Mutagens and Polycyclic Aromatic Hydrocarbons in Ambient Airborne Particles

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Willem Karel de Raat

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Promotores:	Prof. dr ir P. H. M. Lohman Prof. dr G. R. Mohn
Referenten:	Dr R. M. van Aalst, RIVM Prof. dr F. A. de Wolf, Universiteit van Amsterdam
Overige leden:	Prof. dr S. A. L. M. Kooijman, Vrije Universiteit Prof. dr C. J. van Leeuwen, Rijksuniversiteit Utrecht Prof. dr ir A. A. van Zeeland

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CHAPTER 1

INTRODUCTION

Carcinogenicity of ambient airborne particles

Studies on the mutagenic effects of ambient airborne particles and the presence of polycyclic aromatic hydrocarbons (PAH) in these particles are motivated by the concern about possible carcinogenic effects in humans exposed to these particles by inhalation. Both mutagenicity and the presence of PAH may be related with possible carcinogenic effects of the particles; mutagenicity, because it is a toxicological effect which plays a predominant role in carcinogenesis (Mohn, 1980; Varmus, 1984; Barbacid, 1987; Bishop, 1987; IARC, 1988; Rowley, 1990), and the presence of PAH, because members of this group of compounds can have carcinogenic or mutagenic properties (Dipple, 1976; Gelboin and Ts'o, 1978; NRC, 1972 and 1983; IARC, 1973 and 1983; Montizaan et al., 1989).

Concern about the possible carcinogenic effects of ambient airborne particles was not triggered by their mutagenic properties or by the presence of PAH. Before the particles were found to be mutagenic or to contain PAH, other, more direct indications for their carcinogenicity had been obtained. In retrospect, one has to go back as far as the 18th century for the first indication, when the british surgeon Sir Percival Pott attributed the high incidence of scrotum cancer in chimney sweeps to their excessive exposure to soot (Pott, 1775). This was the first study which showed that occupational exposure to chemical substances may have carcinogenic effects. In fact it was also, the first study which pointed to the possibility that chemical substances may have carcinogenic properties. As soot in smoke will generally not be completely retained by the chimney, part of it will be emitted to the ambient air, which makes clear that Pott's study was also the first pointing to the possibility of air pollution by carcinogens, although Pott himself did not reach this conclusion.

This does not mean that in Pott's time there was no awareness of the possible adverse health effects of air pollution caused by combustion processes. The earliest known publication on this subject appeared already in the 17th century (Lodge, 1969; Chambers, 1976). Yet, it took more than 150 years before the importance of Pott's observation was recognized in terms of a possible carcinogenic effect of air pollution. Meanwhile, Pott's observation was confirmed

by other authors (Henry, 1946). The first experimental evidence for the carcinogenicity of soot was obtained in the 1920's by Passey, who showed that skin tumours could be induced in mice by painting the animals with extracts of household chimney soot (Passey, 1922). Comparable results were reported by Campbell in 1934.

In the 1940's Leiter and coworkers were the first to demonstrate that extracts of ambient airborne particles induce tumours in mice after subcutaneous injection (Leiter et al., 1942; Leiter and Shear, 1942/1943). Since then the carcinogenicity of ambient airborne particles for experimental animals has amply been confirmed by other investigators (Kotin et al., 1954A; Hueper et al., 1962; Wynder and Hoffmann, 1965; Epstein et al., 1979; Pott et al., 1980). In addition, the obvious sources for the carcinogens, combustion processes, were found to indeed emit particulate carcinogens to ambient air (Kotin et al., 1954B and 1955; Gurinov et al., 1962; Hoffmann et al., 1965; Grimmer et al., 1983, 1984 and 1985; IARC, 1984A and 1989).

The consequence of these findings was a growing concern about a possible enhancement of the lung-cancer incidence in the human population caused by the inhalation of ambient airborne particles. Some epidemiological studies in the 1950's and 1960's aggravated this concern, as they showed that living in urban areas with heavy air pollution was linked with an increased lung-cancer risk (Kotin, 1956; Eastcott, 1956; Dean, 1959; Stocks, 1960; Wynder and Hammond, 1962; Bluell and Dunn, 1967; McCall and Stenhouse, 1971; Friberg and Cederlöf, 1978). This so-called urban factor in the aetiology of lung cancer has originally been attributed to the higher air-pollution levels in the urban environment. Later its link with air pollution has been seriously disputed and at the moment it is largely ascribed to live-style related factors and occupational factors, although a contribution by air pollution can still not be excluded (Goldsmith and Friberg, 1977; Doll, 1978; IARC, 1976A and 1977). Nevertheless, the urban factor has greatly stimulated research on air pollution with carcinogens. Moreover, the episode of severe sulphurous smog of 1952 in London, with its high numbers of fatalities as well as the concern about the health problems associated with photochemical air pollution in the United States in the 1950's and 1960's (Goldsmith and Friberg, 1977), led to an increasing general awareness that air pollution can have adverse health effects.

The presence of possible carcinogens in ambient airborne particles became an important topic in air-pollution research. Questions arose as to the emission, distribution and fate of the carcinogens, their chemical identity and physical form, and the exposure levels of the human population. To answer these

questions, the investigator had initially to rely on chemical analysis for individual carcinogenic compounds, because carcinogenicity itself could not be used as a flexible toxicological indicator for the presence of carcinogens in the environment. Carcinogenic polycyclic aromatic hydrocarbons (PAH) found in the particles emitted by combustion processes as well as those present in ambient air were obvious candidates for subsequent studies (Waller, 1952; Kotin et al., 1954[a], 1954[b] and 1955; Falk et al., 1956; Stocks, 1960; Hueper et al., 1962; Wynder and Hoffmann, 1965; Sawicki, 1967; NRC, 1972 and 1983; Hoffmann and Wynder, 1977; Grimmer, 1983A).

Later, biological tests for the mutagenicity of chemical compounds were developed, which allowed mutagenicity to be used as a pollution indicator with a certain, albeit limited predictive value as regards the carcinogenicity of the samples tested (IARC, 1976, 1980 and 1986; Mohn, 1980; ICPEMC, 1982; Hoffmann, 1982; De Raat et al, 1985 and 1991). Many studies have since then demonstrated the clear-cut mutagenicity of particles from areas with anthropogenic air pollution (Talcott and Wei, 1977; Pitts et al., 1977; Teranishi et. al., 1978; Commoner et al., 1978; Tokiwa et al., 1980; Alfheim and Møller, 1979; Møller and Alfheim, 1980; Chrisp and Fisher, 1980; Alink et al., 1983; De Raat et al., 1983); again combustion processes were found to be the major, if not the only, source (Lofröth, 1978; Alfheim, 1982; Møller and Alfheim, 1983; Parri et al, 1983; Wei et al., 1982; Selzer Madsen et al., 1982; Xu et al., 1982).

Mutagenicity and carcinogenicity

Mutagenicity can be defined as the ability of chemical or physical agents to induce changes in the genetic information of living organisms as it is laid down at the gene, gene segment or chromosome level. Ample evidence exists that such changes, which are called mutations, play a key role in carcinogenesis. According to current theory, cancer is the result of the induction of mutations which affect the functioning of genes involved in the regulation of cell division or cell differentiation. Changes of the expression of these genes or their gene products may allow the cell to circumvent the internal and external signals which restrict its division or determine its differentiation and may ultimately lead to unrestricted cancerous growth (Varmus, 1984; Bishop, 1987; IARC, 1988; Harris et al., 1989; Marx, 1989; Westin, 1989; Wiedemann and Morgan, 1992). Consequently, mutagenic compounds must in principle be regarded as potential carcinogens. They can induce the changes necessary for the onset, i.e. the initiation of carcinogenesis.

This is not to say that mutagens are always carcinogens or that all carcinogens are mutagens, let alone that a clear quantitative correlation exists between mutagenic potency and carcinogenic potency (McCann et al., 1975A; McCann and Ames, 1976; Rinkus and Legator, 1979; Ashby and Styles, 1978; Mohn, 1980; Brusick, 1983; Zeiger and Tennant, 1986; ICPEMC, 1988; Bridges, 1988; Ashby and Tennant, 1988; Ashby and Purchase, 1988; Tennant et al., 1987; Ashby et al., 1989; Ashby and Tennant, 1991). The predictive value of mutagenicity as an indicator for carcinogenicity can only be limited, for several reasons:

- Whether or not a compound can induce the necessary mutations for the initiation of cancer, depends on its toxicokinetics and biotransformation (metabolic (de)activation) in the exposed organism, the nature of the primary lesions caused by it, their place on the genome, and the response of the organism to the primary lesions (Diwan and Rice, 1989). The compound has to reach sensitive cells in its active form and at high enough concentrations, it has to react with the DNA of the genes involved in cell division and differentiation and the resulting lesions have to be converted to mutations. Consequently, a compound can be a strong carcinogen in one species, organ or tissue, while being devoid of activity in another. This specificity of carcinogenicity should be taken into account when the predictive value of mutagenicity is considered.
- Mutagenicity can be determined with various test methods, which are based on different endpoints in different test organisms. Compounds can strongly differ as regards their activity in these tests. Toxicokinetics, metabolic conversion and primary damage, as well as the response of the test organism to this damage will play a role here. Consequently, the predictive value will depend on the mutagenicity test applied. This test specificity should be taken into account when the predictive value of mutagenicity is considered.
- Cancer is still regarded as a multi-step process, of which initiation (by mutagens) represents only the first step (Berenblum, 1941A and 1941B; Berenblum and Shubik, 1948; Goldsworthy and Hanigan, 1987; IARC, 1988; Pitot, 1989; Peraino and Jones, 1989; Barret et al., 1990; Yamasaki et al., 1992; Hennings et al., 1993). Initiation as such does not necessarily lead to cancerous growth. It has to be followed by two stages which may comprise various steps: promotion, which is the stimulation of the already initiated cell to unrestricted division activity (IARC, 1984; Jongen, 1988; Diwan and Rice, 1989) and progression, when cells start to (de)differen-

tiate and become invasive and malignant (Nicolson, 1987; Pitot, 1989; Dulbecco, 1989; Liotta, 1992). It has repeatedly been shown, that like initiation, promotion can be induced by various classes of chemical compounds, which are not necessarily mutagens. Progression is most probably not to any great extent induced and stimulated by external factors; it seems to be a largely autonomous process.

Recent studies have lead to a reassessment of the conventional multi-step theory. It has been demonstrated for colorectal cancer and some other forms of cancer that the development of the disease from its earliest detectable form to its final malignant stage is accompanied by an accumulation of genetic alterations which affect the genes involved in cell division and differentiation (Fearon and Vogelstein, 1990; Nowell, 1986; Marx, 1989). So, the idea that mutations are only involved in the first stage does not seem a valid one. However, this does not mean that all these mutations have to be induced by external factors. The development of cancer appears to be accompanied by an increasing genetic instability (Nicolson, 1987; Loeb, 1991; Boesen, 1992). Therefore, after the development of the disease has been initiated, the necessary mutations for its proceeding may very well arise spontaneously. This would still mean, that the effect of the mutagenic compound is largely restricted to the induction of the first mutation or mutations, i.e. to the initiation, while further development of the disease does not depend on external factors.

The genetic instability may, however, also be the primary effect, which is not necessarily induced by a mutation and which may, as a secondary effect, lead to the mutations in the genes involved in the regulation of division and differentiation (Boesen, 1992). According to yet another hypothesis cancer can be induced by a stimulation of cell division which in its turn is induced by toxic damage (Ames and Swirsky Gold, 1990A and 1990B; Weinstein, 1992; Travis and Belefant, 1992). Dividing cells are more sensitive to mutagenic agents than non-dividing ones, while potential carcinogenic lesions and mutations may come to a full expression as a result of cell division. Mutagens can also be toxic by their mutagenicity and thus (according to this theory) induce cancer without affecting the genes directly involved in this process.

The assessment of mutagenic factors in environmental samples is not only determined by their link with carcinogenicity. Of equal importance is, what may

be called, the experimental convenience of the methods for the determination of these factors. In contrast with carcinogenicity, the detection of mutagenicity is in general characterized by a short-term nature, easy performance and high sensitivity, due to the fact that the induction and identification of a mutation or related genotoxic effects does not require chronic (lifetime) exposure of mammals and the outgrowth of affected cells to a tumour. Instead, rapidly dividing micro-organisms or cultured mammalian cells may be used as model organisms, in which effects can be scored within a few days after exposure has started.

The Salmonella/microsome test developed by Ames and coworkers (Ames, 1971 and 1972; Ames et al., 1973A and 1973B; Ames et al., 1975; Maron and Ames, 1983) may serve as an example here. In this test bacteria, i.e. specially constructed strains of Salmonella typhimurium, are used for rapid, easy and sensitive detection, while mammalian tissue homogenates are used to simulate the mammalian biotransformation of the test compound, thereby allowing the detection of compounds that are converted into mutagenic metabolites in the mammal. The widespread use of this test in applied toxicology is to a great extent the result of its experimental convenience. Moreover, it has proven to be highly sentitive to many different classes of mutagens (McCann et al., 1975A; Ashby and Tennant, 1991).

Short-term nature, easy performance and sensitivity are crucial properties of the mutagenicity tests when they are used in studies on the presence of mutagens in the environment, in particular when the aims of these studies go further than simply demonstrating that mutagens are present. A reliable impression of the sources and emission of the mutagens, their distribution, spatial as well as temporal, and their fate, requires that large numbers of environmental samples can be compared quantitatively for their mutagenic properties, with a high discriminating power and within a limited time span. Some mutagenicity tests, in particular the Salmonella/microsome test, do fulfil these requirements to a sufficient extent for a meaningful application in environmental studies.

Before the Salmonella/microsome test and other short-term mutagenicity tests became available in the first half of the nineteen seventies, studies on the presence of potential carcinogenic compounds in the environment were largely based on the determination of the concentrations of a very limited number of specific compounds with analytical-chemical techniques (Sawicki, 1967). Emission, distribution and fate of the carcinogens could not be studied directly by testing environmental samples. The long-term nature and high costs of the available

methods for the detection of carcinogenicity prevented this effect being used as a flexible indicator in environmental studies.

Analytical-chemical studies can now be supplemented with mutagenicity tests. The concentrations of a limited number of known active compounds can be supplemented with the integrated effect of many relevant compounds, whether they are known or not, whether they are themselves mutagenic or affect the mutagenicity via interactions. Thereby, a more complete picture of the nature and intensity of actual human exposure can be obtained than would be possible on the basis of analytical-chemical studies alone.

Polycyclic aromatic hydrocarbons (PAH)

The first compounds to be identified as chemical carcinogens were PAH. Their discovery as carcinogens followed from the quest for the so-called coal-tar carcinogen, which ended in 1933 with the identification of the new PAH benzo(a)pyrene as a major, if not predominant carcinogenic component of the tar (Yamagiwa and Ichikawa, 1918, Cook et al., 1933; Kennaway and Hieger, 1930; Kennaway, 1955; Phillips, 1983). Two methylbenz(a)anthracenes were synthesized as model compounds during this research, and, although they were not components of the tar, they were found to be strong carcinogens. In addition, the results indicated that the remainder of the carcinogenicity of the tar was caused by comparable aromatic compounds.

Since the coal-tar work, PAH have featured conspicuously in both toxicological and environmental research. The toxicologist could dispose of model compounds in investigations aimed at the elucidation of the carcinogenesis process. Consequently, many aspects of the carcinogenic properties of PAH have been thoroughly investigated (Santodonato et al., 1981; IARC, 1983; NRC, 1972 and 1983; Dipple, 1976; Gelboin and Ts'o, 1978; Montizaan et al., 1989). Besides the three identified in the coal-tar work, several others have been found to be carcinogenic, via various exposure routes (including inhalation) and in various experimental animals and organs.

Epidemiological evidence strongly suggests that PAH can cause cancer in humans. Enhanced cancer incidences were observed upon occupational exposure to pyrolysis products of coal with extremely high PAH concentrations such as coal tar, coal-tar products and the vapours emitted during coke production and coal gasification (IARC, 1984A; IARC, 1985). Although the possibility can not be excluded that these products contain other compounds with carcinogenic properties besides PAH, the dominant contribution of the latter to the carcinogenicity of comparable products for experimental animals

(Cook et al., 1933; Kotin et al., 1954[b] and 1955; Gurinov et al., 1962; Hoffmann et al., 1965; Grimmer et al., 1983, 1984 and 1985; Holland et al., 1984; Mahlum et al., 1984) suggests that they do contribute to the carcinogenic effects demonstrated in humans.

PAH are so-called indirect carcinogens, i.e. they themselves do not induce cancer directly, but they have to be transformed by the metabolism of the exposed organism to the ultimate carcinogenic compounds (Yang et al., 1978; Bartsch, 1982; IARC, 1983; Glatt and Oesch, 1986; Cavalieri and Rogan, 1992). This metabolic activation depends on their basic molecular skeleton, which consists of a number of conjugated benzene rings, sometimes supplemented with five-membered rings. The rings are oxidized to phenols via epoxides by two groups of enzymes: Cytochrome-P450-dependent mixed-function oxidases and hydratases. The epoxides can be strong mutagens and may, therefore, induce the mutations necessary for the onset of cancer. Furthermore, mutagenic radical cations may be formed by the action of the group of P450 cytochromes. PAH are thus genotoxic carcinogens, i.e. compounds initiating cancer by mutations, a genotoxic event.

Basically the PAH in the environment stem from two sources. They are formed during the incomplete combustion or pyrolysis of fossil fuels and other materials with an organic origin (Hangebrauck et al., 1967; Bjørseth and Ramdahl, 1985). Furthermore, they are already present in some fossil fuels before combustion as a result of the fossilization process (Blumer, 1976; Guerin, 1978 and Bjørseth and Ramdahl, 1985). As combustion is by far the most predominant and most widespread source of anthropogenetic air pollution, PAH are ubiquitous in the air, and after deposition, in surface waters and soil (Slooff et al, 1989; NRC, 1972; Baum, 1978; Borneff and Kunte, 1983; Neff, 1979; Bjørseth and Sortland Olufsen, 1983; CCRX, 1987; Grimmer, 1983B; Verhoeve and Compaan, 1980; Van Aalst et al., 1982; Thijsse and Huygen, 1985). The use or spillage of PAH-rich products, such as coal tar or creosote, a distillate of coal tar, may significantly add to the pollution of the environment with PAH (Slooff et al., 1989). The use of creosote-treated wood in the Netherlands may serve as an example here. It has been recognized as an important source of the more volatile PAH in surface waters and air. Other sources which can be mentioned besides combustion are coke production (emission of PAH-rich coal-tar vapours), aluminium production (the production and use of electrodes made from pitch) and oil spills on sea (Slooff et al., 1989; Neff, 1979; Bjørseth and Ramdahl, 1985).

Since the beginning of the 1950's the presence of PAH in the air and combustion emissions has been subject of numerous environmental studies. The

concentrations of carcinogenic PAH were the sole indicators available for investigations of the emission, distribution and fate in the air of combustion-related carcinogens. As has been explained above, carcinogenicity itself is too inflexible for this purpose. The use of PAH concentrations was in particular stimulated by the important contribution of PAH to the carcinogenic effects of many pyrolysis- and combustion-related complex mixtures of compounds (Kotin et al., 1954B and 1955; Gurinov et al., 1962; Hoffmann et al., 1965; Grimmer et al., 1983, 1984 and 1985). Also the carcinogenicity of ambient airborne particles appeared to be largely the result of the presence of PAH (Kotin et al., 1954A; Hoffmann and Wynder, 1977).

For a long time attention was completely concentrated on PAH as carcinogenic constituents of ambient air in air-pollution research. Hardly any attention was paid to other possible carcinogens, this notwithstanding the clear indications obtained during the 1950's and 1960's that these are present and contribute significantly (Kotin and Falk, 1956; Kotin et al., 1956; Hueper et al., 1962 and Asahina et al., 1972). Most air-pollution studies focused on benzo(a)pyrene, which was demonstrated for the first time in ambient airborne particles by Waller (1952). For a long time this compound was regarded as the carcinogenic representative of the PAH and its concentrations were assumed to be a reliable indicator for air pollution with the complete set of PAH and thus for the carcinogenic risk associated with the presence of PAH.

Later the role of PAH in air-pollution research became less prominent. In the long run, the idea that their concentrations are a (semi) quantitative marker for cancer risk did not find any substantial experimental confirmation. At the moment the concentrations are merely determined to keep an eye on the pollution of the ambient air with combustion-related carcinogens. The presence of these compounds alone is deemed a sufficient reason for their determination, i.e. without the existence of real proof for a direct link with an adverse health effect, because they represent complex mixtures the carcinogenicity of which has been unambiguously proven in epidemiological studies (IARC, 1984A and 1985; Mumford et al., 1987; Xu et al., 1989) and in studies with experimental animals (IARC, 1984A; 1985; 1989; Kotin, 1956). In a way, ambient air in most human environments can be regarded as a very strongly diluted form of these complex mixtures.

Regulatory authorities in some countries have developed air-quality standards for PAH. These are either based on actual concentrations in the

average urban and industrial region[1] (Germany, 10 ng.m^{-3} benzo(a)pyrene, TA-Luft, 1986) or on very rough extrapolations from epidemiological data, in particular data on lung-cancer incidence in coke-oven and gas-works personnel (The Netherlands, 0.5 - 5 ng.m^{-3} benzo(a)pyrene; Slooff et al., 1989; MPV88, 1987; Gezondheidsraad, 1984 and 1990). As the relationship of these standards with real cancer risk can at best be very uncertain, it is difficult to weigh the benefits of measures against their costs in case the standards are exceeded, a situation which occurs often (e.g. De Raat, 1988).

This demonstrates the need for research aimed at a better clarification of the health significance of air pollution with PAH, at least if these standards are taken seriously. An important topic then should be the presence of other possible carcinogens, which was already indicated by earlier carcinogenicity studies and which became particularly immanent by the application of short-term mutagenicity tests.

Links between mutagenicity and PAH concentrations

PAH are mutagens themselves and will, therefore, directly contribute to the mutagenicity of ambient airborne particles. The mutagenicity of the particles cannot, however, be completely explained by the mutagenicity of the PAH present in them. It has amply been shown that the particles contain so-called direct mutagens, i.e. mutagens which do not depend on mammalian bioactivation to exert their effect; in contrast, PAH have to be activated by Cytochrome-P450-dependent oxidation. Furthermore, most of the mutagenicity of the particles can be isolated in chemical fractions which do not contain PAH (Teranishi et al., 1978; Pellizari et al., 1979; De Raat 1983; Pyysalo et al., 1987).

Nevertheless, the mutagenicity not directly caused by the PAH, does seem largely to be associated with the presence of PAH, albeit in a more indirect manner. Several lines of evidence made clear that a substantial part of the non-PAH mutagenicity, including that independent of mammalian bioactivation for its effect, can be explained by the presence of nitrated and, to a less great extent, oxygenated PAH, which are formed from PAH during combustion, emission or residence in the air (Ramdahl, et al., 1982; König et al,. 1983; Nishioka et al., 1986; Tanner and Fajer, 1983; Gibson, 1982; Nielsen et al., 1984; Pitts, 1983 and 1987; Nielsen et al., 1983; Alfheim et al., 1985).

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[1] The philosophy being that concentrations should in any case not increase in the future, this notwithstanding the ignorance about their significance in terms of adverse health effects.

Two types of investigations

The presence of mutagens and PAH in the atmospheric environment has prompted two types of studies: those concentrating on the presence of the compounds itself and those concerned with the possible adverse health effects of their presence. The first type can be characterized as air-pollution studies. The role of toxicology is restricted to the application of a number of standardized tests, which are used together with analytical-chemical methods and methods for collecting and processing samples to investigate the sources of the compounds, their fate after emission, the identity of the compounds determining the mutagenicity and the links between mutagens and PAH. Ultimately these studies try to gain insight into nature and intensity of exposure.

The studies of the second type only start at exposure and try to gain insight into the adverse health effects this exposure may actually cause under real-life conditions. They deal with the so-called extrapolation gap between on one hand mutagenicity of an air sample or its PAH concentrations after intensive processing and on the other the effects of, for instance, ambient airborne particles with mutagens or PAH after inhalation and deposition in the respiratory tract. To what extent is extrapolation possible from what we are measuring to what we are worried about? To bridge this gap toxicokinetics, metabolism and effects of the compounds are investigated in advanced biological experimental systems which are designed to approach the real-life conditions as close as possible. The origins of the mutagens and PAH are taken for granted in these studies which concentrate completely on the toxicological consequences.

Obviously both types of studies strongly depend on each other. The air-pollution studies are ultimately motivated by the results of the toxicological studies. Furthermore, the outcome of the latter determines the toxicological tools to be used as well as well as the compounds to be investigated in the former. On the other hand meaningful toxicological studies can only be carried out if the intensity, variation and nature of exposure has been revealed by air-pollution studies.

CHAPTER 2

OBJECTIVES AND OUTLINE

The core of this thesis consists of a series of experimental studies concerned with the presence of mutagens and PAH in ambient air which belong to the air-pollution type discerned in the foregoing chapter. Their general objective was, therefore, to improve our insight in the nature and intensity of the pollution of ambient air with mutagens and PAH and, thereby, the actual human exposure to these compounds. More specifically they were focussed at the following topics:

- the spatial and temporal distribution of mutagenicity and PAH,
- the chemical diversity and chemical nature of the mutagens,
- the reliability of the methods used for the determination of mutagenicity and PAH concentrations,
- the link between mutagenicity and PAH concentrations.

The studies were mainly restricted to the particulate phase of the air. Attention was only paid to the gaseous mutagens and PAH in sofar this allowed for a more complete picture of the pollution of the air with the particulate mutagens and PAH.

The experimental part is preceded by three chapters of an introductory and theoretical character. The first (chapter 3) is concerned with the limitations and possibilities of mutagenicity tests as analytical tools in environmental studies, with emphasis on the Salmonella/microsome test. The extrapolation problem, i.e. the indicative value of mutagenicity and PAH concentrations with respect to the possible carcinogenic effects of ambient airborne particles in humans, receives attention in chapter 4. Ambient airborne particles, together with their sampling and extraction, form the subject of chapter 5.

The spatial and temporal distribution was covered by three experimental studies. In one of their (chapters 6 and 7) the contribution of the sources in the densely populated and heavily industrialized Rijnmond area of the Netherlands was investigated by comparison of samples taken simultaneously at four sites in the area and at one coastal site upwind, which was assumed to represent the background pollution level of the area. For mutagenicity this

comparison was formalized with a series of mathematical models, which allowed the influence of interactions between site-dependent factors and time-dependent factors on mutagenicity to be singled out. Furthermore qualitative changes were investigated as they are expressed in the proportion of the effects obtained with different types of tester strains with or without tissue homogenate for metabolic activation (the mutagenicity profile) or, in case of the PAH, in the proportion of the concentrations of these compounds (the PAH profile). The nature of the changes of the PAH profile pointed to processes causing them. Chemical conversion was for instance revealed by the specific decrease of more reactive PAH, while changes of the concentrations of more volatile PAH pointed to evaporation or condensation.

The second study (chapters 8 and 9) was concerned with the influence of photochemical air pollution on mutagenicity and PAH concentrations and with the comparison of particles from distant sources and those emitted by traffic in and near the town of Delft. Samples collected simultaneously at sites differently affected by pollution from local traffic were compared for mutagenicity and PAH concentrations. In addition mutagenicity and PAH concentrations were monitored during the daily development of photochemical air pollution, together with concentrations of some gaseous pollutants. As in the previous study, qualitative changes were investigated by analysis of the proportion of the mutagenic effects and the PAH profile.

The central theme of the third study[2] was the development of mutagenicity and PAH concentrations over a year. Samples were simultaneously collected every week at four sites, one in a rural area near the coast, one in Delft and two in the Rijnmond area. As in the previous studies, quantitative as well as qualitative changes were investigated.

The investigations into the chemical diversity and chemical nature of the mutagens are described in chapter 10. So-called bioassay-directed fractionation was applied, which means that the mutagens were concentrated in well defined chemical fractions by repeated chromatographic fractionation combined with biological testing. If the fractions are chemically homogeneous enough, chemical analysis may provide information on the identity of the mutagens. The combination of various chromatographic techniques gave an impression of the number of different groups of compounds which contribute to the mutagenicity.

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[2] This study is not described in detail in this thesis; it is presented in summarized form in chapters 13 and 14; for a more detailed presentation the reader is referred to De Raat et al., 1988.

Furthermore, it was attempted to achieve a reliable quantitative estimate of the contribution by PAH and some of their nitrated derivatives.

The reliability of the technique used for sampling of the particles was the subject of the study presented in chapter 11. Starting point formed the clear indications in the literature that chemical conversion of PAH may occur after the particles have been fixed on the filter and that this artifact strongly depends on the filter material applied (Lee et al., 1980; Grosjean et al., 1983; Van Cauwenberghe et al., 1980; Brorstrøm et al., 1983). In the study the role of the filter material was further elaborated upon. In particular it was investigated whether the artifacts induced by the filter material also affect mutagenicity.

The high concentrations of gaseous PAH in the air reported in the literature (Thrane and Mikalsen, 1981; Yamasaki et al., 1983; Keller and Biddleman, 1984) prompt the question whether the presence of these compounds is accompanied by mutagenicity as it is in the particles. Is the clear link between air pollution with mutagens and PAH restricted to the particles or does it also manifest itself in the gaseous domain? The study that will be described in chapter 12 was aimed at answering this question. To this end sampling has been carried out by filtration for the particles and by adsorption for the gaseous pollutants after the air had passed the filters and both types of samples have been compared for the presence of mutagens and PAH.

Mutagenicity and the presence of PAH were not treated fully independently in the aforementioned experimental studies, but the possible relation between them also received attention. This relation was investigated in two different ways. A large number of samples was investigated for the presence of both groups which allowed for a correlation analysis. Furthermore, the contribution of PAH and their derivatives to the mutagenicity was directly investigated with bioassay directed fractionation. The primary objective was the elucidation of the causal aspects of the relation. However, the overlap of mutagenicity and PAH concentrations as indicators for air pollution with potential carcinogenic compounds was also deemed important. This overlap determines whether the concentrations of one, or a limited number of compounds or effects may give a sufficiently accurate impression of the variation of the pollution with the whole mutagenicity-PAH complex to allow their use as markers for this type of pollution in monitoring programmes. Although detailed investigations of both mutagens and PAH are necessary to arrive at a more complete picture of the nature of the actual human exposure to potential carcinogens (which was the primary objective of this thesis), a more limited approach may suffice to get a quantitative impression of this exposure. In chapters 13 and 14 the relation

between mutagenicity and PAH concentrations is further elaborated upon, aiming at answering the questions as to both causal aspects and overlap.

CHAPTER 3

MUTAGENICITY TESTS AS ANALYTICAL TOOLS IN ENVIRONMENTAL STUDIES

When mutagenicity tests are applied in environmental studies, the samples tested as well as the objectives of testing differ fundamentally from those in most other studies. This can have far-reaching consequences as regards the requirements the tests have to meet, the execution of the tests and the interpretation of their responses. These differences and their consequences are treated in this chapter.

Different samples

In environmental studies the samples tested are taken from the environment or from emissions into the environment. Essentially they are complex mixtures of compounds, which may contain more than one mutagen and numerous other non-mutagenic components. In most cases, hardly any information is available about the chemical nature of the mutagens and other components. In contrast, most other studies are concerned with testing samples which have a much simpler composition and which are much better defined. Often they have the simplest composition possible, when they consist of a pure compound. In that case this one compound fully determines the response of the test, which contrasts sharply with environmental studies, where the response can be the result of the different effects, independent or interactive, of many unknown compounds.

The effects of environmental complex mixtures are not necessarily equal to the sum of the effects of the individual mutagens. Actually, there are many reasons to expect them not to be. Mutagens may affect each others effects or the expression thereof in the response of the test; similar interactions may be the result of the presence of the non-mutagenic components of the mixture, despite the fact that they do not directly contribute to the mutagenicity. Non-mutagens may still be toxic by other mechanisms than mutagenesis and thus affect survival and growth rate of the test organism or interfere with the complex biochemical processes involved in mutagenesis, such as DNA replication and DNA repair. In the same way a mutagen may affect the effect of another by its toxicity, whether or not the origin of this toxicity lies in its mutagenicity.

The presence of other components may alter the solubility of a mutagen and, thereby, its bioavailability. This is in particular important for the *in vitro* tests used in environmental studies, where the indicator organisms, being micro-organisms or tissue-culture cells, are exposed to the test compounds dissolved in a liquid medium. Bioavailability may also be altered if complexation of the mutagens occurs. This may lead to a decreased solubility or make the resulting complex so bulky as to prevent the mutagen which is part of it to reach its target. Furthermore, the mutagen may loose its mutagenic properties while being part of a complex.

Two types of mutagens can be discerned: direct ones and indirect ones. The former are mutagenic themselves. The latter have to be transformed by the metabolism of the exposed organism into mutagenic metabolites. This so-called metabolic activation may be influenced by the test sample, because the mutagen itself, other mutagens or non-mutagenic compounds may interfere with the enzymatic reactions involved. They may alter the activity of the enzymes or they may compete with the indirect mutagen for its metabolic conversion. Also the direct mutagen may be metabolically transformed, which can lead to a decrease or an increase of its activity; as with indirect mutagens this transformation may be influenced by the sample.

These considerations make clear that the effect of a complex environmental sample comes into being at three levels:

- The first level represents the effects of the direct and indirect mutagens as if they were tested as pure compounds. Without further interactions among the components of the complex mixture, the effect would be equal to the sum of these "individual" effects.
- At the second level the interactions among the mutagens become important. The compounds affect each others effects by interference with each others mutagenesis, bioavailability and metabolic conversion and by "non-mutagenic" toxicity.
- The non-mutagenic components add to the interactions at the third level, because they interfere with mutagenesis, bioavailability and metabolic conversion of the mutagens and because of their "non-mutagenic" toxicity.

In contrast to most other studies, where only the first level is important, all three levels play a role in environmental studies.

Consequences for practical applicability

What are the consequences of these extra two levels for the practical applicability of the tests and the interpretation of their responses? Practical applicability will in particular be influenced by "non-mutagenic" toxicity and changes of bioavailability. Mutagenicity is very rarely the only toxic property of mutagens. In general, it is the result of a broad affinity of the mutagen for nucleophilic macromolecules, which may lead not only to mutations, but also to various other types of toxicological effects. DNA lesions may hamper or even block DNA replication and, thereby, cell division. Moreover, they may affect the functioning of a cell by destroying essential information or impairing the proper expression of this information. Depending on the type and intensity of the DNA damage, cell death may occur. Besides DNA, other macromolecules may be damaged leading to the disturbance of other processes and, thereby, toxicity. Electrophilicity is not necessarily the only property which determines the toxicity of a mutagen; the same compound may also cause toxic effects via completely different mechanisms.

In the tests mutagenicity has to be singled out from the various possible toxic effects the test compound may have. If the functioning of the test organism is too severely hampered by "non-mutagenic" toxicity, possible mutagenic effects can no longer be observed with sufficient reliability. In other words: proper testing requires a minimum fitness of the test organism. Whether or not the mutagenicity of a mutagen can be observed with a test, actually depends on the balance between this effect and other toxic effects; if the former outweighs the latter, the test can be performed with success, while no mutagenic response can be observed if the reverse is true. This balance depends on compound, test organism and test conditions.

"Non-mutagenic" toxicity bears hardly any practical relevance for in-vivo tests, i.e. in tests with complete mammals. These tests are carried out to investigate whether the "intrinsic" mutagenicity demonstrated in in-vitro tests with micro-organisms or mammalian tissue-culture cells, expresses itself in the complete animal, i.e. the toxicological significance of the mutagenicity for the exposed human prevails; they are rarely used as screening tests for the "intrinsic" mutagenic properties of compounds, let alone complex mixtures, because of their less short-term character and less easy execution. If "non-mutagenic" toxicity outweighs mutagenicity in the complete animal, it also outweighs mutagenicity in the toxicological evaluation of the compound with respect to the hazard of the compound to humans.

In contrast, the use of in-vitro tests is justified by the fact that they allow a rapid, easy and unambiguous screening for mutagenicity, irrespective of other toxic properties. The other toxic effects are much less clearly defined than the observed mutagenicity; they may have various backgrounds and they may be very test-organism specific. The tests do not allow direct conclusions about the significance of these effects for the complete animal. The other toxic effects only affect the applicability of the test and are not used in the toxicological evaluation of the compounds. If a compound cannot be tested for mutagenicity in an in-vitro test because of other toxic effects, this certainly does not mean that its mutagenicity is not toxicologically relevant, although this is the case with in vivo tests.

Complex mixtures may contain compounds which show "non-mutagenic" toxicity, but do not counterbalance it with mutagenicity. Such a strong reduction of the fitness of the test organism may be the result, that the doses of the mixture that can be tested become too low for a clear expression of the mutagenicity, notwithstanding the fact that the mutagenicity of the mutagens themselves is strong enough to counterbalance their own "non-mutagenic" toxicity. Mutagenicity tests have to be robust against "non-mutagenic" toxicity or/and highly sensitive to mutagenicity. If they are robust, high doses can be tested to demonstrate the mutagenicity; if their sensitivity is high, doses can be tested which are sufficiently low to prevent serious disturbance by other toxic effects. The presence of toxic non-mutagens in complex mixtures requires that either the robustness or the sensitivity is higher than necessary for testing pure mutagens.

As argued above, the complex character of environmental samples may also affect the bioavailability of the mutagens. For practical applicability of the test, only changes of the solubility of the mutagens in the test medium are relevant. Complexation, the other phenomenon which may affect bioavailability, can be regarded as an inherent property of the mixture which is in principle independent of test conditions. It should only be taken into account when the test results are interpretated, but it does not prevent meaningful testing. A reduced solubility does, however, prevent meaningful testing. All the mutagens present in the mixture should be tested completely; the same requirement holds for all the compounds which are not mutagenic themselves, but interact with the mutagens. If these requirements cannot be met, meaningful comparison of effects becomes impossible, because mixtures with high concentrations of mutagens may be less mutagenic than mixtures with lower concentrations, just because a less complete solution is achieved in the specific test medium. Differences might then be introduced which are not necessarily related to the mutagenic potency of the mixture or the sensitivity of the test organism.

Consequences for interpretation

Obviously, the complex character of environmental samples not only influences the practical applicability of the test, but also the interpretability of the response. When the test can be performed, i.e. when all the mutagens in the sample can reach their biological target and when toxicity does not prevent the observation of mutagenicity, the resulting response does not represent a simple addition of the mutagenic effects of the individual mutagens. Although a significant increase of the number of mutations induced unambiguously indicates mutagenicity, interactions may modify the response to a great extent. This does not affect its interpretability if we only want to know whether or not the mixture in question has mutagenic properties, i.e. if we are only interested in a qualitative indication. However, in environmental studies the objective of mutagenicity testing goes beyond that. Samples have to be compared quantitatively, to allow a meaningful description of environmental pollution with the mutagens. If interactions play a role, the samples are not compared for the primary mutagenicity caused by their diverse mutagenic constituents, but for the integrated effect of the whole matrix, which is, as we have seen, not only determined by primary mutagenicity. For instance, two samples with the same composition as regards the mutagens, could give rise to clearly different responses due to the presence of toxic non mutagenic components.

This is only acceptable when it is indeed the effect of the complete mixture in the specific test that counts, and not the primary mutagenic effects of its mutagenic components, i.e. if the interactions add to the toxicological significance of the response. Mutagenicity tests are carried out because they inform us about one specific and well defined toxicological effect in a way that bears relevance in a more general toxicological context. In other words: because the mutagenicity detected is sufficiently test unspecific to bear relevance for other experimental situations and human exposure. However, the interactions are in general ill defined and their broader toxicological relevance is often quite unclear. Consequently, the broader relevance of the changes of the primary mutagenicity which are caused by the interactions remains unclear as well. This leads to the conclusion that in most environmental studies it will be the primary mutagenicity that prevails in stead of the integrated response of the complete mixture. So, either the tests applied to complex mixtures have to be as insensitive as possible to the effects of interactions or it has to be possible to tell the primary mutagenicity apart from the mutagenicity influenced by interactions.

Are all interactions unwanted? In general, it can be stated that the undesirability of the interactions increases with their test specificity. The

interpretability of the response will not be reduced if the primary mutagenicity is influenced in a same manner and to the same extent in various experimental systems. Then the broader toxicological relevance of the interactions is clear because they form an integrated part of the mutagenicity of the mixture, whether are not they are determined by non-mutagenic compounds or "non-mutagenic" effects. It can be speculated that direct interactions with key processes such as DNA replication and DNA repair may belong to this category. Furthermore, chemical interactions in the complex mixture, such as complexation, may not depend on the conditions prevailing in the test and, thereby, leave the interpretability of the test response unharmed. Unwanted are of course all interactions which are clearly test specific. Because they behave independently of the primary mutagenicity, they can in no way be regarded as an integrated part of the mutagenicity. In fact they obscure the mutagenicity and, thereby, the interpretability of the test response.

Returning to the comparison of environmental samples, it can be concluded that interactions will not be problematic as long as they form an integrated part of the mutagenicity. When they behave independently, however, they undermine the interpretability of the differences found. This can be illustrated with our example of two samples which have an equal primary mutagenicity, but different effects due to "non-mutagenic" toxicity. Obviously, the difference looses much of its relevance if the influence of toxicity would be the opposite in another test. If, however, toxicity modifies the difference in the same direction in other tests, it is apparently so tightly linked with mutagenicity that the differences caused by it are relevant in the context of the study, i.e. have to be taken into account when the objective is to describe the presence of mutagenicity in the environment.

Reduction of the influence of interactions

What can be done to reduce the influence of the unwanted interactions? Obviously, tests should be selected with a high specific sensitivity to the primary mutagenicity of the mutagenic components of the mixture and a general low sensitivity to the "non-mutagenic" effects on that mutagenicity by the mutagens and the other components. This is a difficult task, because, although it is easy to select a test with a high sensitivity for certain types of complex mixtures, it is still hard to tell to what extent the response is influenced by interactions. Nevertheless, something can be achieved, because effects on survival and growth rate are easily observed in most tests, which allows the selection of the tests with a low sensitivity to these effects. Reduced survival, can also be determined

concurrently with mutagenicity in some tests, which makes it possible to express the effect in terms of mutations per number of surviving cells, excluding in this way part of the "non-mutagenic" toxicity. Furthermore, dose-response relationships can be analyzed with mathematical models based on mechanistic assumptions and predicting the relationships for the isolated effects (De Raat et al, 1987; Van der Hoeven et al., 1990). However, at the moment the possibilities of this approach are still limited.

The problem of unwanted interactions can be circumvented to some extent by restricting studies to a set of related samples, which means that the samples stem from the same type of sources and processes, implicating a chemical similarity, and, thereby, similarity of interactions. Ambient airborne particles may serve as an example here. Their mutagens most probably originate exclusively from incomplete combustion; the mutagens sofar identified are all PAH and PAH derivatives.

The influence of interactions can be investigated directly by separating the complex mixture into chemically well defined fractions, for instance with chromatographical techniques, and comparing the sum of the effects of the fractions with the original sample. If no artifacts are introduced by the fractionation procedure, interactions are unambiguously indicated by differences between the added effects of the fractions and the effect of the original sample. When no differences are found after various fractionation procedures have been applied, the conclusion is justified, that interactions play no significant role. The absence of interactions in a number of representative samples of the set that has to be investigated, is a clear sign that comparison of the samples of this set for mutagenicity will not be hampered too much by interactions. If interactions do occur, a test may be selected with a reduced sensitivity to their effects.

So the following precautions can be taken against the interactions undermining the application of mutagenicity tests in environmental studies:

- selection of a test method with a specific high sensitivity to the primary mutagenicity of the mutagens in the sample or a specific low sensitivity to unwanted interactions,
- restriction of the study to samples which stem from related sources and processes,
- investigation of the actual occurrence of interactions by preparing fractions of the samples and comparing the added effects of these fractions with the effect of the original sample,

- application of tests which enable the concurrent observation of "non mutagenic" toxicity and mutagenicity and
- isolation of the mutagenicity from other effects from dose-response relationships with the aid of models based on assumptions about the mechanisms behind the response of the test.

The first three possibilities can in fact be regarded as requirements for a meaningful application in environmental studies. The fourth may further add to the applicability, although it makes the performance of the tests less easy and thus hampers applicability for another reason than the occurrence of interactions. The last possibility has not received much attention to date.

Test dependence and qualitative variation

It should be emphasized that even in the absence of interactions, differences in mutagenicity between environmental samples, and thus the outcome of studies into the pollution of the environment with mutagens, can still be test dependent. This, because tests may differ in their sensitivity to the various mutagens present in the samples. The samples should then differ with respect to the types of mutagens present or/and the proportion of their concentrations. If the samples only differ as regards the concentration of an equal set of mutagens with an equal proportion, no clear test dependence of the comparison is to be expected, provided again that interactions play no role.

We are dealing here with a general and fundamental limitation of mutagenicity tests in environmental studies, which is completely detached from the limitation caused by the interactions. If one test is used, we cannot really compensate for this limitation, because every test, at least if we define a test as a combination of one type of test organism and one biological endpoint, knows its specific sensitivity for certain groups of compounds. In any case, tests with a broad sensitivity should be used in environmental studies. If possible, more than one test organism and more than one endpoint should be applied, while the choice of these organisms and endpoints should be based on complementary sensitivities in stead of overlap. Micro-organisms seem to be very suitable test organisms for this purpose, as is demonstrated by the Salmonella/microsome test. They allow the combination of various test organisms and endpoints without affecting too much the mandatory short-term nature of the test.

The test dependence is not only a trivial property of the test. It often reflects a fundamental aspect of mutagenicity. Mutagenicity results from the specific affinity of chemical compounds for certain macromolecules or/and

processes in certain cells, tissues, organs and organisms. This is "translated" in specific mutations, each of them having their own consequences on the organismal level. The specific character of the mutations induced by a compound can be looked upon as a the quality of its mutagenicity. Due to the complex nature of environmental samples the quality of the mutagenicity will often vary, together with the intensity. Qualitative changes are, therefore, an inevitable aspect of studies on the distribution of mutagenicity in the environment. Studies should be designed in such a way, that both changes can be observed. To this end different biological endpoints and test organisms have to be incorporated in testing.

Qualitative variation is not to be regarded a limitation or nuisance that has to be compensated for; instead, it is a relevant aspect of environmental pollution with mutagens, that needs to be elucidated next to quantitative variation. As it is related with the chemical composition of the particles, it may provide valuable clues for the identity of the relevant compounds. It may also tell something about the carcinogenic effects of the particles, as the specific mutagenic quality of a compound may correspond with a high or low carcinogenicity in experimental animals.

Different objectives

If the objectives of mutagenicity testing in environmental studies are compared with those in most other studies, "quantitative comparison" appears to be an important aspect of the former, while it hardly matters in case of the latter. Sufficient numbers of samples have to be compared quantitatively in environmental studies to allow the origin, distribution and fate of the mutagenicity to be investigated with the desired resolution. These samples have to be tested within an acceptable time span and against an acceptable effort, which puts strict demands on the experimental convenience of the test. Moreover, it is essential that differences in mutagenicity between samples from sources and the environment can be observed with a sufficient discriminating power and reproducibility. Whether this is indeed possible, depends on the quality of the test applied, in particular its sensitivity and the variation thereof, and on the differences deemed relevant by the investigator in the light of the specific objectives of his study. The smallest differences the test permits to observe must in any case not be larger than the largest ones occurring in the environment and preferably much smaller than that.

In contrast, quantitative comparison is much less important in most other studies. In general, the objective of testing does not go beyond investigating

whether or not the test sample has mutagenic properties. Sometimes the test results are used in a semi quantitative way, i.e. qualifications as borderline, weak, moderate or strong are used. If the mutagenicity is expressed as a quantitative value, this is not used to its full extent in the interpretation of the results. Furthermore, in most studies comparison of samples is not an explicit objective, which makes it much less important to express the effect scored in the test in quantitative terms.

Test organism and test criterion

The experimental convenience of a test mainly depends on the type of organism used for the detection of the mutagenic effect and the type of mutagenic effect detected, i.e. the test criterion. In general, unicellular organisms allow the highest convenience for a number of obvious practical reasons which follow from the fact that large numbers can be manipulated and exposed together, while each organism can be the starting point for the event that has ultimately to be detected. The sensitivity of tests with unicellular organisms is strongly determined by the division rate because fixation of the initial damage (DNA lesion) in the form of a mutation and expression of the mutation mainly occur during DNA replication. The greater the number of divisions during exposure, the greater the chance that a lesion will result in a mutation that can be observed. Obviously, the division rate also determines the short-term nature of the test, as it takes a minimum number of divisions before the mutation is expressed as an observable event. These considerations make clear why tests with bacteria are almost exclusively used in environmental studies. Because of their small dimensions and high division rate they offer a much greater experimental convenience than other unicellular test organisms, such as yeasts and fungi, or mammalian tissue-culture cells.

Other advantages of bacteria as test organisms follow from the organization of their genetic material. They possess only one set of genetic material, i.e. they are haploid. A change in the base sequence of their DNA cannot be compensated for by another intact DNA molecule. This makes them in principle more sensitive than other organisms with more than one set of genetic material. Furthermore, bacteria are relatively easy subjects for genetic manipulation. Specific properties making them more suitable for mutagenicity testing can be introduced, for instance, by mutations or by the introduction of plasmids. The bacterial strains used in the Salmonella/microsome test may serve as an example here (Ames et al., 1975; Maron and Ames, 1983; McCann et al., 1975B). In most of them an important and reliable DNA-repair process has been

eliminated by a deletion; as a result lesions have to be repaired via a more error-prone repair pathway, leading to a greater chance for mutations. Moreover, the introduction of a resistance plasmid has resulted in affected DNA repair as well, and, thereby, greater sensitivity. Multiplication of the target gene through the incorporation of many copies of a resistance plasmid with that gene is another way of increasing the sensitivity. Only one of those genes needs to be mutated (which means, in the case of the Salmonella/microsome test, to be reverted to wild type) to achieve an event that can be scored. Finally, the sensitivity of the strains in the Salmonella/microsome test is enhanced by the introduction of a mutation which leads to the absence of the lipopolysaccharide sheet around the bacterium, thereby making the cells more permeable for test compounds.

Furthermore, bacteria may offer the advantage of well defined test criteria. Again the Salmonella/microsome test may serve as an example. A short description of the basic principle of this test is necessary to make this clear. Histidine auxotrophic strains of Salmonella typhimurium are used as test organisms; they are exposed in an agar medium lacking sufficient histidine for the outgrowth of the auxotrophic bacteria to scorable colonies; a trace amount of histidine permits the cell divisions which are necessary for the fixation of DNA lesions into mutations and the expression of these mutations. Some of the bacteria will be reverted by a mutation to histidine prototrophy either spontaneously or as a result of the exposure; the reverted bacteria do not stop dividing after histidine depletion and after a while they will grow out to scorable prototrophic colonies. The histidine auxotrophy of the strains is the result of different types of well defined mutations; well defined in the sense that the changes in the base sequence of the DNA leading to auxotrophy are largely known. Consequently, the backward mutations necessary for restoring prototrophy are also known in terms of changes in the base sequence. So, the type of strain reverted by the test compound tells something about the type of mutation that compound is prone to induce. For instance, it may specifically induce frame-shift mutations or base-pair substitution mutations. Furthermore the base sequence of the auxotrophy mutation may indicate a specific chemical affinity of the test compound for the genetic material.

Discriminating power and reproducibility

Experimental convenience is not the sole requirement following from the specific objectives of mutagenicity testing in environmental studies. Of equal importance are a high discriminating power and reproducibility, this to allow a reliable observation of effects and differences between effects deemed relevant by the

researcher. Here we arrive at a major and inherent limitation of mutagenicity tests as analytical tools in environmental studies.

In general a test is composed of a number of treatments with different or replicated doses each leading to a scorable effect. In the Salmonella/microsome test each treatment is represented by a Petri dish with an agar layer in which about 10^8 bacteria are exposed to a certain concentration of the test sample (amount per dish = dose). The basic sensitivity corresponds with the sensitivity of the individual treatment (in case of the Salmonella/microsome test the average sensitivity of the 10^8 bacteria in the agar layer for the mutagenic effects of the test sample). Inevitably, this sensitivity will vary within the test, the so-called intra-test variation. In general, the variation will be higher if treatments from different tests are compared. It should be noted here, that the treatments forming one test share experimental conditions which inevitably differ for treatments from various tests (in case of the Salmonella/microsome test: culture of the test bacteria used to inoculate the agar layer, batches of media components, batch of tissue homogenate etc.).

If series of environmental samples have to be compared two strategies can be followed. The samples can be tested within one test in which case comparison is only affected by the intra-test variation of the sensitivity. This variation can be compensated for by random distribution of the doses over the treatments and by increasing the number of treatments. Even the most convenient test permits only a small number of samples to be compared; not the least because every sample has to be tested at several doses and every dose has to be replicated once or twice, this, to allow a fairly accurate estimate of the effect. If the samples are tested in different tests, the comparison is hampered by the higher inter-test variation. This variation can only be compensated for by repeating the complete test. Obviously, in both cases the compensation of the variation affects the convenience of the test, which was, after all, a predominant reason for its application.

Positive controls

In stead of increasing the number of treatments per test or the number of tests, the variation of the sensitivity could be monitored with the aid of positive controls and the effects of the samples corrected for this, as is done in analytical chemistry[3]. An important question then is, whether the variation is only test dependent or also compound dependent. In the first case the sensitivity can

[3] With external standards, the equivalents of the positive controls in mutagenicity testing.

be monitored with a compound which needs not to be akin to the sample actually tested and the test response could then easily be corrected for the variation observed with the positive control. However, the effects obtained with the positive control do not take the interactions among mutagens and between mutagens and non mutagenic compounds into account (unless it has the same composition as the sample). The variation of these interactions does not necessarily correlate with the primary mutagenicity of the mutagens.

We may safely assume the variation not to be the same for every mutagen in the mixture, the obvious reason being, that they differ as regards their fate and behaviour in the test and the nature of their interaction with the genetic material (or that of their metabolites). The extent to which the variation differs will obviously depend on the chemical similarity of the compounds. For instance, we may expect the effects of two PAH to show a better covariation than the effects of a PAH and a PAH with a significant functional group (e.g. a nitro group). A compound dependence of the variation needs not to be a serious problem if the composition of the samples is not (too) variable. Then one of the samples could be used as positive control in the tests of the other samples. However, in most cases the composition will be variable or it will not be justified to assume them not to be beforehand.

So, we may conclude that the applicability of positive controls will be limited for three reasons.

- They do not take into account the interactions influencing the response of the environmental sample, unless they have the same composition as the sample.
- The variation of the test sensitivity will differ for the individual mutagens in the sample, which only leaves the samples themselves as a reliable positive control.
- The composition of the samples will be variable, which affects the suitability of samples as positive controls.

Consequences and remedies

These considerations make clear that, in a way, experimental convenience and quantitative comparability are conflicting requirements, which can only partly be reconciled by the use of positive controls. A sort of dilemma, that can be solved in two ways. Either by reducing the number of samples to be compared, so that the number of tests or the number of treatments per test (per sample) can be increased, or by restricting the test to samples with a similar composition,

so that the variation of the sensitivity can be monitored in a more reliable way with positive controls. Which of these ways is chosen depends on the specific objectives of the investigator. In most cases it will be something of both.

In any case the following general rules should be followed to optimize discriminating power and reproducibility.

- Obviously, the intra-test and inter-test variation of the sensitivity should be kept as low as possible by pursuing a maximum standardization of the test procedures.
- The investigator should have a notion about the variation of the sensitivity beforehand; it should not exceed the minimum differences deemed relevant.
- Positive controls should always be used. They should represent clearly different classes of compounds and compounds similar to the ones expected to contribute significantly to the effect of the samples. When the composition of the samples is not too variable, a sample or mixture of samples can be used. Only the differences lying outside the variation of the effects of the positive controls (after applying weighing factors to compensate for differences between the mean effects of the controls and the samples) should be taken into account.
- The difference between intra-test and inter-test variation should be taken into account in the design of the study. For instance, if the objective is to compare a restricted number of locations several times, the samples from one time point should, if possible, be tested in one test. The same holds if the comparison of a limited number of test conditions is the objective (in the Salmonella/microsome test: testing with different bacterial strains in the presence and absence of tissue homogenates for metabolic activation).

These general rules are in first instance aimed at optimizing the discriminating power and reproducibility within one study. A quantitative comparison of results from different studies requires a rigorous standardization to be strictly implemented, in particular if the results from different laboratories are to be compared. This standardization should imply test conditions, positive controls and design. It is doubtful whether such a standardization can ever be achieved. In the meantime, a quantitative interpretation of results should only be undertaken within the context of one study or, at the most, studies of one research group or laboratory. This inevitably means that statements about the

mutagenicity in absolute quantitative terms are characterized by a relatively large margin of uncertainty, in particular when compared with concentrations determined with analytical-chemical techniques. However, this does not prevent reliable information being gained about the sources, nature and processes determining the presence of the mutagens in the environment. Individual studies will still reveal trends, proportions and qualitative changes and differences on which this information can be based. Comparison of studies for these types of results is not necessarily hampered by the large margin of uncertainty which is referred to above.

CHAPTER 4

THE EXTRAPOLATION PROBLEM

What does the mutagenicity of extracts of ambient airborne particles determined with a short-term bacterial mutagenicity test, reveal about the ultimate effect that warrants its investigation? Does it reveal anything about the potency of the particles to induce cancer in exposed humans? The same question can be asked for the PAH concentrations in these extracts determined with analytical-chemical techniques. With these questions we touch upon a predominant problem in toxicology, the extrapolation from effects observed in an experimental context to the effects that really count in the "real-life" situation. The investigator will always try to make this extrapolation gap as narrow as possible. However, for obvious ethical and practical reasons he will always have to extrapolate and, therefore, have to accept that the predictive value of his results as regards the "real-life" situation will be limited.

The objectives of the experimental studies presented in this thesis make compromises with respect to the extrapolation from the results to human exposure and effects inevitable. As has been set forth in the first chapter, mutagenicity and PAH concentrations are not only determined to obtain an as realistic as possible impression of the possible carcinogenic effects of the particles for humans. If this was the only objective, it might be better to concentrate on carcinogenicity tests and to choose for more realistic exposure conditions than offered by high-volume filtration and extraction with organic solvents. Of equal importance is the objective to investigate the development of air pollution with mutagenicity and PAH in time and space; this requires an experimental convenience reflected in endpoints and exposure conditions, which inevitably widens the extrapolation gap.

Yet, the extrapolation gap must not become too wide. There must always remain a link between experimental results and relevant effects under "real-life" conditions, which is strong enough to make the effort worthwhile. The link may be direct, i.e. may lead to a quantitative correlation between endpoints and cancer risk. It may also have a more indirect character. For instance, if the determination of mutagenicity and PAH concentrations helps to unravel the processes which

determine the pollution of the air with particulate carcinogens, without directly indicating risk.

In this chapter, an analysis will be presented of the link between on the one hand mutagenicity and PAH concentrations and on the other the possible cancer risk for humans of exposure to the particles. It will be shown that the link belongs largely to the second of the two categories mentioned above. This does not make the effort less worthwhile, because more insight in the temporal and spatial distribution and the processes determining the presence of the possible carcinogens leads to a better understanding of the actual human exposure, and is, thereby, an indispensable complement of the ultimate evaluation of the actual risks.

Mutagenicity

In the case of mutagenicity we have to extrapolate from an effect of extracts of the particles in a bacterial mutagenicity test to effects in humans exposed to the particles via inhalation. Differences in exposure conditions, biological targets and effects will be important here. The mutagenicity is determined in the following way: The particles are isolated from their natural matrix - the air - by filtration, the filters laden with particles are extracted with organic solvents and the extracts are tested in a bacterial short-term test. So particles floating free from each other in the air are brought together, part of their chemical components is subsequently dissolved in one batch of solvent and this solvent is mixed with an aqueous bacterial medium with homogenized mammalian cells (to simulate mammalian biotransformation) and bacteria. Obviously, this experimental procedure cannot be regarded as a good model for human exposure via inhalation.

First of all, because humans inhale complete air and not only its particulate fraction. This means that the target cells are not only exposed to particulate pollutants, but also to their gaseous counterparts. Besides being mutagenic themselves, the latter may also interfere with the action of the particulate compounds. The response of the target cells to the particulate mutagens might be altered by these compounds. Furthermore, the clearing of the particles from the respiratory tract might be impaired, which will most certainly result in an altered exposure of the target cells. In contrast with sampling, the particles are not brought together during inhalation. Depending on their aerodynamic diameter they will deposit separately in different parts of the respiratory tract. The target cells will be exposed to individual particles which are slowly extracted by the aqueous lung surfactant or by fagocytosing

cells and not to an extract of all the particles together, let alone a rigorous extract in an organic solvent.

Both, the effects in the test bacteria and those in the target cells will be influenced by biotransformation of the components in the particles. In the test, biotransformation occurs through the enzymes of the homogenized mammalian tissue outside the bacteria and inside the bacteria by bacterial enzymes. In the respiratory tract, biotransformation occurs in intact cells which may at the same time be the target cells. The resulting difference of metabolic-activation patterns will most probably add to a qualitatively as well as quantitatively different exposure to the ultimately active compounds.

We may expect the effects in the lung cells and the test bacteria not to be equal to the sum of the effects of the mutagenic compounds. Interactions among compounds will probably influence the effects (see previous chapter). In addition to mutagens, the particles can contain compounds which are themselves not mutagenic, but which alter the reaction of the cells or the bacteria to the mutagens; such interactions can also be expected to occur among the mutagens. They may play a role at several levels. The mutagenic response may be altered by other toxic effects, the biotransformation of a compound may be affected by the presence of other compounds (competition, changes of enzyme activity), compounds may become unavailable because they form complexes with other compounds and the reaction to the initial damage (e.g. repair of primary DNA lesions; promotion after initiation of carcinogenesis) may be altered. The marked differences between the exposure conditions in humans and in the test will probably determine these interactions to a great extent.

The extrapolation is not only hampered by these drastic differences in exposure conditions but also by the fact that mutagenesis in special sensitive bacterial strains is compared with carcinogenesis in lung cells. Both, the targets and the effects differ widely. The sensitivity of bacteria and target cells may be expected to differ qualitatively, i.e. as regards the types of mutagens they are sensitive to and the types of mutations that are induced, as well as quantitatively. Furthermore, the initiation of carcinogenesis by mutations is only one, albeit important, stage of the complete process (see chapter 1). The bacterial mutagenicity test tells us nothing about the occurrence of compounds which cause other, non-mutational effects that contribute to carcinogenesis, such as promotion.

This speculative comparison of mutagenicity of the particles as it is determined with a short-term mutagenicity test and carcinogenesis in the respiratory tract of exposed humans leads to the conclusion that, although

mutations play a key role in carcinogenesis, the distance between these two combinations of exposure and effect is so large, that a correlation between the effects can not be expected a priori. The reason for the possible absence of a correlation is not a "general" non-carcinogenicity of the mutagens in the particles. It is the fact that the expression of their carcinogenicity is not necessarily expressed under the specific exposure conditions in the specific cells that causes the uncertainty. In general, tests which as the Salmonella/microsome test are characterized by a broad sensitivity, i.e. they are sensitive to many classes of mutagens, as we may expect, the majority of the mutagens in the particles. So, they indicate with fair certainty whether any mutagens, and thereby possible carcinogens, are present. Whether this presence actually leads to carcinogenesis in the respiratory tract of the exposed human depends on many other factors which are not accounted for in the test or the preparation of the test material.

This is not to say that a correlation can be excluded a priori. Although the correlation may not be deduced from the methods used to determine the mutagenicity, i.e. sampling, extraction and testing, it may, nevertheless, exist and be revealed by further investigations. For example it may directly be investigated by comparing mutagenicity and actual cancer incidence. Sofar, this epidemiological approach has been followed in only one published study (Walker et al., 1982), which did show a correlation between mutagenicity and lung-cancer mortality, while mutagenicity did not correlate with mortality rates for other causes. However, the results of this study should be interpreted with caution. During the last two decades it has become most uncertain to what extent the increase of the lung-cancer incidence which is associated with living in urban and industrial areas (the urban factor) is really caused by air pollution. At the moment, it is generally held, that it can largely be ascribed to differences in smoking habits and, possibly, occupational exposure. If a contribution of air pollution does exist, it is probably so strongly confounded by these other exposures, that it will be very difficult to sort it out in epidemiological studies. Still, the study of Walker et al. is intriguing enough to deserve following; it suggests that mutagenicity opens the possibility to discern a contribution of air pollution to lung-cancer incidence.

Another way to investigate the correlation is to elucidate the chemical nature of the mutagens and, subsequently, investigate their carcinogenicity in experimental animals. A strong point of the short-term tests in this context is, that their short-term nature greatly facilitates investigations on the chemical nature of the mutagens. For example, they can easily be applied in bioassay-directed fractionation (Alfheim et al., 1985; Schuetzle and Lewtas, 1986). This

means that extracts of the particles are fractionated with analytical-chemical techniques, in most cases chromatographical techniques, and that the fractions thus prepared are tested for mutagenicity. The experimental flexibility of the short-term tests even allows mutagenicity to be used as a detection criterion in chromatography. Mutagenic fractions can be fractionated again to achieve further isolation of the mutagens from the non-mutagenic compounds. Finally the chemical homogeneity of the mutagenic fractions becomes high enough for a meaningful chemical analysis aimed at the identification of the mutagens.

The tests can also contribute to the identification of the mutagens without the intervention of analytical-chemical techniques. Bacterial strains have been developed with a specific insensitivity to compounds the mutagenicity of which is determined by bacterial reduction of nitro groups (Rosenkranz and Mermelstein, 1983 and 1985); a reduced effect in these strains compared to that of the parental strains strongly indicates the presence of nitro compounds as mutagens, as was indeed found for the particles (Wang et al., 1980; Alfheim, 1982). Also the comparison of effects obtained in the absence and presence of the tissue homogenates for metabolic activation can support the identification of the mutagens. This is exemplified by the PAH, compounds the mutagenicity of which depends completely on the use of tissue homogenates. The clear effects in the absence of the homogenates unambiguously showed that besides PAH other mutagens must be present in the particles.

The mutagens thusfar identified with bioassay-directed fractionation or otherwise are nitrated derivatives of PAH and related compounds (Alfheim et al., 1985; Lewtas et al., 1990; Nishioka et al., 1988; Harris et al., 1987; Helmig et al., 1992). These compounds are widely studied for their mutagenic and carcinogenic properties as well as their metabolism and toxicokinetics in in vitro and in vivo experimental systems (Onishi and Tokiwa, 1986). The results do not yet allow for unambiguous conclusions about the cancer hazard associated with the inhalation of the particles as far as this hazard is associated with the presence of these mutagens. Together with the scanty character of the epidemiological evidence (Walker et al., 1982), this brings us to the conclusion, that, for the time being, the use of the mutagenicity of the particles in air-pollution research is only justified by the fact that it shows whether or not mutagens (and thereby potential carcinogens) are present and that it can by no means be regarded as a direct indicator for human cancer risk.

Even so, mutagenicity can be put to good use in air-pollution research, in particular in the hands of the researcher interested in the sources and the atmospheric processes which determine the exposure to the potentially

carcinogens in the air. It allows a direct - without chemical analysis of the active compounds - identification of sources and measurement of their contribution. Distribution and changes after emission can directly be followed and the influence of important factors such as meteorological conditions and atmospheric chemical reactions on these processes can directly be investigated. Bioassay-directed fractionation is also important in this context. It allows a direct investigation of the link between changes of mutagenicity and changes of chemical composition. The latter can in their turn be linked with changes in source pattern or with chemical reactions in the atmosphere, thereby, leading to a better understanding of the chemical background of air pollution with particulate mutagens.

Thusfar, mutagenicity has pointed to a diversity of potential carcinogens where there were only PAH in the past and it helped to establish a strong hypothesis about the origin of these compounds. Without exaggeration it can be stated that mutagenicity has lead to a complete reassessment of the "position" of the PAH in air-pollution research. By doing so, it contributed much to our knowledge of human exposure to potential carcinogenic compounds via the inhalation of ambient airborne particles. Obviously, mutagenicity does also, although indirectly, contribute to our knowledge about the actual human cancer risk associated with this exposure, because this risk can only adequately be studied if the exposure is sufficiently known in qualitative as well as quantitative terms.

Polycyclic aromatic hydrocarbons

In the case of the PAH concentrations we are not dealing with extrapolation between two different effects in two different biological systems, but from concentrations to an effect, i.e. extrapolation from concentrations of a series of well defined chemical compounds with proven carcinogenic properties in ambient airborne particles to cancer in the human respiratory tract upon inhalation of these particles. Essentially, the PAH concentrations are only an indicator for exposure because they do not directly indicate possible effects; for this aspect of their indicative value they completely depend on the results of additional toxicological studies. This in contrast with mutagenicity, which indicates an effect and, thereby, exposure; in the case of mutagenicity additional investigations must reveal which compounds cause the effect, which then allows a clarification of exposure in chemical terms and opens the way for studies on the human-toxicological relevance of the effect. The use of mutagenicity may lead to the discovery of new classes of compounds the concentrations of which may subsequently be used as air-pollution indicators in the same way as the PAH concentrations are.

So, if we are considering the extrapolation problem for the PAH we are dealing with two questions. The first reads: what do the measured concentrations tell us about the real exposure levels of the target cells? The second one is concerned with the effects that can be expected upon this exposure. The answer to the first question depends on the extent to which the sampling and extraction techniques used for the chemical analysis of the particles reflect "sampling" and "extraction" as it occurs by and in the respiratory tract.

Sampling has to cover the PAH-containing particles that are inhaled and deposited in the respiratory tract and to exclude other PAH-containing particles. Both demands are easily fulfilled as PAH have only been found in the respirable fraction of the particles (Van Cauwenberghe, 1985; Van Vaeck and Van Cauwenberghe, 1983; Baek et al., 1991), which is fully covered by most sampling devices (see chapter 5). Extraction causes more problems for extrapolation. It differs widely from the processes which make the PAH bioavailable in the respiratory tract. Generally it is aimed at a complete as possible dissolution of all the PAH in organic solvents (see chapter 5), while in the respiratory tract the solvents are aqueous media such as the lung surfactant and those present in the fagocytosing cells. As PAH are rather apolar compounds, they will at least be dissolved less readily, and possibly also less completely in the respiratory tract than during extraction. In the particles the PAH are part of a complex, carbonaceous matrix to which they can be so strongly bonded that it is even difficult to dissolve them completely with organic solvents, let alone that a complete extraction can be achieved with aqueous solvents.

Sofar, it remains obscure in which way and to what extent the PAH are actually made bioavailable to the target cells in the respiratory tract. However, this not necessarily affects the indicative value of the PAH concentrations as this value depends not so much on the actual exposure levels as such, but on the correlation between these levels and the concentrations in the air. This correlation may still be high enough for a meaningful application of the PAH concentrations as air-pollution indicator. It depends on the variation of the bioavailability of the PAH, which in its turn depends on differences among the particles and differences among the exposed population. The sources of the particles as well as their history after emission may be important in this respect. It can, for instance, be imagined that the bioavailability of the PAH in traffic emissions differs from that of the PAH in industrial emissions. This could also be the case for particles immediately downwind of a source and those from the same source after transport over long distances and concurrent alteration by various chemical and physical atmospheric processes.

The individuals of the exposed population are expected to show differences in the clearing efficiency as well as the extraction of the particles. These differences may have a genetic background or may be associated with life style, occupational exposure and nutrition. Tobacco smoking will most certainly be a very important life-style related factor, because it affects functioning of the respiratory tract in many aspects; we may, for instance, think of the reduced clearing of particles in smokers. However, it is doubtful whether these differences are really important in this context. It should be realized that air-pollution research is generally concerned with exposure and effects (e.g. cancer incidence) at the population level. In fact, we are interested in the correlation between concentration and exposure for populations, which implies that individual differences in bioavailability are levelled out to differences at the population level. It may be assumed that the latter will be much less important than the former and it seems improbable that they will seriously affect the reliability of the concentrations as indicators for exposure if we restrict ourselves to the population level. Therefore, the conclusion seems to be justified that the indicative value of the concentrations as far as exposure is concerned, is primarily hampered by the uncertainty about the differences among the particles and not so much by the differences among the exposed population.

A correlation between PAH concentrations and actual exposure does not automatically imply a correlation between these concentrations and lung-cancer incidence. To make this clear we will discern three levels in the aetiology of lung cancer: All "carcinogenic" factors including those not related with air pollution, all air-pollution related factors and PAH without other carcinogenic air pollutants. Obviously we may not expect a clear correlation between the first level and PAH concentrations in ambient air, largely because of the overwhelming contribution of smoking to lung-cancer incidence. However, we may neglect these non-air-pollution factors and restrict ourselves to the correlation with the two other levels, thereby accepting that such correlations are more or less virtual. This virtuality does not, however, make them less important; even an obscured contribution of air pollution may be unacceptable. In the case of "air pollution as a whole", other carcinogenic air pollutants as well as non-carcinogenic ones, e.g. promoters, may affect the correlation. The non-carcinogenic ones are important if we consider the correlation for the "PAH without other carcinogenic air pollutants". We cannot expect beforehand the concentrations of these compounds to correlate with the concentrations of the PAH, as they may have other sources and as they may differ in their physical and chemical fate after emission. Available knowledge about these aspects as well as the relevant effects

of these compounds is far too scanty to allow an evaluation of their significance in this context.

The last important factor in the "extrapolation chain" from PAH concentrations in the particles and lung-cancer incidence is the carcinogenicity of the PAH themselves. Will exposure of the cells in the respiratory tract indeed lead to cancerous growth? The scope of this section does not allow a detailed overview of the literature on the carcinogenicity of PAH (see for reviews: NRC, 1972 and 1983, IARC, 1973 and 1983; Dipple, 1976; Hoffmann and Wynder, 1977; Montizaan et al., 1989). However, it unambiguously reveals that many PAH are indirect genotoxic carcinogens, among them very strong ones, which can induce tumours in various experimental animals, organs and tissues via various exposure routes, including the inhalatory route. Besides experimental evidence, indications are available from occupational-epidemiological studies. It has been observed several times that occupational exposure to the coal-tar vapours and particles emitted by coal gasification and coke production leads to an enhanced lung-cancer incidence (IARC, 1984). We have seen that the presence of PAH can explain at least a large part of the carcinogenicity of coal tar in experimental animals (see chapter 1), which makes it probable that these compounds will also contribute to the lung-cancer incidence in the exposed workers. As coal tar also contains other, albeit related, carcinogens (such as nitrogen-heterocyclic PAH or aza-arenes), the possibility that these, and not the PAH are the causative agents cannot, however, be excluded.

These experimental and epidemiological studies differ from the "real-life" exposure of humans to ambient airborne particles in that the exposure levels were extremely high compared to those prevailing in the "real-life" exposure. We have to extrapolate from clear effects or indications obtained at these extremely high levels to effects that might occur at much lower levels. Are the PAH still carcinogenic at these lower exposure levels? Or to put this question otherwise: Is there a threshold exposure level, below which the PAH no longer induce cancerous growth? If the latter question can be answered in the affirmative and if the threshold lies well above the exposure levels encountered in the "real-life" situation, the presence of the PAH in the air loses its significance for human health. Several mechanism can be suggested which would lead to a threshold level or to an otherwise deviating dose dependence of the carcinogenicity of the PAH at lower exposure levels. They can only be touched upon very briefly here. We have seen that PAH are indirect carcinogens; the biotransformation processes involved in the activation might proceed in another way at lower doses, thereby producing proportionally less (or more) active agent.

It can also be speculated that scavenging of the active agents is more effective at lower doses resulting in a shorter half life and less accumulation. Furthermore, the processes directly involved in the fixation of the initiating mutation, e.g. DNA repair, might be dose dependent. The number of initiating mutations necessary for the onset of cancerous growth per cell may also be important; it has been suggested that one is not enough, which would automatically imply a threshold. Finally, after initiation other processes are necessary for the initiated cell to grow out to a tumour. Promotion is one of them; recently attention has been focussed on the stimulating effect of an increased cell-division rate, which can be a side effect of the exposure caused by general toxicity (see chapter 1). It seems probable that such processes are characterized by a threshold.

We may regard the question as to the threshold level as the key question of the PAH extrapolation problem. The considerations above suggest that a threshold might very well exist. However experimental evidence is very hard to obtain, just because no carcinogenic effects can be induced in experimental frameworks at the exposure levels encountered in the "real-life" situation. In regulatory practice a conservative or worst-case approach is followed, which means that no threshold is accepted and that a linear extrapolation from high experimental to low "real-life" exposure levels is applied; every exposure, low as it may be, is regarded as presenting a carcinogenic risk. It is evident that the regulation of carcinogens in general and that of PAH in ambient air in particular, would benefit much from studies aimed at elucidating carcinogenesis at lower exposure levels.

Obviously, the response of the respiratory tract to the exposure in terms of cancer induction will vary strongly among the exposed population. Again factors such as genetic background, life style (smoking) and nutrition may be important. However, like the differences affecting bioavailability, we may expect this variation to be averaged out at the population level.

The considerations about the indicative value of the PAH concentrations may be summarized as follows:

- The sampling techniques used do not affect the indicative value, as these cover all the inhalable particles and as no PAH are present in the non-inhalable particles.
- The extraction techniques used differ widely from the extraction of the PAH as it occurs in the respiratory tract (the processes which make the PAH bioavailable); the latter will most probably be less rapid and less complete than the former. However, the indicative value does not depend

on completeness but on the extent to which extraction correlates with actual exposure level. This correlation may be weakened by differences between the particles which are associated with their source pattern and their history after emission. Differences among the population as regards the bioavailability of the PAH are most probably not important is this context, because it is the cancer incidence in the population (and thereby the average bioavailability in the population) that counts and not the individual cancer risk.

- As PAH are found to be carcinogenic upon exposure via inhalation, we may expect that the exposure to the PAH in the particles will indeed lead to an enhanced lung-cancer incidence, at least if we accept the absence of a threshold.
- However, this does not automatically imply a high indicative value. The effects of the PAH may be influenced by non-carcinogenic pollutants the presence of which does not necessarily correlate with the PAH.
- If we want to use the PAH concentrations as indicator for the carcinogenicity of air pollution as a whole (instead of air pollution with PAH) the presence of other carcinogens becomes important as well.
- Differences in the carcinogenic response among the exposed population are most probably not important here, again because it is the cancer incidence in the population that counts, instead of individual cancer risk.

It can thus be concluded that the indicative value of the PAH concentrations will be restricted by the following factors:

- Differences among the particles which affect the bioavailability of the PAH.
- Uncertainty about the carcinogenicity of PAH at low levels and the existence of a threshold level.
- The presence of non-carcinogenic pollutants which influence the effects of their carcinogenic counterparts.
- The presence of other carcinogenic pollutants.

Little is known about these factors, which makes it impossible to draw more definitive conclusions about the indicative value of the PAH concentrations. As PAH still play a predominant role in air-pollution research, there is a need for further investigations into these factors and their influence on the indicative

value. Mutagenicity can be put to good use in these investigations, in particular as far as the presence of non-carcinogens and other carcinogens is concerned.

CHAPTER 5

AMBIENT AIRBORNE PARTICLES: ORIGINS, SAMPLING AND EXTRACTION[4]

Air can be regarded as an aerosol, i.e. a mixture of particles and gases that exhibits a degree of stability in a gravitational field. The particles in this aerosol differ widely as regards their origin, composition, dimensions and behaviour. Basically, their presence in the air is the result of the following processes:

- Mechanical processes such as grinding, abrasion and nebulization. Wind, waves, wear, volcanic eruptions, industrial activities or traffic can act as driving forces for these processes. The particles thus produced can have an abiotic nature, e.g. sea-salt particles, volcanic ash and dust from automobile-tyre wear, or a biotic nature, e.g. pollen and spores of fungi. They represent the so-called coarse particles in the air, i.e. the particles with an aerodynamic diameter[5] (ADM) larger than 1-2 μm. Their upperbound ADMs are largely determined by gravitational settling. At larger ADM's their sedimentation rate is so high and, consequently, their residence time so short, that they can no longer be regarded as part of the aerosol.
- The emission of hot vapours which condense after cooling, thereby forming droplets or solid particles. Sources of these vapours are various industrial processes and the combustion of fossil fuels and other organic materials. In general, the ADMs fall in the range of < 0.01 μm to 0.1 μm.

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[4] The contents of this chapter is largely based on the following references: Willeke and Whitby (1975), Corn (1977), Whitby (1978), Haagen-Smit and Wayne (1977), NRC (1979), Hinds (1982), Liu et al. (1984), Finlayson-Pitts and Pits (1986). The reader is referred to these publications for further details. The chapter only presents a very global treatment of a well developed and broad field of air-pollution research.

[5] The diameter of a sphere of unit density which has the same aerodynamic properties as the particle in question.

- Condensation of compounds with low vapour pressures, which are formed in the air by chemical reactions from compounds with high vapour pressures. This process is called homogeneous nucleation or self-nucleation. Numerous compounds and chemical reactions may be involved in the formation of these particles. Well known is the formation of sulphates and nitrates from sulphur oxides and nitrogen oxides. Furthermore, organic compounds may be attacked by reactive oxidizing species, such as OH radicals, leading to oxidized products with a low vapour pressure. The ADM's of the particles formed as a result of these reactions fall in the same range as those of the particles formed after the condensation of hot vapours, i.e. between <0.01 and 0.1 μm.

The particles formed by both condensation processes are called transient nuclei; this to express that their ADM increases rapidly after their formation. They tend to combine (coagulate) to larger particles. Moreover, they will also serve as nuclei for condensation of low vapour pressure compounds emitted or formed by chemical reactions (heterogenous condensation). Finally, they can participate in chemical reactions with gaseous compounds on their surface. Thus, the transient nuclei increase in size until they accumulate in an ADM range from 0.1 to 1 μm, the so-called accumulation range. Together, the transient nuclei and the particles in the accumulation range represent the fine particles. If the number concentration of the fine particles in the air becomes high, low vapour-pressure compounds will completely condense on the surface of the existing particles and the formation of new particles by condensation will become less important, unless they are formed during, or directly upon emission.

So, the distribution of the particles in the air over ADM classes will show a trimodal character: **transient nuclei** (<0.1 μm), **accumulation range** (0.1-1 μm) and **coarse particles** (>1-2μm). The proportion between these three modes in mass, volume, surface or number strongly depends on the sources and the age of the particles as well as the meteorological conditions. In general, the coarse particle will dominate in mass and volume (their share amounts to at least about 50%), the transient nuclei in number and the accumulation-range particles in surface. The latter may also significantly contribute to mass and volume, and, to a less great extent, to number, while the contribution of the coarse particles to number and that of the transient nuclei to mass and volume are generally negligible.

Table 4.1 **Major sources and processes contributing to the particulate fraction of the air on a global scale** (from Whelpdale and Munn, 1977)

		production rate	
		metric ton per day	*% by weight*
natural sources			
*primary**	*wind-blown dust*	2×10^4 to 10^6	9.3
	sea spray	3×10^6	28
	volcanoes	10^4	0.09
	forest fires	4×10^5	3.8
*secondary**	*vegetation*	5×10^5 to 3×10^6	28
	sulphur cycle	10^5 to 10^6	9.3
	nitrogen cycle	2×10^6	14.8
	volcanoes	10^3	0.009
subtotals		10×10^6	94
anthropogenic			
*primary**	*industrial + combustion*	1×10^5 to 3×10^5	2.8
	dust from agriculture	10^2 to 10^3	0.009
*secondary**	*hydrocarbon vapours*	7×10^7	0.065
	sulphates	3×10^4	2.8
	nitrates	6×10^4	0.56
	ammonium salts	3×10^3	0.28
subtotals		6.7×10^5	6
TOTALS		10.7×10^6	100

* Primary particles are particles consisting of solid or liquid material emitted as such, while secondary particles are formed in the air by chemical reactions from gaseous compounds.

Various processes can serve as sinks for the particles. The coarse particles are removed by sedimentation and washout (impaction with rain droplets). In the absence of rain, the transient nuclei will all be removed to the accumulation range by condensation and coagulation. In case of rain, they may be removed by washout. The sinks for the accumulation-range particles are washout, rainout

(the particles serve as nuclei for rain-droplet formation) and dry deposition. Their residence time is much longer than that of either the transient nuclei or the coarse particles. Obviously, it depends strongly on meteorological conditions and is estimated to have a maximum duration of about one week.

The most important sources and processes contributing to the presence of the particles in the air are summarized in table 4.1, together with a rough estimate of their contribution on a global scale. A striking feature of this table is the preponderance of natural sources. However, limitations of sinks and dispersion processes lead to a preponderance of particles with an anthropogenic origin in urban and industrialized regions. Major sources in these regions are fuel combustion, transportation, incineration, industrial emission of gases and vapours and abrasive processes. The ratio between the contributions of these sources will depend on the region; nevertheless, combustion-related sources will always account for a large part. It is the particles emitted by these sources which contain most of the mutagens and the PAH (see chapter 1); furthermore, they are fine particles which are produced by condensation of hot vapour formed during pyrolysis, which means that they fall within the ADM range which can reach the deepest parts of the respiratory tract upon inhalation (Task Group on Lung Dynamics, 1966; ISO, 1983).

It should be noted that combustion also leads to the formation of particles by chemical reactions after emission has taken place, although this is not indicated in the table. Combustion is not only accompanied by the emission of immediately condensating hot vapours, but also by compounds which are sufficiently volatile to remain in the gas phase for a substantial part of their residence in the air. These compounds may participate in gas-phase reactions leading to non volatile products. Among them are a number of PAH. It is now well established (Thrane and Mikalsen, 1981) that some of the volatile PAH, in particular pyrene and fluoranthene, react with OH radicals and nitrogen dioxide to form the mutagens 2-nitropyrene and 2-nitrofluoranthene (Pitts et al., 1985; Nielsen and Ramdahl, 1986; Arey et al., 1986; Pitts, 1987). These nitro-PAH are found in ambient airborne particles and not in the particles emitted by combustion processes, which confirms their formation in the air.

Sampling

Ambient airborne particles can be collected with various techniques based on the following principles: filtration, inertial impaction, gravitational or centrifugal sedimentation, impingement, diffusion, interception and thermal or electrostatic precipitation (Finlayson Pitts and Pitts, 1986). Each principle and technique

is characterized by its weak and strong points and its application depends on requirements dictated by the specific objectives of the study. Basically these requirements have to do with:

- the amount of particulate material that has to be collected per time unit to achieve an acceptable accuracy or experimental convenience (collection rate: high-volume sampling vs. low-volume sampling),
- the number of samples that have to be collected (a high number will demand simpler and cheaper techniques),
- the selection of the particles according to their ADM (e.g. fine vs coarse and respirable vs non respirable),
- the compounds or effects that have to be determined (e.g. certain type of filters are used to prevent chemical conversion of the compounds of interest during collection),
- the techniques that have to be applied for extraction, chemical analysis or biological testing of the collected particles.

In case the objective of a study is to investigate the mutagenicity of the particles, the amount of particulate material that has to be collected for one test sample depends on the sensitivity of the test. The sensitivity of the tests now available requires that relatively large amounts of material have to be collected, in particular if dose-response relationships have to be established for several variants of the test. Practical experience has shown that testing of typical urban particles in a standard Salmonella/microsome test with four to five indicator strains, with and without tissue homogenate for metabolic activation, requires the amount of particulate material from at least several hundred m^3 of air; a clear effect is generally obtained at testing the equivalent of 1 to several m^3 per petri dish. In most cases, the time available for sampling must be short enough to enable the temporal dispersion of the mutagenicity to be investigated with a sufficient resolution. This means that high collection rates have to be maintained during sampling. Furthermore, depending on the desired resolution of the temporal and spatial dispersion, many samples have to be collected, which means that the techniques used must be simple and cheap. Together, these requirements have lead to the choice of high-volume filtration.

In addition to high collection rate and simple application, high-volume filtration offers the advantage that it enables the collection of a large part of the ADM range of the particles. The collection efficiency is determined by the following factors.

- The shape of the inlet determines which particles are small enough to follow the air stream to the filter.
- Very small particles are collected by diffusion followed by sticking to the filter material. The efficiency of this process is determined by the structure of the filter material. Generally particles down to less than 0.01 μm can be collected efficiently.
- Sampling efficiency shows a dip between 0.1 and 1 μm at the linear air speeds generally applied, which is caused by the fact that the contribution of diffusion decreases at larger ADMs, while that of the other major collection process, impaction, decreases at larger ADMs. Its depth depends on the filter material.

The filters generally used in mutagenicity studies are made of glass-fibre mats or Teflon-coated glass-fibre mats, because they combine a high collection efficiency with a low air resistance (a prerequisite for the necessary high collection rates). Their ADM range starts at less than 0.01 μm, at the dip between diffusion and impaction they still reach a efficiency above 90%, while larger particles are completely collected. The construction of the sampler apparatus, wind speed and wind direction determine the upperbound ADM. The inlet of most apparatuses provides for a gradual decrease of sampling efficiency between about 5 and 50 μm. Inlet together with filter guarantees near complete collection of the particles which can enter the respiratory tract.

Sampling artifacts

During sampling each particle is fixed on the filter material in a layer of other particles for the remainder of the sampling time. During this period it is exposed to compounds in other particles, to the filter material and to the air stream passing the filter with its gaseous pollutants. Sampling means that the particles are no longer freely floating in the air, but are placed in an enforced stream of air; that otherwise isolated particles with different origins and chemical composition are brought into contact with each other. After sampling exposure conditions as well as compounds exposed to, differ strongly from those in the air.

This change-over has been found to be the critical step in the preparation of extracts which are suitable for chemical analysis and mutagenicity testing. It will certainly lead to a pattern of chemical reactions which differs from that prevailing in the air and which may affect the PAH and the mutagenicity in another way. Existing mutagens may be deactivated or transformed into new

ones, while new ones may also be formed from non-mutagenic compounds; the composition of the particles as regards compounds which are not mutagenic themselves but influence the effects of the mutagens may also change. The simplest effect can be expected for the PAH concentrations: they can only decrease. Several studies have dealt with this possible artifact (Lee et al., 1980; Grosjean, 1983; Grosjean et al., 1983; Brorström et al., 1983; Van Cauwenberghe et al., 1980; Daisey et al., 1983; Fitz et al., 1984). They suggest that in any case its influence is not so strong as to really undermine the applicability of the two indicators; however, further studies are needed to get a more quantitative impression of this influence and, thereby, the reliability of the two indicators. It was deemed important enough to be one of the main topics of the research presented in this thesis.

Sampling can also introduce artifacts of a more physical nature. Inevitably, the filters used show some air resistance, which increases when they become laden with particles during sampling. The air resistance will result in a pressure drop over the filter which in its turn may lead to the evaporation and, thereby, loss of the more volatile components of the particles. This artifact has been intensively studied for PAH (Van Cauwenberghe et al., 1980 and Van Cauwenberghe 1985; Van Vaeck et al., 1984). The results made clear that it may indeed lead to a serious underestimation of the concentrations of the more volatile PAH in the particles. It remains unclear to what extent it affects the mutagenicity. Contrary to this artifact, another one will lead to an overestimation of PAH concentrations. It has been shown that glass-fibre filters, the filters most often used for high-volume sampling, can efficiently adsorb gaseous organic compounds from the airstream passing the filter (McDow and Huntzicker, 1990; Ligocki and Pankow, 1989). The adsorbed amount may be so large as to easily overcompensate the artifactual evaporation. It is uncertain to which extent this artifact leads to the introduction of mutagens originally not present in the particles. PAH directly adsorbed to glass fibres are very vulnerable to chemical conversion (Pitts et al., 1978 and 1980; De Raat, 1982 and 1983; De Raat et al., 1987A and 1987B) and as the products of these reactions may be strong mutagens, this artifact may in particular be relevant to the mutagenicity in a indirect way. Chapter 11 presents the first study which pays attention to this possibility.

Extraction[6]

After sampling comes extraction, another possible source for artifacts that may affect the value of the two indicators. It means dissolving compounds that were originally components of separate and chemically distinct particles and compounds that were present in one particle but isolated from each other. Compounds that were separated in the air and during sampling are brought in contact with each other. These changes may be accompanied by chemical reactions leading to a new chemical equilibrium in the complex mixture; these reactions may be boosted by the elevated temperatures or other influxes of energy (light and ultra sound) during extraction. In many cases air is not excluded from the extraction system which may lead to additional oxidation. Moreover the solvents need not be inert and may react with the matrix components. Finally the concentration step that follows the dissolution (evaporation of the solvent; the volume of the extract is often reduced more than a hundred fold) may aggravate the chemical artifacts; in many cases the solvent is even completely removed and the remaining tar is dissolved again in a solvent suitable for the biological test or chemical analysis.

Besides being inert (absence of chemical reactions which affect the concentrations of relevant compounds), the extraction needs to be complete. Complete in the sense, that there is no doubt that all the compounds that can in principle be extracted with the applied solvent-technique combination are in practice indeed easily dissolved. Completeness defined in this way is a prerequisite as it determines the comparability of results obtained after extraction with this combination. Ideally completeness should really mean complete dissolution of all relevant compounds. However, it is practically impossible to obtain certainty about this. Inert extraction always leaves an insoluble residue behind which may contain relevant compounds, while complete dissolution can only be achieved with destructive extraction methods. Ambient airborne particles contain PAH and mutagenicity that can easily be extracted with organic solvents. Intensification of the extraction and repeated extraction with the same or other solvents does not yield higher PAH concentrations or does not change mutagenicity (De Raat, 1982 and 1983; De Raat et al., 1987A). It thus appears that it is possible to achieve completeness as regards this readily extractable

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[6] See Stanley et al. (1967), Gordon (1974), Grosjean (1975), Hill et al. (1977), Breuer, 1984, Barkenbus et al., 1983; Watts et al., 1992 and De Raat (1987A) for the extraction of organic compounds (among them PAH) with organic solvents from the particles; the extraction of mutagens with organic solvents has been investigated by Talcott and Wei (1977), Krishna et al. (1983), De Raat (1982 and 1983), Viau et al. (1982), De Raat et al. (1987A) and Lee et al., 1991.

fraction. Relevant compounds may stay behind in the insoluble residue; it is, however, questionable whether these compounds bear any biological relevance as their biological availability will be very limited. In the past some studies on the mutagenicity of the particles have applied extraction techniques not optimal as regards the readily extractable mutagenicity (Alfheim and Møller, 1979; Møller and Alfheim, 1980). The reduced value of these studies is evident, because we may expect a sample dependence of the mutagenicity extracted. This sample dependence should always be avoided because it reduces the comparability of the results to an unacceptable level.

In view of these considerations it is remarkable that the literature mentions hardly any studies about the quality of solvent extraction of ambient airborne particles as regards its inertness and completeness. A few studies are concerned with the extraction of organic carbon and PAH. Their conclusion is that maximum yields can be obtained by the combination of apolar and polar solvents. The author did not find any literature data about possible chemical conversion. In particular the influence of extraction on mutagenicity deserves attention in future research, because small chemical changes may have drastic effects on this effect. This is exemplified by the extremely mutagenic compounds that can be formed from non mutagenic PAH by nitration or oxidation.

CHAPTER 6

MUTAGENICITY OF AMBIENT AEROSOL COLLECTED IN AN URBAN AND INDUSTRIAL AREA OF THE NETHERLANDS

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MUTAGENICITY OF AMBIENT AEROSOL COLLECTED IN AN URBAN AND INDUSTRIAL AREA OF THE NETHERLANDS

W.K. DE RAAT, F.A. DE MEIJERE and S.A.L.M. KOOIJMAN

TNO, P.O. Box 217, 2600 AE Delft (The Netherlands)

ABSTRACT

To investigate the influence of the densely populated and heavily industrialized Rijnmond area of The Netherlands on the genotoxicity of the ambient aerosol, aerosol samples were collected at locations within the area, and at a coastal region located upwind. The mutagenicity of extracts of the samples was compared in the Salmonella/microsome test. The dependence of the effects on sampling time and on sampling location was investigated with the aid of a series of simple mathematical models. These models were also used to estimate the increase in mutagenicity above background levels at the sites in the Rijnmond area due to emissions within that area. Application of the models showed that the clear and significant increases are not merely a result of the additions of mutagens emitted, but that possibly interactions between sampling time- and location-dependent factors play a role. Comparison of the results obtained with the different Ames-test variants (different strains, with and without liver homogenate) indicate that the conclusions concerning the time and location dependence of the effect were not dependent on the variant used.

INTRODUCTION

The presence of mutagens in ambient aerosols has led to many studies during the past 7 years [1—9]. With the realization that almost all aerosol extracts investigated were clearly mutagenic, attention was focused on the origin and the identity of the mutagens and on the variation of the effect in time and space. The results now available point to the combustion of fossil and organic fuels as major sources, and to substituted (and especially nitrated) polycylic aromatic hydrocarbons (PAH) as a possible group of responsible compounds [7,8,10—24]. The effect is clearly related to anthropogenic air pollution. Periods and areas with enhanced levels of air pollution are also characterized by enhanced mutagenicity [4,9,25—27]. The mutagenicity of the aerosol can be considered as a toxicological air quality indicator.

In the present study the contribution to the mutagenicity of aerosol extracts by an urban and industrialized area of The Netherlands was investigated. Figure 1 shows a map of the area under investigation and the five

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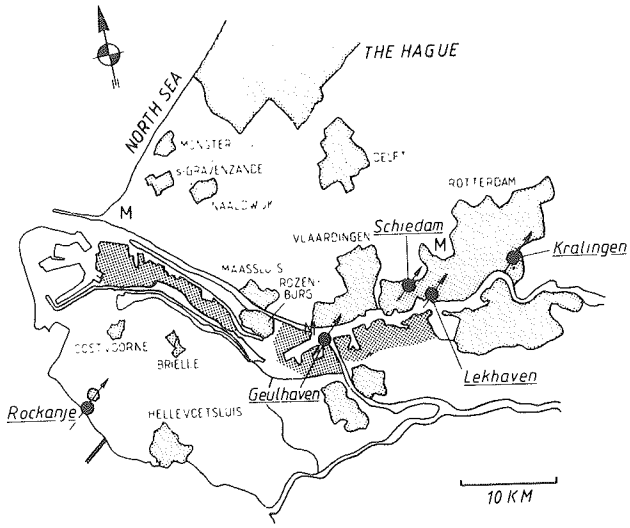


Fig. 1. Map of the Rijnmond area (The Netherlands) with the sampling locations. Light grey: urban area; dark area: (petrochemical) industry; (●) sampling site; (M) weather station. The arrows roughly indicate the prevailing wind direction during the selected sampling periods.

sampling locations. Twenty-three sampling trips were carried out during which the aerosol was simultaneously sampled at these locations with a prevailing wind between SSW and NW. Under these wind conditions, the coastal location (Rockanje) was upwind of the other four locations and the aerosol sampled at this location came directly from over the sea and was thus considered not to be infuenced by local sources. The five aerosol samples from one sampling trip were simultaneously extracted and tested for mutagenicity with the Salmonella/microsome test. Two bacterial strains with differing sensitivity for classes of mutagens were used; the test was carried out in the presence and absence of a rat liver homogenate for metabolic (de)activation. In this way four test conditions (test variants) with differing sensitivities were applied. Comparison of the genotoxicity of aerosol samples from the coastal (upwind) location and the locations within the area, permitted the increase in the effect due to the sources within the area to be estimated. This comparison was formalized by an analysis of the data with a series of simple mathematical models. Differences between the effects for the four test variants can demonstrate whether the proportions between them were influenced by the sources within the area, thus indicating changes in the mutagenic quality of the aerosols.

MATERIALS AND METHODS

Sampling locations

The coastal location (Rockanje) lies just to landward of the dunes at an

altitude of 2—7 m above ground level (the aerosol was sampled at two locations close to each other). When winds are from the south-west, the aerosol sampled at the four other locations can be expected to be influenced by emissions from urban sources (Schiedam and Kralingen) and/or (petro chemical) industrial sources and harbours (Lekhaven and Geulhaven). Sampling altitudes were 2 m for Geulhaven, about 30 m for Schiedam, about 50 m for Lekhaven and about 25 m for Kralingen.

Sampling times

Samples were taken simultaneously at the five locations when winds were forecast to be between SSW and NW for more than 24 h ahead. Data supplied by the Royal Dutch Meteorological Institute showed that winds had indeed been as forecast on 23 occasions at the weather stations in Fig. 1. Sampling commenced between 9 and 11 a.m. and lasted for 24 h. The minimum overlap in time of the five samples was 22 h. None of the sampling times were characterized by enhanced oxidant ($NO_2 + O_3$) concentrations, which indicates the absence of photochemical smog periods during sampling (information from the Institute of Public Health and Environmental Hygiene).

Sampling method

Sartorius HV100 'Staubsammelgeräte' and Sartorius SM13400 glass fibre filters were used for sampling. The flow rate was $100\,m^3\,h^{-1}$, and resulted in an average airspeed through the filter of $0.58\,m\,s^{-1}$. Before sampling the filters were washed for 24 h in a Soxhlet extractor with methanol or a 1:1 (vol) mixture of methanol and acetone (Rathburn HPLC grade or glass-distilled). After sampling the filters were wrapped in aluminium foil and stored at $-80°C$.

Extraction

The five samples derived from one trip were extracted simultaneously in Soxhlet extractions for 8 h with methanol (Rathburn, HPLC grade) and then for 8 h with cyclohexane (nanograde) in a nitrogen atmosphere. The two extracts of a sample were combined and evaporated to dryness at 30°C in a rotating evaporator at reduced pressure. The residues were dissolved in dimethyl sulphoxide (3 ml for the residue from $2400\,m^3$ of air) and stored at $-80°C$.

Mutagenicity testing

All aerosol extracts were subjected to Ames's Salmonella/microsome test for mutagenicity [28]. Four variants of the Ames test were used: namely *Salmonella typhimurium* strains TA98 and TA100 with and without metabolic activation by a liver homogenate (S9 fraction) from rats treated with

Aroclor-1254 [28]. The aerosol extracts of one set of samples were tested simultaneously (in a single test procedure). Four doses of aerosol extract were tested in triplicate; these doses were always the residue from 0, 2.5, 5, 7.5 and 10 m^3 per plate for TA98 and from 0, 5, 10, 15 and 20 m^3 per plate for TA100.

Calculation of the effects

The mutagenic potency of the extracts, (called 'effect' throughout this paper) is assumed to correspond to the response increase per unit of dose. It has been estimated from the numbers of revertant colonies per plate according to the maximum likelihood method assuming Poisson sampling and a linear dose response relation. No significant deviations from these linear relationships were observed.

The dependence of the effect on location and time

A formal analysis of the dependence of the effect on location and sampling time is essentially a comparison of the goodness of fit of mathematically formulated hypothesises (models) in which specific features are included or excluded. For this purpose the data have been analyzed using a series of models with increasing complexity (summarized in Table 1) in such a way that each simple model is a special case of a more complex one. The design of this series of models was possible because of the special set up of the study in which sampling, extraction and testing of the five samples were all carried out simultaneously. The data from the 23 sampling trips (i: 1—23) allow comparison of the effects at the five locations (j: 1—5). Comparison of the goodness of fit of the data to these models allows conclusions regarding the time- and location-dependence of the effects to be reached.

In order to determine if the time-averaged effects at the various locations differ, the simplest model in which the effect is invariant (I) should be compared with that in which the effect is dependent on location only (II); P_j represents the total contribution of upwind sources to the mutagenicity of the aerosol at location j. However, if the variation of the effect of the background aerosol with time is large, the contributions of local emissions may be obscured. The comparison should then be between two models, in one the effect is only dependent on time (i.e. there is a time-dependent background L_i, and no local contribution) (III) and in the other the effect is dependent on both time and location (IV). This model assumes that the contributions of local sources to the mutagenicity at the locations (P_j) are additive to the background aerosol mutagenicity at that particular time (L_i). For possible interaction between location-dependent factors and time-dependent factors, the additive model (IV) should be compared with the simplest interaction model in which interaction *itself* is constant (c), but the contribution due to interaction depends on L_i and P_j. By substantially increasing the numbers of parameters, time (a_i) and/or location (b_j)

TABLE 1
SET OF MODELS OF INCREASING COMPLEXITY DESCRIBING THE EFFECTS OF THE AEROSOL EXTRACTS

Model No.	No. of parameters	Description of the effect	Equations for the effect[a]	Constraint
I	2	Constant	$L + \xi_{ij}$	
II	6	Location specific	$P_j + \xi_{ij}$	
III	24	Time specific	$L_i + \xi_{ij}$	
IV	28	Location and time specific	$L_i + P_j + \xi_{ij}$	$P_1 = 0$[b]
V	29	Constant interaction	$L_i + P_j + cL_iP_j + \xi_{ij}$	$P_1 = 0$
VI	32	Location specific interaction	$L_i + P_j + L_ib_j + \xi_{ij}$	$P_1 = b_1 = 0$[c]
VII	51	Time specific interaction	$L_i + P_j + a_iP_j + \xi_{ij}$	$P_1 = 0$
VIII	53	Location and time specific interaction	$L_i + P_j + a_ib_j + \xi_{ij}$	$P_1 = b_1 = a_+ = 0$ $\Sigma_i a_i^2 = 23$

[a]The value ξ_{ij} represents the difference between the measured effect and the effect calculated from the estimated parameters (L_i, P_j, etc.). These values are assumed to be realizations of an independent random variable following a normal distribution with mean zero and unknown variance. The calculated variance of the ξ-values for each model can be used to judge the goodness of fit of the experimental data to the model, and thus to compare the different models. There are five locations (index j) and 23 sampling times (index i). The location Rockanje is indicated with $j = 1$. The chosen constraints are arbitrary and only serve to define the estimation problem; i.e. determination of the free parameters.
[b]$P_1 = 0$: the contribution of sources at the first location (Rockanje) is zero.
[c]$b_1 = 0$: the sources at Rockanje are not involved in the interaction.

dependent interaction can be included and model V can be compared with models VI or VII, and VI or VII with VIII to determine whether these types of interactions increase the goodness of fit of the models significantly.

Comparison of the goodness of fit of the experimental data to the models described above leads to the choice of the *optimum* model of *this* series, i.e. the model that fits significantly better than the simpler models. This model can then be used to estimate the mean contribution of sources in the area upwind of a location to the mutagenicity of the background aerosol sampled upwind of those sources. This allowed us to make optimal use of the set up of the study for calculating the contribution.

The values of parameters given in Table 1 were estimated according to the least squares criterion from the effects measured. This criterion corresponds with the maximum likelihood criterion if the effects follow a normal distribution with common variance. Since the effects are maximum likelihood estimates themselves, their asymptotic distribution is indeed normal. The assumption of a common variance is a simplified one and should be considered as a good approximation. For a general description of the maximum likelihood theory see Cox and Hinkley [29].

RESULTS

The results of the Ames-tests for the 23 trips with south-westerly wind are depicted in Fig. 2. This figure shows the clear dependence of the effect on location. In nearly *every experiment* the effect was greatest at Lekhaven and smallest at Rockanje; the effects at Schiedam and Kralingen in nearly

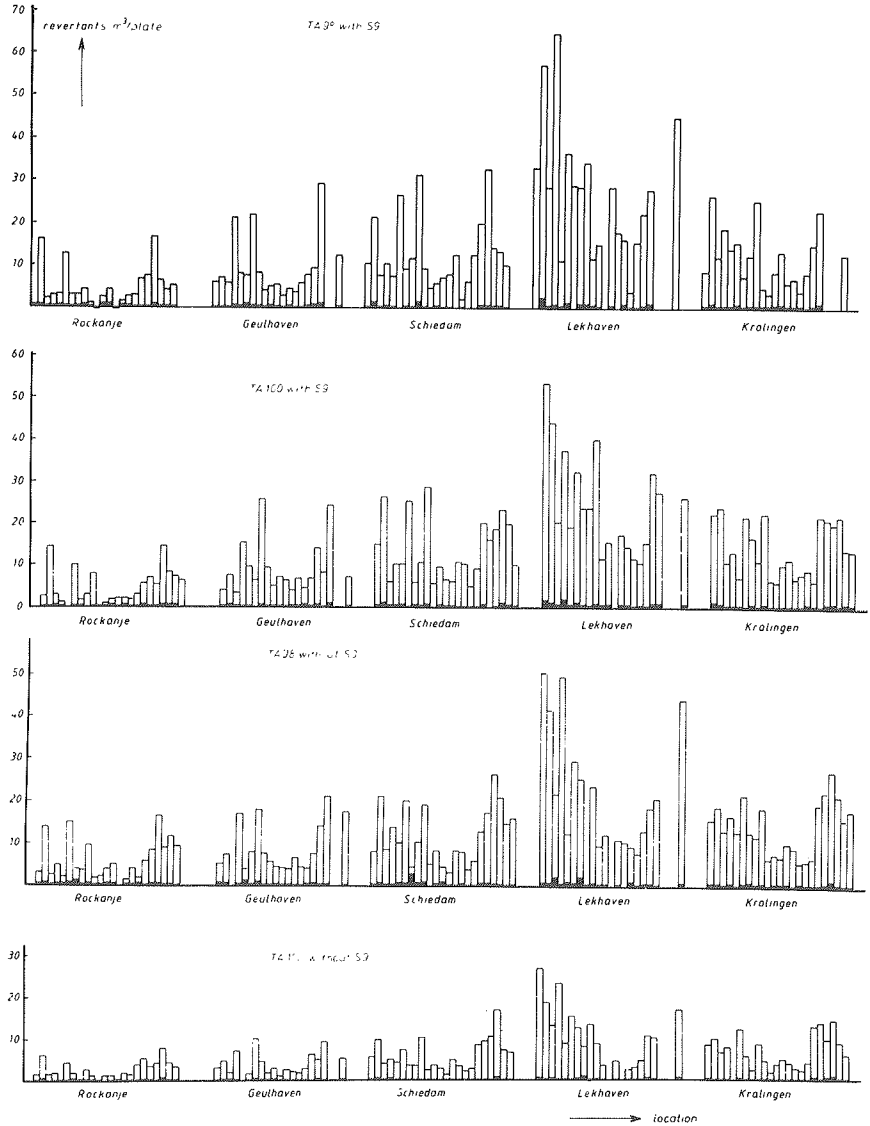


Fig. 2. The mutagenicity of aerosol extracts in the Ames-test (standard deviations in black). The results from the 23 sampling trips in the period March—November 1981 are shown in order for each of the four test variants.

every experiment had values between those at Lekhaven and Geulhaven. The results suggest a gradient exists from Rockanje via Geulhaven and Schiedam to Lekhaven; downwind of Lekhaven the effect decreases again. The contributions of the emissions of the sources upwind of each location increase from Geulhaven to Lekhaven. The difference between Lekhaven and Schiedam, which are quite close to each other, shows that the high effect of the former is to a great extent attributable to sources in the immediate vicinity.

The influence of the wind direction on the effect is indicated by a comparison of results from the 23 trips with south-westerly wind with those of the other 12 trips (results not shown in detail). During most of these trips the wind was north-easterly, during the remaining it was variable. To this end the mean effects were calculated for each location for the two groups of trips. The significance of the differences between the two means for each location was determined with the Wilcoxon test.

The means and the levels of significance are given in Table 2. This table shows consistent clear and significant differences at the three locations west of Lekhaven. Although the Rockanje location is separated from the Rijnmond area by a relatively large rural area, the influence of the wind direction is very clear at this location. The strong contribution of the emissions in the neighbourhood of Lekhaven is illustrated by the high effects also found at this location during the other 12 trips.

The similarity of the results of the four variants of the Ames-test is a striking feature of Fig. 2. Although the effects with TA100 in the absence of liver homogenate are smaller than those with the other variants, all four variants revealed the same trend with respect to variation in time and space. This is illustrated by the high correlation coefficients between results for variant pairs given in Table 3. This table also shows that the angles between the first principal axis and the abcissa generally do not differ very much from each other, indicating that the mean proportions of the Ames-test variants is quite similar for the different locations. Since the four variants have different sensitivities for different classes of compounds, the magnitude of the correlation is somehow a measure of the constancy in 'mutagenic composition' of the aerosol extract.

Two calibration experiments were carried out in order to investigate whether the correlations are indeed high enough to suggest such constancy. An artificial mixture of mutagens and an aerosol extract were tested several times at different, randomly chosen, doses. The results are given in Fig. 3; the experimental details are given in the legend to the figure. Some of the correlation coefficients in Table 3 may indeed suggest a constancy (resemblance) in composition as far as the type of compounds responsible for the mutagenicity is concerned.

TABLE 2

COMPARISON OF THE EFFECTS AT THE DIFFERENT LOCATIONS FOR TWO GROUPS OF EXPERIMENTS WITH DIFFERENT PREVAILING WIND DIRECTION[a]

test variant	Location														
	Rockanje			Geulhaven			Schiedam			Lekhaven			Kralingen		
	$\bar{X}_A$	$\bar{X}_B$	p	$\bar{X}_A$	$\bar{X}_B$	p	$\bar{X}_A$	$\bar{X}_B$	p	$\bar{X}_A$	$\bar{X}_B$	p	$\bar{X}_A$	$\bar{X}_B$	p
TA98+[b]	3.7	14.5	<0.004	8.6	25.4	0.002	11.8	22.4	0.006	26.0	36.5	>0.1	12.0	19.0	>0.1
TA98—	5.3	11.2	<0.002	8.8	17.0	<0.002	10.5	17.4	0.04	21.1	22.6	>0.1	13.1	15.9	0.49
TA100+	3.6	13.2	<0.002	8.6	21.4	<0.002	11.7	21.7	0.04	22.1	26.1	>0.1	12.4	17.9	0.29
TA100—	2.4	7.0	<0.002	4.2	8.8	<0.002	5.1	10.3	0.002	9.9	12.9	>0.1	6.5	9.0	0.34

[a] $\bar{X}_A$ = mean effect (revertants per m^3) of the 23 trips with wind from sea; $\bar{X}_B$ = mean effect of the other 12 trips; $H_0: \bar{X}_B = \bar{X}_A$; $H_1: \bar{X}_B > \bar{X}_A$; Wilcoxon test.

[b] With (+) or without (—) S9-Aroclor 1254.

TABLE 3

THE CORRELATION IN TIME BETWEEN THE EFFECTS IN FOUR VARIANTS OF THE AMES-TEST FOR THE 23 TRIPS WITH SOUTH-WESTERLY WIND

Test pair		Location					All locations
		Rockanje	Geulhaven	Schiedam	Lekhaven	Kralingen	
TA98+S9	r[a]	0.89	0.91	0.85	0.86	0.81	0.91
TA98—S9	p	.45	.38	.37	.41	.38	
TA100+S9	r	0.78	0.92	0.82	0.88	0.86	0.91
TA100—S9	p	.23	.21	.34	.28	.29	
TA98+S9	r	0.90	0.90	0.81	0.73	0.72	0.87
TA100+S9	p	.41	.42	.41	.35	.43	
TA98—S9	r	0.68	0.85	0.86	0.95	0.87	0.93
TA100—S9	p	.18	.23	.27	.25	.29	

[a] r = correlation coefficient; p = angle between the first principal axis and the first variable in degrees.

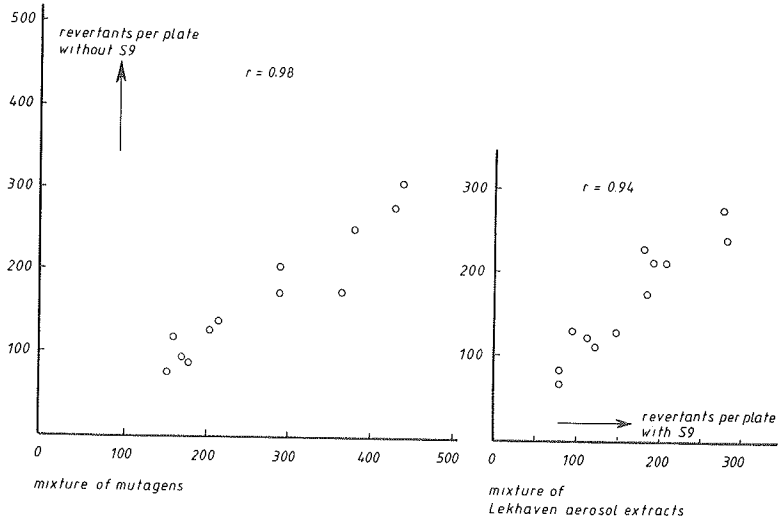
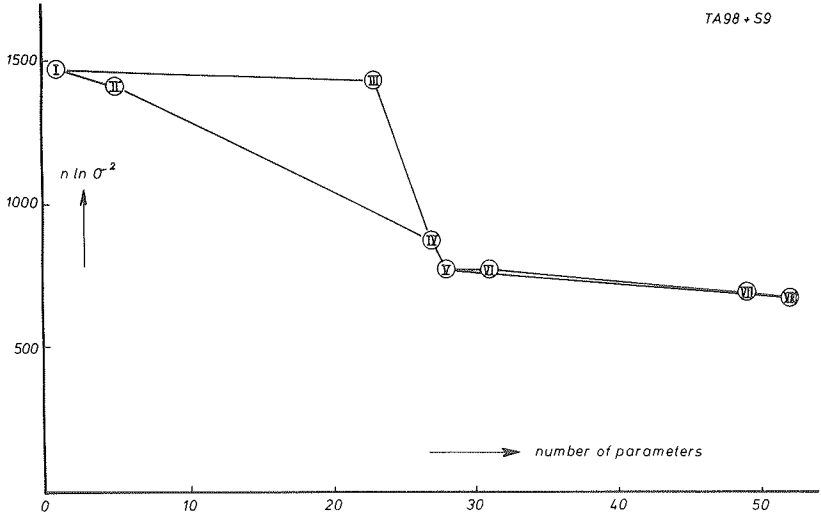
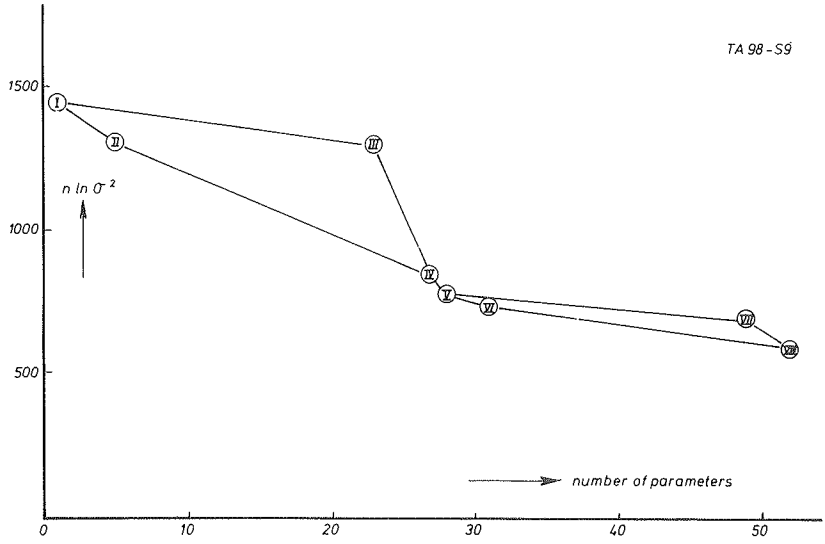


Fig. 3. Correlation of the effect measured with the Ames test variants TA98 with and without S9. Mixture of mutagens in dimethylsulphoxide: benzo(a)pyrene (10 μg ml^{-1}); 3-methylcholanthrene (100 μg ml^{-1}); 3,4-dimethylbenzoanthracene (20 μg ml^{-1}); 2-nitrofluorene (5 μg ml^{-1}); 2,4,7-trinitrofluorenone (0.1 μg ml^{-1}); 2-nitronaphthalene (20 μg ml^{-1}). Highest dose 70 μl per plate. Mixture of Lekhaven aerosol extracts: the extracts of three Lekhaven aerosols (in dimethylsulphoxide) were pooled, highest dose 70 μl (7 m^3) per plate. Each point represents the mean of a triplicate test; the tests were carried out on different dates. Metabolic activation by S9-mix with S9 Aroclor 1254.

ANALYSIS OF THE DATA WITH THE MATHEMATICAL MODELS

The goodness of fit of the models described in Table 1 for the different variants of the Ames test are compared in Fig. 4. Under the hypothesis of a particular model, any decrease on the ordinate ($n\ln \sigma^2$) is asymptotically chisquare distributed with a number of degrees of freedom equal to the





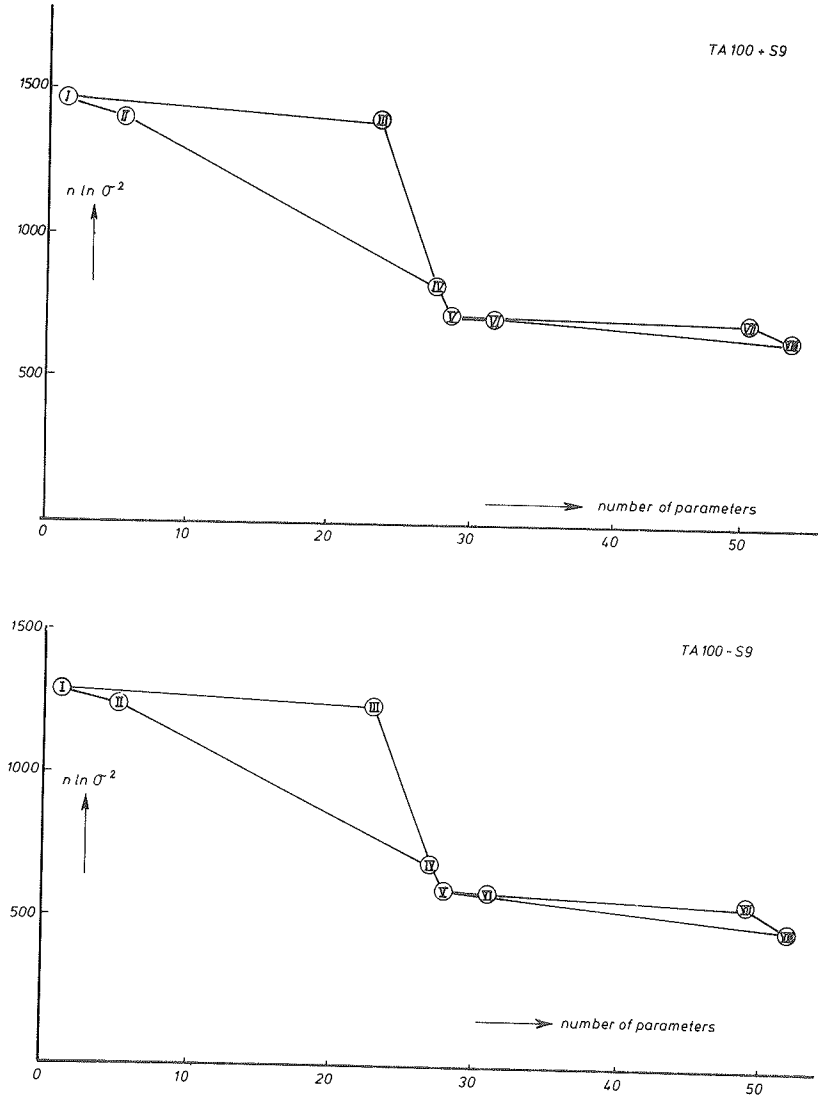


Fig. 4. The dependence of the goodness of fit on the selected model. The product of the number of measured effects and the logarithm of the estimated variance (σ^2 = the variance of ξ_{ij}) is plotted against the number of parameters for the eight models described in Table 1. Comparable models are connected. The number of effects measured in the Ames tests using TA98 + S9, TA98 — S9, TA100 + S9, TA100 — S9 were 104, 105, 106 and 104, respectively.

increase on the abscissa on the basis of the likelihood ratio theory. Models which may validly be compared on this basis are shown connected in Fig. 4. The slopes of the connecting lines therefore indicate the significance of the increase in goodness of fit between the two models. As the number of measured effects is only about twice the number of parameters of the most complex model (VIII), the figure only gives a rough indication of the increase in goodness of fit for the complex models. The results shown in Fig. 4 indicate that:

- a very similar pattern of increase in goodness of fit is shown by the four Ames-test variants
- The mean effect is clearly location dependent
- the variation between the different locations is larger than the variation between the different sampling times
- compared with an addition of the effects of the background and the emitted aerosol, the models which include interactions give significantly better fits
- there are indications that the interaction is both time and location dependent.

TABLE 4

ESTIMATED MEAN INCREASES IN AEROSOL MUTAGENICITY (revertants/m^3) ABOVE BACKGROUND (ROCKANJE) LEVELS DUE TO THE LOCAL EMISSIONS

Ames test	Model No.	Location			
		Geulhaven	Schiedam	Lekhaven	Kralingen
TA 98 with S9	IV	5.4	8.2	23.9	8.5
	V	4.7	7.5	25.8	8.3
	VI	4.4	6.6	25.9	7.9
	VII	5.1	8.2	25.9	8.8
	VIII	5.1	8.3	36.2	10.2
TA 98 without S9	IV	4.1	5.7	17.5	8.0
	V	4.7	7.5	25.6	8.2
	VI	3.8	5.5	19.4	7.2
	VII	3.6	5.5	19.6	7.8
	VIII	3.4	5.6	17.7	7.9
TA 100 with S9	IV	6.3	8.8	21.0	9.3
	V	6.3	8.4	21.5	8.4
	VI	6.9	9.5	22.3	10.1
	VII	6.3	8.5	21.7	9.0
	VIII	5.3	8.6	20.1	9.2
TA 100 without S9	IV	2.4	3.2	9.1	4.2
	V	2.4	3.1	10.1	3.9
	VI	2.8	3.4	10.8	4.1
	VII	2.4	3.2	10.2	4.3
	VIII	2.2	3.2	13.8	4.2

A summary of the estimates (mean) for the time-dependent parameters for models IV—VIII is given in Table 4. Model V is the optimum model for calculating the contribution, because application of more complex models does not lead to a significant improvement in the goodness of fit. The table shows that the estimated mean contributions do not differ very much between the different models.

Part, or all, of the contributions of 'interactions' to the total effect, could be attributable, not to actual interactions affecting the intrinsic mutagenicity of the aerosol, but to the experimental design of the study. Simultaneous sampling, extraction and testing might lead to an artificial variance with time which is equal for all locations and which could result in a better fit of the data to the models with interaction.

To gain an impression of the contribution of any such artificial inter-test variation to the interaction, two model mutagens: furazolidone (Orphahell, Mijdrecht, The Netherlands, without S9) and benzo(*a*)pyrene (Sigma, with S9) were tested in about half of the Ames tests carried out.

As shown in Table 5, the inter-test variation with these compounds was rather large. The effects of the compounds did not correlate significantly with the effects of the aerosol extracts (highest correlation coefficient 0.44; mean of absolute coefficient 0.24) except for the effects measured at Lekhaven with those of benzo(*a*)pyrene (strain TA98 with S9; 0.51, $p < 0.05$ and strain TA100 with S9; 0.67, $p < 0.01$). So a weak effect of the inter-test variation *as measured with the model mutagens* could only be observed at the Lekhaven location.

TABLE 5

THE INTER-TEST VARIATION OF THE EFFECTS OF THE TWO MODEL COMPOUNDS[a]

	TA 98 with S9		TA 98 without S9		TA 100 with S9		TA 100 without S9	
	$\bar{X}$	S	$\bar{X}$	S	$\bar{X}$	S	$\bar{X}$	S
Benzo(*a*)pyrene 5 μg/plate	300	96			642	213		
Furazolidone 0.2 μg/plate							1300	268
Furazolidone 0.5 μg/plate			369	102				

[a] $\bar{X}$ = mean of all experiments; S = standard deviation.

DISCUSSION

The results of this study give a clear impression of the increase of the mutagenicity of the ambient aerosol due to emissions in an urban and industrial area. Depending on the variant of the Ames test used, the mean effect at the various locations ranges from 1.7 to 8.0 times the effect of the aerosol (Table 2) sampled at the upwind coastal location, and the mean contributions of local sources calculated with the simplest interactional model ranges from 2.4 to 25.8 revertants per m^3 (Table 4). A marked variation in time was found at every location. When the wind was north-easterly, the mean effects at the three western locations increased significantly (Table 2), demonstrating the influences of the emissions when the wind is in the opposite direction. The comparison of the goodness of fit of the data to the different models shows: (1) that the effect is significantly dependent on both location and time, a finding which is obvious from Fig. 2; and (2) that the effects are significantly influenced by an interaction between factors dependent on both location (j) and time (i). Close observation of the results presented in Fig. 2 already indicates such an interaction. At every location and with every Ames-test variant the aerosols sampled in the beginning or at the end of the study were more mutagenic than those sampled in between. A number of explanations can be put forward for these significant interactions.

The design of the study implicates simultaneous sampling, extraction and testing. It is improbable that the performance of the sampling or the extraction will show a variation comparable with that of the Ames test. The possible magnitude of the inter-test variation is shown by the Ames-tests with the model mutagens. The almost total lack of correlation between the effects of the model mutagens and the aerosol extracts shows that the time dependence of the effects of the aerosols is not only a result of the inter-test variation as measured with the model mutagens.

However, we cannot exclude that the inter-test variation is (one of) the explanation(s) for the interaction found, because the model describing inter-test variation $(L_i + P_j)S_i$, in which S_i is a term describing the sensitivity of the Ames test, is formally equivalent to interactional model $L_i + P_j + a_iP_j$ (VII). Investigations into the type of inter-test variations shown by aerosol extracts might lead to the possibility of adjusting the effects measured by the simultaneous testing of appropriate aerosol extracts as 'model mutagens'.

If the interaction found is not (only) the result of inter-test variation, the other possible factors are meteorological changes and seasonal variations in the sources. Meteorological changes would result in a different upwind aerosol (air which has followed a different trajectory and contains contributions from different sources) and possibly a different transformation of compounds during their transport in the air, for instance due to photochemical processes. Such an interaction would be in line with the hypothesis that conversion of polycyclic hydrocarbons and related compounds, such as their substituted derivatives, influences the mutagenicity of aerosol extracts [30—34].

A number of studies point to the possibility that the sampling technique might result in such conversions [30—32,35—38].

The explanations so far are all based on a time-dependent interaction. A possible explanation for the interaction described by model V is synergism and antagonism as a result of testing together compounds from aerosol particles of different origin.

The results with the four variants of the Ames-test are so similar that they do not lead to different conclusions with respect to dependence of the effect of location and sampling time. This is illustrated very well by Figs. 2 and 4; the latter shows that the goodness of fit of the models is hardly dependent on the variant. Some of the correlation coefficients may even suggest a resemblance in 'mutagenic composition' of the aerosol extracts.

Consideration of the results reported in the literature shows that the proportions between the effects measured with and without S9-fraction can be quite different from those reported here; the influence of S9 ranges from clear deactivation to no influence at all to clear activation [1—4,10,11, 15,24,26]. Dehnen et al. [24] show that in two industrial towns in Germany a clear activation is found in winter months, while the S9 has no effect in summer or in a non-residential area. These and other studies clearly indicate that the S9-influence depends on sources and meteorological conditions. In the present study the influence of these factors does not result in clear differences, which suggests a relative constancy in the sources responsible for the mutagens in the aerosol and in the meteorological conditions. Meteorological conditions were made more or less similar by selecting trips with one wind direction; furthermore none of the experiments were characterized by photochemical air pollution, which is relevant insofar as such conditions result in enhanced concentrations of the reactive gases as NO_2 and O_3 which are implicated in the conversion of polycyclic aromatic hydrocarbons [30—34]. A similarity in sources would be unexpected since a diverse series of sources is responsible for the air pollution in the area.

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REFERENCES

1 R. Talcott and E. Wei, Airborne mutagens bioassayed in *Salmonella typhimurium*, J. Natl. Cancer Inst., 58 (1977) 449—451.
2 K. Teranishi, K. Hamada and H. Watanabe, Mutagenicity in *Salmonella typhimurium* mutants of the benzene-soluble organic matter derived from airborne particulate matter and its five fractions, Mutat. Res., 56 (1978) 273—280.
3 H. Tokiwa, S. Kitamori, K. Takahashi and Y. Onishi, Mutagenic and chemical assay of extracts of airborne particulates, Mutat. Res., 77 (1980) 99—108.
4 M. Møller and I. Alfheim, Mutagenicity and PAH-analysis of airborne particulate matter, Atmos. Environ., 14 (1980) 83—88.

5 C.E. Chrisp and G.L. Fisher, Mutagenicity of airborne particulates, Mutat. Res., 76 (1980) 143—164.

6 J.M. Lockard, C.J. Vau, C. Lee-Stephens, J.C. Caldwell, J.P. Wojciechowski, H.G. Enoch and P.S. Sabharwal, Induction of sister chromatid exchanges and bacterial revertants by organic extracts of airborne particles, Environ. Mutagenesis, 3 (1981) 671—681.

7 H. Fukino, S. Mimura, K. Inoue and Y. Yamane, Mutagenicity of airborne particles, Mutat. Res., 102 (1982) 237—247.

8 E. Selzer Madsen, P.A. Nielsen and J.C. Pedersen, The distribution and origin of mutagens in airborne particulates, detected by the Salmonella/microsome assay in relation to levels of lead, vanadium and PAH, Sci. Total Environ., 24 (1982) 13—25.

9 G.M. Alink, J.J. van Houdt, J.R. Kalkman and J.S.M. Boley, Mutagenic activity of airborne particulates of non-industrial locations, Mutat. Res., 116 (1983) 21—34.

10 I. Alfheim, Contribution from motor vehicle exhaust to the mutagenic activity of airborne particles, in M. Sorsa and H. Vainio (Eds.), Mutagens in our Environment, Alan R. Liss, New York, 1982.

11 R.E. Talcott and W. Harger, Chemical characterization of direct-acting airborne mutagens: the functional group, Mutat. Res., 91 (1981) 433—436.

12 J.A. Lewtas, A. Austin, L. Claxton, L.R. Burton and R. Jungers, in M. Cooke, A.J. Dennis and G.L. Fisher (Eds.), Polynuclear Aromatic Hydrocarbons, Physical and Biological Chemistry, Sixth International Symposium, Battelle Press, 1981.

13 W.R. Pierson, R.A. Gorse, A. Szkarlat, W.W. Brachaczek, S.M. Japar, F.S.-C. Lee, R.B. Zweidinger and L.D. Claxton, Mutagenicity and chemical characteristics of carbonaceous particulate matter from vehicles on the road, Environ. Sci. Technol., 17 (1983) 31—44.

14 C.Y. Wang, M.-S. Lee, C.M. King and P.O. Warner, Evidence for nitroaromatics as direct acting mutagens of airborne particulates, Chemosphere, 9 (1980) 83—87.

15 H. Tokiwa, S. Kitamori, R. Nakagawa, K. Horikawa and L. Matamola, Demonstration of a powerful mutagenic dinitropyrene in airborne particulate matter, Mutat. Res., 121 (1983) 107—116.

16 C.-J. Wei, O.G. Raabe and L.S. Rosenblatt, Microbial detection of mutagenic nitro-organic compounds in filtrates of coal fly-ash, Environ. Mutagenesis, 4 (1982) 249—258.

17 G. Löfröth, Mutagenicity assay of combustion emissions, Chemosphere, 10 (1978) 791—798.

18 B. Parri, U. Laureni, A. Babrudi, A. Callareta, S. Venturini, R. Ferri, M. Carrozzi, F. Burlini and C. Monti-Bragadin, Mutagenicity test of extracts of airborne dust from the municipal incinerator of Trieste, Environ. Mutagenesis, 5 (1983) 23—32.

19 D.A. Kaden, R.A. Hites and W.G. Thilly, Mutagenicity of soot and associated polycyclic aromatic hydrocarbons to *Salmonella typhimurium*, Cancer Res., 39 (1979) 4152—4159.

20 T. Ramdahl, G. Becher and A. Bjørseth, Nitrated polycyclic aromatic hydrocarbons in urban air particles, Environ. Sci. Technol., 6 (1982) 861—865.

21 T. Ramdahl and G. Becher, Characterization of polynuclear aromatic hydrocarbon derivatives in emissions from wood and cereal straw combustion, Anal. Chim. Acta, 144 (1982) 83—91.

22 X.B. Xu, J.P. Nachtman, Z.L. Jin, E.T. Wei, S.M. Rappaport and A.L. Burlingame, Isolation and identification of mutagenic nitro-PAH in diesel-exhaust particulates, Anal. Chim. Acta, 136 (1982) 163—174.

23 M. Møller and I. Alfheim, Mutagenicity of air samples from various combustion sources, Mutat. Res., 116 (1983) 35—46.

24 W. Dehnen, R. Tomingas and R. Pitz, Studies on the mutagenic effects of extracts derived from airborne particulates within one year in differently burdened areas using the Ames test, Zentralbl. Bakteriol., Parasitenkd., Infecktionskr. Hyg., Abt. 1: Orig., B172 (1981) 351—366.

25 W.-Z. Whong, J. Stewart, M. McCawley, P. Major, J.A. Merchant and T.-M. Ong, Mutagenicity of airborne particles from a non industrial town, Environ. Mutagenesis, 3 (1981) 617—626.
26 J.N. Pitts, W. Harger, D. Lokensgard, D.R. Fitz, G.M. Scorziell and V. Mejia, Diurnal variations in the mutagenicity of airborne particulate matter in California's south-coast airbasins, Mutat. Res., 104 (1982) 35—41.
27 I. Alfheim and M. Møller, Mutagenicity of long-range transported atmospheric aerosols, Sci. Total Environ., 13 (1979) 275—278.
28 B.N. Ames, J. McCann and E. Yamasaki, Methods for detecting carcinogens and mutagens with the Salmonella/mammalian microsome mutagenicity test, Mutat. Res., 31 (1975) 347—364.
29 D.R. Cox and D.V. Hinckley, Theoretical Statistics, Chapman and Hall, London, 1974.
30 J.N. Pitts, K.A. van Cauwenberghe, D. Grosjean, J.P. Schmid, D.R. Fitz, W.L. Belser, G. Knudsen and P.M. Hynds, Atmospheric reactions of polycyclic aromatic hydrocarbons: facile formation of mutagenic nitro derivatives, Science, 202 (1978) 515—519.
31 J.N. Pitts, D.M. Lokensgard, P.S. Ripley, K.A. van Cauwenberghe, L. Vaeck, S.D. Shaffer, A.J. Thill and W.L. Belser, "Atmospheric" epoxidation of benzo(*a*)pyrene by ozone: formation of the metabolite benzo(*a*)pyrene-4,5-oxide, Science, 210 (1980) 1347—1349.
32 J.N. Pitts, Formation and fate of gaseous and particulate mutagens and carcinogens in real and simulated atmospheres, Environ. Health Perspect., 47 (1983) 115—140.
33 T. Nielsen, T. Ramdahl and A. Bjørseth, The fate of airborne polycyclic organic matter, Environ. Health Perspect., 47 (1983) 103—114.
34 R.M. Kamens, G.D. Rives, J.M. Perry, D.A. Bell, R.F. Paylor, R.G. Goodman and L.D. Claxton, Mutagenic changes in dilute wood smoke as it ages and reacts with ozone and nitrogen dioxide: an outdoor chamberstudy. Environ. Sci. Technol., 18 (1984) 523—530.
35 E. Brorström, P. Grennfelt and A. Lindskog, The effect of nitrogen dioxide and ozone on the decomposition of particle-associated polycyclic aromatic hydrocarbons during sampling from the atmosphere, Atmos. Environ., 17 (1983) 601—605.
36 D. Grosjean, Polycyclic aromatic hydrocarbons in Los Angeles air from samples collected on Teflon, Glass and Quartz filters, Atmos. Environ., 17 (1983) 2565—2573.
37 D. Grosjean, K. Fung and H. Harrison, Interactions of polycyclic aromatic hydrocarbons with atmospheric pollutants, Environ. Sci. Technol., 17 (1983) 673—679.
38 D.R. Fitz, D.M. Lokensgard and G.J. Doyle, Investigations on filtration artifacts when sampling ambient particulate matter for mutagen assay, Atmos. Environ., 18 (1984) 205—213.

CHAPTER 7

CONCENTRATIONS OF POLYCYCLIC HYDROCARBONS IN AIRBORNE PARTICLES IN THE NETHERLANDS AND THEIR CORRELATION WITH MUTAGENICITY

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W.K. DE RAAT, S.A.L.M. KOOIJMAN and J.W.J. GIELEN
TNO, P.O. Box 217, 2600 AE Delft (The Netherlands)

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ABSTRACT

The concentrations of 15 polycyclic aromatic hydrocarbons (PAH) were determined in ambient air particles sampled at four sites in an urban and industrialized area in The Netherlands, and at one site near the coast (generally upwind). The contributions of sources in the area to the background concentrations as measured at the coastal site, were investigated. The temporal and spatial variation of the PAH profile, i.e. the relative concentrations of the 15 individual PAH, were also investigated. Variation of the PAH profile was predominantly determined by volatility. The variation of the profile of the less-volatile PAH was small compared with the variation these PAH had in common. Slight indications for the influence of differing sources and chemical conversion on the profile were found. The role of chemical conversion was suggested by a linkage of the variation of the profile with differences in reactivity between the PAH. The similarity of the profiles at the background coastal site and at the sites influenced by local sources was striking.

The PAH concentrations showed weak-to-moderate correlations with mutagenicity. The correlations increased if the results of samples taken during periods with wind from one sector were considered.

INTRODUCTION

Since polycyclic aromatic hydrocarbons (PAH) were first identified as carcinogenic constituents of soot and tar, many studies have considered the presence of these compounds in the ambient air, and the processes, such as chemical conversion, volatilization and condensation, determining their presence and fate [1–4]. Many laboratory studies have demonstrated that PAH can readily react with gaseous air constituents and that these reactions are often photo-chemically catalysed [5–14]. The finding that PAH derivatives and especially the nitrated derivatives can be very potent mutagens has led to increased interest in these conversion processes and their products [15–18].

The initial reason for interest in PAH in ambient air particles (their carcinogenicity) has thus been supplemented by another, and possibly more important one, i.e. the possibility that PAH are precursors of mutagenic derivatives

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of higher potency than the parent compounds. It has clearly been shown that PAH contribute only a part, and probably only a small part, of the mutagenicity of ambient air. The mutagenicity of extracts of ambient air particles is to a great extent independent of mammalian metabolic activation, which is necessary for the expression of the mutagenicity of unsubstituted PAH; furthermore, these so-called direct (activation independent) mutagens depend for the expression of their effect on enzymatic nitroreduction, suggesting that they are indeed nitrated compounds [19–26]. The presence of nitrated and other substituted PAH in ambient air could possibly explain the mutagenic effects of the air [15, 27–32]. As (mutagenic) PAH derivatives have also been demonstrated in important combustion emissions such as motor vehicle exhaust, the contribution of conversion of PAH during atmospheric residence remains unclear [33–42].

The investigation of PAH conversion processes in the ambient air is complicated by the fact that several studies point to the possibility that the sampling techniques for airborne particles may result in PAH conversion [43–49] thus leading to artifacts.

In addition to conversion, volatilization and condensation play an important role in determining PAH levels in ambient air. These processes may strongly influence the distribution of the PAH and their chemical reactivity. A PAH molecule adsorbed on a particle will undergo other reactions than one present in the vapour phase. Furthermore, it has been shown that PAH with less than five rings are only incompletely sampled with the high volume filtration technique that has been used almost exclusively until now [50–53].

It is to be expected that processes such as conversion, volatilization and condensation will influence the relative concentrations of the PAH species (referred to here as the "PAH profile") in ambient air particles sampled by filtration. Varying meteorological conditions could affect the distribution between the vapour and the particulate phases before or during sampling; this, together with variations in the concentrations of the reactive gaseous air constituents, could affect the extent of conversion in the air before, and possibly also during, sampling. Such variations would lead to changes in the relative concentrations of PAH in the sampled air particles. These changes would be observable if variations in source pattern and the common variation of the PAH were not too great.

In the present study the temporal and spatial dependence of the PAH profile of particles sampled by filtration were investigated, to establish the extent to which the profile is determined by processes such as conversion, condensation and volatilization. The concentrations of 15 PAH were determined 12 times at four sites in the industrialized and urban Rijnmond area of The Netherlands and at one site near the Dutch coast. The samples were part of a set of samples which has been tested for mutagenicity in an earlier investigation [54]. Therefore correlation between mutagenicity and PAH concentration was also investigated in the present study.

Sampling sites and time schedule

The particulate fraction of ambient air was sampled at four sites in the urban and industrialized Rijnmond area in the western part of The Netherlands, and at one site near the coast; the sampling sites are indicated in Fig. 1. Sampling was carried out simultaneously on 12 separate occasions at the five sites; malfunctioning of the samplers resulted in the loss of six samples, leaving a total of 54 usable samples.

The coastal site (Oostvoorne) lies landward of the coastal dunes in a residential rural area. When winds are from the southwest (the prevailing direction) the aerosol sampled at the four other sites can be expected to be influenced by emissions from urban sources (Schiedam and Kralingen) and/or (petrochemical) industrial sources and harbours (Lekhaven and Geulhaven). Sampling altitudes were 2 m for Oostvoorne, about 30 m for Schiedam, about 50 m for Lekhaven and about 25 m for Kralingen.

Wind speed and direction data were supplied by the Royal Dutch Meteorological Institute for the weather stations indicated in Fig. 1. The meteorological data are summarized in Table 1. Information supplied by the Institute of Public Health and Environmental Hygiene revealed that none of the sampling trips were characterized by enhanced oxidant ($NO_2 + O_3$) concentrations (> 100 pbb), which indicates low levels of photochemical air pollution. Sampling started between 9 and 11 a.m. and lasted for 24 h. The minimum overlap in time for the five samples was 22 h.

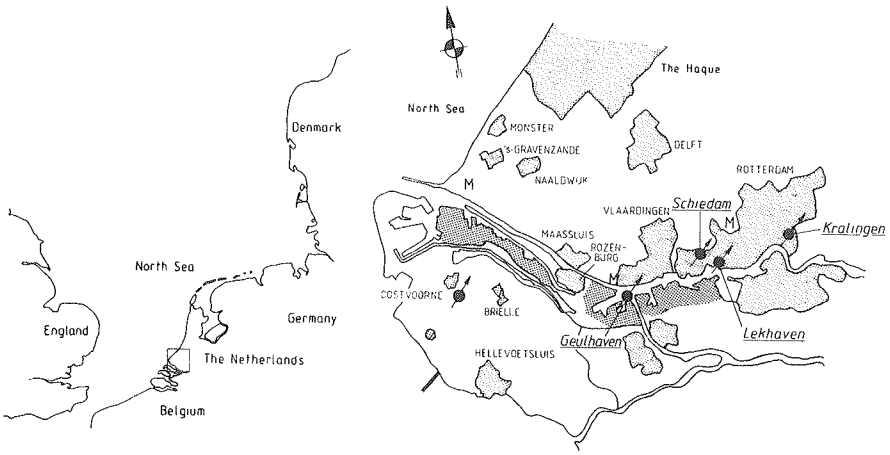

Fig. 1. The Rijnmond area; location of the sampling sites (●) and the weather stations (M).

TABLE 1

Summary of wind direction[a] and wind speed data[b]

Wind direction																	Wind speed	
N	NNE	NE	ENE	E	ESE	SE	SSE	S	SSW	SW	WSW	W	WNW	NW	NNW	Type[c]	$\bar{x}$	s
3	2	7	3	25	9	1					5	7	3	5	2	B	3.3	1.7
										1	12	36	22	1		A	5.5	2.0
									1	19	19	30	3			A	9.4	2.7
						1	22	34	15							B	6.0	2.0
						1	22	17	7	17	8					B	5.5	2.3
								1	3	22	21	7				A	5.6	1.9
								3	5	8	7	37	8	4		A	4.3	1.9
1		2	4	10	4	1	7	15	2	1	3	12	8	1	1	B	3.1	1.6
								3	29	14	13	10	3			A	6.6	2.3
								11	19	26	11	5				A	9.2	2.1
									14	12	10	31	5			A	8.9	1.8
								15	33	24						A	11.8	2.0

[a] Wind direction: the 72-h means (last 10 min of each hour) of the three stations were grouped into the 16 sectors of the compass for each of the 12 sampling trips.

[b] Wind speed: the mean and standard deviation of the 72-h means of the three stations for each sampling trip were calculated.

[c] Type = type of sampling trip: (A) wind predominantly from southwest to west; (B) wind from other directions.

Sampling technique

The particles were collected with Sartorius HV100 "Staubsammelgeräte" (high-volume samplers) on Sartorius SM13400 glass fibre filters, (ϕ = 25.7 cm). The flow rate was 100 $m^3 h^{-1}$ and the samplers were all calibrated with the same flowmeter (Brooks Instruments). The mean linear air speed through the filter was 0.535 $m s^{-1}$. The filters were extracted with methanol for 24 h in a Soxhlet apparatus before being used for sampling. The filters laden with particles were divided into two parts [~ 90% (by weight) for mutagenicity testing and ~ 10% for PAH analysis] and stored at − 80°C. The exact proportion of the parts was determined by weighing after dividing the filters.

Extraction

The extracts for mutagenicity testing were prepared within a few weeks after sampling. The filters were sequentially extracted with 175 ml methanol (Rathburn, HPLC-grade) and 175 ml cyclohexane (Nanograde) for 8 h in a Soxhlet apparatus. The extracts for PAH analysis were prepared 2.5 years after sampling (stored at − 80°C in the dark) and in this case only cyclohexane was used as a solvent because preliminary experiments had revealed that this solvent had an extraction efficiency for benzo[*a*]pyrene comparable to that of methanol. Furthermore, the use of cyclohexane excluded polar compounds which would possibly interfere with the PAH analysis. Extraction was carried out in a nitrogen atmosphere. The solvents were evaporated with a rotary evaporator at 30°C under reduced pressure. The two extracts for mutagenicity testing were combined before solvent evaporation; the residues were dissolved in dimethyl sulphoxide (Merck, A.R.; the residue of 800 m^3 air per ml). The residue of the extract for PAH analysis was dissolved in a 1:1 vol. mixture of methanol and acetone (Rathburn, glass distilled; the residue of 240 m^3 per ml).

PAH analysis

The concentrations of the 15 PAH listed in Table 2 were determined by reversed phase high performance liquid chromatography. The column was eluted with a methanol/water gradient ranging from 74.5 to 100% methanol; detection was based on fluorescence of the compounds (excitation at 250 nm; emission > 389 m). For identification and quantification the chromatograms were compared with a chromatogram of a standard solution produced under identical conditions on the same day. The detection limits are listed in Table 2. The concentrations of dibenzo[*a*, *l*]pyrene must be regarded as maximum possible concentrations, as the peaks were not very clear. Because this compound is generally hardly detectable with GC/MS (J. Nielsen, personal communication) the peak is probably largely the result of another compound.

TABLE 2

PAH determined in the extracts

PAH	Detection limit[a] ($ng\,m^{-3}$)
Anthracene	< 0.01
Fluoranthene	< 0.01
Pyrene	0.04
Benzo[*b*]fluorene	0.06
Benz[*a*]anthracene	0.02
Chrysene	0.04
Benzo[*e*]pyrene	0.08
Perylene	< 0.01
Benzo[*b*]fluoranthene	0.01
Benzo[*k*]fluoranthene	< 0.01
Benzo[*a*]pyrene	0.01
Dibenzo[*a,j*]anthracene	0.04
Dibenzo[*a,l*]pyrene	0.03
Indeno[1,2,3-*cd*]pyrene	0.02
Anthanthrene	0.01

[a] Detection limit is based on the extract derived from 240 m^3 air dissolved in 1 ml acetone/methanol (1:1 v/v).

Mutagenicity testing

The mutagenicity of the dissolved residues was investigated in the Salmonella/microsome test developed by Ames and co-workers [55]. The strains TA98 and TA100 were used. A liver homogenate (S9-fraction) from rats treated with 500 $mg\,kg^{-1}$ Aroclor 1254 was used to allow determination of mutagenic activity dependent on mammalian metabolism. Further details are given in ref. 54.

Statistical analysis

The variation of the relative concentrations of the PAH was analysed using the principal component analysis technique described in detail by Morrison [56]. Principal components are in this case weighted sums of PAH concentrations, the variance of which indicates their linear interdependence. The degree to which the variance is concentrated on one or a few principal components is a measure of the strength of the linear interdependence of the concentrations. If the variance is equally divided over all principal components, then no linear interdependence is present.

The correlation of a PAH concentration with a principal component shows the contribution of this variable to the variance of that principal component, so that the role of a particular PAH in certain patterns of interdependence can be investigated.

TABLE 3

Mean concentrations of PAH ($ng\,m^{-1}$)

		Anthracene	Fluoranthene	Pyrene	Benzo[*b*]fluorene	Benz[*a*]anthracene	Chrysene	Benzo[*e*]pyrene	Perylene	Benzo[*b*]fluoranthene	Benzo[*k*]fluoranthene	Benzo[*a*]pyrene	Dibenzo[*a,j*]anthracene	Dibenzo[*a,l*]pyrene	Indeno[1,2,3-*cd*]pyrene	Anthanthrene
All experiments																
Oostvoorne	$\bar{x}$	0.03	0.93	0.60	0.24	0.40	1.03	0.59	0.08	1.24	0.48	0.45	0.11	0.53	0.72	0.07
	v	72.30	51.38	61.15	105.56	89.24	82.76	93.84	94.18	91.22	87.78	87.80	96.29	82.02	85.02	87.93
Geulhaven	$\bar{x}$	0.19	3.26	2.12	1.42	4.73	7.54	3.07	0.61	6.39	2.24	2.55	0.38	1.51	2.39	0.22
	v	112.86	91.51	103.35	143.14	176.41	169.53	167.05	152.65	167.14	155.24	156.35	118.66	131.15	142.83	112.64
Schiedam	$\bar{x}$	0.08	2.31	1.60	0.61	1.52	2.85	1.29	0.23	2.68	1.03	1.12	0.22	1.03	1.41	0.13
	v	68.19	78.63	87.65	84.13	81.15	72.98	68.82	75.04	81.61	79.44	86.62	64.99	73.94	74.68	71.20
Lekhaven	$\bar{x}$	0.10	2.66	2.03	0.83	2.08	3.52	1.45	0.29	2.81	1.15	1.26	0.23	1.19	1.58	0.19
	v	48.18	65.65	74.86	84.17	83.28	64.17	67.67	74.29	63.80	67.22	74.62	60.87	61.09	61.37	92.02
Kralingen	$\bar{x}$	0.09	2.20	1.88	1.05	1.27	2.53	1.36	0.23	2.27	0.89	1.15	0.21	1.12	1.40	0.23
	v	118.28	45.16	76.07	161.03	71.68	53.66	82.03	63.74	59.26	58.12	79.93	58.24	56.27	53.66	117.05
A experiments																
Oostvoorne	$\bar{x}$	0.02	0.83	0.54	0.20	0.28	0.95	0.58	0.05	1.20	0.45	0.32	0.09	0.51	0.67	0.05
	v	74.43	42.58	61.40	92.64	87.87	97.90	106.34	76.70	107.50	103.76	86.42	95.18	97.26	100.71	56.54
Geulhaven	$\bar{x}$	0.14	1.84	1.18	0.51	0.97	1.94	1.02	0.19	1.95	0.77	0.87	0.17	0.73	1.01	0.12
	v	154.67	82.47	96.85	111.31	121.39	114.68	143.15	127.67	123.91	119.34	127.09	122.53	123.26	126.32	122.14
Schiedam	$\bar{x}$	0.09	2.38	1.76	0.56	1.57	2.78	1.22	0.22	2.64	1.02	1.08	0.21	1.04	1.40	0.12
	v	72.79	90.17	93.67	108.14	90.97	87.96	82.76	90.68	97.66	94.99	104.61	76.99	87.19	88.95	88.94
Lekhaven	$\bar{x}$	0.11	2.97	2.20	0.78	2.46	4.23	1.64	0.32	3.33	1.35	1.36	0.26	1.34	1.79	0.15
	v	43.54	57.17	46.31	57.69	83.12	58.39	66.50	76.96	58.61	64.60	78.52	57.32	59.04	60.79	34.85
Kralingen	$\bar{x}$	0.06	1.73	1.34	0.48	1.08	2.29	1.07	0.18	2.23	0.86	0.96	0.17	1.01	1.30	0.15
	v	45.30	44.39	52.69	83.13	54.96	58.97	61.49	55.87	71.82	70.67	77.80	65.89	53.95	59.90	71.89

$\bar{x}$ = Mean concentration of a PAH ($ng\,m^{-3}$); v = coefficient of variation (i.e. 100 × standard deviation/$\bar{x}$); see Table 1 for definition of A experiments.

RESULTS

The results of the PAH analysis are summarized in Table 3. The table reveals clear temporal and spatial variation in PAH concentrations. The lowest concentrations are found at the coastal location Oostvoorne. The concentrations found at Oostvoorne during the B experiments (for definition see Table 1), which are not shown in detail, were generally only slightly higher than those found during the A experiments; the mean concentrations of benz[*a*]anthracene, perylene, benzo[*a*]pyrene and anthanthrene were however more significantly elevated (0.64, 0.15, 0.71 and 0.11 ng m^{-3} respectively). Oostvoorne can be regarded as a background location during the A experiments in which onshore winds do not pass any local sources of PAH before sampling. The differences between the concentrations encountered at Oostvoorne and those at the other sites give a clear indication of the extent to which local sources in the Rijnmond area contribute to the concentrations of PAH in the ambient air particles.

In the B-experiments a number of samples from the Geulhaven site show much higher PAH concentrations than the other samples from that site. It is clear that a local source near Geulhaven strongly contributes under the conditions prevailing. No "abnormally" high concentrations were found at Geulhaven during the A experiments; under these conditions the highest values are found at Lekhaven.

Table 4 shows the mean relative PAH concentrations, i.e. the mean PAH profile; the concentrations have been normalized to yield a sum of 100 for each sample. In this way the influence of the samples is independent of the sum. This normalization reveals a striking feature: the mean profiles are nearly identical for all five locations. Differences in contribution of background sources and local sources between the locations do not influence the mean PAH profile to any great extent. The largest differences are found with benz[*a*]anthracene. As will be discussed below, close observation of Table 4 reveals differences which suggest conversion of PAH. Although the mean PAH profile seems to be largely *independent of location*, it is possible that the PAH profile shows a clearer *temporal dependence*; this possibility was investigated with principal components analysis. The results of this analysis are summarized in Tables 5 and 6.

Table 5 lists the variances of the first two principal components expressed as percentage of total* variance. This table shows that the first principal component contributes strongly to the total variance when the concentrations of all experiments are analysed. Consideration of the A experiments alone results in a slightly larger contribution of the first principal component. If this principal component analysis is carried out for the data of the separate locations, caution in interpreting the results is necessary because of the very small number of samples involved. Selection of the A experiments markedly increased the share of the first principal component in the variance at Kralingen and Oostvoorne; changes in wind direction thus had a clear effect on the PAH profile of these locations.

* Sum of the variances of all components.

TABLE 4

The mean PAH profiles

		Anthracene	Fluoranthene	Pyrene	Benzo[*b*]fluorene	Benz[*a*]anthracene	Chrysene	Benzo[*e*]pyrene	Perylene	Benzo[*b*]fluoranthene	Benzo[*k*]fluoranthene	Benzo[*a*]pyrene	Dibenzo[*a,j*]anthracene	Dibenzo[*a,l*]pyrene	Indeno[1,2,3-*cd*]pyrene	Anthanthrene
All experiments																
Oostvoorne	$\bar{x}$	0.5	14.9	9.1	3.0	4.9	13.3	7.3	1.1	15.5	6.1	5.8	1.3	6.9	9.3	1.0
	v	46.1	38.0	44.3	50.2	34.7	10.1	36.6	43.0	24.4	18.4	31.1	57.8	22.0	16.7	56.7
Geulhaven	$\bar{x}$	0.8	16.3	9.5	4.6	7.5	15.1	6.8	1.2	14.1	5.4	5.2	0.9	5.0	6.9	0.7
	v	83.1	54.8	45.8	57.5	59.1	28.6	32.4	51.0	21.4	14.0	47.5	74.4	30.9	28.2	63.2
Schiedam	$\bar{x}$	0.5	13.1	8.9	3.3	8.3	15.7	7.1	1.3	14.2	5.6	6.0	1.2	5.8	8.0	0.8
	v	66.1	36.1	36.8	42.6	27.8	16.0	22.9	24.7	11.9	9.2	24.0	17.7	19.1	12.7	33.8
Lekhaven	$\bar{x}$	0.6	14.0	9.6	4.1	8.5	15.5	6.8	1.3	13.1	5.3	5.8	1.1	5.6	7.6	0.9
	v	72.0	37.9	44.4	47.1	39.7	20.7	26.6	24.8	20.3	20.7	25.4	33.5	10.7	15.9	52.4
Kralingen	$\bar{x}$	0.5	13.1	10.2	4.7	7.1	14.5	7.3	1.3	13.1	5.1	6.2	1.2	6.5	8.1	1.1
	v	70.9	30.9	36.7	114.4	32.3	19.7	39.5	25.4	27.1	23.5	32.4	18.5	20.3	19.0	59.2
A experiments																
Oostvoorne	$\bar{x}$	0.4	16.1	10.0	2.8	4.2	13.3	7.4	0.8	15.5	6.0	5.0	1.2	7.2	9.3	0.8
	v	50.1	40.5	42.8	50.4	27.4	12.4	38.6	36.8	27.3	19.1	24.7	52.8	25.2	19.2	40.2
Geulhaven	$\bar{x}$	1.0	19.1	11.1	4.9	5.6	13.4	6.5	1.0	13.5	5.3	4.6	0.9	5.4	6.9	0.7
	v	85.6	50.0	32.9	63.0	54.0	15.7	34.3	65.6	20.2	15.0	60.5	80.7	25.2	24.8	62.6
Schiedam	$\bar{x}$	0.6	13.6	9.7	3.0	8.6	15.3	6.8	1.2	13.9	5.5	5.9	1.2	5.9	8.0	0.7
	v	65.8	39.9	35.6	46.9	27.4	18.0	24.2	29.1	13.1	9.8	28.3	19.3	21.5	14.3	39.5
Lekhaven	$\bar{x}$	0.5	13.8	10.3	3.7	9.3	16.6	6.2	1.3	13.0	5.2	5.6	1.0	5.4	7.2	0.8
	v	43.6	44.4	39.4	51.4	38.6	16.8	29.0	33.4	16.7	17.4	35.5	22.0	11.0	11.6	46.8
Kralingen	$\bar{x}$	0.4	12.4	9.5	3.1	7.5	15.3	7.0	1.2	14.1	5.5	6.2	1.1	6.8	8.7	1.0
	v	30.7	33.0	38.9	60.4	29.0	17.3	20.8	30.8	18.3	13.7	31.4	20.5	14.4	8.9	49.4

$\bar{x}$ = Mean share of a PAH in percent; v = coefficient of variation (i.e. 100 × standard deviation/$\bar{x}$); the concentrations of each sample were normalized such that the sum was 100.

TABLE 5

Results of the principal component analysis; variance of the principal components

	All experiments					A experiments				
	N	All PAH		Except four volatile PAH		*N*	All PAH		Except four volatile PAH	
		I	II	I	II		I	II	I	II
All locations	54	78.1	90.5	89.8	97.0	35	81.9	89.5	91.8	96.3
Oostvoorne	12	81.8	92.0	83.9	96.0	8	92.7	96.4	95.8	97.8
Geulhaven	10	81.6	96.0	92.9	98.8	6	81.6	99.1	91.4	99.9
Schiedam	11	90.3	94.0	96.4	98.0	8	92.5	96.0	97.8	99.2
Lekhaven	9	70.7	90.8	86.5	96.2	5	69.7	93.2	93.0	99.2
Kralingen	12	68.4	89.6	79.2	89.3	8	87.0	93.2	92.7	96.9

N = number of samples analysed;
I = variance of first principal component expressed as percentage of total variance;
II = variance of first and second principal component expressed as percentage of total variance.

Table 6 shows the correlations of the PAH concentrations with the first principal components. With the exception of anthanthrene, all higher molecular weight PAH correlate very well with the first principal component. The lower molecular weight PAH, anthracene, fluoranthene, pyrene and benzo[*b*]-fluorene, only show weak-to-moderate correlations with the first principal component.

Table 5 also gives the results of an analysis in which the concentrations of the four lower molecular weight PAH are excluded. These results confirm that molecular weight and wind direction are the most important factors causing variation of the PAH profile with time.

The results of the principal component analysis are apparently very strongly determined by the changes of the concentration of the total set of higher molecular weight PAH with time. This factor is so strong compared with others that, besides wind direction, no other factors can be detected, even if they would have a significant influence on the profile of these PAH.

The normalization procedure described above eliminates the common variation of the concentrations. It also introduces an interdependence among the concentrations of the PAH because an increase in the relative concentration of one necessarily implies a decrease of the concentrations of the others. The larger the number of PAH considered, the smaller the chance that the normalization procedure will lead to erroneous conclusions. Principal component analysis were carried out on normalized concentrations of six PAH selected because they strongly differ in sensitivity towards nitration [57]. However, to minimize the interdependence, the normalization procedure was carried out for *all 15 PAH*. The results of this analysis for the complete set of samples showed the following correlations with the first principal component (51.5% of the total variance): −0.65 for benzo[*e*]pyrene (not reactive), 0.61 for perylene (reactive), −0.81 for benzo[*b*]fluoranthene (not reactive), −0.69 for benzo[*k*]-

TABLE 6

Correlation of the PAH concentrations with the first principal components; all experiments

Location	All loc.	Oostvoorne	Geulhaven	Schiedam	Lekhaven	Kralingen
Variance, percentage of total	78.1	81.8	81.6	90.3	70.7	68.4
Anthracene	0.63	0.87	0.57	0.77	0.68	0.44
Fluoranthene	0.77	0.87	0.76	0.91	0.57	0.83
Pyrene	0.66	0.91	0.63	0.87	0.62	0.86
Benzo[*b*]fluorene	0.80	0.87	0.95	0.93	0.48	0.47
Benz[*a*]anthracene	0.95	0.95	0.96	0.96	0.93	0.90
Chrysene	0.95	0.96	0.96	0.99	0.98	0.95
Benzo[*e*]pyrene	0.94	0.88	0.95	0.96	0.95	0.62
Perylene	0.97	0.87	0.99	0.98	0.92	0.95
Benzo[*b*]fluoranthene	0.95	0.92	0.96	0.99	0.93	0.78
Benzo[*k*]fluoranthene	0.96	0.95	0.97	0.99	0.94	0.82
Benzo[*a*]pyrene	0.97	0.95	0.98	0.98	0.92	0.93
Dibenzo[*a,j*]anthracene	0.97	0.95	0.98	0.98	0.89	0.94
Dibenzo[*a,l*]pyrene	0.96	0.93	0.97	0.98	0.99	0.93
Indeno[1,2,3-*cd*]pyrene	0.97	0.95	0.98	0.99	0.98	0.91
Anthanthrene	0.66	0.71	0.79	0.93	0.57	0.84

fluoranthene (not reactive), 0.68 for benzo[*a*]pyrene (reactive), and 0.84 for anthanthrene (reactive). Similar results were obtained with the data of the separate sites. The three reactive PAH tend to behave as one group, and the three non-reactive PAH as another group. In some cases the two groups correlated with different principal components. This can be attributed to a smaller influence of the weighing procedure giving rise to larger differences in total concentration between the samples for the six PAH; this again leads to higher correlations due to day-to-day variation.

These results suggest that variation of the PAH profile reflects differences in reactivity among the PAH, resulting from time-dependent chemical conversion. The interdependence introduced by the normalization procedure does, however, demand cautious evaluation of the results; furthermore, the influence of differences in source patterns cannot be excluded. The weakness of the correlation of anthanthrene with the first principal components, shown in Table 6, may also be an indication of conversion of this very reactive PAH. However, the low concentration of this PAH could introduce an extra source of variation.

It must be pointed out that all the results presented originated from the principal components analysis with the correlation matrix. Analysis with the co-variance matrix gave comparable results, although in this case the share of the first principal component of the total variances was even larger. The calculation of the correlation between mutagenicity and PAH concentrations was restricted to the PAH showing a high correlation ($\geqq 0.9$) with the first principal component, i.e. the PAH whose concentrations were most strongly determined by the day-to-day variation of the total set of high-molecular weight PAH. In this case the results of the analysis with the co-variance matrix were used.

Some of the results of the calculation of the correlation between mutagenicity and PAH concentrations are shown in Table 7. When all experiments are considered the correlations are weak. Restriction to the A experiment increases the correlation markedly; in the case of TA98 a value of $r = 0.78$ is found. When the results are considered per location, even higher correlations

TABLE 7

Correlation between PAH concentration and mutagenicity

	N Samples	*N* PAH	TA98+		TA98−		TA100+		TA100−	
			$\bar{X}r$	Sr	$\bar{X}r$	Sr	$\bar{X}r$	Sr	$\bar{X}r$	Sr
All experiments	54(TA98+:50)	10	0.43	0.06	0.41	0.07	0.37	0.04	0.31	0.06
A experiments	35(TA98+:32)	6	0.78	0.04	0.56	0.09	0.59	0.03	0.51	0.07

N samples = number of samples; *N* PAH = number of PAH (correlation with the first principal component: $\geqq 0.9$); $\bar{X}r$ = mean correlation coefficient; Sr = standard deviation of $\bar{X}r$.
All correlation coefficients were significantly different from zero ($p < 0.05$).
TA100+,−; TA98+,− = strain with (+) or without (−) S9 fraction.

are sometimes found, although these are not very significant because of the low number of samples considered.

DISCUSSION

The results of this study give a clear impression of the variation in the PAH composition of airborne particles in an industrialized and urban area. They show the contribution of local sources to the PAH load of the particles and the variation of the PAH profile of the particles. The results listed in Table 3 reveal the significant increase of the PAH load between the background site Oostvoorne and the other sites. The contribution shows a clear spatial, temporal and PAH dependence.

The variation of the PAH profile was investigated in two different ways: the spatial variation using the mean profiles (Table 4; i.e. proportion of means after normalization) and the temporal variation using principal component analysis (with real and normalized concentrations). Both methods show the PAH profile to be fairly constant. The mean profiles are quite similar and principal component analysis shows the first principal component to contribute a large part to the total variance. This linkage of the PAH is not surprising, since all compounds predominantly originate from one group of anthropogenic sources, i.e. the combustion of fossil and organic fuels. The strong time-dependent linkage shows that the common variation of the PAH is much greater than the variation of the PAH profile.

Volatility

The dominance of the common variation of PAH is restricted to 11 of the 15 PAH considered. The others (see Table 6) often show a much lower correlation with the first principal component. The most probable explanation for the "deviating" behaviour of these four PAH is the fact that they are present to a significant extent as vapour in the ambient air. This type of occurrence yields an extra source of variation because it can be expected that the vapour–solid ratio will depend on temperature and on the age of the particles. It is possible that a stable vapour–solid ratio be reached in an air mass free of recent emissions, while this ratio will fluctuate in air sampled immediately downwind of the source. Furthermore, it has been shown that sampling itself influences the vapour–solid ratio due to the formation of a low pressure under the filter which causes an artifactual evaporation [49, 58]. The vapour–solid ratio has been determined in a number of studies, and these studies all show that the four "deviating" PAH are to a large extent present in the vapour phase [50–53].

In a recent study by one of the present authors, the HPLC analysis technique used here was used to compare the PAH concentrations in particles sampled on a filter and in the organics adsorbed on polyurethane foam downstream of the filter. About 90% of pyrene was shown to be present in the vapour phase [53].

Chrysene was the most volatile PAH which was collected (almost) totally in the particulate phase. The extraction procedure used possibly implies an extra loss of the more volatile PAH. In any case this study confirms the conclusion of other studies that high volume sampling with a glass fibre filter is only useful for the analysis of PAH with more than four rings, and the less volatile four-ring PAH, such as chrysene and benz[*a*]anthracene. In some principal component analyses even the concentrations of the four volatile compounds show a good correlation with the first principal component. This was the case at Schiedam and Oostvoorne, especially when the wind direction was south-west. This might indicate that the vapour–solid ratio was not greatly influenced by local sources in these cases. When the concentrations of the four volatile PAH were omitted from the principal components analysis, the share of the first principal component in the total variance did, however, increase dramatically at Lekhaven, Kralingen and Geulhaven, suggesting that the presence of local sources did have a strong influence on the vapour–solid ratio at these sites. The variation implied by the volatility of the four PAH hinders the identification of other factors possibly influencing their profile. For this, the sampling of the particles by filtration would have to be supplemented by sorption-sampling of the volatile components passing the filter (for instance with polyurethane foam).

Source pattern and conversion

Considering only the 11 PAH which are totally collected on the filter, Table 5 shows that the variation of the profile at the Oostvoorne and Kralingen sites is markedly lower when the A experiments are selected. To a lesser extent this is also the case at Lekhaven. Selection of the A experiments did not reduce the variation at Geulhaven or Schiedam. Oostvoorne and Kralingen are on the borders of the area under investigation. At Oostvoorne a change in wind direction results in a change in sample from background particles from over the sea to particles originating from the urban and industrial areas nearby. This shift clearly influences the PAH profile. The B experiments can be regarded as the background experiments for Kralingen because the local sources lie to the west of this site.

Two factors could explain the differences: contribution of emissions with a different PAH profile, or chemical conversion of PAH. The latter factor would then be related to the age of the particles, to the differences in meteorological conditions linked with different wind directions, or to differences in concentrations of reactive gaseous air constituents. It is difficult to distinguish the influences of these two factors.

The possible role of differences in reactivity in the variation of the PAH profile can be investigated in laboratory experiments. The results of Nielsen [57] indicate that conversions should result in a decrease in the concentration of anthanthrene, benzo[*a*]pyrene and perylene (compounds highly susceptible to nitration) compared with those of benzo[*e*]pyrene, benzo[*b*]fluoranthene and

benzo[*k*]fluoranthene. The principal component analysis with the normalized concentrations indeed suggests that conversion takes place at all sites. Furthermore, if the mean PAH profile of those six PAH is studied, it appears that the ratio of reactive to unreactive compounds increases in the A experiments from Oostvoorne eastwards, suggesting that the longer residence time in the air of the particles sampled at Oostvoorne results in an increased conversion. The mean profiles for these six PAH with the largest differences in reactivity are given in Table 8.

Comparison of the results from the two sets of experiments also suggests a larger share of the more reactive PAH at Geulhaven and Oostvoorne when locally emitted particles predominate. Although it is possible that these shifts in the PAH profile result from differences in source pattern, the authors regard the linkage between these shifts and differences in reactivity to be strong enough to suggest that conversion takes place.

Conversion of PAH does not only take place in the ambient air; a number of studies have clearly indicated that high volume filtration of airborne particles leads to an artifactual conversion. On the basis of the results of the present study it is not possible to distinguish between conversion in the air and conversion during sampling. However, it is unlikely that differences between the PAH profiles at the different sites are a result of artifactual conversion as the samples of each experiment were taken simultaneously and thus under nearly identical meteorological conditions. If the inter-site variation was a result of sampling artefacts, then higher concentrations of reactive gaseous air constituents such as ozone and nitrogen oxides would have to be present in the air at Oostvoorne, which is unlikely. It is tempting to conclude that the slight difference between Oostvoorne and the other sites is the result of the longer residence time in the air of the particles collected at this first site.

During this study the meteorological conditions did not give rise to strong photochemical air pollution; this is demonstrated by the low oxidant levels measured. Levels of ozone, in particular, can thus be much higher than was the

TABLE 8

Mean profiles of the three least reactive and the three most reactive PAH in the A experiments

		Benzo[*e*]-pyrene	Perylene	Benzo[*b*]-fluoranthene	Benzo[*k*]-fluoranthene	Benzo[*a*]-pyrene	Anthanthrene
Oostvoorne	I	20.2	2.4	43.1	16.8	15.0	2.5
	II	22.9	1.9	45.3	17.0	12.1	1.9
Schiedam	I	19.8	3.7	40.9	16.2	17.1	2.2
	II	19.4	3.5	41.9	16.2	17.1	1.9
Kralingen	I	19.9	3.6	40.1	15.7	17.7	3.0
	II	19.6	5.3	40.9	15.8	17.6	2.8

I = Mean proportion (as calculated in Table 4, i.e. the concentrations of each sample were normalized such that the sum was 100).

II = Proportions of the mean (proportion of means of the real concentration times 100).

case during this study. The incorporation of samples taken during periods with enhanced oxidant levels should form an interesting extension of the current work.

Influence of methods

The influence of the methods (especially extraction and analysis) used on the results of the principal component analysis forms an important point of discussion. The PAH concentrations in any one sample were all analysed with one single chromatogram. Day-to-day variation in sensitivity of detection could lead to enhancement of the variance of the first principal component. The authors consider this artefact to be unimportant, since every analysis was calibrated with a chromatogram of a standard mixture of PAH.

Extraction and solvent evaporation may lead to conversion of the PAH during sample preparation and thus invalidate conclusions concerning conversion during residence in the air. One of the present authors (W.K. de Raat) has carried out experiments in which filters impregnated with benzo[*a*]pyrene were extracted, and the benzo[*a*]pyrene concentration of the extracts determined. No indications for conversion were found.

Relation between PAH concentrations and mutagenicity

The strong mutagenicity of many substituted (especially nitro) PAH present in the ambient aerosol indicates that these compounds may well give rise to the major part of the mutagenicity. A possible relation between PAH concentration and mutagenicity must therefore be based on the formation of substituted PAH from PAH, especially as PAH themselves contribute only a minor part.

This substitution might occur prior to emission as many studies have demonstrated the presence of substituted PAH in emissions. Additional substitution might occur during residence of the PAH in the air or on the filter during sampling. Correlation between PAH concentrations and mutagenicity will be weaker if the proportions between PAH and mutagenic derivatives vary because of varying emissions or because of time- or site-dependent conversion in the air or during sampling. It can be expected that samples taken in an area with one dominating source, and during periods with comparable meteorological conditions, will show high correlations. The present study shows weak to moderate correlations indicating the influence of varying sources and/or conversion in the air. The correlations increase when only the A experiments are considered, indicating a reduced variation in source pattern and meteorological conditions. It must be pointed out that the mutagenicity of the PAH derivatives is often so high that conversions which hardly change the PAH concentration could lead to marked increases of the mutagenicity. Another factor disturbing the hypothesized correlation between PAH and mutagenicity could be further conversion of the PAH derivatives themselves. Furthermore,

it can be expected that the Ames test shows a stronger inherent variation than the PAH analysis.

CONCLUSIONS

The present study demonstrates the contribution of an urban and industrialized area to the concentration of a number of PAH in the particulate fraction of ambient air. The variation of the PAH profile with time and space was predominantly a consequence of volatility of the PAH. The variation of the profile of the less volatile PAH was very small compared with their common variation. Slight indications were found for the influence of source pattern and conversion of PAH. The PAH concentrations correlated weakly to moderately with mutagenicity and this correlation was lowered by variation of sources and/or conversion.

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REFERENCES

1 D.H. Phillips, Fifty years of benzo(a)pyrene, Nature, 303 (1983) 468–472.
2 A. Dipple, Polynuclear aromatic carcinogens, in C.E. Searle (Ed.), Chemical Carcinogens, ACS Monograph, 173, 1976.
3 D. Hoffmann and E.L. Wynder, Organic particulate pollutants — chemical analysis and bioassays for carcinogenicity, in A.C. Stern (Ed.), Air Pollution, Vol. II, 3rd edn, Academic Press, New York, 1977.
4 National Research Council, Polycyclic Aromatic Hydrocarbons, Evaluation of Sources and Effects, National Academy Press, Washington DC (1983).
5 J.F. Thomas, M. Mukai and B.D. Tebbens, Fate of airborne benzo(a)pyrene, Environ. Sci. Technol., 2 (1968) 33–39.
6 B.D. Tebbens, M. Mukai and J.F. Thomas, Fate of arenes incorporated with airborne soot; effect of irridation, Am. Ind. Hyg. Assoc. J., June (1971) 365–372.
7 H.L. Falk, I. Markul and P. Kotin, Aromatic hydrocarbons, IV. Their fate following emission into the atmosphere and experimental exposure to washed air and synthetic smog, AMA Arch. Ind. Hyg. Occup. Med., 13 (1956) 13–17.
8 B.D. Tebbens, J.F. Thomas and M. Mukai, Fate of arenes incorporated with airborne soot, Am. Ind. Hyg. Assoc. J., 27 (1966) 415–422.
9 J. Jäger and V. Hanus, Reaction of solid carrier-adsorbed polycyclic aromatic hydrocarbons with gaseous low-concentrated nitrogen dioxide, J. Hyg., Epidemiol., Microbiol. Immunol., 24 (1980) 1–2.
10 T. Nielsen, T. Ramdahl and A. Bjørseth, The fate of polycyclic organic matter, Environ. Health Perspect., 47 (1983) 103–114.
11 L.D. Butler and P. Crossly, Reactivity of polycyclic aromatic hydrocarbons adsorbed on soot particles, Atmos. Environ., 15 (1981) 91–94.

12 F. Valerio, P. Bottino, D. Ugolini, M.R. Cimberle, G.A. Tozzi and A. Frigerio, Chemical and photochemical degradation of polycyclic aromatic hydrocarbons in the atmosphere, Sci. Total Environ., 40 (1984) 169–188.
13 A.H. Miguel, "Atmospheric" reactivity of particulate polycyclic aromatic hydrocarbons collected in an urban tunnel, Sci. Total Environ., 36 (1984) 305–311.
14 K. Nikolao, P.M. Masclet and G. Mouvier, Sources and chemical reactivity of polynuclear aromatic hydrocarbons in the atmosphere — a critical review, Sci. Total Environ., 32 (1984) 102–132.
15 H.S. Rosenkranz and R. Mermelstein, Mutagenicity and genotoxicity of nitroarenes. All nitro-containing chemicals were not created equal, Mutat. Res., 114 (1983) 217–267.
16 J.N. Pitts, Jr., Formation and fate of gaseous and particulate mutagens and carcinogens in real and simulated atmospheres, Environ. Health Perspect., 47 (1983) 115–140.
17 J.N. Pitts, Jr., K. van Cauwenberghe, D. Grosjean, J.P. Schmid, D.R. Fitz, W.L. Belser, G.B. Knudson and P.M. Hynds, Atmospheric reactions of polycyclic aromatic hydrocarbons: facile formation of mutagenic nitroderivatives, Science, 202 (1978) 515–518.
18 J.N. Pitts, Jr., D.M. Lokensgard, P.S. Ripley, K.A. van Cauwenberghe, L. van Vaeck, S.D. Shaffer, A.J. Thilland and W.L. Belser, "Atmospheric" epoxidation of benzo(a)pyrene by ozone: formation of the metabolite benzo(a)pyrene 4,5 oxide, Science, 210 (1980) 1347–1349.
19 H. Tokiwa, S. Kitamori, K. Takahishi and Y. Onishi, Mutagenic and chemical assay of extracts of airborne particulates, Mutat. Res., 77 (1980) 99–108.
20 K. Teranishi, K. Hamada and H. Watanabe, Mutagenicity in *Salmonella typhimurium* mutants of the benzene-soluble organic matter derived from airborne particulate matter and its five fractions, Mutat. Res., 56 (1978) 273–280.
21 R. Talcott and E. Wei, Airborne mutagens bioassayed in *Salmonella typhimurium*, J. Natl. Cancer Inst., 58 (1977) 449–451.
22 C.E. Chrisp and G.L. Fisher, Mutagenicity of airborne particles, Mutat. Res., 76 (1980) 143–164.
23 W. Dehnen, N. Pitz and R. Tomingas, The mutagenicity of airborne particulate pollutants, Cancer Lett., 4 (1977) 5–12.
24 M. Møller and I. Alfheim, Mutagenicity and PAH-analysis of airborne particulate matter, Atmos. Environ., 14 (1980) 83–88.
25 C.Y. Wang, M.S. Lee, C.M. King and P.O. Warner, Evidence for nitroaromatics as direct-acting mutagens of airborne particulates, Chemosphere, 9 (1980) 83–87.
26 R.E. Talcott and W. Harger, Chemical characterization of direct-acting airborne mutagens: the functional group, Mutat. Res. 93 (1981) 433–436.
27 T. Ramdahl, G. Becher and A. Bjørseth, Nitrated polycyclic aromatic hydrocarbons in urban air particles, Environ. Sci. Technol., 16 (1982) 861–865.
28 T.L. Gibson, Nitro derivatives of polynuclear aromatic hydrocarbons in airborne and source particulate matter, Atmos. Environ. 16 (1982) 2037–2040.
29 H. Tokiwa, R. Kitamori, R. Nakawaga, K. Horikawa and L. Matamale, Demonstration of a powerful mutagenic dinitropyrene in airborne particulate matter, Mutat. Res., 121 (1983) 107–116.
30 J. Jäger, Detection and characterization of nitro-derivatives of some polycyclic aromatic hydrocarbons by fluorescence quenching after thin layer chromatography: applications to air pollution analysis, J. Chromatogr., 152 (1978) 575-578.
31 J. König, E. Balfanz, W. Funcke and Th. Romanowski, Determination of oxygenated aromatic hydrocarbons in airborne particulate matter by capillary gas chromatography and gas chromatography/mass spectrometry, Anal. Chem., 55 (1983) 599–603.
32 R.L. Tanner and R.F. Fajer, Determination of nitropolynuclear aromatics in ambient aerosol samples, Int. J. Environ. Anal. Chem., 14 (1983) 231–241.
33 X.B. Xu, J.P. Nachtman, Z.L. Jin, E.T. Wei and S.M. Rappaport, Isolation and identification of mutagenic nitro-PAH in Diesel-exhaust particulates, Anal. Chim. Acta, 136 (1982) 163–174.
34 I. Salmeen, R.A. Gorse, T. Riley and D. Schuetzle, Identification and quantification of direct-acting Ames assay mutagens in diesel particulate extracts, in M. Cooke and A.J. Dennis (Eds),

Polynuclear Aromatic Hydrocarbons; Formation, Metabolism and Measurement, Battelle Press, Columbus, Ohio, 1983.

35 T.J. Mast, D.P.H. Hsieh and J.N. Seiber, Mutagenicity and chemical characterization of organic constituents in rice straw smoke particulate matter, Environ. Sci. Technol., 18 (1984) 338–348.

36 M. Møller and I. Alfheim, Mutagenicity of air samples from various combustion sources, Mutat. Res., 116 (1983) 35–46.

37 J. Alfheim, G. Becher, J.K. Hongslo and T. Ramdahl, Mutagenicity testing of high performance liquid chromatography fractions from wood stove emission samples using a modified Salmonella assay requiring smaller sample volumes, Environ. Mutagen., 6 (1984) 91–102.

38 C.J. Wei, O.G. Raabe and L.S. Rosenblatt, Microbial detection of mutagenic nitro-organic compounds in filtrates of coal fly ash, Environ. Mutagen., 4 (1982) 249–258.

39 T. Ramdahl, I. Alfheim, S. Rustad and T. Olsen, Chemical and biological characterization of emissions from small residential stoves burning wood and charcoal, Chemosphere, 11 (1982) 601–611.

40 G. Löfroth, Mutagenicity assay of combustion emissions, Chemosphere, 10 (1978) 791–798.

41 W.R. Harris, E.K. Chess, D. Okamoto, J.F. Remsen and D.W. Later, Contribution of nitropyrene to the mutagenic activity of coal fly ash, Environ. Mutagen., 6 (1984) 131–144.

42 I. Alfheim and T. Ramdahl, Contribution of wood combustion to indoor air pollution as measured by mutagenicity in Salmonella and polycyclic aromatic hydrocarbon concentration, Environ. Mutagen., 6 (1984) 121–130.

43 F.S.C. Lee, W.R. Pierson and J. Ezike, The problem of PAH degradation during filter collection of airborne particulates – an evaluation of several commonly used filter media, in A. Bjørseth and A. Dennis (Eds), Polynuclear Aromatic Hydrocarbons: Chemistry and Biological Effects, Battelle Press, Columbus, Ohio, 1980.

44 D. Grosjean, K. Fung and J. Harrison, Interactions of polycyclic aromatic hydrocarbons with atmospheric pollutants, Environ. Sci. Technol., 17 (1983) 673–679.

45 D. Grosjean, Polycyclic aromatic hydrocarbons in Los Angeles air from samples collected on Teflon, glass and quartz filters, Atmos. Environ., 17 (1983) 2565–2573.

46 E. Brorström, P. Grennfelt and A. Lindskog, The effect of nitrogen dioxide and ozone on the decomposition of particle-associated polycyclic aromatic hydrocarbons during sampling from the atmosphere, Atmos. Environ., 17 (1983) 601–605.

47 J. Peters and B. Seifert, Losses of benzo(a)pyrene under conditions of high volume sampling, Atmos. Environ., 14 (1976) 117–119.

48 D.R. Fitz, D.M. Lokensgard and G.J. Doyle, Investigation of filtration artifacts when sampling ambient particulate matter for mutagen assay, Atmos. Environ., 18 (1984) 205–213.

49 K. van Cauwenberghe, L. van Vaeck and J.N. Pitts, Jr., Physical and chemical transformations of organic pollutants during aerosol sampling, in B. Versino and H. Ott (Eds), Proceedings of the First European Symposium Physico-Chemical Behaviour of Atmospheric Pollutants, Commission of the European Communities, 1980.

50 K.E. Thrane and A. Mikalsen, High-volume sampling of airborne polycyclic aromatic hydrocarbons using glass fibre filters and polyurethane foam, Atmos. Environ., 15 (1981) 909–918.

51 H. Yamasaki, K. Kuwata and H. Miyato, Effect of ambient temperature on aspects of airborne polycyclic aromatic hydrocarbons, Environ. Sci. Technol., 16 (1983) 189–194.

52 C.D. Keller and T.F. Biddleman, Collection of airborne polycyclic aromatic hydrocarbons and other organics with a glass fibre filter-polyurethane foam system, Atmos. Environ. 18 (1984) 837–845.

53 W.K. de Raat, F.L. Schulting, E. Burghardt and F.A. de Meijere, Application of polyurethane foam for sampling volatile mutagens from ambient air, Sci. Total Environ., 63 (1987) 175–190.

54 W.K. de Raat, F.A. de Meijere and S.A.L.M. Kooijman, Mutagenicity of ambient aerosol collected in an urban area of the Netherlands, Sci. Total Environ., 44 (1985) 17–33.

55 B.N. Ames, J. McCann and E. Yamasaki, Methods for detecting carcinogens and mutagens with the Salmonella mammalian microsome mutagenicity test, Mutat. Res., 31 (1975) 347–364.

56 D.F. Morrison, Multivariate Statistical Methods, McGraw Hill Series in Probability and Statistics, McGraw Hill Book Company, New York, 1972.
57 T. Nielsen, Reactivity of polycyclic aromatic hydrocarbons towards nitrating species, Environ. Sci. Technol., 18 (1984) 157–163.
58 L. van Vaeck, K. van Cauwenberghe and J. Janssens, The gas-particle ditribution of organic aerosol constituents: measurements of the volatilization artefact in hi-vol cascade impactor sampling, Atmos. Environ., 18 (1984) 417–430.

CHAPTER 8

THE MUTAGENICITY OF AMBIENT AIRBORNE PARTICLES FROM LOCAL TRAFFIC AND DISTANT SOURCES DURING EPISODES WITH MODERATE PHOTOCHEMICAL AIR POLLUTION

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THE MUTAGENICITY OF AMBIENT AIR PARTICLES FROM LOCAL TRAFFIC AND DISTANT SOURCES DURING EPISODES WITH MODERATE PHOTOCHEMICAL AIR POLLUTION

W. K. DE RAAT and F.A. DE MEIJERE

TNO, P.O. Box 217, 2600 AE Delft (The Netherlands)

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ABSTRACT

The mutagenicity of ambient air particles in the Ames test was investigated during episodes with moderate photochemical air pollution. The particles were sampled on 11 occasions, each consisting of at least four sampling periods of 3 h during the day and one period of not more than 12 h during the following night. During the last 4 days, sampling was carried out at three locations, which allowed observation of the contribution from local sources (traffic in a town and on a motorway) to the mutagenicity.

The local sources caused an increase in the indirect (S9-dependent) mutagenicity; the direct mutagenicity was not significantly affected. The particles sampled upwind dominated the mutagenicity in the area; in contrast to the locally emitted particles, their effects decreased in the presence of S9 fraction. This difference may indicate a qualitative change in the mutagenicity during the residence of the particles in the air.

The direct mutagenicity depended very much on bacterial nitro reduction, as was demonstrated by using strain TA98NR; the effect in this strain was substantially increased in the presence of S9 fraction. This increase shows that, in the presence of S9 fraction, the effect in the TA98 strain is to a great extent the result of indirect mutagens and not only the result of partially deactivated direct mutagens.

No typical quantitative or qualitative diurnal variation could be demonstrated. This is most probably due to the overwhelming influence of long-range transported mutagens which had already undergone maximum conversion during their residence in the air.

Multivariate analysis of the effects and concentrations of gaseous pollutants indicated a rather strong link between mutagenicity and SO_2, which confirms the important contribution of long-range transported mutagenicity.

INTRODUCTION

Many studies (Dehnen et al., 1977; Pitts et al., 1977; Talcott and Wei, 1977; Tokiwa et al., 1977; Commoner et al., 1978; Teranishi et al., 1978; Møller and Alfheim, 1980) have demonstrated that ambient air particles contain mutagenic compounds, which largely originate from the emission of combustion processes. Extracts of particles present in flue gases from oil, wood, coal and municipal waste combustion, and in the exhaust gases of diesel and gasoline engines, are all mutagenic (Löfroth, 1978; Lewtas, 1982; Ramdahl et al., 1982a;

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Claxton, 1983; Gorse et al., 1983; Li et al., 1983; Møller and Alfheim, 1983; Pani et al., 1983). Both ambient air particles and particles from combustion emissions contain polycyclic aromatic hydrocarbons (PAH) and oxygenated and nitrated PAH derivatives, compounds which are considered to be responsible for the major part of the mutagenicity (Bjørseth et al., 1979; Gibson, 1982; Ramdahl et al., 1982b; Schuetzle et al., 1982; Xu et al., 1982; Hanson et al., 1983; Harris et al., 1984; Nielsen et al., 1984; Mast et al., 1984). The nitrated PAH, particularly, appear to be very mutagenic, at least when they are examined using bacterial test systems (Rosenkranz and Mermelstein, 1983). To date, no important sources of mutagens in ambient air particles, other than combustion processes, have been identified.

The fate of PAH after emission has been studied intensively. Several studies have clearly demonstrated that, in the laboratory, PAH can undergo chemical conversion upon exposure to reactive air constituents such as ozone and nitrogen oxides (Falk et al., 1956; Pitts et al., 1978, 1980; Fox and Olive, 1979; Jäger and Hanus, 1980; Butler and Crossley, 1981; Nielsen et al., 1983; Pitts, 1983; Miquel, 1984; Nielsen, 1984; van Vaeck and van Cauwenberghe, 1984). A recent study suggests that conversion also takes place in ambient air (de Raat et al., 1987). Furthermore, it is likely that conversion products of PAH are mutagenic, and that emitted PAH derivatives are sensitive to comparable conversions. Chemical conversion can thus affect the mutagenicity of the particles.

During episodes with photochemical air pollution (PA), conversion should be stimulated: PA is associated with enhanced concentrations of reactive compounds and radicals, and with high temperatures and low dispersion of pollutants. If the residence of mutagens and PAH in the ambient air results in their conversion, this should be especially manifest during PA. We therefore investigated the mutagenicity of ambient air particles originating from local sources (traffic) and distant sources during episodes with PA. The particles were sampled on 11 days, each day divided into at least four sampling periods of 3 h during the daytime and one period of not more than 12 h during the following night, which enabled the observation of the diurnal variation of the mutagenicity. In the last four experiments, sampling was carried out at three locations, which enabled the examination of the contribution of local sources to long-range transported mutagenicity.

METHODS AND MATERIALS

Sampling technique

The particles were sampled by filtration using a Sartorius HV 100 high volume sampler and collected on Sartorius SM13400 glass fibre filters. The flowrate was $100\,m^3\,h^{-1}$; the average linear air speed through the filter was $0.54\,m\,s^{-1}$. Before sampling the filters were washed by Soxhlet extraction with

methanol for 24 h. After sampling the filters with particles were wrapped in aluminium foil and stored at −80°C.

Sampling, time schedule and locations

On 11 days, at least four samples of 3 h each were taken between 8.00 and 20.00 h and one sample of not more than 12 h during the following night. The sampling dates (further also called experiments) and the time schedule of sampling are given in Fig. 1. Sampling days were selected when, on the basis of the weather forecast by the Royal Dutch Meteorological Institute, PA could be expected in the area under investigation. In the Netherlands, PA generally occurs with easterly winds. When this was the case, and when low wind speeds, high temperatures and a clear sky were also forecast, sampling was started at 5.00 or 8.00 h.

In all experiments, sampling was carried out at a location (referred to as TNO–ZP) on the TNO site in Delft, which is immediately downwind of the major highway between The Hague and Rotterdam when the wind is easterly. During the last four experiments, sampling was also carried out at a location (referred to as Delft) on the roof of the Fire Department building in downtown Delft, located downwind of TNO–ZP, and at a rural location (referred to as Delfgauw) upwind of TNO-ZP. Sampling at TNO-ZP and Delfgauw was carried out at ground level, and at Delft at an altitude of ~20 m. The sampling locations are indicated in Fig. 2.

It was assumed that the samples collected at Delfgauw were not influenced by major sources within a distance of at least 20 km; those collected at TNO–ZP differed from the Delfgauw samples only in the contribution from traffic on the highway. The Delft samples were locally influenced by downtown sources (at

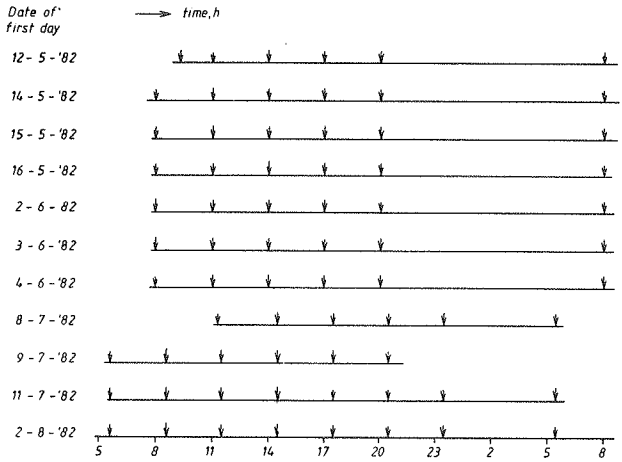


Fig. 1. Dates and time schedule of sampling. (↓) Replacement of filter.

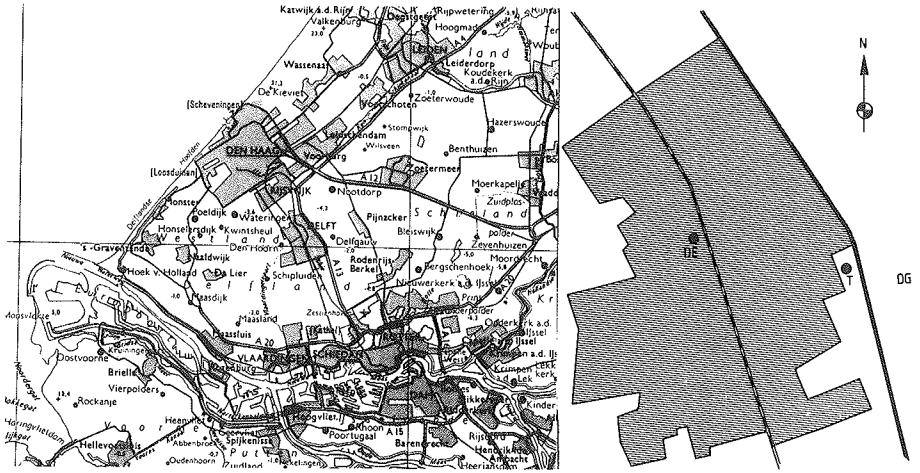


Fig. 2. Map of Delft; indicated are the locations TNO-ZP (T), Delfgauw (DG) and Delft (DE).

this time of the year mainly traffic). The study thus enabled the characterization of the contribution of local sources to the background mutagenicity of the ambient air particles.

Concentrations of gaseous pollutants

The concentrations of NO, NO_2 and peroxyacetylnitrate (PAN) were determined at TNO–ZP. Ozone and SO_2 concentrations were not available for TNO–ZP, so data provided by the National Institute for Public Health and Environmental Hygiene for Voorschoten, located just west of the highway between The Hague and Leiden, were used. Although these concentrations are not directly linked to atmospheric processes in the area under investigation, the widespread nature of PA when occurring in the Netherlands, the importance of long-range transport for the SO_2 concentrations and the similarity between the sampling area and the area where the concentrations were measured, warrants their use in this study. The concentrations were received in the form of hourly means. With these values splines were calculated for each experiment and the parts of the splines covering the sampling periods were integrated to obtain a mean value for each sampling period.

Extraction of the particles

The filters with particles were extracted in a Soxhlet apparatus with methanol (Rathburn HPLC-grade) for 8 h ($\sim$ 20 cycles). The air in the apparatus was replaced by nitrogen and the extraction was carried out in the dark. Several experiments with longer extraction times and/or additional solvents

showed that this procedure is highly effective with respect to extraction of mutagenicity and concentration of polycyclic aromatic hydrocarbons in the extract. The solvent was removed by evaporation (rotory evaporator, reduced pressure, 30°C, in the dark) until 5 ml remained. Dimethylsulphoxide (Merck, AR) was then added and the remaining methanol evaporated. One millilitre of the final solution represented 200 m^3 of sampled air. The dissolved residues were immediately stored at −80°C and tested for mutagenicity within a few weeks.

The Ames test

The Salmonella/microsome test (Ames test) was carried out according to the procedure laid down by Ames et al. in 1975. Two strains of *Salmonella typhimurium* were used: TA98 and TA98NR. The latter is a mutant strain isolated from the former and showing a reduced sensitivity to the mutagenicity of many nitro-compounds (Rosenkranz and Mermelstein, 1983) and was obtained from Dr. H.S. Rosenkranz, Cleveland, Ohio. For detection of metabolic (de)activation, a liver S9-fraction from rats treated with Arochlor 1254 was used; this S9-fraction was obtained from CIVO Toxicology and Nutrition, TNO, Zeist, the Netherlands and prepared according to Ames et al. (1975). Each extract thus yielded four effects, obtained using two strains with or without S9-fraction.

In each of the first seven experiments, all extracts were examined in one test procedure, i.e. on one day, with one culture, one S9-fraction and one batch of plates, to optimize comparability of the responses from the different extracts. The extracts of the last four experiments were examined in two test procedures; for these the comparability of the results from the different locations and with the different strains were given priority. The two tests were carried out within 2–4 days.

All test samples (extracts and positive control compounds) were dissolved in 100 μl of dimethylsulphoxide. The extracts were tested in duplicate or in triplicate. Two or three concentrations were tested (excluding zero); the highest was the amount of extract derived from 10 m^3 of air. Straight lines were calculated from the dose–response relationships according to the maximum likelihood method, assuming Poisson sampling of the responses of the test. The mutagenicities of the extracts were summarized as the slopes of the lines. In most of the tests a certain systematic deviation from linearity was observed. Some examples are depicted in Fig. 3. It appears that, in the presence of S9-fraction, the effect increases more than expected, and in the absence, less than expected. This phenomenon was not restricted to the higher doses. The calculated effects can thus be regarded as an averaged effect for the doses tested. The greater the deviation, the more the effect will have been underestimated or overestimated for respectively the absence or presence of S9-fraction, at least if we assume the "real" mutagenicity to be linearly dependent on the dose and the deviations to be due to phenomena such as toxicity and shifts in metabolism by the S9-enzymes. Microscopic observation of the background histidine auxotrophic colonies, however, did not indicate

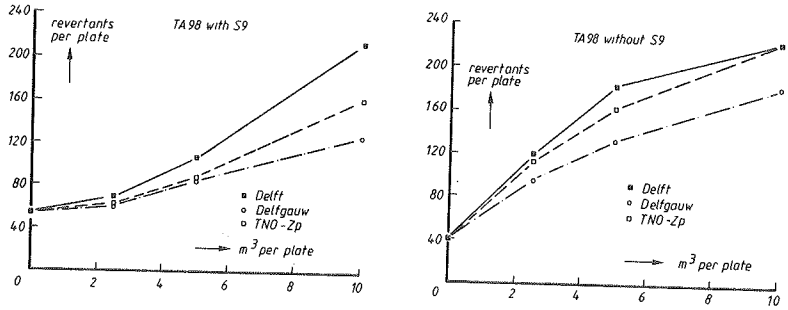


Fig. 3. Mutagenicity of ambient air particles sampled between 17.00 and 20.00 h in Experiment 8. The points represent means of duplicate determinations.

toxicity. Since there is no model available which can isolate the assumed linear dose–mutagenicity relation from other factors, the effect can only be assessed by linear regression. In some cases the effects of the highest dose were omitted because the dose–response relation clearly deviated from linearity.

The sensitivity of the test was monitored by simultaneously (with the extracts) testing benzo[*a*]pyrene (with S9-fraction; Sigma) and 2-nitrofluorene (without S9-fraction; Aldrich). To check the difference in sensitivity to nitro compounds between the two strains, the following compounds were tested in a separate experiment: 2,4,7-trinitrofluoren-9-one (Aldrich), 2-nitrofluorene (Aldrich), 2-nitronaphthalene (Aldrich), daunomycin (Sigma), furazolidone (Orphahell, Mijdrecht, the Netherlands), benzo[*a*]pyrene (Sigma), 3-methylcholanthrene (Sigma), 2-aminoanthracene (Sigma) and 2-acetylaminofluorene (Fluka).

RESULTS

Table 1 summarizes some relevant meteorological parameters. Nearly all experiments were carried out during meteorological conditions which facilitate the development of PA in the area under investigation: easterly winds, low wind speeds and high temperatures.

Table 2 lists the mean and highest concentrations of gaseous pollutants measured between 8.00 and 20.00 h. The concentrations of PAN and O_3 exhibited the characteristic daily trend in this area, which is determined by the differences in mixing height between day and night. Both compounds reached their peak values in the afternoon. These values indicate moderate levels of PA for the area under investigation.

The results of the mutagenicity tests are given in Fig. 4. This figure shows that the mutagenicity of the samples collected varies strongly from day to day and during the day. The sampling days are not characterized by a common trend for the mutagenicity. This observation was tested with the analysis of variance (ANOVA) for those combinations of experiment and Ames-test variant which had a complete set of effects between 8.00 and 20.00 h. The ANOVA was also carried out with the proportions between the effects, i.e.

TABLE 1

Summary of meteorological data

Experiment No.	Wind direction																Wind speed ($m s^{-1}$)	Max. temp. (°C)
	N	NNE	NE	ENE	E	ESE	SE	SSE	S	SSW	SW	WSW	W	WNW	NW	NNW		
1			11	9	3	1											3.3	18.7
2			8	8	9												3.6	22.2
3		1	1	6	8	1	1						1	1		1	2.7	25.4
4	2	1		1									7	3	3		1.9	22.8
5	4	1	3	2	8	1			1					1	2	1	2.8	27.7
6	4	1	4	4		1			4	1	1		2		1	1	2.4	28.0
7	2	3	8	3			1		1					1	1	2	2.1	27.6
8				1	14	3	1										3.8	25.8
9					4	9	3										5.5	30.9
10		1	17	6	1												5.5	25.7
11		1	3	6	2	8	4								1		2.6	29.6

Wind direction = frequency of hourly averages.
Wind speed = average of hourly averages.
Max. temp. = maximum hourly average.

TABLE 2

Concentrations of gaseous pollutants between 08.00 and 20.00 h

A: per experiment

Experiment	SO_2 ($\mu g\,m^{-3}$) $\bar{x}$	NO ($\mu g\,m^{-3}$) $\bar{x}$	NO_2 ($\mu g\,m^{-3}$) $\bar{x}$	O_3 ($\mu g\,m^{-3}$)		PAN (ppb)	
				$\bar{x}$	H	$\bar{x}$	H
1	27.0	37.0	73.6	103.8	145		
2	11.2	42.4	68.7	77.1	92		
3	30.9	19.0	73.0	122.6	216		
4	19.8	5.6	49.4	129.4	167		
5	20.4	16.4	83.6	153.0	197	1.0	1.3
6	31.3	2.1	45.8	179.4	225	2.3	7.5
7	30.3	9.3	87.0	144.3	189	1.8	2.7
8	27.8	5.2	75.9	187.0	225	2.3	3.1
9	55.4	17.5	88.1	144.5	216	1.3	2.0
10	11.7	6.8	32.7	116.3	142	0.9	1.3
11	36.2	15.9	74.0	135.5	212	1.1	1.7

B: per sampling period

Period	O_3 ($\mu g\,m^{-3}$)		PAN (ppb)	
	$\bar{x}$	95%	$\bar{x}$	95%
8–11	78.4	55.3–101.5	0.8	0.2–1.5
11–14	129.8	107.7–152.8	1.9	1.3–2.4
14–17	167.0	145.0–189.1	1.8	1.2–2.3
17–20	157.8	135.7–179.9	1.4	0.9–2.0

$\bar{x}$ = average of the concentrations during the sampling periods or experiments.
H = highest value *measured*.
95% = 95% confidence interval of average.

TA98/TA98NR etc. There were no indications of a significant common trend ($p > 0.05$). Therefore it appears that any common temporal development of PA during the sampling days is not reflected in the mutagenicity of the ambient air particles.

The experimental variation of the mutagenicity test is probably an important factor influencing the variation of the effects. The fluctuation in the sensitivity of the tests carried out during this study was investigated by simultaneously testing standard mutagens together with the extracts of the samples in each test. The results using the standard mutagens are summarized in Table 3.

The mutagenicity of the extracts is characterized by a clear dependence on nitro-reduction, the effects with TA98NR being markedly lower than those with TA98 in the absence of S9-fraction. In the presence of S9-fraction the reduction is much less manifest because the response with TA98 decreases and the response with TA98NR increases. The results of correlation calculations

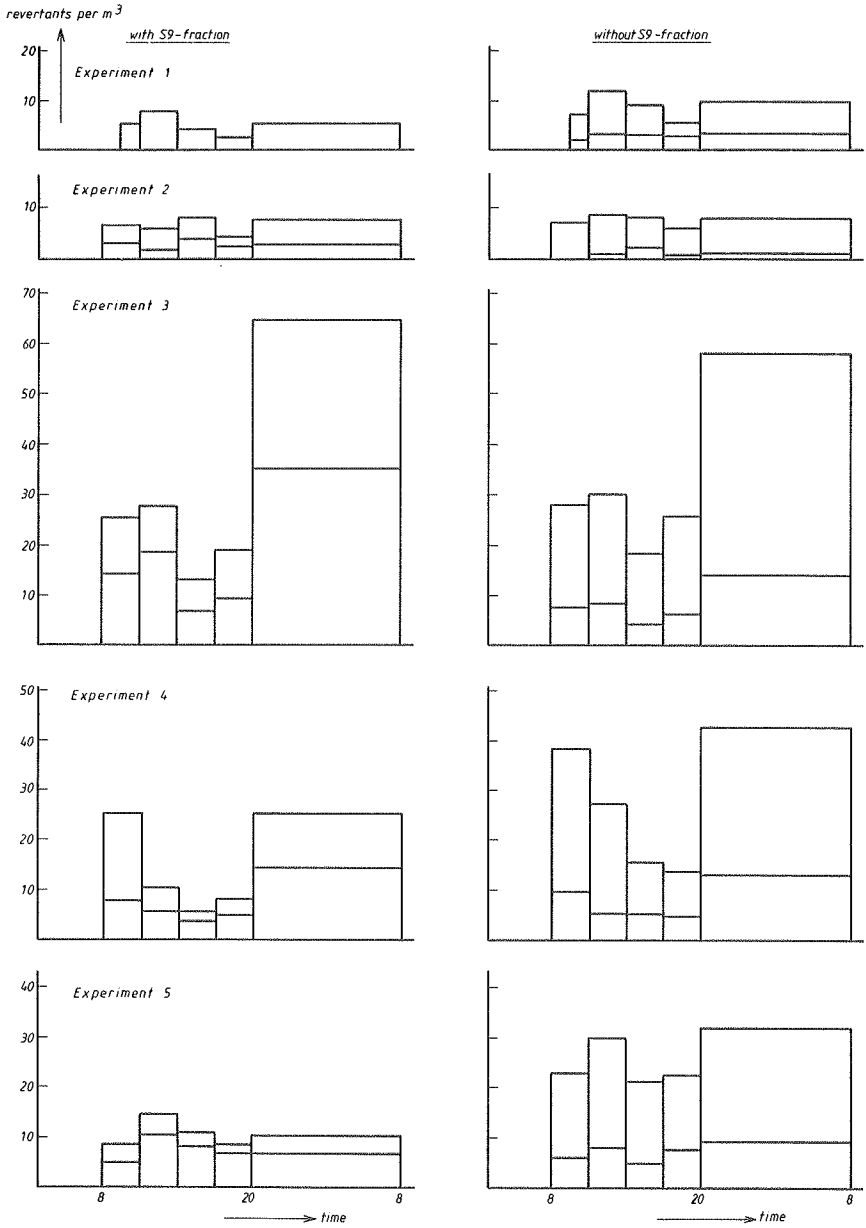


Fig. 4. Results of the Ames test on the extracts of the particles. The figure indicates the calculated slopes of the dose–response relations with strains TA98 and TA98NR. In nearly all cases the effect obtained with TA98NR was lower than that with TA98; if not, the difference is shaded.

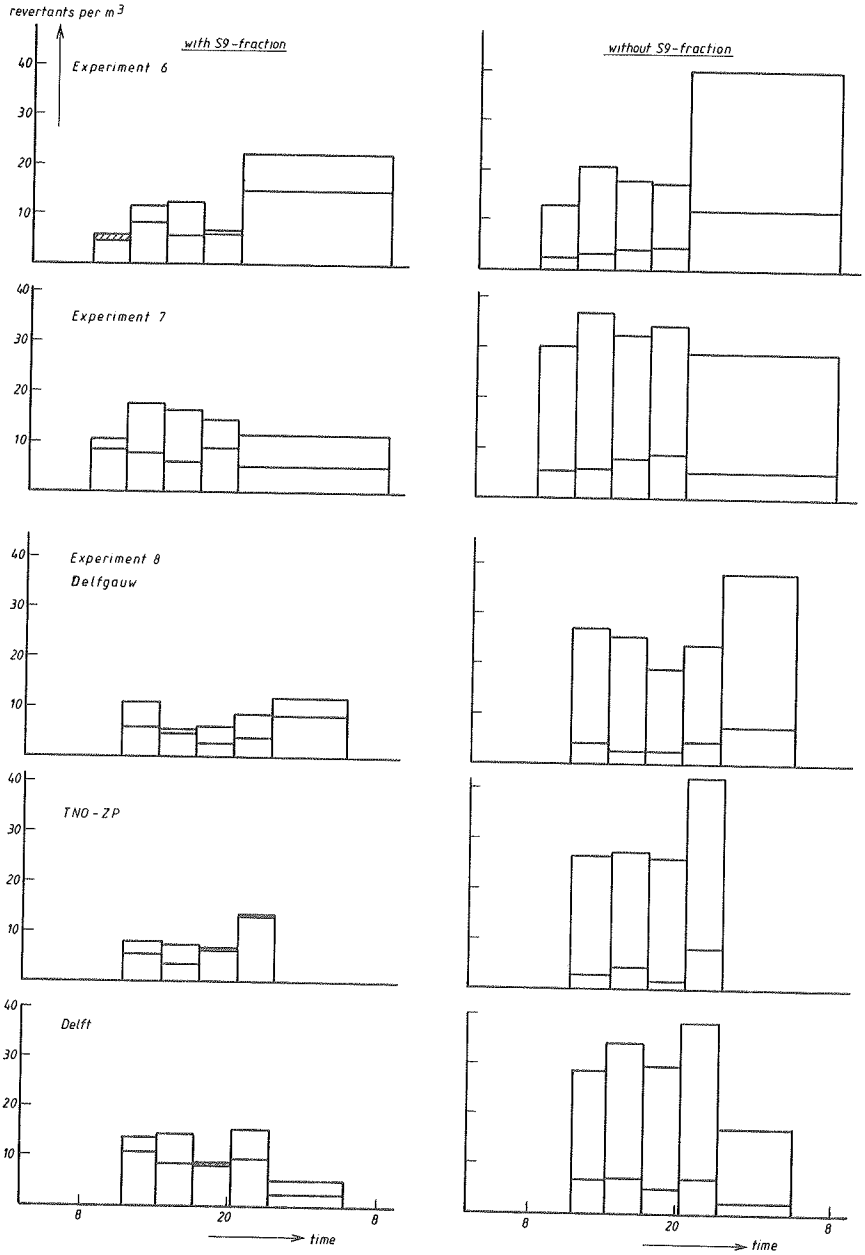
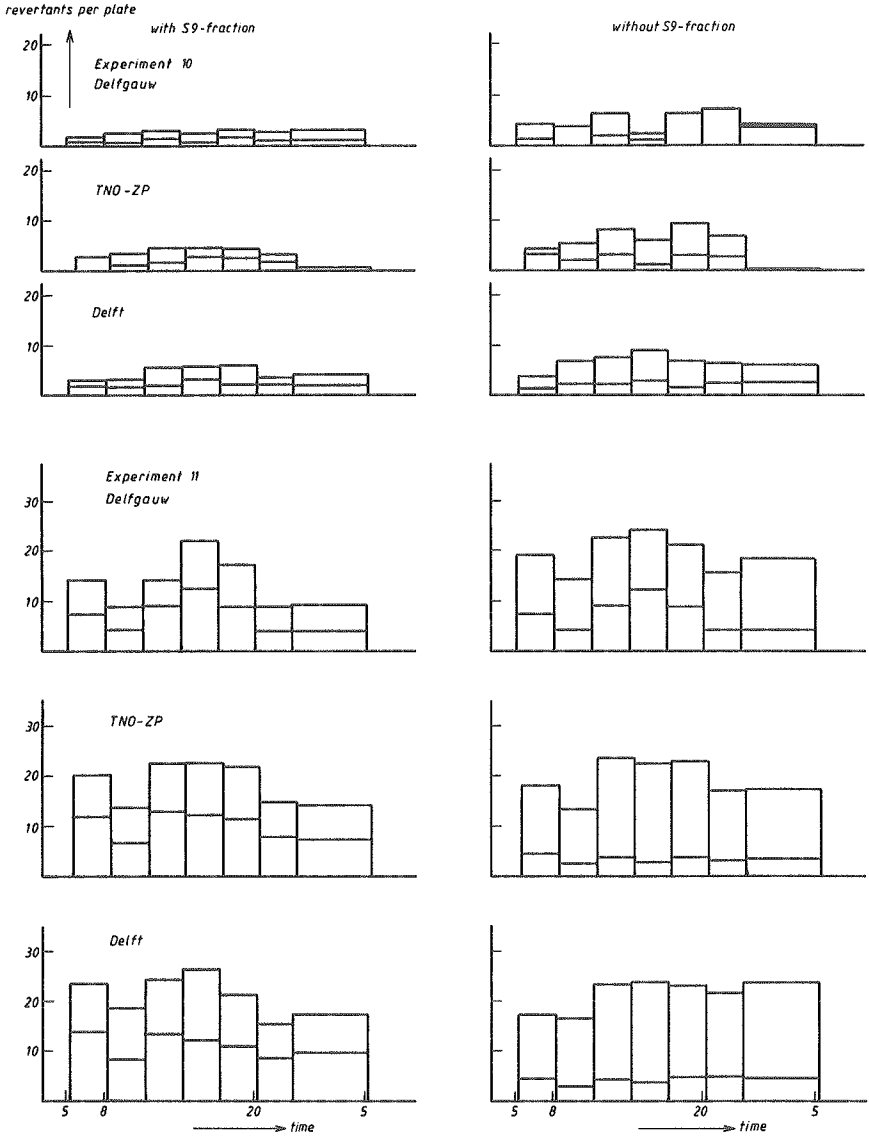


Fig. 4 (*continued*).



with the responses of the four variants of the Ames test are listed in Table 4 and plotted in Fig. 5. Quite high correlations are obtained for the two responses with S9 fraction ($r > 0.9$); the two responses with TA98NR gave the lowest correlation. Table 4 also lists the gradient of the first principal axis, which can be regarded as a measure of the mean proportion between the responses. The clear dependence on nitro-reduction is confirmed by this table; in the absence

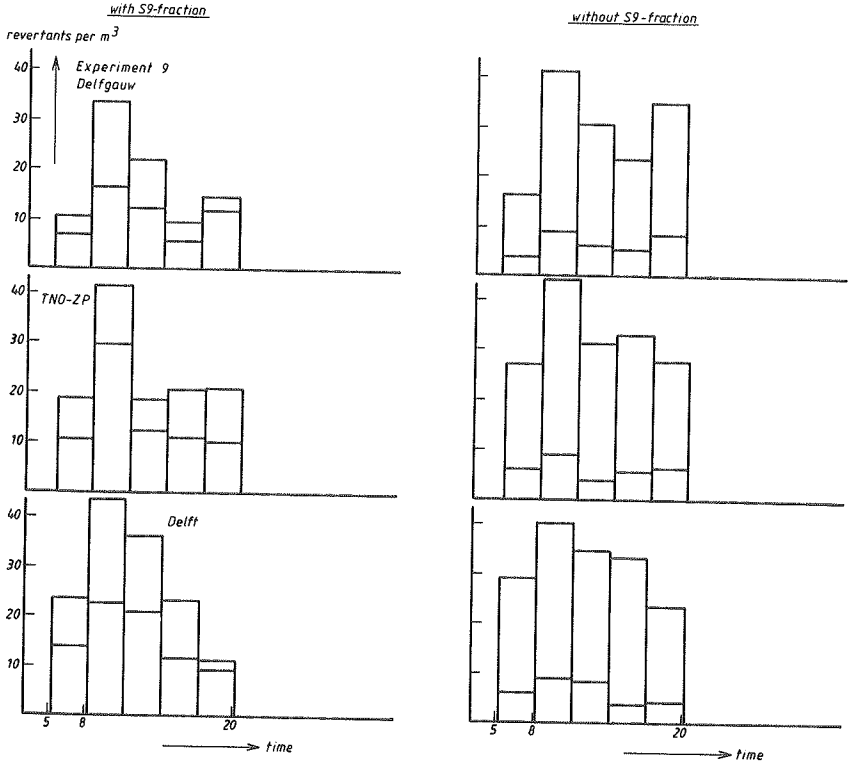


Fig. 4 (*continued*).

TABLE 3

Summary of the effects of the standard mutagens

	2-Nitrofluorene (2 μg per plate)		Benzo[*a*]pyrene (5 μg per plate)	
	TA98	TA98NR	TA98	TA98NR
$\bar{x}$	691	95	296	223
s (% of $\bar{x}$)	99 (14)	22 (23)	69 (23)	71 (32)
v	289	67	172	243
n	8	8	9	10

$\bar{x}$ = average of the average test results.
s = standard deviation.
v = difference between highest and lowest value.
n = number of tests, each test comprised two plates.

TABLE 4

Correlations and mean proportions of the effects obtained with the four Ames test variants

			A (n = 53)	B (n = 39)	C (n = 9)			
TA98 − S_9	TA98 + S_9	r	0.77	0.74	0.86	0.72	0.79	0.75
		Pa	0.84	0.77	0.91	0.69	0.60	0.70
TA98 − S_9	TA98NR − S_9	r	0.86	0.79	0.94	0.74	0.85	0.88
		Pa	0.23	0.20	0.24	0.18	0.18	0.23
TA98 − S_9	TA98NR + S_9	r	0.80	0.73	0.85	0.68	0.67	0.85
		Pa	0.46	0.43	0.58	0.43	0.25	0.31
TA98 + S_9	TA98NR − S_9	r	0.71	0.64	0.79	0.63*	0.40*	0.45*
		Pa	0.22	0.21	0.23	0.21	0.14	0.18
TA98 + S_9	TA98NR + S_9	r	0.96	0.94	0.96	0.96	0.92	0.96
		Pa	0.58	0.62	0.67	0.71	0.49	0.45
TA98NR − S_9	TA98NR + S_9	r	0.72	0.62	0.74	0.68	0.24*	0.59*
		Pa	2.53	2.98	3.06	3.07	4.35	1.59

r = coefficient of correlation. Pa = gradient of the first principal axis; $y = ax + b$; y = second variant. A = all samples except those of experiment 1. B = samples taken between 8.00 and 20.00 h, except experiment 1. C = samples of the four separate sampling periods between 8.00 and 20.00 h, except those of experiments 1 and 8. *$r \neq 0$; $p > 0.05$.

of S9 fraction the response in TA98NR is about one-fifth of the response in TA98, and this difference is hardly dependent on the sampling periods selected.

Figure 6 depicts the results of the tests carried out to check the difference in sensitivity between the two strains. The two strains hardly differ in sensitivity to the compounds without nitro-groups, while large differences were found between the responses of the tests with nitro-compounds. The differences found with the extracts of the particles seem to be large enough to indicate a strong contribution of nitro-compounds.

The mean proportions can be regarded as characteristic for the total study, with the exception of those in which an effect with and without S9-fraction was apparent. The correlation coefficients and the mean proportions for selected groups of samples indicate a rather constant proportion.

The results of the last four experiments are summarized in Table 5 to enable comparison of the three locations with respect to the mutagenicity of the particles. The contribution from local sources depends on the experiment and on the sampling period. Indirect mutagens, i.e. mutagens dependent on S9-fraction, predominate in this contribution. Both highway traffic and the downtown area contribute. However, the results show clearly that the major part of the mutagenicity found downwind of the local sources is due to distant upwind sources.

Figure 7 shows the relationship between wind direction and mutagenicity (TA98 without S9-fraction). To this end, the hourly means were subjected to the

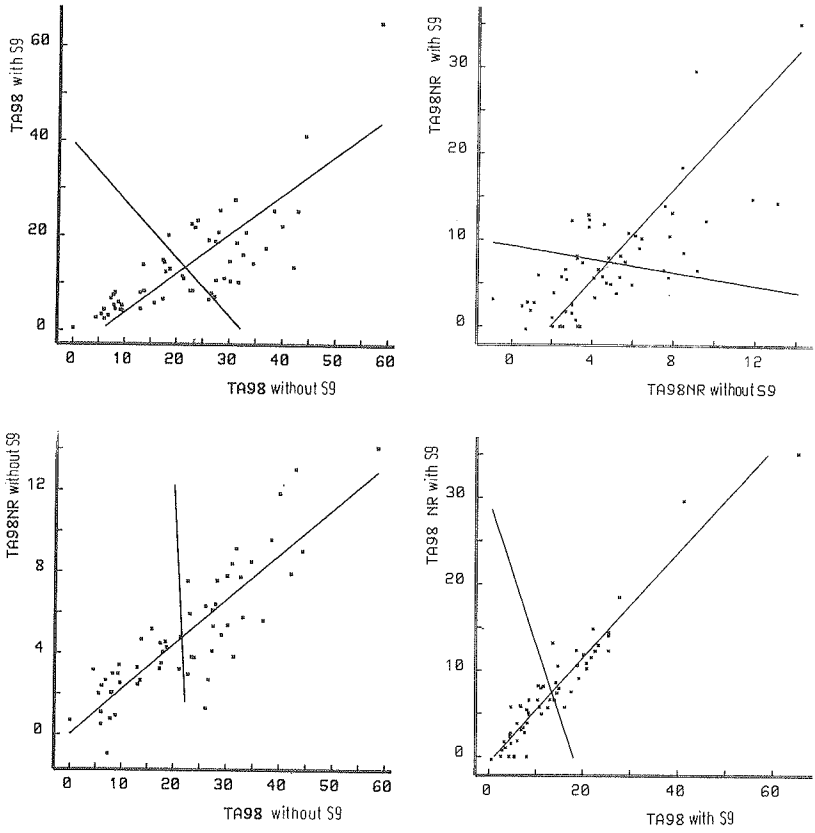


Fig. 5. Plots of the effects obtained with the different Ames test variants (effects are depicted in Fig. 3; see also Table 4). The lines represent the two principal axes.

same spline/integration procedure as the concentrations of the gaseous compounds. This figure reveals that the effect tends to a maximum with south-easterly winds. In this case sampling occurred downwind of Rotterdam, and the dependence of the effect on wind direction most probably reflects contribution of sources in Rotterdam. The contribution from the highway will then also be stronger. The effects with the other variants of the Ames test showed a comparable wind direction dependence.

The relation between the effects and the concentrations of the gaseous pollutants (studied by correlation analysis, principal component analysis of the correlation matrix and factor analysis with varimax rotation) is shown in Table 6. The SO_2 concentrations show a strong link with the effects obtained with S9-fraction. Two factors seem to largely determine mutagenicity: one is strongly related to the effects obtained with S9-fraction and the other to those obtained without S9-fraction. It cannot be excluded that this result is (partly)

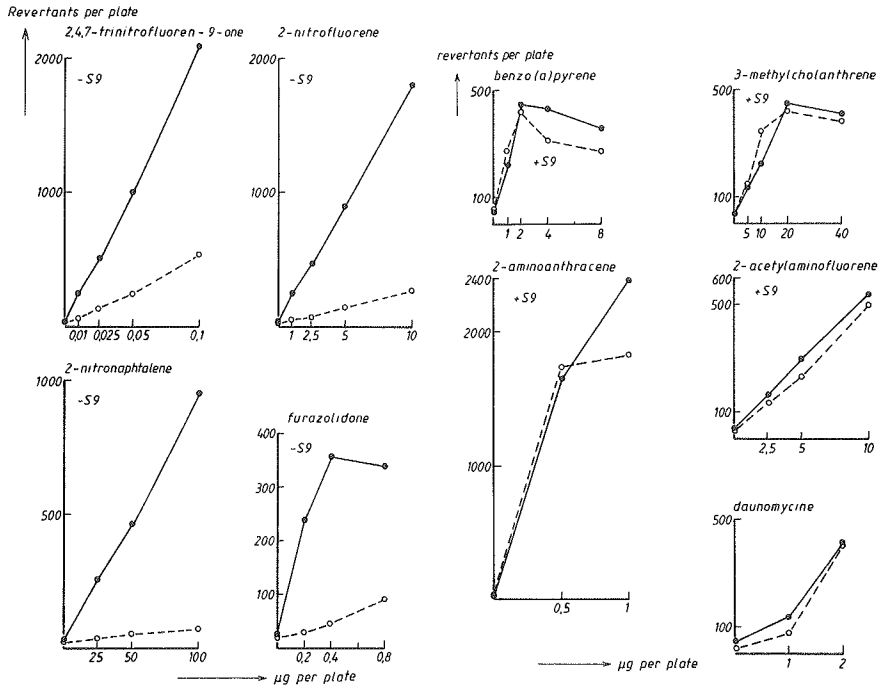


Fig. 6. Comparison of the two strains with respect to their sensitivity to a number of mutagens. (●—●) TA98; (○—○) TA98NR. The test with 2-aminoanthracene was carried out with 5 µl of S9-fraction per plate instead of 50 µl.

due to an independent variation of the test sensitivity for both types of effects, although, in that case, the link with SO_2 concentrations would not have been expected.

DISCUSSION

The results of the present study show the contribution of a small urban area and traffic on a motorway to the mutagenicity of ambient air particles. A striking result is the qualitative difference between the increase in the mutagenicity caused by the local sources and the mutagenicity of the particles upwind. The former was largely dependent on the presence of S9-fraction in the test; i.e. is indirect, while the latter decreased in the presence of S9-fraction. In the absence of S9-fraction the influence of the local sources is rather small. If it is assumed that interactions between the upwind particles and the local particles do not influence the mutagenicity, it can be concluded that emissions from traffic predominantly influence the indirect mutagenicity, at least when

TABLE 5

Comparison of the three locations with respect to the effects of the samples in the Ames test

		All samples	Samples taken between 08.00 and 20.00 h	Experiment 8	9	10	11	08.00–11.00	11.00–14.00	14.00–17.00	17.00–20.00
TA98 – S9											
Delfgauw	$\bar{x}$	18.71	20.15	26.53	28.98	4.92	19.59	19.70	21.62	18.82	20.33
	S_x	11.12	11.50	6.99	9.74	1.77	3.66	19.14	10.61	11.09	11.48
TNO-ZP	$\bar{x}$	20.06	21.86	30.43	32.70	5.70	19.49	21.03	22.43	22.23	21.57
	S_x	12.03	11.21	7.77	6.87	2.99	3.86	20.46	10.04	11.61	8.45
Delft	$\bar{x}$	21.13	22.89	29.57	32.24	6.81	21.51	21.25	23.89	25.17	20.83
	S_x	11.21	11.05	7.94	6.24	1.68	3.17	17.06	11.23	11.78	9.74
TA98 + S9											
Delfgauw	$\bar{x}$	10.29	11.71	8.61	17.81	2.72	13.68	15.00	12.51	9.87	10.29
	S_x	7.68	8.94	2.76	10.00	0.48	4.95	16.21	7.80	8.56	6.73
TNO-ZP	$\bar{x}$	13.47	14.79	8.85	24.05	3.38	18.72	19.54	13.62	13.79	13.42
	S_x	9.69	10.62	3.15	9.65	1.45	4.14	19.47	8.75	9.20	9.16
Delft	$\bar{x}$	15.56	17.46	11.34	27.60	4.52	21.03	21.81	19.96	17.51	11.64
	S_x	10.82	11.84	4.49	12.54	1.32	3.99	20.36	13.26	9.24	6.68

$\bar{x}$ = average.
S_x = standard deviation.

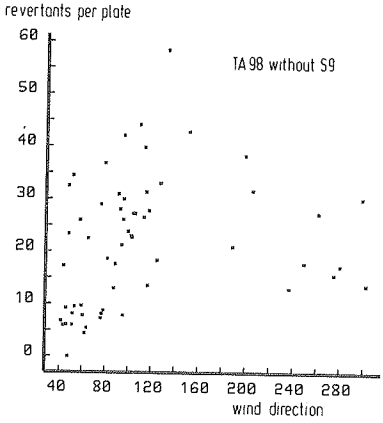


Fig. 7. Plot of wind direction versus mutagenicity at the location TNO-ZP.

sampling is carried out at the same short distance from emissions as was the case in the present study.

Several studies have indicated the sources possibly contributing to the *upwind* mutagenicity in the present study. De Raat (1983) and de Raat et al.

TABLE 6

Results of the principal component analysis

Principal component analysis (factor matrix)

Variable	1	2	3	4	5	6
TA98 – S9	−0.93	0.07	0.08	−0.19	−0.00	−0.27
TA98 + S9	−0.92	−0.20	−0.24	0.07	0.07	0.12
TA98NR – S9	−0.75	0.02	0.22	−0.61	−0.05	0.10
TA98NR + S9	−0.89	−0.24	−0.27	0.07	0.22	0.03
SO_2	−0.83	0.22	−0.27	0.30	−0.32	0.00
NO	−0.12	−0.92	0.20	0.26	0.04	−0.08
NO_2	−0.62	0.19	0.69	0.31	−0.00	0.08
O_3	−0.14	0.94	−0.01	0.18	0.19	−0.04
Variance (% of sum)	0.52	0.24	0.10	0.09	0.02	0.01

Factor analysis (varimax rotated matrix)

Variable	1	2	3	4	5	6
TA98 – S9	−0.62	0.08	0.32	−0.57	−0.13	−0.40
TA98 + S9	−0.92	−0.12	0.16	−0.28	−0.11	−0.01
TA98NR – S9	−0.32	0.02	0.17	−0.93	−0.03	0.00
TA98NR + S9	−0.94	−0.14	0.11	−0.25	−0.03	−0.03
SO_2	−0.72	0.21	0.24	−0.11	−0.61	−0.05
NO	−0.17	−0.94	0.15	0.10	0.04	−0.01
NO_2	−0.21	0.06	0.95	−0.20	−0.08	−0.04
O_3	−0.05	0.93	0.25	0.07	−0.06	−0.03
Eigenvalue	2.8	1.8	1.2	1.4	0.4	0.1

(1985) demonstrated the influence of local sources on the mutagenicity in the Rijnmond area. This area extends from the southeast to the southwest of Delft. The dependence of the effect on wind direction (see Fig. 7) is probably strongly determined by sources in this area, especially in the city of Rotterdam. At this time of the year the most important source in Rotterdam will be traffic. Nevertheless, deactivation by S9-fraction was also found in experiments assumed to be influenced by sources in Rotterdam (see Table 1). It thus seems that after a longer residence of the particles in the air, their mutagenicity changes from activation to deactivation by S9-fraction. However, the increase in the effect with southeasterly winds could also be the result of emissions from further distances than Rotterdam. These might obscure the indirect mutagenicity attributed to emissions in Rotterdam.

The importance of long range transport of mutagens in the Netherlands was clearly demonstrated by Alink et al. (1983) and van Houdt et al. (1987). Especially with southeasterly winds, sources outside the Netherlands seem to contribute; trajectory studies revealed that high effects coincide with the

sampling of air masses which have passed industrial areas, for instance the Ruhr area (van Houdt et al. 1987). The sources in such an area would not be restricted to traffic; other combustion processes (oil, coal) may contribute. This makes it difficult to compare the upwind particles collected at Delfgauw with the traffic emissions on the motorway and in the town, and prevents the drawing of definite conclusions about changes in mutagenic quality based on such a comparison.

The study of Dehnen et al. (1981) provides extensive information about the mutagenicity of air particles in two towns in the Ruhr area. In the summer, when the contribution of space heating is negligible, mutagenicity was not influenced by the inclusion of S9-fraction; with samples from a rural site, S9-fraction induced a decrease in the effect. If such particles contribute to the effects found at the upwind location, Delfgauw, which is probably the case with easterly winds, the influence of S9-fraction already reflects the situation near the source areas.

The predominant contribution of particles from distant sources provides a major explanation for the lack of a common diurnal variation of the mutagenicity for the different sampling days. It can be expected that this mutagenicity will show strong variations during the day depending on wind direction (source area) and wind speed (dilution). The contribution of the local sources did not show a typical diurnal pattern; possibly the sampling periods were too long to detect variations in traffic intensity. Photochemical air pollution (PA) shows a typical diurnal variation in the concentrations of radicals and oxidants. A clear influence of PA on mutagenicity should therefore lead to typical diurnal variation of the mutagenicity. Detection of the variation requires a rather constant concentration and quality of the primary mutagenic particles. In the present study the larger part of the mutagenicity originated from particles which had possibly been exposed to reactive compounds for a longer time and had probably already undergone maximum conversion. The particles of the local emissions were possibly too strongly dominated by the particles from distant sources, while the residence time might have been too short to allow them to attain a substantial conversion.

A number of studies have been carried out in which comparison of relatively 'young' and 'old' particles have indicated an influence of the residence time in the air on the mutagenicity. Pitts et al. (1982) studied the diurnal variation of the mutagenicity and found a clear relation between traffic density and mutagenicity, demonstrating the importance of local sources in their study. A remarkable finding was that the relation between intensity of emission, influence of S9-fraction and residence in the air, pointed to deactivation with longer residence time. Similar indications were found by Möller et al. (1982) and Alfheim (1982) in their street canyon experiments. Selzer Madsen et al. (1982) also suggested a relative decrease of activatable mutagenicity after emission; in addition, they showed that relatively apolar activatable mutagens (extracted with cyclohexane) stem from 'fresh' traffic emissions, whilst relatively polar deactivatable mutagens stem from more distant sources. These

results point to the possibility that the difference in quality between the two types of particles encountered in this study is indeed the result of residence time in the air.

Both the TA98 strain and its nitro-reductase-deficient derivative TA98NR were utilized in all tests. In the absence of S9-fraction the effects in the latter reached only 20% of the effects in the former, showing the strong dependence of the mutagenicity on bacterial nitroreduction. The difference between the two strains in tests with model compounds even suggests that, in the absence of S9-fraction, non-nitro-reductase-dependent mutagenicity contributed hardly, if at all, to the mutagenicity of the particles. The inclusion of S9-fraction in the test reduces the difference markedly, which leads to an increase in the correlation between the effects in the two strains. The presence of two groups of compounds is suggested by these results; direct mutagenic nitro-compounds, the effect of which is strongly reduced by S9-fraction, and indirect mutagens. The two strains are assumed to differ only in their sensitivity to the first group. The situation is made more complicated by the fact that some nitro-compounds can belong to both groups and others only to one. 6-Nitrobenzo[*a*]pyrene is only mutagenic in the presence of S9-fraction, while the effects of dinitropyrenes are almost totally reduced by it (Rosenkranz and Mermelstein, 1983). An influence of residence time in the air on the mutagenicity would then be due to an increase in the first group and/or a decrease in the second group, possibly by conversion of the second into the first.

The multivariate analyses with the effects and the concentrations of the gaseous pollutants show a rather strong link in the factor analysis between the SO_2 concentrations and the effects obtained in the presence of S9-fraction. When easterly winds prevail, the SO_2 concentrations in the Netherlands are largely due to long-range transported air pollution. Although the limited number of samples warrants caution, the correlation strongly suggests that the indirect mutagenicity is for a large part the result of long-range transported air pollutants. A weaker link was found in the case of direct mutagenicity. It is tempting to hypothesize that this reflects conversion after emission, a process which would be independent of variations in SO_2 concentrations.

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REFERENCES

Alfheim, I., 1982. Contribution from motor vehicle exhaust to the mutagenic activity of airborne particles. In: M. Sorsa and H. Vainio (Eds), Mutagens in Our Environment: Alan R. Liss, New York, pp. 235–248.

Alink, G.M., J.J. van Houdt, J.R. Kolkman and J.S.M. Boley, 1983. Mutagenic activity of airborne particles of non-industrial locations. Mutat. Res., 116: 21–34.

Ames, B.N., J. McCann and E. Yamasaki, 1975. Methods for detecting carcinogens and mutagens with the *Salmonella* mammalian microsome mutagenicity test. Mutat. Res., 31: 347–364.

Björseth, A., G. Lunde and A. Lindskog, 1979. Long-range transport of polycyclic aromatic hydrocarbons. Atmos. Environ. 13: 45–53.

Butler, L.D. and P. Crossley, 1981. Reactivity of polycyclic aromatic hydrocarbons adsorbed on soot particles. Atmos. Environ. 15: 91–94.

Claxton, L.D., 1983. Characterization of automotive emissions by bacterial mutagenesis bioassay; a review. Environ. Mutagen., 5: 609–631.

Commoner, B., P. Madyastha, A. Bronsdon and A.J. Vithayathil, 1978. Environmental mutagens in urban air particulates. J. Toxicol. Environ. Health, 4: 59–77.

Dehnen, W., N. Pitz and R. Tomingas, 1977. The mutagenicity of airborne particulate pollutants. Cancer lett., 4: 5–12.

Dehnen, W., R. Tomingas and R. Pitz, 1981. Studies on the mutagenic effects of extracts derived from airborne particulates within one year in differently burdened areas using the Ames test. Zentralbl. Bakteriol. Hyg., Abt. I. Orig., Reihe B, 172: 351–366.

Falk, H.L., I. Markul and P. Kotin, 1956. Aromatic hydrocarbons. IV Their fate following emission into the atmosphere and experimental exposure to washed air and synthetic smog. AMA Arch. Ind. Hyg. Occup. Med. 13: 13–17.

Fox, M.A. and S. Olive, 1979. Photo oxidation of anthracene on atmospheric particulate matter. Science, 205: 582–583.

Gibson, T.L., 1982. Nitro derivatives of polynuclear aromatic hydrocarbons in airborne and source particulate matter. Atmos. Environ., 16: 2037–2040.

Gorse, R.A., T.L. Riley, F.C. Ferris, A.M. Pero and L.M. Skewes, 1983. 1-Nitropyrene concentration and bacterial mutagenicity in on-road vehicle particulate emissions. Environ. Sci. Technol., 17: 198–202.

Hanson, R.L., T.R. Henderson, C.H. Hobbs, C.R. Clark, R.L. Carpenter, J.S. Dutcher, T.M. Harvey and D.F. Hunt, 1983. Detection of nitroaromatic compounds on coal combustion particles. J. Toxicol. Environ. Health, 11: 971–980.

Harris, W.R., E.K. Chess, D. Okamoto, J.F. Remsen and D.W. Later, 1984. Contribution of nitropyrene to the mutagenic activity of coal fly ash. Environ. Mutagen., 6: 131–144.

Houdt, J.J. van, G.M. Alink and J.S.M. Boleij, 1987. Mutagenicity of airborne particles related to meteorological and air pollution parameters. Sci. Total Environ., 61: 23–36.

Jäger, J. and V. Hanus, 1980. Reaction of solid carrier-adsorbed polycyclic aromatic hydrocarbons with gaseous low concentrated nitrogen dioxide. J. Hyg., Epidemiol., Microbiol., Immunol., 24: 1–12.

Lewtas, J., 1982. Comparison of the mutagenic and potentially carcinogenic activity of particle bound organics from wood stoves, residential oil furnaces and other combustion sources. In: J.A. Cooper and D. Malek (Eds), Residential Solid Fuels. Oregon Graduate Center, Beaverton, pp. 606–619.

Li, A.P., C.R. Clark, R.L. Hanson, T.R. Henduson and C.H. Hobbs, 1983. Comparative mutagenicity of a coal combustion flyash extract in *Salmonella typhimurium* and Chinese hamster overy cells. Environ. Mutagen. 5: 263–272.

Löfroth, G., 1978. Mutagenicity assay of combustion emissions. Chemosphere, 10: 791–798.

Mast, T.J., D.P.H. Hsieh and J.N. Seiber, 1984. Mutagenicity and chemical characterization of organic constituents in rice straw smoke particulate matter. Environ. Sci. Technol., 18: 338–348.

Miquel, A.H., 1984. "Atmospheric" reactivity of particulate polycyclic aromatic hydrocarbons collected in an urban tunnel. Sci. Total Environ., 36: 305–311.

Møller, M. and I. Alfheim, 1980. Mutagenicity and PAH-analysis of airborne particulate matter. Atmos. Environ., 14: 83–88.

Møller, M. and I. Alfheim, 1983. Mutagenicity of air samples from various combustion sources. Mutat. Res., 116: 35–46.

Møller, M., I. Alfheim, S. Larssen and A. Mikalsen, 1982. Mutagenicity of airborne particles in relation to traffic and air pollution parameters. Environ. Sci. Technol., 16: 221.

Nielsen, T., 1984. Reactivity of polycyclic aromatic hydrocarbons towards nitrating species. Environ. Sci. Technol., 18: 157–163.

Nielsen, T., Th. Ramdahl and A. Bjørseth, 1983. The fate of airborne polycyclic organic matter. Environ. Health Perspect., 47: 103–114.

Nielsen, T., B. Seitz and T. Ramdahl, 1984. Occurrence of nitro PAH in the atmosphere in a rural area. Atmos. Environ., 18: 2159–2165.

Pani, B., U. Laureni, L.N. Babrudi, A. Collareta, S. Venturini, R. Ferri, M. Carrozzi, F. Burilini and C. Monti-Bragadin, 1983. Mutagenicity test of extracts of airborne dust from the municipal incinerator of Trieste. Environ. Mutagen., 5: 23–32.

Pitts, J.N., Jr, 1983. Formation and fate of gaseous and particulate mutagens and carcinogens in real and simulated atmospheres. Environ. Health Perspect., 47: 115–140.

Pitts, J.N., D. Grosjean, T.M. Mischke, V.F. Simmon and D. Poole, 1977. Mutagenic activity of airborne particulate organic pollutants. Toxicol. Lett., 1: 65–70.

Pitts, J.N., Jr., K.A. van Cauwenberghe, D. Grosjean, J.P. Schmid, D.R. Fitz, W.L. Belser, G.B. Knudson and P.M. Hynds, 1978. Atmospheric reactions of polycyclic aromatic hydrocarbons: facile formation of mutagenic nitroderivates. Science, 202: 515–518.

Pitts, J.N., Jr., D.M. Lokensgard, P.S. Ripley, K.A. van Cauwenberghe, L. van Vaeck, S.D. Shaffer, A.J. Thilland and W.L. Belser, 1980. "Atmospheric" epoxidation of benzo(a)pyrene by ozone: formation of the metabolite benzo(a)pyrene 4, 5 oxide. Science, 210: 1347–1349.

Pitts, J.N., W. Harger, D.M. Lokensgard, D.R. Fitz, G.M. Scorziell and V. Mejia, 1982. Diurnal variations in the mutagenicity of airborne particulate organic matter in California's south coast air basin. Mutat. Res., 104: 35–41.

Raat, W.K. de, 1983. Genotoxicity of aerosol extracts; Some methodological aspects and the contribution of urban and industrial locations. Mutat. Res., 116: 47–64.

Raat, W.K. de, F.A. de Meijere and S.A.L.M. Kooijman, 1985. Mutagenicity of ambient aerosol collected in an urban and industrial area of the Netherlands. Sci. Total Environ., 44: 17–33.

Raat, W.K. de, S.A.L.M. Kooijman and J.W.J. Gielen, 1987. Concentrations of polycyclic hydrocarbons in airborne particles in the Netherlands and their correlation with mutagenicity. Sci. Total Environ., 66: 95–114.

Ramdahl, T., I. Alfheim, S. Rustad and T. Olsen, 1982a. Chemical and biological characterization of emissions from small residential stoves burning wood and charcoal. Chemosphere, 11: 601–611.

Ramdahl, T., G. Becher and A. Bjørseth, 1982b. Nitrated polycyclic aromatic hydrocarbons in urban air particles. Environ. Sci. Technol., 16: 861–865.

Rosenkranz, H.S. and R. Mermelstein, 1983. Mutagenicity and genotoxicity of nitro arenes. All nitro-containing chemicals were not created equal. Mutat. Res., 114: 217–267.

Schuetzle, D., T.J. Prater, T. Riley, T.M. Harvey and D. Hunt, 1982. Analysis of nitrated derivatives of PAH in diesel particulates. Anal. Chem., 54: 265.

Selzer Madsen, E., P.A. Nielsen and J.C. Pedersen, 1982. The distribution and origin of mutagens in airborne particulates detected by the Salmonella/microsome assay in relation to levels of lead, vanadium and PAH. Sci. Total Environ., 24: 13–25.

Talcott, R. and W. Wei, 1977. Airborne mutagens bioassayed in *Salmonella typhimurium*. J. Natl Cancer Inst., 58: 449–451.

Teranishi, K., K. Hamada and H. Watanabe, 1978. Mutagenicity in *Salmonella typhimurium* mutants of the benzene-soluble organic matter derived from airborne particulate matter and its five fractions. Mutat. Res., 56: 273–280.

Tokiwa, H., K. Morita, H. Takeyashi, K. Takahashi and Y. Onishi, 1977. Detection of mutagenic activity in particulate air pollutants. Mutat. Res., 48: 237–248.

Vaeck, L. van, and K. van Cauwenberghe, 1984. Conversion of polycyclic aromatic hydrocarbons on diesel particulate matter upon exposure to ozone. Atmos. Environ., 18: 323–328.

Xu, X.B., J.P. Nachtman, Z.L. Jin, E.T. Wei and S.M. Rappaport, 1982. Isolation and identification of mutagenic nitro-PAH in Diesel-exhaust particulates. Anal. Chim. Acta, 136: 163–174.

CHAPTER 9

POLYCYCLIC AROMATIC HYDROCARBON (PAH) CONCENTRATIONS IN AMBIENT AIR-BORNE PARTICLES FROM LOCAL TRAFFIC AND DISTANT SOURCES; VARIATION OF THE PAH PROFILE

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Polycyclic aromatic hydrocarbon (PAH) concentrations in ambient airborne particles from local traffic and distant sources; variation of the PAH profile

W.K. de Raat and F.A. de Meijere

Institute of Environmental Sciences, TNO, P.O. Box 6011, 2600 JA Delft, Netherlands

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ABSTRACT

The temporal and spatial dependence of the PAH profile, i.e. the relative concentrations of polycyclic aromatic hydrocarbons, was investigated for ambient airborne particles during a period with moderate photochemical air pollution. The concentrations of 14 PAH were measured; they differed in volatility, sensitivity to atmospheric chemical conversion and contributing sources. Multivariate analysis (principal-component analysis and factor analysis) revealed that temporal dependence was predominantly determined by five factors clearly linked with volatility, reactivity and sources of the PAH, the first being by far the most important. The results, therefore, indicate that volatilization, conversion and a varying contribution of local sources were the major causes of the variation of the profile with time. The contribution of local sources was investigated by comparison of samples that were taken simultaneously at three different sites, one a background site and two sites downwind of traffic. A marked site dependence was found. The comparison suggested that the differences were not only determined by sources, but also by volatilization and/or conversion during residence of the particles in the air.

INTRODUCTION

This paper is concerned with an analysis of the temporal and spatial dependence of the relative concentrations of polycyclic aromatic hydrocarbons in ambient airborne particles, i.e. the PAH profile of the particles. Such an analysis can yield information on the sources contributing to the PAH in the particles and the chemical and physical processes affecting the concentrations of the PAH after emission. Sources are characterized by a particular profile and variations in the contribution of individual sources may be detected in the variation of the profile in the ambient air (Cretney et al., 1985; Daisey et al., 1986; de Raat et al., 1987a). Chemical reaction of PAH after

emission will affect the concentrations of reactive PAH more than those of inert PAH and will therefore result in a decrease of the relative concentrations of the reactive PAH (de Raat et al., 1987a; Nielsen, 1988).

Likewise, evaporation of PAH from, and condensation on, particles will be reflected by a variation of the proportion of the more volatile PAH in the profile (de Raat et al., 1987a). In addition, sampling of the particles may induce an artificial change of the profile. The almost inevitable pressure drop across the filter used in high-volume samplers will accelerate evaporation of the more volatile PAH (Pupp et al., 1974; van Vaeck and van Cauwenberghe, 1978; van Cauwenberghe et al., 1980; König et al., 1980). The exposure of a particle on a filter surface to other particles and an air stream containing reactive gaseous components may result in chemical conversion (Lee et al., 1980; Peters and Seifert, 1980; Brorström et al., 1983; Grosjean, 1983; Alfheim and Lindskog, 1984; Miguel and Andrade, 1986; Lindskog et al., 1987).

In the present study, we investigated the variation of the PAH profiles of a set of samples collected during a period with moderate photochemical air pollution in the western part of the Netherlands. The aim was to determine whether or not sources, chemical conversion and volatilization could be detected from the variation of the profile. We collected at least four 3-h samples during the day to evaluate the possible influence of the diurnal trend of the reactive gaseous pollutants typical of photochemical episodes (Finlayson-Pitts and Pitts, 1986). During part of the study, samples were simultaneously collected at three locations, thereby allowing the influence of local traffic over distant sources to be singled out.

METHODS AND MATERIALS

Sampling

Airborne particles were collected with the aid of Sartorius HV100 high-volume samplers on Sartorius SM13400 glass-fibre filters. Prior to sampling the filters were cleaned by Soxhlet extraction (24 h, methanol). The particle-bearing filters were wrapped in aluminium foil and stored at $-80°$ C. The flow rate was 100 $m^3 h^{-1}$, equivalent to a linear air speed of 0.54 $m s^{-1}$. During the first 8 days, samples were taken on the premises of TNO (TNO-ZP) in the southeastern corner of Delft, about 100 m west of the busy motorway between the Hague and Rotterdam, at ground level. During the last 4 days, additional samples were taken on the roof of the Fire Service building near Delft city centre, about 20 m above ground level; and at Delfgauw, 1.5 km east of TNO-ZP in the grounds of a market gardener, at ground level. For the locations, see Fig. 1.

Sampling began at 08.00 or 05.00 h and continued for 24 h; filters were

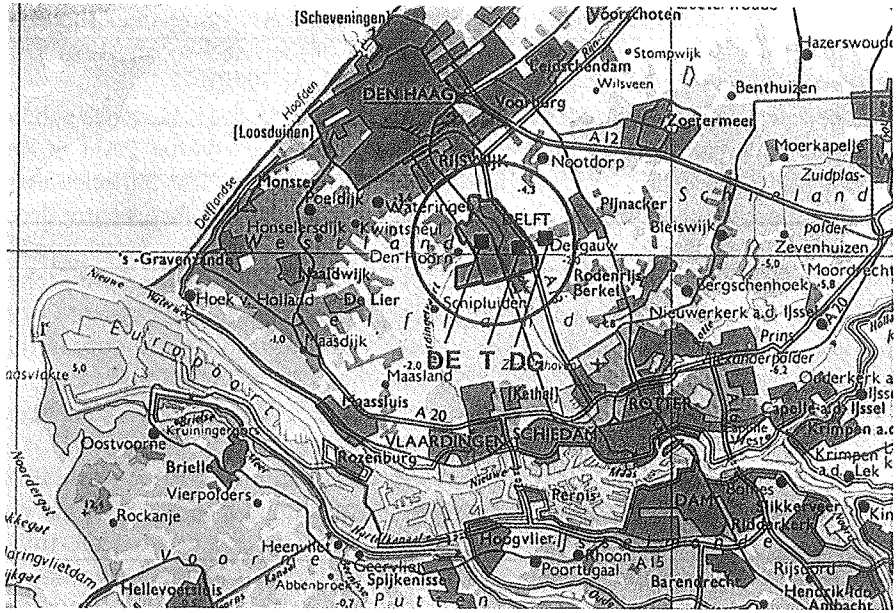

Fig. 1. Map of Delft; locations TNO-ZP (T), Delfgauw (DG) and Delft (DE) are indicated.

changed at 08.00 (if sampling was started at 05.00 h), 11.00, 14.00, 17.00, 20.00 and on some sampling days at 23.00 h. Sampling dates and schedules are shown in Fig. 2. The decision of whether or not to take samples was based on the weather forecast issued by the Royal Dutch Meteorological Institute; when weather favouring photochemical air pollution was predicted (high temperatures, low wind speeds from easterly directions and a clear sky) sampling was commenced. The relevant meteorological conditions are listed in Table 1, which shows that conditions favouring photochemical air pollution occurred on most sampling days; no rain occurred on sampling days.

Extraction

After sampling, the filters were extracted with methanol (Rathburn, HPLC grade) in a Soxhlet apparatus for 8 h (20 cycles) in an atmosphere of nitrogen in the dark. The volume of solvent was reduced to 5 ml with the aid of a rotatory evaporator at 30° C in the dark. Dimethyl sulphoxide (Merck, A.R.) was added to the concentrated extract and the remaining methanol was evaporated. The extracts were tested for mutagenicity within a few days (see De Raat and De Meijere, 1988, for details and results). The remainder of the

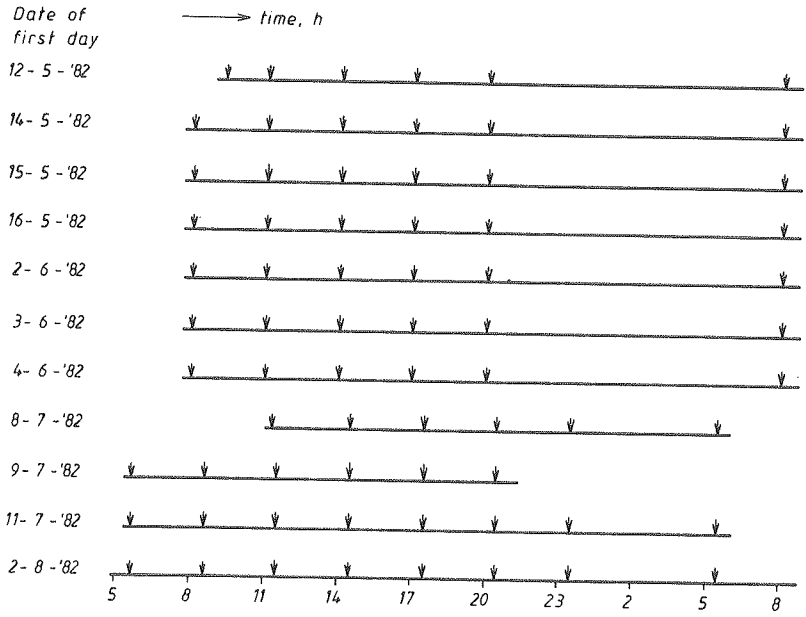

Fig. 2. Date and sampling schedule. (↓) Replacement of filter.

extracts was stored for ~ 3 years at − 80° C in glass scintillation vials wrapped in aluminium foil. Although the storage time is rather long, significant conversion of PAH under these storage conditions is highly improbable. It was expected that the very low temperature would have slowed down chemical reactions and microbiological degradation of the PAH to a negligible level. In addition, the absence of light would have prevented any photochemical conversion of the PAH.

Determination of PAH

The PAH listed in Table 2 were determined with the aid of reversed-phase HPLC. The extracts were injected directly onto the column without prior purification. The dimethyl sulphoxide had no effect on the outcome of the analysis. Stationary phase: Supelco-PAH (250 × 4.6 mm). Mobile phase: 75 w/w % methanol in water to 100 % methanol (Rathburn, HPLC grade). Detection: based on fluorescence, excitation at 250 nm and emission at > 390 nm. The PAH were identified and their concentrations determined with the aid of a mixture of external reference compounds consisting of PAH purchased from various commercial firms. The identities and concentrations of 10 PAH in this mixture were thoroughly checked with a standard mixture

TABLE I

Summary of meteorological data

Experiment No.	Wind direction																Wind speed ($m\,s^{-1}$)	Maximum temperature (°C)
	N	NNE	NE	ENE	E	ESE	SE	SSE	S	SSW	SW	WSW	W	WNW	NW	NNW		
1			11	9	3	1											3.3	18.7
2			8	8	9												3.6	22.2
3		1	1	6	8	1	1						1	1		1	2.7	25.4
4	2	1		1									7	3	3		1.9	22.8
5	4	1	3	2	8	1			1					1	2	1	2.8	27.7
6	4	1	4	4		1			4	1	1		2		1	1	2.4	28.0
7	2	3	8	3			1		1					1	1	2	2.1	27.6
8				1	14	3	1										3.8	25.8
9					4	9	3										5.5	30.9
10		1	17	6	1												5.5	25.7
11		1	3	6	2	8	4								1		2.6	29.6

Wind direction, frequency of hourly averages.
Wind speed, average of hourly averages.
Maximum temperature, maximum hourly average.

TABLE 2

PAH for which concentrations were determined

PAH	Abbreviation
Phenanthrene	FE
Fluoranthene	FL
Pyrene	PY
Triphenylene[a]	TF
Benz[*a*]anthracene	BA
Chrysene	CH
Benzo[*e*]pyrene[a]	BeP
Perylene[a]	PE
Benzo[*b*]fluoranthene	BbF
Benzo[*k*]fluoranthene	BkF
Benzo[*a*]pyrene	BaP
Benzo[*g,h,i*]perylene	BG
Indeno[*1,2,3-c,d*]pyrene	IN
Anthanthrene[a]	AN

[a]Analysis not based on NBS SRM 1647.

from the National Bureau of Standards (NBS SRM 1647). The NBS mixture did not contain triphenylene, benzo[e]pyrene, perylene and anthanthrene, therefore analysis for these four PAH was not based on this standard.

Multivariate analysis

The variation of the PAH profile with time was analyzed using principal-component analysis and factor analysis with orthogonal varimax rotation.

RESULTS

Table 3 lists the results of the principal-component analyses for all samples. It shows that the PAH fall into two groups, one of lower molecular weight, and the other of higher molecular weight. The concentrations of the former correlate with the first two principal components, whereas those of the latter correlate only with the first principal component. As will be discussed in the next section, this difference between the two groups reflects differences in volatility. Apparently, volatility strongly affects the concentrations of PAH in the first group. We therefore restricted further analysis of the results to the PAH of the second group, viz. from BA to AN. The concentrations of these PAH are strongly correlated.

The relative invariability of the profile of the less volatile PAH implies that variation in concentration of one or a few PAH gives a reasonable indication

TABLE 3

Results of principal-component analysis of PAH concentrations

	PAH	TNO-ZP			Delfgauw			Delft		
		1	2	3	1	2	3	1	2	3
	FE	0.43	0.86	− 0.16	0.37	0.88	0.18	0.54	0.79	− 0.17
	FL	0.84	0.49	0.11	0.49	0.79	− 0.25	0.79	0.57	0.10
	PY	0.86	0.28	0.38	0.47	0.83	− 0.28	0.78	0.62	0.01
	TF	0.88	0.34	− 0.02	0.73	0.34	0.50	0.86	0.24	0.33
	BA	0.89	− 0.17	0.40	0.95	− 0.02	0.21	0.98	0.01	− 0.15
	CH	0.99	− 0.05	− 0.08	0.99	− 0.04	− 0.05	0.98	− 0.07	0.06
	BeP	0.93	− 0.16	− 0.24	0.92	− 0.17	− 0.29	0.95	− 0.26	0.11
	PE	0.90	− 0.23	0.35	0.97	− 0.17	0.03	0.96	− 0.10	− 0.18
	BbF	0.96	− 0.11	− 0.22	0.94	− 0.24	− 0.19	0.94	− 0.27	0.10
	BkF	0.97	− 0.13	− 0.15	0.96	− 0.24	− 0.09	0.97	− 0.23	0.03
	BaP	0.97	− 0.18	0.11	0.97	− 0.22	0.02	0.97	− 0.20	− 0.10
	BG	0.95	− 0.11	− 0.10	0.98	0.04	0.16	0.99	− 0.13	− 0.02
	IN	0.96	− 0.08	− 0.27	0.98	− 0.17	− 0.07	0.97	− 0.22	0.05
	AN	0.92	− 0.18	− 0.12	0.96	− 0.18	0.07	0.96	− 0.15	− 0.19
Cumulative variance percentage		80.90	90.82	95.85	74.04	91.79	96.28	82.85	95.14	97.13

The analysis was carried out using all samples.
Listed are the cumulative variance of the first three principal components as a percentage of the summed variance and the correlation of the components with the PAH concentrations.

of the variations in concentrations of the whole group. A detailed presentation of results has therefore been restricted to the concentrations of BaP and BbF. The concentrations of these PAH are indicated in Fig. 3 for location TNO-ZP. These two PAH were chosen because of their relatively high concentrations and for their different reactivities; they represent extremes in the range of reactivities of the PAH generally found in the atmosphere (Nielsen, 1984). Figure 3 shows that the concentrations vary widely from day to day and also during the day. No clear-cut trend during the day is noticeable. The very high concentrations in one sample taken at night are striking.

Mean concentrations and coefficients of variation are given in Tables 4 and 5, which clearly show the wide variation of concentrations. They also reveal the mean contribution from local sources when Delfgauw is compared with the other locations and when the four sampling periods between 08.00 and 20.00 h are compared. However, the contribution differs for different PAH. Large contributions were found for BA and BG, whereas the BeP contribution from local sources is negligible. The contributions from local sources are also evidenced in Fig. 4, which clearly shows that they are characterized by a PAH profile differing from that present at Delfgauw. This difference between

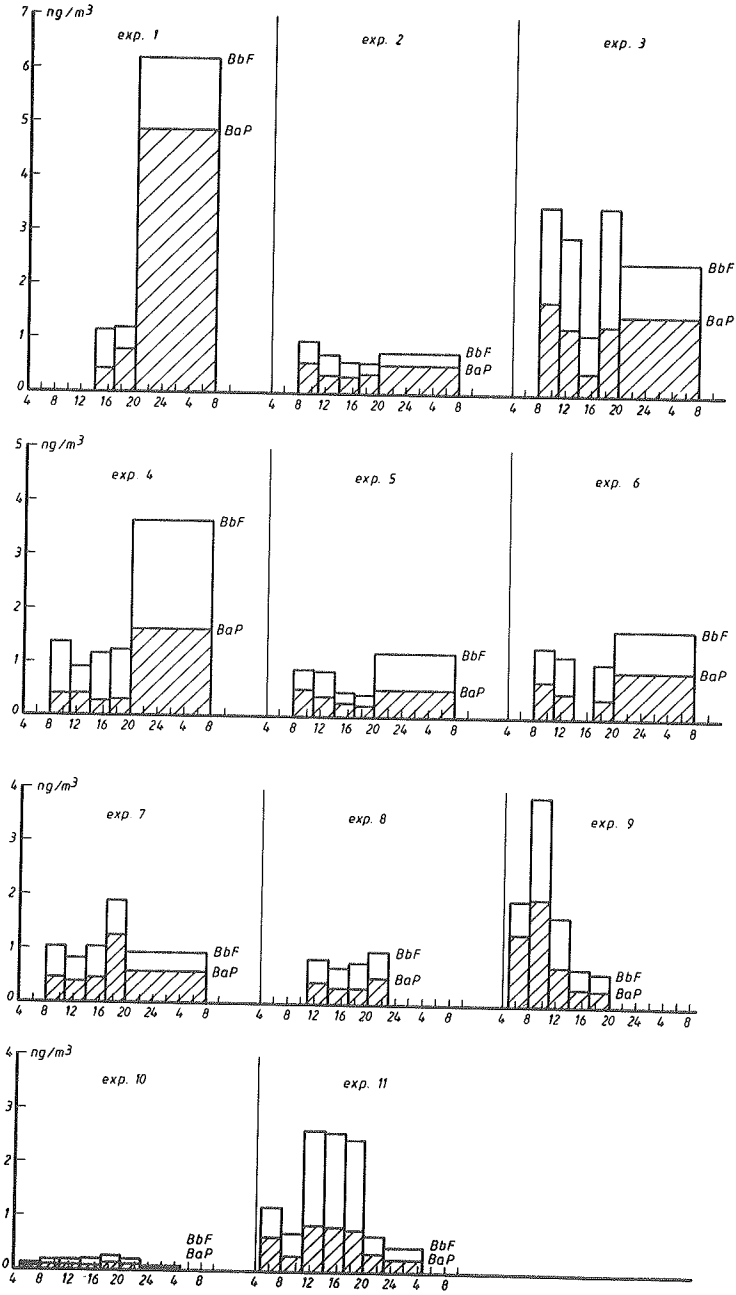

Fig. 3. Concentrations of benzo[*a*]pyrene (BaP) and benzo[*b*]fluoroanthene (BbF) in ambient airborne particles at TNO-ZP.

TABLE 4

Mean PAH concentrations and coefficients of variation

Sampling location		All samples									
		BA	CH	BeP	PE	BbF	BkF	BaP	BG	IN	AN
TNO-ZP	$\bar{x}$	60.3	143.8	82.4	11.5	130.9	48.6	64.3	130.8	94.7	8.7
All experiments	v	148	83	87	157	89	92	113	86	80	91
TNO-ZP											
Experiments 9,	$\bar{x}$	38.5	110.6	70.4	7.7	105.6	37.8	46.9	92.4	72.1	6.9
10 and 11	v	87	81	99	95	96	97	100	79	87	99
Delfgauw											
Experiments 9,	$\bar{x}$	30.6	97.2	60.3	6.3	93.9	34.2	40.3	65.3	59.7	6.1
10 and 11	v	102	87	108	109	107	109	114	106	101	112
Delft											
Experiments 9,	$\bar{x}$	59.8	144.7	65.9	9.5	113.1	43.6	56.0	115.8	80.7	9.0
10 and 11	v	74	67	89	89	84	85	92	79	82	97
		Samples taken between 08.00 and 20.00 h									
		BA	CH	BeP	PE	BbF	BkF	BaP	BG	IN	AN
TNO-ZP	$\bar{x}$	48.5	140.5	78.5	8.7	129.2	46.9	56.7	117.9	91.1	7.8
All experiments	v	65	66	79	73	77	76	79	63	69	77
TNO-ZP											
Experiments 9,	$\bar{x}$	41.7	134.0	85.0	8.7	135.6	46.8	54.4	103.5	86.2	7.3
10 and 11	v	87	80.5	102	95	93	96	102	80	88	102
Delfgauw											
Experiments 9,	$\bar{x}$	38.3	127.3	82	8.1	130.4	47	54.3	81.7	79.7	7.8
10 and 11	v	106	84	101	109	98	102	110	111	97	113
Delft											
Experiments 9,	$\bar{x}$	65.2	166.5	79.1	10.7	138.9	52.6	66.0	132.1	96.2	10.1
10 and 11	v	79	72	93	95	86	88	97	84	87	102

$\bar{x}$, mean, nanograms per 100 m^3.
v, coefficient of variation of $\bar{x}$ (standard deviation expressed as percentage of mean).

profiles was found for all individual sampling periods, even when samples of the imcomplete Experiment 8 were taken into account.

The mean PAH profiles are shown in Fig. 5, in which the concentrations are normalized to give a sum of 100 for each sample. This procedure renders the mean profile independent of differences in the sum between experiments. The figure shows the rather marginal effect of local sources on the mean profile.

TABLE 5

Mean PAH concentrations[a] in the particles for the separate sampling periods between 08.00 and 20.00 h at TNO-ZP

	BA	CH	BeP	PE	BbF	BkF	BaP	BG	IN	AN
08.00–11.00 h	65.7	166.4	92.7	11.9	154.6	57.7	74.3	152.9	112.8	11.0
11.00–14.00 h	43.2	143.2	80.8	8.3	133.0	47.7	54.2	106.2	89.4	7.6
14.00–17.00 h	36.0	115.3	62.0	6.4	102.4	35.9	40.9	85.8	69.1	5.6
17.00–20.00 h	54.4	148.7	82.8	8.9	134.7	49.4	61.0	135.2	99.6	7.7

[a]Mean concentrations per 100 m^3

Principal-component analysis indicated a very marked effect of a common factor. To detect any other factors that might be obscured by the common factor, a factor analysis was performed. The results are listed in Table 6. Three to four factors can be distinguished. The rotated matrices reveal a clear-cut effect of the isolation of the results obtained during the day (samples taken between 08.00 and 20.00 h). When the results for all samples are taken into account, the following groups of PAH can be discerned: CH, BeP, BbF, BkF and IN; BA, PE and BaP; AN. The first group consists of rather inert PAH, whereas those of the latter two groups are more reactive (Nielsen, 1984). Exclusion of the samples taken at night leads to one group of reactive PAH, and a group containing BG.

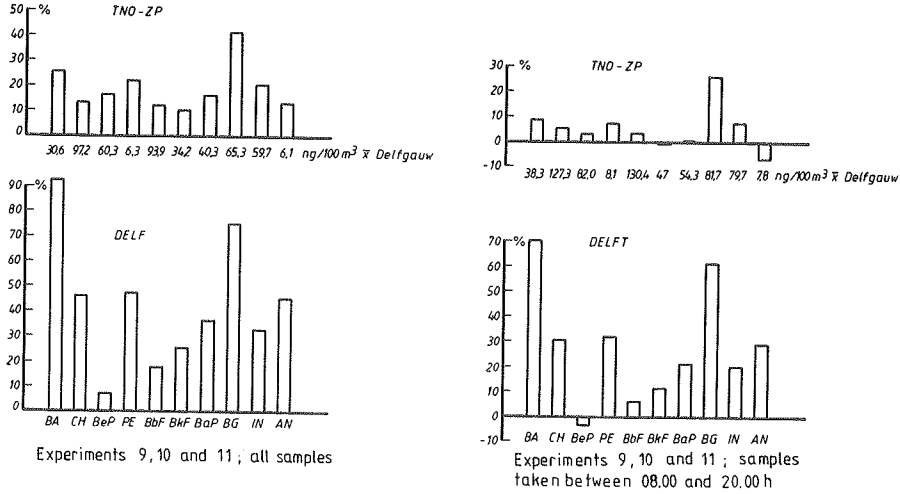

Fig. 4. Contribution of local sources to PAH concentrations in ambient airborne particles. The contributions are depicted as a percentage of the concentrations at Delfgauw ($\bar{x}$).

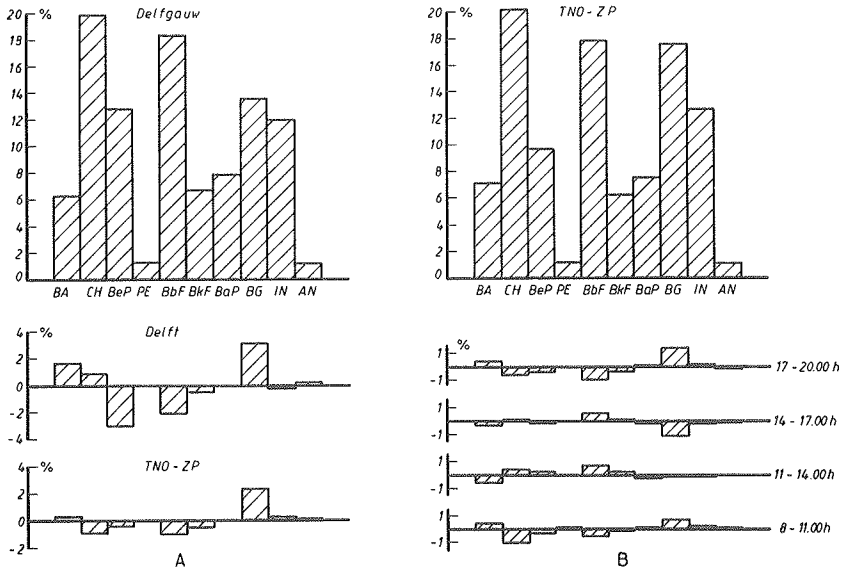

Fig. 5. Mean proportions of PAH and influence of local sources. (A) Mean proportions at Delfgauw and the differences from the mean proportions at the other locations. All samples of Experiments 9, 10 and 11. (B) Mean proportion at TNO-ZP, and influence of sampling period. Samples taken between 08.00 and 20.00 h for all experiments.

The three locations were compared by carrying out a factor analysis for the concentrations of all samples of the last four experiments. The results (Table 7) were very similar for the three locations. The results for Delfgauw and TNO-ZP were almost identical. Although PAH concentrations of the samples taken at night were included, only two clear-cut factors were found: one is more strongly related to the chemically reactive PAH, and the other to the more inert PAH.

DISCUSSION

Principal-component analysis of the PAH concentrations reveals a clear-cut effect of two factors on the concentrations of the lighter PAH (four rings or less), whereas only one factor affects the concentrations of the heavier PAH. A similar result was found for the PAH concentrations in the industrial and urban area of Rijnmond, the Netherlands (de Raat et al., 1987a). An obvious explanation for this division of PAH into two groups is variation of the vapour/solid ratio of the lighter PAH in ambient air. Another explanation may be the evaporation to which PAH are subject during sampling (Pupp et al., 1974; Vaeck and van Cauwenberghe, 1978; van Cauwenberghe et al., 1980;

TABLE 6

Results of factor analysis for location TNO-ZP

PAH	All samples				Samples taken between 08.00 and 20.00 h[a]			
BA	− 0.38	0.89	− 0.23	0.08	− 0.39	0.79	0.46	0.05
CH	− 0.78	0.55	− 0.25	0.10	− 0.75	0.49	0.44	− 0.05
BeP	− 0.86	0.40	− 0.29	0.01	− 0.87	0.43	0.22	0.05
PE	− 0.43	0.86	− 0.25	0.07	− 0.55	0.79	0.25	0.05
BbF	− 0.86	0.44	− 0.26	0.05	− 0.83	0.45	0.32	− 0.04
BkF	− 0.80	0.51	− 0.28	0.08	− 0.77	0.54	0.34	− 0.05
BaP	− 0.61	0.71	− 0.35	0.07	− 0.63	0.69	0.35	− 0.05
BG	− 0.64	0.51	− 0.46	0.33	− 0.53	0.58	0.62	− 0.01
IN	− 0.82	0.38	− 0.38	0.21	− 0.72	0.52	0.45	− 0.03
AN	− 0.59	0.46	− 0.66	0.04	− 0.52	0.80	0.25	− 0.12
Eigen value	4.87	3.58	1.31	0.19	4.52	3.90	1.49	0.03

The table shows the factor matrix and Eigen values of the factors. Varimax rotated.
[a]Only experiments with four concentrations for each PAH.

TABLE 7

Results of factor analysis for the three locations and the samples from the last four experiments

PAH	Delfgauw		TNO-ZP		Delft	
	1	2	1	2	1	2
BA	0.92	0.39	0.92	0.39	0.51	0.82
CH	0.65	0.74	0.59	0.80	0.73	0.59
BeP	0.37	0.92	0.39	0.91	0.83	0.55
PE	0.80	0.57	0.83	0.54	0.53	0.84
BbF	0.46	0.89	0.45	0.89	0.86	0.49
BkF	0.59	0.80	0.58	0.81	0.80	0.58
BaP	0.75	0.65	0.77	0.63	0.69	0.72
BG	0.88	0.46	0.86	0.47	0.69	0.69
IN	0.65	0.76	0.64	0.76	0.81	0.57
AN	0.82	0.54	0.82	0.54	0.55	0.83

Factor matrices, varimax rotated.

König et al., 1980). The extent of this evaporation depends on the pressure drop across the filter and on the sampling temperature, and therefore varies with the climatic conditions and with the concentration of particles in ambient air.

In several studies, the PAH were sampled by combining filters and adsorbents, usually polyurethane foam (Thrane and Mikalsen, 1981; Keller and Biddleman, 1984; de Raat et al., 1987b). The results confirm those of the present study, in that the PAH contributing most strongly to the variation of the second PC are those adsorbed by the foam. The present study therefore shows indirectly that filtration is an efficient sampling technique for PAH with more than four rings as well as some four-ring PAH, e.g. benz[*a*]anthracene and chrysene.

A comparison of the samples collected simultaneously at the three locations shows that local emissions (produced exclusively by traffic) are characterized by a PAH profile differing from that of the particles sampled up wind. Delfgauw is much further away from source areas than is TNO-ZP from the motorway, or the Fire Service from the city centre. Table 1 clearly shows that Delfgauw can, in fact, be regarded as an upwind location for Delft and TNO-ZP. Several explanations can be put forward for the difference between the profiles of local and the upwind emissions. One obvious explanation is that the sources are different. In the Netherlands, the most important source is traffic, at least during the period of year when the sampling was carried out. It has, however, been shown that particulate PAH are transported over long distances (Lunde and Björseth, 1977; Björseth and Lunde, 1979), therefore the atmosphere over the Netherlands is polluted with PAH from various European industrial areas. The combustion of coal and oil for industrial purposes is a major source in these areas, emitting a PAH profile differing from that of traffic (Cretney et al., 1985; Daisey et al., 1986).

Another factor that could give rise to differences in PAH profiles is volatility. It is to be expected that vapour/solid ratios depend on the distance between source and sampler. This phenomenon may be responsible for the strong increase of benz[a]anthracene and chrysene concentrations. If so, a longer residence time would result in more vaporization.

The residence time may also affect the extent of conversion of the PAH. Several laboratory studies lead to the conclusion that PAH in ambient air are subject to conversion (Falk et al., 1956; Tebbens et al., 1966; Butler and Crossley, 1981; Nielsen et al., 1983; Pitts, 1983; Miguel, 1984; Ramdahl and Bjørseth, 1984). Evidence supporting this has recently been presented. A comparison of PAH profiles of samples taken at different locations showed that concentrations of relatively reactive PAH are subject to an additional source of variation (de Raat et al., 1987a and Nielsen, 1988). In addition, Nielsen and Ramdahl (1986) and Pitts (1987) showed the presence of 2-nitropyrene and 2-nitrofluoranthene, compounds that do not occur in emissions, and therefore must be formed by nitration of pyrene and fluoranthene in ambient air; most probably by reactions with hydroxyl radicals in the vapour phase.

It is difficult to determine which of these factors (source patterns, volatility and conversion) cause the differences between upwind and downwind PAH profiles and, if they do, what the extent of their effect is. If the increase was positively related to reactivity, a conversion effect would be very probable. However, the results shown in Fig. 5 do not point to such a relation, which implies that differences in source patterns also play a part. According to the literature, the PAH profile of local emissions is, in fact, rather characteristic of traffic emissions; the different profile found at Delfgauw suggests background pollution by industrial oil and coal combustion (Cretney et al., 1985; Daisey et al., 1986), confirming that differences in source pattern do indeed play a part. In this context, the strong increase in concentration of benzo[*g*,*h*,*i*]perylene is of particular importance. Its concentrations in local emissions are some 10 times those of benzo[*e*]pyrene. It follows that most of the PAH-containing particles polluting the air of the study area are of two very different types: rather old particles of industrial origin, and young particles emitted by local traffic.

Principal-component analysis does no more than reveal the major effect of PAH volatility on PAH profiles. The concentrations of the less volatile PAH were all strongly correlated with those of the first principal components. Accordingly, the profile of these PAH is fairly stable. In spite of this, factor analysis revealed that three or four factors had an effect on the profile of these PAH. One of these is linked with the concentrations of benzo[*g*,*h*,*i*]perylene. In view of the above considerations, it is likely that this factor represents the varying contribution from local sources.

Another factor is linked with the concentrations of anthanthrene, and is prominent only when the samples taken at night are taken into account. The most important factors are linked with two groups of PAH: one comprising the reactive PAH, benz[*a*]anthracene, perylene, benzo[*a*]pyrene and anthanthrene, and the other comprising chrysene, benzo[*e*]pyrene, the benzofluoranthenes and indeno[*c*,*d*]pyrene. This division into two groups suggests that PAH profiles are affected by chemical conversion, because it agrees with the grouping according to chemical reactivity on the basis of laboratory experiments. The separate anthanthrene factor may also be the result of conversion because, among the PAH studied, anthanthrene is the most readily nitrated (Nielsen, 1984). If the anthanthrene factor does result from conversion, two conversion processes may be responsible, one of which proceeds at night, and affects the concentration of anthanthrene in particular.

It must be emphasized that sampling may also cause conversion. The multivariate analysis gives an insight into the factors determining the temporal variation of the profile and can therefore not discriminate between this artifactual conversion and conversion taking place before sampling

(atmospheric conversion). Variation in concentrations of reactive gaseous components may result in variation of the extent of artifactual conversion.

Comparison of locations is necessary to demonstrate atmospheric conversion. Using location comparisons, atmospheric conversion was indicated by two previous studies (de Raat et al., 1987a; Nielsen, 1988). Comparison of locations in the present study is too strongly affected by differences in sources to distinguish atmospheric conversion. Any comparison of the three locations on the basis of factor analysis must be interpreted with a great deal of caution, because of the small number of samples investigated. Factor analysis gives almost the same results for both Delfgauw and TNO-ZP. This suggests that the PAH profiles are affected by the same factors to the same extent at these locations. The profiles do not reflect any contribution from local sources, and no anthanthrene factor is found. This analysis included all samples in the last four experiments; during these experiments more samples were taken at night, samples being taken between 05.00 and 08.00 h and between 20.00 and 23.00 h. Table 4 shows that inclusion of the samples taken at night obscures the benzo[*g*,*h*,*i*]perylene factor, which is postulated to represent the varying contributions of local traffic. The absence of a separate anthanthrene factor may be due to the absence of very high PAH concentrations such as those found in Experiments 1, 3 and 4. Factor analysis for Delfgauw and TNO-ZP revealed only two factors. The PAH clearly fell into two groups: one of relatively reactive PAH and one of relatively inert PAH. It thus seems that chemical conversion still affects the profile of the older particles sampled upwind, which is unexpected because of their long residence time in the air. This leaves conversion caused by sampling as an explanation; it is, apparently, not restricted to freshly emitted PAH. The factor matrix for Delft differs only slightly from those for the other two sites. Surprisingly, no separate benzo[*g*,*h*,*i*]perylene factor is found at Delft, notwithstanding the clear contribution of local sources at this location.

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REFERENCES

Alfheim, I. and A. Lindskog, 1984. A comparison between different high volume sampling systems for collecting ambient airborne particles for mutagenicity testing and for analysis of organic compounds. Sci. Total Environ., 34: 203–222.

Bjørseth, A. and G. Lunde, 1979. Long-range transport of polycyclic aromatic hydrocarbons. Atmos. Environ., 13: 45–53.

Brorström, E., P. Grennfelt and A. Lindskog, 1983. The effect of nitrogen dioxide and ozone on the decomposition of particle-associated polycyclic aromatic hydrocarbons during sampling from the atmosphere. Atmos. Environ., 17: 601–605.

Butler, J.D. and P. Crossley, 1981. Reactivity of polycyclic aromatic hydrocarbons adsorbed on soot particles. Atmos. Environ., 15: 91–94.

Cretney, J.R., H.L. Lee, G.J. Wright, W.H. Swallow and M.C. Taylor, 1985. Analysis of polycyclic aromatic hydrocarbons in air particulate matter from a lightly industrialized urban area. Environ. Sci. Technol., 19: 397–404A.

Daisey, J.M., J.L. Cheney and P.L. Lioy, 1986. Profiles of organic particulate emissions from air pollution sources: status and needs for receptor source apportionment modeling. J. Air Pollut. Control Assoc., 36: 17–33.

De Raat, W.K., S.A.L.M. Kooijman and J.W.J. Gielen, 1987a. Concentrations of polycyclic hydrocarbons in airborne particles in the Netherlands and their correlation with mutagenicity. Sci. Total Environ., 66: 95–114.

De Raat, W.K., F.L. Schulting, E. Burghardt and F.A. de Meijere, 1987b. Application of polyurethane foam for sampling volatile mutagens from ambient air. Sci. Total Environ., 63: 175–189.

De Raat, W.K. and F.A. de Meijere, 1988. The mutagenicity of ambient air particles from local traffic and distant sources during episodes with moderate photochemical air pollution. Sci. Total Environ., 73: 159–179.

Falk, H.L., I. Markul and P. Kotin, 1956. Aromatic hydrocarbons. IV. Their fate following emission into the atmosphere and experimental exposure to washed air and synthetic smog AMA Arch. Ind. Hyg. Occup. Med., 13: 13–17.

Finlayson-Pitts, B.J. and J.N. Pitts Jr, 1986. Atmospheric Chemistry. Wiley Interscience Publication, John Wiley and Sons, New York.

Grosjean, D., 1983. Polycyclic aromatic hydrocarbons in Los Angeles air from samples collected on Teflon, glass and quartz filters. Atmos. Environ., 17: 2565–2573.

Keller, D. and T.F. Biddleman, 1984. Collection of airborne polycyclic aromatic hydrocarbons and other organics with a glass fiber filter–polyurethane foam system. Atmos. Environ., 17: 837–845.

König, J., W. Funcke, E. Balfanz, B. Grosch and F. Pott, 1980. Testing a high volume air sampler for quantitative collection of polycyclic aromatic hydrocarbons. Atmos. Environ., 14: 609–613.

Lee, F.S.C., W.R. Pierson and J. Ezike, 1980. The problem of PAH degradation during filter collection of airborne particles — an evaluation of several commonly used filter media. In: A. Bjorseth and A. Dennis (Eds), Polynuclear Aromatic Hydrocarbons: Chemistry and Biological Effects, Batelle Press, Columbus, Ohio, pp. 543–564.

Lindskog, A., E. Brorström-Lundén, I. Alfheim and I. Hagen, 1987. Chemical transformation of PAH on airborne particles by exposure to NO_2 during sampling: a comparison between two filter media. Sci. Total Environ., 61: 51–57.

Lunde, G. and A. Bjørseth, 1977. Polycyclic aromatic hydrocarbons in long-range transported aerosols. Nature, 268: 518–519.

Miguel, A.H., 1984. "Atmospheric" reactivity of particulate polycyclic aromatic hydrocarbons collected in an urban tunnel. Sci. Total Environ., 36: 305–311.

Miguel, A.H. and J.B. de Andrade, 1986. Desorptivity versus chemical reactivity of polycyclic aromatic hydrocarbons (PAHs) in atmospheric aerosols collected on quartz fiber filters. Int. J. Environ. Anal. Chem., 26: 265–278.

Nielsen, T., 1984. Reactivity of polycyclic aromatic hydrocarbons towards nitrating species. Environ. Sci. Technol., 18: 157–163.

Nielsen, T., 1988. The decay of benzo[*a*]pyrene and cyclopenteno[*c*,*d*]pyrene in the atmosphere. Atmos. Environ., 22: 2249–2254.

Nielsen, T. and T. Ramdahl, 1986. Discussion. Determination of 2-nitrofluoranthene and 2-nitropyrene in ambient particulate matter: Evidence for atmospheric reactions. Atmos. Environ., 20: 1507

Nielsen, T., Th. Ramdahl and A. Bjørseth, 1983. The fate of airborne polycyclic organic matter. Environ. Health Perspect., 47: 103–114.

Peters, J. and B. Seifert, 1980. Losses of benzo[*a*]pyrene under conditions of high volume sampling. Atmos. Environ., 14: 117–119.

Pitts, J.N. Jr, 1983. Formation and fate of gaseous and particulate mutagens and carcinogens in real and simulated atmospheres. Environ. Health Perspect., 47: 115–140.

Pitts, J.N. Jr, 1987. Nitration of gaseous polycyclic aromatic hydrocarbons in simulated and ambient urban atmospheres: a source of mutagenic nitroarenes. Atmos. Environ., 21: 2531–2547.

Pupp, C., R.C. Lao, J.J. Murray and R. Pottie, 1974. Equilibrium vapour concentration of some polycyclic aromatic hydrocarbons, As_4O_6 and SeO_2 and the collection efficiencies of these air pollutants. Atmos. Environ., 8: 915–925.

Ramdahl, T. and A. Bjorseth, 1984. Nitration of polycyclic aromatic hydrocarbons adsorbed to different carriers in a fluidized bed reactor. Chemosphere, 13: 527–534.

Tebbens, B.D., D. Jerome and M. Mukai, 1966. Fate of arenes incorporated with airborne soot. Am. Ind. Hyg. Assoc. J., 27: 415–422.

Thrane, K.E. and A. Mikalsen, 1981. High-volume sampling of airborne polycyclic aromatic hydrocarbons using glass fibre and polyurethane foam. Atmos. Environ., 15: 909–918.

Van Cauwenberghe, K., L. van Vaeck and J.N. Pitts, 1980. Physical and chemical transformations of organic pollutants during aerosol sampling. In: B. Versino and H. Ott (Eds). Proc. First European Symposium Physico-Chemical Behaviour of Atmospheric Pollutants. EUR 6621. Commission of the European Communities, Luxembourg, pp. 194–209.

Van Vaeck, L. and K. van Cauwenberghe, 1978. Cascade impactor measurements of the size distribution of the major classes of organic pollutants in atmospheric particulate matter. Atmos. Environ., 12: 2229–2239.

CHAPTER 10

CONTRIBUTION OF PAH AND SOME OF THEIR NITRATED DERIVATIVES TO THE MUTAGENICITY OF AMBIENT AIRBORNE PARTICLES AND COAL FLY ASH

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CONTRIBUTION OF PAH AND SOME OF THEIR NITRATED DERIVATIVES TO THE MUTAGENICITY OF AMBIENT AIRBORNE PARTICLES AND COAL FLY ASH

W. K. de Raat[1], J. P. Boers[2], G. L. Bakker[2], F. A. de Meijere[2], A. Hooimeijer[2], P. H. M. Lohman[3] and G. R. Mohn[3and4]

1. Medical Biological Laboratory TNO, P.O. Box 5815 Rijswijk, the Netherlands 2. Institute of Environmental Sciences TNO, P.O. Box 6011, 2600 JA Delft, the Netherlands; 3. MGC-Department of Radiation Genetics and Chemical Mutagenesis, University of Leiden, Wassenaarseweg 72, 2333AL Leiden, the Netherlands; 4. Laboratory of Carcinogenesis and Mutagenesis, National Institute of Public Health and Environmental Protection, P.O. Box 1, 3720 Bilthoven, the Netherlands

ABSTRACT

In order to investigate the chemical nature and diversity of the mutagens in ambient airborne particles (AAP) and coal fly ash (CFA), extracts of these particles were subjected to bioassay-directed fractionation. Open-column as well as high-pressure liquid chromatography were used as fractionation techniques after purification by means of liquid-liquid partition. Extracts and fractions were tested with the Salmonella/microsome test using various tester strains, among them TA98NR and analysed for polycyclic aromatic hydrocarbons (PAH) and some nitrated PAH.

Seven distinct groups of mutagens could be discerned in AAP. Two of them were identified as PAH and mono-nitro PAH; the first accounted for about 5% of the total effect in TA98 with S9 and about 20% of the total effect in TA100 with S9, while these percentages were for the latter about 12, 24, 14 and 13 for respectively TA98 with and without S9 and TA100 with and without S9. A significant contribution of dinitropyrenes (possibly together with other multiply nitrated PAH) could be ruled out. Five more polar groups of mutagens could be isolated in AAP, which showed clearly nitroreductase-dependent direct mutagenicity. Besides direct-acting nitrocompounds, these groups also comprised indirect-acting compounds (S9 dependent), possibly, but not necessarily, these are also nitrocompounds.

The distribution of the mutagenicity over the various active chromatographical fractions was clearly strain and S9 dependent. The use of strains TA100 and TA97 pointed to the importance of indirect mutagens, also for the more polar fractions. PAH did not significantly contribute to the mutagenicity of CFA. These particles contained two groups of

mutagens that were not found in AAP: a group the effect of which is most probably caused by dinitropyrenes and comparable compounds and a polar group containing nitro-reductase dependent direct mutagens.

INTRODUCTION

More than 15 years ago the first studies were published which showed that ambient airborne particles (AAP) possess mutagenic properties and many have followed since (Talcott and Wei, 1977; Pitts et al., 1979; Dehnen et al., 1977; Teranishi et al., 1978; Commoner et al., 1978; Tokiwa et al., 1980; Alfheim and Møller, 1979; Møller and Alfheim, 1980; Alink et al., 1983; De Raat, 1983). In most of these studies extracts of the particles in organic solvents were tested with the Salmonella/microsome test developed by Ames and coworkers (Ames et al., 1975). The effects in this test pointed to the presence of at least two groups of mutagens: direct ones, i.e. compounds that need not to be activated by the mammalian tissue homogenate (S9 fraction) used in this test, and indirect ones, i.e. compounds which depend for their effect on the presence of S9 fraction. In general a predominant contribution of the direct mutagens was observed. Thus, the effects did not indicate an important contribution of the purely indirectly mutagenic polycyclic aromatic hydrocarbons.

An indication about the identity of the direct mutagens was obtained by using tester strains with a specific insensitivity to compounds which are activated via bacterial reductive metabolism of nitrogroups (Rosenkranz and Mermelstein, 1983 and 1985; Tokiwa and Onishi, 1986). The extracts of the particles showed markedly reduced effects in these strains, compared to the normally sensitive parental strains, which indicated a substantial contribution of such nitrocompounds (Wang et al., 1980; Alfheim, 1982; De Raat et al., 1988). Other authors investigated the effect of a specific reduction of nitro groups with sodium borohydride on the mutagenicity and found a substantial decrease of the effect, thus confirming the results obtained with the insensitive strains (Talcott and Harger, 1981). The identity of the mutagens was further investigated by mutagenicity testing and chemical analysis of fractions of the extracts which were prepared by liquid-liquid partitioning and/or chromatography. This so-called bioassay-directed fractionation directly showed the small contribution of the PAH; various classes of more polar, directly mutagenic compounds were found to be present, the effects of which appeared to depend on the presence of a nitrogroup, among them the nitrated derivatives of PAH (Alfheim et al., 1985).

Obviously, these results led to a re-evaluation of the rôle of PAH as carcinogenic constituents of AAP. This notwithstanding, there exists clear-cut evidence for, another, albeit less direct rôle of the PAH. It has amply been demonstrated that oxygenated and nitrated PAH derivatives can be strong direct mutagens (Rosenkranz and Mermelstein, 1983; Mermelstein and Rosenkranz, 1985; Tokiwa and Onishi, 1986). PAH can be converted to such compounds during combustion, emission and residence in ambient air (Pitts, 1983; Pitts, 1987; Nielsen et al., 1983; Van Cauwenberghe, 1987; Valerio et al., 1984). Moreover, the directly mutagenic groups identified sofar in the extracts are nitrated and oxygenated PAH (Ramdahl et al., 1982; König et al., 1983; Nishioka et al., 1986 and 1988; Tanner et al., 1983; Gibson, 1982; Nielsen et al., 1984). Taken together, these results suggest that the mutagenicity of the particles ultimately finds its origin in the formation of PAH and other polycyclic compounds during combustion. These compounds are subsequently converted to directly mutagenic derivatives, either before emission or during their residence in the air.

Sofar, the number of different classes of mutagens in AAP remains obscure. Moreover, it is uncertain whether all the directly mutagenic classes consist of compounds the effects of which are determined by the presence of nitrogroups, although the strong nitroreductase dependence of the complete extracts suggests this to be the case (De Raat, 1988). Both aspects were adressed in the present study. By means of bioassay-directed fractionation based on various fractionation techniques we tried to isolate as many mutagenic classes as possible and tested these for mutagenicity with *Salmonella typhimurium* TA98NR, a nitroreductase-deficient strain, alongside with its parental strain TA98.

The mutagenicity of the particles has sofar nearly exclusively been investigated with Salmonella strains TA1538 and, in particular, TA98. It is well known that these strains are relatively sensitive to directly mutagenic nitrocompounds (Rosenkranz and Mermelstein, 1983). However, other strains may show a higher sensitivity to other directly mutagenic compounds or to indirect mutagens, e.g. PAH. The use of these strains might relativize the importance of the directly mutagenic nitrocompounds. Therefore, strains TA100 and TA97 were used in the present study in addition to the TA98 strains.

The presence of PAH and their mononitroderivatives in AAP is well established. However, although it has been demonstrated that both groups of mutagens do contribute to the mutagenicity of the particles, no accurate estimations of this contributions are as yet available. We tried to achieve this, by testing well defined isolated fractions with these compounds.

It is also suggested that the extremely mutagenic dinitropyrenes (Tokiwa and Onishi, 1986) contribute to the mutagenicity of the particles (Tokiwa et al., 1983). In this study the actual contribution of these compounds to the mutagenicity of AAP was investigated by comparing AAP samples with coal fly ash (CFA) samples containing high dinitropyrene concentrations.

METHODS AND MATERIALS

Samples

Ambient airborne particles (AAP)

AAP were collected with Sartorius HV100 high-volume samplers on Sartorius SM13400 glass-fibre filters. Filter diameter: 25.7 cm; filters cleaned by Soxhlet extraction with methanol; flow rate: 100 $m^3.h^{-1}$; duration: 24 h.

Sampling locations: Delft, on the TNO Zuidpolder terrain to the South-East of Delft, at ground level; Schiedam, just West of Rotterdam, at an altitude of about 2.5 m; Lekhaven, on the roof of an old factory in the seaport area of Rotterdam, at an altitude of about 50 m; Blijdorp, in the zo-ological garden of Rotterdam, at ground level; Petten, on the terrain of the Energy Research Centre of the Netherlands, in a rural area on the coast, at ground level. Samples were taken during 1985 and the first four months of 1986.

Filters with particles were wrapped in aluminium foil and immediately stored at -80°C.

Within a few days of sampling the filters with particles were extracted for 8 h (about 20 cycles) in a Soxhlet apparatus with methanol (Rathburn, HPLC-grade) in the dark. During extraction the air in the Soxhlet apparatus was replaced by nitrogen. The solvent was removed from the extract with a rotation evaporator (at 30°C, in the dark, under reduced pressure). Original extracts or residues dissolved in methanol, acetone (Nanograde) or dimethyl sulphoxide (Merck, A.R.) were stored at -30°C or -80°C.

Strongly mutagenic extracts (>50 revertants per m^3 eq. in TA98 without S9) were selected for the fractionation experiments; a number of experiments were carried out with extracts combined from various samples.

Coal fly ash (CFA)

Two CFA samples, A and B, were taken from the bag filters of a small underfeed boiler (3.8 MWth) heating a greenhouse. The CFA samples were extracted with acetone. Extracts were prepared and treated in a similar way as the AAP extracts.

Mutagenicity testing

Extracts and fractions were tested for mutagenicity with the Salmonella/microsome test developed by Ames and coworkers (Ames et al., 1975 and Maron and Ames, 1983). The following *Salmonella typhimurium* strains were used: TA98, TA98NR, TA98-1.8DNP$_6$ (TA98DNP), TA100 and TA97. Most tests were carried out in the presence and absence of a rat liver homogenate (S9-fraction) prepared from rats treated with 500 mg.kg^{-1} Aroclor 1254, according to the procedure of Ames *et al.* (1975).
Testing was carried out in such a way, that comparison of the fractions from one chromatographical fractionation procedure and comparison of results obtained with and without S9 fraction or with different tester strains was not hampered by inter-test variation, i.e. the variation caused by the day of testing, differences between cultures and differences between batches of materials. Doses were tested in duplicate or triplicate. Test samples were always added to the plates in 0.1 ml when organic solvents were used as solvent (in most cases dimethyl sulphoxide) and in 1 ml when water was used as a solvent.

Determination of the concentrations of PAH and nitrated PAH

The concentrations of PAH in extracts and fractions were determined with reversed-phase high-performance liquid chromatography (RP-HPLC). Stationary phase: Supelcosil LC-PAH (250*4.6 mm); mobile phase: gradient from 75 to 100% methanol in water; detection: fluorescence, excitation 250 nm, emission >390 nm; identification and quantification on the basis of chromatograms with external standards.
Determined were the concentrations of the following PAH: Phenanthrene (PHE), anthracene (AC), fluoranthene (FL), pyrene (PY), benz(a)anthracene (BAA), chrysene (CH), benzo(e)pyrene (BEP), perylene (PE), benzo(b)fluoranthene (BBF), benzo(k)fluoranthene (BKF), benzo(a)pyrene (BAP), benzo(g,h,i)perylene (BGH), indeno(c,d)pyrene (IP) and anthanthrene (AN).

The concentrations of mono-nitro PAH and dinitropyrenes in extracts and fractions were determined with RP-HPLC. Stationary phase: Vydac-201 TP (particle size: 5 micrometer; 250*4.6 mm); mobile phase: gradient elution with acetonitril and Tris buffer. The eluate passed a zinc column for reduction of the nitro compounds. Detection was based on chemiluminescence with peroxyoxalate. Identification and quantification was based on the comparison of chromatograms obtained with and without reduction prepared from the sample and a mixture of reference compounds.

Analyses were carried out with the original extracts or fractions, or residues dissolved in acetone, methanol or dimethyl sulphoxide.

Chromatographical fractionation

Extracts of AAP or CFA were first fractionated with liquid-liquid partitioning (LLP) with methoxyisobutane (MIB) and water *or* with straight-phase open-column liquid chromatography (OCLC-I) with MIB and methanol as eluents.

The MIB-layer of LLP *or* the MIB-fraction of OCLC I were further fractionated either with straight-phase OCLC (OCLC-II) or with semi-preparative straight-phase HPLC (SP-HPLC) with gradient eluation.

The OCLC-II fractions were in some experiments fractionated with straight phase SP-HPLC.

One relatively polar SP-HPLC fraction was in some instances fractionated with OCLC-II.

A schematic representation of the fractionation procedures followed is depicted by Fig. 1. The extracts were not subjected to the complete scheme; the figure represents a compilation of the fractionation procedures followed for each extract separately.

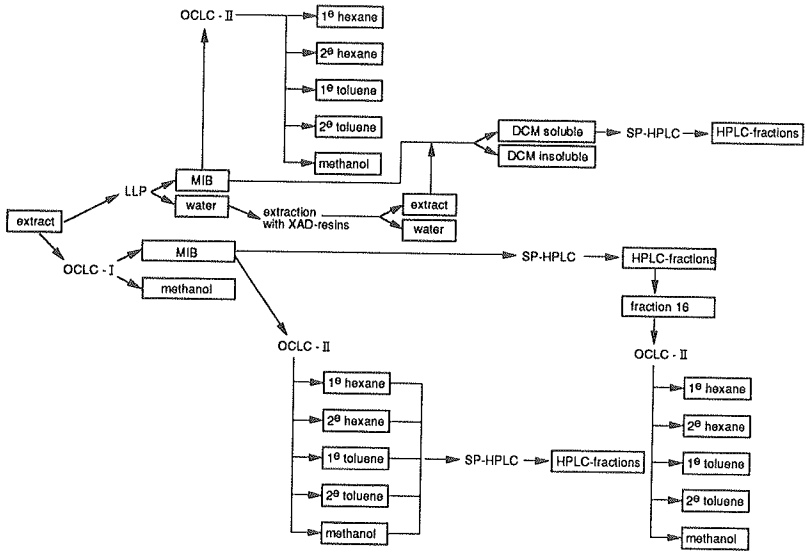

Fig. 1 *Schematic representation of the fractionation procedures*

LLP : *Liquid-Liquid Partition*

OCLC-I : *Open Column Liquid Chromatography; straight phase; with MIB and methanol*

OCLC-II : *Open Column Liquid Chromatography; straight phase; with hexane, toluene and methanol*

SP-HPLC : *Semi-Preparative High Pressure Liquid Chromatography; straight phase; gradient elution with hexane, dichloromethane and actenonitrile*

[ ] : *tested for mutagenicity or/and analysed for PAH or/and nitro PAH*

LLP

Residues of extracts were dissolved in 100 ml of methoxyisobutane (MIB) and 100 ml of distilled water by ultrasonic vibration at 0°C. The mixture was manually shaken for five minutes in a separation funnel; then the two layers were separated ; in several experiments water was again added to the MIB layer and the separation procedure repeated. In some experiments the volume of the water layer was reduced by evaporation; in others the organics were extracted from the water with two resins: Amberlite XAD2 and XAD7 (Rohm and Haas) according to a procedure similar to that described by Yamasaki and Ames (1977). Two separate columns (h: 9cm, bed volume: 9 ml) were used and the eluate of the XAD2 column was extracted with XAD7. The adsorbed organics were desorbed with 30 ml acetone.

OCLC I

Residues were dissolved in acetone. 200 microliters of this solution were applied to Sep-Pak Silica cartridges (Waters Associates; cartridges were not pre-treated) in small aliquots; after addition of each aliquot the column was dried with a counter-current stream of nitrogen. The column was eluted twice with MIB (20 ml) and with methanol (20 ml).

OCLC II

Like OCLC I except that LLP MIB layers were fractionated and that elution was carried out with 10 to 20 ml of the following solvents hexane (twice), toluene (twice) and methanol.

SP HPLC

SP HPLC was applied to the MIB layer of LLP and the fractions of OCLC I (only MIB fraction) and OCLC II. Residues were dissolved in dichoromethane by ultrasonic vibration and eluted on an Alltech Silica semi-preparative column (25cm * 1cm, particle size: 10 micrometer) by gradient elution. The gradients applied are shown in Fig. 2. The eluate was collected in fractions of 7 or 3.5 ml.

The column was chemically reactivated (Bredeweg et al., 1979 and Stray et al., 1984); to this end the column was eluted with a mixture of hexane, acetic acid and 2,2-dimethoxypropane (90:10:2.5, Vol.). This results in a reproducible activity of the stationary phase (0.2% water). After reactivation the column was eluted respectively with acetonitril, DCM and hexane.

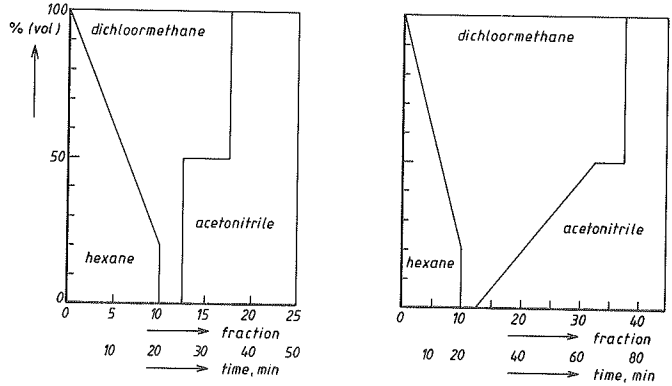

Fig. 2 *Elution schemes used in the semi preparative HPLC fractionation.*

RESULTS

LLP

Most AAP extracts were subjected to LLP. In 11 experiments the MIB and water layers were both tested with strain TA98 fraction (the water layer was tested as such or after partial evaporation of the water). These tests showed that only traces of mutagenicity were left behind in the water layer and these could be easily extracted with XAD.

Five experiments allowed for the calculation of the recovery of the mutagenicity (the sum of the mutagenic effects of the fractions divided by the effect of the original extract). In tests with S9 the mean recovery was 99% with S9, without S9 it was 117%; standard deviations: 14.8 and 8.3% respectively. Recoveries were always highest for TA98 without S9.

The mean recovery of the amount of extracted material (residue) was 99.3% (sd: 20.8%) in these experiments. The mean share of the MIB layer in the recovered residue was 22.2% (sd: 8.7%).

OCLC-I

A number of extracts, among them the two CFA extracts were prefractionated with OCLC-I. This resulted in two mutagenic fractions: the first MIB fraction and the methanol fraction; no mutagenicity was found in the second MIB fraction.

Mean recoveries for five AAP extracts were: 109% (TA98 with S9) and 145% (TA98 without S9; both sd's 20%). Again recovery was higher for TA98 without S9 in every experiment.

76% (sd: 12%) and 74% (sd: 15%) of the mutagenicity of the original extracts was recovered in the MIB fractions for strain TA98 with and without S9 respectively; these percentages were 37 (sd: 5) and 62 (sd: 10) respectively for the methanol fractions.
For two extracts (an AAP extract and a CFA extract) we reconstituted the extract from the fractions and compared the mutagenicity of this reconstituted extract with that of the original extract. The effects of these reconstituted extracts amounted from 65 to 86% of the original extracts depending of sample and presence of S9.
The absence of mutagenicity in the second MIB layer indicates, that the groups of mutagens in the other two fractions do not overlap; OCLC-I thus allows the separation of two distinct groups of mutagens in the extracts.
The possibility of overlap was further investigated, by reapplying the MIB fraction to a Sep-pak cartridge and elution with MIB and methanol. Virtually all mutagenicity was found back in the MIB fraction then, indicating, that the first MIB fraction does not contain any polar mutagens which were eluted by MIB because of overloading.

OCLC-II

MIB layers of LLP and MIB fractions of OCLC-I were subjected to OCLC-II. The second hexane and second toluene fractions never showed significant mutagenicity, which indicates that three distinct groups of mutagens can be separated with this technique. Re-elution of the first hexane fraction on a new column made clear that this fraction did not contain polar compounds eluted in this apolar solvent due to overloading. If the eluate of the first hexane fraction was collected in aliquots of 2.5 ml, nearly all mutagenicity appeared in the first aliquot, which again indicates the clear separation between the first hexane and the first toluene fraction.
The results of an OCLC-II fractionation with CFA A are presented in detail in Fig. 3.
In nearly all experiments the methanol fraction showed the strongest mutagenicity and toluene fraction the weakest. The effect of the hexane fraction generally showed a clear increase upon the use of S9 fraction while that of the methanol fraction was often decreasing.
Recoveries could be calculated for 9 extracts; TA98 with S9: 84% (sd: 27%); TA98 without S9: 121% (sd: 28%). No reconstituted extracts were prepared.
The separation characteristics of the OCLC-II technique were investigated by eluting 22 compounds, comprising PAH, nitro PAH, PAH with oxygen containing groups, amino PAH, nitrogen-heterocyclic PAH and oxygen-heterocyclic PAH. No toluene fractions were prepared. The presence of these compounds in a certain fraction was determined by a spot check with TLC of the fraction and a solution of the pure compound. Only PAH, mono-

nitro PAH, 9-fluorenon and 2,4,7-trinitrofluorenon were exclusively eluted in the hexane fractions; all other compounds could only be demonstrated in the methanol fractions.

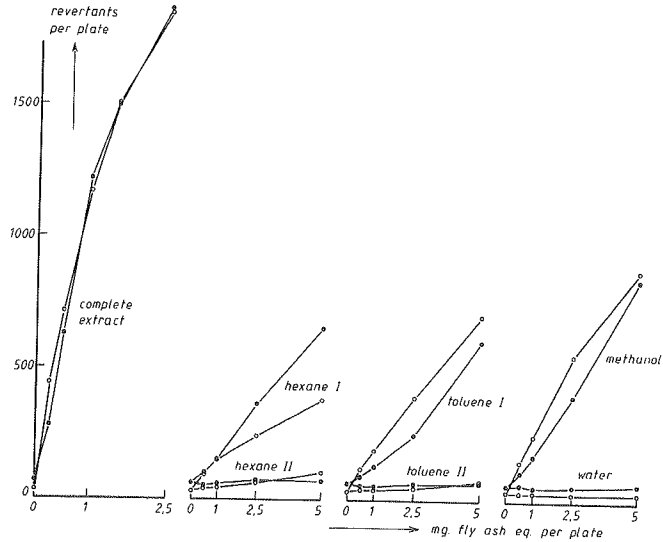

Fig. 3 *Mutagenicity of OCLC-II fractions of CCFA sample A.*
- • *with S9 fraction*
- o *without S9 fraction*

Benzo(a)pyrene, 3-methylcholanthrene, 2-nitrofluorene and 1-nitropyrene were subjected to OCLC-II and the fractions obtained were tested with strains TA98 and TA100 with and without S9. The mutagenicity of the first two compounds, which could only be demonstrated in the presence of S9, was completely recovered in the first hexane fraction while 80% of that of 2-nitrofluorene was found back in this fraction, the remaining part being divided over the second hexane and the first toluene fraction. 1-Nitropyrene yielded mutagenic first hexane and first toluene fractions; chemical analysis demonstrated that the effect of the first toluene fraction could be attributed to a contamination with the extremely mutagenic dinitropyrenes. Separate experiments with dinitropyrenes confirmed the elution of these compounds in the first toluene fraction. In all these experiments recoveries of about 100% were found.

In two experiments the fractions of AAP extracts were analysed for a number of PAH, two mono-nitro PAH and dinitropyrenes. The results are listed in Table 1; they show, that PAH and their mono-nitro derivatives elute in the first hexane fraction, confirming the results obtained with the pure compounds; no dinitropyrenes were found.

In many experiments we have tested fractions of AAP extracts with the NR and DNP mutants of TA98 together with their parental strain. The direct (S9-independent) mutagenicity of all fractions was always found to be strongly reduced in the mutants as compared with that in the parental strain, indicating the predominant rôle of nitrogroup-dependent mutagenicity.

Table 1 *PAH and mono nitropyrenes in two AAP samples*

Compound	Hexane 1	Hexane 2	Toluene 1	Methanol
chrysene	1120	-	-	-
benzo(e)pyrene	830	-	-	-
benzo(b)fluoranthene	1110	41	23	14
benzo(a)pyrene	620	26	15	11
benzo(g,h,i)perylene	1130	-	-	-
1-nitropyrene	6	0.6	-	-
4-nitropyrene	5	-	-	-
chrysene	1260	-	230	72
benzo(e)pyrene	1020	-	-	-
benzo(b)fluoranthene	1390	23	22	-
benzo(a)pyrene	820	15	11	-
benzo(g,h,i)perylene	1500	-	-	-
1-nitropyrene	7	0.5	-	-
4-nitropyrene	6	-	-	-

Concentrations in pg per m^3 eq.
Toluene 2 fraction was not analysed.
No dinitropyrenes could be demonstrated.
- : concentration below detection limit.

SP-HPLC

Separation characteristics and reproducibility of the SP-HPLC were investigated in a series of experiments in which a mixture of PAH and various PAH derivatives was fractionated with this technique. Reproducibility appeared to be strongly dependent of the application of chemical reactivation. Mean retention times obtained with the chemically reactivated column are listed in Table 2. The low sd's of these retention times (about 3%) illustrate the high reproducibility of the technique. The table shows that the groups of PAH and mono-nitro PAH each elute within a narrow retention range (fraction 6 for the PAH and fractions 8, 9 and 10 for the mono-nitro PAH); these groups do not show any overlap.

The results obtained with a mixture of nitro PAH are listed in Table 3. In this case the fractions were analysed. Again all mono-nitro eluted in fractions 8, 9 and 10, while the dinitropyrene eluted in fraction 12.

A comparable experiment with 24 PAH showed, that these compounds were all eluted in fraction 6, with small amounts of the lower molecular ones in fraction 5 and small amount of the ones with higher molecular weights in fraction 7. In this experiment we found substantial losses for a number of PAH, in particular the ones most sensitive to chemical conversion, i.e. perylene, benzo(a)pyrene and anthanthrene (Nielsen, 1984); a strong indication that conversion of PAH occurs during elution.

Table 2 *Retention times of a number of reference compounds in SP HPLC.*

Compound	Retention time [a)]
fluoranthene	0.96
benz(a)anthracene	1.00
chrysene	1.00
9-nitroanthracene	1.32
7-nitrobenz(a)anthracene	1.37
6-nitrobenzo(a)pyrene	1.39
6-nitrochrysene	1.54
3-nitrofluoranthene	1.59
9-hydroxycarbazole	1.59
1-nitropyrene	1.62
13-hydroxydibenzo(a,i)carbazole	1.64
9-hydroxyfluorenone	2.46
benzo(a)acridine	2.89
acridine	2.94

a : retention time divided by the retention time of chyrsene (11.2 min.: 39.2 ml).

Table 3 *Distribution of reference nitro PAH over SP HPLC fractions.*

Fraction 8	Fraction 9	Fraction 10	Fraction 11	Fraction 12
1-nitronaphtalene	2-nitronaphtalene	3-nitroperylene		1,8-dinitropyrene
6-nitrobenzo(a)pyrene	4-nitropyrene			
9-nitroanthracene	1-nitropyrene			
7-nitrobenz(a)anthracene	3-nitrofluoranthene			
	6-nitrochrysene			
	1-nitrochrysene			

In two experiments with AAP extracts, fractions 5, 6 and 7, all other fractions together and the original extract were analysed for 15 PAH; in one experiment a reconstituted extract was also analysed. The results (see Table 4) confirm the elution pattern found with the mixture of pure PAH, while losses were very small.

Comparable experiments were carried out for nitro PAH in the same extracts and in CFA sample A. The results obtained with CFA sample A are presented in Table 5; they confirm the elution pattern found with the mixture of pure nitrocompounds.

Table 4 *Concentrations PAH in SP HPLC fractions of two AAP samples.*

PAH	ng.m^{-3}				
sample 2	Fr. 5	Fr. 6	Fr. 7	Reconstitution	Complete extract
Anthracene	0.17	0.11	< 0.01	0.40	0.49
Fluoranthene	0.22	8.36	< 0.01	10.69	10.30
Pyrene	2.71	3.83	< 0.03	7.69	7.03
Benzo(b)fluorene	0.70	1.31	< 0.05	2.03	1.84
Benz(a)anthracene	0.07	3.26	< 0.01	3.50	3.27
Chrysene	0.34	5.62	< 0.03	5.63	6.08
Benzo(e)pyrene	0.47	4.44	< 0.08	4.10	4.37
Benzo(j)fluoranthene	0.10	3.77	< 0.11	3.03	2.75
Perylene	< 0.01	0.51	< 0.01	0.49	0.57
Benzo(b)fluoranthene	< 0.01	5.42	< 0.01	5.24	5.49
Benzo(k)fluoranthene	< 0.01	2.27	< 0.01	2.15	2.24
Benzo(a)pyrene	< 0.01	3.20	< 0.01	2.99	3.01
Benzo(g,h,i)perylene	< 0.04	4.16	0.10	3.28*	3.46*
Ideno(1,2,3-c,d)pyrene	< 0.02	3.75	0.05	3.17	3.78
Anthanthrene	< 0.01	0.43	0.02	0.42	0.61
PAH	**ng.m^{-3}**				
sample 3	**Fr. 5**	**Fr. 6**	**Fr. 7**	**Recovery**	**Complete extract**
Phenanthrene	2.39	2.37	< 0.03	4.76	6.29
Anthracene	0.20	0.13	< 0.01	0.33	0.38
Fluoranthene	0.90	9.79	< 0.01	10.69	11.30
Pyrene	4.06	4.65	< 0.03	8.71	9.34
Benzo(b)fluorene	1.01	1.23	< 0.05	2.24	2.08
Benz(a)anthracene	0.06	2.60	< 0.01	2.66	2.67
Chrysene	0.45	4.83	< 0.03	5.28	5.48
Benzo(e)pyrene	0.56	3.29	< 0.08	3.85	4.55
Benzo(j)fluoranthene	< 0.11	2.62	< 0.11	2.62	3.19
Perylene	0.02	0.34	< 0.01	0.36	0.41
Benzo(b)fluoranthene	< 0.01	3.86	< 0.01	3.86	4.15
Benzo(k)fluoranthene	< 0.01	1.52	< 0.01	1.52	1.64
Benzo(a)pyrene	< 0.01	2.18	< 0.01	2.18	2.38
Benzo(g,h,i)perylene	< 0.04	2.98	0.09	2.98	2.35
Ideno(1,2,3-c,d)pyrene	< 0.02	2.44	< 0.02	2.44	2.61
Anthanthrene	< 0.01	0.23	0.08	0.23	0.48

Reconstitution : analysis of an extract reconstituted from fractions 5, 6 and 7.
Recovery : addition of the concentrations in fractions 5, 6 and 7.

Table 5 *Nitro PAH in SP HPLC fractions of CFA sample A.*

Fraction	1-nitropyrene	6-nitro-chrysene	1-nitro-perylene	6-nitro-benzo(a)pyrene	1,8-dinitro-pyrene*
9	500	730	160	27	-
10	-	-	-	45	-
11	-	-	-	-	16
12	-	-	-	-	12

Concentrations in $pg.mg^{-1}$
- : concentration below detection limit
* : the dinitropyrenes have the same retention time; 1,8-dinitropyrene was used as standard.

A typical UV-absorption chromatogram prepared with SP-HPLC of an MIB fraction from OCLC-I with AAP is depicted in Fig. 4. Many of such chromatograms were produced and although relative heights of the peaks fluctuated, their retention times were almost constant.

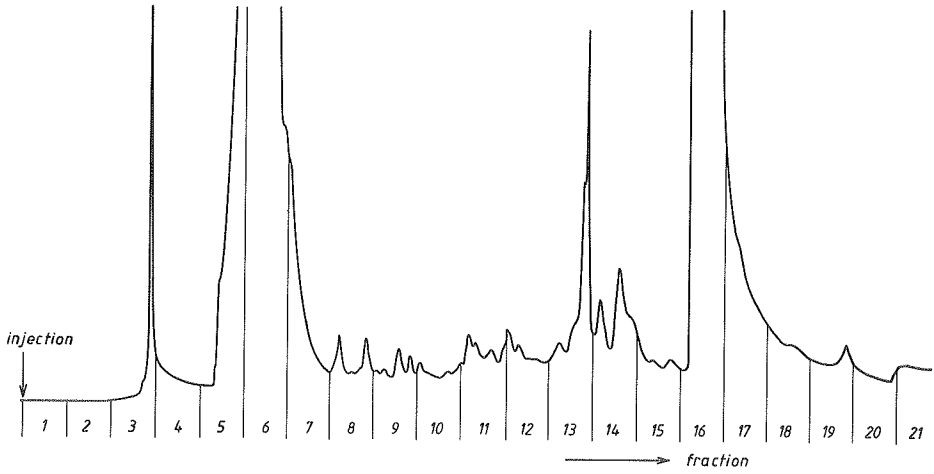

Fig. 4 *Typical UV absorption SP HPLCC chromatogram; AAP. Elution scheme A, see Fig. 2*

Fig. 5 depicts the results of mutagenicity tests with SP-HPLC fractions of AAP extracts after pre-fractionation with OCLC-I (so the MIB fraction of OCLC-I was subjected to SP-HPLC) and LLP. We will refer to "chromatograms" as are depicted in this Fig. as mutagrams because mutagenicity is used for detection in chromatography.

We can distinguish three clearly mutagenic peaks and one weakly mutagenic peak in the mutagrams, which points to the presence of four distinct groups of mutagens.
The most apolar group elutes in the same fraction (fraction 6) as the mainstay of the PAH and its effect, like that of the mutagenic PAH, depends completely on the presence of S9.

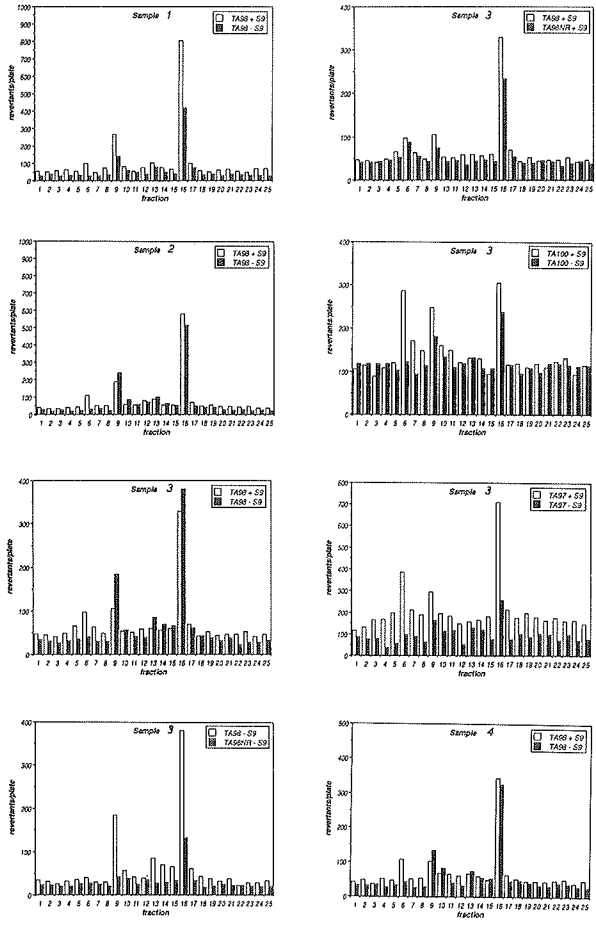

Fig. 5

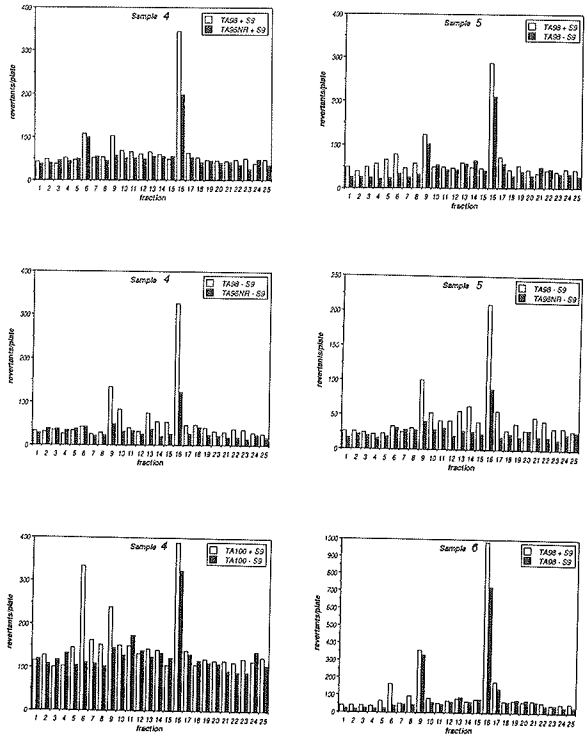

Fig. 5 *Mutagenicity of SP HPLC fractions; AAP samples; MIB fractions of OCLC-I prefractionations (samples 1 to 5) and MIB layer of LLP (sample 6); dose: 50 m^3 eq. per plate; means of duplicates; background not substracted.*

The next mutagenic group elutes in the same fraction as the mono-nitro PAH (fraction 9). Its direct effect strongly depends on bacterial nitroreduction.

Weak mutagenic effects are sometimes observed in fractions 12, 13 or/and 14. The tests with TA98NR again point to nitrocompounds.

By far the strongest effects were observed in fraction 16. Again the much weaker effects in the nitroreductase-dependent strain point to a predominant contribution of nitrocompounds. If strains TA100 or TA97 were used in stead of strain TA98 markedly different mutagrams were obtained. The relative importance of the PAH fraction increases dramatically if the first strain is applied. To a lesser extent this holds for strain TA97. The effect of fraction 16 appears to be strongly dependend on the presence of S9 in this strain. In general it can be

stated that indirect mutagenicity is more important than its direct counterpart in strains TA100 and TA97.

Mutagrams of CFA extracts are depicted in Fig. 6 (MIB fractions of OCLCI). These mutagrams differ markedly from those of the AAP extracts. No clear-cut contribution of the PAH-containing fraction was observed. In contrast to the AAP extracts, the CFA extracts yielded clearly mutagenic fractions 11 and 12. The effects of these fractions, which contain dinitropyrenes, virtually disappeared when the test was carried out with S9. The NR mutation did affect the mutagenicity of the fractions, although much less strongly than the mutagenicity of the mono-nitro PAH fraction.

Fraction 13 also shows some mutagenicity in the CFA mutagrams; its effect differs clearly from that of the two preceding fractions, in the sense, that the presence of S9 does *not* lead to a reduction of the effect.

Like AAP, CFA yielded strongly mutagenic fractions 16; the mutagenicity was markedly reduced upon the use of the nitroreductase-deficient strain.

On the far polar end of the mutagram of CFA sample A a clearly mutagenic fraction 21 was observed, which effect disappeared when the test was carried out with S9.

In two experiments OCLC-II fractions of an AAP extract (MIB layer of LLP) were fractionated with SP-HPLC. The results of one experiment are presented in Fig. 7. The other experiment lead to essentially the same mutagrams. The mutagrams of the second hexane and toluene fractions are not shown in the Fig., as these did not yield any mutagenic fractions, which confirms the lack of overlap between the three mutagenic fractions.

Only the hexane-1 fractions yielded mutagenic PAH and mono-nitro PAH fractions (fractions 6 and 9). Clearly mutagenic fractions 16 were observed in the mutagrams of *all three* OCLC-II fractions. This is a remarkeble finding, as fraction 16 contains the more polar mutagens which are not expected to elute in hexane from a Sep-Pak Silica cartridge. So, we can discerne three distinct groups of mutagens in fraction 16 of the AAP extracts.

In other experiments, fractions 16 were subjected to OCLC-II. The results (see Table 6) confirm the presence of three distictly different groups of mutagens in fraction 16.

By changing the elution scheme of SP-HPLC (scheme B, see Fig. 2) it was attempted to separate the fraction-16 mutagens in one mutagram. The mutagram is depicted in Fig. 8; it shows a series of peaks on the polar side of the chromatogram, which originate from the "fraction-16 mutagens" of the other mutagrams.

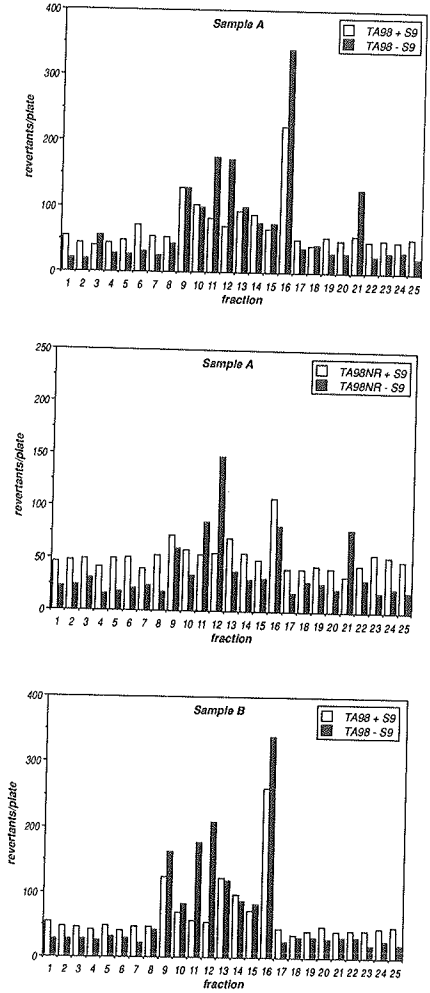

Fig. 6 *Mutagenicity of SP HPLC fractions; CFA samples A and B; MIB fraction of OCLC-I; dose: 2 mg fly ash eq. per plate; means of duplicates; background not substracted.*

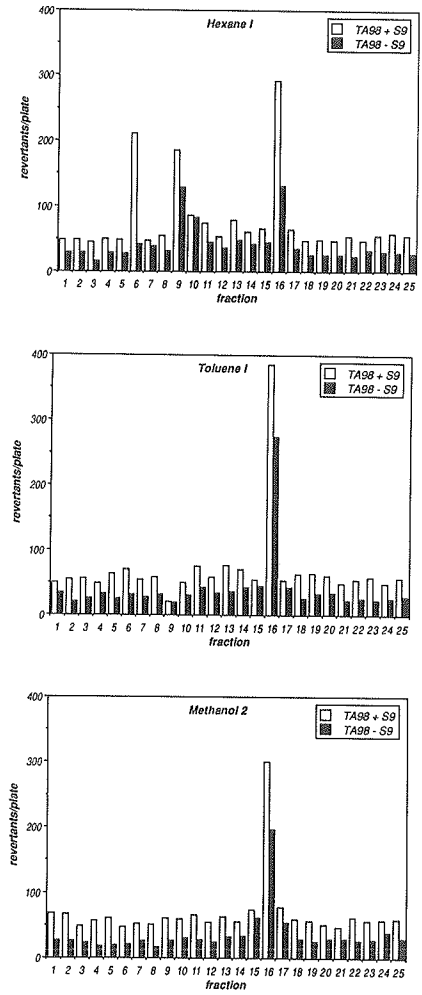

Fig. 7 *Mutagenicity of SP HPLC fractions; OCLC-II fractions AAP sample 7; dose: 50 m^3 eq. per plate; means of duplicates; background not substracted.*

Table 6 *Mutagenicity of the OCLC-II fractions prepared from SP HPLC fraction 16.*

Sample dose	Strain	S9	Fraction 16	Hexane1	Hexane2	Toluene1	Toluene2	Methanol	Back-ground	Re-covery %	Recon-stitution %
2	TA98	+	583	176	26	133	58	112	40	64	
50 m^3		-	514	164	18	173	52	198	18	104	
4	TA98	+		108	74	109	61	96	43		
50 m^3		-		140	57	243	57	183	25		
	TA98NR	+		70	44	61	36	71	39		
		-		63	30	106	22	66	19		
A	TA98	+	196	95	43	71	45	61	40	100	
(fly ash)		-	214	87	26	90	40	77	21	123	
2 mg											
B	TA98	+	253	112	42	78	41	71	41	85	72
(fly ash)		-	232	78	20	52	33	79	18	89	60
2 mg											

Listed are: means of duplicates
recovery (added effects as percentage of original fraction 16)
reconstitution (effects of reconstituted fractions as percentage of original fraction 16)

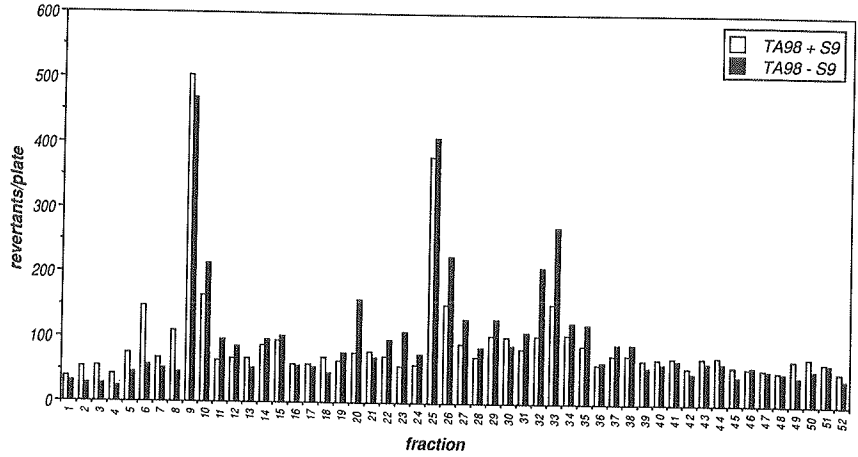

Fig. 8 *Mutagenicity of SP HPLC fractions; sample 7; MIB layer of LLP; 50 m^3 eq. per plate; means of duplicates; background not substracted; elution scheme B, see Fig. 2.*

For a series of SP-HPLC fractionations the recoveries could be calculated because the OCLC-I MIB fraction or the LLP MIB layer from which the mutagram was prepared was tested simultaneously (in the same test procedure) with the SP-HPLC fractions. In two

experiments a reconstituted (from the SP-HPLC fractions) MIB fraction was also tested simultaneously. The recovered effects and the effects of the reconstituted fraction are listed in table 7.

Table 7 *Recovery and reconstitution of mutagenicity: SP HPLC fractionation.*

Sample	Strain	S9	Fraction	Recovery (%)	Reconstitution (%)
AAP					
1	TA98	+	hexane1	70	
		-		241	
2	TA98	+	OCLC-I	70	
		-		131	
3	TA98	+	OCLC-I	59	
		-		109	
	TA98NR	+		65	
		-		100	
	TA97	+		240*	
		-		69	
	TA100	+		68	
		-		144	
4	TA98	+	OCLC-I	69	
		-		136	
	TA98NR	+		59	
		-		155	
	TA100	+		115	
		-		144	
5	TA98	+	OCLC-I	57	126
		-		178	115
	TA98NR	+		68	117
		-		81	160
6	TA98	+	LLP	103	
		-		134	
7	TA98	+	LLP	103	
		-		228	
8	TA98	+	hexane1	119	
		-		191	
CFA					
A	TA98	+	OCLC-I	66	
		-		239	
	TA8NR	+		65	96
		-		203	91
B	TA98	+	OCLC-I	69	
		-		124	

Recovery : added effects of the HPLC fractions as percentage of the effect of the original fraction.
Reconstitution : effect of a sample reconstituted of the HPLC fractions as percentage of the effect of the original fraction.
hexane1 : first hexane fraction of the OCLC fractionation.
* : effect of original fraction severely affected by toxicity.

Table 8 lists effects of the SP HPLC fractions 6 and 9, containing resp. the PAH and the mono-nitro PAH. The effects are expressed as revertants per m^3 and as percentage of the effect of the original complete extract. The table shows the clear strain dependence of the contribution of the PAH; if TA100 is used in stead of TA98 it increases from about 5% to about 20%. Also the contribution of the mono-nitro PAH is affected by the use of TA100: a clear increase in tests with S9 and a clear decrease in tests without S9.

Table 8 *The contribution of the fractions with PAH and mono-nitro PAH to the mutagenicity of the extracts of AAP.*

Sample	Strain	PAH fraction		Mono-nitro PAH fraction				Complete extract	
		with S9		with S9		without S9		with S9	without S9
		rev./m^3	%	rev./m^3	%	rev./m^3	%	rev./m^3	rev./m^3
2	TA98	1.44	4.0	3.04	8.5	4.40	19.6	35.65	22.50
3	TA98	0.98	3.9	1.14	4.5	3.00	19.2	25.45	15.60
	TA98NR	1.02	7.2	0.76	0.5	0.36	8.9	14.20	4.05
	TA100	3.52	17.1	2.76	13.4	1.56	20.4	20.65	7.65
4	TA98	1.14	5.0	1.04	4.6	2.14	17.4	22.75	12.30
	TA98NR	1.04	9.8	0.20	1.9	0.48	15.2	10.60	3.15
	TA100	4.52	25.3	2.64	14.8	0.60	5.9	17.90	10.25
5	TA98	0.58	2.2	1.46	5.7	1.50	9.8	22.85	15.35
	TA98NR	0.62	5.3	0.38	3.2	0.46	12.6	11.80	3.65
6	TA98	2.44	6.2	6.30	1.61	6.06	27.4	39.15	22.10
7*	TA98	3.30	7.6	13.02	30.1	12.88	48.8	43.20	26.40
$\bar{x}$	TA98		4.8		11.6		23.7		
s			1.9		10.1		13.5		

rev./m^3	:	revertants per m^3.eq.
%	:	percentage of the effect of the complete extract
$\bar{x}$	:	average for strain TA98
s	:	standard deviation
PAH fraction	:	SP HPLC fraction 6
mono-nitro PAH fraction	:	SP HPLC fraction 9
*	:	PAH: 5+6+7; mono-nitro PAH: 8+9+10; elution scheme B

DISCUSSION

By combining a number of chromatographical techniques we could discern seven clearly distinct groups of mutagens in AAP and six of these groups together with two other in CFA. The most apolar group elutes in the same SP HPLC fraction as the mainstay of the unsubstituted PAH and its effect, like that of these compounds (and their alkylated deriva-

tives, compounds which are expected to elute in the same SP HPLC fraction), is completely S9 dependent. Chlorinated PAH may have been eluted in the same fraction or in adjacent fractions. However these compounds will not have contributed significantly to the mutagenicity as this group of compounds comprises strong *direct* mutagens (Leon et al., 1985; Rannug et al., 1986; Ball and Young, 1987; Pallet et al., 1987; Bhatia et al., 1987).

The next group of mutagens elutes in the same SP HPLC fraction as the mono-nitro PAH. The direct effect of this fraction is strongly nitro-reductase dependent. Therefore, it will be most probably caused by nitro compounds. From the mutagenic nitro compounds thus far identified in comparable extracts only the mono-nitro PAH are expected to elute in this fraction. Multiple nitrated PAH or compounds with, besides nitro groups, oxygen-containing groups are expected to elute later, which leaves the mono-nitro PAH as the sole compounds to which the effect of the fraction can be ascribed.

SP HPLC with CFA yielded a group of mutagens coeluting with the dinitropyrenes. The extremely strong mutagenicity of dinitropyrenes in the *Salmonella*/microsome test (Rosenkranz and Mermelstein, 1983; Tokiwa and Onishi, 1986) and their rather high concentrations in the CFA extracts strongly suggest that these compounds indeed contribute to the effect of the SP HPLC fraction in question. The effect is strongly reduced when the fraction was tested in the presence of S9, which is in line with a contribution of dinitropyrenes, as these compounds are strongly inactivated by S9 (Shah et al., 1990). However, we cannot fully exclude a contribution of other mutagens, e.g. other mutagenic multiply nitrated PAH or oxygen-containig nitro PAH.

The lack of any significant effect of the "dinitropyrene fraction" of the AAP extracts excludes a significant contribution of dinitropyrenes to the mutagenicity of AAP. It may be expected that other multiple nitrated PAH will elute in the same SP HPLC fraction as the dinitropyrenes or in adjacent fractions, so our conclusion applies to these compounds as well.

The effects obtained with the other fractions (the three subfractions prepared from SP HPLC fraction 16, SP HPLC fraction 21 of CFA sample A and the methanol layer of OCLC I) were also clearly nitroreductase-dependent. So, it seems that all mutagenicity is caused by nitro compounds. However, it should be emphasized that the reduced effect of a fraction in strain TA98NR only tells us something about the identity of the direct mutagens. Effects of the same fraction in the presence of S9 may point to indirect mutagens, even if this effect does not exceed that in the absence S9. In fact, close observation of the mutagrams makes clear that S9 in some cases causes a decrease of the effect in TA98 and an *increase* of the effect in TA98NR, which unambiguously points to the presence of indirect mutagens. Obviously, this indirect effect will also contribute to the effect in TA98,

but then it is more than compensated by the inactivation of the direct effect. This combination of inactivation of direct, nitro-reduction dependent compounds and activation of indirect compounds was also observed by us in an earlier study, in which we investigated the mutagenicity of AAP during moderate photochemical air pollution. That study suggested a relative large contribution of the inactivatable direct mutagens during such episodes, in particular when particles from distant sources were sampled (De Raat and De Meijere, 1988).

The indirect mutagens present in the particles may be the same nitrocompounds that cause the direct effect. A nitro PAH may be mutagenic in *Salmonella* via bacterial reduction of the nitrogroup and/or bacterial esterification of the resulting hydroxylamine (Tokiwa and Onishi, 1986); apparantly this activation pathway can be disturbed by S9, possibly by metabolic conversion before activation by the bacterium can take place. The S9 in its turn may activate the same compound via ring epoxidation (Tokiwa and Onishi, 1986); this is the common activation pathway for unsubstituted and alkylated PAH. Obviously, nitro PAH which are not directly mutagenic may still be activated by S9.

As we did not undertake a thorough analytical-chemical characterization of the more polar fractions we cannot draw any conclusions about their chemical homogeneity, let alone conclusions about the chemical identity of the mutagens in them. We may safely assume, that the mutagenicity is not caused by one compound; rather a group of nitro compounds among them direct and indirect mutagens, with similar physico-chemical properties will be concentrated in the fractions. It is evident, that a definitive inventory of the relatively more polar mutagens in AAP and CFA requires further fractionation, possibly by reversed-phase HPLC, of the mutagenic more polar fractions, followed by mutagenicity testing and chemical analysis. Our study shows that at least five distinct more polar groups of nitroreductase-dependent direct mutagens are present in the extracts.

Strain TA98 is generally used in mutagenicity studies because of its high sensitivity. However, there is no fundamental toxicological reason why we should use this strain instead of other. The effects of the types of compounds present in our extracts in TA98 do not necessarily correlate better with carcinogenicity than those obtained with the other strains. That is why we have carried out tests with two other strains, namely TA100 and TA97. The mutations detected with those strains have another molecular-biological background than those detected with TA98 and will, therefore, show a different qualitative (as regards the type of compounds detected by the strains) and quantitative sensitivity. If TA100 is used in stead of TA98, the PAH become much more important, as is revealed by comparison of otherwise strictly comparable mutagrams. In another study carried out by us we have used TA100 next to TA98 to investigate the contribution of an industrial and

urban area to the mutagenicity of the aerosol. This study consequently shows clear activation upon the inclusion of S9 in tests with TA100, while S9 hardly affected the overall effect obtained with TA98 (de Raat et al., 1985). The present study makes clear that this is the result of a relatively stronger contribution of the strictly indirect PAH and the lack of sensitivity of this strain to the direct mutagenicity of mono-nitro PAH. Also strain TA97 showed a higher sensitivity to the indirect mutanicity and a lower sensitivity to the direct mutagenicity than TA98. Based on the effects in this strain, the nitroreductase-dependent mutagenicity of AAP extracts is much less important than the indirect mutagenicity.

The CFA extracts yielded two extra mutagenic SP HPLC fractions, one of them containing the dinitropyrenes and possibly other multiple nitro PAH. The other fraction elutes to the far polar side of the mutagram. The presence of S9 leads to a complete annihilation of its effect. The identity of these mutagens remains obscure, but for the fact, that also in this case their mutagenicity depends on nitroreduction. PAH do not contribute to the mutagenicity of the fly ash samples.

Comparable extracts were subjected to bioassay-directed fractionation by many other authors (Alfheim et al., 1985). In earlier studies classical acid/base liquid/liquid partitioning schemes were used, in most cases combined with straight-phase open-column liquid chromatography (Teranishi et al., 1978; Tokiwa et al., 1980; de Raat, 1983; Reali et al., 1984; Lewtas et al., 1982 and 1990, Crebelli et al., 1988, Schleibinger and Rüden, 1986). In general, mutagenicity is found in the acidic and the neutral fractions. The mutagens of the neutral fraction can be separated into two or three mutagenic fractions with column chromatography. In some cases slight effects are found in the basic fraction. These results point to the predominant rôle of the more polar mutagens. We may expect that PAH and their mono-nitro derivatives, possibly accompanied by multiple-nitro derivatives will have been isolated in the apolar neutral fraction. The mutagenicity of one or more of our more polar fractions will be caused by acidic mutagens. An obvious follow up of our study would be the combination of acid/base LLP with our fractionation scheme.

The importance of more polar mutagens was also revealed by sequential fractionation of the particles with a series of solvents with increasing polarity. Selzer Madsen (1982) used cyclohexane and methanol; the cyclohexane extract was subjected to a LLP procedure leading to a PAH fraction which contained all the mutagenicity of the cyclohexane extract. The major part (about 80%) of the mutagenicity was found back in the methanol extract; the effect of the PAH fraction could not solely be attributed to unsubstituted and alkylated PAH as a significant effect was found in the absence of S9 (see also Møller and Alfheim, 1980 and Alfheim and Møller, 1979). Other authors that used this approach were Daisey et

al. (1980) and Lioy et al. (1985), who used hexane, dichloromethane and acetone which resulted in three clearly mutagenic extracts.

Thin-layer chromatography was used by Siak et al. (1985) for the separation of mutagens in AAP. Also their results showed the importance of the more polar mutagens; about 50% of the TA98 mutagenicity could be demonstrated in the fractions containing mutagens that are more polar than mononitro and dinitro PAH. The contribution of PAH to the effect in TA98 was only small. Like ours, their results point to a negligible contribution of the dinitropyrenes. Application of strains TA98NR and TA98DNP strongly suggested that all direct mutagenicity was dependent on bacterial metabolism of nitro groups.

HPLC has been used by several groups to separate the mutagens in AAP and emissions of combustion processes (Rappaport et al., 1980; Alfheim, 1982; Salmeen et al., 1984; Harris et al., 1984 and 1987; Mast et al., 1984; Alfheim et al., 1984; Nishioka et al., 1986 and 1988). In general these studies show the importance of the more polar nitro compounds as direct mutagens in emission particles and AAP. In some studies various fractionation procedures were combined to produce fractions homogeneous enough for meaningful analyses with GC/MS. By testing the same fractions indications were obtained about the identity of the compounds actually causing the mutagenicity. Besides PAH and their mono and multiply nitrated derivatives, these studies point to oxygenated nitro PAH, in particular hydroxy-nitro PAH, nitrated ketones and acetoxy-nitro PAH (Nishioka et al., 1988 and Harris et al., 1987). The presence of these compounds still only partly explains the more polar mutagenicity. Some of the groups of mutagens that were isolated in the present study could very well consist of these compounds.

Bioassay-directed fractionation techniques such as used in the present study are, sofar, solely used for identification purposes. However they may as well be used for answering other questions related with the presence of mutagens in AAP. The difference in shape between the mutagrams prepared with different test variants (type of strain and absence or presence of S9) point to one possibility. Besides the Salmonella strains, we may use other test organisms, such as mammalian tissue-culture cells and besides point mutations we may use other test criteria (genetic end points), such as cytogenetic effects. The more general toxicological significance of the strong effects found in the Salmonella/microsome test can be investigated. These effects are most probably largely caused by nitrocompounds. The Salmonella-strains are in particular sensitive to these compounds because they themselves are able to activate them. So, the rôle of these strains is not restricted to the detection of the mutagen or mutagenic metabolite. Metabolism of these compounds in mammals differs notably from that in bacteria; in general it can be stated, that oxidative metabolism is at least as important as reductive metabolism (Tokiwa and

Onishi, 1986). *In vivo* hardly any signs of reductive metabolism by mammalian tissues was found up to now; then reduction is carried out by the gut micro flora and it is questionable whether this bears any relevance with respect to exposure to the AAP via the respiratory tract. So, the active compounds generated by S9 and by tissue-culture cells used for detection may be more relevant as regards the possible health effects than the compounds that are directly mutagenic in *Salmonella*. This may suggest that the results obtained with the strains less sensitive to the direct mutagens are more relevant than those obtained with strain TA98. However, this does not imply a less important rôle for the more polar compounds; the tests with TA98NR and TA97 do show, that these play also a predominant rôle with respect to the indirect mutagenicity. The direct effects gave the nitro mutagens in AAP an important place in experimental toxicology; the considerations above suggest that they are, at least as far as their direct effects are concerned, overexposed and that the compounds activated by mammalian metabolism deserve more attention. Our results suggest, that these are nitrocompounds as well, although not activated via their nitro group and that because of their indirect effects, nitro compounds are still more important than PAH.

Bioassay-directed fractionation may also help to investigate the influence of atmospheric conversion of PAH and other relevant compounds (or artifactual conversion during sampling and extraction) on the mutagenicity. The formation of oxygenated and nitrated derivatives from PAH under atmospheric conditions has been amply demonstrated, not only in the laboratory but also in the atmosphere itself; furthermore, some studies show that mutagenicity of the particles is changing, from indirect to direct, during the residence of the particles in the air (Van Cauwenberghe, 1987; Alfheim, 1982; Pitts et al., 1982; Selzer Madsen et al., 1982; De Raat en De Meijere, 1988). Bioassay-directed fractionation might give insight into the actual shifts taking place, which (groups of) mutagens disappear and which appear. If changes of conditions during sampling or extraction result in a shift, it may be concluded, that chemical conversion, affecting mutagenicity, takes place; such artifacts may well compensate each other in the effect of the complete extract and thus remain obscure without fractionation.

The quality of the fractionation was investigated by comparing original extracts with extracts reconstituted from the fractions. In general, it can be concluded that this comparison did not suggest the occurrence of severe losses or artifactual chemical reactions influencing mutagenicity during the various fractionation steps. The recoveries tended to differ clearly from the effects of the reconstituted extracts, which suggests that the results of fractionation were not only affected by losses and, possibly, artifactual chemical reactions, but also by changing interactions among components of the extracts. It is

obvious that the differences between on one hand the original extracts and on the other the reconstituted extracts and the recoveries make it impossible to estimate the real contribution of the various groups of mutagens. The calculated values represent in any case the effects of these groups when they are isolated. In view of the analytical-chemical results, it seems improbable that the effects of the PAH fraction and the mono-nitro PAH fraction are affected by losses or chemical reactions.

CONCLUSIONS

The following conclusions can be drawn from the present study:

- Seven distinct groups of mutagens could be discerned in AAP. Two of them were identified as PAH and mono-nitro PAH.
- A rather exact estimation of the effects of these two groups was possible. In *isolated* form and in the presence of S9 PAH accounted for about 5% of the total effect in TA98 and about 20% of the total effect in TA100. For mono-nitro PAH these percentages were about 12, 24, 14 and 13% of the total effects in respectively TA98 with and without S9 and TA100 with and without S9.
- A significant contribution of dinitropyrenes (probably together with other multiple nitrated PAH) could be ruled out.
- The direct effects of the five more polar groups of mutagens were nitroreductase dependent, which confirms the predominant rôle of nitrocompounds as mutagens in AAP. Besides direct-acting nitrocompounds, these groups also comprise indirect-acting compounds (S9 dependent), possibly, but not necessarily, these are also nitro compounds.
- The distribution of the mutagenicity over the various active chromatographical fraction was clearly strain and S9 dependent. The use of strains TA100 and TA97 pointed to the importance of indirect mutagens, also for the more polar fractions.
- No significant contribution of PAH to the mutagenicity of CFA could be demonstrated, while these particles contained two groups of mutagens that were not found in AAP: a group the effect of which is most probably caused by dinitropyrenes and comparable compounds and a polar group containing nitroreductase-dependent mutagens.

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LITERATURE

Alfheim, I, (1982)
Contribution from motor vehicle exhaust to the mutagenic activity of airborne particles
In: Mutagens in Our Environment, Eds.: M. Sorsa and H. Vainio, 235-248, Alan R. Liss, New York.

Alfheim, I. and M. Møller (1979)
Mutagenicity of long-range transported atmospheric aerosols
The Science of the Total Environment, 13, 275, 278.

Alfheim, I., G. Becher, J.K. Hongslo and T. Ramdahl (1984)
Mutagenicity testing of high performance liquid chromatography fractions from wood stove emission samples using a modified Salmonella assay requiring smaller sample volumes
Environmental Mutagenesis 6, 91-102.

Alfheim, I., A. Bjørseth and M. Møller (1985)
Characterization of microbial mutagens in complex samples - methodology and application
CRC Critical Reviews in Environmental Control, 14 (2), 91-150.

Alink, G.M., H.A. Smit, J.J. van Houdt, J.R. Kolkman and J.S.M. Boley (1983)
Mutagenic activity of airborne particulates at non-industrial locations
Mutation Research, 116, 21-34.

Ames, B.N., J. McCann and E. Yamasaki (1975)
Methods for detecting carcinogens and mutagens with the Salmonella mammalian microsome mutagenicity test
Mutation Research, 31, 347-364.

Ball, J. C. and W. C. Young, 1987
Mutagenicity studies of alpha,alpha'-dihalomethylbenzenes, alpha-methylbenzylhalides, and 1-chloromethylpyrene in the Ames assay
Mutation Research, 191, 79-84

Ball, J. C., W. C. Young and I. T. Salmeen, 1987
Catalyst-free sodium borohydride reduction as a guide in the identification of direct-acting Ames assay mutagens in diesel-particle extracts
Mutation Research, 192, 283-287

Bartsch, H., T. Kuroki, M. Roberfroid and C. Malaveille, 1982
Metabolic activation systems *in vitro* for carcinogen/mutagen screening tests
In: Chemical Mutagens - Principles and Methods for their Detection, vol. 7, F. J. de Serres and A. Hollaender eds., 95-161, Plenum, New York

Bhatia, A. L., H. Tausch and G. Stehlik
Mutagenicity of chlorinated polycyclic aromatic compounds
Ecotoxicology and Environmental Safety, 14, 48-55

Bredeweg, R.A., L.D. Rothman and C.D. Pfeiffer (1979)
Chemical reactivation of Silica columns
Analytical Chemistry, Vol. 51, 12, 2061-2063.

Cauwenberghe, K. A., van (1987)
Atmospheric reactions of PAH
In: Handbook of Polycyclic Aromatic Hydrocarbons, vol. 2, Emissions Sources and Recent Progress in Analytical Chemistry, A. Bjørseth and Th. Ramdahl eds., 351-384, Dekker, New York

Commoner, B., P. Madyastha, A. Brousdon and A.J. Vithayathil (1978)
Environmental mutagens in urban air particualtes
J. Toxicol. Environ. Health, 4, 59.

Crebelli, R., S. Fuselli, A. Meneguz, G. Aquilina, L. Conti, P. Leopardi, A. Zijno, F. Baris and A. Carere (1988)
In vitro and in vitro mutagenicity studies with airborne particulate extracts
Mutation Research, 204, 565-575.

Daisey, J.M., T.J. Kneip, I. Hawryluk and F. Mukai (1980)
Seasonal variations in bacterial mutagenicity of airborne matter in New York City
Env. Sci. & Technol. 14, 1487-1490.

Dehnen, W., N. Pitz and R. Tomingas (1977)
The mutagenicity of airborne particulate pollutants
Cancer Letters, 4, 5-12.

Gibson, T.L. (1982)
Nitro derivatives of polynuclear aromatic hydrocarbons in airborne and source particulate matter
Atmospheric Environment 16, 2037-2040.

Harris, W.R., E.K. Chess, D. Okamoto, J.F. Remsen and D.W. Later (1984)
Contribution of nitropyrene to the mutagenic activity of coal fly ash
Environmental Mutagenesis 6, 131-144.

Harris, W.R., J.F. Remsen, E.K. Chess and D.W. Later (1987)
Correlation of nitroaromatic compounds with mutagenic activity of coal fly ash
Journal of Toxicology and Environmental Health, 20, 81-103.

Hoeven, N. van der, S.A.L.M. Kooijman and W.K. de Raat (1990)
Salmonella test: relation between mutagenicity and number of colonies
Mutation Research, 234, 289-302.

König, J., E. Balfanz, W. Funcke and T. Romanowski (1983)
Determination of oxygenated polycyclic aromatic hydrocarbons in airborne particulate matter by capillary gas chromatography and gas chromatography/mass spectrometry
Analytical Chemistry, 55, 599.

Leon, A. A., J. C. Ball, S. Foxall-van Aken, G. H. Daub and D. L. Vanderjagt
Chemical reactivities and mutagenicities of a series of chloromethylbenzo(a)pyrenes
Chem.-Biol. Interactions, 56, 101-111

Lewtas, J., J. Chuang, M. Nishioka and B. Petersen (1990)
Bioassay-directed fractionation of the organic extract of SRM 1649 urban air particulate matter
Intern. J. Environ. Anal. Chem., 39, 245-256.

Lioy, P.J., M. Avdenko, R. Harkov, T. Atherholt and J.M. Daisey (1985)
A pilot indoor-outdoor study of particulate matter and particulate mutagenicity
Journal of the Air Pollution Control Association, 35, 653-657.

Maron, D.M. and B.N. Ames (1983)
Revised methods for the Salmonella mutagenicity test
Mutation Research, 113, 173-215.

Mast, T.J., D.P.H. Hsieh and J.N. Sieber (1984)
Mutagenicity and chemical characterization of organic constituents in rice straw smoke particulate matter
Environmental Science and Technology, 18, 338-348.

Møller, M. and I. Alfheim (1980)
Mutagenicity and PAH - analysis of airborne particulate matter
Atmospheric Environment, 14, 83-88.

Nielsen, T. (1984)
Reactivity of polycyclic aromatic hydrocarbons towards nitrating species
Environmental Science and Technology 18, 157-163.

Nielsen, T., B. Seitz and T. Ramdahl (1984)
Occurrence of nitro-PAH in the atmosphere in a rural area
Atmospheric Environment, 18, 2159-2165.

Nielsen, T., T. Ramdahl and A. Bjørseth (1983)
The fate of airborne polycyclic organic matter
Atmospheric Health Perspectives, 47, 103-114.

Nishioka, M.G., C.C. Howard and J. Lewtas (1986)
Detection of hydroxy-nitro PAH's and nitro-PAH's in ambient air particulate matter using bioassay directed formation
In: Polynuclear Aromatic Hydrocarbons: Chemistry, Characterization and Carcinogenesis; eds. M. Cooke and A.J. Dennis, Battelle Press, 1986.

Nishioka, M.G., C.G. Howard, D.A. Contos, L.M. Ball, J. Lewtas (1988)
Detection of hydroxylated nitro aromatic and hydroxylated nitro polycyclic aromatic compounds in an ambient air particulate extract using bioassay-directed fractionation,
Env. Sci. & Technol., 22, 908-915.

Pitts, J. N. Jr. (1987)
Nitration of gaseous polycyclic aromatic hydrocarbons in simulated and ambient urban atmospheres: a source of mutagenic nitroarenes
Atmospheric Environment, 21, 2531-2547

Pitts, J.N. Jr. (1983)
Formation and fate of gaseous and particulate mutagens and carcinogens in real and simulated atmospheres
Environmental Health Perspectives, 47, 103-114.

Pitts, J.N. Jr., D. Grosjean and T.M. Mischke (1979)
Mutagenic activity of airborne particulate organic pollutants
In: Assessing Toxic Effects of Environmental Pollutants; S.D. Lee and J.B. Mudd Eds.; 1979, 219-235.

Pitts, J.N., W. Harger, D.M. Lokensgard, D.R. Fitz, G.M. Scorziell and V. Meija (1982)
Diurnal variations in the mutagenicity of airborne particulate organic matter in California's south air basin
Mutation Research 104, 35-41.

Raat, W.K., de (1983)
Genotoxicity of aerosol extracts. Some methodological aspects and the contribution of urban and industrial locations
Mutation Research, 116, 47-64.

Raat, W.K. de, F.A. de Meijere and S.A.L.M. Kooijman (1985)
Mutagenicity of ambient aerosol collected in an urban and industrial area of the Netherlands
The Science of the Total Environment 44, 17-33.

Raat, W.K. de, N. v.d. Hoeven, S.A.L.M. Kooijman, P.I. Muttzall and M.I. Willems (1987)
Studies on some factors influencing the response of the *Salmonella*/microsome test
Report of the Dutch Organization for Applied Scientific Research (TNO), Division of Technology for Society (MT): R 87/177[a], Delft, The Netherlands.

Raat, W.K. de and F.A. de Meijere (1988)
The mutagenicity of ambient air particles from local traffic and distant sources during episodes with moderate photochemical air pollution
The Science of the Total Environment, 73, 159-179.

Rannug, U., U. Nilsson and A. Colmsjö, 1986
The mutagenicity and chemical structure of certain chloroderivatives of polynuclear aromatic hydrocarbons
In: Genetic Toxicology of Environmental Chemicals, part B: Genetic Effects and Applied Mutagenesis, 157-164, Alan R. Liss, New York

Rappaport, S.M., Yi.Y. Wang, E.T. Wei, R. Sawyer, B.E. Watkins and H. Rapoport (1980)
Environment Science and Technology 12, 1505-1509 (1980)

Reali, D., H. Schlitt, C. Lohse, R. Barale and N. Loprieno (1984)
Mutagenicity and chemical analysis of airborne particulates from a rural area in Italy
Environmental Mutagenesis, 6, 813-823.

Rosenkranz, H.S. and R. Mermelstein (1983)
Mutagenicity and genotoxicity of nitroarenes: all nitro-containing chemicals were not created equal
Mutation Research 114, 217.

Rosenkranz, H.S. and R. Mermelstein (1985)
The genotoxicity, metabolism and carcinogenicity of nitrated polycyclic aromatic hydrocarbons
Journal of Environmental Science and Health, C3, 221-272.

Salmeen, I.T., A.M. Pero, R. Zator, D. Schuetzle and T.L. Riley (1984)
Ames assay chromatograms and the identification of mutagens in diesel particle extracts
Environmental Science and Technology, 18, 375-383.

Schleibinger, H. and H. Rüden (1986)
Mutagenicity of fractionated organic extracts derived from suspended particulate matter
J. Aerosol. Sci., 17, 365-369.

Selzer Madsen, E., P.A. Nielsen and J.C. Pedersen (1982)
The distribution and origin of mutagens in airborne particulates detected by the Salmonella/microsome assay in relation to levels of lead, vanadium and PAH
The Science of the Total Environment, 24, 13-25.

Shah, A. B., R. D. Combes and I. R. Rowland, 1990
Activation and detoxification of 1,8-dinitropyrene by mammalian hepatic fractions in the *Salmonella* mutagenicity assay
Mutagenesis, 5, 45-49

Siak, J., T.L. Chan, T.L. Gibson and G.T. Wolff (1985)
Contribution of bacterial mutagenicity from nitro-PAH compounds in ambient aerosols
Atmospheric Environment, 19, 369-376.

Stray, H., S. Mano, A. Mikalsen and M. Oehme (1984)
Selective determination of substituted PAH in aerosols using liquid CO_2 extraction, HPLC prefractionation on chemically activated silica and HRGC combined with negative ion mass spectrometry
Journal of High Resolution Chromatography & Chromatography Communications, 1984, 74-82.

Talcott, R. and W. Wei (1977)
Airborne mutagens bioassayed in *Salmonella typhimurium*
Journal of the National Cancer Institute 58, 449-451.

Talcott, R. E. and W. Harger, 1981
Chemical characterization of direct-acting airborne mutagens: the functional group
Mutation Research, 91, 433-436

Tanner, R.L. and R.F. Fajer (1983)
Determination of nitro-polynuclear aromatics in ambient aerosol samples
International Journal of Environmental Analytical Chemistry 14, 231-241.

Teranishi, K., K. Hamada and H. Watanabe (1978)
Mutagenicity in *Salmonella typhimurium* mutants of the benzene-soluble organic matter derived from airborne particulate matter and its five fractions
Mutation Research 56, 273-280.

Tokiwa, H., S. Kitamori, K. Takahishi and Y. Onishi (1980)
Mutagenic and chemical assay of extracts of airborne particulates
Mutation Research 77, 99-108.

Tokiwa, H., S. Kitamori, R. Nakagawa, K. Horikawa and L. Matamala (1983)
Demonstration of a powerful mutagenic dinitropyrene in airborne particulate matter
Mutation Research, 121, 107-116.

Tokiwa, H. and Y. Onishi, 1986
Mutagenicity and carcinogenicity of nitroarenes and their sources in the environment
CRC Critical Reviews in Toxicology, 17, 23-60

Valerio, F., P. Bottino, D. Ugolini, M.R. Cimberle, G.A. Tozzi and A. Frigerio (1984)
Chemical and photochemical degradation of polycylic aromatic hydrocarbons in the atmosphere
The Science of the Total Environment 40, 169-188.

Wang, C.Y., M.S. Lee, C.M. King and P.O. Warner (1980)
Evidence for nitroaromatics as direct-acting mutagens of airborne particulates
Chemosphere 9, 83-87.

Yamasaki, E. and B.N. Ames (1977
Concentration of mutagens from urine by absorption with the non-polar resin XAD-2: cigarette smokers have mutagenic urine
Proceedings of the National Academy of Sciences U.S.A. 74, 3555-3559.

CHAPTER 11

COMPARISON OF FILTER MATERIALS USED FOR SAMPLING OF MUTAGENS AND POLYCYCLIC AROMATIC HYDROCARBONS IN AMBIENT AIRBORNE PARTICLES

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W. K. DE RAAT, G. L. BAKKER and F. A. DE MEIJERE
T.N.O., P.O. Box 217, 2600 AE Delft, Schoemakerstraat 97, 2628 VK Delft, The Netherlands

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Abstract—The filter material used in high-volume sampling of ambient airborne particles was investigated for its influence on mutagenicity and PAH content of the particles.

The particles were collected simultaneously with glass-fibre filters (G filters) and Teflon filters (T filters) or Teflon-coated filters (Tc filters). Sampling was carried out 17 times at different locations in The Netherlands.

The use of G filters lead to slightly higher mutagenicity in almost all experiments. In addition every sample collected with this filter material contained substantially higher concentrations of the lower molecular weight PAH which are partly present in the vapour phase. In contrast some experiments revealed slightly lower concentrations of non-volatile PAH which are sensitive to chemical conversion under atmospheric conditions.

A multivariate analysis of the concentrations did hardly show any influence of the filter material. The main factor affecting the PAH profile was volatility; factor analysis revealed chemical reactivity as a second factor.

The differences between the concentrations of the volatile PAH can be explained by the lower pressure drop across the G filters, resulting in less artifactual evaporation. In addition, adsorption of these compounds from the gas phase on this filter material was found.

The latter phenomenon may also explain the differences in mutagenicity, as it has been shown that PAH adsorbed on glass-fibre material react with gaseous air components to form mutagens. The lower concentrations of non-volatile PAH on the G filters points directly to conversion which may also have contributed to the differences in mutagenicity.

It is not possible to decide whether the chemical conversion on the T and the Tc filters (demonstrated with factor analysis) has an artifactual origin or takes place in the ambient air before the air reaches the filter.

It is concluded, that the differences in mutagenicity between the filter materials are too small to support the use of T or Tc filters instead of G filters in mutagenicity studies.

The adsorption of volatile PAH on filter material makes it uncertain to what extent high-volume sampling by filtration leads to over- or underestimation of the volatile PAH present in the particulate phase of the air.

Key word index: Airborne particles, sampling artifacts, filter material, mutagenicity, PAH.

INTRODUCTION

In studies on the mutagenic properties of ambient airborne particles, the particles are collected by filtration at relatively high flow rates (so-called high-volume sampling). Each particle is fixed on the filter material in a layer of other particles for the remainder of the sampling time. The particles are exposed to each other, to the filter material and to the gaseous components of the air passing the filter. They are no longer freely floating in the air, but exposed to an enforced stream of air. So exposure conditions as well as compounds exposed to are changed compared to the situation in the air.

We have to reckon with the possibility that this change will cause a quantitative as well as qualitative change of the reactions which affect the composition of the particles. Artifacts are the result if mutagens are inactivated, transformed into other mutagens or if new mutagens are formed from non-mutagenic compounds. Moreover, the composition may be changed with respect to the compounds that are not mutagenic themselves, but influence the response of the mutagenicity test.

Concern about these artifacts is in first instance caused by the finding, that polycyclic aromatic hydrocarbons (PAH) are apt to artifactual conversion during sampling (Lee *et al.*, 1980; Grosjean *et al.*, 1983; van Cauwenberghe *et al.*, 1980; Brorström *et al.*, 1983). Artifactual conversion of PAH bears relevance to mutagenicity for three reasons.

Firstly, a number of PAH are clear-cut mutagens or/and carcinogens (NRC, 1983; Dipple, 1976). However, it seems improbable, that a decrease of the contribution of PAH to mutagenicity will significantly affect mutagenicity of the particles. Studies aimed at the identification of the mutagens have shown that the PAH contribution is only small (Siak *et al.*, 1985; Alfheim, 1982; de Raat, 1988).

Secondly, the products arising from the PAH are of importance. Upon exposure to ambient air, PAH can be transformed into nitrated and oxygenated species

(Nielsen *et al.*, 1983; Pitts, 1983, 1987; Valerio *et al.*, 1984; Nikolao *et al.*, 1984; Miguel, 1984). These compounds, in particular the nitrated ones, can be strong mutagens (Rosenkranz and Mermelstein, 1983). A number of authors have reported their presence in the particles and nowadays it is generally believed that they at least strongly contribute to the mutagenicity. Therefore, it cannot be ruled out, that artifactual conversion of PAH leads to strongly mutagenic compounds, and so affects the mutagenicity of the extracts.

The similarity in chemical structure of PAH and mutagens brings us to the third reason. Similarity in structure may imply similarity in reactivity. Conversion of mutagens can be expected if conversion of PAH occurs.

The present paper describes a study in which artifactual changes of mutagenicity were investigated alongside artifactual conversion of PAH.

So far two studies have provided clear indications that artifactual conversion of PAH depends on the filter material used (Lee *et al.*, 1980; Grosjean *et al.*, 1983). Higher concentrations are found when particles are sampled with Teflon filters (T filters) or Teflon-coated glass filters (Tc filters) instead of the traditional glass-fibre filters (G filters) or quartz-fibre filters. These results strongly suggest (stronger?) artifactual conversion in the latter two types of filters.

We carried out a series of experiments in which the particles were sampled simultaneously with G filters and with T or Tc filters. So a sampling condition known to affect PAH concentrations was varied. The objective was to confirm this artifactual conversion of PAH and to investigate whether it is accompanied by a change of mutagenicity.

METHODS AND MATERIALS

Sampling

The particles were collected with Sartorius HV 100 high volume samplers and three types of filters; Sartorius SM 13400 glass-fibre filters (G filters), Sartorius SM 11842 Teflon filters (T filters) and Pallflex T60 A20 filters made of glass-fibre material with a Teflon coating (Tc filters). G filters were cleaned by Soxhlet extraction with methanol; other filters were used without any pretreatment. The diameter of the filters was 25.7 cm.

Preliminary experiments showed that the T and Tc filters had to be supported by G filters because of their fragility. For this reason every sample was taken with a double filter, i.e. every filter with particles was supported with a G filter. In a number of experiments these under filters were also extracted for further investigation.

Sampling was carried out at three locations: Delft, on the TNO-Zuidpolder terrain to the south-east of the town, at ground level; Blijdorp, in Rotterdam Zoological Garden, at ground level; Lekhaven, on the roof of an old factory in the seaport area of Rotterdam, at an altitude of about 50 m. The sampling locations are indicated on the map (Fig. 1). The filters with particles were stored at −80°C.

Further details about sampling are given in Table 1.

Extraction

Within a few days of sampling the filters with particles were extracted for 8 h (about 20 cycles) in a Soxhlet apparatus with methanol (Rathburn, HPLC-grade) in the dark. During extraction the air in the Soxhlet apparatus was replaced by nitrogen. The solvent was removed from the extract with a rotation evaporator (30°C, in the dark and under reduced pressure). The residues were dissolved in dimethyl sulphoxide (Merck, A.R.) for mutagenicity testing. The original extracts, or in some experiments the residues dissolved in methanol, acetone (Nanograde) or dimethyl sulphoxide, were analyzed for PAH. The dissolved residues were stored at −30°C or −80°C.

Mutagenicity testing

The residues dissolved in dimethyl sulphoxide were tested for mutagenicity with the *Salmonella*/microsome test as described by Ames *et al.* (1975) and Maron and Ames (1983).

All samples were tested with strain TA98; in some experiments we also applied strain TA98NR, a nitroreductase deficient derivative of TA98 (Rosenkranz and Mermelstein, 1983) and strain TA100. All samples were tested with and without a liver S9 fraction prepared from rats treated with 500 mg kg^{-1} of Aroclor 1254, according to the procedure of Ames *et al.* (1975).

Testing was carried out in such a way that comparison of filter materials and comparison of results with and without S9 fraction is not hampered by the inter-test variation, i.e. the variation of results obtained on different days, with different cultures and different batches of materials. All doses were tested in duplicate and were added to the plates in the same volume (0.1 ml). The colonies of histidine prototrophics were counted with a New Brunswick Biotran II automated colony counter.

Determination of PAH concentrations

The concentrations of PAH were determined with reversed-phase high-performance liquid chromatography (RP-HPLC). Stationary phase: Supelcosil LC-PAH (250 × 4.6 mm); mobile phase: gradient from 75 to 100% methanol in water; detection: fluorescence, excitation 250 nm, emission > 390 nm; identification and quantification on the basis of chromatograms with external standards.

The concentrations of the following PAH were determined: Phenanthrene (PHE), anthracene (AC), fluoranthene (FL), pyrene (PY), benz(*a*)anthracene (BAA), chrysene (CH), benzo(*e*)pyrene (BEP), perylene (PE), benzo(*b*)fluoranthene, benzo(*k*)fluoranthene (BKF), benzo(*a*)pyrene (BAP), benzo(*g,h,i*)perylene (BGH), indeno(*c,d*)pyrene (IP) and anthanthrene (AN).

Multivariate analysis

The PAH profile, i.e. the proportion of the PAH concentrations, was investigated for its fluctuation with two multivariate techniques: principal-component analysis with the correlation matrix and factor analysis with orthogonal rotation according the varimax criterion (Morrison, 1972; Thomas, 1986).

RESULTS

Mutagenicity

In all 17 experiments both types of upper filters as well as the G filters under the T or Tc filters have been tested for mutagenicity. In addition other extracts have been tested in several experiments: those of the filters under the G filters and combined extracts of upper and under filters for the "G samples" as well as for the "T or Tc samples".

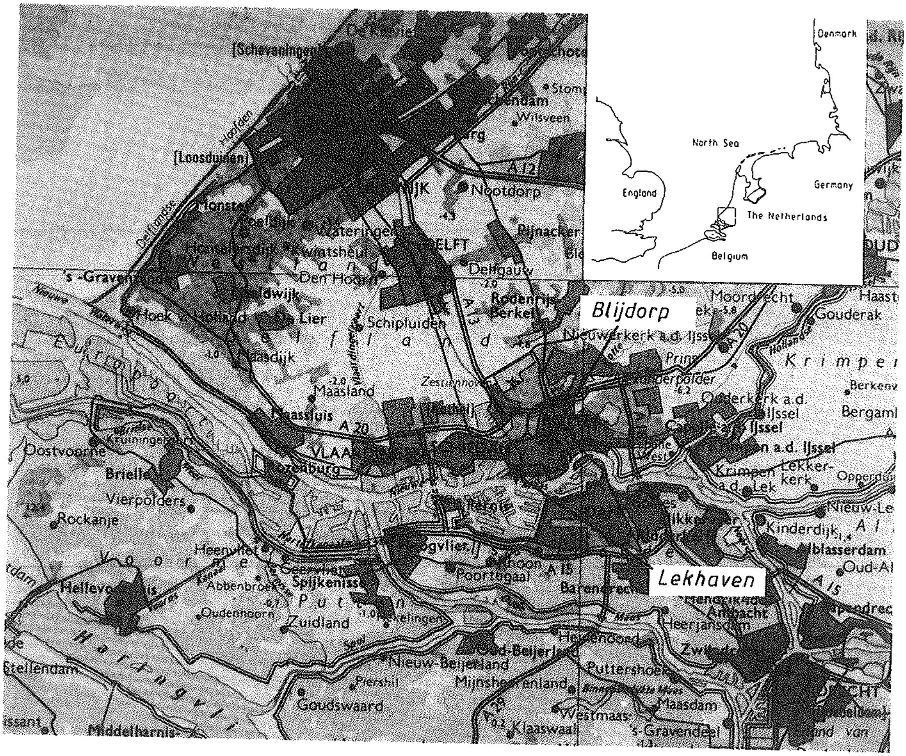

Fig. 1. Location of the sampling sites.

Table 1. List of experiments carried out

Experiment	Start Date	Start Time	Location	Filter*	Air flow rate ($m^3 h^{-1}$)
1	19–04–84	16.30	Delft	Tc	50
2	25–04–84	10.45	Delft	Tc	100
3	09–05–84	11.00	Delft	Tc	80
4	22–01–85	11.00	Lekhaven	Tc	60
5	23–01–85	11.00	Lekhaven	Tc	60
6	24–01–85	11.00	Lekhaven	Tc	60
7	23–10–85	11.00	Blijdorp	Tc	100
8	30–10–85	11.15	Blijdorp	Tc	100
9	06–11–85	10.50	Blijdorp	Tc	100
10	13–11–85	11.15	Blijdorp	Tc	100
11	20–11–85	11.15	Blijdorp	Tc	100
12	27–11–85	10.55	Blijdorp	Tc	100
13	04–12–85	11.20	Blijdorp	Tc	100
14	20–11–84	10.45	Lekhaven	T	90
15	21–11–84	10.45	Lekhaven	T	60
16	09–01–85	11.00	Lekhaven	T	80
17	21–01–85	11.00	Lekhaven	T	60

* Filter: in every experiment the indicated filter (Tc for Teflon-coated glass-fibre and T for Teflon) was compared with a glass-fibre filter. Sampling lasted 24 h.

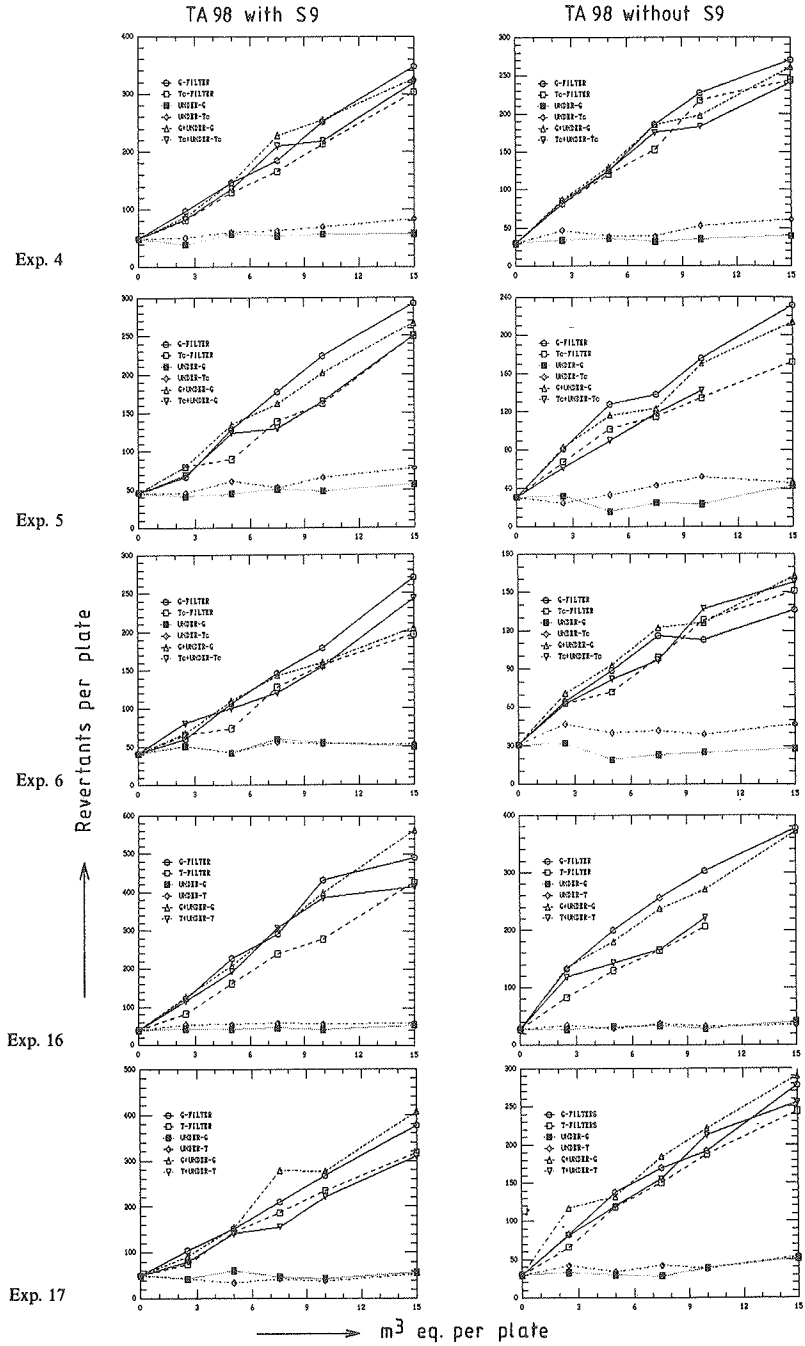

Fig. 2. Results of the *Salmonella*/microsome tests with the extracts of the filters; the five complete experiments (see text).

Five experiments were complete in the sense that all six extracts were tested; Fig. 2 depicts the results of these experiments obtained with strain TA98.

At first observation the figure reveals that the mutagenicity does not strongly depend on the type of filter material, whether determined in the presence or absence of S9 fraction. At a closer look, extracts of T or Tc upper filters appear to be somewhat less mutagenic than those of the G upper filters. In some experiments this difference decreases if the extracts of the under filters are added to those of the upper ones; in others the combination of extracts does not seem to affect the difference. No significant mutagenic effects were found with the extracts of the under filters.

In general, the other ('incomplete') experiments confirm the results of the complete ones. In some of them larger differences between the effects of the upper filters were found; results of these are depicted in Fig. 3. Others surprisingly produced clear mutagenic effects for under extracts; this is exemplified by results depicted in Fig. 4.

The results obtained with the two other strains did not differ much from those obtained with strain TA98 with respect to the comparison of the filter materials. The use of the nitro-reductase deficient strain lead to substantially lower effects, in particular in the absence of the S9 fraction. This clearly points to an important contribution of compounds the mutagenicity of which is due to bacterial reduction of their nitro group. As expected (see de Raat *et al.*, 1988) we found that the S9 fraction affects the response differently if TA100 is used; a clear increase of the effect in this strain, whereas a decrease or no influence was found for strain TA98.

We have tested blank extracts of the filter materials on several occasions. The results are not presented in detail. Only the extracts obtained with the Tc filters showed a slight mutagenicity. However, the doses needed for a clear effect were so high that blank mutagenicity cannot have influenced the comparison of the materials.

PAH concentrations

Table 2 lists the PAH concentrations for the six experiments in which the extracts of the two upper filters as well as those of the two under filters were analyzed. We did not analyze the extracts of the second and the third experiment.

A striking finding is the presence of the more volatile PAH in the under filters. Relatively (compared to the upper filters) large amounts were found. The T and Tc material differed from the G material with respect to this phenomenon. Larger amounts of benz(*a*)anthracene and chrysene (in some experiments also pyrene and/or fluoranthene) were found in the extracts of the under filters of the T or Tc samples;

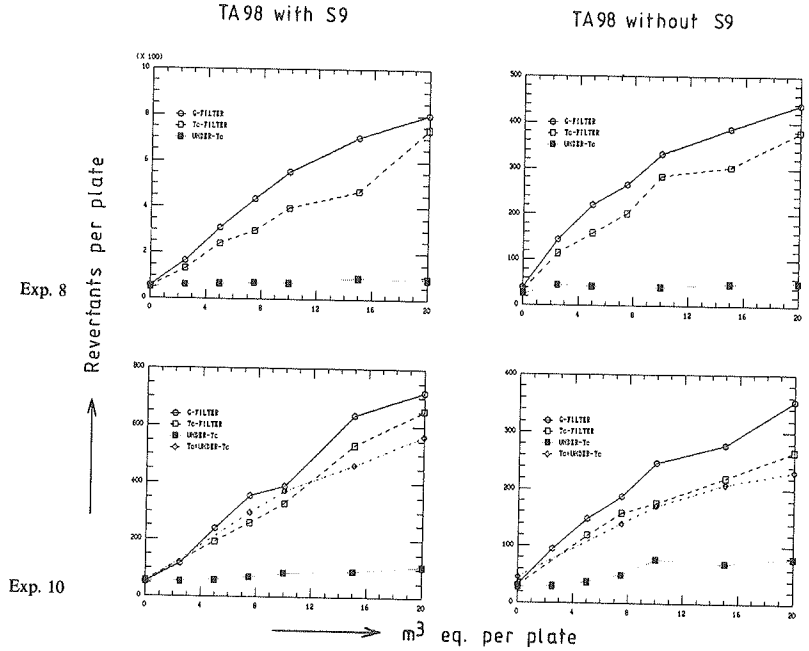

Fig. 3. Results of the *Salmonella*/microsome tests with the extracts of the filters; incomplete experiments (see text) showing clear differences between upper G and upper TC or upper T filters.

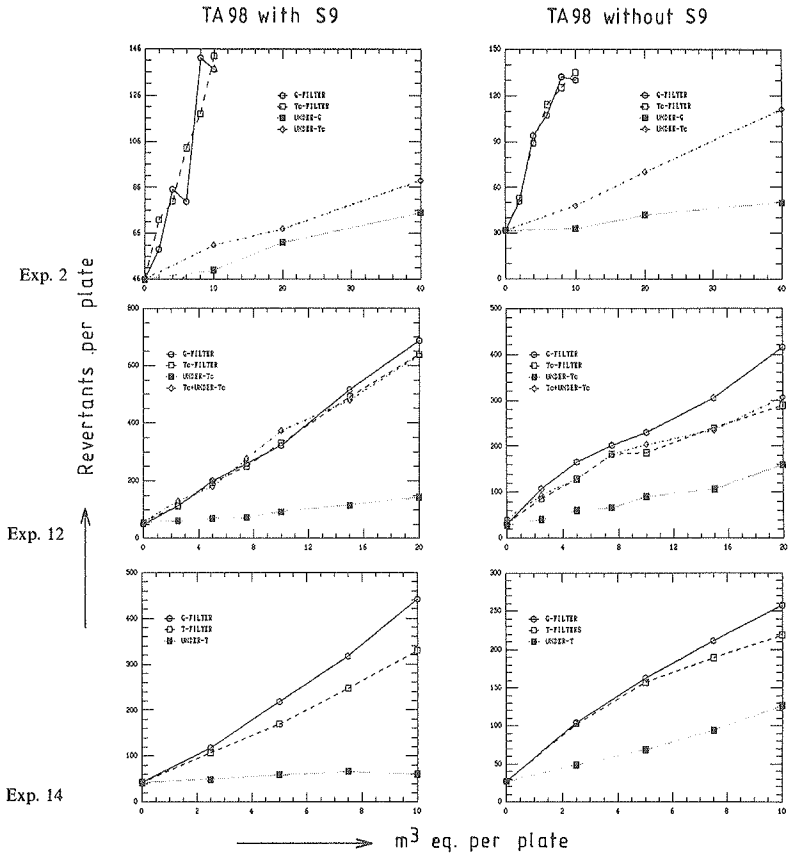

Fig. 4. Results of the *Salmonella*/microsome tests with the extracts of the filters; incomplete experiments (see text) showing clear effects of under filters (G filters supporting the upper filters).

traces of the non-volatile PAH were also found in these extracts.

In general the upper G filters contained larger amounts of the more volatile PAH, in particular fluoranthene and pyrene, than the upper T and Tc filters.

The analysis of the under filters of the G samples was omitted in the other experiments. The results of these experiments are not presented in detail. They confirm those of the 'complete' ones in the sense that relatively high concentrations of the more volatile PAH were found in the under filters of the T or Tc samples and that the concentrations of these PAH were higher in the upper G filters.

The comparison of the filter materials is of course affected by any difference in flow rate between the samples or by any differences in dilution between the extracts. We may use the concentrations of a supposedly non-volatile and stable PAH to correct for such differences. In that case we assume, that volatility and reactivity are the only factors affecting the differences between the amounts collected by the two samples of an experiment. Furthermore we assume that the amounts sampled of this 'correction' PAH are not affected by these factors. We used benzo(*b*)fluoranthene (BBF) for this purpose. The concentrations of this PAH can be easily determined with HPLC and relatively high concentrations of it are found in the particulate phase of ambient air. Several studies (de Raat *et al.*, 1987a) made clear, that it is not present in the gas phase passing the filter. Nielsen (1984) showed that it belongs to the group of very inert PAH. A possible influence of the filter material can be expected to be much stronger for PAH like BAP, which is much more sensitive to chemical conversion, and PY, which is present in the gas phase as well as the particulate phase of the air.

The concentrations were divided for each sample by the BBF concentration of that sample. Then we calculated the ratios of these 'normalized' concentra-

Table 2. Concentrations of the PAH in the extracts of the filters

PAH	Experiment 1				Experiment 4				Experiment 5				Experiment 6				Experiment 16				Experiment 17			
	Tc	U-Tc	G	U-G	Tc	U-Tc	G	U-G	Tc	U-Tc	G	U-G	Tc	U-Tc	G	U-G	T	U-T	G	U-G	T	U-T	G	U-G
PHE	2.86	0.25	3.08	0.34	2.52	2.10	2.75	2.22	0.20	1.02	1.48	1.03	1.69	1.90	3.26	2.73	2.75	3.96	7.25	3.66	3.60	1.48	4.67	1.56
AC	0.17	0.01	0.18	0.01	0.14	0.10	0.20	0.10	0.10	0.09	0.25	0.07	0.08	0.09	0.18	0.15	0.16	0.26	0.47	0.25	0.06	0.08	0.12	0.06
FL	4.53	0.48	4.49	0.54	3.18	8.52	10.73	5.25	1.39	3.24	5.81	2.95	2.03	5.63	8.08	5.64	7.31	9.22	17.86	2.33	1.81	6.16	7.75	6.59
PY	3.18	0.19	2.26	0.24	2.42	6.06	7.66	3.77	0.92	2.65	4.39	0.06	1.00	3.68	4.90	3.41	5.01	5.67	11.91	1.15	0.94	3.99	4.03	4.11
BAA	1.91	0.60	2.23	0.04	3.07	0.38	2.98	0.12	0.87	0.17	1.44	0.06	1.30	0.24	1.94	0.07	2.18	0.22	2.71	0.04	1.83	0.55	2.92	0.07
CH	3.82	1.17	4.59	0.07	4.54	0.40	4.01	0.09	1.27	0.21	2.21	<	2.72	0.47	3.85	0.06	6.24	0.37	7.45	<	3.37	0.91	5.42	0.11
BEP	3.16	0.13	3.09	<	3.08	0.16	2.45	<	0.57	<	0.64	<	1.85	<	2.26	<	3.71	<	4.00	<	2.69	<	3.39	<
PE	0.44	<	0.30	<	0.67	0.02	0.59	<	0.13	<	0.19	<	0.21	0.01	0.27	<	0.33	<	0.39	<	0.55	<	0.62	<
BBF	4.71	0.12	4.37	0.01	4.87	0.18	4.16	<	1.08	0.01	1.54	<	3.11	0.15	3.96	<	6.38	0.01	7.32	0.01	4.54	0.04	5.67	<
BKF	1.96	0.04	1.80	0.01	2.08	0.06	1.64	<	0.46	0.07	0.66	<	1.29	0.06	1.64	<	2.41	0.01	2.72	0.01	1.84	0.01	2.45	<
BAP	2.45	0.01	1.64	<	3.07	0.08	3.01	<	0.71	0.03	1.00	<	1.11	0.03	1.26	<	2.91	0.01	3.37	0.01	2.57	0.01	2.87	<
BGH	5.43	0.18	4.82	<	4.54	0.15	3.89	<	1.35	0.09	2.03	<	2.56	0.14	3.06	<	4.20	<	4.91	<	3.86	<	4.52	<
IP	3.48	0.08	3.36	<	3.18	0.12	2.89	<	0.96	0.06	1.34	<	1.97	0.09	2.67	<	3.89	<	4.40	<	3.22	<	3.72	<
AN	0.32	<	0.15	<	0.38	<	0.31	<	0.12	<	0.15	<	0.11	<	0.13	<	0.50	<	0.54	<	0.31	<	0.24	<

Concentrations in ng m^{-3}.
< : Concentration below detection limit.
Tc : Teflon-coated glass fibre.
T : Teflon.
G : Glass fibre.
U : Support (under) filter.

tions for each experiment, i.e. the G/T or G/Tc ratios. These are listed in Table 3. The dependence on filter material of the PAH concentrations can be easily observed from this table.

For most of the non-volatile PAH the ratios do not differ much from unity, which indicates the absence of a dependence. Only in the case of AN, which is very sensitive to chemical conversion, clearly higher amounts were found in some T and Tc extracts. High ratios were found for the more volatile PAH.

The normalized concentrations are summarized in Table 4.

Multivariate techniques were used for comparison of the filter materials with regard to the variation of the PAH profile (the relative concentrations of the PAH). The results are listed in Table 5. Principal-component (PC) analysis reveals a high correlation of the concentrations of the less volatile PAH (PAH with more than four rings) with the first PC. The significant variance of the second PC appears to be mainly the result of an extra source of fluctuation for the more volatile PAH. The results of the PC analysis do not differ much for the two filter materials.

Factor analysis with varimax rotation points to a clear separate factor affecting the concentrations of the more volatile PAH. In case of T and Tc filters, this factor is also manifest for BAA and CH, whereas the PHE concentrations seem to be more strongly affected by this factor when a G filter is used.

If we consider the results of the factor analysis for the less volatile PAH we find a clear separate factor in case a G filter is used, which affects the concentrations of PE, BAP and AN. As the results are influenced by the strong 'volatility factor', a separate analysis was carried out for the concentrations of the less volatile PAH. Then the two groups of samples yield nearly identical results.

DISCUSSION

Mutagenicity

The results of this study show that the use of T or Tc filters instead of G filters consistently leads to slightly lower mutagenic effects of the collected samples. We may call the difference reassuringly small, as the great majority of the studies on the mutagenicity of airborne particles have been carried out with G filters. Although the differences do point to artifacts (see below), they do not justify a drastic re-evaluation of the results obtained with G filters so far. It can even be questioned whether they provide convincing arguments for the use of T and Tc filters in future studies. As these filters have a much greater air resistance and are more difficult to handle because of their fragility (at least in our hands), G filters may even be preferred.

The value of these conclusions depends of course on the representativeness of the particles sampled and the conditions during sampling. Although 17 experiments were carried out, spread out over three locations in

Table 3. The ratios of the PAH concentrations after normalization for the BBF concentrations

PAH	Exp. 1	Exp. 4	Exp. 5	Exp. 6	Exp. 7	Exp. 8	Exp. 9	Exp. 10	Exp. 11	Exp. 12	Exp. 13	Exp. 14	Exp. 15	Exp. 16	Exp. 17
PHE	1.24	1.28	5.19	1.51	1.08	1.55	1.36	1.66	1.53	1.87	1.20	1.86	1.31	2.30	1.04
AC	1.14	1.67	1.75	1.77	1.25	2.19	1.30	2.17	1.34	1.68	1.70	2.10	1.06	2.56	1.60
FL	1.07	3.95	2.93	3.13	1.52	2.01	2.77	2.25	1.49	3.68	3.00	1.66	2.63	2.12	3.43
PY	0.77	3.71	3.35	3.85	2.06	2.17	8.00	3.24	1.66	*	*	1.49	2.79	2.07	3.43
BAA	1.26	1.14	1.16	1.17	1.23	1.11	1.60	1.12	0.96	1.20	1.81	1.55	1.83	1.08	1.28
CH	1.30	1.03	1.22	1.11	1.21	1.07	1.61	1.12	0.91	1.18	1.69	1.32	1.79	1.04	1.29
BEP	1.05	0.93	0.79	0.96	1.03	0.94	1.00	0.98	0.84	0.88	0.94	0.95	0.94	0.94	1.01
PE	0.73	1.03	1.02	1.01	1.08	1.03	0.92	1.08	0.99	0.97	0.99	0.93	0.81	1.03	0.90
BBF	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
BKF	0.99	0.92	1.00	1.00	1.10	1.02	1.03	1.02	1.01	0.93	1.00	1.02	1.03	0.98	1.07
BAP	0.72	1.15	0.99	0.90	1.15	0.98	0.87	1.04	0.98	0.93	0.98	0.90	0.74	1.01	0.89
BGH	0.96	1.00	1.05	0.94	1.12	1.02	1.02	1.04	0.97	1.00	0.99	1.02	0.99	1.01	0.94
IP	1.04	1.06	0.98	1.06	1.10	0.97	1.00	1.03	0.97	0.93	0.98	0.99	0.98	0.99	0.93
AN	0.51	0.96	0.88	0.93	1.12	1.06	0.69	1.07	0.92	0.85	0.95	0.64	0.55	0.94	0.62

The table lists the ratio between the PAH concentrations in the extracts of the G filters and those in the extracts of the Tc or T filters after they had been divided by the concentrations of BBF.

Table 4. Means of the PAH concentrations normalized for the BBF concentrations

PAH	PHE		AN		FL		PY		BA		CH		BEP		PE		BBF		BKF		BAP		BGH		IP		AN	
filter	T	G	T	G	T	G	T	G	T	G	T	G	T	G	T	G	T	G	T	G	T	G	T	G	T	G	T	G
$\bar{x}$	0.68	1.04	0.05	0.08	0.95	2.13	0.46	1.30	0.56	0.67	0.95	1.12	0.61	0.57	0.11	0.11	1	1	0.43	0.43	0.63	0.62	1.05	1.06	0.77	0.78	0.11	0.11
Exp. 1, 4–13																												
s	0.28	0.35	0.04	0.06	0.49	0.87	0.34	0.71	0.19	0.15	0.25	0.17	0.05	0.07	0.02	0.03	0	0	0.02	0.03	0.13	0.17	0.15	0.17	0.10	0.10	0.02	0.06
$\bar{x}$	0.49	0.74	0.02	0.04	0.63	1.44	0.41	0.89	0.39	0.56	0.75	0.98	0.69	0.65	0.10	0.09	1	1	0.40	0.41	0.47	0.42	0.82	0.81	0.70	0.68	0.08	0.05
Exp. 14–17																												
s	0.21	0.24	0.01	0.02	0.37	0.70	0.20	0.50	0.04	0.15	0.17	0.04	0.11	0.10	0.04	0.03	0	0	0.01	0.03	0.06	0.08	0.11	0.10	0.06	0.06	0.01	0.01
$\bar{x}$	0.63	0.96	0.04	0.07	0.86	1.94	0.45	1.19	0.52	0.64	0.89	1.08	0.62	0.60	0.11	0.11	1	1	0.42	0.42	0.59	0.57	0.99	0.99	0.75	0.75	0.10	0.09
Exp. 1, 4–13, 14–17																												
s	0.27	0.35	0.04	0.05	0.47	0.86	0.32	0.67	0.18	0.16	0.24	0.16	0.08	0.09	0.03	0.03	0	0	0.02	0.03	0.13	0.18	0.17	0.19	0.10	0.10	0.05	0.06

T : T or Tc filter.
G: G filter.
$\bar{x}$: Mean.
s : Standard deviation of $\bar{x}$.
See text and subscript of Table 3 for normalization procedure.

The Netherlands and a period of 20 months, we cannot exclude the possibility that other source areas and meteorological conditions would have lead to greater differences in mutagenicity between the samples collected simultaneously. No sampling occurred during episodes of photochemical air pollution. Then concentrations of reactive compounds such as nitrogen oxides, ozone and hydroxyl radicals are substantially increased. The resulting greater reactivity of the air passing the filter might have lead to greater differences.

So far three other studies dealt with the influence of the filter material on the mutagenicity of the particles.

Fitz *et al.* (1984) followed an experimental set-up comparable to ours and they did not find any dependence of mutagenicity on filter material.

Minor effects were found by Daisey *et al.* (1983) when they compared G and Tc filters. Their filters were extracted with a series of solvents with increasing polarity. Only the mutagenicity of the most polar fractions appeared to be dependent of the filter material. A detailed inspection of their mutagenicity results strongly suggest that both materials affect the mutagenicity.

Alfheim and Lindskog (1984) compared various sampling techniques among them filtration with different filter materials. They obtained very clear differences, even between G filters of different brands or with different pretreatments. Their Tc filters gave higher effects than their G filters!

The results of the first two studies agree with ours. They do not point to important differences between the effects obtained with different materials and therefore do not support a preference for T or Tc filters over G filters. It is hard to explain the big discrepancy between, on the one hand, the results of Alfheim and Lindskog and, on the other hand, ours and those of Daisey *et al.* and Fitz. Clear differences with related materials found by the former and slight differences with very different materials by the latter.

An absence of substantial differences between the effects obtained with the different materials does not automatically imply an absence of substantial artifacts. Artifacts may also occur when using the supposedly less reactive materials. The results of Daisey *et al.* clearly point to this possibility. A more complete impression of the influence of the artifacts, therefore, requires other experimental set-ups. These were followed in several studies. None of them were, however, carried out with T or Tc filters.

The duration of the exposure, i.e. the period during which the particles are exposed on the filter, can be varied. Pitts *et al.* (1982) compared one 24 h sample with eight samples of 3 h covering the same period and taken on the same place. They did not find any difference in mutagenicity and they conclude, that, if sampling influences mutagenicity, it does so within a period of 3 h. An important aspect of their study was that they collected rather freshly emitted particles (emitted by traffic) during photochemical air pollution

Table 5. Multivariate analysis with the PAH concentrations
All PAH and all experiments

	Principal-component analysis				Factor analysis with varimax rotation					
	PC 1		PC 2		Factor 1		Factor 2		Factor 3	
	T	G	T	G	T	G	T	G	T	G
PHE	0.86	0.87	−0.13	−0.40	0.50	0.43	0.38	0.62	0.71	0.41
AC	0.57	0.66	−0.69	−0.64	0.02	0.08	0.68	0.64	0.30	0.35
FL	0.69	0.69	−0.70	−0.65	0.16	0.23	0.94	0.94	0.19	0.19
PY	0.59	0.59	−0.67	−0.69	0.16	0.15	0.98	0.98	−0.03	0.10
BA	0.86	0.90	−0.34	0.10	0.43	0.64	0.63	0.23	0.24	0.42
CH	0.86	0.95	−0.35	0.02	0.49	0.81	0.81	0.47	0.23	0.26
BEP	0.83	0.79	0.42	0.51	0.97	0.99	0.17	0.05	−0.04	0.06
PE	0.83	0.84	0.43	0.31	0.84	0.62	−0.03	0.04	0.24	0.63
BBF	0.88	0.89	0.37	0.32	0.95	0.94	0.25	0.26	0.11	0.19
BKF	0.93	0.94	0.33	0.30	0.94	0.91	0.25	0.22	0.17	0.30
BAP	0.94	0.93	0.22	0.03	0.81	0.58	0.23	0.33	0.36	0.71
BGH	0.92	0.93	0.31	0.28	0.90	0.80	0.20	0.12	0.20	0.51
IP	0.85	0.96	0.27	0.24	0.91	0.85	0.27	0.22	0.22	0.41
AN	0.88	0.80	−0.12	−0.27	0.53	0.25	0.39	0.36	0.33	0.84
Variance of PCs (%) 70.0, 71.6, 18.0, 15.9										
PAH with more than four rings; all experiments										
BEP					0.90	0.95	0.25	0.10	0.34	0.25
PE					0.50	0.48	0.41	0.49	0.76	0.73
BBF					0.89	0.92	0.33	0.29	0.28	0.20
BKF					0.82	0.86	0.41	0.39	0.36	0.30
BAP					0.55	0.48	0.62	0.71	0.46	0.43
BGH					0.66	0.70	0.49	0.54	0.44	0.36
IP					0.75	0.79	0.53	0.51	0.36	0.29
AN					0.29	0.18	0.92	0.96	0.24	0.19

T: T and Tc filters; G: glass-fibre filters. The table lists the factor matrices and the variance of the PCs as percentage of the summed variance.

and that they could demonstrate changes of mutagenicity due to residence of the particles in the air. Comparable results (as regards the influence of exposure time) were obtained by de Raat (1983) and Fitz *et al.* (1984).

The influence of artifacts can also be investigated by exposing particles on a filter to particle-free air or by adding reactive gases to the air before it reaches the filter. The first set-up was followed by Fitz *et al.* (1984) who did not find any influence. Brorström *et al.* (1983) reported a dramatic increase of the mutagenicity upon adding 1 ppm of NO_2, probably contaminated with traces of nitric acid. However, they did not present detailed mutagenicity data.

So, these set-ups have lead to conflicting results. The absence of any indications for artifacts in the studies of Pitts *et al.*, de Raat and Fitz *et al.* and clear effects in the study of Brorström *et al.*

In our opinion, the evidence for substantial artifacts is still very meagre and up to now there is no reason to expect that studies on the mutagenicity of the particles are seriously hampered by such artifacts. However more studies are needed for definitive conclusions.

It is hard to think of any other explanation for the slight differences encountered in the present study besides artifactual conversion of compounds which determine the mutagenicity.

Differences in flow rate can be excluded because this variable was thoroughly checked at the beginning and the end of sampling.

The possibility that T and Tc filters need a more intensive extraction was investigated by extracting some filters twice and separately testing the extracts. This did not result in significant effects for the second extracts, which indicates that all relevant compounds were present in the first extracts (results not shown).

The mutagenicity of support-filter extracts might point to an incomplete retention of the mutagens by the upper (T or Tc) filters. The PAH analysis of the support-filter extracts renders this possibility highly improbable. Hardly any non-volatile PAH could be demonstrated in these extracts. As these PAH and the mutagens are found in particles with the same aerodynamic-diameter range the absence of these PAH implies the absence of mutagenic particles.

The presence of mutagens in the support filters may be explained in another way. For this we have to anticipate on the discussion of the results of the PAH determinations for a second time. A most striking

outcome of these determinations were the relatively high concentrations of volatile PAH in the support filters. Also in this case the absence of non-volatile PAH rules out the possibility that the presence of the volatile ones is the result of incomplete retention of particles, which leaves adsorption to the filter material from the gas phase as the sole explanation.

Several studies (Pitts *et al.*, 1978, 1980; de Raat, 1983) have revealed, that benzo(*a*)pyrene adsorbed to glass-fibre material is very sensitive to chemical conversion upon exposure to particle-free air, the resulting products being highly mutagenic. The same may hold for the volatile PAH adsorbed to the glass fibres of the support filter, which would make these filters mutagenic.

The presence of the volatile PAH themselves does not provide an explanation for the mutagenicity. These compounds are generally very weak mutagens and their effects are strictly dependent of the presence of a rat-liver homogenate in the mutagenicity test.

Direct adsorption of mutagens from the gas phase by the glass fibres is improbable. de Raat *et al.* (1987a) have investigated the mutagenicity of the volatiles passing the filter by mounting the adsorbent polyurethane foam under the filter. No volatile mutagens could be demonstrated, while the volatile PAH were completely sampled with the foam.

The mutagenicity of the support filters may provide an explanation for the differences in mutagenicity between the upper filters. Adsorption and conversion of volatile PAH will also occur in the glass-fibre upper filters. The resulting mutagenicity then explains the slightly higher effects obtained with these filters. We did not use T or Tc filters as support filters. Therefore, it remains unknown whether or not adsorption and conversion contributes to the effects of these filters as well.

PAH

The present study shows striking differences between on one hand the G filters and on the other the T and Tc filters for the concentrations of the volatile PAH (anthracene, phenanthrene, fluoranthene and pyrene). An obvious explanation is offered by the greater air resistance of the latter two types which results in a greater pressure drop and consequently a greater loss of particulate PAH due to evaporation. The greater the air resistance of the filter the less suitable it is for the determination of the volatile PAH present in the particulate phase.

Another explanation is offered by the relatively high concentrations in the support filters. As argued above this is most probably caused by adsorption of gases and will also take place in the upper filters. The lack of knowledge about the adsorption by the T and the Tc filters makes it impossible to distinguish the contributions of both artifactual evaporation and adsorption. Up to now it was generally assumed that sampling by filtration leads to an underestimation of the volatile PAH in the particles. The adsorption shown by us questions this assumption.

The multivariate analysis clearly revealed the influence of volatility on the PAH profile. This may be a reflection of both a fluctuating artifactual evaporation and fluctuation of the gas/solid ration before sampling. It is not possible to sort these causes out. However, the large differences in the concentrations between the filter types, which are undoubtedly the result of artifacts, are not at all reflected in the multivariate analysis. This suggests that the 'volatility factor' found with this analysis is caused by 'pre-sampling fluctuations'.

The concentrations of the less volatile PAH, i.e. those that are completely sampled by filtration (Thrane and Mikalsen, 1981; Keller and Biddleman, 1984; de Raat *et al.*, 1987a), are, as the present study shows, hardly influenced by the choice of the filter material. The normalization for the concentration of the rather inert benzo(*b*)fluoranthene brings small differences to light. These can only be caused by conversion after they have been trapped on the filter. This is confirmed by the link with chemical reactivity of the differences. The results, therefore, confirm those of Lee *et al.* (1980), Grosjean (1983) and Alfheim and Lindskog (1984), although in our case the differences between the filter materials were only marginal. However, these authors did not report a link between chemical reactivity of the PAH and the influence of filter material. Some authors could not demonstrate conversion of PAH due to the filter material (Grosjean *et al.*, 1983; Fitz *et al.*, 1984).

Clear evidence for artifactual conversion is provided by the studies of Miguel and Andrade (1986) and Brorström *et al.* (1983). The former exposed the filter after sampling to particle-free air; they did find a clear link with reactivity of the PAH. The latter added NO_2, probably with traces of nitric acid, to the air before it passed the filter. Experiments of Grosjean *et al.* (1983) also pointed to the importance of the presence of nitric acid in the air.

The multivariate analysis provided an additional indication for conversion. As we have found in another study (de Raat, 1987b), one clear separate factor affected the concentrations of the more reactive PAH in particular. However, we did not find a dependence of this factor on filter material. Therefore, we cannot conclude it to be linked with artifacts. It might as well be the result of conversion in the air before sampling. Nielsen *et al.* (1988) and de Raat *et al.* (1987b) have demonstrated that non-volatile PAH are indeed converted during their residence in the air. They compared PAH profiles of samples collected at different distances from source areas and found a decreasing share of the more reactive PAH in the profile with an increasing distance.

Nevertheless, the fact that a 'conversion factor' is found with the T and Tc filters must make us alert to the possibility of artifactual conversion on these filters.

Definite conclusions can only be drawn after the influence of these materials on the PAH concentrations is further investigated.

CONCLUSIONS

The study leads to the following main conclusions.

Sampling of ambient airborne particles with T or Tc filters instead of G filters leads to slightly weaker mutagenic effects.

The authors find the differences in mutagenicity too small to justify a drastic reevaluation of the results obtained with G filters so far. Neither do they provide a convincing argument for the use of T or Tc filters in future studies.

The differences are probably caused by conversion of components on the filter.

Gaseous PAH are adsorbed by glass-fibre material. The conversion of these adsorbed PAH to mutagenic derivatives during their exposure to particle-free air provides a probable explanation for the differences in mutagenicity.

G filters lead to much higher concentrations of volatile PAH due to adsorption by the G filters and stronger artifactual evaporation (blow-off) from the other types.

Concentrations of more reactive PAH can be slightly lower if G filters are used instead of the other types, which points to artifactual conversion.

The variation of the PAH profile points to conversion for all three types of filter materials. It is unclear to which extent this is caused by artifactual conversion or conversion in the air before sampling.

The possibility that artifactual conversion of PAH takes place on the T and the Tc filters cannot be excluded.

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REFERENCES

Alfheim I. (1982) Contribution from motor vehicle exhaust to the mutagenic activity of airborne particles. In *Mutagens in our Environment* (edited by M. Sorsa and H. Vainio), pp. 235–248. Liss, New York.

Alfheim I. and Lindskog A. (1984) A comparison between different high volume sampling systems for collecting ambient airborne particles for mutagenicity testing and for analysis of organic compounds. *Sci. total Envir.* **34**, 203–222.

Ames B. N., McCann J. and Yamasaki E. (1975) Methods for detecting carcinogens and mutagens with the Salmonella mammalian microsome mutagenicity test. *Mutat. Res.* **31**, 347–364.

Brorström E., Grennfelt P. and Lindskog A. (1983) The effect of nitrogen dioxide and ozone on the decomposition of particle-associated polycyclic aromatic hydrocarbons during sampling from the atmosphere. *Atmospheric Environment* **17**, 601–605.

Cauwenberghe K. A. var, van Vaeck L. and Pitts J. N. Jr (1980) Physical and chemical transformations of organic pollutants during aerosol sampling. In *Proceedings of the First European Symposium Physico-Chemical Behaviour of Atmospheric Pollutants* (edited by B. Versino and H. Ott). Commission of the European Communities, 1980.

Daisey J. M., Allen C. F., McGarrity G., Atherholt T. and Loy P. J. (1983) Effects of filter type on the organic composition and mutagenicity of inhalable particulate matter. *Aerosol Sci. Technol.* **2**, 295.

Dipple A. (1976) Polynuclear aromatic carcinogens. In *Chemical Carcinogens* (edited by C. E. Searle). ACS Monograph, 173.

Fitz D. R., Lokensgard D. M. and Doyle G. J. (1984) Investigation of filtration artifacts when sampling ambient particulate matter for mutagen assay. *Atmospheric Environment* **18**, 205–213.

Grosjean D. (1983) Polycyclic aromatic hydrocarbons in Los Angeles air from samples collected on Teflon, glass and quartz filters. *Atmospheric Environment* **17**, 2565–2573.

Grosjean D., Fung K. and Harrison J. (1983) Interactions of polycyclic aromatic hydrocarbons with atmospheric pollutants. *Envir. Sci. Technol.* **17**, 673–679.

Keller D. and Biddleman T. F. (1984) Collection of airborne polycyclic aromatic hydrocarbons and other organics with a glass-fiber filter–polyurethane foam system. *Atmospheric Environment* **17**, 837–845.

Lee F. S. C., Pierson W. R. and Ezike J. (1980) The problem of PAH degradation during filter collection of airborne particles—an evaluation of several commonly used filter media. In *Polynuclear Aromatic Hydrocarbons: Chemistry and Biological Effects* (edited by A. Bjørseth and A. Dennis). Battelle Press, Columbus, Ohio.

Maron D. M. and Ames B. N. (1983) Revised methods for the Salmonella mutagenicity test. *Mutat. Res.* **113**, 173–215.

Miguel A. H. (1984) 'Atmospheric' reactivity of particulate polycyclic aromatic hydrocarbons collected in an urban tunnel. *Sci. total Envir.* **36**, 305–311.

Miguel A. H. and de Andrade J. B. (1986) Desorptivity versus chemical reactivity of polycyclic aromatic hydrocarbons (PAHs) in atmospheric aerosols collected on quartz fiber filters. *Int. J. Envir. Analyt. Chem.* **26**, 265–278.

Morrison D. F. (1972) *Multivariate Statistical Methods.* McGraw Hill Series in Probability and Statistics. McGraw Hill, New York.

National Research Council (1983) *Polycyclic Aromatic Hydrocarbons, Evaluation of Sources and Effects.* National Academy Press, Washington, D.C.

Nielsen T. (1984) Reactivity of polycyclic aromatic hydrocarbons towards nitrating species. *Envir. Sci. Technol.* **18**, 157–163.

Nielsen T., Ramdahl T. and Bjørseth A. (1983) The fate of airborne polycyclic organic matter. *Envir. Hlth. Perspect.* **47**, 103–114.

Nikolao K., Masclet P. M. and Mouvier G. (1984) Sources and chemical reactivity of polynuclear aromatic hydrocarbons in the atmosphere—a critical review. *Sci. total Envir.* **32**, 102–132.

Pitts J. N., Jr, Grosjean D. and Mischke T. M. (1979) Mutagenic activity of airborne particulate organic pollutants. In *Assessing Toxic Effects of Environmental Pollutants* (edited by S. D. Lee and J. B. Mudd), pp. 219–235.

Pitts J. N., Jr, Lokensgard D. M., Ripley P. S., van Cauwenberghe K. A., van Vaeck L., Shaffer S. D., Thilland A. J. and Belser W. L. (1980) 'Atmospheric' epoxidation of benzo(*a*)-pyrene by ozone: formation of the metabolite benzo(*a*)-pyrene-4,5-epoxide. *Science* **210**, 1347–1349.

Pitts J. N., Jr (1983) Formation and fate of gaseous and particulate mutagens and carcinogens in real and simulated atmospheres. *Envir. Hlth Perspect.* **47**, 115–140.

Pitts J. N., Jr, Harger W., Lokensgard D. M., Fitz D. R.,

Scorziell G. M. and Meija V. (1982) Diurnal variations in the mutagenicity of airborne particulate organic matter in California's south coast air basin. *Mutat. Res.* **104,** 35–41.

Pitts J. N., Jr (1987) Nitration of gaseous polycyclic aromatic hydrocarbons in simulated and ambient urban atmospheres: a source of mutagenic nitroarenes. *Atmospheric Environment* **21,** 253–2547.

Raat W. K. de (1983) The genotoxicity of aerosol extracts: some methodological aspects and the contribution of urban and industrial locations. *Mutat. Res.* **116,** 47–64.

Raat W. K. de (1988) Polycyclic aromatic hydrocarbons and mutagens in ambient air particles. *Toxicol. Envir. Chem.* **16,** 259–279.

Raat W. K. de, Kooijman S. A. L. M. and Gielen J. W. J. (1987b) Concentrations of polycyclic hydrocarbons in airborne particles in The Netherlands and their correlation with mutagenicity. *Sci. total Envir.* **66,** 95–114.

Raat W. K. de and de Meijere F. A. (1988) The mutagenicity of ambient particles from local traffic and distant sources during episodes with moderate photochemical air pollution. *Sci. total Envir.* **73,** 159–179.

Raat W. K. de, de Meijere F. A. and Kooijman S. A. L. M. (1985) Mutagenicity of ambient aerosol collected in an urban and industrial area of the Netherlands. *Sci. total Envir.* **44,** 17–33.

Raat W. K. de, Schulting F. L., Burghardt E. and de Meijere F. A. (1987a) Application of polyurethane foam for sampling volatile mutagens from ambient air. *Sci. total Envir.* **63,** 175–189.

Rosenkranz H. S. and Mermelstein R. (1983) Mutagenicity and genotoxicity of nitroarenes. All nitro-containing chemicals were not created equal. *Mutat. Res.* **114,** 217–267.

Siak J., Chan T. L., Gibson T. L. and Wolff G. T. (1985) Contribution of bacterial mutagenicity from nitro-PAH compounds in ambient aerosols. *Atmospheric Environment* **19,** 369–376.

Talcott R. and Wei R. T. (1977) Airborne mutagens bioassayed in *Salmonella typhimurium*. *J. Nat. Canc. Inst.* **58,** 449–451.

Thomas W. (1986) Principal component analysis of trace substance concentrations in rainwater samples. *Atmospheric Environment* **20,** 996–1000.

Thrane K. E. and Mikalsen A. (1981) High-volume sampling of airborne polycyclic aromatic hydrocarbons using glass-fibre filters and polyurethane foam. *Atmospheric Environment* **15,** 909–918.

Valerio F., Bottino P., Ugolini D., Cimberle M. R., Tozzi G. A. and Frigerio A. (1984) Chemical and photochemical degradation of polycyclic aromatic hydrocarbons in the atmosphere. *Sci. total Envir.* **40,** 169–188.

CHAPTER 12

APPLICATION OF POLYURETHANE FOAM FOR SAMPLING VOLATILE MUTAGENS FROM AMBIENT AIR

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APPLICATION OF POLYURETHANE FOAM FOR SAMPLING VOLATILE MUTAGENS FROM AMBIENT AIR

W.K. DE RAAT, F.L. SCHULTING, E. BURGHARDT and F.A. DE MEIJERE
TNO, P.O. Box 217, 2600 AE Delft (The Netherlands)

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ABSTRACT

This paper reports the use of polyurethane foam (PUF) in the sampling of airborne mutagens too volatile to be retained by glass fibre filters. Air was sampled simultaneously with a glass fibre filter and PUF plugs, and the two extracts of the samples were tested for mutagenicity and analysed for polycyclic aromatic hydrocarbons (PAH). As PUF itself was found to contain mutagens, it was thoroughly rinsed, and a blank extract of the same PUF used for sampling was tested as a control. The resulting blank extracts were not mutagenic.

In the first series of experiments, in which methanol was used as the extraction solvent, sampling induced clear-cut mutagenic effects in the PUF extracts. As the mutagenicity of the PUF itself appeared only with the use of methanol or other alcohols as extraction solvents, a second series of experiments was carried out in which acetone was used as the extraction solvent. In these experiments sampling induced no mutagenicity in the extracts. The discrepancy between the two series of experiments may be due to the absence of volatile mutagens in the second series, but another explanation may be an artefact induced by the solvent in the first series. Additional experiments suggest that sampling induces the formation, from PUF, of compounds mutagenic upon extraction with methanol. The study leads to the provisional conclusion that *no* volatile mutagens could be demonstrated in ambient air sampled by the use of PUF as an absorbent, notwithstanding the clear-cut effects of the filter extracts and the presence of volatile PAH in the PUF extracts.

INTRODUCTION

The past decade has seen the publication of many studies showing that ambient air particles contain mutagenic compounds [1–8]. These compounds probably originate from the combustion of fossil and organic fuels. Combustion emissions have been shown to contain mutagens [9–16], and the mutagens in airborne particulate matter, as well as those in the emissions, have been identified as polycyclic aromatic hydrocarbons (PAH) and their nitro or oxygen derivatives [9, 10, 12, 14, 16–25].

Sampling of mutagens in ambient air is normally done with the aid of (Teflon-coated) glass fibre filters, which do not retain volatile compounds. As far as we know, scant attention has so far been paid to the sampling of the more volatile mutagens in ambient air. For combustion emissions, such compounds are sampled with the aid of adsorbents, and samples obtained by this method have often been shown to contain mutagens [9, 10, 12, 13, 26].

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It has also been shown [27–33] that glass fibre filters do not fully retain PAH of low molecular weight (with fewer than five rings). Several studies have shown however, that these compounds are trapped by plugs of polyurethane foam (PUF) [32–34].

The presence of volatile mutagens in combustion emissions, in addition to solid ones, and the presence of volatile PAH in ambient air, warrants a search for volatile mutagens in the latter. The present paper describes such an investigation. Compounds not trapped on a glass fibre filter during the sampling of ambient air were passed through PUF, and the extracts of the PUF and the filter were tested for mutagenicity separately with the Ames test and analysed for PAH. Our choice of PUF as the sorbent was based on its successful use in the sampling of PAH, chlorinated pesticides and polychlorinated biphenyls [32–40, 44]. Its low air resistance and large sorption surface make it very suitable for the sampling of large volumes of air.

METHODS AND MATERIALS

Polyurethane foam

The PUF used was of the flexible polyether type and was not reticulated. This type of PUF is normally used in upholstery, and has a density of $\sim 25\,g\,dm^{-3}$. The PUF was cut into cylindrical pieces 7.5 cm long and 5 cm in diameter. The pieces were cleaned by extraction with methanol or acetone in a Soxhlet apparatus for 24 h.

Sampling

The air to be sampled was pumped first through a round glass fibre filter (ϕ = 15 cm, Sartorius SM 13400) and then through one or two PUF plugs. The glass fibre filters were also cleaned with methanol or acetone. The flow rate of the air was $\sim 10\,m^3\,h^{-1}$. Sampling was carried out at two locations: *Lekhaven*, on the roof of a building near the seaport of Rotterdam, and at *Delft*, at ground level at the TNO measuring station for air pollution in the south east of Delft. An earlier study [8] showed that particles in ambient air near Lekhaven are highly mutagenic.

Extraction of the samples

After sampling, the filter and the PUF plug were extracted separately with methanol (Rathburn, HPLC grade) or acetone (Rathburn, glass-distilled) in a Soxhlet apparatus for 16 h. The solvent volume was reduced to 5–7 ml under nitrogen. Shortly before sampling, the previously cleaned PUF plug was extracted in the same way to produce a blank extract, the possible mutagenicity of which could be compared with that of the extract of the *same* PUF plug after sampling.

As the present study showed that PUF itself contains mutagens, several solvents were compared for their efficiency in extracting these mutagens.

Chemical analysis

A number of extracts were analysed for PAH using reversed phase high pressure liquid chromatography (HPLC). The extracts were *not* subjected to a clean-up method but analysed as such. A gradient elution was applied ranging from 20% distilled water in methanol (Rathburn HPLC grade) to 100% methanol. The PAH were detected by their fluorescence. They were further identified and quantified by comparison of the chromatogram with that of a standard mixture of PAHs prepared on the same day.

In one experiment the PAH (and some other compounds) were analysed by combined gas chromatography and mass spectrometry. The mass spectrometer was a VG 7070 F, and the gas chromatograph a Varian 1400 with a 50 m × 0.25 mm fused silica capillary column coated with CP-sil 5. A solid injection system was used for introduction of samples. The temperature of the injection block was 350°C, and the oven was programmed to raise the temperature from 120 to 320°C at a rate of 4°C min^{-1}. Compounds were identified by their gas-chromatographic retention indexes and by comparison of their spectra with those in the literature.

Testing for mutagenicity

The extracts were tested using the Salmonella microsome test developed by Ames and co-workers [41]. *Salmonella typhimurium* TA98 was used as the indicator strain, and a liver homogenate (S9 fraction) of male Spf bred rats (Cpb: Wn; Wistar random; 200 g) treated with Aroclor 1254 was used for metabolic activation [41]; 50 μl of liver homogenate was applied per plate. The extracts derived from one sampling period were tested simultaneously (within one test procedure). If necessary, the extracts were diluted with acetone or methanol. The volume dosed was always 0.1 ml. All doses were tested in triplicate.

RESULTS

Effect of the blank PUF extracts

Preliminary experiments, in which methanol was used as the extraction solvent, showed that blank extracts of previously cleaned PUF plugs were sometimes weakly mutagenic at concentrations expected in air sampling experiments. The effect ranged from zero to three times the background when the extract of $\sim 10\,cm^3$ of PUF was tested, and was apparent only in the presence of S9 fraction (metabolic activation). These results prompted us to investigate the blank mutagenicity of the PUF more closely. The results, which we plan to

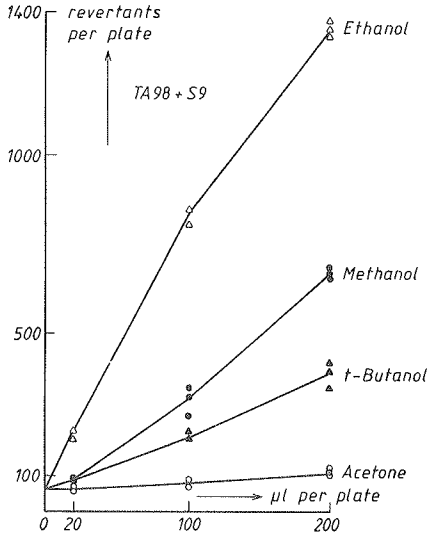


Fig. 1. Mutagenicity of four PUF extracts prepared with different solvents. Concentrations of the residues (mg ml^{-1}): methanol, 24.7; acetone, 21.0; ethanol, 36.3; t-butanol, 22.2.

publish in more detail elsewhere, can be summarized as follows: The blank mutagenicity of the methanol extracts is completely dependent on metabolic activation. Liquid/liquid fractionation of the extract and testing of the fractions indicated the presence of basic mutagens. The effect was much less prominent in the TA98 1, 8 DNP 6 strain isolated by Rosenkranz and co-workers [42]. This finding may indicate the presence of compounds metabolized to a hydroxylamine that is acetylated by the bacteria to give a genotoxic compound. All these results taken together led us to believe that the PUF extract contains a mutagenic amine.

In the course of the investigation, we decided that the extract of cleaned PUF and the extract of the *same* PUF after sampling should be compared for mutagenicity. This procedure enables a correction to be made for any mutagenicity of the blank. It will be shown in the sequel that the blank extracts were not mutagenic.

Another way of circumventing the problem of the mutagenicity of the blank extract is to use a solvent of better cleaning power than methanol. We tested distilled water, hexane, methanol, isopropanol, ethanol and acetone for suitability in this respect. The results of an experiment with the four last-mentioned solvents are shown in Fig. 1. Surprisingly, the effects of mutagenicity of blanks was found only in the extracts with water and alcohols. It seems to be quite independent of the extractive power of the solvents, and may thus well be due to water and alcohols reacting with a PUF component to form mutagenic compounds. We therefore decided to use acetone as the extractant for the ambient air mutagenicity experiments carried out in a later phase of this study.

An additional experiment showed that extraction with acetone removes a large part of the mutagens or "premutagens" extractable with ethanol.

Mutagenicity of PUF extracts and filter extracts after sampling

Figure 2 shows the results of mutagenicity tests with methanol extracts of PUF plugs and filters after sampling. It also shows the results for the blank extracts of the PUF plugs. Preliminary experiments revealed that extracts of cleaned glass fibre filters were not mutagenic. In one experiment two PUF plugs mounted one behind the other were used. This was also the experiment in which the extracts were analysed with GC/MS. Both the extracts of the filters and those of the PUF plugs showed clear mutagenic activity. In none of the four experiments was an effect of the blank extract found, which clearly indicates that the PUF-associated mutagenicity is due to sampling and not to mutagens already present in the PUF plug before sampling. In one experiment the PUF-derived mutagenicity was even greater than the filter-derived mutagenicity. The second PUF plug also contained a considerable amount of mutagenic extractables. Metabolic activation either had no effect at all on mutagenicity, or increased it markedly. Although these experiments suggest that the mutagens present in ambient air were absorbed by the PUF plug, it cannot be ruled out that PUF-derived mutagenic activity is in part the result of an artifact induced by sampling. The sampled air may well have contained compounds capable of reacting with components of PUF to produce mutagenic compounds or compounds that react with the solvent to give mutagens. In two experiments, cleaned PUF plugs were stored in clean air and in nitrogen. The results (Table 1) suggest that the PUF so treated liberates mutagens upon extraction with methanol. For the experimental details, see the footnote to Table 1.

These experiments may thus suggest that even the storage of PUF plugs leads to the formation of (pre)mutagens, possibly by degradation of the PUF. It cannot be ruled out that comparable processes take place in accelerated form when PUF is exposed to polluted air. Another reason for believing that the mutagenicity of extracts is due to artifacts rather than mutagens absorbed from air follows from the experiments in which the PUF plugs were extracted with acetone after sampling. In none of these experiments did sampling result in clear PUF-derived mutagenic activity at doses which (Fig. 3) induced a clear effect when the filter extracts were tested. This is despite the fact that clear-cut effects were found for the filter extracts. The results of the experiments with acetone as the extraction solvent therefore indicate the absence of mutagens absorbed from air by the PUF plugs.

Chemical analysis of the PUF filter extracts after sampling

In one of the experiments with methanol as extraction solvent, the extracts were analysed using GC/MS. The results of this experiment (Table 2) give a

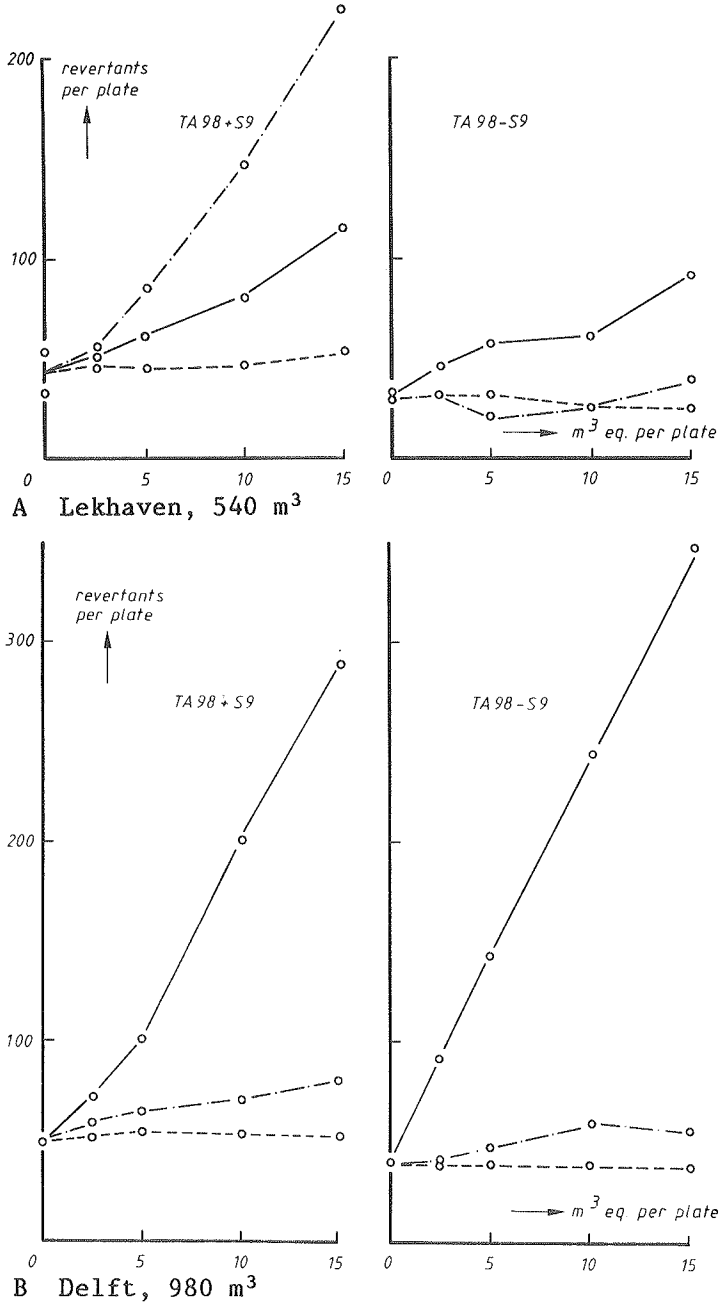
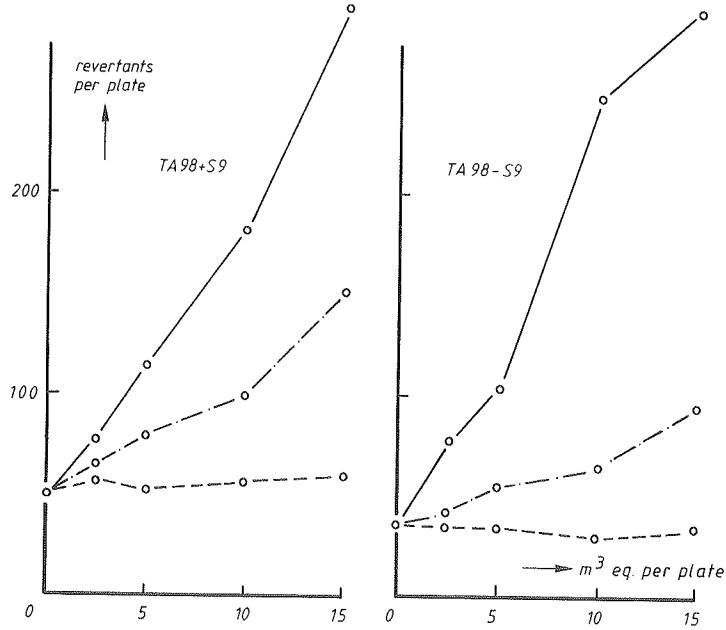
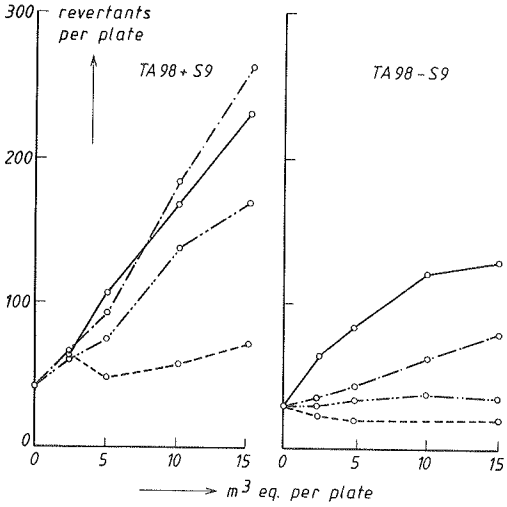


Fig. 2. Results of four experiments (A–D) in which the mutagenicities of extracts of the glass fibre filter and of the polyurethane foam plug were compared. Extraction solvent: methanol. (– – – –) Blank extract of the cleaned PUF plug; (–·–·–·) extract of the PUF plug after sampling; (–··–··–··) extract of a second PUF plug after sampling; this plug was mounted behind the first one (experi-

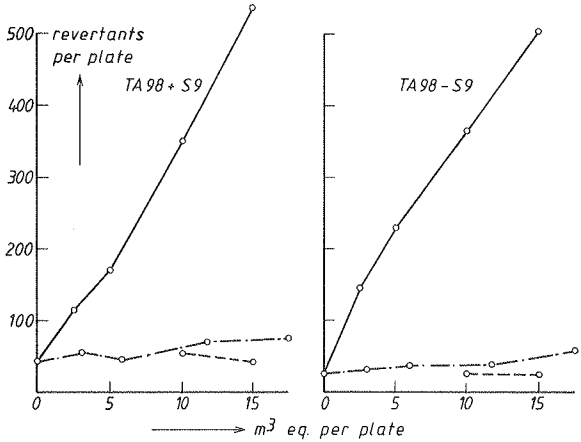


C Lekhaven, 840 m³

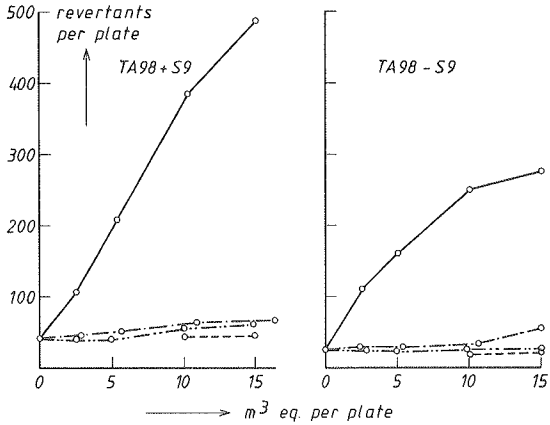


D Lekhaven, 580 m³

ment D only); (——) extract of the glass-fibre filter after sampling. The blank extract had the same volume as the extract after sampling; the same amounts (ml) were tested for both extracts. Each point represents the mean of a triplicate. The amount of air sampled in each experiment is indicated.



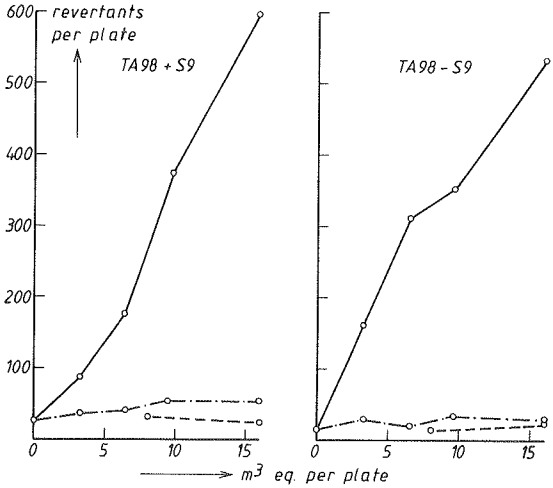
A Lekhaven 2035 m^3



B Lekhaven 1987 m^3

Fig. 3. Results of three experiments (A–C) in which mutagenicities of extracts of the glass fibre filter and of the polyurethane foam plug were compared. Solvent: acetone. (– – – – –) Blank extract of the cleaned PUF plug; (–·–·–) extract of the PUF plug after sampling; (–·–··–··) extract of a second PUF plug after sampling; this plug was mounted behind the first one (experiment B only); (——) extract of the glass-fibre filter after sampling. The blank extract had the same volume as the extract after sampling; the same amounts (ml) were tested for both extracts. Each point represents the mean of a triplicate. The amount of air sampled in each experiment is indicated.

clear impression of the components trapped by PUF plugs and the filter. Comparable results were obtained for the PAHs by HPLC analysis of the acetone extracts (Table 3). PAHs with five or more rings are virtually absent in the volatile phase passing the filter. Substantial amounts of the more volatile PAHs pass the filter and are trapped by the PUF plug. A second PUF plug



C Lekhaven 1597 m³

mounted behind the first still traps substantial amounts of phenanthrene and fluorene. For the other PAHs, application of a filter and one plug is probably sufficient for complete sampling. Table 2 also shows the ability of the PUF foam to trap other PAH-like compounds, too volatile to be completely trapped by a filter. A filter does not fully retain alkanes with fewer than 26 carbon atoms.

TABLE 1

Effect of storage in clean air or nitrogen upon the mutagenicity of PUF plugs

A	Dose mleq. of foam	Plug I				Plug II			
		Before storage		After storage		Before storage		After storage	
	0	22	31						
	3.6	33	32	40	38	31	25	41	39
	7.1	32	50	43	71	46	31	66	60
	14.3	58	47	90	86	53	49	86	85
B		Air		Nitrogen		Control I		Control II	
	0	32	38			31	40		
	3.6	47	55	50	47	35	30	24	21
	7.1	48	62	52	63	21	38	39	30
	14.3	76	82	82	58	29	36	47	49

Experiment A: comparison of mutagenicity of methanol extracts of two cleaned PUF plugs before and after storage for 23 h in glass containers in clean air.
Experiment B: mutagenicity of methanol extracts of cleaned PUF plugs stored for 24 h in clean air or nitrogen in glass containers and of PUF plugs (controls I and II) which had only been cleaned. The table lists numbers of revertants per plate; test strain: TA98; test was carried out with S9-fraction.

TA LE

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r	)$_{21}$									3				7.3					0		
r	)$_{22}$									3				1.3					0		
r	)$_{23}$									2				7.8					0		
r	)$_{24}$									3				3.2					0		
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TABLE 3

Concentrations of PAHs in air samples taken with glass fibre filters and polyurethane foam plugs and extracted with acetone

	Experiment A		Experiment B		Experiment C		
	Filter	PUF	Filter	PUF	Filter	PUF I	PUF II
Phenanthrene	2.89	23.43	0.69	12.00	0.42	24.00	4.90
Anthracene	0.05	1.64	0.04	0.65	0.77	2.10	0.32
Fluoranthene	2.06	15.85	1.00	7.10	1.70	8.50	0.13
Pyrene	1.29	11.36	0.53	4.60	1.20	5.10	< 0.12
Benzo[*b*]fluorene	0.20	0.11	0.13	< 0.05	0.25	< 0.06	< 0.06
Benzo[*a*]anthracene	2.95	0.53	1.30	0.40	1.50	0.55	< 0.05
Chrysene	4.59	0.55	2.20	0.20	2.20	0.30	< 0.08
Benzo[*e*]pyrene	3.12	< 0.09	1.70	< 0.21	1.40	< 0.22	< 0.22
Benzo[*j*]fluoranthene	1.72	< 0.13	< 0.99	< 0.99	< 1.00	< 1.00	< 1.00
Perylene	0.51	0.01	0.19	< 0.01	0.25	< 0.01	< 0.01
Benzo[*b*]fluoranthene	5.05	< 0.01	2.40	< 0.02	2.20	< 0.02	< 0.02
Benzo[*k*]fluoranthene	2.21	< 0.01	0.98	< 0.01	0.92	< 0.01	< 0.01
Benzo[*a*]pyrene	2.08	< 0.01	0.96	< 0.02	1.40	< 0.02	< 0.02
Benzo[*g,h,i*]perylene	5.29	< 0.05	1.70	< 0.13	1.40	< 0.14	< 0.14
Indeno[1,2,3-*cd*]pyrene	3.96	0.04	1.70	< 0.09	1.90	< 0.09	< 0.09
Anthanthrene	0.13	< 0.01	0.06	< 0.02	0.26	< 0.02	< 0.02

The table lists the concentrations in $ng\,m^{-3}$; the experiments correspond to those for which the mutagenicity results are given in Fig. 3; during sampling the air consecutively passed through the glass fibre filter (Filter) and one or two PUF plugs (PUF I and II). Analysis was based on HPLC and fluorescence detection.

DISCUSSION

Polyurethane foam was used in two series of experiments for the sampling of mutagens too volatile to be fully retained by a glass fibre filter. The most important difference between the series was the use of two different solvents for extraction: methanol for the first series and acetone for the second. In the experiments with methanol, sampling induced clear-cut mutagenicity of the PUF; in those with acetone, no significant mutagenicity was found at the doses tested. The discrepancy between the two series is the more remarkable because in both it was demonstrated that the PUF sorbed PAHs, a group of compounds often associated with airborne mutagenicity, either because they themselves contribute to the effect or because their nitrated and oxygenated derivatives do [6, 16, 18, 19, 21, 24, 25, 43]. So, in both series of experiments, volatile compounds were sampled that are related to the compounds held responsible for the mutagenicity of airborne particles.

The discrepancy might be explained by the simple assumption that volatile mutagens were only present during the first series. Since samples were taken at Lekhaven in both series, this explanation is not very plausible. If we assume that the volatile mutagens stem from the same sources as the solid ones, we might have expected these compounds to be present in the second series as well,

in view of the marked effects of the filter extracts. An explanation which is, in our opinion, more justified is that the mutagens are not sampled as such from the air but are formed from the PUF during sampling. The results of this study clearly point to the presence of compounds in PUF that react with alcohols or water to give mutagens; a first attempt to identify these suggests the presence of mutagenic amines in the methanol extracts. Although testing of the blank extraction for the first series of experiments did not reveal any effect, it cannot be ruled out that sampling somehow leads to the formation of mutagens as artifacts. Control experiments suggest that PUF becomes mutagenic upon storage when methanol is used as extraction solvent. The sampling procedure may bring about comparable processes, or may accelerate processes leading to mutagenicity upon storage. From the differences between the two series of experiments, and the difference in mutagenicity between blank methanol and blank acetone extracts, it follows that the experiments do not unambiguously demonstrate the presence of volatile mutagens in the sampled air. The tainting of blank PUF extracts with mutagens and the formation of mutagens during storage mean that PUF is unsuitable as a sorbent for airborne mutagens if it is extracted with methanol. If PUF is to be used in further studies as a sorbent for volatile mutagens, the mutagens in the blanks must be identified, and the mechanisms leading to their formation elucidated. Is the formation of mutagens only a result of the use of methanol or other alcohols as extractants, and can it be prevented by the use of acetone? If further experiments with acetone as extractant confirm the absence of volatile mutagens in ambient air, the formation of artifacts becomes a more likely explanation, and studies on the usefulness of PUF in sampling ambient air for mutagens lose their relevance.

Although sampling did not induce clear mutagenicity in the acetone PUF extracts compared with that of the filter extracts, they did contain considerable amounts of low molecular weight PAH. Tables 2 and 3 show that the PAH concentrations in the two series of experiments fall in the same range. If it is assumed that the methanol extracts contain artifact mutagens, it remains to be explained why the acetone extracts, while containing PAH, are not mutagenic in the dose range tested. Several studies strongly suggest that the mutagenicity of the particulate fraction is to a large extent due to nitrated and oxygenated derivatives of PAH, which contribute more to the mutagenic effect than unsubstituted PAH. Nitration of a PAH makes the substance less volatile, because of the increased polarity. The vapour pressure of nitrated PAH derivatives, even of low molecular weight, may be so low that they are not present in the vapour phase, and not vapourized by the pressure drop across the filter during sampling. Their parent compounds do, however, pass the filter. This study shows, for instance, that most of the pyrene (over 90%) is present in the vapour phase. Although the vapour phase has so far not been investigated for mononitro pyrenes, the recent literature [43], as well as HPLC analyses carried out in the laboratory of one of the authors, show that 1-nitropyrene is a prominent mononitro PAH in the particulate phase. These results therefore suggest that PAH-related mutagenicity is restricted to the *particulate* phase of ambient air.

As stated in the Introduction, PUF has already been widely used for the sampling of organics from ambient air in analytical chemical air pollution studies. In some of these, attempts were made to establish the distribution of PAH over the particulate and vapour phase. The results agree with those of the present study. All PAH can be trapped when three PUF plugs of the kind used in this study are mounted in series. Other organic compounds can also be sampled efficiently. Therefore, PUF remains a valuable and cheap supplement to the conventional glass fibre filter in high-volume sampling. Although the human toxicity of PUF is beyond the scope of this paper, it must be pointed out that the widespread use of PUF in the human environment warrants further investigation into the possible mutagenicity of the material itself or any of its constituents.

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REFERENCES

1 R. Talcott and W. Wei, Airborne mutagens bioassayed in *Salmonella typhimurium*, J. Natl Cancer Inst., 58 (1977) 449–451.
2 K. Teranishi, K. Hamada and H. Watanabe, Mutagenicity in *Salmonella typhimurium* mutants of the benzene-soluble organic matter derived from airborne particulate matter and its five fractions, Mutat. Res., 56 (1978) 273–280.
3 H. Tokiwa, S. Kitamori, K. Takahashi and Y. Onishi, Mutagenic and chemical assay of extracts of airborne particulates, Mutat. Res., 77 (1980) 99–108.
4 C.E. Chrisp and G.L. Fisher, Mutagenicity of airborne particles, Mutat. Res., 76 (1980) 143–164.
5 W. Dehnen, N. Pitz and R. Tomingas, The mutagenicity of airborne particulate pollutants, Cancer lett., 4 (1977) 5–12.
6 M. Møller and I. Alfheim, Mutagenicity and PAH-analysis of airborne particulate matter, Atmos. Environ., 14 (1980) 83–88.
7 E. Selzer Madsen, P.A. Nielsen and J.C. Pedersen, The distribution and origin of mutagens in airborne particulates detected by the Salmonella/microsome assay in relation to levels of lead, vanadium and PAH, Sci. Total Environ., 24 (1982) 13–25.
8 W.K. de Raat, F.A. de Meijere and S.A.L.M. Kooyman, Mutagenicity of ambient aerosol collected in an urban area of the Netherlands, Sci. Total Environ., 44 (1985) 17–33.
9 M. Møller and I. Alfheim, Mutagenicity of air samples from various combustion sources, Mutat. Res., 116 (1983) 35–46.
10 J. Alfheim, G. Becher, J.K. Hongslo and T. Ramdahl, Mutagenicity testing of high performance liquid chromatography fractions from wood stove emission samples using a modified Salmonella assay requiring smaller sample volumes, Environ. Mutagen., 6 (1984) 91–102.
11 C-J. Wei, O.G. Raabe and L.S. Rosenblatt, Microbial detection of mutagenic nitro-organic compounds in filtrates of coal fly ash, Environ. Mutagen., 4 (1982) 249–258.
12 T. Ramdahl, I. Alfheim, S. Rustad and T. Olsen, Chemical and biological characterization of emissions from small residential stoves burning wood and charcoal, Chemosphere, 11 (1982) 601–611.
13 G. Löfroth, Mutagenicity assay of combustion emissions, Chemosphere, 10 (1978) 791–798.
14 W.R. Harris, E.K. Chess, D. Okamoto, J.F.Remsen and D.W. Later, Contribution of nitropyrene to the mutagenic activity of coal fly ash, Environ. Mutagen., 6 (1984) 131–144.

15 I. Alfheim and T.Ramdahl, Contribution of wood combustion to indoor air pollution as measured by mutagenicity in Salmonella and polycyclic aromatic hydrocarbon concentration, Environ. Mutagen., 6 (1984) 121–130.
16 I. Salmeen, R.A. Gorse, T. Riley and D. Schuetzle, Identification and quantification of direct acting Ames assay mutagens in diesel particulate extracts, In M. Cooke and A.J. Dennis (Eds), Polynuclear Aromatic Hydrocarbons; Formation, Metabolism and Measurement, Battelle Press, Columbus, OH, 1983.
17 T.J. Mast, D.P.H. Hsieh and J.N. Seiber, Mutagenicity and chemical characterization of organic constituents in rice straw smoke particulate matter, Environ. Sci. Technol., 18 (1984) 338–348.
18 T. Ramdahl, G. Becher and A. Bjœrseth, Nitrated polycyclic aromatic hydrocarbons in urban air particles, Environ. Sci. Technol., 16 (1982) 861–865.
19 T.L. Gibson, Nitro derivatives of polynuclear aromatic hydrocarbons in airborne and source particulate matter, Atmos. Environ., 16 (1982) 2037–2040.
20 D. Schuetzle and J.M. Perez, Factors influencing the emissions of nitrated-polynuclear aromatic hydrocarbons (Nitro-PAH) from Diesel engines, J. Air Pollut. Control Assoc., 33 (1983) 751–755.
21 H. Tokiwa, S. Kitamori, R. Nakagawa, K. Horikawa and L. Matamale, Demonstration of a powerful mutagenic dinitropyrene in airborne particulate matter, Mutat. Res., 121 (1983) 107–116.
22 X.B. Xu, J.P. Nachtman, Z.L. Jin, E.T. Wei and S.M. Rappaport, Isolation and identification of mutagenic nitro-PAH in Diesel-exhaust particulates, Anal. Chim. Acta, 136 (1982) 163–174.
23 R.L. Hanson, T.R. Henderson, C.H. Hobbs, C.R. Clark, R.L. Carpenter, J.S. Dutcher, T.M. Harvey and D.F. Hunt, Detection of nitroaromatic compounds on coal combustion particles, J. Toxicol. Environ. Health, 11 (1983) 971–980.
24 C.Y. Wang, M.S. Lee, C.M. King and P.O. Warner, Evidence for nitroaromatics as direct acting mutagens of airborne particulates, Chemosphere, 9 (1980) 83–87.
25 R.E. Talcott and W. Harger, Chemical characterization of direct acting mutagens: the functional group, Mutat. Res., 91 (1981) 433–436.
26 I. Alfheim and L. Wikström, Air pollution from aluminium smelting plants I. The emission of polycyclic aromatic hydrocarbons and of mutagens from an aluminium smelting plant using the Söderberg process, Toxicol. Environ. Chem., 8 (1984) 55–72.
27 L. Van Vaeck, K. van Cauwenberghe and J. Janssens, The gas-particle distribution of organic aerosol constituents: measurements of the volatilization artefact in hi-vol cascade impactor sampling, Atmos. Environ., 18 (1984) 417–430.
28 D. Rondia, Sur la volatilité des hydrocarbures polycycliques, Int. J. Air. Water Pollut., 9 (1965) 113–121.
29 C. Pupp, R.C. Lao, J.J. Murray and R.F. Pottie, Equilibrium vapour concentrations of some polycyclic aromatic hydrocarbons, As_4O_6 and SeO_2 and the collection efficiencies of these air pollutants, Atmos. Environ., 8 (1974) 915–925.
30 F. De Wiest and D. Rondia, Sur la validité des determinations du benzo (a) pyrene atmospherique pendant les mois d'été, Atmos. Environ., 10 (1976) 487–489.
31 L. Van Vaeck, G. Broddin, W. Cautreels and K. van Cauwenberghe, Aerosol collection by cascade impaction and filtration: influence of different sampling systems on the measured organic pollutant levels, Sci. Total Environ., 11 (1979) 41–52.
32 K.E. Thrane and A. Mikalsen, High-volume sampling of airborne polycyclic aromatic hydrocarbons using glass fibre filters and polyurethane foam, Atmos. Environ., 15 (1981) 909–918.,
33 H. Yamasaki, K. Kuwata and H. Miyato, Effects of ambient temperature on aspects of airborne polycyclic aromatic hydrocarbons, Environ. Sci. Technol., 16 (1983) 189–194.
34 C.D. Keller and T.F. Biddleman, Collection of airborne polycyclic aromatic hydrocarbons and other organics with a glass fibre filter-polyurethane foam system, Atmos. Environ., 18 (1984) 837–845.
35 T. Braun, Trends using resilient polyurethane foams as sorbents in analytical chemistry, J. Anal. Chem., 39 (1984) 596–604.

36 T.F. Biddleman and C.E. Olney, High-volume collection of atmospheric polychlorinated biphenyls, Bull. Environ. Contam. Toxicol., 11 (1974) 442–450.
37 M. Oehme and H. Stray, Quantitative determination of ultra-traces of chlorinated compounds in high-volume air samples from the Artic using polyurethane foam as collection medium, Fresenius Z. Anal. Chem., 311 (1982) 665–673.
38 C. Turner and D.E. Glofelty, Field air sampling of pesticide vapours with polyurethane foam, Anal. Chem., 49 (1977) 7–10.
39 R.G. Lewis, A.R. Brown and M. Jackson, Evaluation of polyurethane foam for sampling of pesticides, polychlorinated biphenyls and polychlorinated naphthalenes in ambient air, Anal. Chem., 49 (1977) 1668–1669.
40 W.N. Billings and T.F. Biddleman, High-volume collection of chlorinated hydrocarbons in urban air using three solid adsorbents, Atmos. Environ., 17 (1983) 383–391.
41 B.N. Ames, J. McCann and E. Yamasaki, Methods for detecting carcinogens and mutagens with the Salmonella mammalian microsome mutagenicity test, Mutat. Res., 31 (1975) 347–364.
42 H.S. Rosenkranz and R. Mermelstein, Mutagenicity and genotoxicity of nitroarenes. All nitro-containing chemicals were not created equal, Mutat. Res., 114 (1983) 217–267.
43 T. Nielsen, B. Seitz and T. Ramdahl, Occurrence of nitro-PAH in the atmosphere in a rural area, Atmos. Environ., 18 (1984) 2139–2165.
44 R. Guicherit and F.L. Schulting, The occurrence of organic chemicals in the atmosphere of the Netherlands, Sci. Total Environ., 43 (1985) 193–219.

CHAPTER 13

POLYCYCLIC AROMATIC HYDROCARBONS AND MUTAGENS IN AMBIENT AIR PARTICLES

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Polycyclic Aromatic Hydrocarbons and Mutagens in Ambient Air Particles

W. K. DE RAAT

MT-TNO, Department of Biology, P.O. Box 217, 2600 AE Delft, The Netherlands

The presence of mutagenic compounds in ambient air particles has led to a reassessment of the role of polycyclic aromatic hydrocarbons (PAH) in air pollution research. Besides PAH, other air pollutants were associated with carcinogenicity, although by means of an effect which indirectly and preliminarily indicates carcinogenicity. While PAH themselves are mutagens, they only marginally contribute to the mutagenicity of the particles. However, a close relation exists between PAH and the other mutagenic compounds. Both are emitted in the flue gases of combustion processes and they show a clear structural relationship, i.e. the mutagens most probably are oxygenated and nitrated PAH-derivates. Furthermore PAH might give rise to mutagens via atmospheric reactions.

The present paper deals with several aspects of the relation between PAH and mutagenicity. The contribution of PAH, nitrated PAH and more polar compounds to the mutagenicity was studied by mutagenicity testing and chemical analysis of chromatographic fractions prepared with TLC and HPLC. The contribution of PAH and mono-nitro PAH could be clearly established. The marginal contribution of the former was confirmed, the contribution of the latter is small compared to that of more polar nitro-compounds.

The atmospheric conversion of PAH was studied by analysing the variations of PAH-profiles, i.e. the proportion among PAH-concentrations. Clear indications for conversions were found but the influence of sampling artifacts could not be excluded.

PAH-concentrations showed moderate to reasonable correlations with mutagenicity, no relation between variations of PAH-profile and mutagenicity could be demonstrated, possibly due to methodological factors.

KEY WORDS: Ambient air, particles, polycyclic aromatic hydrocarbons, mutagenicity.

INTRODUCTION

The important role of polycyclic aromatic hydrocarbons (PAH) in investigations on the carcinogenic effects of air pollution was brought into dispute about ten years ago by the finding that ambient air particles contain mutagens.[1–8] The first Salmonella-microsome (Ames) tests with extracts of these particles already showed that PAH do not contribute to any great extent to the mutagenicity. The effects were largely independent of metabolic activation, i.e. of the presence of rat-liver homogenates (S9-fractions), which is a prerequisite for the expression of the mutagenicity of PAH in this test.

If mutagenicity is accepted as a first indication for carcinogenicity, which is the main reason for the use of mutagenicity tests in air pollution research, then we have to conclude that two (different) groups of possible carcinogens are present in ambient air particles. One group consists of PAH of which the carcinogenicity has been established directly in animal experiments and the other group consists of compounds of which indirect evidence for carcinogenicity has been obtained, i.e. the mutagens.

This paper deals with the relation between PAH and mutagens present in ambient air particles.

Shortly after the discovery that ambient air particles contain mutagens, investigations into the origin of these compounds clearly pointed to the sources emitting PAH, i.e. combustion processes. Particles in combustion emissions are clearly mutagenic [9–21] and up to now no other important sources of mutagens have been detected. The high sensitivity and relative speed of mutagenicity tests, especially the Ames test, made it possible to use mutagenicity for detecting purposes in chromatography, and this in turn enabled direct investigations into the identity of the mutagens. These and other studies showed, that both the mutagenicity of the emission particles and that of the ambient air particles could be attributed largely to derivatives of PAH.[12,14–16,19,22–34]

Already during the 1950's the chemical conversion of PAH under atmospheric conditions was investigated in experimental systems. It was shown subsequently that exposure of PAH to air with reactive compounds such as ozone and nitrogen dioxide can lead to a substantial conversion of PAH, especially when such an exposure is carried out in the presence of light.[35–44] More recently it has

been shown, that the exposure products often are mutagenic.[45–48] Especially nitration can result in extremely mutagenic compounds. The foregoing indicates that the presence of PAH and the presence of mutagens in ambient air particles are somehow related. The relation shows the following characteristics: (1) common sources, (2) small direct contribution of PAH to mutagenicity, (3) similarity in chemical structure and (4) possible change of PAH into other mutagens by chemical conversion after emission. This paper gives some results of investigations on the relation between PAH-content and mutagenicity of ambient air particles. These results concern the contribution of PAH and mono-nitrated PAH to the mutagenicity, the influence of chemical conversion on the PAH-content of samples of the actual particles and the correlation between PAH-content and mutagenicity.

METHODS AND MATERIALS

Sampling and extraction

The particulate fraction of the ambient air was sampled on Sartorius SM 13400 glass-fibre filters with Sartorius HV100 high volume samplers. The flow rate was $100\,m^3.h^{-1}$, which resulted in a linear air speed of $0.535\,m.sec^{-1}$. Sampling dates and locations are indicated in the legends of tables and figures. Before sampling the filters were cleaned by extracting them for 24 h with methanol in a Soxhlet-apparatus. Sampling lasted for 24 h unless stated otherwise. The filters loaded with particles were stored at −80 °C before extraction. Unless stated otherwise the mutagens and PAH were extracted with methanol (Rathburn, HPLC-grade) in a Soxhlet-apparatus for 8 h. Extraction was carried out in the dark in a nitrogen atmosphere. The solvent was removed with a rotary evaporator under reduced pressure at 30 °C. The residue was dissolved in dimethylsulphoxide (Merck, AR, DMSO) or in a solvent (methanol and/or acetone, Rathburn HPLC-grade) more suitable for the HPLC-analysis of PAH and nitro-PAH. Dissolved residues were stored at −80 °C.

Chromatographical fractionation

Extracts of the ambient air particles were chromatographically fractionated with the following techniques:

The residue as such derived from about 200 m^3 of air, was dissolved in 50 ml of methoxyisobutane (MIB; Rathburn HPLC-grade) by ultrasonic vibration. Then 50 ml of distilled water was added and after about 2 × 3 min of manual shaking the two layers were separated. The water-layer as such was tested for mutagenicity *or* the water was removed by evaporation (rotary evaporator, 30 °C reduced pressure), *or* organics were extracted from the water with a combination of Amberlite XAD2 and XAD7 following the method normally used for extracting mutagens from urine.[62]

The MIB was removed from the MIB-layer by evaporation (rotary evaporator 30 °C, reduced pressure) and the residue solved in acetone (Rathburn, HPLC-grade). The acetone solution was put on one or two Seppak Silica cartridges (Waters). About one third of the silica in the cartridge was brought into contact with the solution. Then the acetone was removed by blowing nitrogen through the cartridge (opposite the elution direction) for 5 min. The cartridge was eluted respectively with hexane (Nanograde) (twice, about 15 ml), toluene (HPLC-grade) (once or twice, about 15 ml) and methanol (once, about 15 ml). After evaporation of the solvents (see above), the residues were dissolved in DMSO and tested or dissolved in acetone and brought on Merck PSC kieselgel 60F 254s thin layer plates with concentration zone (TLC-plates). The hexane-fraction residue was eluted on the TLC-plates with a mixture of chloroform and hexane (1:1 Vol). The silica was scraped off the plates in 1 cm broad bands, extracted by ultrasonic vibration in acetone and the extracts were tested for mutagenicity and/or analysed. Figure 1 summarizes the procedure described above.

In addition to this procedure some extracts were fractionated with semi-preparative HPLC (high pressure liquid chromatography). The crude extracts (solutions in acetone of the residue) were prefractionated with Seppak Silica cartridges: the residue of about 200 m^3 per cartridge was eluted twice with MIB (15 ml) and once with methanol (15 ml). The residue of the first layer was dissolved in dichloromethane (Baker, HPLC reagent) and subjected to semi-preparative HPLC. Stationary phase: Alltech RSiL-Silica (10 μm) column ($L = 250$ mm, $\phi = 10$ mm); mobile phase: gradient elution with hexane (Rathburn, HPLC-grade), dichloromethane and acetonitrile (Rathburn, HPLC-grade). The gradient is indicated in Figure 2. The flow was 3.5 ml/min. The stationary phase was chemically activated

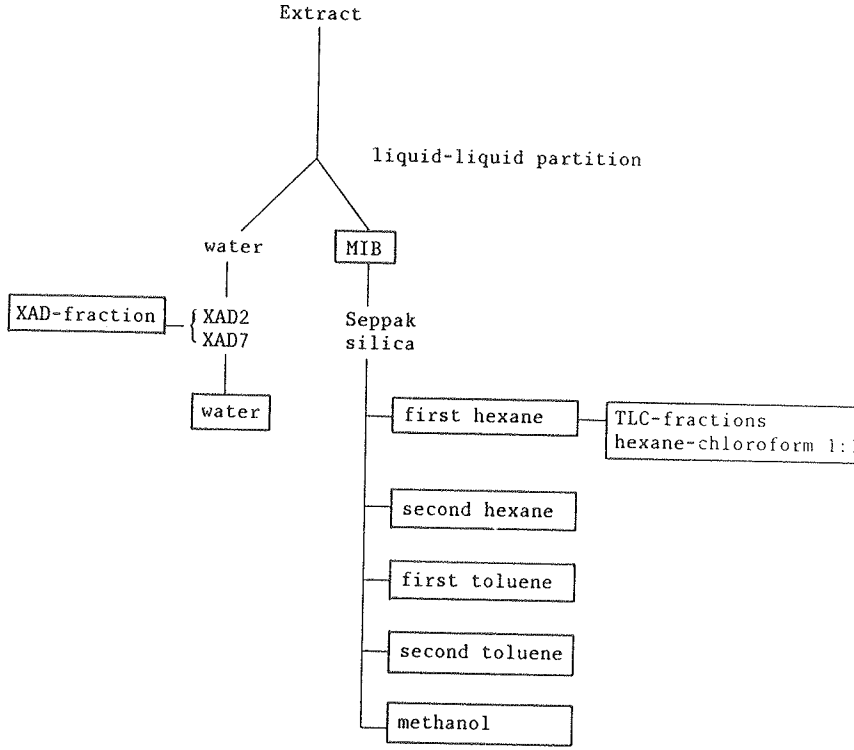

Figure 1 Schematic representation of a chromatographical fractionation procedure applied on extracts of ambient air particles.

before each run with a mixture of hexane, acetic acid (Baker, AR) and dimethoxypropane (Merck, AR), 90:10:2.5, Vol. A similar HPLC-fractionation procedure has been described by Stray *et al.*[49] Fractions of 7 ml (2 min) were collected and tested or/and analysed after evaporation (see above) and resolving the residue in a suitable solvent.

Mutagenicity testing

The residues of the extracts or fractions were dissolved in DMSO and tested in the Salmonella-microsome test following the procedure described by Ames *et al.*[50] The strains TA98, TA98NR and TA98DNP were used; the first strain was obtained from Dr. I. E. Mattern, Rijswijk, the Netherlands and the latter two from Dr. H. S. Rosenkranz, Cleveland, Ohio, USA. For metabolic activation a liver

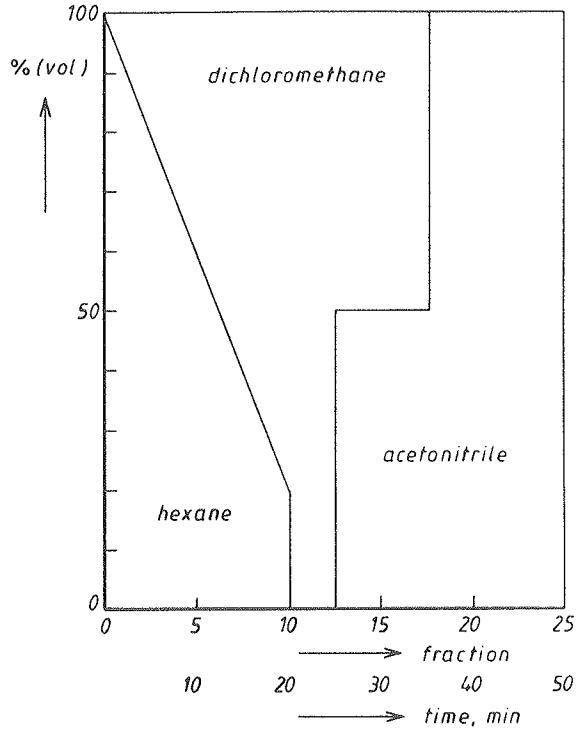

Figure 2 Elution scheme of the semi preparative HPLC-fractionation. The fractions are retarded 5.5 min compared to changes in the elution pattern.

S9-fraction prepared from rats treated with Aroclor 1254 was used. The tests were carried out in duplicate or triplicate and the test substances were always dosed in the same volume of DMSO (0.1 ml).

Analysis of PAH and nitro-PAH

PAH were analysed with RP-HPLC. Stationary phase: Supelco-PAH (250 mm × 4.6 mm); Mobile phase: 75% v/v methanol in water to 100% methanol; detection: fluorescence, excitation 250 nm and emission >390 nm. The PAH were identified and quantified with external reference compounds. The mono-nitro PAH 1- and 4-nitropyrene and 3-nitrofluoranthene were also analysed with RP-HPLC. Stationary phase: Vydac-201TP (5 μm) (250 mm × 4.6 mm); mobile phase: gradient of acetonitrile and 75 mM Tris.HCL (pH 6.0); post-column reduction with zinc; detection: chemiluminescence with

peroxyoxalate. Detection and quantification was based on chromatograms with external reference compounds and on comparison of chromatograms prepared with and without reduction.

Multivariate analysis

Of a series of samples the variation of PAH-concentrations and mutagenic effects was analysed with the principal component analysis[51] and the results of this analysis were subjected to orthogonal rotation according to the varimax criterium.[52] In this way the factors determining the proportion among concentrations (PAH-profile) and the relation of mutagenicity with variations of the PAH-profile could be studied.

RESULTS AND DISCUSSION

Contribution of PAH and mono-nitro PAH to the mutagenicity

Figure 3 shows the results of a fractionation according to the procedure of Figure 1. These results are representative for comparable experiments with other extracts: The major part of the mutagenicity is concentrated in the MIB-layer. The small to negligible mutagenic activity in the water layer can be extracted with the XAD-columns. The Seppak separation always results in three different mutagenic fractions: the first hexane fraction, the first toluene fraction and the methanol fraction. The analysed PAH and mono-nitro-PAH can only be demonstrated in first hexane fractions, so the contribution of these compounds to the total mutagenicity does not exceed the mutagenicity of the first hexane fraction. Figure 4 shows the results of an Ames test with TLC-fractions from the first hexane fraction of Figure 3. The mutagenicity of fraction 8 is totally dependent of the presence of S9-mix. This mutagenicity probably reflects the contribution of the PAH to the total mutagenicity as PAH are generally concentrated on or near the same place of TLC-plates (Figure 5 for example). As is shown in Figure 5 the mono-nitro PAH are present in more polar fractions and they could very well determine the mutagenicity of these fractions. These fractions show direct, nitro-reductase dependent mutagenicity. The first

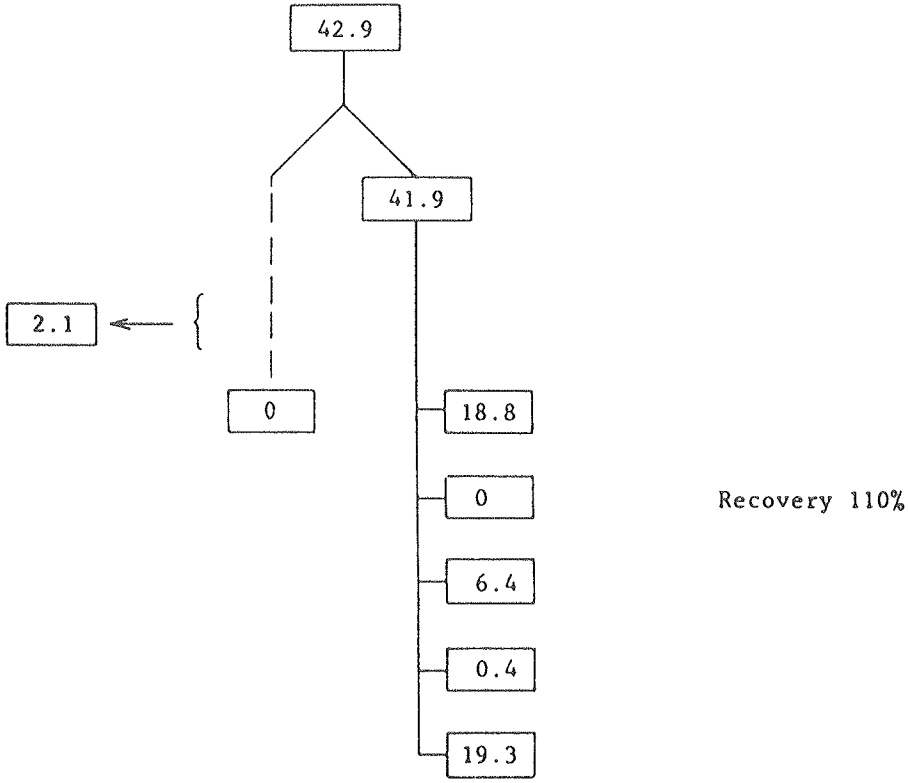

Figure 3 Mutagenicity (TA98+S9) of LC-fractions and XAD-fraction prepared according to the scheme depicted in Figure 1. The layout of this figure is strictly comparable to that of Figure 1. The values (revertants per m^3) represent means of duplicates. All values were obtained in one test-procedure. Mixture of two samples; one from the TNO-site in Delft (9-1-'86, 29 h) and one from the zoological garden (Blijdorp) in Rotterdam (1-1-'86, 21 h).

hexane fraction often gives rise to mutagenic fractions with a higher polarity than mono-nitro PAH. This indicates that the mutagenicity of the total first hexane fraction exceeds the contribution of the PAH and the mono-nitro PAH to the mutagenicity. Figure 6 shows results of a series of experiments in which ambient air particles were sampled once a week during one year at the TNO-site in Delft, the Netherlands, (sampling started on Wednesday at 11.00 h and lasted 24 h). The extracts were fractionated according to the procedure depicted in Figure 1. The water layer was in this case tested as such or after concentration by evaporation. No second toluene fraction was prepared.

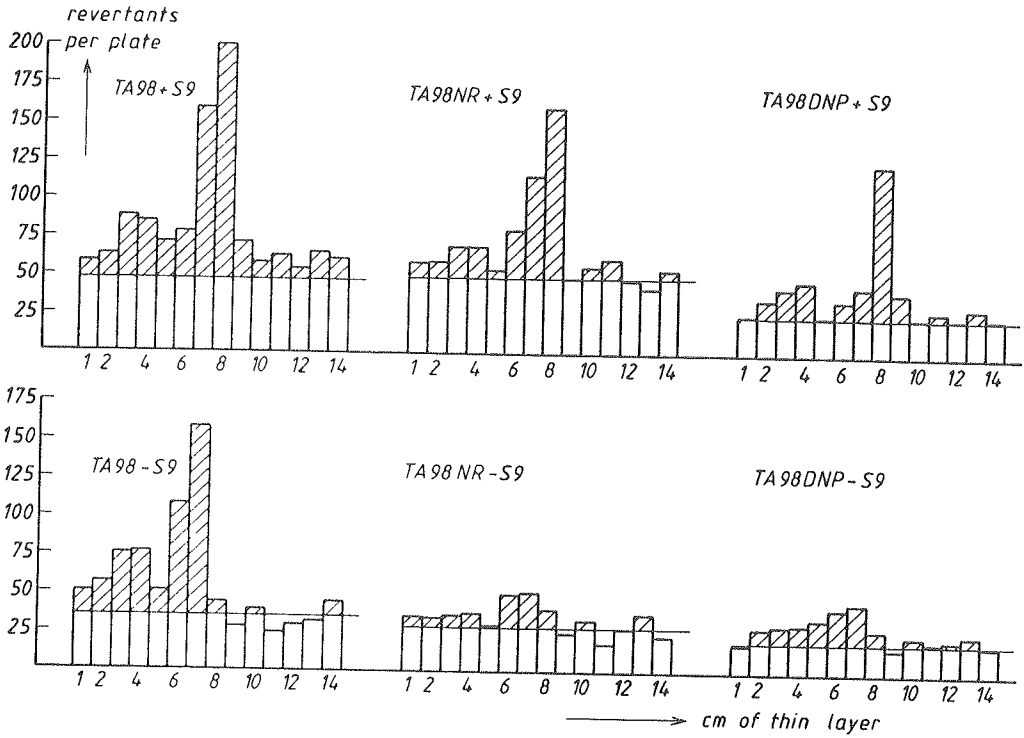

Figure 4 Mutagenicity of TLC-fractions of a first hexane layer (see Figure 1). The bars represent means of duplicates; hatched part: mutagenicity above background. Fraction 1 is the first fraction (cm of thin layer) after the concentration zone of the TLC-plate. All values were obtained in one test-procedure. The hexane fraction of Figure 3 was used.

The importance of compounds more polar than the PAH and the mono-nitro PAH appears clearly from Figure 6. The slight influence of the S9-fraction indicates the unimportance of the contribution of the PAH.

A much more exact estimation of the contribution of the PAH and the mono-nitro PAH can be obtained with the semi-preparative HPLC-fractionation. Figure 7 shows "mutagrams" for two extracts. As can be concluded from Figure 8, the analysed PAH are exclusively present in the fractions 5–7 and for the major part in fraction 6; the mono-nitropyrenes and 2-nitrofluoranthene could only be demonstrated in the fractions 9 and 10 (results not shown). Fractions 5–7 show only a small mutagenic effect which is totally dependent on the presence of S9-fraction; the effect of fraction 9 is

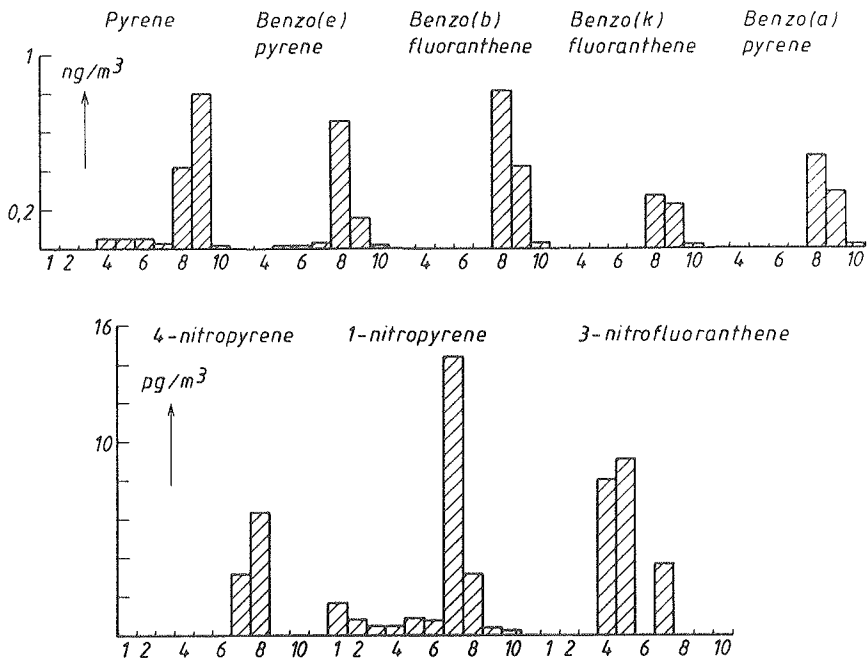

Figure 5 Concentration of PAH and mono-nitro PAH in TLC-fractions of a first hexane layer. All compounds were analysed in one TL-chromatogram. Fraction 1 is the first fraction (cm of thin layer) after the concentration zone of the TLC-plate. Sample from zoological garden (Blijdorp) in Rotterdam (31-10-'85, 24 h).

the result of direct, nitro-reductase dependent compounds. These effects, combined with the results of chemical analysis and the finding that more polar PAH-derivatives elute in fraction 12 and beyond make it very probable that the two relatively apolar peaks in the "mutagram" represent the contribution of PAH and mono-nitro PAH. The dinitropyrenes elute, whether or not mixed with an extract, in fraction 12. Until now we did not find clear cut mutagenicity of this fraction when extracts of ambient air particles were fractionated, indicating that the contribution of these extremely mutagenic compounds to the mutagenicity is negligible. The largest part of the mutagenicity appears to be associated with relatively polar compounds, which depend for their direct mutagenicity on nitro-reduction. In the "mutagram" these compounds are brought together in fraction 16. However, since much polar mutagenicity is removed already from the extract by the Seppak-prefractionation,

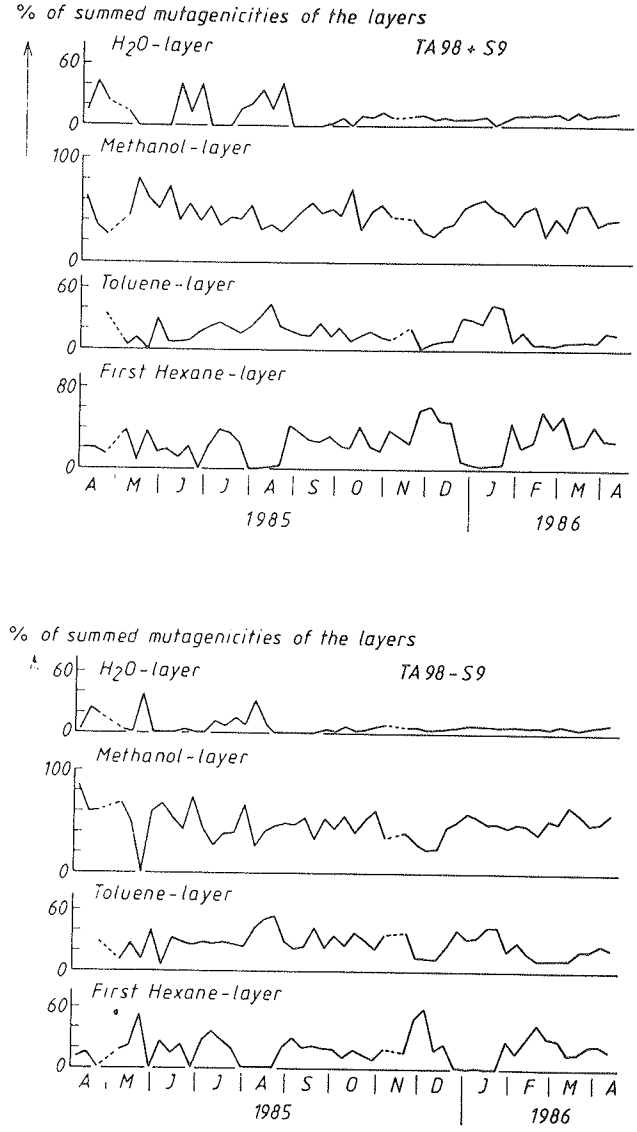

Figure 6 Mutagenicity of LC-fractions prepared from ambient air particles. Samples were taken every 24 h during a one year period once a week (sampling started on Wednesday at about 11.00 h). Location: TNO-site in Delft. Fractions were prepared according to the scheme depicted in Figure 1, no second toluene was prepared and the water layer was concentrated by evaporation of the water or tested as such. The indicated values are based on least squares calculations of six plate counts obtained with three doses (0, 10 and 20 m^3 per plate). A broken line indicates, that a sample or test result was not available.

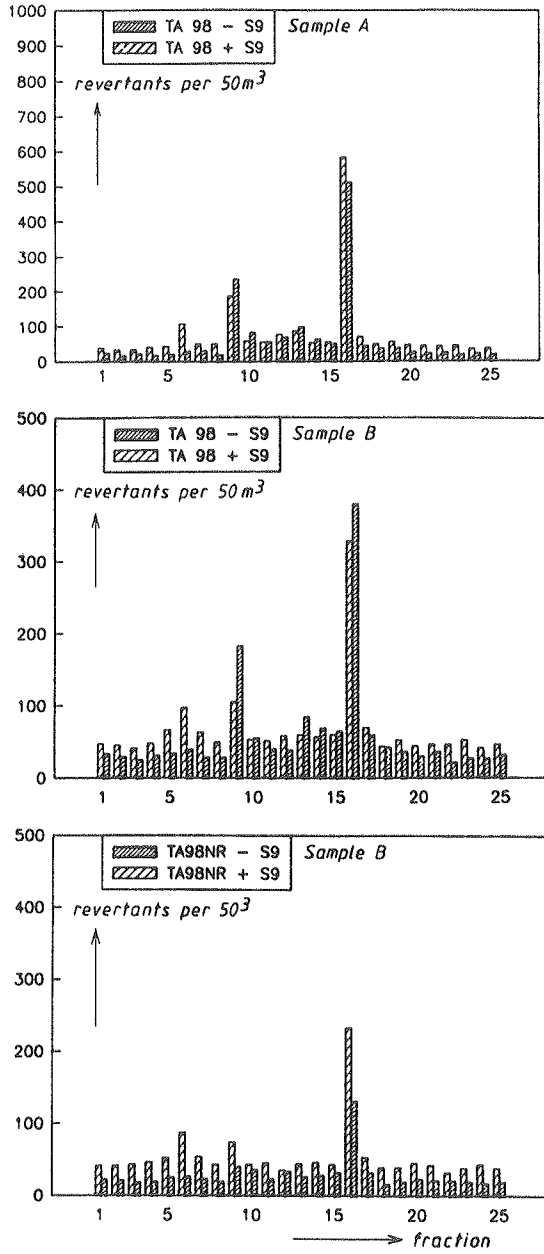

Figure 7 Mutagenicity of semi-preparative HPLC-fractions of two extracts prepared from ambient air particles (samples A and B). The values represent means of duplicates. The fractions of one sample were tested in one test procedure. Each fraction represents 2 min of the elution time (7 ml of eluent). See Figure 2 for elution scheme. The background mutations were not subtracted from the effects. Sample A: Mixture of several 24 h samples taken during winter 1985/1986 at the zoological garden (Blijdorp) in Rotterdam and at a location near the harbour of Schiedam (near Rotterdam). Sample B: Mixture of several 24 h samples from the same period and locations as sample A.

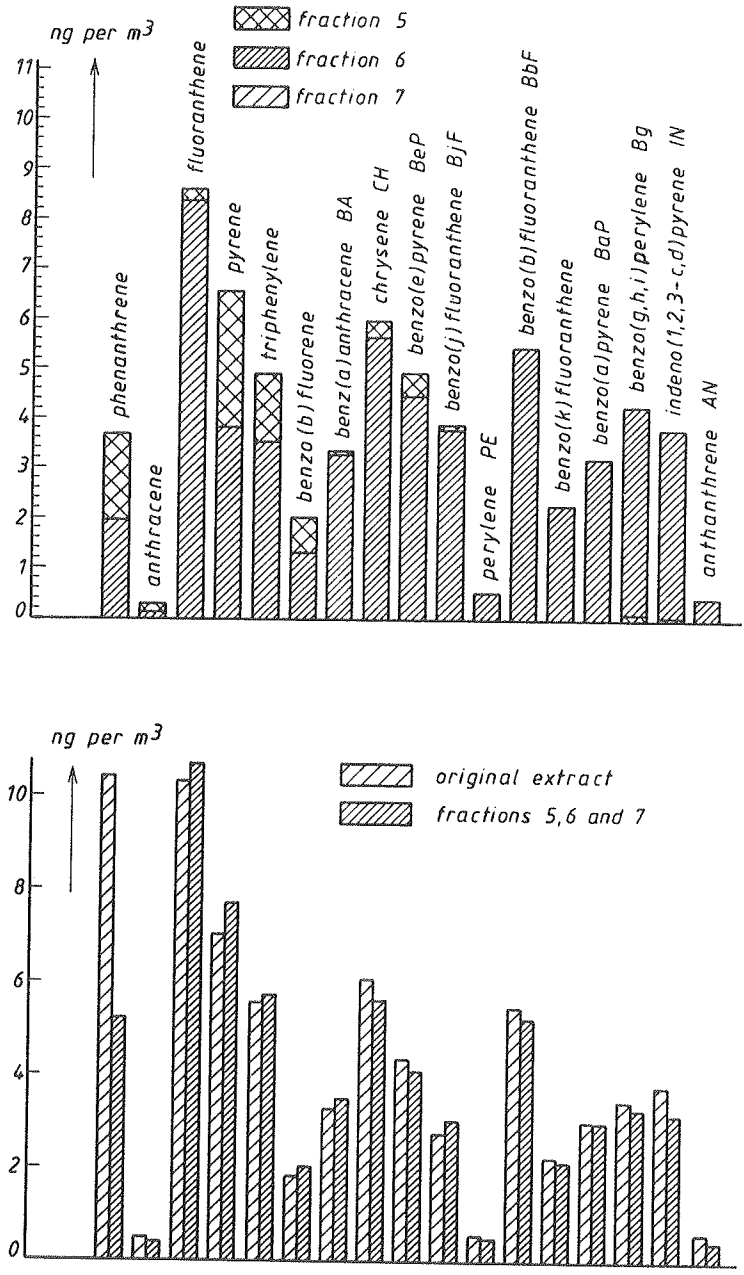

Figure 8 Concentrations of PAH in an extract of ambient air particles and fractions of it prepared with semi-preparative HPLC. The mutagenicities of the same fractions are depicted in Figure 7, sample A.

the effect of fraction 16 represents only a part of the mutagenicity of the relatively polar compounds.

In any case these experiments clearly visualize the contribution of the PAH and confirm the negligible contribution of the PAH to the mutagenicity. Even the contribution of the mono-nitro PAH does not seem to be large.

An important question arising from these experiments is the influence of the analytical procedure itself on the observed effect(s). The semi-preparative HPLC-fractionation was subjected to recovery experiments. The mutagenicities of the MIB-fraction and of a sample reconstituted from the HPLC-fractions were compared with the sum of the mutagenicities of the HPLC-fractions. We only found small decreases of the effects due to the fractionation. As emerges from Figure 8, the PAH concentrations did hardly decrease, so the HPLC-fractionation is rather inert to these compounds when present in the complex matrix of an extract.

Influence of chemical conversion on the PAH-profile

The chemical conversion of PAH during their residence in the air can be studied in two different ways: PAH can be exposed to simulated atmospheres in experimental systems or it can be investigated whether differences between PAH with respect to sensitivity for chemical conversion are somehow reflected in the variation of the actual ambient PAH-concentrations.

Here the results of investigations following the second approach are given. In two investigations into the mutagenicity of ambient air particles we have analysed the extracts for a number of PAH. These PAH can differ in origin (contributing sources), volatility and sensitivity to chemical conversion. Differences between samples in degree of chemical conversion will result in an extra source of variation for the more sensitive PAH, which will be detectable if it is not overwhelmed by other factors such as origin and volatility. In a study, which will be published in detail elsewhere[53] the particles were simultaneously collected eight times on four locations in an urban and industrialized area in the Netherlands (Rijnmond area) and on one location near the coast, lying upwind of that area at Southwestern winds. The principal component analysis showed that volatility strongly influences the proportion between the PAH-

concentrations (PAH-profile). The concentrations of the *less* volatile PAH (>4 rings) are very strongly determined by one factor, i.e. are very strongly correlated ($r > 0.9$). However, if the results of this study are analysed with the rotation procedure (see Table 1), two groups of less volatile PAH can be distinguished. This dichotomy seems to reflect differences in sensitivity towards chemical conversion; on the one hand benz(a)anthracene, perylene, benzo(a)pyrene and anthanthrene, on the other hand benzo(e)pyrene and benzo(k) and (b)fluoranthenes.[54] A comparable result (see Table 1) was found in another study in which the particles were sampled during episodes of photochemical air pollution. In this case the particles were sampled 8 to 10 times from 8.00 h to 20.00 h in four periods of 3 h at the TNO-site in Delft. During these periods the concentrations of ozone and peroxyacetyl nitrate showed the time dependency characteristic of photochemical air pollution. In this study the same dichotomy was found, while one factor was influencing benzo(g, h, i)perylene, a PAH linked with traffic emissions which are the only important local emissions of PAH. The results of the two studies suggest that conversion is influencing the variation of the PAH-concentrations. It can however not be excluded that this conversion is the result of sampling artifacts. In the first of the two studies (in the Rijnmond area) it appeared that the share of the sensitive PAH increased with an increasing contribution of local sources, indicative of a stronger conversion upon longer exposure to the reactive constituents of the air.[53] It is hard to imagine that this phenomenon would be the result of sampling artifacts.

Correlation between mutagenicity and PAH-content

The relation between PAH-content and mutagenicity of the particles is determined by their common sources, the direct contribution of PAH to the mutagenicity and the conversion of PAH. The first two factors will give rise to correlation between PAH-content and mutagenicity. Furthermore it has been demonstrated that PAH and mutagens are both predominantly present in particles with an aerodynamic diameter of less than 1 μm. They will thus show a similar distribution due to meteorology. It can be expected that this factor will already lead to considerable correlations. The correlation will be reduced as a result of chemical conversion of PAH and

Table 1 Results of a multivariate analysis with PAH concentrations in ambient air particles.[a]

PAH	*Rijnmond*			
BA	−0.49	−0.81	−0.32	−0.00
CH	−0.74	−0.57	−0.33	−0.01
BeP	−0.85	−0.39	−0.31	−0.16
PE	−0.44	−0.73	−0.51	−0.06
BbF	−0.84	−0.39	−0.38	0.01
BkF	−0.80	−0.46	−0.39	0.01
BaP	−0.55	−0.59	−0.58	0.01
DBA	−0.71	−0.46	−0.49	−0.18
DBP	−0.84	−0.40	−0.36	0.06
IN	−0.83	−0.40	−0.39	0.03
AN	−0.41	−0.37	−0.83	−0.01
Eigen values	5.40	3.05	2.41	0.07
PAH	*Delft*			
BA	−0.39	−0.79	−0.46	−0.05
CH	−0.75	−0.49	−0.44	−0.05
BeP	−0.87	−0.43	−0.22	−0.05
PE	−0.44	−0.79	−0.25	−0.05
BbF	−0.83	−0.45	−0.32	−0.04
BkF	−0.77	−0.54	−0.34	−0.05
BaP	−0.63	−0.69	−0.35	−0.05
Bg	−0.53	−0.58	−0.62	−0.01
IN	−0.72	−0.52	−0.45	−0.03
AN	−0.52	−0.80	−0.25	−0.12
Eigen values	4.52	3.90	1.49	0.03

[a]Principal components analysis with correlation matrix and orthogonal rotation according the varimax criterium. Indicated are the correlation of the concentrations with the factors and the Eigen values of the factors.
Rijnmond: 35 samples (24 h) taken at 4 locations in the industrialized and urban Rijnmond area (in and near Rotterdam) in the Netherlands and at one coastal location upwind of that area; from July to November 1981.
Delft: 32 samples taken at the TNO site in Delft (the Netherlands) during episodes with photochemical air pollution; from May to August 1982; samples of 3 h between 8 and 20 h.
DBA: dibenzo[a,j]anthracene; DBP: dibenzo[a,l]pyrene or other compound eluting at the same place.

mutagens after emission and by variation of the proportion between PAH and mutagens at the sources. The correlation is possibly even more strongly affected by the methods (mutagenicity and analysis from PAH) used: The mutagenicity can possibly be influenced by non-mutagenic compounds, which have no relation with the PAH; the PAH-concentration can unambiguously be determined. Furthermore PAH-concentrations are simultaneously determined (one sample, one extraction, one chromatogram) and will *together* show an experimental variation which is largely independent from that of the mutagenicity which will be largely determined by the Ames test. It is also reasonable to assume that the experimental variation of the mutagenicity is larger than that of the PAH-concentrations because no adequate positive control and calibration compounds can be used in the mutagenicity test.

Table 2 shows correlation coefficients for the mutagenic effects and the PAH-concentrations obtained in the study in which samples were collected during photochemical air pollution (see the preceding section, second study, samples taken at Delft). It is striking that clearly higher correlations are found if the test is carried out in the presence of metabolic activation. Comparable results were obtained in the other study (Rijnmond area). The PAH did not differ much with respect to their correlation with mutagenicity. So the dichotomy of the PAH which is possibly caused by conversion cannot be observed in the correlation matrix. Conversion does not increase or decrease the mutagenicity systematically enough and strongly enough to affect the correlations. Varimax rotation yielded such strong separate factors for mutagenicity, that an influence of conversion could not be discerned (results not shown). Altogether this

Table 2 Correlation between mutagenicity and PAH-concentrations.[a]

	Ba	*CH*	*BeP*	*PE*	*BbF*	*Bkf*	*BaP*	*Bg*	*IN*	*AN*
TA98 – S9	0.50	0.55	0.56	0.55	0.55	0.58	0.56	0.59	0.57	0.53
TA98 + S9	0.65	0.78	0.84	0.77	0.82	0.81	0.77	0.73	0.79	0.72
TA98NR – S9	0.39	0.45	0.42	0.41	0.43	0.47	0.47	0.52	0.47	0.43
TA98NR + S9	0.68	0.77	0.81	0.82	0.80	0.81	0.79	0.73	0.79	0.76

[a]Samples taken during episodes with photochemical air pollution; Delft, TNO site; see Table 1. (See Figure 8 for abbreviations.)

suggests that any significant influence of PAH-conversion on mutagenicity is absent. Important, PAH-independent processes influence mutagenicity, which might very well be related with the great experimental variations of Ames test results.

The contribution of the mutagens to the carcinogenicity of ambient particles

Investigations into the mutagenicity and the PAH-content of ambient air particles are ultimately motivated by the carcinogenicity of the particles[55–56] and the controversial relation between human cancer and air pollution.[57–58] The experiments presented in this paper were in part aimed at determining the *contribution of PAH* and other compounds *to the mutagenicity*. More important however is the *contribution of the mutagens to the carcinogenicity* of the particles. An extensive treatment on this subject goes beyond the scope of this paper. Only three short remarks will be made.

— In the past a number of studies dealt with the contribution of PAH to the carcinogenic effects of the particles in experimental animals. PAH-fractions were isolated from the extracts and these and other fractions tested. If we assume that the effects of the PAH-fraction were indeed the result of PAH, these studies lead to the conclusion, that PAH are important, if not the most important carcinogens in the particles.[59] In any case, the contribution of the PAH to the carcinogenicity is most certainly much greater than the contribution of these compounds to mutagenicity. Mutagenicity thus seems to lead to an underestimation of the contribution of the PAH and so to an overestimation of the contribution of the (other than PAH) mutagens to the carcinogenicity.

— The mutagenicity in the absence of S9-fraction, i.e. the direct mutagenicity, is for the greatest part, if not totally, dependent on nitro-reduction. This mutagenicity is clearly expressed in bacteria; many nitro-PAH are extremely mutagenic in the Ames test. The question arises how far *this* type of mutagenicity is bacteria-specific to such an extent, that it leads to an overestimation of effects that can be expected in mammals and thus of carcinogenicity. Comparative studies in which chromatographical fractions are tested with *bacterial* and *mammalian in vitro* tests are

necessary for a better evaluation of the Ames test effects of the extracts which represent the mainstay of the data collected thusfar.

— The detection of very mutagenic nitro-PAH in ambient air particles and particles present in combustion emissions has led to investigations into the carcinogenicity of these compounds in animal experiments.[60–61] So far the results clearly point to carcinogenic activity for mono-nitro PAH and dinitropyrenes. However, even if these compounds are as carcinogenic as PAH on a "per weight" basis, it must be noted that their concentrations are *much* lower than those of the PAH. The possibility that PAH are more important, just because of their higher concentrations must not be excluded.

As this and other studies show that the more polar compounds, i.e. oxidized nitro compounds, are much more important contributors to mutagenicity than non-oxidized nitro compounds, the investigations into their carcinogenicity should be intensified.

Acknowledgements

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References

1. C. E. Chrisp and G. L. Fisher, *Mutation Research* **76**, 143 (1980).
2. W. Dehnen, N. Pitz and R. Tomingas, *Cancer Letters* **4**, 5 (1977).
3. M. Møller and I. Alfheim, *Atmospheric Environment* **14**, 83 (1980).
4. J. N. Pitts *et al.*, *Toxicology Letters* **1**, 65 (1977).
5. E. Selzer Madsen, P. A. Nielsen and J. C. Pedersen, *The Science of the Total Environment* **24**, 13 (1982).
6. R. Talcott and E. T. Wei, *Journal of the National Cancer Institute* **58**, 449 (1977).
7. K. Teranishi, K. Hamada and H. Watanabe, *Mutation Research* **56**, 273 (1978).
8. H. Tokiwa, S. Kitamori, K. Takahashi and Y. Onishi, *Mutation Research* **77**, 99 (1980).
9. B. Pani, L. N. Babrudi, A. Collareta, S. Venturini, R. Ferri, M. Carrozzi, F. Burilini and C. Monti-Bragadin, *Environmental Mutagenesis* **5**, 23 (1983).
10. R. M. Kamens, G. D. Rives, I. M. Perry, D. A. Bell, R. F. Paylor, Jr., R. G. Goodman and L. D. Claxton, *Environmental Science and Technology* **18**, 523 (1984).

11. D. A. Kaden, R. A. Hites and W. G. Thilly, *Cancer Research* **39**, 4152 (1979).
12. I. T. Salmeen, A. M. Pero, R. Zator, D. Schuetzle and T. L. Riley, *Environmental Science and Technology* **18**, 375 (1984).
13. J. L. Mumford and J. Lewtas, *Journal of Toxicology and Environmental Health* **10**, 565 (1982).
14. I. Alfheim, G. Becher, J. K. Hongslo and T. Ramdahl, *Environmental Mutagenesis* **6**, 91 (1984).
15. X. B. Xu, J. P. Nachtman, Z. L. Jin, E. T. Wei and S. M. Rappaport, *Analytica Chimica Acta* **136**, 163 (1982).
16. T. J. Mast, D. P. H. Hsieh and J. N. Seiber, *Environmental Science and Technology* **18**, 338 (1984).
17. G. Löfroth, *Chemosphere* **10**, 791, 798 (1978).
18. T. Ramdahl, I. Alfheim, S. Rustad and T. Olsen, *Chemosphere* **11**, 601 (1982).
19. C.-J. Wei, O. G. Raabe and L. S. Rosenblatt, *Environmental Mutagenesis* **4**, 249 (1982).
20. M. Møller and I. Alfheim, *Mutation Research* **116**, 35 (1983).
21. Yi. Y. Wang, S. M. Rappaport, R. F. Sawyer, R. E. Talcott and E. T. Wei, *Cancer Letters* **5**, 39 (1978).
22. S. M. Rappaport, Yi. Y. Wang, E. T. Wei, R. Sawyer, B. E. Watkins and H. Rapoport, *Environmental Science and Technology* **12**, 1505 (1980).
23. R. Nakagawa, S. Kitamori, K. Horikawa, K. Nakashima and H. Tokiwa, *Mutation Research* **124**, 201 (1983).
24. R. L. Hanson, T. R. Henderson, C. H. Hobbs, C. R. Clark, R. L. Carpenter, J. S. Dutcher, T. M. Harvey and D. F. Hunt, *Journal of Toxicology and Environmental Health* **11**, 971 (1983).
25. T. Ramdahl, G. Becher and A. Bjørseth, *Environmental Science and Technology* **16**, 861 (1982).
26. R. L. Tanner and R. Fajer, *International Journal of Environmental Analytical Chemistry* **14**, 231 (1983).
27. L. D. Claxton, *Environmental Science Research*, Vol. 25 (Plenum Press, New York, 1982).
28. T. Nielsen, B. Seitz and T. Ramdahl, *Atmospheric Environment* **18**, 2159 (1984).
29. C. Y. Wang, M. S. Lee, C. M. King and P. O. Warner, *Chemosphere* **9**, 83 (1980).
30. R. Talcott and W. Harger, *Mutation Research* **91**, 433 (1981).
31. I. Alfheim, *Mutagens in Our Environment* (Alan R. Liss, New York, 1982).
32. J. Siak, T. L. Chan, T. L. Gibson and G. T. Wolff, *Atmospheric Environment* **19**, 369 (1985).
33. W. R. Harris, E. K. Chess, D. Okamoto, J. F. Remsen and D. W. Later, *Environmental Mutagenesis* **6**, 131 (1984).
34. H. Tokiwa, S. Kitamori, R. Nakagawa, K. Horikawa and L. Matamale, *Mutation Research* **121**, 107 (1983).
35. J. F. Thomas, M. Mukai and B. D. Tebbens, *Environmental Science and Technology* **2**, 33 (1968).
36. B. D. Tebbens, M. Mukai and J. F. Thomas, *American Industrial Hygiene Association Journal* **June 1971**, 365 (1971).
37. H. L. Falk, I. Markul and P. Kotin, *A.M.A. Archives of Industrial Hygiene and Occupational Medicine* **13**, 13 (1956).

38. B. D. Tebbens, J. F. Thomas and M. Mukai, *American Industrial Hygiene Association Journal* **27**, 415 (1966).
39. J. Jäger and V. Hanus, *Journal of Hygiene, Epidemiology, Microbiology and Immunology* **24**, 1 (1980).
40. T. Nielsen, T. Ramdahl and A. Bjørseth, *Environmental Health Perspectives* **47**, 103 (1983).
41. L. D. Butler and P. Crossly, *Atmospheric Environment* **15**, 91 (1981).
42. F. Valerio, P. Bottino, D. Ugolini, M. R. Cimberle, G. A. Tozzi and A. Frigerio, *The Science of the Total Environment* **40**, 169 (1984).
43. A. H. Miguel, *The Science of the Total Environment* **36**, 305 (1984).
44. K. Nikolao, P. M. Masclet and G. Mouvier, *The Science of the Total Environment* **32**, 102 (1984).
45. H. S. Rosenkranz and R. Mermelstein, *Mutation Research* **114**, 217 (1983).
46. J. N. Pitts, Jr., *Environmental Health Perspectives* **47**, 115 (1983).
47. J. N. Pitts, Jr., K. van Cauwenberghe, D. Grosjean, J. P. Schmid, D. R. Fitz, W. L. Belser, G. B. Knudson and P. M. Hynds, *Science* **202**, 515 (1978).
48. J. N. Pitts, Jr., D. M. Lokensgard, P. S. Ripley, K. A. van Cauwenberghe, L. van Vaeck, S. D. Shaffer, A. J. Thilland and W. L. Belser, *Science* **210**, 1347 (1980).
49. H. Stray, S. Manø, A. Mikalsen and M. Oehme, *Journal of High Resolution Chromatography and Chromatography Communications* **7**, 74 (1984).
50. B. N. Ames, J. McCann and E. Yamasaki, *Mutation Research* **31**, 347 (1975).
51. D. F. Morrison, *Multivariate Statistical Methods* (McGraw-Hill Book Company, New York, 1972).
52. W. Thomas, *Atmospheric Environment* **20**, 995 (1986).
53. W. K. de Raat, S. A. L. Kooijman and J. W. J. Gielen, *The Science of the Total Environment* **66**, 95 (1987).
54. T. Nielsen, *Environmental Science and Technology* **18**, 157 (1984).
55. S. S. Epstein, K. Fujii and S. Asahina, *Environmental Research* **19**, 163 (1979).
56. J. Leiter and M. J. Shear, *Journal of the National Cancer Institute* **3**, 167 (1942/43).
57. R. Doll, *Environmental Health Perspectives* **22**, 23 (1978).
58. F. E. Speizer, *Environmental Health Perspectives* **47**, 32 (1983).
59. F. Pott, R. Tomingas, A. Brockhous and F. Huth, *ZB Bakt., I. Abt. Orig.* **B170**, 17 (1980).
60. S. Nesnow, L. L. Triplett and T. J. Slaga, *Cancer Letters* **23**, 1 (1984).
61. M. Hirose, M. S. Lee, C. Y. Wang and C. M. King, *Cancer Research* **44**, 1158 (1984).
62. E. Yamasaki and B. N. Ames, *Proceedings of the National Academy of Science, USA* **74**, 3555 (1977).

CHAPTER 14

DISCUSSION

Variation of the mutagenic quality

The proportion of the effects observed in different mutagenicity tests can be regarded as a sort of measure for the mutagenic quality of a sample of ambient airborne particles (see chapter 3). Obviously, this proportion (or mutagenicity profile) depends on the composition of the samples as regards the compounds that determine its mutagenic properties and the specific sensitivities of the tests for these compounds. Mutagens as well as non-mutagens play a role here, because the primary effect of the former may be modulated by a secondary effect of the latter (e.g. toxicity not based on interaction with the genetic material). Both groups determine the responses of the mutagenicity tests and, thereby, the mutagenicity profile.

Obviously, the mutagenic quality of ambient airborne samples needs not to be constant. It will depend on the sources and on the atmospheric physical and chemical processes that determine the composition of the particles. Therefore, changes of the mutagenic quality should be taken into account when air pollution with particulate mutagens is studied, at least if we strive for an anyhow complete picture of this type of air pollution.

In the experimental part of this thesis the mutagenic quality of the particles was investigated by testing the extracts of the particles with several variants of the Salmonella/microsome test, i.e. with different tester strains, each of them with their specific sensitivity to certain classes of mutagens, with or without a rat-liver post-mitochondrial fraction (S9 fraction) for simulation of mammalian metabolic transformation. The results unambiguously reveal the valuable extra information that can be gained by applying these variants together. Variation of the mutagenicity profile indicated changes in the composition of the particles as regards the compounds which determine their mutagenicity. These changes may be interpreted in terms of chemical conversion during the residence of the particles in the air or variation of source pattern. Moreover, the mutagenicity profile provided direct indications about the nature and the diversity of the mutagens in the particles.

The Rijnmond study (chapter 6) was characterized by a clear common mutagenicity profile of the samples tested. The effects in both strains increased

upon the use of S9 fraction, the increase being much stronger for strain TA100 than for strain TA98. The "with S9 effects" in TA100 were nearly as strong as the "without S9 effects" in TA98, while the "without S9 effects" were much weaker. The stability of the profile was revealed by strong correlations among the effects obtained with the four variants of the test. Several factors may contribute to this stability. We may assume the aerodynamic-diameter range of the particles causing the four effects not to differ very much. They will belong to the accumulation mode and their aerodynamic diameter will not be larger than a few μm (chapter 5). Consequently, they are expected to have an identical dispersion and deposition behaviour, which means that the correlations among the effects and, thereby, the stability of the profile can only increase when dispersion and deposition influence the concentration of the particles in the air. The stable profile can also be the result of a stable source pattern and a similar influence of chemical conversion after emission. Furthermore, the stability can have an artifactual background, as the four effects are determined in one test procedure and will thus be influenced similarly by inter-test variation, which cannot be corrected for by the use of positive controls. The effects of model mutagens tested simultaneously with the extracts indicate that a substantial inter-test variation can be expected (see also chapter 8, De Raat et al., 1987C and De Raat et al., 1988). It is not possible to assess the importance of these diverse possible causes of stability on the basis of the available data. The influence of dispersion and deposition, i.e. of changes in the concentration of the sampled material per volume of air, can be simply eliminated by expressing the effects as revertants per amount of sampled material or extract. However, the effects were only expressed as revertants per volume of air in this study, which makes it impossible to get an impression of the other factors.

The correlations between the effects were not perfect; a substantial variation in the profile remained. This can be the result of variant-specific inter-test variation, intra-test variation (chapter 2), and variation of source pattern and atmospheric chemical conversion. Closer examination of the results reveals that the variation of the profile is not only due to experimental noise. They point to a stronger metabolic activation for particles emitted by local emissions than for those sampled upwind. The latter had been airborne longer, which indicates that chemical conversion during their residence in the air leads to changes in their composition which affect their mutagenicity. A comparable shift from indirect to direct mutagenicity has been reported by Selzer-Madsen et al. (1982) and Pitts et al. (1982A).

A somewhat less stabile mutagenicity profile was observed during the Photochemistry Study (chapter 8[7 and 8]). This difference might be the result of the shorter sampling periods (24 versus 3 to 12 hours), because they allow short-term fluctuations of the mutagenic quality to have a stronger effect. Moreover, a much stronger dependence of the mutagenicity profile on residence time was indicated by the results of this study, leaving less room for the common variation. In general, a clear-cut inactivation of the background mutagenicity was found upon the use of S9 fraction, whereas emissions of local sources (traffic) lead to an increase of the mutagenicity which was not inactivated, but, in stead, clearly activated by S9 fraction. This strong effect of residence time will most probably be associated with the meteorological conditions during this study. Apparently, the chemical reactions causing the necessary changes of the composition of the particles are stimulated by photochemical air pollution, which is not unexpected as this form of air pollution is accompanied by enhanced concentrations of many reactive compounds.

Testing with strain TA98NR together with its parent strain pointed to a strong nitroreductase dependence of the direct mutagenicity. In fact, this dependence was as strong as that of the nitro PAH which were tested as model compounds with the same strains. This makes it improbable that compounds not mutagenic via the reduction of a nitro group have significantly contributed to the mutagenicity. In contrast to its parental strain, no inactivation by S9 fraction was observed in strain TA98NR; in fact a clear activation was often found. As this activation will also occur if strain TA98 is applied[9], the real inactivation of the direct mutagenicity in this strain by S9 fraction will be even stronger than appears from the comparison of the effects in this strain with and without S9 fraction. Even the correction for this compensation of inactivation by

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[7] Strain TA100 was not used for testing in this study. So if we compare the two studies for this aspect, we have to restrict ourselves to the effects scored with TA98 in the absence and the presence of S9 fraction. Than we get correlation coefficients of 0.89 and 0.77 for the Rijnmond study and the study in chapter 5 respectively. However, much stronger correlation (0.96) was observed for the effects scored with strains TA98 and TA98NR in the presence of S9 fraction, which reflects the similarity of the sensitivity patterns of these strains for non-nitro compounds.

[8] Again, the influence of common distribution and deposition cannot be estimated because the effects were only expressed as revertants per m^3.

[9] There is no reason to assume the nitroreductase deficient strain to be less sensitive than its parent strain for indirect mutagenicity. This assumption is confirmed by comparative testing of indirect mutagens with both strains in the Photochemistry Study.

activation may still leave inactivation undetected, as the remaining direct mutagenicity observed with strain TA98NR may be caused by the same inactivatable compounds[10], and may thus be inactivated by S9 fraction and subsequently compensated for by indirect mutagenicity.

So, the conclusion is justified, that under the conditions prevailing in this study, a substantial part of the mutagenicity (possibly all) is inactivated by S9 fraction and that this loss is compensated for by indirect mutagenicity. Indirect mutagenicity may thus be a much more important phenomenon than appears from comparing effects scored with the parental strain in the presence and absence of S9 fraction. These effects suggest that the presence of S9 fraction causes only inactivation and that indirect mutagenicity plays no role; however, the use of both strains together shows that the mutagenicity found in the presence of S9 fraction is not just direct mutagenicity remaining after inactivation; it is largely caused by the activation of indirect mutagens!

The shift from indirect to direct mutagenicity upon the residence of the particles in the air may be the result of the formation of direct mutagens, the removal of indirect mutagens and the formation of direct mutagens which are sensitive to inactivation by S9 fraction. The results of the Photochemistry Study unambiguously indicate the importance of the third process; however, substantial contribution of the other two processes cannot be excluded. Although the effects obtained with strain TA98 in the Rijnmond Study do not directly point to inactivation (nearly all effects increased upon the use of S9 fraction), they do not allow for the conclusion that they were indeed not influenced by this phenomenon. The observed activation can be the resultant of inactivation and activation, the latter dominating the former. The nitroreductase-deficient strain was not used in this study, which made it impossible to investigate the influence of S9 fraction when a substantial part of the direct mutagenicity was eliminated by nitroreductase deficiency while leaving the sensitivity for indirect mutagenicity intact. Thus, it is also impossible to investigate whether the formation of inactivatable compounds contributed to the shift from indirect to direct mutagenicity.

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[10] The reduction of the direct nitro-dependent mutagenicity will never be complete in strain TA98NR because some nitroreductase activity remains in this strain. This is shown by the effects of the model compounds in this strain. Furthermore, it has been demonstrated that the mutagenicity of some nitro compounds, for instance the extremely mutagenic dinitropyrenes, is only marginally affected by the deficiency in this strain, this notwithstanding the fact that expression of their mutagenicity requires nitro reduction.

Another study carried out by the author will be touched upon here for further elucidation of the qualitative variation of the mutagenicity, although it is not described in detail in the experimental part of this thesis. The reader is referred to De Raat (1988) for a detailed report in Dutch[11]. In this study, sampling was not restricted to selected meteorological conditions, as it was in

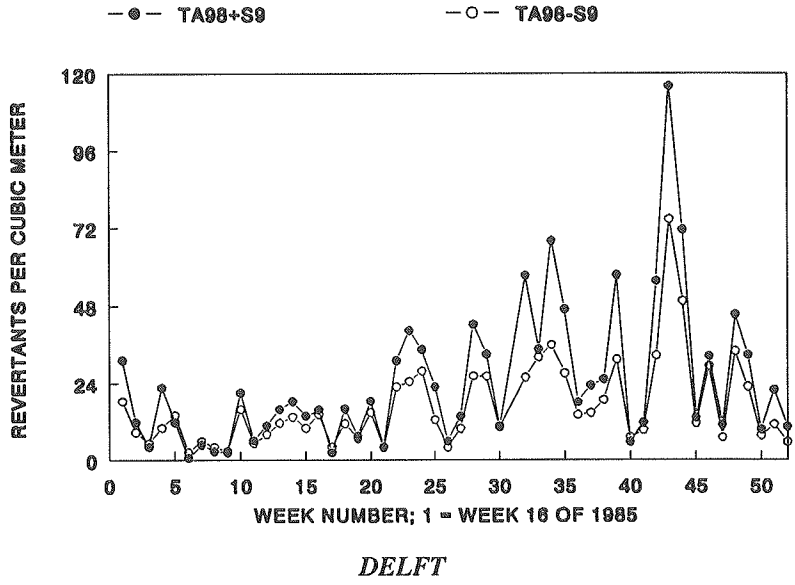
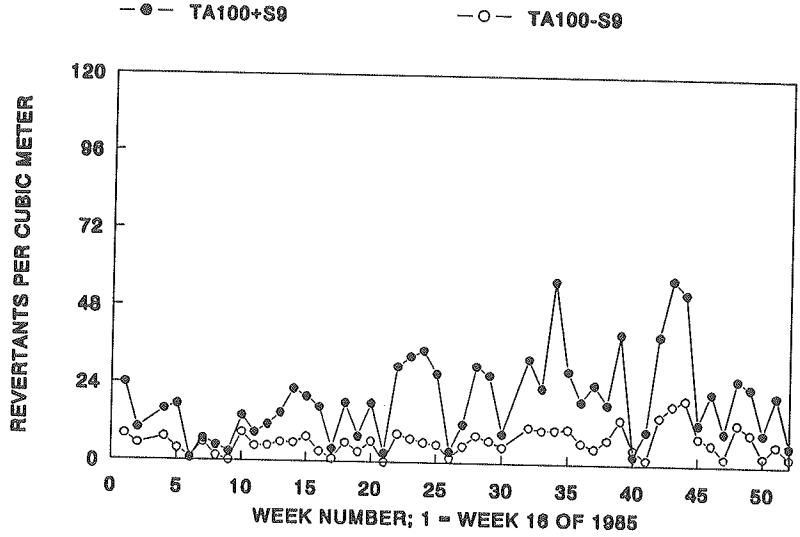


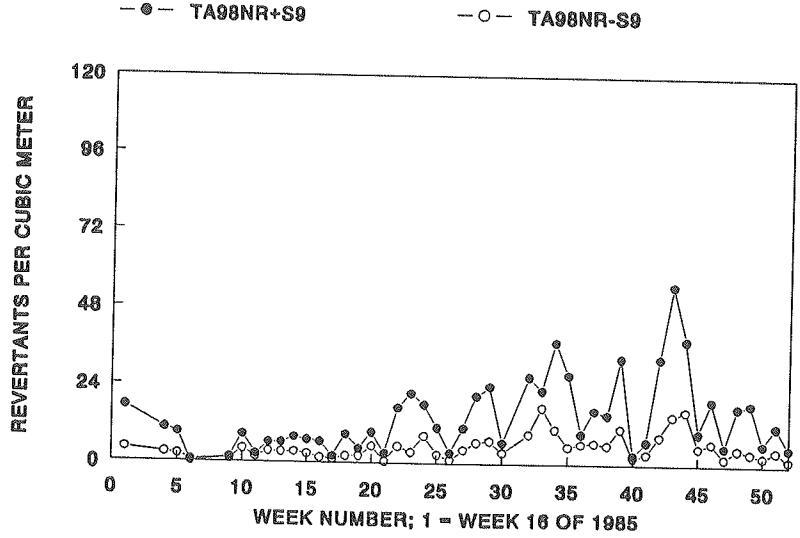
Figure 14.1 *Mutagenicity of ambient airborne particles; development during one year on four locations in the Netherlands (One-Year Study, see text).*

[11] Some results have been presented in chapters 11 and 13, while samples collected during this study were subjected to bioassay-directed fractionation in the study described in chapter 12. The particles were collected with Sartorius HV100 high-volume samplers on glass-fibre filters; the filters were subjected to Soxhlet extraction with methanol; the extracted material was dissolved in methanol and tested in the *Salmonella typhimurium* strains TA98, TA98NR and TA100. Sampling started every wednesday morning of one year between 10.00 and 11.00 a.m. and lasted 24 hours. Delft: on the TNO Zuidpolder terrain at Delft on ground level; Schiedam: near the yacht harbour of Schiedam at 10 m; Petten: directly behind the dunes on ground level on the terrain of the Dutch Energy Research Centre; Blijdorp: in the zo-ological garden of Rotterdam on ground level.



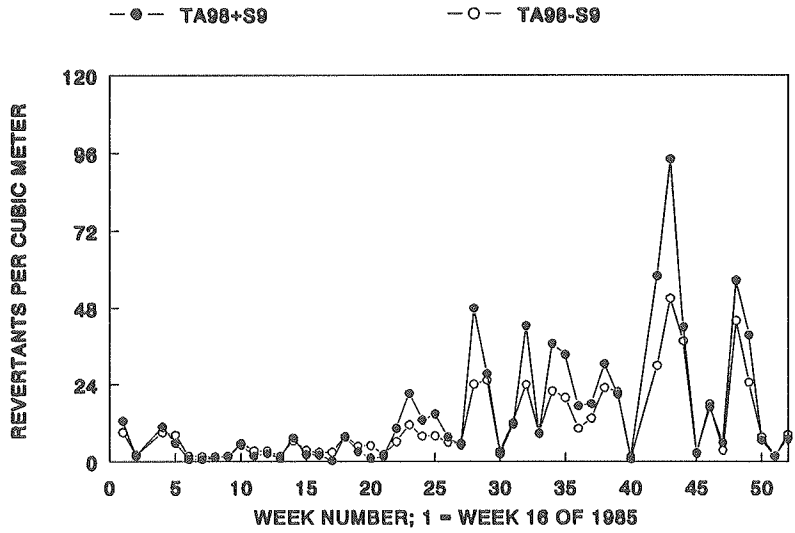
DELFT

Figure 14.1 *Continued*



DELFT

Figure 14.1 *Continued*



PETTEN

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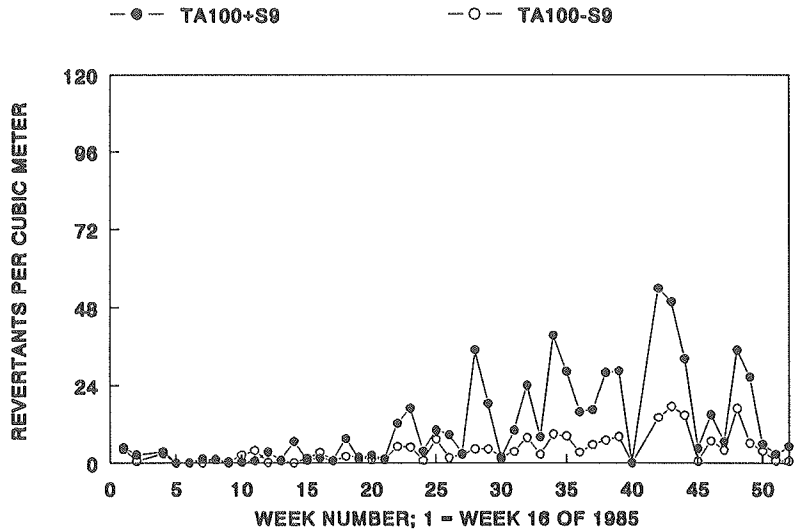
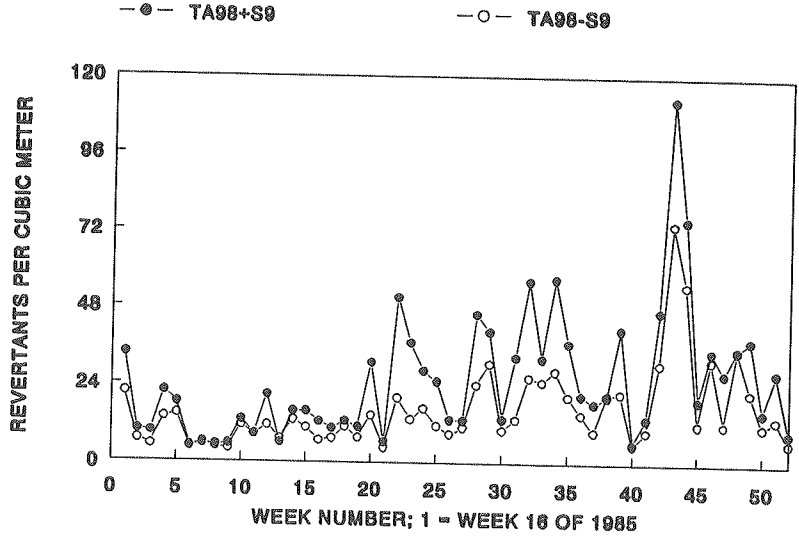


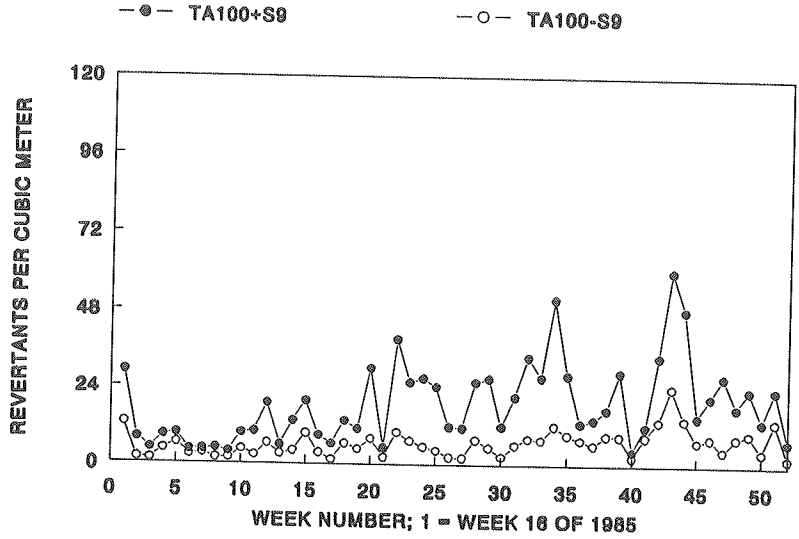
PETTEN

Figure 14.1 *Continued*



SCHIEDAM

Figure 14.1 *Continued*



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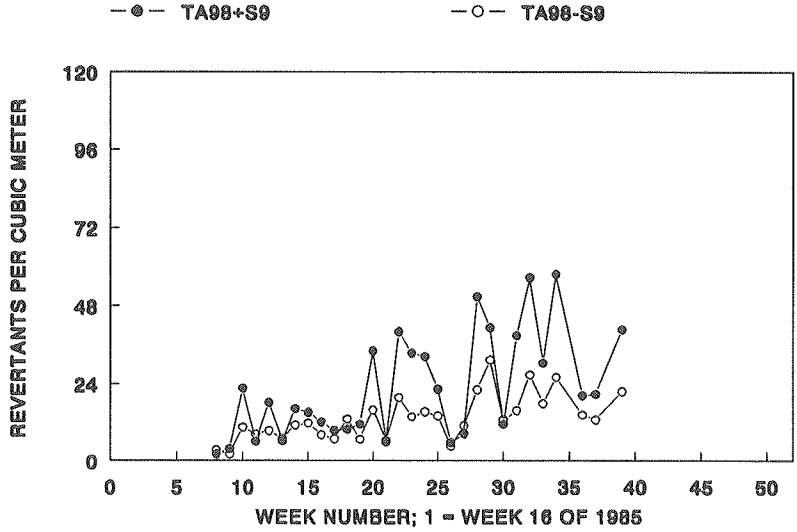

BLIJDORP

Figure 14.1 *Continued*

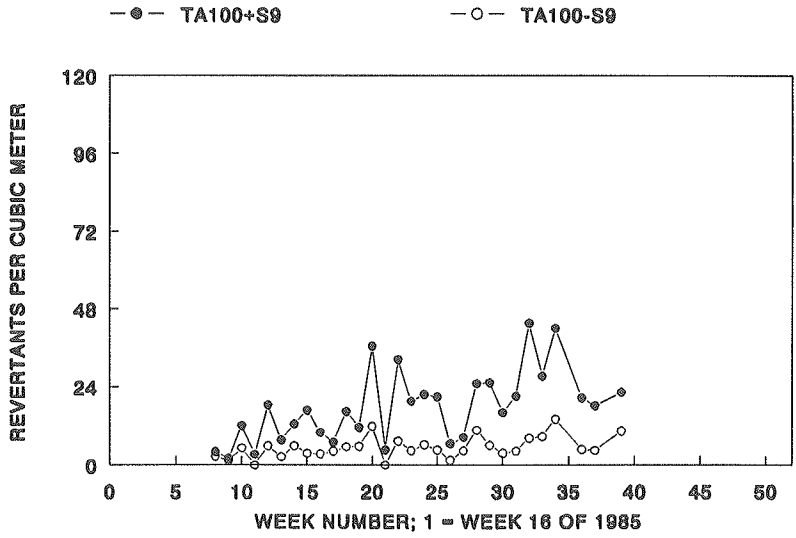

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Figure 14.1 *Continued*

the Rijnmond Study and the Photochemistry Study. The particles were collected every wednesday over a period of one year (the study is referred to here as the One-Year Study) at four locations, three of them in an urban area (two in the Rijnmond area and one near Delft, a town lying close to this area) and one in a rural area near the coast (Petten, in the northern part of the province of North Holland). Tester strains TA98 and TA100 were applied to all samples, while the 50 samples collected near Delft (at the ZP-TNO location of the Photochemistry Study) were also tested with strain TA98NR. Some results are presented in Figure 14.1.

Again a rather stabile mutagenicity profile was observed[12], which did not differ much from that of the Rijnmond Study. Comparison of the effects at the coastal location with those at the other locations did point to a shift from indirect to direct mutagenicity upon residence of the particles in the air. In addition, the effects of S9 fraction were found to differ for winter and summer samples. While the former were activated, no activation or slight inactivation was observed in some of the latter (see for instance Dehnen et al., 1981, for similar differences between winter and summer). This effect of season can be attributed to differences in source pattern and chemical conversion. In winter residential heating will be an important source at this latitude, while the contribution of this source will be negligible during summer; possibly the particles emitted by this source are more indirectly mutagenic. The obvious differences in meteorological conditions will undoubtedly influence atmospheric chemistry and, thereby, lead to differences in mutagenic quality. More and other reactive compounds may be present during summer due to higher temperatures and longer as well as more intensive irradiation with sunlight, thus stimulating the chemical conversion which causes the shift from indirect to direct mutagenicity during the residence of the particles in the air. The meteorological conditions in winter often lead to a lower mixing height and, thereby, a higher concentration of air pollutants. As a result, higher mutagenic effects per m^3 may be expected, which were indeed observed in both the Rijnmond Study and the One-Year Study during winter. The higher concentration of pollutants may also be accompanied by a different atmospheric chemistry and, thereby, a different mutagenic quality.

In contrast to the effects of the Rijnmond Study and the Photochemistry Study, those of the One-Year Study were calculated on both, a per-μg-extract basis as well as on the usual per-m^3-air basis. This permitted answering the

[12] Correlation coefficients ranged from 0.86 to 0.94 for all samples (all sampling times and sampling locations).

question as to the influence of common dispersion and deposition on the stability of the mutagenicity profile. Surprisingly, only a slight decrease of stability was observed when the influence of common dispersion and common deposition was eliminated[13], which means that the high correlations among the mutagenic effects are largely determined by stability of source pattern and similarity of chemical conversion.

A less strong nitroreductase dependence was found in the One-Year Study than in the Photochemistry Study[14]. This difference will most probably be associated with differences in meteorological conditions. Sofar, the Photochemistry Study was the only one which specifically dealt with nitroreductase dependence of mutagenicity during episodes of photochemical air-pollution, while during the One-Year Study this form of air pollution was hardly manifest. The difference could be the result of the formation of more nitroreductase-dependent mutagenicity during photochemical air pollution, i.e. a stronger dilution of the non-nitroreductase-dependent mutagenicity by nitroreductase-dependent mutagenicity. However, the effects obtained with model compounds point to a virtually complete nitroreductase dependence of the direct mutagenicity for both studies (70% reduction upon the use of the NR strain is still very much). Moreover, the results presented in chapter 10 made clear that the mutagenicity of all isolated groups of direct mutagens in the mutagrams depended on nitroreduction, confirming the insignificant contribution of other direct mutagens[15]. So, a dilution of non nitroreductase-dependent mutagenicity during the Photochemistry Study is improbable, as this type of mutagenicity does not contribute to a significant extent from the start. This leaves the possibility that, overall, residence in the air during photochemical air pollution leads to a larger contribution of compounds with a weaker residual mutagenicity in TA98NR to the direct mutagenicity, due to the formation of such compounds or the chemical elimination of nitro compounds with a higher residual effect in TA98NR.

It may be speculated, that the formation of direct mutagens with a weaker residential effect in TA98NR as well as the inactivatable direct mutagens is the result of the reaction of gaseous PAH with OH radicals followed by their

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[13] Investigated with principal-component analysis; see section 13.3 for results.

[14] An average reduction of 70% instead of 80%.

[15] The samples investigated in the study of chapter 10 were all collected during the One-Year Study.

nitration (Pitts et al., 1985; Arey et al., 1986; Nielsen and Ramdahl, 1986; Pitts, 1987). Being dependent on OH radicals, the formation of these compounds will in particular be manifest during photochemical air pollution and possibly the products formed differ in their nitroreductase and S9 dependence from nitrocompounds directly emitted by combustion processes. Two products have been discovered sofar: 2-nitropyrene and 2-nitrofluoranthene (Pitts et al., 1985; Nielsen and Ramdahl, 1986). These compounds are not present in combustion emissions and their presence in the air is a sure sign that atmospheric gas-phase nitration via the reaction with OH radicals takes place.

This hypothesis can easily be checked by simultaneously determining the concentrations of these nitro PAH and the effects in TA98 and TA98NR for samples collected during periods with different levels of photochemical air pollution. First, it should be proven that these compounds do indeed differ as regards their residual effect and their inactivatability from the direct mutagens in the particles collected during periods without photochemical air pollution.

With the exception of some summer samples, most of the samples collected during the One-Year Study showed a clear activation upon the use of S9 fraction. No differences in metabolic activation between TA98 and TA98NR[16] were found for these samples, which makes it highly unlikely that inactivation has been obscured by activation. The slight inactivation in some of the summer samples was accompanied by activation of the effect in strain TA98NR, which thus partly compensated the inactivation. The mutagenicity profile of these summer samples was thus similar to that of the samples collected during the Photochemistry Study, which suggests that they were also collected during photochemical air pollution.

Taken together, the results of the Photochemistry Study and the One-Year Study point to a clear dichotomy. Inactivation of direct mutagenicity by S9 fraction seems to be restricted to episodes of photochemical air pollution. No inactivation occurs when the meteorological conditions do not favour photochemical air pollution. We may safely extrapolate from these findings to the Rijnmond Study, which leads to the conclusion that also the results of this study were not influenced by inactivation of direct mutagenicity. This does imply that in case of the Rijnmond Study and the major part of the One-Year Study the formation of inactivatable compounds did not contribute to the shift from indirect to direct mutagenicity, as it did in case of the Photochemistry Study. So, in the absence of photochemical air pollution S9 fraction adds indirect

[16] In terms of absolute numbers of revertants.

mutagenicity to **unaltered** direct mutagenicity, while the proportion of these two types of mutagenicity is changed in favour of direct mutagenicity upon residence in the air. In contrast, photochemical air pollution is accompanied by the formation of direct mutagenicity which is inactivated by S9 fraction. The inactivation can be so strong, that it exceeds the activation, leading to an overall decrease of the response.

Variation of the PAH profile

The Rijnmond Study and the Photochemistry Study were characterized by rather stabile PAH profiles[17 and 18]. High relative variances of the first principal components were found (they explained 80% or more of the variance) and the concentrations of most PAH, in particular those with a not too high volatility, correlated very well ($r>0.9$) with the first principal component. There are two main explanations for this stability. First, it will be due to a similarity of the PAH profiles of diverse combustion emissions (Tomingas et al., 1978; Grimmer, 1983B; Daisey et al., 1986; Masclet et al., 1986; Miguel and Pereira, 1989; Alsberg et al., 1989; Beak et al., 1992) and the stability of the relative contributions of combustion emissions with different profiles (stability of source pattern). Traffic emissions will strongly dominate in the Netherlands, resulting in a stabilizing effect on the profile of the locally emitted particles. Background particles in the Netherlands are emitted over large distances and will represent an average of various sources (traffic, industrial combustion and residential heating) in relatively large source areas. It may be expected that the differences between the profiles of these source areas will in general be limited. Such differences may, for instance be associated with the ubiquitous use of coal and lignite combined with poor stack-gas cleaning and obsolete combustion technology in eastern European countries.

The second reason for the stability of the PAH profile is provided by the fact that all PAH containing particles fall within a narrow aerodynamic-diameter range (Van Vaeck and Van Cauwenberghe, 1978; Katz and Chan, 1980; Van Cauwenberghe and Van Vaeck, 1983; Van Cauwenberghe, 1985) and will

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[17] The PAH profile is defined as the proportion among the concentrations of the PAH in a sample of particles, or, in other words their relative concentrations in this sample.

[18] The same was found for the One-Year Study; the results of PC analyses with the PAH concentrations are presented in section 13.3; see De Raat (1988) for a detailed report in Dutch.

thus be influenced in the same way by distribution and deposition. The stronger this common variation of the particles, the greater the stability of the PAH profile. It can be eliminated by calculating the concentrations per amount of extract or sample in stead of volume of air. The profile of the concentrations thus calculated is solely determined by source pattern or by chemical conversion, sorption, condensation and evaporation after emission. Both types of concentrations were calculated during the One-Year Study. Principal-component analysis showed that the stability was hardly affected by calculation of the concentrations per amount of extract instead of volume of air (see section 14.3). So, it can be concluded, that the contribution of distribution and deposition to the common variation of the PAH concentrations is of minor importance.

It should be emphasized that these considerations refer to a general trend. They do not imply that significant changes of the PAH profile do not occur or that such changes are not due to variation of the source pattern or processes as chemical conversion, sorption, evaporation or condensation. It is merely concluded that the common variation strongly predominates the independent variation. As has amply been demonstrated (see chapters 7 and 9, and the report of the One-Year Study (De Raat et al, 1988)), a more detailed analysis of results does indeed reveal systematic changes of the PAH profile that can be linked with certain processes or sources.

First, there is the clear dichotomy associated with the volatility of the PAH. The variation of the more volatile particulate PAH, i.e. the PAH which are also present in the gas phase for a substantial part, differed clearly from that of less volatile PAH. Several explanations can be put forward for this phenomenon. The ratio of gas-phase concentrations and particulate-phase concentrations of these PAH will vary as a result of a varying contribution of sources emitting different ratios (see for instance Westerholm et al., 1988). Moreover, condensation and evaporation may add to the fluctuation (Van Vaeck et al, 1984). In addition, it can be speculated, that the more volatile particulate PAH will be present in the outer layers of the particles, because they will condensate later than their less-volatile counterparts and will thus be more available for chemical reactions with reactive gaseous compounds or reactive compounds dissolved in the water layer that surrounds the particle. Finally artifactual evaporation and adsorption have to mentioned (see chapter 11 and section 14.4); they will vary from sample to sample due to the variation of sampling conditions, concentrations of the PAH in the air and concentrations of the particles in the air. Moreover, gaseous PAH adsorbed on the filter material and the particles will be sensitive to chemical reaction with gaseous components

in the air stream passing the filter, depending on sampling conditions and the composition of the air (Pitts et al., 1978 and 1980; De Raat, 1982, 1983 and 1987A). As the exposure of these PAH differs from those originally present in the particles, these chemical reactions are expected to lead to a specific volatility-related variation of the profile.

It is difficult to estimate the importance of each of these explanations for the stability of the profile. An impression about the importance of non-artifactual causes can be gained by comparing locations at which samples were collected simultaneously. In the Rijnmond Study, multivariate analyses revealed a clear location dependence of the volatility dichotomy, as is reflected by much weaker correlations of the concentrations of the more volatile PAH with the first principal component at the locations Geulhaven, Lekhaven and Kralingen, compared to the background location and the location Schiedam. In the case of the first two locations the presence of local sources may explain this difference (see discussion of chapter 7). In the case of Kralingen, selection of sampling trips with similar meteorological conditions leads to a less clear volatility dichotomy, which may point to the influence of a fluctuation of the background source pattern (see discussion of chapter 7). In the One-Year Study factor analysis[19] revealed clear-cut volatility-related factors; the locations clearly differed as to the PAH the concentrations of which were influenced by these factors (De Raat et al., 1988; see Fig. 14.2 for concentrations of benzo(a)pyrene and pyrene on the Delft location). It is hard to see how in these cases location dependence of the dichotomy could be caused by artifacts, which leads to the provisional conclusion that non-artifactual causes as mentioned above, will also contribute to the instability of the profile.

Besides volatility, changes of the PAH profile also appear to be related with reactivity. The sensitivity of PAH upon exposure to the gas-phase components of ambient air has been demonstrated in a great number of studies (Falk et al., 1956; Jäger and Hanus, 1980; Butler and Crossley, 1981; Nielsen et al., 1983; Pitts, 1983; Nikolaou et al., 1984; Miguel, 1984; Valerio et al., 1984), although it has been difficult to prove that particulate PAH which are actually part of the complex matrix ambient particles are indeed converted during their residence in the air. In most studies pure PAH were brought onto an artificial matrix and subsequently exposed to reactive gaseous components. It is generally accepted, that under such conditions matrix-dependent protection of the PAH

[19] Orthogonal rotation of axis according to the varimax criterion.

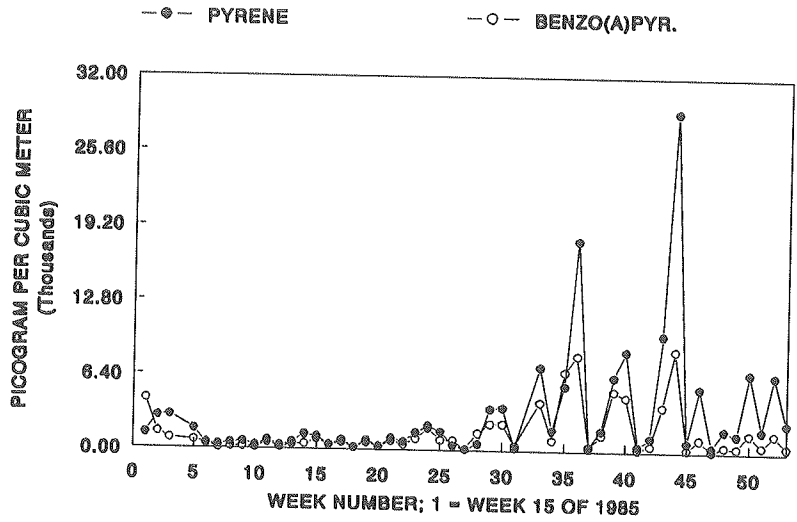

Figure 14.2 *Concentrations of particulate benzo(a)pyrene and pyrene in the air on the Delft location during the One-Year Study.*

is much less than when they are part of the real particles. Moreover, the artificial matrices made of silica or glass fibres are suspected to exert a catalytic activity. Definite proof for chemical conversion of the particulate PAH has to come from studies which are focussed on possible correlations between the spatial and temporal distribution of PAH concentrations and the sensitivity of these compounds to chemical conversion.

In our studies, multivariate analysis consistently shows that the concentrations of reactive PAH correlate less strongly with the first principal component than those of the less reactive ones[20]. This is reflected in separate factors if factor analysis is applied (chapter 9 and De Raat et al., 1988; see also the results of the principal-component analysis with normalized concentrations in chapter 7). This reactivity dependence of the profile strongly suggests that chemical conversion of PAH occurs. Again, the question as to the contribution of sampling artifacts can be posed. A number of studies indicate, that sampling does indeed lead to artifactual conversion of PAH already present on the filter (chapter 11).

[20] Classification according Nielsen (1984).

Also in this case comparison of locations may indicate the importance of non-artifactual causes. The two studies described in this thesis and the One-Year Study showed an increasing share of the more reactive PAH in the profile upon an increasing contribution of local sources, indicating that ageing of particles is accompanied by chemical conversion and that the reactivity-dependent variation of the profile is by no means only the result of sampling artifacts. Comparable results were obtained by Nielsen (Nielsen, 1988). It seems unlikely, that such conversion will serve as a complete sink for the more reactive PAH, as it has been demonstrated that PAH, including the reactive ones, can be transported over very long distances (Lunde and Bjørseth, 1977; Bjørseth et al, 1979). It may be speculated that part of the amounts present in the particles are effectively protected against chemical breakdown.

Finally, the influence of sources can be recognized in the variation of the PAH profile, in particular when the results of the Photochemistry Study are considered. Benzo(e)pyrene appeared to be a nearly exclusive marker PAH for the distant sources, while the contribution by local sources was characterized by relatively high concentrations of benzo(g,h,i)perylene. The latter has been recognized as a marker PAH for traffic emissions (Katz and Chan, 1980; Daisey et al., 1986), which is in line with traffic being the most important source in the Delft area; factor analysis showed its concentrations to be influenced by a separate factor, which was not associated with reactivity.

No separate factor for benzo(g,h,i)perylene was found during the One-Year Study, while comparison
of locations did not reveal a relatively high contribution of local sources to the concentrations of this PAH. This difference between the two studies is hard to explain. Possibly it has to do with the fact that in the Photochemistry Study only a motorway or part of a small town was lying between the two locations to be compared. Thus, the samples to be compared differed only with respect to a pure and fresh contribution of traffic, whereas the great distance between the locations as well as the varying wind direction in the One-Year Study will have lead to a much less monotypic difference between the samples to be compared. Furthermore, the short sampling periods of the Photochemistry Study allowed periods with different traffic densities to be compared, whereas sampling in the One-Year Study lasted 24 hour, thus integrating the traffic of one day.

Relatively small increases of the concentrations of benzo(e)pyrene due to local sources were also found in the Rijnmond Study and the One-Year Study. Apparently, the particles emitted in the investigated regions are characterized

by relatively low benzo(e)pyrene concentrations compared to particles emitted by distant sources.

PAH as indicator compounds for air-pollution monitoring

PAH, in particular benzo(a)pyrene, fulfil a role as indicator compounds in many air-pollution monitoring programmes. The objective of monitoring may be restricted to their own group, i.e. the unsubstituted polycyclic aromatic hydrocarbons. It may also comprise the other polycyclic compounds emitted by combustion processes. In both cases it is the concern about the possible carcinogenic properties of the compounds to be indicated by the indicator PAH that motivates monitoring.

A number of unsubstituted PAH are proven animal carcinogens (Santodonato et al., 1981; Montizaan et al., 1989) and are ranked as probable human carcinogens by the IARC (IARC, 1983). Although chemical analysis of these compounds is relatively easy, not all relevant PAH can be determined in routine monitoring programmes. In particular so, as their number is greatly increased by substitution with alkyl groups. Alkylated PAH **as a group** show very similar toxicological properties to the unsubstituted PAH, although alkylation may lead to drastic changes of potency for individual PAH.

In the earlier days only one PAH, namely benzo(a)pyrene, was selected as indicator compound (Waller, 1952), mainly because the carcinogenic properties of the particles were largely attributed to the presence of this compound. Later it was realized that other PAH may also contribute, which led in some cases to an increase in the number of indicator PAH. This broader approach was facilitated by the improvement of analytical chemical techniques. Well known are the six of Borneff, which were chosen because they could easily be detected by thin-layer chromatography (Kunte and Borneff, 1976). Nowadays gas chromatography and high-performance liquid chromatography allow the concentrations of larger numbers to be determined on a routine basis (Sortland Olufsen and Bjørseth, 1983; Wise, 1983 and 1985; Bartle, 1985). Again, the selection is mainly determined by analytical-chemical convenience, while their representativeness for the compounds to be indicated, i.e. their real indicative value, played no role at all.

If monitoring is only aimed at PAH themselves, the representativeness of the indicator compound (its value as indicator compound) is solely determined by the correlations among the PAH concentrations, i.e. their common variation. Ideally, the composition of the particles as regards the PAH, including the

indicator, should be constant. However, reality differs from this ideal situation. As we have seen before, the composition will vary for several reasons. First of all, it will vary at the source level. For instance, it depends on fuel type and a great number of combustion conditions (Grimmer, 1983B; Daisey et al., 1986). Furthermore, physical and chemical processes occurring in the air will cause variation of the composition. Which sources actually contribute to the concentrations on a particular site and time, depends on distribution, and, thereby, on meteorological conditions. Moreover, the composition of the particles is influenced by evaporation, condensation and chemical conversion during their residence in the air.

So, a high indicative value of PAH towards their own group cannot be taken for granted. It depends on the common variation of the complete group of PAH and the correlation of the concentrations of the indicator PAH with that common variation. An ultimate validation, therefore, demands a multivariate analysis of the concentrations of a large number of PAH in a large number of representative samples. This provides answers to the questions as to whether one PAH, preferably benzo(a)pyrene, can be taken as indicator compound and, if such would lead to a too wide margin of uncertainty, which other PAH should be added.

PAH are not the only possible carcinogenic polycyclic components of the particles. During combustion, many other polycyclics are also formed, among them different types of PAH derivatives, heterocyclics and derivatives of these compounds. Also these compounds may have carcinogenic properties, and nowadays, it is generally accepted that they contribute significantly to the carcinogenicity of the particles. Consequently, monitoring should also cover the presence of these compounds in the air. PAH are the obvious indicator compounds for monitoring these other polycyclics for the following reasons.

- They are emitted by the same sources and their presence will, therefore, be linked with the other polycyclics.
- It would permit the interpretation of the already vast existing data base of atmospheric PAH concentrations in terms of air pollution with the polycyclics.
- Analytical-chemical techniques of proven reliability and convenience are available.
- It is hard to select other representative polycyclics, because of their diversity, their often unknown chemical nature and the incomplete knowledge of their effects.

It may be expected that the composition of the complete group of polycyclics will depend even stronger on source pattern and physical and chemical processes after emission than the composition of the group of unsubstituted PAH. Implicitly, their common variation as well as the correlations of the common variation with the concentrations of the indicator PAH will be less. So, validation of the PAH as indicator compounds for monitoring the other polycyclics is certainly required. To this end, a large number of representative concentrations of a large and representative number of relevant compounds, including PAH should be subjected to multivariate analyses. Two problems arise here. First, not all relevant compounds have been identified yet[21]. Second, it is hard to see how such an enormous analytical-chemical task could be accomplished. Sofar, only PAH and a few related compounds have been identified and can be analyzed with convenient techniques. It is questionable whether they form a sufficiently reliable basis for the validation.

However, the concentrations of this too limited set of compounds may be supplemented with an effect, i.e. mutagenicity, which may partly undo the imperfect character of a validation based on their concentrations alone. Because of the mechanistic relation of mutagenicity with carcinogenicity, the composition of the particles as regards the relevant polycyclic compounds[22] will in some way be reflected by their mutagenic quality (see chapter 3). Consequently, changes in the mutagenic quality point to changes in the composition. The changes themselves are not important, because they cannot be interpretated in chemical or toxicological terms. It is the stability of the mutagenic quality that counts, as this stability points to stability of composition, or, in other words, predominance of common variation. So, the impossible task of multivariate analysis with the concentrations of a large number of other polycyclics may not be necessary. Instead, the mutagenic effects of a large number of samples should be subjected to multivariate analysis.

A lack of common variation of the mutagenicity does not necessarily indicate a lack of common variation of the relevant compounds. As has been set forth in chapter 3, the response of a mutagenicity test may be strongly influenced by non-mutagenic compounds. These compounds may bear no relevance in the context of our subject, i.e. they do not necessarily have a polycyclic

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[21] For instance, the majority of the direct as well as the indirect mutagenicity is caused by unidentified nitrocompounds (chapter 10).

[22] The compounds which contribute to the carcinogenic properties of the particles.

nature, neither do they have to occur in combustion emissions. However, their effects will in many cases be test-specific, which means that a variation of their relative concentrations results in a variation of the mutagenic quality not associated with a variation of the relative concentrations of relevant compounds. This problem could partly be addressed by choosing mutagenicity tests which do not differ much with respect to their sensitivity to non-mutagenic compounds. The Salmonella/microsome test may serve as an example here. It allows the comparison of diverse types of genetic endpoints in one organism. However, it should be realized, that some test-specific influence of non-mutagenic compounds will remain.

These considerations might give rise to the question as to why mutagenicity itself is not proposed here as an indicator for the complete group of possible carcinogenic polycyclic compounds in the particles. This would indeed be possible, if multivariate analyses point to a strong common variation of a series of mutagenic effects. Then one easy mutagenic effect may be used as an integrative value indicating the intensity of air pollution with the complete group. However, mutagenicity tests still lack the reproducibility, sensitivity and convenience of the analytical-chemical methods used to determine PAH concentrations, which does not make them attractive indicators. If PAH concentrations correlate with the common variation of the mutagenicity, there is no reason to use mutagenicity in stead of the more convenient and reliable PAH concentrations. In case the correlations of the PAH concentrations are too low, it may be a better strategy to look for another polycyclic compound which does show the required correlation.

In summary, validation of PAH as indicator compounds requires a two-step procedure. First, the common variation of the compounds which are to be indicated has to be determined. To enable a meaningful monitoring of these compounds with one or a few PAH, this common variation should be strong enough. If validation is aimed at monitoring of the PAH themselves, the common variation can be investigated by means of multivariate analyses with the concentrations of a large number of PAH. In case of the other polycyclics, the mutagenic quality can be used as starting point. Then effects in different mutagenicity tests have to be subjected to multivariate analysis. A stable quality is a sure sign of a stable composition of the compounds which have to be indicated. However, lack of stability of the mutagenic quality does not necessarily point to a lack of stability of the composition. It only means that mutagenicity cannot be used to validate PAH as indicator compounds. As the second step of the two-step procedure, the value of the prospective indicator compounds

is established by investigating the correlation of the concentrations of these compounds with the common variation of the compounds which have to be indicated.

The studies presented in this thesis allow this two-step procedure to be followed. Large numbers of samples were analyzed for a series of polycyclic aromatic hydrocarbons and tested for mutagenicity with at least four variants of the Salmonella/microsome test. So, the common variation of the PAH and the mutagenic effects and the correlation of the concentrations of indicator PAH with these common variations could be investigated.

Obviously, the validity of the two-step procedure depends on the extent to which the samples can be regarded as representative for the pollution of the air with particulate PAH and mutagens in general. They should not be taken downwind of predominating sources or under selected meteorological conditions. This can be expected to result in an overestimation of the assessed indicative value of the PAH concentrations, because the composition of the particles is less affected by variations of source pattern and variations of atmospheric chemical and physical processes. It is clear that the Rijnmond Study and the Photochemistry Study do not fulfil this requirement. The samples taken during both studies can hardly be regarded as a random representative sample of the samples that can be taken in the Netherlands.

In contrast, no meteorological conditions were selected during the One-Year Study. The particles were collected each week, one year long, during a fixed period of 24 hours. The samples will represent a truly random and representative collection. Moreover, the samples were taken at four different locations, one real background location on the coast and three in an urban and industrial area and not down wind of specific sources or source areas. It may, therefore, be assumed that, in the case of the One-Year Study, an assessment of the indicative value of the PAH concentrations is not affected by a lack of representativeness of the samples, at least if air pollution in the Netherlands is considered. No overestimation of the indicative value will have occurred due to a similarity of the samples imposed by the set-up of the study. For this reason, the two-step procedure will be followed here in detail for this study.

The common variation was investigated with the principal-component analysis. Analyses were carried out for effects and concentrations expressed per volume of air and per amount of extract and for the samples of all sampling times and locations together. The two ways of expressing the effects and concentrations were used to investigate the influence of dispersion and deposition on the common variation. The results are presented in Table 14.1.

Table 14.1 **Principal component analysis with PAH-concentrations and mutagenicity of the One-Year Study**

A CONCENTRATIONS AND EFFECTS PER CUBIC METER AIR

	CORRELATION WITH FIRST PC		
PAH			
PHENANTHRENE	0.91		0.91
ANTHRACENE	0.91		0.90
FLUORANTHENE	0.66		0.64
PYRENE	0.84		0.82
TRIPHENYLENE	0.87		0.85
BENZ(A)ANTHRACENE	0.99		0.98
CHRYSENE	0.95		0.94
BENZO(E)PYRENE	0.88		0.89
PERYLENE	0.97		0.96
BENZO(B)FLUORANTHENE	0.97		0.97
BENZO(K)FLUORANTHENE	0.98		0.97
BENZO(A)PYRENE	0.97		0.97
BENZO(G,H,I)PERYLENE	0.92		0.91
INDENO(C,D)PYRENE	0.98		0.98
ANTHANTHRENE	0.91		0.91
MUTAGENICITY			
TA98 WITH S9		0.98	0.90
TA98 WITHOUT S9		0.96	0.84
TA100 WITH S9		0.95	0.83
TA100 WITHOUT S9		0.94	0.80
PERCENTAGE OF VARIANCE	84.3	91.9	80.5

TABLE 14.1 **Continued**

B CONCENTRATIONS AND EFFECTS PER MICROGRAM EXTRACT

	CORRELATION WITH FIRST PC		
PAH			
PHENANTHRENE	0.85		0.83
ANTHRACENE	0.82		0.79
FLUORANTHENE	0.60		0.57
PYRENE	0.72		0.68
TRIPHENYLENE	0.82		0.79
BENZ(A)ANTHRACENE	0.98		0.97
CHRYSENE	0.93		0.92
BENZO(E)PYRENE	0.96		0.96
PERYLENE	0.95		0.95
BENZO(B)FLUORANTHENE	0.96		0.96
BENZO(K)FLUORANTHENE	0.94		0.94
BENZO(A)PYRENE	0.90		0.90
BENZO(G,H,I)PERYLENE	0.89		0.90
INDENO(C,D)PYRENE	0.96		0.96
ANTHANTHRENE	0.85		0.85
MUTAGENICITY			
TA98 WITH S9		0.95	0.87
TA98 WITHOUT S9		0.94	0.79
TA100 WITH S9		0.95	0.82
TA100 WITHOUT S9		0.86	0.71
PERCENTAGE OF VARIANCE	77.6	85.6	73.7

The table shows the results of PC analyses for mutagenic effects and PAH concentrations expressed per cubic meter air (A) and per microgram extract (B). Listed are the correlations of the variables with the first principal component and the share of the first principal component (percentage) in the summarized variance of all components.

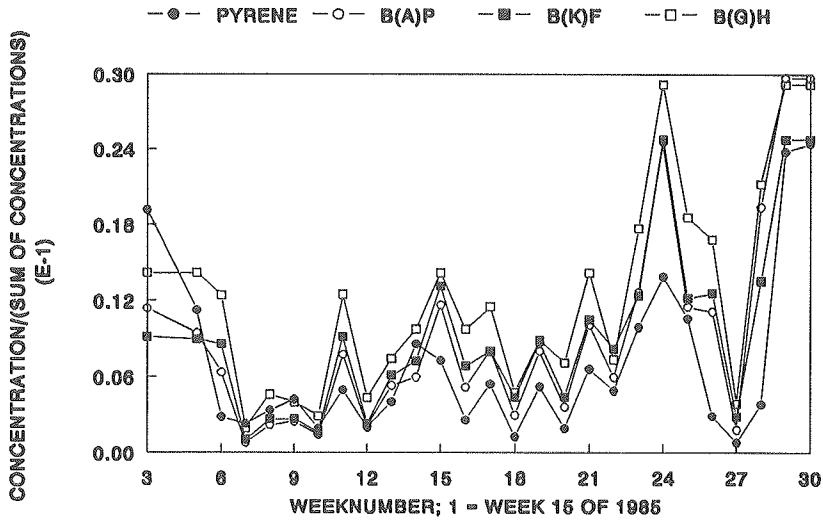

Figure 14.3 *Concentrations of particulate benzo(a)pyrene, benzo(k)fluoranthene and pyrene in the air at the Delft location during the One-Year Study; spring-summer samples; X-axis: weeknumber, 1 = week 15 of 1985.*

The results can be summarized as follows:

1. The variation of the four mutagenic effects is predominantly determined by a common factor. 86 to 92% of the variation of the data set is explained by the first principal component (PC) and all four effects show strong correlations with the first PC.
2. The variation of the concentrations of the fifteen PAH is predominantly determined a common factor. 78 to 84% of the variation of the data set is explained by the first PC and most concentrations show strong correlations with the first PC.
3. The analyses with effects and concentrations leads to a reduction of the relative variance of the first PC to 74 and 81%, which can be explained by a less strong correlation of the effects with the first PC.
4. Expression of effects and concentrations on a per-amount-extract basis leads to a slight reduction of the relative variance of the first PC and the correlations with the first PC.

The One-Year Study was characterized by much higher effects and concentrations in winter than in summer (see Figure 14.1). To investigate the influence of this difference on the outcome of the analyses, separate analyses were carried out with the summer samples. The results, which are not presented in detail here, show that this selection did not lead to a decrease of the common variation (see Figure 14.3 for the concentrations of three PAH during spring-summer at the Delft location).

The results reveal strong common variations for all three data sets. They can even be expected to be stronger in reality than indicated by the PC analyses. In the case of mutagenicity the reason for this will be that the intra- and inter-test variation cannot be corrected for by using positive controls. The influence of this artifactual variation can be substantial (see chapters 6 and 8, De Raat et al, 1987A and De Raat et al., 1988) and will certainly lead to a marked decrease of the correlation of the effects with the common variation of effects plus concentrations. Thus, the already high common variation indicated by the PC analyses represents an underestimation of the actual common variation.Sampling artifacts may also significantly weaken the common variation. The concentrations of the more volatile PAH will be affected by artifactual evaporation and adsorption (chapter 11 and section 14.4), which will result in an extra variation, as these artifacts will certainly depend on meteorological conditions and the composition of the air during sampling. The literature even indicates that the influence of these artifacts can even be so strong as to make the determination of the volatile particulate PAH largely unreliable. It may safely be assumed that without artifactual evaporation and adsorption, the common variation of the PAH would have been higher. Moreover, artifactual chemical conversion of PAH in the particles may occur and affect the concentrations of the more reactive PAH. Also this artifact will depend on meteorological conditions and composition of the air and will thus affect the common variation. However, as the correlations with the first PC of the relatively inert and reactive PAH (fluoranthenes versus benzo(a)pyrene and perylene (Nielsen, 1984; Butler and Crossley, 1981)) indicate, the influence of this artifact is not very important.

Obviously, the common variations cannot be expected to become complete if the influence of these artifacts could be corrected for. The stability of the profiles is also affected by causes which are fully detached of the methods used. They have already been treated in the first two sections of this chapter. The contribution of sources with different profiles and the profiles of the contributing sources will vary. Furthermore condensation, evaporation and chemical conversion will lead to an extra non-common variation; however, these

processes cannot be distinguished from their artifactual counterparts on the basis of these analyses.

The results of the Rijnmond Study and the Photochemistry Study will not be treated in detail in this context. They deviate on one important point from those of the One-Year Study: The correlations between the PAH concentrations and the common variation of the mutagenicity were less high. In the case of the Rijnmond Study they were only moderate for all four test variants (see chapter 6), while in the case of the Photochemistry Study correlations approaching those of the One-Year Study were only found for the with-S9 variants, the without-S9 variants yielded only moderate correlations (see chapter 8). It is hard to explain these differences with the One-Year Study. In view of the selection of meteorological conditions, higher correlations would have been expected. It can be speculated that the strong influence of residence in the air on the mutagenicity and the sharp difference between the PAH profiles of distant and local sources may have lowered the correlations in the Photochemistry study. No such explanations are available for the Rijnmond Study.

Taken together, the results of all three studies show that one or a few PAH can be used as reliable indicators for the complete group of PAH. The strong common variation of the mutagenicity observed in all three studies suggests a stable composition of the particles as regards the other possibly carcinogenic polycyclic compounds. The One-Year Study indicates that the same PAH can be used to indicate these compounds as well; the indicative value of PAH in this respect is less clearly indicated by the other studies.

These conclusions bring us to the question which PAH should be chosen as indicator compounds. In view of the predominant role of benzo(a)pyrene in air-pollution studies, this PAH would be an attractive choice. A break with the past would be prevented; the results of future studies could be compared with vast data sets collected in the past. Benzo(a)pyrene belongs to the group of relatively reactive PAH and its concentrations may thus be influenced by artifactual and atmospheric chemical conversion. However, what really counts is the extent to which this conversion affects the common variation. The results clearly indicate that this extent is only limited; correlations with first PC's approach those of the relatively inert PAH. So, there is no real reason to use other PAH in stead of benzo(a)pyrene as indicator compound.

Obviously, the ultimate evaluation of PAH as indicator compounds for air pollution with possible combustion-related polycyclics cannot only be based on the common variations revealed by multivariate analyses. Besides, the objectives of monitoring should be taken into account. These objectives may

demand such high predictive reliability that even the common variations observed during the One-Year Study do not suffice. However, for two reasons, such an approach would be meaningless. First, the common variation among the mutagenic effects indicates to what extent the group of polycyclics behaves as a tightly linked group. Monitoring cannot be more accurate than this intra-group variation allows, at least if monitoring is based on the concentrations of one compound. Second, the ultimate goal of monitoring is protection of the human population against too high exposure levels. However, it is fully unclear which levels are indeed too high. Air-pollution threshold values for PAH are based on very rough extrapolations from epidemiological data[23], which are inevitably characterized by a great margin of uncertainty. In the opinion of the author, uncertainty caused by a lack of common variation will soon be overshadowed by this toxicological uncertainty. As long as the real human risk of lifetime exposure cannot be indicated with more certainty, the common variations observed during the One-Year Study would enable a sufficient accurate monitoring.

However, it is possible to improve the indicative value by using more than one indicator compound. In the ideal situation, one PAH should be used besides other polycyclics representing classes known to be really toxicologically relevant. However, such classes have not been identified yet. Possible candidates are the mono-nitro PAH; however more research is necessary to establish their toxicological relevance to the full. Meanwhile, other PAH could be used. Two categories form obvious choices. PAH representing different sources, for instance benzo(g,h,i)perylene and benzo(e)pyrene and PAH differing in chemical reactivity, for instance perylene and benzo(a)pyrene versus one of the benzofluoranthenes.

At this point, it is important to emphasize that the validity of our conclusions is restricted to the Dutch situation, because the samples represent air pollution in The Netherlands. Extension to regions with other types of air pollution would in the first place require that differences in source pattern are taken into account by using source-linked PAH as indicator compounds.

Finally, it should be emphasized that the conclusions can only have a provisional character because, the three studies were not designed to answer the questions posed in this section. This holds in particular for the conclusions based on the mutagenic effects. The combination of four variants of the

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[23] Which do not directly apply to human exposure to ambient airborne particles; they are concerned with occupational or residential indoor exposure.

Salmonella/microsome test is but a meagre basis for conclusions on the stability of the composition of the particles as regards the relevant compounds. A follow-up of this research should include more variants of this test and also other tests. Furthermore, the correlation between the common variation of the mutagenicity and the PAH concentrations may have been affected by inter-test variation, which problem should be addressed in future studies by utmost standardization and the use of positive controls.

Sampling artifacts

Polycyclic aromatic hydrocarbons

The results presented in chapter 11 clearly demonstrate that the concentrations of the more volatile PAH are affected by artifactual evaporation and adsorption. Lower concentrations were found when filters with a higher air resistance were used (Teflon filters and Teflon-coated glass-fibre filters) instead of glass-fibre filters, which can be explained by evaporation induced by a greater pressure drop under these filters. These PAH were also found in the glass-fibre filters which were mounted under the filters used for the collection of the particles. As the possibility of an incomplete retention of particles could be excluded[24], adsorption of gaseous PAH from the air stream remains the only explanation. Both, PAH originally present in the gas phase of the air and those evaporated from the particles may be adsorbed by the filter material, while the evaporation artifact will also affect the adsorbed gaseous PAH. As the adsorption of glass-fibre upper filters will probably be equal to that of the glass-fibre under filters (McDow and Huntzicker, 1990), it will be possible to correct the PAH concentrations for the adsorption artifact by subtracting the amounts on the under filter from those on the upper filter. However, as our results do not allow the evaporation artifact to be quantified, it remains unclear whether these two artifacts together cause an underestimation or an overestimation of the real concentrations of the volatile particulate PAH in the air.

Several workers have tried to quantify the influence of the evaporation artifact. The investigations of a Belgian group has lead to the definite conclusion that evaporation does indeed significantly affect the PAH concentrations determined by the analysis of high-volume sampling. They found clear differences between samples collected with cascade impactors and high-volume samplers

[24] Because of the absence or very low amounts of the less volatile PAH in the support filters.

as regards the concentrations of the more volatile PAH, which can only be explained by artifactual evaporation (Van Vaeck et al., 1978 and 1979). Confirmation of this finding was obtained by leading pure nitrogen gas through loaded filters (with particles); this resulted in clear decreases of the concentrations of the more volatile PAH (Van Vaeck et al., 1984). Furthermore, they compared on one hand sampling with one filter and an adsorbent to collect the volatiles passing the filter, and on the other sampling with a series of filters and one adsorbent. They found larger amounts of the more volatile PAH in the adsorbent of the one-filter sample than in that of the multi-filter sample. They concluded, that this difference could only be the result of artifactual evaporation, induced by a greater pressure drop over the more heavily laden filter of the one-filter sample. By rapidly changing the filters they could reduce the evaporation loss to negligible proportions.

Coutant et al. (1988) used a set of denuder tubes to remove the PAH from the air before it passed the filter, whereafter the volatile PAH were sampled with an adsorbent. So, the PAH in the sorbent could only stem from the particulate material collected by the filter and were evaporated during sampling. Evaporation appeared to be a very important artifact for the more volatile PAH: 13% and 71% of the total amount collected for benz(a)anthracene and anthracene respectively. Comparison of the amounts of the volatile PAH in filter samples with and without denuder showed that under normal conditions they will be the resultant of both absorption and evaporation as they were greater in the without- than the with-denuder samples. The results of Coutant et al. only give an impression about the evaporation of the real particulate PAH; the total evaporation will also involve adsorbed gaseous PAH and will, therefore, be greater than the evaporation measured by Coutant et al.

The results presented in chapter 11 suggest that also less volatile PAH, such as benzo(a)pyrene may be lost by evaporation, although to a much lesser extent than their more volatile counterparts, as they were observed in larger amounts in the extracts of the support filters of the T and Tc filters than in those of the G filters. Peters and Seifert (1980) demonstrated the evaporation of these PAH in another way. They impregnated filters with ^{14}C-benzo(a)pyrene. After sampling they had lost about 10% of the radioactivity, which could only be explained by artifactual evaporation.

It is hard to see how strategies aimed at the quantification of artifactual evaporation or evaporation sofar developed, could be used on a routine basis to correct the concentrations of the more volatile PAH obtained with high-volume sampling. Possibly they might be used to develop models which predict

the influence of the artifact on the basis of sampling conditions, such as temperature, relative humidity, wind speed, flow rate, linear air velocity and filter material and the amount of organic material collected on the filter. However, up to now, no attempts have been made to develop such models. So, for the time being, it has to be concluded that high-volume sampling yields unreliable results with respect to the concentrations of the more volatile particulate PAH.

A third artifact that may affect the PAH concentrations is chemical conversion. The influence of this artifact was investigated by comparing the concentrations of relatively reactive and relatively inert PAH in samples collected simultaneously with different sampling materials, whereby it was assumed that chemical conversion proceeds more rapidly on glass-fibre filters due to a supposed catalytic effect. In some experiments slightly lower concentrations were observed when glass-fibre filters were used, which made it probable that sampling with these filters is indeed accompanied by chemical conversion of PAH. However, on the whole, the difference caused by this artifact is only marginal. It should be emphasized that this result does not necessarily mean that the influence of the artifact itself is marginal. The possibility should be accounted for that chemical conversion also occurs on the other filter types, albeit to a (somewhat) smaller extent. It is by no means certain, that chemical conversion is only determined by a specific catalytic effect of the glass-fibre filters. Another mechanism might play a role on the other filter types.

There are various other approaches to investigate artifactual conversion of PAH during sampling, besides comparing different filter materials. Basically they all comprise comparison of PAH concentrations obtained under sampling conditions which differ in supposed intensity of exposure to reactive gaseous compounds. Sampling time and linear air velocity can be varied; particles can be exposed to particle-free air after sampling; supposed reactive components such as nitrogen dioxide or ozone can be added to the air before sampling, and so on (De Raat et al, 1987A). All experiments following these approaches have been carried out with glass-fibre filters or quartz fibre filters. Sofar, Teflon filters and teflon-coated glass-fibre filters have only been used in comparative studies such as the one described in chapter 11. Therefore, it remains unclear whether or not the difference between the filters observed in the study of chapter 11 represents the complete conversion artifact or the top of an iceberg with unknown dimensions. If the first is true, under the prevailing sampling conditions the conversion artifact is of minor importance; a conclusion, which, because of the extensive character of our study, can most probably be extended to most

of the other studies presented in these thesis which are concerned with the concentrations of the PAH.

In view of the wide spread use of high-volume sampling in studies on the presence of particulate PAH in the air, it is surprising that the artifactual conversion of these compounds during sampling has received only sporadic attention. Obviously, the reliability of the concentrations of the less volatile PAH as they are determined after high-volume sampling is predominantly determined by this artifact. As is revealed by the discussion section of chapter 11, the studies carried out sofar have yielded rather conflicting results. It has unambiguously been shown that pure PAH adsorbed on a glass-fibre filter are converted chemically upon exposure to the gas phase of ambient air (Pitts et al., 1978 and 1980; De Raat, 1983). Furthermore, the conversion artifact will seriously affect investigations into the PAH content of particles from combustion emissions (Lindskog, 1983; Risby and Lestz, 1983; Schuetzle and Perez, 1983; Brorström-Lunden and Lindskog, 1985; Hartung et al., 1986). Whether it is really important when ambient airborne particles are sampled, remains to be clarified. It should be pointed out that the PAH in these particles are part of a complex matrix which may provide protection towards reactive gaseous species. Furthermore, in contrast to particles directly sampled from combustion emissions, ambient airborne particles have already been exposed to the air for some time before they are sampled. While the flue gases of combustion processes have been shown to be dynamic, instable systems (Kamens et al., 1984; Bell and Kamens, 1986), it may be assumed that ambient air has reached a certain equilibrium, which may be reflected in a less high sensitivity of the particulate PAH to sampling-mediated conversion.

As a follow-up of the study presented in chapter 11, it should be investigated whether variation of the intensity of exposure during sampling does also yield indications for conversion if Teflon filters or Teflon-coated glass-fibre filters are used. If the results show this not to be the case[25], or if conversion is much less on these filter materials, the influence of the artifact can be easily investigated by comparison of filter materials. Furthermore it is clear, that in that case these filters should replace glass-fibre filters in future studies on the concentrations of particulate PAH in ambient air.

[25] That is to say, if the artifact really depends on a specific catalytic effect of glass-fibre filters.

Mutagenicity

Chapter 11 shows that the use of teflon filters or teflon-coated glass-fibre filters instead of glass-fibre filters leads to only slightly lower mutagenic effects. In view of the fact that the great majority of studies on the mutagenicity of ambient airborne particles have been carried out with glass-fibre filters, this difference can be called reassuringly small. Therefore, the results do not justify a re-evaluation of the mutagenic effects obtained sofar with glass-fibre filters, nor do they provide convincing arguments for using the other filters in future studies. They clearly indicate that the conclusions of this thesis with respect to the spatial and temporal distribution of the mutagenicity would not have differed much if the other filters had been used instead of glass-fibre filters.

Concern about the influence of sampling on mutagenicity is in the first place caused by the proven artifactual conversion of PAH induced by sampling. As the mutagens identified sofar in the particles are mainly nitrated and oxygenated PAH and as PAH can react with air components to form such mutagenic derivatives, it seems probable that the conversion of PAH during sampling results in an increase of mutagenicity. Moreover, the similarity in chemical structure of PAH and mutagens in the particles implies that the latter may be apt to comparable reactions, leading to quantitative and qualitative changes of the mutagenicity. These considerations make clear that the influence of the filter material on mutagenicity should in the first place be interpreted in connection with its influence on artifactual conversion of the PAH. The first question to be answered is: Is artifactual conversion of PAH indeed accompanied by changes of mutagenicity? An answer requires experiments which clearly confirm artifactual conversion of PAH. The fact that the study described in chapter 11 does not clearly provide such confirmation, may be regarded as its major limitation. Only marginal decreases of PAH concentrations were accompanied by slight effects on mutagenicity, which leaves the question as to the effects on mutagenicity that would have occurred if the decreases of PAH had been more substantial. The amounts of PAH that could have been converted into new mutagens are only small. Moreover, these small amounts suggest that also PAH-like molecules, such as the mutagens already present in the particles, will not have reacted with reactive air components to any great extent.

If the results reported in the literature are taken seriously, notwithstanding their sporadic character, we have to conclude that other sampling conditions[26] than those prevailing during our study, may lead to more artifactual conversion of the PAH. Episodes of photochemical air pollution may for instance be accompanied by such conditions. The question as to the effect of these conditions on the mutagenicity still remains to be answered. However, the sporadic character of the available evidence for the artifactual conversion of PAH should be emphasized and weighed against the extensive character of our study. Moreover, the influence of filtermaterial on mutagenicity was meticulously investigated by two other groups with similar results, i.e. they found no or only slight changes of the mutagenicity (Fitz et al., 1984 and Daisey et al., 1983).

Ours is the first study to show the presence of mutagens in filters mounted under the filters on which the particles have been collected. In view of the results presented in chapter 12 it is highly improbable that this mutagenicity is caused by the adsorption of gaseous mutagens from the air. Furthermore, the very low amounts of higher molecular weight PAH excludes the possibility that the presence of the mutagens is due an incomplete retention of the particles by the upper filter (see also McDow and Huntzicker, 1990). This leaves the chemical activation of adsorbed non-mutagenic compounds as the sole acceptable explanation. The same extracts contained substantial amounts of more volatile PAH, which may very well be the parent compounds of the mutagens, in particular as it has been shown that PAH adsorbed to glass-fibre are rather sensitive to chemical conversion upon the exposure to the gaseous phase of the air (Pitts et al., 1978 and 1980; De Raat, 1983). The same adsorption and conversion may be expected to occur on the upper filter, which provides an explanation for the differences in mutagenicity between the samples collected with different types of filter material.

It can be concluded that the studies carried out sofar do not point to serious sampling artifacts for mutagenicity. However, the fact that these artifacts may strongly affect the validity of mutagenicity studies on the particles in a clearly adverse manner, justifies a greater research effort than has been made sofar. The approach to be followed in future research depends on whether or not the indications for artifactual conversion of PAH can be confirmed. If they are, it should be investigated to what extent conversion of PAH correlates with changes

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[26] Important in this context are meteorological conditions, in particular temperature and humidity, as well as the chemical composition of the air, in particular the presence of reactive gaseous compounds.

of mutagenicity. Such studies should be concerned with quantitative as well as qualitative changes of mutagenicity, as conversion of PAH and related compounds could affect one type of mutagenicity while leaving the other unchanged. Moreover, bioassay-directed fractionation could be helpful, as it allows the observation of changes of the mutagenicity for separate groups of compounds which would be obscured in the complete extract. If artifactual conversion of PAH appears not to be of any importance, artifactual changes of mutagenicity should be investigated when concentrations of supposedly reactive compounds in the air are high, for instance during episodes of photochemical air pollution. Furthermore, particles with a relatively short residence time in the air should be involved in such studies, in order to exclude the possibility of reactions affecting the mutagenicity being completed before sampling takes place. For instance, it can be expected that the particles emitted by local traffic in the Photochemistry Study will be more sensitive to artifacts than those emitted upwind by distant sources.

The importance of the indirect mutagens

The first studies on the mutagenicity of ambient airborne particles pointed to a predominant direct mutagenicity. This was a rather surprising result, as a predominant indirect effect had been expected, because of the presence of indirectly mutagenic PAH. However, the results seemed to indicate that these compounds contribute only marginally. The direct mutagenicity unambiguously pointed to a new class of particulate air pollutants with possible carcinogenic properties. Obviously, all attention was focussed on these compounds and the fact that the tests also pointed to indirect mutagenicity was more or less neglected. Soon, directly mutagenic nitroderivatives of PAH were identified in several particulate materials produced by combustion processes and pyrolysis (Gibson, 1982; Xu et al., 1982; Gorse et al., 1983; Hanson et al., 1983; Wei and Shu, 1983; Rosenkranz and Mermelstein, 1985; Beije and Møller, 1990; Mücke and Fiedler, 1990) and in the particulate fraction of ambient air (Ramdahl et al., 1982; Nielsen et al., 1984; Tanner and Fajer, 1983; Rosenkranz and Mermelstein, 1985; De Raat et al., 1988; Arey et al., 1989; Beije and Möller, 1990). In addition, the use of strains with a specifically reduced sensitivity for nitro-group dependent mutagenicity (TA98NR and TA98DNP) revealed that the direct mutagenicity could at least largely be attributed to nitrocompounds (Wang et al., 1980; Pitts et al., 1982A and 1982B; Kado et al., 1986; Siak et al., 1985; De Raat et al., 1988; chapters 8 and 10). It was hypothesized that this

mutagenicity is formed during combustion or after emission by nitration of PAH and PAH-like compounds. Nitro PAH received a prominent place in toxicology (Rosenkranz and Mermelstein, 1985; Tokiwa and Onishi, 1986; Beije and Møller, 1990) and they even seem to overshadow the "good old" PAH. Much research has been, and still is aimed at answering the question as to the real toxicological relevance of these direct bacterial mutagens, which depend on bacterial reduction and esterification for their often extremely mutagenic effects in the Salmonella/microsome test.

The emphasis on direct mutagenicity caused by nitro PAH may lead to the impression that no indirect mutagenicity is present in the particles, or when present, that it does not bear any relevance. Close observation of the results published sofar as well as those presented in this thesis refutes this impression. It turns out, that the predominance of the direct mutagenic effects of nitro compounds depends heavily on the tester strain used. From the beginning, virtually all tests have been carried out with strains TA1538 and its plasmid-bearing derivative TA98, because these were found to be the strains most sensitive to the direct mutagenicity of the particles. However, even in these strains clear increases of the effect may be obtained upon the use of S9 fraction (Talcott and Wei, 1977; Teranishi et al., 1978; Dehnen et al., 1981; Pitts et al., 1982A; Kado et al., 1986; Barale et al., 1991), which points to a significant contribution of indirect mutagens. As was set forth in the first section of this chapter, decreases may also occur, in particular when meteorological conditions during sampling favour photochemical air pollution. The loss of direct mutagenicity is, however, compensated for to a substantial extent by indirect mutagenicity. Thus the combination of photochemical air pollution and S9 fraction may in these strains lead to a predominance of indirect mutagenicity over direct mutagenicity, as a result of the activation of the former and the inactivation of the latter.

The importance of indirect mutagenicity is also illustrated by the effects obtained with strain TA100. In the Rijnmond Study and the One-Year Study all samples were tested with this strain. The application of S9 fraction resulted in an activation, which always exceeded the direct mutagenicity. So, indirect mutagenicity predominates over direct mutagenicity in this strain. This difference with TA98 can be explained by the lower sensitivity of TA100 for the direct effects of many nitroaromatic compounds (Rosenkranz and Mermelstein, 1983). Furthermore, TA100 shows in general a higher sensitivity for the indirect mutagenicity of unsubstituted PAH. Another strain with a relatively high

sensitivity to indirect mutagenicity is TA97, as became clear from the application of this strain in the bioassay-directed fractionation experiments (chapter 10).

These considerations make clear that neglect of the contribution of the indirect mutagens to the mutagenicity is not justified because it leads to an underestimation of the mutagenic potential of the particles. The mutagenicity of the particles is not solely determined by the direct activity of nitrocompounds; compounds with indirect effects may contribute substantially. This is not to say, that these indirect mutagens are not nitrocompounds. On the molecular level, nitro PAH can be mutagenic by reduction of the nitro group and by oxidation of their aromatic rings, in which case they are activated in a similar way as the unsubstituted PAH (Fu et al., 1982; Rosenkranz and Mermelstein, 1983 and 1985; El-Bayoumy and Hecht, 1984; Bond et al., 1985; King et al., 1984). The results of bioassay-directed fractionation strongly suggest that the indirect mutagenicity is indeed caused by nitro compounds. With the exception of the PAH fraction, all indirect mutagenicity eluted in the same fractions as the direct nitroreductase-dependent mutagenicity. Obviously, direct and indirect mutagenicity of individual nitrocompounds needs not to be correlated as it is determined by totally different metabolic and genetic mechanisms. However, certain classes of nitro compounds, as they are supposed to be isolated in chromatographical fractions, may very well combine indirect and direct mutagenicity.

The experimental part of this thesis was not concerned with the link between mutagenic quality and relevant effect in humans. However the results give rise to some questions on this point. First of all the question as to the toxicological relevance of the fact that the direct mutagenicity of the particles depends largely on bacterial nitroreduction. Does a comparable activation play a significant role in mammals after inhalatory exposure? Studies on the metabolic conversion of nitro PAH in mammals suggest that nitrocompounds are not reduced to a great extent by mammalian tissues, but that oxidative metabolism prevails. Nitroreduction may largely be confined to the microbial gut flora and exposure of the mammal to the reduced bacterial metabolites occurs by resorption from the gut (Rosenkranz and Mermelstein, 1985; Tokiwa and Onishi, 1986; Beije and Møller, 1988; Møller et al., 1990; Richardson et al., 1988). It is questionable whether this activation pathway bears relevance if effects in the respiratory tract are considered.

These considerations point to the possibility, that the indirect mutagenicity of the particles and the nitro PAH therein may be more important than the direct mutagenicity, which is ironic, as the overwhelming interest of toxicologists for nitro PAH stems in the first place from their often extremely direct

mutagenicity in bacterial mutagenicity tests. It is a well established fact that lung tissues are capable of carrying out the metabolic conversions which lead to the activation of PAH and their derivatives via ring epoxidation. Lung S9 fractions have also been used in the Salmonella/microsome test and were found to be nearly as efficient activators of ambient airborne particles as liver S9 fractions (De Raat, 1982; Jongen et al., 1984; Van Houdt et al., 1988). This could mean that, in fact, the effects determined in the presence of S9 fraction with strains less sensitive to the direct mutagenicity of the particles, such as TA100, TA97 and TA98NR are more relevant from a human-toxicological viewpoint than the effects in strain TA98 and its parent strain TA1538. This does not automatically imply that the presence of nitrocompounds is less relevant as well, because these compounds may very well be responsible for the indirect mutagenicity next to their well known direct effect.

Non bacterial tests

Many other tests and test variants can in principle be applied in studies on the mutagenicity of the particles, next to the Salmonella/microsome test with its different strains and S9 fractions. As argued in chapter 2, most of these will affect the experimental convenience of mutagenicity testing to a prohibitive extent. However, the question as to the extra information that would have been provided by a more extensive qualitative differentiation than is possible within the Salmonella/microsome test remains. Very little information is available that might contribute to an answer to this question. Some of the samples of the Rijnmond study have been tested with an in vitro test for the induction of sister chromatid exchanges (SCE test; De Raat, 1982 and 1983). Comparison of the results obtained with this test and those obtained with the Salmonella/microsome test suggests that the application of the SCE test does not lead to clearly deviating global conclusions about the spatial and temporal distribution. The results of Alink et al. (1983) lead to a comparable conclusion. The word "global" in this conclusion has to be emphasized, as the limited number of SCE tests carried out in both studies prevents qualitative changes to be observed with the same discriminating power as could be achieved with the variants of the Salmonella/microsome test. For this, it would have been necessary to apply the in vitro SCE test on a much larger scale. Then it might be possible to trace qualitative changes linked with processes and sources which escape attention when using the Salmonella/microsome test.

The induction of SCE by extracts of the particles has also been demonstrated by Schürer et al. (1980), Lockard et al. (1981), Krishna et al.

(1984), Seemayer et al. (1984), Pyysalo et al. (1987) and Hadnagy et al. (1989). In addition the particles were also found to be clastogenic (Krishna et al., 1984; Hadnagy et al., 1986 and Hadnagy et al.; 1989) and to induce mutations in mammalian tissue-culture cells (Crespi et al, 1985; Seemayer et al, 1987). It can thus be concluded that the mutagenicity of the particles in tests with other test organisms and test criteria is a well established fact. It appears that the in vitro SCE test is at least sensitive enough to be used as a toxicological tool in studies on the pollution of the air in a comparable way as the Salmonella/microsome test. However, whether its convenience allows this, remains to be seen. Most other mutagenicity test will certainly lack the necessary experimental convenience. Nevertheless, they still could add to our insight into the human-toxicological relevance of the effects observed with the Salmonella/microsome test, in particular if they are based on organisms, conditions and criteria which approach the "real-life" situation more clearly. For instance, they could clarify the relevance of the nitroreductase-dependent direct mutagenicity, the indirect mutagenicity, the deactivation caused by S9 fraction etc. Applied to the fractions of bioassay-directed fractionation, they could directly show which groups of compounds are really important. Subsequently, air-pollution research and research on the adverse effects in humans upon inhalation of the particles could be concerned with these compounds. Thus, an obvious follow-up of the studies described in this thesis would be to study the mutagenicity of the particles with a more extensive qualitative differentiation. This would lead to a more purposeful approach in both the air-pollution type of studies as well as the studies aimed at the elucidation of human risk.

REFERENCES TO CHAPTERS 1 - 5 AND 14

Alfheim, I. and M. Møller, 1979 / Mutagenicity of long-range transported atmospheric aerosols / Sci. Total Environ. 13, 275-278

Alfheim, I., 1982 / Contribution from motor vehicle exhaust to the mutagenic activity of airborne particles / In: Sorsa, M. and H. Vainio, eds., Mutagens in our Environment / Alan R. Liss, New York, 1982

Alfheim, I, A. Bjørseth and M. Møller, 1985 / Characterization of microbial mutagens in complex samples - methodology and application / CRC Critical Reviews in Environmental Control, 14, 91-150

Alink, G. M., J. J. van Houdt, J. R. Kalkman and J. S. M. Boley, 1983 / Mutagenic activity of airborne particulates of non-industrial locations / Mutat. Res. 116, 21-34

Alsberg, T., S. Håkansson, M. Strandell and R. Westerholm, 1989 / Profile analysis of urban air pollution / Chemometrics and Intelligent Laboratory Systems 7, 143-152

Ames, B. N., 1971 / The detection of chemical mutagens with enteric bacteria / In: Hollaender, A., ed., Chemical Mutagens, Principles and Methods for Their Detection, Vol. 1 / Plenum, New York

Ames, B. N., 1972 / A bacterial system for detecting mutagens and carcinogens / In: Sutton, H. E. and M. J. Harris, eds, Mutagenic Effects of Environmental Contaminants / Academic Press, New York

Ames, B. N., W. E. Durston, E. Yamasaki and F. D. Lee, 1973A / Carcinogens are mutagens: a simple test system combining liver homogenates for activation and bacteria for detection / Proc. Natl. Acad. Sci. USA 70, 2281-2285

Ames, B. N., F. D. Lee and W. E. Durston, 1973B / An improved bacterial test system for detection and classification of mutagens and carcinogens / Proc. Natl. Acad. Sci. USA 70, 782-786

Ames, B. N., J. McCann and E. Yamasaki, 1975 / Methods for detecting carcinogens as mutagens with the Salmonella/mammalian-microsome mutagenicity test / Mutat. Res. 31, 347-364

Ames, B. N. and L. Swirsky Gold, 1990A / Chemical carcinogenesis: too many rodent carcinogens / Proc. Natl. Acad. Sci. USA 87, 7772-7776

Ames, B. N. and L. Swirsky Gold, 1990B / Too many rodent carcinogens: mitogenesis increases mutagenesis / Science 249, 970-971

Arey, J., B. Zielinska, R. Atkinson, A. M. Winer, T. Ramdahl and J. N. Pitts, 1986 / The formation of nitro-PAH from the gas-phase reaction of fluoranthene and pyrene with the OH radical in the presence of NO_x / Atmos. Environ. 20, 2339-2345

Arey, J., R. Atkinson, B. Zielinska and P. A. McElroy, 1989 / Diurnal concentrations of volatile polycyclic aromatic hydrocarbons and nitroarenes during photochemical air pollution episode in Glendora, California / Environ. Sci. Technol. 23, 321-327

Asahina, S., J. Andrea, A. Carmel, E. Arnold, Y. Bishop, S. Joshi and S. S. Epstein, 1972 / Carcinogenicity of organic fractions of particulate pollutants in New York City and administered subcutaneously to infant mice / Cancer Res. 32, 2263-2268

Ashby, J. and J. A. Styles, 1978 / Does carcinogenic potency correlate with mutagenic potency in the Ames assay? / Nature 271, 452-455

Ashby, J. and I. F. H. Purchase, 1988 / Reflections on the declining ability of the Salmonella assay to detect rodent carcinogens as positive / Mutat. Res. 205, 51-58

Ashby, J. and R. W. Tennant, 1988 / Chemical structure, Salmonella mutagenicity and extent of carcinogenicity as indicators of genotoxic carcinogenesis among 222 chemicals tested in rodents by the U.S. NCI-/NTP / Mutat. Res. 204, 17-115

Ashby, J., R. W. Tennant, E. Zeiger and S. Stasiewicz, 1989 / Classification according to chemical structure, mutagenicity to Salmonella and level of carcinogenicity of a further 42 chemicals tested for carcinogenicity by the U.S. National Toxicology Program / Mutat. Res. 223, 73-103

Ashby, J. and R. W. Tennant, 1991 / Definitive relationships among chemical structure, carcinogenicity and mutagenicity for 301 chemicals tested by the U.S. NTP / Mutat. Res. 257, 229-306

Baek S. O., M. E. Goldstone, P. W. W. Kirk, J. N. Lester and R. Perry, 1991 / Phase distribution and particle size dependency of polycyclic aromatic hydrocarbons in the urban atmosphere / Chemosphere 22, 503-520

Baek, S. O., M. E. Goldstone, P. W. W. Kirk, J. N. Lester and R. Perry, 1992 / Concentrations of particulate and gaseous polycyclic aromatic hydrocarbons in London air following a reduction in the lead content of petrol in the United Kingdom / Sci. Total Environ. 111, 169-199

Barale, R., L. Giromini, G. Ghelardini, C. Scapoli, N. Loprieno, M. Pala, F. Valerio and I. Barrai, 1991 / Correlations between 15 polycyclic aromatic hydrocarbons (PAH) and the mutagenicity of the total PAH fraction in ambient air particles in La Spezia / Mutat. Res. 249, 227-241

Barbacid, M., 1987 / Ras-genes / Ann. Rev. Biochem. 56, 779-827

Barkenbus, B. D., W. H. Griest, J. J. Huntzicker, E. K. Heyerdahl and C. S. MacDougall, 1983 / Recovery of organic carbon from atmospheric particulate matter using Soxhlet extraction with benzene/methanol azeotrope / J. Environ. Sci. Health A(18), 297-310

Barrett, J. C., T. Tsutsui, T. Tlsty and M. Oshimura, 1990 / Role of genetic instability in carcinogenesis / Genetic Mechanisms in Carcinogenicity and Tumour Progression 114, 97-114

Bartle. K. D., 1985 / Recent advances in the analysis of polycyclic aromatic compounds by gas chromatography / In: Bjørseth, A. and Th. Ramdahl, eds, Handbook of Polycyclic Aromatic Hydrocarbons, Vol. 2, Emission Sources and Recent Progress in Analytical Chemistry / Marcel Dekker, New York

Bartsch, H., T. Kuroki, M. Robertfroid and C. Malaveille, 1982 / Metabolic activation systems *in vitro* for carcinogen/mutagen screening tests / In: De Serres, F. J. and A. Hollaender, eds, Chemical Mutagens Principles and Methods for their Detection, Vol. 7 / Plenum, New York

Baum, E. J., 1978 / Occurrence and surveillance of polycyclic aromatic hydrocarbons / In: Gelboin, H. V. and P. O. P. Ts'o, eds., Polycyclic Hydrocarbons and Cancer, Vol. 1 / Academic Press, New York

Beije, B. and L. Møller, 1988 / 2-Nitrofluorene and related compounds: prevalence and biological effects / Mutat. Res. 196, 177-209

Bell, D. A. and R. M. Kamens, 198. / Photodegradation of wood smoke mutagens under low NO_x conditions / Atmos. Environ. 20, 317-321

Berenblum, I., 1941A / The cocarcinogenic action of croton resin / Cancer Res. 1, 44-48

Berenblum, I., 1941B / A study of the significance of cocarcinogenic action and related phenomena / Cancer Res. 1, 807-814

Berenblum, I. and P. Shubik, 1949 / An experimental study of the initiating stage of carcinogenesis and a re-examination of the somatic cell mutation theory / Br. J. Cancer 3, 109-118

Bishop, J. M., 1987 / The molecular genetics of cancer / Science 235, 305-311

Bjørseth, A., G. Lunde and A. Lindskog, 1979 / Long-range transport of polycyclic aromatic hydrocarbons / Atmos. Environ 13, 45-53

Bjørseth, A. and B. Sortland Olufsen, 1983 / Long-range transport of polycyclic aromatic hydrocarbons / In: Bjørseth, A., ed., Handbook of Polycyclic Aromatic Hydrocarbons / Marcel Dekker, New York

Bjørseth, A. and T. Ramdahl, 1985 / Sources and emissions of PAH / In: Bjørseth, A. and T. Ramdahl, eds, Handbook of Polycyclic Aromatic Hydrocarbons, Vol. 2 / Marcel Dekker, New York

Bluell, P. and J. E. Dunn, 1967 / Relative impact of smoking and air pollution on lung cancer / Arch. Environ. Health, 15, 291-297

Blumer, M., 1976 / Polycyclic aromatic compounds in nature / Sci. Amer. 234, 34-45

Boesen, J. J. B., 1992 / Stress Response and Genetic Instability in Mammalian Cells / Thesis State University of Leiden

Bond, J. A., J. L. Mauderly, R. F. Henderson and R. O. McClellan, 1985 / Metabolism of 1-[^{14}C]nitropyrene in respiratory tract tissue of rats exposed to diesel exhaust / Toxicol. Appl. Pharmacol. 79, 461-470

Borneff, J. and H. Kunte, 1983 / Polycyclic aromatic hydrocarbons in river and lake water, biota and sediments / In: Bjørseth, A., ed., Handbook of Polycyclic Aromatic Hydrocarbons, Vol. 1 / Marcel Dekker, New York

Breuer, G. M., 1984 / Solvents and techniques for the extraction of polynuclear aromatic hydrocarbons from filter samples of diesel exhaust / Anal. Lett. 17, 1293-1306

Bridges, B. A., 1988 / Genetic toxicology at the cross roads - a personal view on the deployment of short-term tests for predicting carcinogenicity / Mutat. Res. 205, 25-31

Brorström, E., P. Grennfelt and A. Lindskog, 1983 / The effect of nitrogen dioxide and ozone on the decomposition of particle-associated polycyclic aromatic hydrocarbons during sampling from the atmosphere / Atmos. Environ. 17, 601-605

Brorström-Lunden, E. and A. Lindskog, 1985 / Degradation of polycyclic aromatic hydrocarbons during simulated stack gas sampling / Environ. Sci. Technol. 19, 313-316

Brusick, D., 1983 / Evaluation of chronic rodent bioassays and Ames assay tests as accurate models for predicting human carcinogens / In: Milman and Sell, eds, Application of Biological Markers to Carcinogen testing / Plenum Publishing Corporation, New York

Butler, J. D. and P. Crossley, 1981 / Reactivity of polycyclic aromatic hydrocarbons adsorbed on soot particles / Atmos. Environ. 15, 91-94

Campbell, 1934 / Cancer of the skin and increase in incidence of primary tumours of lung in mice exposed to dust obtained from tarred roads / Br. J. Exp. Pathol. 15, 287-294

Cavalieri, E. L. and E. G. Rogan, 1992 / The approach to understanding aromatic hydrocarbon carcinogenesis; the central role of radical cations in metabolic activation / Pharmacol. Ther. 55, 183-199

CCRX, 1987 / Polycyclische aromatische koolwaterstoffen in het Nederlandse milieu / Coördinatiecommissie voor Metingen van Radioactiviteit en Xenobiotische Stoffen

Chambers, 1976 / Classification and extent of air pollution problems / In: Stern, A. C., ed., Air Pollution Volume I, Air Pollutants, Their Transformation and Transport / Academic Press, New York

Chrisp, C. E. and G. L. Fisher, 1980 / Mutagenicity of airborne particulates / Mutat. Res. 76, 143-164

Commoner, B., P. Madyasta, A. Bronsdon and A. Vithayathil, 1978 / Environmental mutagens in urban air particulates/ J. Toxicol. Environ. Health 4, 59-77

Cook, J. W., C. L. Hewett and I. Hieger, 1933 / The isolation of a cancer-producing hydrocarbon from coal tar; parts I, II and III / J. Chem. Soc. 1, 395-405

Corn, M., 1977 / Aerosols and the primary air pollutants - nonviable particles / Their occurrence, properties, and effects / In: Stern, A. C., ed., Air Pollution, Vol. I, Air Pollutants, Their Transformation and Transport / Academic Press, New York

Coutant, R. W., L. Brown, J. C. Chuang, R. M. Riggin and R. G. Lewis, 1988 / Phase distribution and artifact formation in ambient air sampling for polynuclear aromatic hydrocarbons / Atmos. Environ. 22, 403-409

Crespi, C. L., H. L. Liber, T. D. Behymer, R. A. Hites and W. G. Thilly, 1985 / A human cell line sensitive to mutation by particle-born chemicals / Mutat. Res. 157, 71-75

Daisey, J. M., C. E. Allen, G. McGarrity, T. Atherhold and P. J. Lioy, 1983 / Effects of filter type on the organic composition and mutagenicity of inhalable particulate matter / Aerosol Sci. Technol. 2, 295

Daisey, J. M., J. L. Cheney and P. L. Lioy, 1986 / Profiles of organic particulate emissions from air pollution sources: status and needs for receptor source apportionment modelling / J. Air Pollut. Control Assoc. 36, 17-33

De Raat, W. K., 1982 / De Genotoxiciteit van Buitenluchtaerosol / Report CL 82/5 / Institute of Environmental Sciences TNO, Delft

De Raat, W. K., 1983 / Genotoxicity of aerosol extracts; some methodological aspects and the contribution of urban and industrial locations / Mutation Research 116, 47-63

De Raat, W. K., A. O. Hanstveit and F. J. de Kreuk, 1985 / The role of mutagenicity testing in the ecotoxicological evaluation of industrial discharges into the aquatic environment / Food Chem. Toxicol., 23, 33-41

De Raat, W. K., F. A. de Meijere and G. L. Bakker, 1987A / De Mutageniteit van de Deeltjes in de Buitenlucht; Eindrapport Deel I; Invloed van Monsterneming en Extractie / Report R 87/201 / Institute of Environmental Sciences TNO, Delft

De Raat, W. K., G. L. Bakker and F. A. de Meijere, 1987B / De Mutageniteit van de Deeltjes in de Buitenlucht; Eindrapport Deel II; De Identificatie van de Mutagenen / Report R 87/255 / Institute of Environmental Sciences TNO, Delft

De Raat, W. K., N. van der Hoeven, S. A. L. M. Kooijman, P. I. Mutzall and M. I. Willems, 1987C / Studies on some factors influencing the *Salmonella*/microsome test / Institute of Environmental Sciences TNO, report R87/177[a]

De Raat, W. K., F. A. de Meijere and G. L. Bakker, 1988 / De Mutageniteit van de Deeltjes in de Buitenlucht; Eindrapport Deel III; Fluctuatie van het Effect in de Tijd (Longitudinale Studie) / Report R 88/118 / Institute of Environmental Sciences TNO, Delft

De Raat, W. K., G. J. Vink and A. O. Hanstveit, 1991 / The significance of mutagenicity testing as a criterion in ecotoxicological evaluations / In: Waters, M. D. et al., eds, Genetic Toxicology of Complex Mixtures / Plenum Press, New York

Dean, G., 1959 / Lung cancer among white South Africans / Br. Med. J., 2, 852-857

Dehnen, W., R. Tomingas and N. Pitz, 1981 / Studies on the mutagenic effect of extracts derived from airborne particles within one year in differently burdened areas using the Ames test / Zbl. Bakt. Hyg., I. Abt. Orig., B172 351-366

Dipple, A., 1976 / Polynuclear aromatic carcinogens / In: Searle, C., ed, Chemical Carcinogens / ACS Monograph, 173

Diwan, B. A. and J. M. Rice, 1989 / Organ and species specificity in chemical carcinogenesis and tumour promotion / In: Sirica, A. E., ed., Pathobiology of Neoplasia / Plenum, New York

Doll, R., 1978 / Atmospheric pollution and lung cancer / Environ. Health Perspect. 22, 23-31

Dulbecco, R., 1989 / Cancer progression: the ultimate challenge / Int. J. Cancer, supplement 4, 6-9

Eastcott, D. F., 1956 / The epidemiology of lung cancer in New Zealand / The Lancet, Jan. 7th, 1956, 37-39

El-Bayoumy, K. and S. S. Hecht, 1984 / Identification of *trans*-1,2-dihydro-1,2-dihydroxy-6-nitrochrysene as a major metabolite of 6-nitrochrysene / Cancer Res. 44, 3408-3413

Epstein, S. S., K. Fuji and S. Asahina, 1979 / Carcinogenicity of a composite organic extract of urban particulate atmospheric pollutants following subcutaneous injection in infant mice / Environ. Res. 19, 163-176

Falk, H. L., I. Markul and P. Kotin, 1956 / Polycyclic aromatic hydrocarbons IV; their fate following emission into the atmosphere and experimental exposure to washed air and synthetic smog / Proc. Am. Assoc. Cancer Research 2, 15

Fearon, E. R. and B. Vogelstein, 1990 / A genetic model for colorectal tumorigenesis / Cell 61, 759-767

Finlayson-Pitts, B. J. and Pitts, 1986 / Atmospheric Chemistry / Wiley Interscience Publication / Wiley, New York

Fitz, D. R., D. M. Lokensgard and G. J. Doyle, 1984 / Investigation of filtration artifacts when sampling ambient particulate matter for mutagen assay / Atmos. Environ. 18, 205-213

Friberg, L. and R. Cederlöf, 1978 / Late effects of air pollution with special reference to lung cancer / Environ. Health Perspect., 22, 45-66

Fu, P. P., M. W. Chou, S. K. Yang, F. A. Beland, F. F. Kadlubar, D. A. Casciano, R. H. Heflich and F. E. Evans, 1982 / Metabolism of the mutagenic environmental pollutant 6-nitrobenzo[a]pyrene: metabolic activation via ring oxidation / Biochem. Biophys. Res. Commun. 105, 1037-1043

Gelboin, H. V. and P. O. Ts'o, 1978 / Polycyclic Hydrocarbons and Cancer / Academic Press, New York

Gezondheidsraad, 1984 / Advies inzake polycyclische aromatische koolwaterstoffen (PAK) in de buitenlucht; advieswaarden voor de kwaliteit van de buitenlucht / Gezondheidsraad, rapportnummer 40, Den Haag, 1984

Gibson, T. L., 1982 / Nitro derivatives of polynuclear aromatic hydrocarbons in airborne and source particulate matter / Atmos. Environ. 16, 2037-2040

Glatt, H. R. and F. Oesch, 1986 / Structural and metabolic parameters governing the mutagenicity of polynuclear aromatic hydrocarbons / In: De Serres, F. J., eds., Chemical Mutagens; Principles and Methods for their Detection, Vol. 10 / Plenum, New York

Goldsmith, J. R. and L. Friberg, 1977 / Effects of air pollution on human health / In: Stern, A. C., ed., Air Pollution Vol. II / Academic Press, New York

Goldsworthy, T. L. and M. H. Hanigan, 1987 / Models of hepatocarcinogenesis in the rat; contrasts and comparisons / CRC Reviews in Toxicology 17

Gordon, R. J., 1974 / Solvent selection in extraction of airborne particulate matter / Atmos. Environ. 8, 189-191

Gorse, R. A., T. L. Riley, F. C. Ferris, A. M. Pero and L. M. Skewes, 1983 / 1-Nitropyrene concentration and bacterial mutagenicity in on-road vehicle particulate emissions / Environ. Sci. Technol. 17, 198-202

Grimmer, G., 1983A / Environmental Carcinogens: Polycyclic Aromatic Hydrocarbons / CRC Press, Boca Raton, Florida

Grimmer, G., 1983B / Profile analysis of polycyclic aromatic hydrocarbons in air / In: Bjørseth, A., ed., Handbook of Polycyclic Aromatic Hydrocarbons / Marcel Dekker, New York

Grimmer, G., K.-W. Naujack and G. Dettbarn, 1983 / Characterization of polycyclic aromatic hydrocarbons as essential carcinogenic constituents of coal combustion and automobile exhaust using mouse-skin-painting as a carcinogenic-specific detector / Toxicol. Environ. Chem., 6, 97-107

Grimmer, G., H. Brune, R. Deutsch-Wenzel, G. Dettbarn, J. Misfeld, U. Abel and J. Timm, 1984 / The contribution of polycyclic aromatic hydrocarbons to the carcinogenic impact of emission condensate from coal-fired residential furnaces evaluated by topical application / Cancer Lett., 23, 167-176

Grimmer, G., H. Brune, R. Deutsch-Wenzel, G. Dettbarn, J. Misfeld, U. Abel and J. Timm, 1985 / The contribution of polycyclic aromatic hydrocarbon fractions with different boiling ranges to the carcinogenic impact of emission condensate from coal fired residential furnaces as evaluated by topical application to the skin of mice / Cancer Lett., 28, 203-211

Grosjean, D., 1975 / Solvent extraction and organic carbon determination in atmospheric particulate matter: the organic extraction - organic carbon analyzer (OE-OCA) technique / Anal. Chem. 47, 797-805

Grosjean, D., 1983 / Polycyclic aromatic hydrocarbons in Los Angeles air from samples collected on Teflon, glass and quartz filters / Atmos. Environ. 17, 2565-2573

Grosjean, D., K. Fung and J. Harrison, 1983 / Interactions of polycyclic aromatic hydrocarbons with atmospheric pollutants / Env. Sci. Technol. 17, 673-679

Guerin, M. R., 1978 / Energy sources of polycyclic aromatic hydrocarbons / In: Gelboin, H. V. and P. O. P. Ts'o, Polycyclic Aromatic Hydrocarbons and Cancer, Vol. 1 / Academic Press, New York

Gurinov, B. P., V. N. Tugarinova, O. J. Vasilieva, M. V. Nivontova and L. M. Shabad, 1962 / Carcinogenic properties of the motor transport exhaust soot / Gig. Sanit., 28, 19-23

Haagen-Smit, A. J. and L. G. Wayne, 1977 / Atmospheric reactions and scavenging processes / In: Stern, A. C., ed., Air Pollution, vol. I, Air Pollutants, Their Transformation and Transport / Academic Press, New York

Hadnagy, W., N. H. Seemayer, and R. Tomingas, 1986 / Cytogenetic effects of airborne particulate matter in human lymphocytes in vitro / Mutat. Res. 175, 97-101

Hadnagy, W., N. H. Seemayer, R. Tomingas and K. Ivanfy, 1989 / Comparative studies of sister chromatid exchanges and chromosomal aberrations induced by airborne particulates from an urban and a highly industrialized location in human lymphocyte cultures / Mutat. Res. 225, 27-32

Hangebrauck, R. P., D. J. von Lehmden and J. E. Meeker, 1967 / Sources of polynuclear hydrocarbons in the atmosphere / U.S. Public Health Service, Publication no. 999-AP-33

Hanson, R. L., T. R. Henderson, C. H. Hobbs, C. R. Clark, R. L. Carpenter, J. S. Dutcher, T. M. Harvey and D. F. Hunt, 1983 / Detection of nitroaromatic compounds on coal combustion particles / J. Toxicol. Environ. Health 11, 971-980

Harris, W. R., J. F. Remsen, E. K. Chess and D. W. Later, 1987 / Correlation of nitroaromatic compounds with mutagenic activity of coal fly ash / J. Toxicol. Environ. Health 20, 81-103

Harris, C. C., R. Reddel, A. Pfeifer, P. Amstad, G. Mark, A. Weston, R. Modali, D. Iman, M. McMenamin, E. Kaighn, E. Gabrielson, R. Jones and B. F. Trump, 1990 / Oncogenes and tumour suppressor genes in human lung carcinogenesis / In: Harris, C. C. and L. Liotta, Genetic Mechanisms in Carcinogenesis and Tumour Progression / UCLA Symposia on Molecular and Cellular Biology New Series, Vol. 114 / Wiley-Liss, New York

Hartung, A., J. Schulze, H. Kiess and K.-H. Lies, 1986 / Nitro-derivatives of PAH as artifacts during diesel exhaust sampling / Staub Reinhal. Luft 46, 132-135

Helmig, D., J. Arey, W. P. Harger, R. Atkinson and J. Lopez-Cancio, 1992 / Formation of mutagenic nitrodibenzopyranones and their occurrence in ambient air / Environ. Sci. Technol. 26, 622-624

Hennings, H., A. B. Glick, D. A. Greenhalgh, D. L. Morgan, J. E. Strickland, T. Tannebaum and S. H. Yuspas, 1993 / Critical aspects of initiation, promotion and progression in multistage epidermal carcinogenesis / Proc. Soc. Exp. Biol. Med. 202, 1-8

Henry, S. A., 1946 / Cancer of the Scrotum in Relation to Occupation / Oxford University Press, London

Hill, H. H., K. W. Chan and F. W. Karasek, 1977 / Extraction of organic compounds from airborne particulate matter for gas chromatographic analysis / J. Chromatogr. 131, 245-252

Hinds, W. C., 1982 / Aerosol Technology; Properties, Behaviour, and Measurement of Airborne Particles / Wiley, New York

Hoffmann, D., E. Theisz and E. L. Wynder, 1965 / Studies on the carcinogenicity of gasoline exhaust / J. Air Pollut. Control Assoc., 15, 162-165

Hoffmann, D., E. Theisz and E. L. Wynder, 1972 / Studies on the carcinogenicity of gasoline exhaust / J. Air Pollut. Control Assoc. 15, 162-165

Hoffmann, D. and E. L. Wynder, 1977 / Organic particulate pollutants; chemical analysis and bioassays for carcinogenicity / In: Air Pollution Vol. II, Ed.:A. C. Stern / Academic Press, New York

Hoffmann, G. R., 1982 / Mutagenicity testing in environmental toxicology / Environ. Sci. Technol., 16, 560A-574A

Holland, J. M., F. W. Larimer, T. K. Rao, J. L. Epler, C.-H. Ho, M. V. Buchanan and M. R. Guerin, 1984 / The distribution of dermal tumorigens in coal liquids: relationship of tumorigenicity and microbial mutagenicity / J. Appl. Toxicol., 4, 117-123

Hueper, W. C., P. Kotin, E. C. Tabor, W. W. Payne, H. Falk and E. Sawicki, 1962 / Carcinogenic bioassays on air pollutants / Arch. Pathol. 74, 11-38

IARC, 1973 / Certain Polycyclic Aromatic Hydrocarbons and Heterocyclic Compounds / IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Man, Vol. 3 / International Agency for Research on Cancer, Lyon

IARC, 1976A / Environmental Pollution and Carcinogenic Risks / IARC Scientific Publications, Vol. 13 / International Agency for Research on Cancer, Lyon

IARC, 1976B / Screening Tests in Chemical Carcinogenesis / IARC Scientific Publications, Vol. 12 / International Agency for Research on Cancer, Lyon

IARC, 1977 / Air Pollution and Cancer in Man / IARC Scientific Publications, Vol. 16 / International Agency for Research on Cancer, Lyon

IARC, 1980 / Long-Term and Short-Term Screening assays for Carcinogens: a Critical Appraisal / IARC Monographs, Supplement 2 / International Agency for Research on Cancer, Lyon

IARC, 1983 / Polynuclear Aromatic Compounds, Part 1 / IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Humans, Vol. 32 / International Agency for Research on Cancer, Lyon, France

IARC, 1984A / Polynuclear Aromatic Compounds, Part 3, Industrial Exposures in Aluminium Production, Coal Gasification, Coke Production, and Iron and Steel Founding / IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans, Vol. 34 / International Agency for Research on Cancer, Lyon

IARC, 1984B / Models, Mechanisms and Etiology of Tumour Promotion / IARC Scientific Publications, Vol. 56 / International Agency for Research on Cancer, Lyon

IARC, 1985 / Polynuclear Aromatic Hydrocarbons, Part 4, Bitumens, Coal-tars and Derived Products, Shale Oils and Soots / IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans, Vol. 35 / International Agency for Research on Cancer, Lyon

IARC, 1986 / Long-Term and Short-Term Assays for Carcinogens: a Critical Appraisal / IARC Scientific Publications, Vol. 83 / International Agency for Research on Cancer, Lyon

IARC, 1988 / Cell Differentiation, Genes and Cancer / IARC Scientific Publications, Vol. 92 / International Agency for Research on Cancer, Lyon

IARC, 1989 / Diesel and Gasoline Engine Exhausts and Some Nitroarenes / IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Vol. 46 / International Agency for Research on Cancer, Lyon ICPEMC, 1982 / Committee 2 Report; Mutagenesis Testing as an Approach to Carcinogenesis / International Commission for Protection Against Environmental Mutagens and Carcinogens / Elsevier, Amsterdam

ICPEMC, 1988 / Testing for mutagens and carcinogens; the role of short-term genotoxicity assays / Mutat. Res. 205, 3-12

ISO, 1983 / Air Quality-Particle Size Fraction Definitions for Health Related Sampling / ISO /TR 7708-1983 (E) / International Standards Organization

Jäger, J. and V. Hanus, 1980 / Reaction of solid carrier-adsorbed polycyclic aromatic hydrocarbons with gaseous low-concentrated nitrogen dioxide / J. Hyg. Epidemiol. Microbiol. Immunol. 24, 1-12

Jongen, W. M. F., B. Prins and J. J. van Houdt, 1984 / Bioactivation of premutagenic aerosol extracts by liver- and lung homogenates of different species / Mutat. Res. 130, 231

Jongen, W. M. F., 1988 / Co-cultivation of Cells as a Promising Tool in Cancer Research *In Vitro* / Thesis, Agricultural University Wageningen

Kado N. Y., G. N. Guirguis, C. P. Flessel, R. C. Chan, K.-I. Chang and J. J. Wesolowski, 1986 / Mutagenicity of fine (<2.5-μm) airborne particles: diurnal variation in community air determined by a Salmonella micro preincubation (microsuspension) procedure / Environ. Mutag. 8, 53-66

Kamens, R. M., G. D. Rives, J. M. Perry, D. A. Bell, R. Flynn Paylor, R. G. Goodman and L. D. Claxton, 1984 / Mutagenic changes in dilute wood smoke as it ages and reacts with ozone and nitrogen dioxide: an outdoor chamber study / Environ. Sci. Technol. 18, 523-530

Katz, M. and C. Chan, 1980 / Comparative distribution of eight polycyclic aromatic hydrocarbons in airborne particulates collected by conventional high-volume sampling and by size fractionation / Environ. Sci. Technol. 14, 838-843

Keller, C. D. and T. F. Biddleman, 1984 / Collection of airborne polycyclic aromatic hydrocarbons and other organics with a glass fibre filter-polyurethane foam system / Atmos. Environ. 18, 837-845

Kenneway, E. L. and I. Hieger, 1930 / Carcinogenic substances and their fluorescence spectra / Br. Med. J. 1, 1044-1046

Kennaway, E. 1955 / The identification of a carcinogenic compound in coal-tar / Br. Med. J., Sept. 24, 1955, 749-752

King, L. C., M. J. Kohan, L. M. Ball and J. Lewtas, 198. / Mutagenicity of 1-nitropyrene metabolites from lung S9 / Cancer Lett. 22, 255-263

König, J., E. Balfanz, W. Funcke and T. Romanowski, 1983 / Determination of oxygenated polycyclic aromatic hydrocarbons in airborne particulate matter by capillary gas chromatography and gas chromatography/mass spectrometry / Anal. Chem. 55, 599-603

Kotin, P., H. Falk, P. Mader and M. Thomas, 1954A / Aromatic hydrocarbons I; presence in the Los Angeles atmosphere and the carcinogenicity of atmospheric extracts / A.M.A. Arch. Indus. Hyg. Occup. Med., 9, 153-163

Kotin, P., H. L. Falk and M. Thomas, 1954B / Aromatic hydrocarbons II; presence in the particulate phase of gasoline-engine exhausts and the carcinogenicity of exhaust particles / A.M.A. Arch. Indus. Hyg. Occup. Med., 10, 164-177

Kotin, P., H. L. Falk and M. Thomas, 1955 / Aromatic hydrocarbons III, presence in the particulate phase of Diesel-engine exhausts and the carcinogenicity of exhaust extracts / A.M.A. Arch. Indus. Health, 11, 113-120

Kotin, P, 1956 / Role of atmospheric pollution in pathogenesis of pulmonary cancer; review / Cancer Res. 16, 375-393

Kotin, P. and H. L. Falk, 1956 / II. The experimental induction of pulmonary tumours in strain-A mice after their exposure to an atmosphere of ozonized gasoline / Cancer 9, 910-917

Kotin, P., H. L. Falk and M. Thomas, 1956 / I. Production of skin tumours in mice with oxidation products of aliphatic hydrocarbons / Cancer 9, 905-909

Krishna, G., T. Ong, W.-Z. Whong and J. Nath, 1983 / Mutagenicity studies of ambient airborne particles; I comparison of solvent systems / Mutat. Res. 124 113-120

Krishna, G., J. Nath and T. Ong, 1984 / Correlative genotoxicity studies of airborne particles in *Salmonella typhimurium* and cultured human lymphocytes / Environ. Mutag. 6, 585-592

Kunte, H. and J. Borneff, 1976 / Nachweisverfahren für polycyclische aromatische Kohlenwasserstoffe in Wasser / Z. Wasser Abwasser Forsch. 9, 35-

Lee, F. S. C., W. R. Pierson and J. Ezike, 1980 / The problem of PAH degradation during filter collection of airborne particles an evaluation of several commonly used filter media / In: Bjørseth, A. and A. Den nis, eds, Polynuclear Aromatic Hydrocarbons: Chemistry and Biological Effects / Batelle Press, Columbus, Ohio

Lee, H., S. M. Law and S. T. Lin, 1991 / The effect of extraction solvent on the mutagenicity of airborne particles / Toxicolog. Lett. 58, 59-67

Leiter, J., M. B. Shimkin and M. J. Shear, 1942 / Production of subcutaneous sarcomas in mice with tars extracted from atmospheric dusts / J. Nat. Cancer Inst., 3, 155-165

Leiter, J. and M. J. Shear, 1942/1943 / Production of tumours in mice with tars from city air dusts / J. Nat. Cancer Inst., 3, 167-175

Lewtas, J., J. Chuang, M. Nishioka and B. Petersen, 1990 / Bioassay-directed fractionation of the organic extract of SRM 1649 urban air particulate matter / Intern. J. Environ. Anal. Chem. 39, 245-256

Ligocki, M. and J. F. Pankow, 1989 / Measurements of the gas/particle distributions of organic compounds / Envir. Sci. Technol. 23, 75-83

Lindskog, A., 1983 / Transformation of polycyclic aromatic hydrocarbons during sampling / Environ. Health Perspect. 47, 81-84

Liotta, L. A., 1992 / Cancer cell invasion and metastasis / Scientific American, Febr., 1992, 34-40

Liu, B. Y. H., D. Y. H. Pui and H. J. Fissan (eds.), 1984 / Aerosols; Science, Technology, and Industrial Applications of Airborne Particles / Elsevier, Amsterdam

Lockard, J. M., C. J. Viau, C. Lee-Stephens, J. C. Caldwell, J. P. Wojciechowski, H. G. Enoch and P. S. Sabharwal, 1981 / Induction of sister chromatid exchanges and bacterial revertants by organic extracts of airborne particles / Environ. Mutag. 3, 671-681

Lodge, J. P., 1969 / Selections of *The Smoake of London, Two Prophecies* / Maxwell Reprint Co., Elmsford, NY

Loeb, L. A., 1991 / Mutator phenotype may be required for multistage carcinogenesis / Cancer Res. 51, 3075-3079

Lofröth, G., 1978 / Mutagenicity assay of combustion emissions / Chemosphere 10, 791-798

Lunde, G. and A. Bjørseth, 1977 / Polycyclic aromatic hydrocarbons in long-range transported aerosols / Nature 268, 518-519

Mahlum, D. D., C. W. Wright, E. K. Chess and B. W. Wilson, 1984 / Fractionation of skin tumour-initiating activity in coal liquids / Cancer Res. 44, 5176-5181

Maron, D. M. and B. N. Ames, 1983 / Revised methods for the Salmonella mutagenicity test / Mutat. Res. 113, 173-215

Marx, J., 1989 / Many gene changes found in cancer / Science 246, 1886-1388

Masclet, P., G. Mouvier and K. Nikolaou, 1986 / Relative decay index and sources of polycyclic aromatic hydrocarbons / Atmos. Environ. 20, 439-446

McCall, M. G. and N. S. Stenhouse, 1971 / Deaths from lung cancer in Australia / The Medical Journal of Australia, March 6th, 1971, 524-525

McCann, J., E. Choi, E. Yamasaki and B. N. Ames, 1975A / Detection of carcinogens as mutagens in the *Salmonella*/microsome test: assay of 300 chemicals / Proc. Nat. Acad. Sci. USA 72, 5135-5139

McCann, J., N. E. Spingarn, J. Kobori and B. N. Ames, 1975B / Detection of carcinogens as mutagens: bacterial strains with R factor plasmids / Proc. Nat. Acad. Sci. (USA) 72, 979-983

McCann, J. and B. N. Ames, 1976 / Detection of carcinogens as mutagens in the *Salmonella*/microsome test: assay of 300 chemicals: discussion / Proceedings of the National Academy of Sciences USA 73, 950-954

McDow, S. R. and J. J. Huntzicker, 1990 / Vapour adsorption artifact in the sampling of aerosol: face velocity effects / Atmos. Environ. 24A, 2563-2571

Miguel, A. H., 1984 / "Atmospheric" reactivity of particulate polycyclic aromatic hydrocarbons collected in an urban tunnel / Sci. Total Environ. 36, 305-311

Miguel A. H. and P. A. P. Pereira, 1989 / Benzo(k)fluoranthene, benzo(ghi)perylene, and indeno(1,2,3-cd)pyrene: new tracers of automotive emissions in receptor modelling / Aerosol Sci. Technol. 10, 292-295

Miller, 1970 / Carcinogenesis by chemicals: an overview - G. H. A. Clowes memorial lecture / Cancer Res. 30, 559-576

Mohn, G. R., 1980 / On the correlation between mutagenicity and carcinogenicity / In: Cleton F. J. and J. W. I. M. Simons, eds., Genetic Origins of Tumour Cells / Martinus Nijhoff, The Hague

Møller, L., J. Rafter, S. Törnquist, L. Eriksson, Beije, B., R. Toftgård, T. Midvedt, M. Corrie and J.-Å. Gustafsson, 1990 / In vivo metabolism and genotoxic effects of the air pollutant and marker for nitro-PAH's, 2-nitrofluorene / In: Howard, P. C. et al., ed., Nitroarenes / Plenum, New York

Møller, M. and I. Alfheim, 1980 / Mutagenicity and PAH-analysis of airborne particulate matter / Atmos. Environ. 14, 83-88

Møller, M. and I. Alfheim, 1983 / Mutagenicity of air samples from various combustion sources / Mutation Res. 116, 35-46

Montizaan, G. K., P. G. N. Kramers, J. A. Janus and R. Posthumus, 1989 / Integrated Criteria Document PAH: Effects of 10 Selected Compounds / RIVM (National Institute of Public Health and Environmental Protection), appendix to report 758474-011

MPV88, 1987 / Milieuprogramma 1988-1991, voortgangsrapportage / Tweede Kamer, vergaderjaar 1987-1988, 20202, nrs. 1-2

Mücke, W. and H. Fiedler, 1990 / Nitro-PAK - Bildung, Umweltverhalten und Wirkungen / Z. Umweltchem. Ökotox. 2, 170-176

Mumford, J. L., Z. Z. He, R. S. Chapman, R. S. Rao, D. B. Harris, X. M. Li, Y. L. Xian, W. Z. Jiang and C. W. Xu, 1987 / Lung cancer and indoor air pollution / Science 235, 217-220

Neff, J. M., 1979 / Polycyclic Aromatic Hydrocarbons in the Aquatic Environment: Sources, Fates and Biological Effects/ Applied Science Publ., London

Nicholson, G. L., 1987 / Tumour cell instability, diversification and progression to the metastatic phenotype: from oncogene to oncofetal expression / Cancer Res. 47, 1473-1487

Nielsen, T., T. Ramdahl and A. Bjørseth, 1983 / The fate of airborne polycyclic matter / Environ. Health Perspect. 47, 103-114

Nielsen, T., 1984 / Reactivity of polycyclic aromatic hydrocarbons towards nitrating species / Environ. Sci. Technol. 18, 157-163

Nielsen, T., B. Seitz and T. Ramdahl, 1984 / Occurrence of nitro-PAH in the atmosphere in a rural area / Atmos. Environ. 18, 2159-2165

Nielsen, T. and T. Ramdahl, 1986 / Discussion on "Determination of 2-nitrofluoranthene and 2-nitropyrene in ambient particulate matter: evidence for atmospheric reactions" / Atmos. Environ. 20, 1507

Nielsen, T., 1988 / The decay of benzo(a)pyrene and cyclopenta(c,d)pyrene in the atmosphere / Atmos. Environ. 22, 2249-2254

Nikolaou, K., P. Masclet and G. Mouvier, 1984 / Sources and chemical reactivity of polynuclear aromatic hydrocarbons in the atmosphere - a critical review / Sci. Total Environ. 32, 103-132

Nishioka, M. G., C. C. Howard and J. Lewtas, 1986 / Detection of hydroxy-nitro PAH's and nitro-PAH's in ambient air particulate matter using bioassay directed fractionation / In: Cooke, M. and A. J. Dennis, eds, Polynuclear Aromatic Hydrocarbons: Chemistry, Characterization and Carcinogenesis / Batelle Press

Nowell, P. C., 1976 / The clonal evolution of tumour cell populations / Science 194, 23-28

Nowell, P. C., 1986 / Mechanisms of tumour progression / Cancer Res. 46, 2203-2207

NRC (National Research Council), 1972 / Particulate Polycyclic Organic Matter / National Academy of Sciences, Washington

NRC (National Research Council) 1979 / Airborne Particles / University Park Press, Baltimore

NRC (National Research Council), 1983 / Polycyclic Aromatic Hydrocarbons, Evaluation of Sources and Effects / National Aca demy Press, Washington

Parri, B., U. Laureni, A. Babrudi, A. Callareta, S. Venturini, R. Ferri, M. Carrozzi, F. Burlini and C. Monti-Bragadin, 1983 / Mutagenicity test of extracts of airborne dust from the municipal incinerator of Trieste / Environ. Mutag. 5, 23-32

Passey, R. D., 1922 / Experimental soot cancer / Br. J. Med. 2, 1112-1113

Pellizari, E. D., L. W. Little, C. Sparacino, T. J. Hughes, L. Claxton and M. D. Waters, 1978 / Integrating microbiological and chemical testing into the screening of air samples for potential mutagenicity / In: Application of Short-Term Bioassays in the Fractionation and Analysis of Complex Mixtures / EPA Publications, 600/9-78-027

Peraino, C. and C. A. Jones, 1989 / The multistage concept of carcinogenesis / In: Sirica, A. E., ed., The Pathobiology of Neoplasia / Plenum, New York

Peters, J., and B. Seifert, 1980 / Losses of benzo(a)pyrene under the conditions of high-volume sampling / Atmos. Environ. 14, 117-119

Phillips, D. H., 1983 / Fifty years of benzo(a)pyrene / Nature, 303, 468-472

Pitot, H. C., 1989 / Progression; the terminal stage in carcinogenesis / Japanese Journal of Cancer Research 80, 599-607

Pitts, J. N., D. Grosjean and T. M. Mischke, 1977 / Mutagenic activity of airborne particulate organic pollutants / Toxicol. Let. 1, 65-70

Pitts, J. N., K. A. van Cauwenberghe, D. Grosjean, J. P. Schmid, D. R. Fitz, W. L. Belser, G. Knudsen and P. M. Hynds, 1978 / Atmospheric reactions of polycyclic aromatichydrocarbons: facile formation of mutagenic nitro derivatives / Science 202, 515-519

Pitts, J. N., D. M. Lokensgard, P. S. Ripley, K. A. van Cauwenberghe, L. van Vaeck, S. D. Shaffer, A. J. Thill and W. Belser, 1980 / "Atmospheric" epoxidation of benzo(a)pyrene by ozone: formation of the metabolite benzo(a)pyrene-4,5-oxide / Science 210, 1347-1349

Pitts, J. N., W. Harger, D. Lokensgard, D. R. Fitz, G. M. Scorziell and V. Mejia, 1982A / Diurnal variations in the mutagen icity of airborne particulate matter in Calif ornia's south-coast air basins / Mutat. Res. 104, 35-41

Pitts, J. N., J. W. Sweetman, W. Harger, D. R. Fitz, H.-R. Paur and A. M. Winer, 1982B / Diurnal variations of airborne particulate organic matter adjacent to a heavily travelled west Los Angeles freeway / J. Air Pollut. Control Assoc. 35, 638-643

Pitts, J. N., 1983 / Formation and fate of gaseous and particulate mutagens and carcinogens in real and simulated atmospheres / Environ. Health Perspect. 47, 103-114

Pitts, J. N., J. A. Sweetman, B. Zielinska, A. M. Winer and R. Atkinson, 1985 / Determination of 2-nitrofluoranthene and 2-nitropyrene in ambient particulate matter: evidence for atmospheric reactions / Atmos. Environ. 19, 1601-1608

Pitts, 1987 / Nitration of gaseous polycyclic aromatic hydrocarbons in simulated and ambient urban atmospheres: a source of mutagenic nitroarenes / Atmos. Environ. 21, 2531-2547

Pott, F., R. Tomingas, A. Brockhaus and F. Huth, 1980 / Studies on the tumorigenicity of extracts and their fractions of airborne particulates with the subcutaneous test in the mouse / Zbl. Bakt., I. Abt. Orig. B 170, 17-34

Pott, P., 1775 / Chirurgical Observations Relative to the Cataract, the Polypus of the Nose, the Cancer of the Scrotum, the Different Kinds of Ruptures, and the Mortification of the Toes and Feet / Printed by T. J. Carnegie, London

Pyysalo, H., J. Tuominen, K. Wickström, E. Skytta, L. Tikkanen, S. Salomaa, M. Sorsa, T. Nurmela, T. Matiila and V. Pohjola, 1987 / Polycyclic organic material (pom) in urban air; fractionation, chemical analysis and genotoxicity of particulate and vapour phases in an industrial town in Finland / Atmos. Environ. 21, 1167-1180

Ramdahl, T., G. Becher and A. Bjørseth, 1982 / Nitrated polycyclic aromatic hydrocarbons in urban air particles / Environ. Sci. Technol. 16, 861-865

Ramdahl, T., B. Zielinska, J. Arey, R. Atkinson, A. M. Winer and J. N. Pitts, 1986 / Ubiquitous occurrence of 2-nitrofluoranthene and 2-nitropyrene in air / Nature 321, 425-427

Richardson, K. E., P. P. Fu and C. E. Cerniglia, 1988 / Metabolism of 1-, 3-, and 6-nitrobenzo[a]pyrene by intestinal flora / J. Toxicol. Environ. Health 23, 527-537

Rinkus, S. J. and M. S. Legator, 1979 / Chemical characterization of 465 known or suspected carcinogens and their correlation with mutagenic activity in the *Salmonella typhimurium* system / Cancer Res. 39, 3289-3318

Risby, T. H. and S. S. Lestz, 1983 / Is the direct mutagenic activity of diesel particulate matter a sampling artifact? / Environ. Sci. Technol. 17, 621-624

Rosenkranz, H. S. and R. Mermelstein, 1983 / Mutagenicity and genotoxicity of nitroarenes: all nitro-containing chemicals were not created equal / Mutat. Res. 114, 217-267

Rosenkranz, H. S. and R. Mermelstein, 1985 / The genotoxicity, metabolism and carcinogenicity of nitrated aromatic hydrocarbons / J. Environ. Sci. Health C3, 221-272

Rowley, J. D., 1990 / Molecular cytogenetics: Rosetta stone for understanding cancer; twenty-ninth G. H. A. Clowes Memorial Award Lecture / Cancer Res. 50, 3816-3825

Santodonato, J., P. Howard and D Basu, 1981 / Health and ecological assessment of polynuclear aromatic hydrocarbons / J. Environ. Pathol. Toxicol. 5, 1-372

Sawicki, E., 1967 / Airborne carcinogens and allied compounds / Arch. Environ. Health, 14, 46-53

Schuetzle, D. and J. M. Perez, 1983 / Factors influencing the emissions of nitrated-polynuclear aromatic hydrocarbons (nitro-PAH) from diesel engines / J. Air Pollut. Control Assoc. 33, 751-755

Schuetzle, D. and J. Lewtas, 1986 / Bioassay-directed chemical analysis in environmental research / Anal. Chem., 58, 1060A-1075A

Schürer, C.-C, N. Manojlovic and N. H. Seemayer, 1980 / Induction of sister chromatid exchange in human cells in vitro by mutagenic effect of city smog / Mutat. Res. 74, 164-165

Seemayer, N. H., N. Manojlovic, C.-C. Schürer and R. Tomingas, 1984 / Cell cultures as a tool for detection of cytotoxic, mutagenic and carcinogenic activity of airborne particulate matter / J. Aerosol Sci 15, 426-430

Seemayer, N. H., W. Hadnagy, R. Tomingas and N. Manojlovic, 1987 / Cell- and genotoxic activities of airborne particulates from a highly industrialized region: a survey over a period of 11 years (1975-1986) / J. Aerosol Sci. 18, 721-724

Selzer Madsen, E., P. A. Nielsen and J. C. Pedersen, 1982 / The distribution and origin of mutagens in airborne particulates, detected by the Salmonella/microsome assay in relation to levels of lead, vanadium and PAH / Sci. Total Environ. 24, 13-25

Siak, J., T. L. Chan, T. L. Gibson and G. T. Wolff, 1985 / Contribution to bacterial mutagenicity from nitro-PAH compounds in ambient aerosols / Atmos. Environ. 19, 369-376

Slooff, W., J. A. Janus, A. J. C. M. Matthijsen, G. K. Montizaan and J. P. M. Ros, 1989 / Integrated Criteria Document PAHs / RIVM (National Institute of Public Health and Environmental Protection), report 758474011

Sortland Olufsen, B. and A. Bjørseth, 1983 / Analysis of Polycyclic aromatic hydrocarbons by gas chromatography / In: Bjørseth, A., ed., Handbook of Polycyclic Aromatic Hydrocarbons / Marcel Dekker, New York

Stanley, T. W., J. E. Meeker and M. J. Morgan, 1967 / Extraction of organics from airborne particulates / Effects of various solvents and conditions on the recovery of benzo(a)pyrene, benz(a)acridine and 7H-benz(d,e)-anthracene-7-one / Env. Sci. Technol. 1, 927-931

Stocks, P., 1960 / On the relations between atmospheric pollution in urban and rural localities and mortality from cancer, bronchitis and pneumonia, with particular refer ence to 3:4 benzopyrene, beryllium, molybdenum, vanadium and arsenic / Br. J. Cancer, 14, 397-418

TA-Luft, 1986 / Technische Anleitung zur Reinhaltung der Luft / Bonn, 27 februar 1986

Talcott, R. and E. T. Wei, 1977 / Airborne mutagens bioassayed in *Salmonella typhimurium* / J. Natl. Cancer Inst. 58, 449-451

Tanner, R. L. and R. F. Fajer, 1983 / Determination of nitro-polynuclear aromatics in ambient aerosol samples / Intern. J. Environ. Anal. Chem. 14, 231-241

Task Group on Lung Dynamics, 1966 / Deposition and retention models for internal dosimetry of the human respiratory tract / Health Phys. 12, 173-207

Tennant, R. W., J. W. Spalding, S. Stasiewicz, W. D. Casparay, J. M. Mason and M. A. Resnick, 1987 / Comparative evaluation of genetic toxicity patterns of carcinogens and noncarcinogens: strategies for predictive use of short-term assays / Environ. Health Perspect. 75, 87-95

Teranishi, K., K. Hamada and H. Watanabe, 1978 / Mutagenicity in *Salmonella typhimurium* mutants of the benzene-soluble organic matter derived from air-borne particulate matter and its five fractions / Mutat. Res. 56, 273-280

Thijsse, Th. R. and C. Huygen, 1985 / Onderzoek naar de grootschalige achtergrondconcentraties van spoorelementen- en verbindingen in de Nederlandse buitenlucht / Luchtverontreiniging ten gevolge van de uitworp van kolengestookte centrales, deelrapport 2 / (In Dutch with an English summary) / Report R85/272 of Institute of Environmental Sciences TNO, Delft / PEO, Utrecht

Thrane, K. E. and A. Mikalsen, 1981 / High-volume sampling of airborne polycyclic aromatic hydrocarbons using glass-fibre filters and polyurethane foam / Atmos. Environ. 15, 909-918

Tokiwa, H., S. Kitamori, K. Takahashi and Y. Onishi, 1980 / Mutagenic and chemical assay of extracts of airborne particulates / Mutat. Res. 77, 99-108

Tokiwa, H. and Y. Onishi, 1986 / Mutagenicity and carcinogenicity of nitroarenes and their sources in the environment / CRC Critical Reviews in Toxicology 17, 23-60

Tomingas, R., F. Pott and G. Voltmer, 1978 / Profiles of polycyclic aromatic hydrocarbons in suspended particles of different cities in the western federal republic of Germany / Zbl. Bakt. Hyg., I. Abt. Orig. B166 322-331

Travis, C. C. and H. Belefant, 1992 / Promotion as a factor in carcinogenesis / Toxicol. Lett. 60, 1-9

Valerio, F., P. Bottino and D. Ugolini, 1984 / Chemical and photochemical degradation of polycyclic aromatic hydrocarbons in the atmosphere / Sci. Total Environ. 40, 169-188

Van Aalst, R. M., R. A. M. van Ardenne, J. F. de Kreuk and Th. Lems, 1982 / Pollution of the North Sea from the atmosphere / Report CL 82/152 / Institute of Environmental Sciences TNO, Delft

Van Cauwenberghe, K. A., L. van Vaeck and J. N. Pitts, 1980 / Physical and chemical transformations of organic pollutants during aerosol sampling / In: Versino, B. and H. Ott, eds, Proceedings of the First European Symposium on the Physico-Chemical Behaviour of Atmospheric Pollutants / Commission of The European Communities

Van Cauwenberghe, K. and L. van Vaeck, 1983 / Toxicological implications of the organic fraction of aerosols; a chemist's view / Mutat. Res. 116 1-20

Van Cauwenberghe, K. A., 1985 / Sampling, size distribution, reactivity and analysis of organic aerosol constituents / In: Lee, S. D., T. Schneider, L. D. Grant and P. J. Verkerk, eds., Aerosols: Research, Risk Assessment and Control Strategies / Lewis

Van der Hoeven, N., S. A. L. M. Kooijman and W. K. de Raat, 1990 / Salmonella test: relation between mutagenicity and number of revertant colonies / Mutat. Res. 234, 289-302

Van Houdt, J. J., P. W. H. G. Coenen, G. M. Alink, J. S. M. Boleij and J. H. Koeman, 1988 / Organ specific metabolic activation of five extracts of indoor and outdoor particulate matter / Arch. Toxicol. 61, 213-217

Van Vaeck, L. and K. van Cauwenberghe, 1978 / Cascade impactor measurements of the size-distribution of the major classes of organic pollutants in atmospheric particulate matter / Atmos. Environ. 12, 2229-2239

Van Vaeck, L., G. Broddin, W. Cautreels and K. van Cauwenberghe, 1979 / Aerosol collection by cascade impaction and filtration: influence of different sampling systems on the measured organic pollutant levels / Sci. Total Environ 11, 41-52

Van Vaeck, L., K. van Cauwenberghe and J. Janssens, 1984 / The gas-particle distribution of organic aerosol constituents: measurement of the volatilisation artifact in hivol cascade impactor sampling / Atmos. Environ. 18, 417-430

Varmus, H. E., 1984 / The molecular genetics of cellular oncogenes / Ann. Rev. Genet. 18, 553-612

Verhoeve, P and H. Compaan, 1980 / Polycyclische aromatische koolwaterstoffen in het Nederlandse milieu (In Dutch) / Report CL 80/37 / Institute of Environmental Sciences TNO, Delft

Viau, C. J., S. M. Sherman and P. S. Sabharwal, 1982 / Comparative extraction of genotoxic components of air particulates with several solvent systems / Mutat. Res. 103, 133-137

Walker, R. D., T. H. Conner, E. J. MacDonald, N. M. Trieff, M. S. Legator, K. W. MacKenzie and J. G. Dobbins, 1982 / Correlation of mutagenic assessment of Houston air particulate extracts in relation to lung cancer mortality rates / Environ. Res. 28, 303-312

Waller, R. E., 1952 / The benzpyrene content of town air / Br. J. Cancer, 6, 8-21

Wang, C. Y., M. S. Lee, C. M. King and P. O. Warner, 1980 / Evidence for nitroaromatics as direct-acting mutagens of airborne particulates / Chemosphere 9, 83-87

Watts, R. R., A. J. Hoffman, M. C. Wilkins, D. E. House, R. M. Burton, L. R. Brooks and S. H. Warren, 1992 /Evaluation of high volume particle sampling and sampling protocols for ambient urban air mutagenicity determinations / J. Air Waste Manage. Assoc. 42, 49-55

Wei, C.-J., O. G. Raabe and L. S. Rosenblatt, 1982 / Microbial detection of mutagenic nitro-organic compounds in filtrates of coal fly-ash / Environ. Mutag. 4, 249-258

Wei, E. T. and H. P. Shu, 1983 / Nitroaromatic carcinogens in diesel soot: a review of laboratory findings / Am. J. Public Health 73, 1085-1088

Weinstein, I. B., 1992 / Toxicity, cell proliferation, and carcinogenesis / Molecular Carcinogenesis 5, 2-3

Westerholm, R., U. Stenberg and T. Alsberg, 1988 / Some aspects of the distribution of polycyclic aromatic hydrocarbons (PAH) between particles and gas phase from diluted gasoline exhausts generated with the use of a dilution tunnel, and its validity for measurement in ambient air / Atmos. Environ. 22, 1005-1010

Westin, 1989 / Oncogenes / In: Sirica, A. E., ed., The Pathobiology of Neoplasia / Plenum, New York

Whelpdale, D. M. and R. E. Munn, 1977 / Global Sources, Sinks and Transport of Air Pollution / In: Stern, A. C., ed, Air Pollution, Air Pollutants, Their Transformation and Transport / Academic Press, New York

Whitby, K. T., 1978 / The physical characteristics of sulphur aerosols / Atmos. Environ. 12, 135-159

Wiedemann, L. M. and G. J. Morgan, 1992 / How are cancer associated genes activated or inactivated / Eur. J. Cancer 28, 248-251

Willeke, K. and K. T. Whitby, 1975 / Atmospheric aerosols: size distribution interpretation / J. Air Pollut. Control Assoc. 25, 529-534

Wise, S. A., 1983 / High-performance liquid chromatography for the determination of polycyclic aromatic hydrocarbons In: Bjør seth, A., ed., Handbook of Polycyclic Aro matic Hydrocarbons / Marcel Dekker, New York

Wise, S. A., 1985 / Recent progress in the determination of PAH; high performance liquid chromatography / In: Bjørseth, A. and Th. Ramdahl, eds, Handbook of Polycyclic Aromatic Hydrocarbons, Vol. 2, Emission Sources and Recent Progress in Analytical Chemistry / Marcel Dekker, New York

Wynder, E. L. and E. Hammond, 1962 / A study of air pollution carcinogenesis; I. Analysis of epidemiological evidence / Cancer 15, 79-92

Wynder, E. L. and D. Hoffmann, 1965 / Some laboratory and epidemiological aspects of air pollution carcinogenesis / J. Air Pollut. Control Assoc. 15, 155-158

Xu, X. B., J. P. Nachtman, Z. L. Jin, E. T. Wei and S. M. Rappaport, 1982 / Isolation and identification of mutagenic nitro-PAH in diesel-exhaust particles / Anal. Chim. Acta 136, 163-174

Xu, Z.-Y., W. J. Blot, H.-P. Xiao, A. Wu, Y.-P. Feng, B. J, Stone, J. Sun, A. G. Ershaw, B. E. Henderson and J. F. Fraumeni, 1989 / Smoking, air pollution, and the high rates of lung cancer in Shenyang, China / J. Nat. Cancer Inst. 81, 1800-1806

Yamagiwa, K. and K. Ichikawa, 1918 / Experimental study of the pathogenesis of carcinoma / J. Cancer. Res. 3, 1-21

Yamasaki, H., M. Mesnil and H. Nakazawa, 1992 / Interaction and distinction of genotoxic and non-genotoxic events in carcinogenesis / Toxicol. Lett. 64-65, 597-604

Yamasaki, H., K. Kuwata and H. Miyato, 1983 / Effects of ambient temperature on aspects of airborne polycyclic aromatic hydrocarbons / Environ. Sci. Technol. 16, 189-194

Yang, S. K., J. Deutsch and H. V. Gelboin, 1978 / Benzo(a)pyrene metabolism: activation and detoxification / In: Gelboin, H. V. and P. O. P. Ts'o, Polycyclic Hydrocarbons and Cancer, Vol. 1 / Academic Press, New York

Zeiger, E. and R. W. Tennant, 1986 / Mutagenesis, clastogenesis and carcinogenesis: expectations, correlations and relations / In: Genetic Toxicology of Environmental Chemicals, Part B: Genetic Effects and Applied Mutagenesis / Alan R. Liss, New York

SUMMARY

Introduction

The studies presented in this thesis find their starting point and motivation in the concern about the possible carcinogenic properties of ambient airborne particles. Several lines of evidence point to the pollution of ambient air with particulate carcinogens by combustion processes. Clear carcinogenic effects are observed when extracts of the particles emitted by combustion processes and those present in the air are tested in experimental animals, while epidemiological studies suggest a carcinogenic effect of the former in humans as well. The carcinogenic effects of both types of particles are attributed to the presence of polycyclic aromatic hydrocarbons (PAH) and related compounds, which are products of the incomplete combustion of fossil fuels and other organic materials. Consequently, PAH have received much attention in air-pollution research, first, because of their own contribution to the carcinogenicity of the particles and second, because they are regarded as indicators for the presence in the air of the complete group of combustion-related and possibly carcinogenic polycyclic compounds.

Ambient airborne particles are also shown to have mutagenic properties, the significance of which is largely determined by the predominant role of mutations in the process of carcinogenesis. Cancer is in most, if not all cases initiated by mutations and mutagenic compounds may, therefore, have carcinogenic effects as well. Mutagenicity can thus be used as a sort of indicator for the presence of possible carcinogens in air-pollution research, supplementary to the concentrations of known or suspected carcinogens. Its practical use is in particular determined by the experimental convenience of a number of test methods for mutagenicity, which makes it possible to investigate the temporal and spatial distribution of mutagenicity in the air alongside the concentrations of possible carcinogens.

The present thesis is concerned with both, the mutagenicity of ambient airborne particles and the presence of PAH in these particles. It consists of a series of theoretical and experimental studies focussed on one or more of the following aspects:

- human-toxicological significance and indicative value,
- possibilities and limitations of methods,

- spatial and temporal distribution in The Netherlands,
- nature and diversity of the mutagens, and
- relation between mutagenicity and presence of PAH.

In the experimental studies ambient airborne particles were collected by high-volume filtration on glass-fibre filters; extracts of the particles in organic solvents were tested for mutagenicity with the Salmonella/microsome test and analyzed for PAH with high-performance liquid chromatography.

Human-toxicological significance

The obvious conclusion is reached, that a direct correlation between the mutagenicity, as it is determined by testing an extract of the particles in a bacterial short-term mutagenicity test, and respiratory-tract cancer after exposure of humans to the complete particles via inhalation, cannot be deduced from a comparison of the experimental situation and the human in vivo situation. Important reasons for this are the test specificity of mutagenicity, the species, organ and tissue specificity of carcinogenicity, and the multi-step character of carcinogenesis, initiation by mutation being the first step, followed by promotion and progression. Furthermore, differences in exposure conditions are important. Although the respirable particles can completely be collected by filtration, mutagenicity testing requires them to be extracted with organic solvents, followed by mixing of the extract with an aqueous bacterial medium. Obviously, this experimental procedure cannot be regarded as a good model for human exposure via inhalation of the complete particles present in their natural matrix: ambient air.

However, this is not to say that a correlation can be excluded a priori; it may, nevertheless, exist. It may directly be revealed by epidemiological studies or in a more indirect way by testing the particles or their mutagenic components in advanced experimental systems aimed at bridging the extrapolation gap between effects of organic extracts in the Salmonella/microsome test and induction of human respiratory-tract cancer upon inhalation of the particles. Although both types of studies have been carried out, they do not allow for definitive conclusions about the existence of the correlation.

The uncertainty about the correlation does not really undermine the validity of mutagenicity of the particles as an air-pollution indicator. It contributes in a more indirect way to our understanding of the risk. It helps to elucidate the nature and intensity of exposure to a complex mixture of polycyclic compounds with proven experimental and human carcinogenicity,

thereby, it helps to define starting points for regulatory measures and further research aimed at bridging the extrapolation gap. In any case, it has lead to a complete reassessment of the "position" of the PAH in air-pollution research by demonstrating the importance of other compounds, most notably, nitrated derivatives of PAH.

As for the risk caused by the presence of the PAH themselves, it is concluded, that inhalation of the particles most probably represents a human respiratory cancer risk due to the presence of these compounds alone, at least if we accept the absence of a threshold dose level. A better understanding of risk requires in the first place investigations into the carcinogenic effects of PAH at very low dose levels in experimental biological systems.

Possibilities and limitations of mutagenicity tests

Two important differences between mutagenicity testing in environmental studies and most other studies are identified. First, most environmental samples consist of complex mixtures with an unknown and variable composition, while well defined samples, mostly pure compounds are tested in other studies. Second, quantitative comparison of series of samples is required in environmental studies, while most other applications of the tests are aimed at getting a qualitative indication of the mutagenicity of isolated samples. The first difference implies, that in environmental studies the effects may much stronger be influenced by interactions among the mutagens and between mutagens and non-mutagenic compounds. If these interactions are too test specific and vary too independently of the primary mutagenicity, mutagenicity testing in environmental studies loses its sense. Various strategies to adress this limitation of mutagenicity testing are proposed, among them direct investigation of interactions by means of bioassay-directed fractionation[27], restriction of testing to samples with a similar composition and selection of tests with a specific low sensitivity to the non-mutagenic toxicity of the samples that have to be investigated. Quantitative comparison of samples (the second difference) implies that the tests have to fulfil certain requirements as regards discriminating power and reproducibility. Here we arrive at an inherent weakness of mutagenicity tests in this context. No real positive controls can be applied, as the composition of the samples to be compared is in principle unknown and, what is more important, variable. So intra-test, inter-test and inter-laboratory

[27] By comparing the mutagenic effects of original extracts, extracts reconstituted from fractions and the sums of the effects of fractions.

variation cannot completely be compensated for by monitoring the sensitivity of the test for the specific sample to be tested. This problem severely restricts the applicability of mutagenicity in air-pollution research. Statements about the mutagenicity in absolute quantitative terms are characterized by a wide margin of uncertainty and in this respect mutagenicity is clearly inferior to analytical chemical indicators, such as PAH concentrations.

PAH as indicator compounds for monitoring purposes

The results of three experimental studies, in which the temporal and spatial distribution of PAH concentrations and mutagenicity was investigated (see below), allow for a provisional evaluation of particulate PAH as indicator compounds for monitoring purposes. It is concluded, that in The Netherlands the common variation of the PAH is strong enough to select only one of them as indicator compound, for instance benzo(a)pyrene. The common variation of the mutagenic effects was taken as a measure for the common variation of the complete group of possibly carcinogenic combustion-related polycyclics and could thus be used to validate the PAH as indicator compounds with respect to these compounds. It is suggested by one study that the indicative value of the PAH can indeed be extended to these compounds. However, the other two studies yielded a less clear indicative value in this respect. It must be emphasized, that the four effects determined with the Salmonella/microsome test are but a meagre basis for the estimation of the common variation of the other polycyclic compounds. A more extensive qualitative differentiation of the mutagenicity is required for this purpose.

Sampling artifacts

The technique used to collect the particles (high-volume filtration over glass-fibre filters) leads to a drastic change in their exposure to air components which may in its turn lead to physical as well as chemical changes. Artifactual evaporation of particulate compounds and adsorption of gaseous compounds have been well established for the PAH; they may lead to under- or overestimation of the concentrations of the actual concentrations in the particles. Furthermore, chemical conversion of compounds may take place. Several studies suggest that the PAH concentrations based on this sampling technique may also be affected by this artifact. Only sporadic attempts have been made to investigate the influence of sampling on mutagenicity, with contradictory results. However, artifactual conversion of PAH makes such research urgently needed, because the conversion products can be highly

mutagenic and because the original mutagens are probably chemically related to the PAH and may, therefore, be sensitive as well.

In general, sampling artifacts are investigated by comparison of samples taken under sampling conditions which are supposed to influence evaporation, adsorption or chemical conversion differently. One of these conditions is the filter material used. It has been shown, that artifactual chemical conversion of a number of compounds, among them PAH, is stimulated or induced by the use of glass-fibre filters and quartz-fibre filters. Some studies strongly suggest that sampling with these filters leads indeed to lower concentrations of PAH than supposedly more inert filters made of Teflon or Teflon-coated glass fibres. If the conversion of PAH is indeed accompanied by a change of mutagenicity, sampling with these filter types must lead to different mutagenic effects. This was investigated in the study presented in this thesis by simultaneously sampling with glass-fibre (G) filters and Teflon (T) or Teflon-coated (Tc) filters. A detail which appeared to be important afterwards, was the use of G filters to support the fragile T and Tc filters.

Slightly higher mutagenicity was found for the G filters. The difference was too small to justify a drastic re-evaluation of the results obtained with G filters so far, and it is improbable that the use of these filters would have lead to significantly other results in the studies presented in this thesis. The difference could be explained by adsorption of gaseous PAH to the filter material and subsequent chemical conversion, as gaseous PAH as well as mutagenicity were found in the support filters, the presence of which could not be the result of incomplete retention by the upper filter. A slight conversion of PAH on G filters was indicated by the lower amounts of the more reactive PAH on these filters. So, it remains unclear whether a stronger artifactual conversion of these compounds, as has been reported in the literature, would have been accompanied by changes of mutagenicity.

Gaseous mutagens and PAH

It has amply been demonstrated, that, besides particulate PAH, gaseous PAH may be present in the air in relatively high concentrations. The objective of one study was to investigate whether the presence of these compounds is, like that of their particulate counterparts, accompanied with mutagenicity. To this end sampling was carried out with a filter to collect the particles and a polyurethane plug to adsorb the gaseous compounds passing the filter. Substantial amounts of PAH were indeed collected with the adsorbent. The mutagenicity results were less unambiguous, due to a considerable background

mutagenicity of the adsorbent, which increased during sampling. However, the background mutagenicity appeared to depend on the extraction solvent. It could completely be evaded by the use of acetone. With this solvent no mutagenicity could be demonstrated in the adsorbent, notwithstanding high mutagenicity on the filter and large amounts of gaseous PAH in the adsorbent. The interpretation of this result depends strongly on the adsorption characteristics of the adsorbent. However, chemical analysis makes clear that most gaseous polycyclic compounds associated with the presence of PAH will to a great extent be trapped by the adsorbent.

Temporal and spatial distribution

The temporal and spatial distribution of mutagenicity and PAH concentrations was investigated in three studies. In the first, (the Rijnmond Study) the contribution of sources in the urban and industrialized Rijnmond area was investigated by comparison of samples taken simultaneously at four locations in the area and at one coastal location upwind, which was assumed to represent the background level of the area. A substantial contribution to mutagenicity and PAH concentrations by sources in the area was observed; at the prevailing wind direction (south west) this contribution clearly exceeded the background levels. The mutagenic effects obtained with different variants of the test were strongly correlated; in general marginal to strong activation by S9 fraction was found depending on the tester strain. It may be concluded that the temporal and spatial distribution did not strongly depend on the variant of tester strain or the use of S9 fraction. Nevertheless, a weaker activation by S9 fraction was observed upwind than in the area, indicating a change of the mutagenicity upon residence of the particles in the air, which can most probably ascribed to atmospheric chemical conversion. The analysis of the temporal and spatial variation of the mutagenicity was formalized with a series of mathemathical models. These models suggested that the effects in the area are not solely the result of the addition of local emissions to the background, but that they are also influenced by interactions. The best-fitting model was used to calculate the increase of the mutagenicity due to the emissions in the area.

Multivariate analysis revealed a strong common variation for the different PAH. Deviations from this pattern were specifically associated with volatility and, to a lesser extent, with chemical reactivity. The concentrations of the more volatile PAH may be influenced by an extra variation due to sampling artifacts, variations in source pattern and atmospheric evaporation,

condensation and adsorbation. However, these possible causes cannot be singled out on the basis of the results. The variation associated with chemical reactivity may point to artifactual conversion during sampling and atmospheric conversion. The importance of the latter possibility is unambiguously indicated by the relatively lower background concentrations of the more reactive PAH.

Notwithstanding the fact that mutagens and PAH are both emitted by the same group of sources, they were only moderately correlated in this study. Selection of sampling trips with similar wind directions lead to an increase of the correlations, which shows that changes of source pattern or atmospheric chemical reactions weaken the link between mutagenicity and carcinogenicity.

In the second study (the Photochemistry Study), the particles were collected upwind and downwind of a motorway and part of the town of Delft during photochemical air pollution. Short samples were taken to allow the development of mutagenicity and PAH concentrations over the day to be followed, alongside the development of chemical parameters of photochemical air pollution. A striking difference was observed between the mutagenicity profiles (proportion of the effects determined with the different test variants) of the particles collected upwind and those emitted by the local sources (only traffic). The former showed strong inactivation upon the use of S9 which was partly compensated by activation in case of the latter, which suggests that during photochemical air pollution, atmospheric conversion has a drastic effect on the mutagenicity. The use of the nitroreductase strain TA98NR next to its parental strain TA98 unambiguously demonstrated that virtually all mutagenicity was caused by nitrocompounds, i.e. depended on the reduction of nitrogroups. Because of its drastically reduced sensitivity to the S9-independent or direct mutagenicity, this strain allowed to investigate whether S9-dependent or indirect mutagenicity was present which was obscured by the inactivation of the effect in TA98. A clear activation was observed.

No typical diurnal variation of mutagenicity was observed, quantitatively nor qualitatively, suggesting that mutagenicity was not affected by photochemical air-pollution as it developed during the day in the region of investigation. This might be explained by an already maximum chemical conversion for the particles upwind before they reach the area and a too short residence time of the particles emitted in the area. The mutagenicity observed in the presence of S9 showed a stronger link with the SO_2 concentrations than the direct mutagenicity, which suggests that the former is less strongly affected during transport in the air.

The PAH profile did not differ much from that observed in the Rijnmond Study. Again, a rather strong common variation of the PAH concentrations was indicated by multivariate analysis; the non-common variation was specifically associated with volatility and, to a lesser extent, with chemical reactivity and sources. The contribution of the local sources was characterized by relatively higher concentrations of more reactive PAH and benzo(g,h,i)-perylene. In case of the reactive PAH, this points to conversion of PAH during their residence in the air, while benzo(g,h,i)perylene signifies the contribution of traffic. The average contributions were ranging from 0% for benzo(e)pyrene to 90% for benz(a)anthracene of the concentrations observed upwind.

The correlations between mutagenicity and PAH concentrations depended on S9 fraction. In the absence of S9 fraction, only moderate correlations were observed, while its presence lead to a substantial increase. It seems probable, that this difference reflects a much stronger effect of atmospheric chemical conversion on the direct mutagenicity. This is in line with the observation that the indirect mutagenicity is stronger linked with the variation of the So_2 concentrations.

The third study[28] (the One-Year Study) differed in one important aspect from the other two. No meteorological conditions were selected for. The particles were collected weekly during one year at four locations, among them one presumed background location near the coast. The samples can, therefore, be regarded as a more or less random and aselective sample of samples that can be collected in the Netherlands over a year. On many points the results confirmed the results of the other two studies. The nitroreductase dependence of the effects was somewhat less than it was in the Photochemistry Study (70% in stead of 80%). However, this dependence is still strong enough to suggest that all direct mutagenicity depends on the reduction of nitrogroups. The difference is attributed to differences in meteorological conditions; photochemical air pollution is apparently accompanied by the formation of nitrocompounds with a less strong residual mutagenicity in TA98NR or the elimination of other nitrocompounds. Most of the samples showed activation upon the use of S9 fraction, thereby, resembling the samples of the Rijnmond Study. Activation was not stronger in the nitroreductase-dependent strain than in its parental strain, which reveals that no inactivation occurred which is

[28] This study is not described completely and in detailed form in a separate chapter in this thesis. Results which provide further elucidation of the other studies, are presented in a supplementary discussion (chapter 14). See De Raat et al. (1988) for a detailed report in Dutch.

obscured by activation. So, photochemical air pollution appears to go specifically with the formation of inactivatable compounds. It is speculated that these compounds are products of the nitration of gaseous PAH via the reaction with OH radicals.

Diversity and nature of the mutagens

In order to investigate the diversity and chemical nature of the mutagens, extracts of the particles were fractionated with a number of chromatographical techniques. The fractions were tested with different variants of the Salmonella/microsome test and analyzed for the presence of PAH and several nitro PAH. This combination of fractionation, testing and chemical analysis is often referred to as "bioassay-directed fractionation". Seven distinct groups of mutagens could be discerned. Two of them were identified as PAH and mono-nitro PAH. A significant contribution was excluded for the well known and extremely mutagenic dinitropyrenes, compounds which have been identified in ambient airborne particles by other authors as well as in a number of combustion emissions. In the presence of S9 fraction and depending on the tester strain, 5 to 20% of the mutagenicity could be attributed to PAH. The presence of mono-nitro PAH explained 13 to 24% of the mutagenicity, depending on strain as well as presence of S9 fraction.

The direct effects of the other five fractions showed a clear nitroreductase dependence. The results, therefore, strongly suggest that indeed all the direct mutagenicity in the particles is caused by nitrocompounds. The same fractions often showed a clear-cut indirect mutagenicity. Similar types of nitrocompounds could contribute to this effect, because nitro PAH can in principal be activated via two routes: reduction of the nitrogroup by the bacteria, causing the direct effect and epoxidation of aromatic rings by the S9 fraction, causing the indirect effect. In view of the probable insignificance of the first route in mammals, it is concluded that the indirect mutagenicity deserves more attention than it has received sofar. Effects in strains with a high relative sensitivity to the indirect mutagenicity, such as strain TA97, might be more relevant, than those obtained with the widely used strain TA98.

Obviously, bioassay-directed fractionation may be a powerful tool to investigate the general toxicological relevance of the mutagenicity of the particles. By testing the fractions with a series of tests, the really important groups of compounds can be distinguished from the groups with a specifically strong bacterial mutagenicity. This will lead to more purposeful investigations on the identity of mutagens that may pose a real threat to human health.

Furthermore, bioassay-directed fractionation may be used to investigate the influence of atmospheric as well as artifactual chemical conversion on the mutagenicity. These changes can directly and sensitively be visualized as shifts in the "mutagrams"[29], even when they compensate each other in the effects of the complete extract.

[29] Chromatograms prepared with mutagenicity as detection criterion.

SAMENVATTING

Inleiding

Buitenlucht bestaat niet alleen uit gasvormige stoffen, maar bevat daarnaast ook vaste stoffen en vloeistoffen, die tezamen de "deeltjesfractie" vormen. Het onderzoek dat in dit proefschrift is beschreven vindt zijn aanleiding en motivering in de zorg om de kankerverwekkende of carcinogene eigenschappen van de deeltjesfractie. Verschillende onderzoekslijnen laten zien dat de deeltjesfractie wordt verontreinigd met carcinogene stoffen door emissies van verbrandingsprocessen. Extracten van de deeltjes in buitenlucht en in emissies van verbrandingsprocessen blijken ondubbelzinnig carcinogeen te zijn wanneer ze worden onderzocht met proefdieren, terwijl epidemiologische studies hebben laten zien dat verbrandingsemissies ook voor de mens carcinogene eigenschappen kunnen hebben. De carcinogeniteit van de deeltjes in buitenlucht en verbrandingsemissies kan worden toegeschreven aan de aanwezigheid van dezelfde groep chemische stoffen, waartoe de polycyclische aromatische koolwaterstoffen (doorgaans afgekort als PAK) behoren en die verder een groot aantal andere polycyclische aromatische stoffen bevat. Deze stoffen worden gevormd tijdens de onvolledige verbranding van fossiele brandstoffen en andere materialen van organische oorsprong.

PAK hebben veel aandacht gekregen in het luchtverontreinigingsonderzoek vanwege hun bijdrage aan de carcinogeniteit van de deeltjesfractie. Ze worden verder beschouwd als indicatorstoffen voor de aanwezigheid in de buitenlucht van de complete groep polycyclische en mogelijk carcinogene stoffen die door verbrandingsprocessen worden geëmitteerd.

De deeltjes in de buitenlucht blijken ook mutagene eigenschappen te hebben. Het belang van deze bevinding is vooral gelegen in de belangrijke rol van mutaties bij het ontstaan van kanker. Kanker blijkt in de meeste, zo niet alle gevallen te worden geïnitieerd door mutaties en mutagene stoffen dienen om die reden als mogelijk carcinogene stoffen te worden beschouwd. Dit betekent dat mutageniteit in principe kan worden gebruikt als een soort van indicator voor de aanwezigheid van carcinogene stoffen in de buitenlucht, in aanvulling op de concentraties van stoffen met bewezen carcinogene eigenschappen. De praktische toepassing in deze zin in luchtverontreinigings onderzoek wordt vooral bepaald door de gevoeligheid en de experimentele eenvoud van een aantal biologische toetsmethoden voor de mutageniteit van

stoffen. Hierdoor is het mogelijk de verdeling in ruimte en tijd van de mutageniteit van de deeltjesfractie met bijna dezelfde flexibiliteit te onderzoeken als die van de concentraties van carcinogene stoffen.

In dit proefschrift komen zowel de mutageniteit van de deeltjes als de aanwezigheid van PAK en verwante verbindingen in de deeltjes aan de orde. Het is opgebouwd uit een aantal theoretische en experimentele studies die met name gericht zijn op de volgende onderwerpen:

- humaan-toxicologische betekenis,
- mogelijkheden en beperkingen van methoden,
- verspreiding in ruimte en tijd,
- chemische identiteit en diversiteit van de mutagenen en
- relatie tussen mutageniteit en aanwezigheid van PAK.

De deeltjesfractie werd in de experimentele studies verzameld door filtratie van grote hoeveelheden lucht ("high-volume sampling") over glasvezelfilters. De filters met afgevangen deeltjes werden geëxtraheerd met organische oplosmiddelen. De extracten werden vervolgens onderzocht op mutagene eigenschappen met de Salmonella/microsoomtoets ("Ames test") en geanalyseerd op de aanwezigheid van PAK met hoge-druk vloeistofchromatografie (HPLC).

Humaan-toxicologische betekenis

Een theoretische beschouwing leidt tot de volgende voor de hand liggende conclusie: een directe correlatie tussen enerzijds mutageniteit, zoals die is bepaald door het toetsen van een extract van de deeltjes in een kortdurende mutageniteitstoets met bacteriën, en anderzijds kanker in de ademhalingswegen na de blootstelling van de mens aan de complete deeltjes in de buitenlucht, niet kan worden afgeleid uit een vergelijking van de experimentele situatie en de menselijke *in-vivo* situatie. Belangrijke redenen hiervoor zijn de toetsspecificiteit van de mutageniteit, de soort-, orgaan- en weefselspecificiteit van de carcinogeniteit, en het "meer-staps-karakter" van carcinogeniteit. Verder zijn verschillen tussen de experimentele blootstelling en de blootstelling van de mens van belang. Hoewel de deeltjesfractie kan worden verzameld door filtratie, is extractie noodzakelijk om mutageniteitstoetsing mogelijk te maken, terwijl de extracten vervolgens vermengd worden met een waterig microbiologisch medium. Deze experimentele procedure mag vanzelfsprekend niet worden beschouwd als een goed model voor de blootstelling van de mens

door inademing van de complete deeltjes in hun natuurlijke matrix, de buitenlucht.

Dit betekent echter niet dat daarmee een correlatie *a priori* kan worden uitgesloten; hij zou ondanks alles toch kunnen bestaan. Of er sprake is van correlatie kan direct worden onderzocht door middel van epidemiologisch onderzoek of, op een indirecte manier, door onderzoek met geavanceerde experimentele systemen, specifiek gericht op het overbruggen van de extrapolatiekloof tussen effecten van extracten in de Salmonella/microsoomtoets en het ontstaan van kanker bij de mens tengevolge van de inademing van de deeltjes. Hoewel beide benaderingen aandacht hebben gekregen, is het (nog) niet mogelijk een definitieve conclusie te trekken over het bestaan van een correlatie.

De waarde van de mutageniteit van de deeltjes als luchtverontreinigingsindicator wordt niet volledig bepaald door het al of niet bestaan van een correlatie. Mutageniteit draagt ook op een meer indirecte wijze bij aan het inzicht in de risico's voor de mens. Door mutageniteitsonderzoek kan duidelijkheid worden verkregen over de aard en de intensiteit van de blootstelling aan een complex mengsel van polycyclische verbindingen met bewezen experimentele en humane carcinogeniteit. Daardoor helpt mutageniteit de uitgangspunten vast te stellen voor enerzijds beleidsmaatregelen en anderzijds verder onderzoek dat gericht is op het overbruggen van bovengenoemde extrapolatiekloof. Mutageniteit heeft in ieder geval geleid tot een ingrijpende herwaardering van de rol van PAK in het luchtverontreinigingsonderzoek, door te wijzen op het belang van andere verbindingen, met name de genitreerde derivaten van PAK.

Wat betreft de PAK zelf, wordt in dit proefschrift geconcludeerd dat de inhalatie van buitenlucht alleen al een kankerrisico met zich meebrengt door de aanwezigheid van deze stoffen, afgezien van de aanwezigheid van andere mogelijk carcinogene stoffen. Dit, als het ontstaan van kanker bij de mens tengevolge van de blootstelling aan PAK, zoals algemeen wordt aangenomen, geen drempelwaarde ("no-effect level") vertoont. Tegelijkertijd wordt echter deze aanname ter discussie gesteld. Er zijn redenen om te veronderstellen, dat een "no-effect level" mogelijk is, of dat de dosisafhankelijkheid in ieder geval sterk zal afwijken bij de lage concentraties waaraan de mens wordt blootgesteld bij het inademen van buitenlucht. Een beter begrip van het risico vereist dan ook in de eerste plaats onderzoek naar de carcinogeniteit van PAK bij lage blootstellingsniveaus in experimentele systemen.

Mogelijkheden en beperkingen van mutageniteitstoetsen

De toepassing van mutageniteitstoetsen in milieuonderzoek verschilt in twee opzichten fundamenteel van de toepassing in de meeste andere studies. In de eerste plaats is van belang dat de te toetsen monsters in het milieuonderzoek vrijwel altijd complexe mengsels van stoffen zijn met een onbekende en wisselende samenstelling, terwijl in de meeste andere studies goed gedefinieerde monsters of zuivere stoffen worden getoetst. Verder vereist milieuonderzoek meestal een kwantitatieve vergelijking van monsters wat betreft hun mutageniteit, terwijl in ander onderzoek doorgaans een kwalitatieve indicatie over de mutageniteit van geïsoleerde monsters volstaat.

Het eerste verschil impliceert dat in milieustudies de waargenomen effecten sterker worden beïnvloed door interacties tussen de mutagenen onderling en tussen mutagenen en niet mutagene stoffen dan in andere studies. Als deze interacties te toetsspecifiek zijn en bovendien onafhankelijk variëren van de primaire mutageniteit, verliest mutageniteitstoetsing in milieustudies zijn betekenis. Er kan op verschillende manieren met deze beperking worden rekening gehouden, bijvoorbeeld door de interacties direct te onderzoeken met "bioassay-directed fractionation"[30], door het onderzoek te beperken tot monsters die voldoende in samenstelling overeenkomen of door het gebruik van toetsen met een relatief geringe gevoeligheid voor andere toxische effecten (dan de mutageniteit).

Kwantitatieve vergelijking van de monsters (het tweede verschil) houdt in dat strengere eisen gesteld moeten worden aan het onderscheidend vermogen van de toetsen en de reproduceerbaarheid van hun uitkomsten. Een belangrijke beperkende factor hierbij is dat de variatie van de gevoeligheid van de toetsen niet goed met positieve controles kan worden onderzocht, omdat de samenstelling van de monsters onvermijdelijk onbekend zal zijn en, wat nog belangrijker is, in veel gevallen zal variëren. De intra-test-, inter-test- en interlaboratoriumvariatie van de gevoeligheid zal altijd een zekere stofafhankelijkheid vertonen, en het is daarom moeilijk een serie toetsresultaten met verschillende monsters te corrigeren voor variaties in de gevoeligheid door middel van een externe standaard in de vorm van een positieve-controlestof. Dit probleem beperkt de toepasbaarheid van de toetsen in luchtverontreinigingsonderzoek in ernstige mate. Uitspraken over de mutageniteit in

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[30] Door het vergelijken van mutagene effecten van oorspronkelijke extracten, extracten die zijn samengesteld uit analytisch-chemisch bereide fracties en de gesommeerde effecten van zulke fracties.

kwantitatieve termen worden dan ook gekenmerkt door een grote onzekerheidsmarge en in dit opzicht is mutageniteit als indicator inferieur aan analytisch-chemische indicatoren, zoals de concentraties van PAK.

PAK als indicatorstoffen voor monitoring

Een voorlopige evaluatie van de PAK als indicatorstoffen (ook wel gidsstoffen genoemd) voor monitoringsdoeleinden werd uitgevoerd aan de hand van de resultaten van een drietal studies waarin de verspreiding van deze stoffen in ruimte en tijd werd onderzocht. Dit leidde tot de conclusie dat in Nederland de gemeenschappelijke variatie van de PAK als groep zo sterk is, dat één of een beperkt aantal PAK als indicatoren voor de complete groep kunnen dienen. De gemeenschappelijke variatie van de mutagene effecten werd beschouwd als maat voor de gemeenschappelijke variatie van de gehele groep van mogelijk carcinogene polycyclische verbindingen die door verbrandingsprocessen worden geëmitteerd. Dit maakte het mogelijk de waarde van de PAK als indicatorstoffen voor deze groep te beoordelen. Eén van de studies suggereert dat de indicatieve waarde van de PAK zich inderdaad tot de gehele groep uitstrekt. De resultaten van de andere twee studies wijzen daarentegen op een meer beperkte indicatieve waarde.

Benadrukt moet worden dat de vier mutagene effecten die met de Salmonella/microsoomtoets werden bepaald, een mager uitgangspunt vormen voor het schatten van de gemeenschappelijke variatie van de gehele groep polycyclische stoffen. Een meer gedifferentieerd beeld van de mutageniteit (meer en sterker uiteenlopende effecten) is vereist, voordat op grond van de gemeenschappelijke variatie van de effecten definitieve conclusies kunnen worden getrokken over de indicatieve waarde van de PAK.

Monsternameartefacten

Tijdens de monsterneming vindt een drastische verandering plaats van de blootstelling van de deeltjes aan de lucht. Veranderingen in de samenstelling van de deeltjes zijn hiervan het gevolg. Vaste stoffen in de deeltjes verdampen en gasvormige stoffen in de lucht adsorberen aan de deeltjes of het filter. Beide processen kunnen de uiteindelijke concentraties van de PAK in de monsters beïnvloeden en kunnen leiden tot aanzienlijke over- en onderschattingen van de werkelijke concentraties in de lucht. Verder kunnen de veranderde blootstellingsomstandigheden chemische omzettingen tot gevolg hebben. Verschillende studies suggereren dat de PAK-concentraties ook voor dit artefact gevoelig zijn.

De invloed van monsternameartefacten op de mutageniteit van de deeltjes is slechts sporadisch onderzocht, met tegenstrijdige resultaten. De artefactuele omzetting van de PAK maakt zulk onderzoek echter dringend gewenst, omdat de omzettingsprodukten van PAK sterk mutageen kunnen zijn en omdat de oorspronkelijke mutagenen waarschijnlijk chemisch verwant zijn met de PAK, en daarom gevoelig zouden kunnen zijn voor vergelijkbare omzettingen.

Monsternameartefacten worden in het algemeen onderzocht door monsters te vergelijken die **tegelijkertijd** op **dezelfde locatie** zijn genomen, waarbij de monsternametechniek zodanig verschilt dat verschillen in verdamping, adsorptie of chemische omzetting verwacht mogen worden. Verschillen tussen deze monsters wat betreft PAK-concentraties of mutageniteit vormen een duidelijke aanwijzing voor het optreden van artefacten. Een belangrijk aspect van de monsternametechniek is in dit verband het toegepaste filtermateriaal. De artefactuele omzetting van PAK blijkt te worden gestimuleerd indien de deeltjes worden verzameld op glasvezel- of kwartsfilters. Sommige studies hebben aangetoond dat Teflon-filters en met Teflon geïmpregneerde filters, waarvan wordt verondersteld dat ze meer inert zijn, hogere PAK-concentraties opleveren. Als de omzetting van PAK inderdaad gepaard gaat met veranderingen van de mutageniteit, zal de monsterneming met de meer inerte filtermaterialen andere mutagene effecten tot gevolg hebben dan met de gebruikelijke glasvezelfilters.

Deze veronderstelling werd getoetst door simultane monsterneming met glasvezelfilters (G-filters) en Teflon-geïmpregneerde filters (Tg-filters) of Teflonfilters (T-filters). Een experimenteel detail dat achteraf belangrijk is gebleken, is het gebruik van onderfilters van glasvezel ter ondersteuning van de fragiele T- en Tg-filters. De mutageniteit bleek in geringe mate filterafhankelijk te zijn. Iets hogere effecten werden doorgaans gevonden met de G-filters. Het verschil was zo klein, dat geen ingrijpende herwaardering nodig is van de tot dusver vrijwel uitsluitend met G-filters verkregen gegevens over de mutageniteit van de deeltjes. Het is onwaarschijnlijk dat het gebruik van T- of Tg-filters i.p.v. G-filters bij het in dit proefschrift beschreven onderzoek tot significant verschillende resultaten zou hebben geleid.

Het verschil in mutageniteit kon worden verklaard door adsorptie van vluchtige PAK aan glasvezelfilters, gevolgd door chemische omzetting, aangezien zowel gasvormige PAK als mutageniteit aanwezig waren in de onderfilters en deze aanwezigheid niet het gevolg kon zijn van een onvolledige retentie van de deeltjes door het bovenfilter. Lagere hoeveelheden van de meer reactieve PAK op de glasvezelfilters wezen op een geringe omzetting van

PAK in de deeltjes tijdens de monsterneming. Onduidelijk blijft of een sterkere artefactuele omzetting van PAK, zoals beschreven is in de literatuur, gepaard zou zijn gegaan met een significante verandering van de mutageniteit van de deeltjes.

Gasvormige mutagenen en PAK

Naast de PAK in de deeltjesfractie, komen in de buitenlucht ook gasvormige PAK voor. De concentraties van deze stoffen kunnen aanzienlijk zijn in vergelijking met die van de PAK in de deeltjes. Onderzocht werd of de aanwezigheid van deze stoffen, evenals het geval is met hun soortgenoten in de deeltjes, gepaard gaat met mutageniteit. Daartoe werden monsters genomen met combinaties van filters om de deeltjes te verzamelen en polyurethaanschuimpluggen om de gasvormige stoffen te adsorberen. In het polyurethaanschuim werden de verwachte hoeveelheden meer vluchtige PAK aangetroffen. De uitkomsten van de mutageniteitstoetsen met extracten van de filters en het polyurethaanschuim waren minder duidelijk. Dit, vanwege de hoge achtergrondmutageniteit van het polyurethaanschuim. Wel nam de mutageniteit toe door de monsterneming. Echter, zowel de achtergrondmutageniteit als de door de monsterneming geïnduceerde mutageniteit bleek sterk afhankelijk van de keuze van het oplosmiddel. Bij toepassing van aceton in plaats van methanol of andere alcoholen bleven beide effecten achterwege, terwijl met dit oplosmiddel wel duidelijke mutageniteit en hoge PAK-concentraties in het filter werden gevonden. Geconcludeerd wordt dat de achtergrondmutageniteit en de door de monsterneming geïnduceerde mutageniteit hoogstwaarschijnlijk te wijten zijn aan een monsternameartefact; gedacht kan worden aan de vorming van verbindingen in het schuim die omgezet worden in mutagenen door reactie met alcoholen. De met aceton verkregen resultaten suggereren in sterke mate dat de gasfase van de buitenlucht in vergelijking met de deeltjes niet of nauwelijks mutagene stoffen bevat die verwant zijn met PAK.

Verspreiding in ruimte en tijd

De verspreiding van de mutageniteit en de PAK-concentraties in ruimte en tijd was onderwerp van een drietal studies. In de eerste, de Rijnmond-studie, werd de bijdrage onderzocht van bronnen in de Rijnmond aan mutageniteit en PAK-concentraties door het vergelijken van monsters die simultaan genomen werden op vier plaatsen in de Rijnmond en één plaats bovenwinds van de

Rijnmond aan de kust. Aangenomen werd dat bij wind van zee op de laatste locatie de achtergrond-deeltjesfractie van het gebied werd bemonsterd.

Er werd een aanzienlijke bijdrage van de bronnen in de Rijnmond waargenomen aan zowel de mutageniteit als de PAK-concentraties. Bij de overheersende windrichting bleken de lokale bronnen veel belangrijker dan de achtergrond. De met de vier verschillende varianten van de Salmonella/microsoomtoets waargenomen effecten vertoonden een sterke onderlinge correlatie. Afhankelijk van de Salmonella-stam, werd een zwakke tot sterke activering door S9-fractie gevonden. De verspreiding in ruimte en tijd bleek nauwelijks op systematische wijze af te hangen van de toegepaste toetsvariant. Uitzondering hierop was de zwakkere metabole activering door S9-fracties in de bovenwindse monsters, wat toegeschreven zou kunnen worden aan chemische omzettingen tijdens het verblijf van de deeltjes in de lucht.

De verspreiding in ruimte en tijd van de mutageniteit werd geanalyseerd met behulp van een aantal modellen. Daarbij werden aanwijzingen gevonden dat de effecten in de Rijnmond niet uitsluitend het gevolg zijn van additie van achtergrond en lokale emissies, maar dat ook tijd en plaatsafhankelijke interacties een rol spelen. De bijdrage van de lokale bronnen werd onderzocht met het best passende model.

Door toepassing van multivariate analyse kon een sterke gemeenschappelijke variatie van de PAK-concentraties worden aangetoond. Afwijkingen van de gemeenschappelijkheid bleken samen te hangen met vluchtigheid en chemische reactiviteit. Monsternameartefacten, veranderende bronsamenstelling en verdamping, adsorptie en condensatie tijdens het verblijf van de deeltjes in de buitenlucht zouden de extra variatie voor de meer vluchtige PAK kunnen veroorzaken. De met reactiviteit samenhangende extra variatie wijst op monsternameartefacten en chemische omzettingen in de buitenlucht. Dat de laatste oorzaak inderdaad van belang is, blijkt uit de verhoudingsgewijs lagere achtergrondconcentraties van de meer reactieve PAK.

Hoewel mutagenen en PAK door dezelfde groep van bronnen worden geëmitteerd, bleken mutageniteit en PAK-concentraties slechts matig sterke correlaties te vertonen. Wel namen de correlaties toe als monsters werden geselecteerd die bij dezelfde windrichtingen waren genomen, wat wijst op een invloed van veranderingen in bronpatroon of chemische omzettingen op de relaties tussen PAK-concentraties en mutageniteit.

In de tweede studie (fotochemie-studie) werden de deeltjes verzameld boven en benedenwinds van een snelweg en een deel van Delft tijdens perioden met fotochemische luchtverontreiniging. De monsternameduur was

kort (3 uur); dit om een eventuele samenhang tussen de ontwikkeling over de dag van enerzijds fotochemie en anderzijds mutageniteit en PAK-concentraties waar te kunnen nemen. De mutageniteitsprofielen (verhouding tussen effecten bepaald met verschillende toetsvarianten) verschilde sterk voor de bovenwinds verzamelde deeltjes en de bijdrage van de lokale bronnen (voornamelijk verkeer). De eerste vertoonden een sterke metabole **deactivering**, die benedenwinds grotendeels werd gecompenseerd door **activering** van de mutageniteit van de lokaal geëmitteerde deeltjes. Dit verschil vormt een duidelijke aanwijzing dat de mutageniteit drastische veranderingen ondergaat gedurende het verblijf van de deeltjes in de lucht.

Vergelijking van de effecten in de nitroreductasedeficiënte mutant TA98NR en de nitroreductaseproficiënte oorspronkelijke stam, liet zien dat nagenoeg alle directe, mutageniteit, dat wil zeggen de mutageniteit in de afwezigheid van S9-fractie, afhankelijk was van nitroreductie en dus veroorzaakt werd door nitroverbindingen. Door de sterk verminderde gevoeligheid voor de directe mutageniteit van stam TA98NR kon S9-afhankelijke (of indirecte) mutageniteit worden waargenomen die in TA98 meer dan gecompenseerd werd door de inactivering van de directe mutageniteit in de aanwezigheid van S9-fractie.

De mutageniteit vertoonde geen vaste dagelijkse kwalitatieve of kwantitatieve trend, wat suggereert dat de mutageniteit niet werd beïnvloed door de fotochemische luchtverontreiniging, zoals die zich ontwikkelde over de dag in het onderzoeksgebied. Dit zou kunnen worden verklaard door een reeds maximale chemische omzetting van relevante stoffen in de bovenwinds verzamelde deeltjes voordat zij in het onderzoeksgebied arriveren of een te korte verblijftijd voor substantiële chemische omzetting in de deeltjes die in het onderzoeksgebied worden geëmitteerd. De indirecte mutageniteit bleek sterker gecorreleerd met SO_2 dan de directe mutageniteit, wat aangeeft dat de eerste minder sterk wordt beïnvloed door chemische omzettingen tijdens het verblijf in de lucht dan de indirecte mutageniteit.

Het PAK-profiel verschilde niet veel van dat in de Rijnmondstudie. Weer was er sprake van een sterke gemeenschappelijke variatie, dat wil zeggen een constant PAK-profiel. De niet-gemeenschappelijkheid hing specifiek samen met vluchtigheid, chemische reactiviteit en bronnen. De bijdrage van het lokale verkeer werd gekenmerkt door relatief hoge concentraties van de meer reactieve PAK en benzo(g,h,i)peryleen. In het geval van de meer reactieve PAK wijst dit op chemische omzetting van PAK gedurende het verblijf van de deeltjes in de lucht, terwijl de laatstgenoemde PAK als een

marker voor de bijdrage van het verkeer kan worden opgevat. De gemiddelde bijdragen liepen uiteen van 0% voor benzo(e)pyreen tot 90% van de bovenwinds waargenomen concentraties.

De correlaties tussen mutageniteit en PAK-concentraties bleken S9-afhankelijk te zijn. Zonder S9-fractie werden matige correlaties gevonden; toepassing van S9-fractie leidde tot een aanzienlijke toename. Het lijkt waarschijnlijk dat dit verschil een veel sterkere invloed weerspiegelt van chemische omzettingen op de indirecte mutageniteit. Dit stemt overeen met de sterkere samenhang tussen indirecte mutageniteit en SO_2-concentraties.

Het derde onderzoek[31] verschilde in één belangrijk opzicht van de andere twee. De deeltjes werden **niet** verzameld tijdens specifieke meteorologische omstandigheden. De monsterneming vond wekelijks plaats gedurende een jaar op vier lokaties waarvan er één aan de kust als achtergrondlokatie werd beschouwd. De monsters vertegenwoordigen daardoor een aselecte trekking van de monsters die in Nederland gedurende één jaar kunnen worden genomen. In het algemeen werden de resultaten van de andere twee studies bevestigd. De nitroreductaseafhankelijkheid was wat minder groot dan in de fotochemiestudie (70% in plaats van 80%). maar nog steeds groot genoeg om te suggereren dat alle directe mutageniteit door nitroverbindingen wordt veroorzaakt. Het verschil tussen de twee studies wordt toegeschreven aan de verschillende meteorologische omstandigheden. Fotochemische luchtverontreiniging gaat klaarblijkelijk gepaard met de vorming van nitroverbindingen met een minder grote residuele mutageniteit in TA98NR of met de eliminatie van andere nitroverbindingen.

Het grootste deel van de monsters vertoonde een duidelijke indirecte mutageniteit, en kwam in dit opzicht overeen met de monsters van de Rijnmondstudie. Het feit dat de metabole activering door S9-fractie even sterk was in de twee TA98-stammen geeft aan dat er geen inactivering optreedt die wordt overschaduwd door activering. Het lijkt er dus op, dat fotochemische luchtverontreiniging **specifiek** tot de vorming leidt van inactiveerbare stoffen. Gespeculeerd wordt dat deze verbindingen de produkten zijn van de nitrering van gasvormige PAK via de reactie met OH-radicalen.

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[31] Deze studie is niet in detail beschreven in een afzonderlijk hoofdstuk van dit proefschrift. Resultaten die verhelderend kunnen werken bij de interpretatie van de andere studies zijn gepresenteerd in een aanvullende discussie (hoofdstuk 14). Voor een gedetailleerde rapportage wordt verwezen naar De Raat et al. (1988).

Aard en diversiteit van de mutagenen

Aard en diversiteit van de mutagene stoffen in de deeltjes zijn onderzocht door extracten van de deeltjes te fractioneren met behulp van verschillende chromatografische technieken en vervolgens de fracties te onderzoeken op mutagene eigenschappen en de aanwezigheid van PAK en een aantal nitro-PAK. Voor deze combinatie van chromatografie, mutageniteitstoetsing en chemische analyse wordt vaak de Engelse term "bioassay-directed fractionation" gebruikt.

Zeven verschillende groepen van mutagenen konden worden aangetoond in de deeltjes, waarvan er twee werden geïdentificeerd als PAK en hun mono-nitroderivaten. Een bijdrage van de bekende en uiterst mutagene dinitropyrenen kon worden uitgesloten, ondanks het feit dat andere onderzoekers deze verbindingen in verschillende verbrandingsemissies hebben aangetoond. Afhankelijk van de toegepaste bacteriestam in de Salmonella/microsoomtoets, kon 5 tot 20% van de mutageniteit in de aanwezigheid van S9-fractie aan PAK worden toegeschreven. De aanwezigheid mononitro-PAK verklaarde 13 tot 24% van de mutageniteit, afhankelijk van bacteriestam en S9-fractie.

Zonder S9-fractie bleek de mutageniteit van alle fracties sterk nitroreductaseafhankelijk, wat erop wijst dat alle directe mutageniteit wordt veroorzaakt door nitroverbindingen. Direct-mutagene fracties vertoonden echter ook indirecte mutageniteit. Beide soorten mutageniteit zouden door dezelfde of verwante nitroverbindingen veroorzaakt kunnen worden, omdat nitro-PAK in principe zowel mutageen kunnen zijn door de bacteriële reductie van de nitrogroep en de S9-afhankelijke oxydatie van aromatische ringen. Gezien het feit dat vooral de laatste route van belang is in zoogdieren, verdient met name de indirecte mutageniteit van de nitroverbindingen aandacht bij verder onderzoek. In dit licht bezien zijn de effecten in stammen met een relatief hoge gevoeligheid voor de indirecte mutageniteit, zoals TA97 en TA100, mogelijk relevanter dan de effecten in TA98.

"Bioassay-directed fraction" biedt veelbelovende mogelijkheden in het onderzoek naar de humaan-toxicologische relevantie van de mutageniteit van de deeltjes. Onderzoek van de fracties met uiteenlopende mutageniteitstoetsen maakt het mogelijk werkelijk relevante stofgroepen te onderscheiden van groepen met een specifiek sterke mutageniteit voor bacteriën. Daardoor wordt een meer doelgericht onderzoek mogelijk naar mutagenen die bij de mens effecten zouden kunnen veroorzaken en naar de intensiteit en de aard van deze effecten bij de mens onder natuurlijke blootstellingsomstandigheden.

Tenslotte kan "bioassay-directed fractionation" een bijdrage leveren in het onderzoek naar de invloed van chemische omzettingen die op kunnen treden tijdens het verblijf van de deeltjes in de buitenlucht en tijdens de monsterneming van de deeltjes. Veranderingen van de mutageniteit kunnen direct zichtbaar worden gemaakt in de "mutagrammen", ook als deze veranderingen elkaar compenseren in de effecten van het complete extract.

CURRICULUM VITAE

De schrijver van dit proefschrift werd op 20 juni 1950 geboren in het Noord-Brabantse Sprang-Capelle. In het nabijgelegen Waalwijk bezocht hij het Willem van Oranje College, waar hij in 1968 het diploma HBSb behaalde. Vervolgens studeerde hij van 1968 tot 1975 biologie aan de Vrije Universiteit te Amsterdam. In juli 1972 werd het kandidaatsexamen B4 afgelegd. Het doktoraaldiploma, met als hoofdvak microbiologie en als bijvakken geschiedenis der natuurwetenschappen en plantenfysiologie, behaalde hij in augustus 1975. Tijdens zijn studie vervulde hij studentenassistentschappen bij de vakgroep Microbiologie van de Vrije Universiteit en was hij als deeltijdleraar verbonden aan het Willem van Oranje College te Waalwijk.

Na korte tijd als wetenschappelijk medewerker onderzoek te hebben verricht bij de vakgroep Microbiologie van de Vrije Universiteit, trad hij in december 1975 in dienst bij het Centraal Laboratorium TNO. Aanvankelijk als wetenschappelijk medewerker en later als projectleider was hij betrokken bij het milieutoxicologische onderzoek dat binnen de afdeling Biologie werd uitgevoerd, waarbij hij zich met name bezig hield met de aanwezigheid en identiteit van genotoxische stoffen in afvalstromen, oppervlaktewater en lucht. Daarnaast werd onder andere de waarde onderzocht van de Salmonella/microsoomtoets voor de biologische monitoring van de beroepsmatige blootstelling aan mogelijk carcinogene stoffen en werd aandacht besteed aan de ontwikkeling van een mathematisch model voor de Salmonella/microsoomtoets.

In 1988 werd hij hoofd van de werkgroep Signaalstoffen van de hoofdgroep Maatschappelijke Technologie van TNO. Zijn onderzoeksterrein verschoof daarbij van de toxicologie naar de chemische ecologie. Doel van het onderzoek was de ontwikkeling van methoden voor de plaagbestrijding op basis van de verstoring van de chemische communicatie met behulp van feromonen.

In 1992 keerde hij weer terug naar de toxicologie. Hij trad in dienst van de afdeling Arbeid en Gezondheid van het Medisch Biologisch Laboratorium TNO, waar hij zich ging bezig houden met het beoordelen van de arbeidstoxicologische risico's van bestrijdingsmiddelen en andere stoffen. Per 1 januari 1994 vervult hij deze functie binnen het nieuwe instituut TNO Voeding.

STELLINGEN
behorende bij het proefschrift
MUTAGENS AND POLYCYCLIC AROMATIC HYDROCARBONS IN AMBIENT AIRBORNE PARTICLES

1. De toepassing van mutageniteitstoetsen in het milieuonderzoek is te veel gebaseerd op gevoeligheid en gemakkelijke uitvoerbaarheid en te weinig op bruikbaarheid van resultaten.

R. Hoffmann, 1982, Environ. Sci. Technol., 16, 560A-571A; W. K. de Raat et al., 1990, in: M. D. Waters et al., eds., The Genetic Toxicology of Complex Mixtures, 249-269, Plenum, New York; F. E. Würgler en P. G. N. Kramers, Mutagenesis 7, 321-327

2. Absoluut kwantitatieve uitspraken over de mutageniteit van complexe mengsels van stoffen uit het milieu zijn onmogelijk vanwege het ontbreken van geschikte standaarden. Om deze reden kan de mutageniteit van deze mengsels niet als uitgangspunt dienen voor milieunormering.

Dit proefschrift

3. Voor monitoring van luchtverontreiniging met het carcinogene complex van polycyclische stoffen dat door verbrandingsprocessen wordt geëmitteerd, volstaat het de concentraties van benzo(a)pyreen te bepalen.

Dit proefschrift

4. Vanwege de geringe discrepantie tussen de concentraties van benzo(a)pyreen in de buitenlucht en de voorgestelde gezondheidskundige grenswaarden voor deze concentraties, en vanwege de grote maatschappelijke consequenties van de handhaving van deze grenswaarden, is dringend onderzoek gewenst dat gericht is op het beantwoorden van de vraag naar het bestaan van een drempelwaarde voor de carcinogeniteit van PAK.

W. Slooff et al., 1989, RIVM, rapport 758474011; Thijsse, Th. R. en C. Huygen, 1985, TNO Milieuwetenschappen, rapport R85/272, Delft; W. K. de Raat et al., 1988, TNO Milieuwetenschappen, rapport R 88/118, Delft

5. Polyurethaanschuim is een potentiële bron voor blootstelling van de mens aan mutagene, mogelijk carcinogene stoffen.

Dit proefschrift

6. Er zijn geen doorslaggevende redenen om bij het onderzoek naar de mutageniteit van de deeltjes in de buitenlucht, in plaats van glasvezelfilters, gebruik te maken van Teflon-filters of met Teflon geïmpregneerde glasvezelfilters.

J. M. Daisey, et al., 1983, Aer. Sci. Technol. 2, 295; I. Alfheim en A. Lindskog, 1984, Sci. Total Environ. 34, 203-222; D. R. Fitz et al., 1984, Atmos. Environ. 18, 205-213; A. Lindskog en E. Brorström-Lunden, 1984, Sci. Total Environ. 61, 51-57; R. R. Watts, et al., 1992, J. Air Waste Manage. Assoc. 42, 49-55; dit proefschrift

7. Zowel de bestrijders als de aanhangers van alternatieve geneeswijzen veronachtzamen de volkswijsheid die zegt dat uiteindelijk alleen het resultaat telt, en zijn geneigd uitsluitend het resultaat te accepteren dat binnen de kaders van hun, al dan niet natuurwetenschappelijke, modellen past.

8. Het afleiden van oorzakelijke verbanden uit analogieën is een essentiëel kenmerk van de antroposofische natuurwetenschap, dat echte communicatie en vruchtbare samenwerking met de reguliere natuurwetenschap ten zeerste bemoeilijkt, zo niet uitsluit.

L. F. C. Mees, 1984, Dieren zijn wat mensen hebben, Vrij Geestesleven, Zeist

9. In tegenstelling tot wat Stephen J. Gould stelt in zijn boek "Wonderful Life", is het contingente karakter van de evolutie niet in strijd met de opvatting dat de evolutie te vergelijken is met een film die, hoe vaak ook afgedraaid, telkens weer hetzelfde verhaal oplevert.

S. J. Gould, 1989, Wonderful life, W. W.Norton & Company, New York

10. Gezien het uitzonderlijke belang van borstvoeding voor de volksgezondheid, dient het milieubeleid onvoorwaardelijk gericht te zijn op de toxicologische veiligheid van dit voedingsmiddel.

11. De ontwikkeling van de wetenschap in Nederland wordt meer geremd door de strijd om geld dan door het gebrek aan geld.

12. In het huidige wetenschappelijke bedrijf wordt fotokopiëren vaak verward met lezen.

Karel de Raat