologists often are required to make, predicting from animal data health effects of environmental pollutants on man – nevertheless can be substantial. Furthermore, they can have pronounced effects on the economic as well as technical impacts of air pollution control strategies. Potential errors can be reduced by employing several experimental systems capable of covering concentrations ranging from parts/10^3 to parts/10^9 of the pollutants, their precursors and their reaction products. This approach will be illustrated in the following discussion.

3.1. *Chemical inhibition of photochemical smog*

Recently, a proposal for controlling the products of photochemical smog such as O_3, peroxyacetyl nitrate (PAN), secondary aerosols, etc., by adding to urban air 100 parts/10^9 of an inhibitor, diethylhydroxylamine (DEHA), $(C_2H_5)_2NOH$, has been strongly advocated (Heicklen 1976). This chemical approach, it is stated, would eliminate the need for exhaust control devices on motor vehicles, and save billions of dollars. Actual field trials involving introduction of DEHA into the air of several major cities in the world suffering from photochemical smog have been suggested.

The research on which the proposal was originally based was carried out on hydrocarbon–NO_x systems containing DEHA at concentration levels over 1 part/10^6 and generally conducted under 'laboratory', not simulated ambient conditions. Thus our group, among others (Maugh 1976), was concerned not only with the possible health effects of photooxidation products of DEHA–NO_x–HC mixtures generated in ambient photochemical smog, but also with the atmospheric uncertainties involved in this extrapolation. It seemed unlikely that such low levels of an amine (100 parts/10^9) could continually and effectively inhibit ozone, PAN and secondary aerosol formation as a smog front travelled in some cases hundreds of kilometres over a period of many hours, and even days.

To test the idea under realistic conditions, we studied the reactions of DEHA at 100 and 500 parts/10^9 in ambient air, or in hydrocarbon surrogate mixtures simulating 6 to 9 a.m.

Los Angeles air (Pitts, Smith, Fitz & Grosjean 1977*b*), in a large outdoor environmental chamber constructed from FEP Teflon film 50 μm thick.

Typically, the chamber was first covered with a black plastic light shield and filled with ambient air to a volume of about 50 m³. It was then divided into two compartments of equal volume and a known amount of DEHA in high-purity N_2 added to one of the compartments. The black cover was then removed, exposing both compartments to sunlight for at least 6 h. Since identical samples of polluted air, one with and one without DEHA, were subjected simultaneously to the same environmental conditions (i.e. solar irradiation, temperature and humidity), differences in the time–concentration profiles of the smog parameters measured in the two compartments could unambiguously be attributed to the DEHA in one compartment.

Results from an experiment in which DEHA at 100 parts/10^9 was present in Riverside ambient air are shown in figure 3. Clearly, the DEHA did *not* inhibit formation of photochemical smog; in fact, it substantially *accelerated* the conversion of NO to NO_2 and the production of O_3, PAN and light-scattering secondary aerosols.

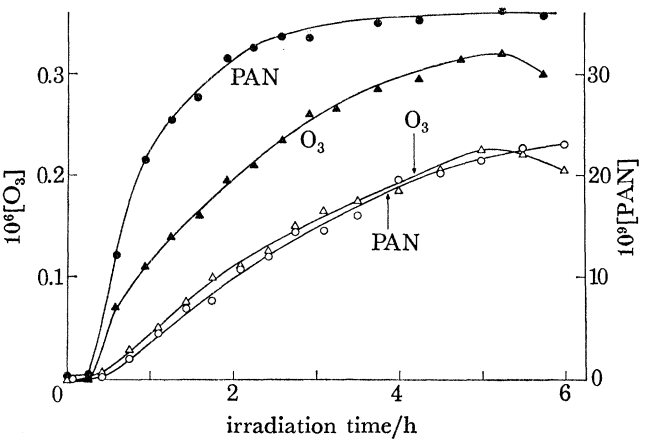


FIGURE 3. Time–concentration profiles of ozone and PAN formed upon irradiation of Riverside ambient air with (closed symbols) and without (open symbols) the addition of 100 parts/10^9 of diethylhydroxylamine, showing acceleration of smog formation by DEHA when added at that level.

With 0.5 parts DEHA/10^6 added to ambient air 'doped' with the HC surrogate mixture, an 'intermediate' behaviour was observed. Inhibition was predominant during the first hour of irradiation, but at that point a marked acceleration of smog formation took place. This was evident from the much higher rates of formation and concentrations of O_3, PAN and light-scattering particles in the compartment with DEHA.

In short, DEHA is indeed an inhibitor of certain symptoms of photochemical smog for several hours when present at concentrations around 1 part/10^6. However, it is an *accelerator* of O_3, PAN, aerosol formation and NO–NO_2 conversion when present at the recommended level of 100 parts/10^9. Furthermore, even if higher levels of DEHA (odour threshold 0.5 parts/10^6) could be added to ambient air (not considering the health implications, which could be serious), its dilution as the air mass was being transported would soon lead to *increased* photochemical smog in suburban, and even rural areas downwind.

Clearly, proposals for the control of photochemical smog by chemical additives cannot be based only on extrapolations from experiments conducted in the 'laboratory' torr pressure to

parts/10^9 concentration ranges. They must be experimentally validated at realistic pollutant levels in ambient air.

3.2. *Formation of nitrosamines and nitramines*

In the gas phase, even under 'laboratory' conditions in the torr pressure range, relatively little has been published on the chemistry of amine–NO_x–HONO–air systems. The most definitive work is that of Hanst, Spence & Miller (1977), who recently reported that 1 part/10^6 of gaseous dimethylamine (DMA) reacts in air at a rate of *ca.* 4 %/min with 0.5 part/10^6 of gaseous HONO (in equilibrium with 2 parts/10^6 NO, 2 parts/10^6 NO_2, and 13000 parts/10^6 H_2O) to produce dimethylnitrosamine (DMN) with an approximate 10–30 % yield. They also showed that DMN is rapidly photolysed in sunlight and that a new, 'unknown' compound is a major product.

We have used two quite different smog chambers and analytical techniques such as gas chromatography – mass spectrometry (g.c.–m.s.) and long-path Fourier-transform infrared (F.t.–i.r.) spectroscopy, to answer such questions as:

Can nitrosamines be formed when levels of secondary alkylamines below 1 part/10^6 are introduced into air containing typical ambient levels of NO_x and small amounts of HONO?

Do tertiary aliphatic amines also react with NO_x and HONO under similar conditions to form nitrosamines?

What are the rates of conversion of the amines, and what are their products and yields?

What is the relative importance of thermal (dark) versus photochemical processes in nitrosamine formation and decay?

In the range below 1 part/10^6, do simple aliphatic amines inhibit or accelerate formation of photochemical oxidant?

Are compounds other than nitrosamines being formed that may also represent possible environmental hazards?

3.2.1. *Experimental*

Two outdoor environmental chambers were employed, one similar to that used in the DEHA experiments and the other designed for spectroscopic studies. Each allowed the study of simulated polluted atmospheres under realistic conditions of solar irradiation (May–June 1977, latitude 33° N), temperature and humidity.

In a typical experiment, the large 50 m^3 chamber (not subdivided in these experiments) was initially covered with black plastic film and filled with particle-free air at 20–40 % relative humidity (r.h.) from our air purification system (Doyle, Bekowies, Winer & Pitts 1977). A measured volume of NO_2 was injected into a dilution flask and flushed into the chamber with 30 l O_2; NO in N_2 was injected in the same way 3 min later and the contents of the chamber thoroughly mixed. Under these conditions, some nitrous acid was formed, but its concentration could not be determined. Samples of diethylamine (DEA) or triethylamine (TEA) (vacuum-distilled, purity-verified by g.c.–m.s. and i.r.) were injected 15 min later. Initial amine concentrations, as determined by g.c. with the use of Tenax as a preconcentration step, were *ca.* 0.45 part/10^6 for DEA and 0.35 part/10^6 for TEA.

The amine–NO_x–HONO mixtures were allowed to react for 2 h in the dark. The black cover was then removed and the chamber exposed to sunlight for another 2 h. Ozone, NO, NO_2, PAN, acetaldehyde and the aerosol light-scattering coefficient (b_{scat}) were continuously monitored. Gas samples for g.c.–m.s. analysis were collected at regular intervals on Tenax

g.c. cartridges and analysed on a Finnigan Model 3100 instrument coupled to a Model 6100 mass spectral data system. Concentrations of DEA and TEA were monitored. Small (less than 10 %) corrections were made for artefact formation of diethylnitrosamine (DEN) on the Tenax.

The other environmental chamber contained a multiple reflexion i.r. cell having an optical path up to *ca.* 1.2 km (figure 4). Four rectangular mirrors, cut from a single 41 cm diameter Pyrex blank, composed the in-focus end of this cell, originally developed by Hanst and associates of the E.P.A. and modified in our laboratory. The out-of-focus optical assembly consisted of four 30 cm diameter mirrors set 22.5 m (the average radius of curvature of the eight mirrors) from the nesting mirror assembly. All mirrors were optically polished and gold coated for maximum reflectivity in the infrared.

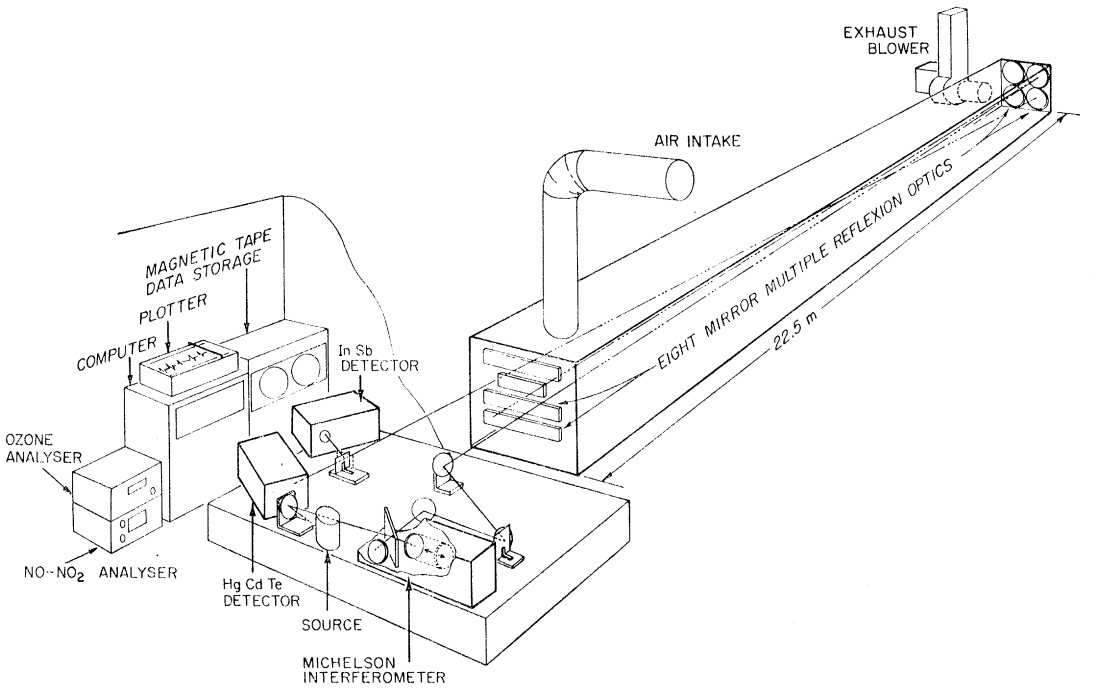


FIGURE 4. One kilometre optical pathlength Fourier-transform infrared spectroscopic facility.

The cell housing (i.e. the chamber) consisted of a rectangular aluminum frame ($0.76 \times 0.76 \times 23$ m) enclosed by 50 μm FEP Teflon walls whose measured transmittance was more than 80 % in the actinic u.v. For dark reactions a removable sun screen could be installed over the entire length of the cell.

Coupled with the cell was a Digilab Model 296 Michelson i.r. interferometer with a variable spectral resolution up to 0.5 cm^{-1}. The beam leaving the cell was sent to either of two liquid N_2 cooled detectors: a photovoltaic InSb detector for the 2000–3900 cm^{-1} region or a photoconductive HgCdTe detector for the 600–2000 cm^{-1} region. Data collection and processing were performed by a Data General Nova 1200 computer (Tuazon, Winer, Graham & Pitts 1977; Tuazon *et al.* 1978*a*, *b*). Initial concentrations employed with the two experimental systems were *ca.* 0.1–0.2 part $NO_x/10^6$ and *ca.* 0.3–0.5 part amine/10^6 in the 50 m^3 chamber and *ca.* 4 parts/10^6 NO, NO_x and amine in the F.t.–i.r. cell (r.h. *ca.* 40–50 %).

3.2.2. *Results*

(*a*) *Large outdoor chamber.* Solar irradiation of diethylamine-NO_x-air and triethylamine–NO_x–air mixtures at concentrations below 1 part/10^6 in the 50 m^3 chamber resulted in typical manifestations of severe photochemical smog, i.e. rapid conversion of NO to NO_2 and the formation of substantial amounts of O_3, PAN, CH_3CHO and light-scattering particles. Time–concentration profiles for DEA are shown in figure 5. Clearly, at these concentrations, both DEA and TEA behave as organics of high reactivity in producing photochemical smog.

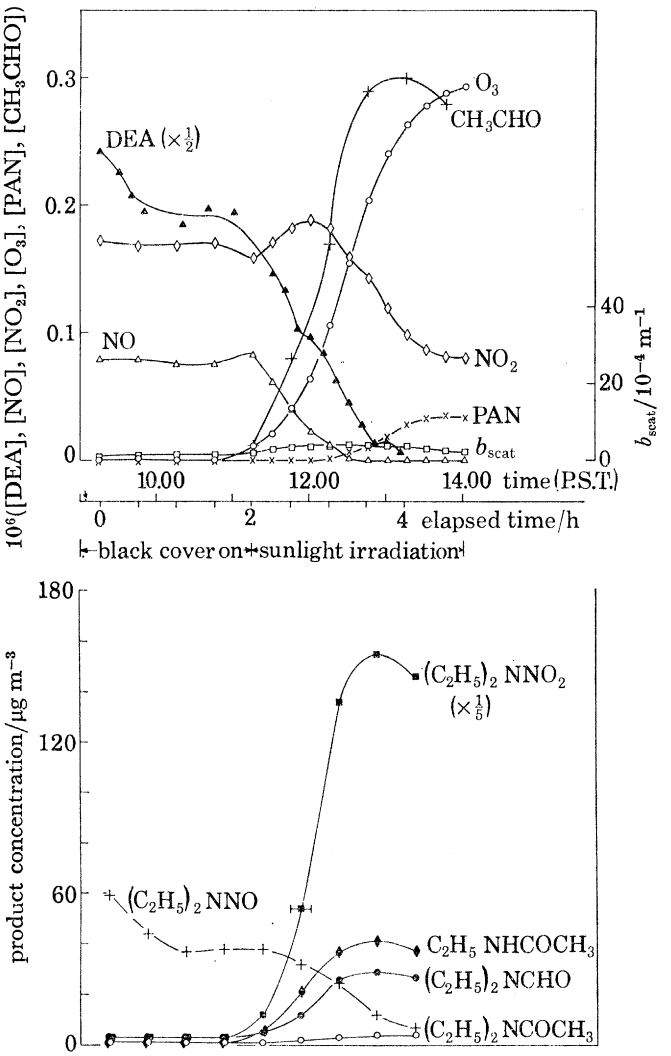


FIGURE 5. Time–concentration profiles of diethylamine, NO, NO_2, PAN, acetaldehyde and other nitrogenous compounds in the diethylamine–NO_x–HONO–air system under simulated atmospheric conditions in the dark and in sunlight.

As seen in figure 5, diethylnitrosamine was rapidly formed in the dark from the secondary amine DEA, but with a rather low yield of 59 μg/m^3 (maximum = 2.8 %). It was destroyed in sunlight with $t_{\frac{1}{2}} \approx 1$ h. A small amount of DEN was also formed in the dark from TEA (yield *ca.* 0.8 %). However, in contrast to DEA, on irradiation of the TEA–NO_x mixture with sunlight, *additional* DEN was formed initially with a maximum yield of 38 μg/m^3 (*ca.* 1.8 %).

Clearly, the idea that dialkylnitrosamines cannot be formed in sunlight from simple tertiary amines and NO_x is incorrect.

Four major gas phase products (other than acetaldehyde and PAN) were formed upon irradiation of both DEA–NO_x and TEA–NO_x mixtures: diethylnitramine [$(C_2H_5)_2NNO_2$], *N*,*N*-diethylformamide [$(C_2H_5)_2NCHO$], *N*-ethylacetamide [$C_2H_5NHCOCH_3$] and *N*,*N*-diethylacetamide [$(C_2H_5)_2NCOCH_3$]. Subsequently, an authentic sample of diethylnitramine was prepared and further confirmation was obtained by g.c.–m.s. and i.r. spectroscopy.

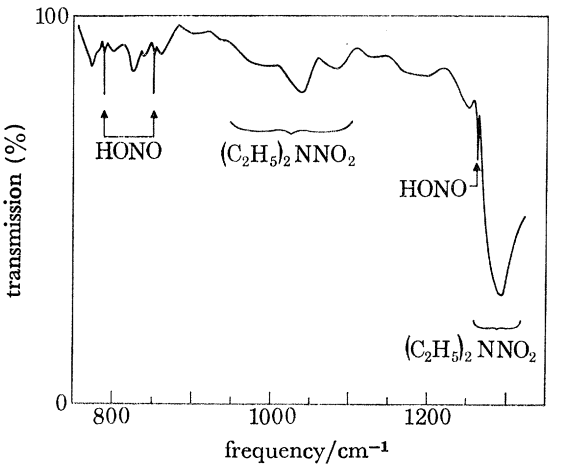


FIGURE 6. I.r. spectrum of diethylnitramine found upon irradiation of a diethylamine–NO_x–HONO mixture in air; optical pathlength 720 m.

Diethylnitramine was the major product in the DEA–NO_x system, reaching a maximum concentration of 162 parts/10^9 (780 μg m^{-3}). This corresponds to a yield of 32 % of the measured initial DEA concentration (0.45 part/10^6). The two major products from TEA–NO_x irradiations were diethylformamide (178 μg m^{-3}, 8.6 % yield), and diethylnitramine (177 μg m^{-3}, 7.4 % yield). Two additional gas phase products, not seen with DEA, were also formed in the gas phase. The major one (molecular ion m/e = 87) had a yield estimated at *ca.* 2 % and a mass spectrum compatible with an amide-like structure. The second product was formed in low yield and was identified as diacetamide $(CH_3CO)_2NH$.

G.c.–m.s. analysis of high volume aerosol samples collected at the end of the experiments revealed the presence of small amounts of acetamide in both the DEA run (3 μg/m^3) and the TEA run (9 μg/m^3). Diethylhydroxylamine was also formed (*ca.* 8 μg/m^3) in the TEA experiment.

(*b*) *Long-path F.t.–i.r. studies.* The F.t.–i.r. spectroscopic facility (figure 4) was employed at a path length of 720 m and a resolution of 1 cm^{-1} to investigate the infrared spectra of the products formed on irradiating with sunlight DMA–NO_x and DEA–NO_x mixtures (and associated HONO) in air at *ca.* 50 % r.h. In particular, *in situ* i.r. spectroscopic confirmation of the formation of nitramines and identification of the 'unknown' major photolysis product of dimethylnitrosamine in air observed by Hanst *et al.* (1977) were desired.

(i) *Dimethylamine*–NO_x. After several minutes of solar irradiation of DMA–NO_x mixtures ([NO] ≈ [NO_x] ≈ [DMA] ≈ 5 parts/10^6), and associated HONO, a very strong absorption band was observed at 1308 cm^{-1}. This band, as well as those appearing at 773, 985 and 1132 cm^{-1}, became more intense as the experiment progressed. Irradiations lasted for 2–4 h.

Comparison of these four major absorption frequencies with those reported by Davies & Jonathan (1958) for dimethylnitramine showed good agreement, both for the positions and relative intensities of the bands. The dimethylnitramine formed in these experiments was also identified by g.c.–m.s. analysis of samples adsorbed on Tenax cartridges. From the approximate positions of the major bands in the spectrum of the 'unknown' product of Hanst *et al.* formed in the photolysis of dimethylnitrosamine in air, we identify it as dimethylnitramine.

(ii) *Diethylamine–NO_x.* Irradiations of DEA and NO_x (and associated HONO) were carried out in the F.t.–i.r. facility in the same manner as those described above for DMA. As was the case for DMA runs, no significant amounts of O_3 or PAN were formed during these irradiations. This was due to inhibition by the high concentrations of NO and amine present; carbon monoxide and formaldehyde were, however, observed.

Contours of the product spectrum for 95 min of irradiation are shown in figure 6. For clarity, contributions from the unreacted DEA and water interferences were removed. By comparison of this spectrum with that of an authentic sample of $(C_2H_5)_2NNO_2$ prepared in our laboratory and taken under similar conditions (25°, 0.48 parts/10^6, 720 m, 1 cm^{-1} resolution), we can assign the very strong band observed at 1288 cm^{-1} to the NO_2 symmetric stretch in diethylnitramine. The absorptivity of this band is 37 cm^{-1} atm^{-1}† (Tuazon *et al.* 1978*a*).

3.2.3. *Discussion*

(*a*) *Nitrosation in the dark.* The only gaseous product identified in the dark experiments with either DEA or TEA was DEN. Its formation from DEA can be explained in terms of DEA reacting with the relatively small amounts of nitrous acid formed immediately following injection of NO and NO_2 into the 50 m^3 chamber:

$$NO + NO_2 + H_2O \underset{b}{\overset{a}{\rightleftharpoons}} 2HONO, \tag{1}$$

$$(C_2H_5)_2NH + HONO \longrightarrow (C_2H_5)_2NNO + H_2O. \tag{2}$$

The degree to which (1) and (2) occur by homogeneous versus heterogeneous paths in gas phase systems is still controversial (Chan, Nordstrom, Calvert & Shaw 1976; Kaiser & Wu 1977).

The maximum DEN yield is only 2.8 %, despite the fact that the concentrations of NO_x and DEA remain large throughout the 2 h period in the dark. One explanation is based on the fact that reaction (1) is very slow in the range 0.1–0.2 parts/10^6 (Chan *et al.* 1976; Kaiser & Wu 1977). Thus the HONO formed in the dark at the start of the experiment, when relatively high concentrations of NO and NO_2 were added consecutively to the chamber, would not significantly be regenerated after the initial amount present is consumed in (2). If this is indeed the case, the initial HONO present when the amine was injected would limit the DEN yield. Results of subsequent experiments, in which the initial HONO concentration was varied, are consistent with this hypothesis.

This is another example of the extrapolation problem in atmospheric chemistry referred to earlier. Clearly, when one considers the $NO + NO_2 + H_2O \rightleftharpoons HONO$ equilibrium in air, an assumption that the rapid rate of HONO formation observed at relatively high concentrations of reactants (e.g. 5 parts/10^6 NO and NO_2) is also true for concentrations around 10 parts/10^9 would be in serious error.

† 1 atm = 101325 Pa.

In the case of DEN formation in the dark from TEA, it is difficult to formulate a reasonable homolytic gas phase reaction mechanism. Heterolytic nitrosation mechanisms, perhaps on the chamber walls, may need to be invoked.

(*b*) *Mechanism of photo-oxidation.* The nature and distribution of the products observed for the TEA–NO_x system can be reasonably well explained in terms of initial OH attack on the secondary H atoms (reaction 3), followed by a series of reactions well established for photochemical smog (Demerjian, Kerr & Calvert 1974; Finlayson & Pitts 1976; Finlayson-Pitts & Pitts 1977):

$$(C_2H_5)_2NCH_2CH_3 + OH \longrightarrow \underset{\mathbf{1}}{(C_2H_5)_2N\dot{C}HCH_3} + H_2O. \tag{3}$$

Sources of OH radicals include the photolysis of nitrous acid at the onset of irradiation and photochemical reactions of acetaldehyde and ozone as the reaction proceeds.

By analogy with reactions of alkyl radicals in photochemical smog, one postulates that radical **1** reacts with molecular oxygen to yield the peroxy radical **2**; this then reacts with NO to yield NO_2 and the amine-alkoxy radical **3**:

$$(C_2H_5)_2N\dot{C}HCH_3 + O_2 \longrightarrow \underset{\mathbf{2}}{(C_2H_5)_2N\overset{\displaystyle O-O\cdot}{\overset{|}{C}}HCH_3}, \tag{4}$$

$$(C_2H_5)_2N\overset{\displaystyle O-O\cdot}{\overset{|}{C}}HCH_3 + NO \longrightarrow \underset{\mathbf{3}}{(C_2H_5)_2N\overset{\displaystyle O\cdot}{\overset{|}{C}}HCH_3} + NO_2. \tag{5}$$

Decomposition of **3** by two intramolecular pathways, (6) and (7), gives the major product acetaldehyde and the major nitrogenous product (other than PAN) diethylformamide:

$$(C_2H_5)_2N\overset{\displaystyle O\cdot}{\overset{|}{C}}HCH_3 \longrightarrow CH_3CHO + \overset{\mathbf{4}}{(C_2H_5)_2N\cdot}, \tag{6}$$

$$\longrightarrow (C_2H_5)_2NCHO + \cdot CH_3. \tag{7}$$

Reaction (6) is the favoured pathway. Alternatively, molecular oxygen may abstract the H atom α to the oxygen atom. This would form HO_2 and another observed product, diethylacetamide:

$$(C_2H_5)_2N\overset{\displaystyle O\cdot}{\overset{|}{C}}HCH_3 + O_2 \longrightarrow (C_2H_5)_2NCOCH_3 + H\dot{O}_2. \tag{8}$$

Further photo-oxidation of acetaldehyde in the presence of NO_x would lead to the formation of PAN, another major product.

Little is known about the atmospheric reactions of the diethylamino radical **4**. However, from the product distribution, and by analogy with the very slow reaction of the amino radical NH_2 with O_2 (Levine & Calvert 1977; Lesclaux, Khê, Desauzier & Soulignac 1975), one can assume that the reaction of $(C_2H_5)_2N\cdot$ with molecular oxygen is slow. Thus, presumably **4** can react efficiently with NO and NO_2, even though they are present at much lower concentrations than O_2:

$$(C_2H_5)_2N\cdot + NO \underset{b}{\overset{a}{\rightleftharpoons}} (C_2H_5)_2NNO \tag{9}$$

$$\Big\downarrow \text{direct oxidation, } h\nu \text{ and/or thermal} \tag{11}$$

$$(C_2H_5)_2N\cdot + NO_2 \underset{b}{\overset{a}{\rightleftharpoons}} (C_2H_5)_2NNO_2. \tag{10}$$

The efficient photochemical formation and photostability of nitramines compared with nitrosamines is of some interest. If indeed they are formed in the competitive reactions (9) and (10), their relative concentrations will depend in part upon the ratio of NO to NO_2, the rate constants for (9*a*), (10*a*) and (11), and their relative stabilities in sunlight as determined by the photochemical reactions (9*b*) and (10*b*). The long wavelength absorption cut-offs for DEN and diethylnitramine in methanol are 390 and 310 nm respectively. Thus, even though in condensed phases nitramines react photochemically at 253.7 nm to give the corresponding nitrosamines (Suryanarayanan & Bulusu Suryanarayanana 1972), their absorption cross sections in the actinic ultraviolet ($295 < \lambda < 400$ nm) are low. Thus their loss by direct photodecomposition (10*b*) is expected to be correspondingly small. Nitrosamines, however, absorb over a wide range of actinic u.v. and photodecompose efficiently (Hanst *et al.* 1977; Bamford 1939).

Thus, the observed relative yields of DEN versus diethylnitramine, and their time concentration profiles (figure 5) are consistent with their expected stabilities in sunlight. Furthermore, our mechanism is consistent with the results of Hanst *et al.* (1977), who, as noted above, found that photolysis of DMN gave large yields of a 'new compound' which we have identified as dimethylnitramine. The relative importance of (9*a*), (9*b*), (10*a*), (10*b*) and (11) remains to be determined, as do many other aspects of amine photo-oxidation in air; so our mechanism is clearly tentative.

For diethylamine, an analogous sequence of photo-oxidation reactions involving initial OH attack on the secondary C—H bond, would give the products acetaldehyde and ethylacetamide. However, the large yield of diethylnitramine from DEA (*ca.* 4.5 times more than from TEA) indicates that additional efficient reactions also produce the diethylamino radical, **4**. A likely candidate is initial OH attack on the N—H bond (reaction 12), which would compete with attack on the secondary C—H bonds (reaction 3):

$$(C_2H_5)_2NH + OH \longrightarrow (C_2H_5)_2N\cdot + H_2O. \qquad (12)$$

Indeed, recent measurements of the absolute rate constants for OH attack on dimethylamine and trimethylamine, 6.5×10^{-11} and 6.1×10^{-11} cm^3 molecule^{-1} s^{-1} respectively (Atkinson, Perry & Pitts 1978*b*) suggest that in secondary alkylamines, H atom abstraction by OH from the N—H bond is competitive with H atom abstraction from the secondary C—H bonds.

(*c*) *Environmental implications*. As we noted earlier, diethylnitrosamine and dimethylnitrosamine are potent carcinogens (Fahmy & Fahmy 1976; Walters 1977). Both dimethylnitramine (Goodall & Kennedy 1976; Druckrey, Preussman, Schmahl & Muller 1961) and acetamide (Jackson & Dessau 1961; Weisburger, Yamamoto, Glass & Frankel 1969) also show carcinogenic activity in test animals, though with less potency. The possible carcinogenicity in experimental animals of diethylnitramine and of the substituted amides identified in this study has not apparently been determined.

Amines are emitted into industrial atmospheres as a result of a wide variety of activities; some of these are secondary and tertiary aliphatic amines or related compounds such as ethanolamines. Little is known about their concentrations in ambient air, but probably they are usually very small. Thus, even though levels of NO_x and HONO from mobile and stationary sources may be appreciable (Chan *et al.* 1976), the risk of forming significant amounts of nitrosamines or nitramines in air to which the general public is exposed seems correspondingly small. However, in industrial situations in which amines are released into such polluted atmos-

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY A MATHEMATICAL, PHYSICAL & ENGINEERING SCIENCES

pheres, within and immediately downwind from the facility, formation of significant amounts of nitrosamines in the dark and nitramines and amides in sunlight seems possible. Clearly in these cases measurement of the actual ambient levels of amines, NO_x, HONO, nitrosamines and nitramines would be useful.

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PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY A MATHEMATICAL, PHYSICAL & ENGINEERING SCIENCES

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PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY A MATHEMATICAL, PHYSICAL & ENGINEERING SCIENCES