INDOOR AND OUTDOOR AIRBORNE PARTICLES

AN *IN VITRO* STUDY ON MUTAGENIC POTENTIAL AND TOXICOLOGICAL IMPLICATIONS

Department of Toxicology
Department of Air Pollution
Wageningen Agricultural University
De Dreijen 12, 6703 BC WAGENINGEN
The Netherlands

Promotor: dr. J. H. Koeman,
hoogleraar in de toxicologie

Co-promotor: dr. J. S. M. Boleij,
universitair hoofddocent

J. J. van Houdt

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Proefschrift
ter verkrijging van de graad van
doctor in de landbouwwetenschappen,
op gezag van de rector magnificus,
dr. C. C. Oosterlee,
in het openbaar te verdedigen
op dinsdag 3 mei 1988
des namiddags te vier uur in de aula
van de Landbouwuniversiteit te Wageningen

ter herinnering aan
Willem Jacobus van Houdt

STELLINGEN

1. De mutagene potentie van binnenluchtaerosolen verschilt niet alleen kwantitatief, maar ook kwalitatief van die gemonsterd in de buitenlucht.
[dit proefschrift]

2. Gezien het geringe aandeel in de totale blootstelling aan mutagene aerosolen, is het op zijn minst merkwaardig dat de aandacht van onderzoekers zich vrijwel volledig richt op de mutageniteit van buitenlucht.
[dit proefschrift]

3. De methode die Alfheim en Ramdahl voor de monstername van binnenluchtaerosolen gebruiken is niet adequaat.
[Alfheim & Ramdahl, Environ Mutagen 6: 121-130 (1984).]

4. Met het oog op kankerpreventie is de eliminatie van initiërende komponenten, die diffuus in lage concentraties in het milieu voorkomen, in het algemeen minder effectief dan de verlaging van blootstelling aan factoren met een promotorwerking.
[Cohen LA, Scientific American 257(3):42-48 (1988).]

5. De opvatting dat door de geringe aktiviteit van nitroreduktase in dierlijke cellen de mens niet gevoelig is voor nitrogesubstitueerde verbindingen is onjuist, gezien de hoge nitroreduktase aktiviteit in darmflora.
[Rowland et al., Xenobiotica 13(4): 251-256 (1983).]
[Goldstein et al.,Tox Appl Pharm 75:547-553 (1984).]

6. Bij het onderzoek naar het werkingsmechanisme van ozon in de plantecel dient meer gebruik gemaakt te worden van de reeds beschikbare kennis die toxicologisch onderzoek met dierlijke cellen heeft opgeleverd.

7. Er hebben zich tot op heden geen milieuproblemen voorgedaan die het voortbestaan van de mensheid zo ernstig bedreigen als de afbraak van de ozonlaag.

8. De huidige EG landbouwpolitiek vermindert niet alleen de inkomensmogelijkheden voortvloeiend uit de teelt van marktordeningsprodukten, die van vrije produkten wordt eveneens negatief beïnvloed.

9. Het denkbeeld dat in Nederland pas wordt gestraft als de schuld is vastgesteld is onjuist, gezien de behandeling die een verdachte in de politiecel ten deel valt.

10. De toenemende vertrossing komt tot uiting in het ledenaantal van Veronica.

J J van Houdt

Indoor and outdoor airborne particles, an <u>in vitro</u> study on mutagenic potential and toxicological implications

23 maart 1988

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Woord vooraf

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Aim and structure of this thesis

In this thesis mutagenic activity of indoor and outdoor particulate matter is subjected to a detailed study. The study is divided in two parts; one concerning the occurrence of airborne mutagens in indoor and outdoor air and one in which some aspects of bioavailability are studied.
In part I chapter 1 provides a summary of literature data on physical and chemical characteristics of airborne particulate matter. The occurrence of mutagens in the environment and their sources is discussed. Chapter 2 presents data on *in vitro* testing of airborne particles collected outdoors. Chapter 3 and chapter 4 deal with the comparison of particle bound mutagens in indoor and outdoor air. In chapter 3 the contribution of smoking and cooking and in chapter 4 the contribution of wood combustion to mutagenicity of indoor aerosols is established.
In part II chapter 5 reviews a number of studies which deal with some aspects of biological fate of mutagenic compounds and their 'carrier' particles. The experiments described in part II are all carried out with 3 typical samples of airborne particulate matter: an outdoor sample collected in winter and two indoor samples, one polluted with wood smoke and one polluted with cigarette smoke. In chapter 6 the reduction in phagocytic activity of rat alveolar macrophages by extracts of airborne particulate matter is determined. In chapter 7 solubility of particle bound organics in physiological fluids and in chapter 8 metabolic activation of extracts of airborne particulate matter by homogenates from different organs is presented.
Finally part III summarizes all data and conclusions.

PART I
Mutagenic activity of airborne particulate matter in outdoor and indoor environments

1 Introduction

1.1. AEROSOL CHARACTERISATION AND DISTRIBUTION

Sources of aerosols

Airborne particulate matter has long been recognized as an atmospheric constituent. Clouds of particles have names which segregate their characteristics by size and colour, like smoke, fog, haze, mist and smog. The term aerosol was introduced early this century by Donnan as an analogy to colloidal suspensions in water (hydrosols).
The term aerosol describes a gas particle suspension with colloidal stability to gravitational settling. On earth the gravitational settling restricts the particle size to densities near unity of 10-20 μm in diameter or less (63).
Environmental concern for the adverse effects of aerosols dates back to the twelfth century, when the production of smoke by burning soft coal in London was reported to be a capital offence. Although in later times people became more tolerant of such pollution, concern was maintained as expressed by investment in immission control during the last half century (63).
Particulate matter can be classified according to origin - that is whether it is primary or secondary. Primary particles are brought directly into the atmosphere. Primary man-made particles are predominantly produced by combustion and industrial processes, incineration and refuse burning. Wind blown dust, sea spray, volcanoes and forest fires may be regarded as primary aerosols of natural origin. One of the most important developments in recent years has been the recognition of the significance of chemical reactions by which secondary particles are formed in the atmosphere. Innumerable compounds, generally classified as acid sulfates, nitrates and organic matter are formed by gas to particle conversion processes of originally emitted gaseous trace elements. Nitrate for example is found in large quantities in ambient aerosol, while for nitrate direct sources are not known (168).
The yearly total of primary aerosol emissions in the Netherlands amounts to 200 k tons, of which combustion of fossil fuels constitutes a major source of 40% (26). A survey of aerosol sources in the earth atmosphere is presented in table I (168). The table gives information on the contribution to total tropospheric aerosol production rates of natural and anthropogenic sources on the one hand and primary and secondary sources on the other hand. The majority of the global particulate load proves to be of natural origin. The bulk of human activities arises from combustion and industrial processes.

TABLE I : Estimates of tropospheric aerosol production rates (taken from 168).

	Source	production rate tons/day	weight of total in %
		A. natural sources	
1.	primary		
	wind blown dust	2×10^4 - 1×10^6	9.3
	sea spray	3×10^6	28
	volcanic	1×10^4	0.09
	forest fires	4×10^5	3.8
2.	secondary		
	vegetation	5×10^5 - 3×10^6	28
	sulfur cycle	1×10^5 - 1×10^6	9.3
	nitrogen cycle	2×10^6	14.8
	volcanic (gases)	1×10^3	0.009
	Subtotals	10×10^6	94
		B. man-made sources	
1.	primary combustion and industrial dust	1×10^5 - 3×10^5	2.8
	from cultivation	1×10^2 - 1×10^3	0.009
2.	secondary		
	hydrocarbon vapors	7×10^3	0.065
	sulfates	3×10^5	2.8
	nitrates	6×10^4	0.56
	ammonia	3×10^3	0.028
	Subtotals	6.7×10^5	6
	Totals	10.7×10^6	100

Chemical and physical characterisation of aerosols

Although only 6% of the total atmospheric burden originates from anthropogenic sources, the conclusion is not justified that, with regard to human exposure, this contribution is of minor importance. Differences in chemical and physical composition may give anthropogenic particles quite different health implications from natural aerosols.
Organic substances are, next to airborne sulfates the second major constituents of fine airborne particles in the atmosphere. Although estimated to constitute only a small fraction of airborne pollutants by mass, organic substances represent 26 to 47% of the total fine particulate fraction in the U.S. (123). An enormous variety of organic compounds has been analysed in the atmosphere (47, 80, 84, 85, 100, 156). Polycyclic aromatic hydrocarbons (PAH), as well as nitro substituted aromatics, among them a number of known animal carcinogens have been detected in airborne particulate matter

(15, 25, 74, 132).
Organic compounds are emitted by anthropogenic sources like stationary and mobile combustion appliances as well as from natural sources like vegetation and forest fires.
In the atmosphere there are three mechanisms by which aerosol formation takes place: nucleation (167), condensation (102) and coagulation (65). More than 50% (weight) of the submicron atmospheric particles is of a secondary nature. It is obvious that main sources of secondary aerosols are directly related to the sources of the precursor gases (43). Due to processes of formation or transformation size distributions of aerosols may differ considerably with different atmospheric sources. Coarse aerosols are often generated by natural sources like storms, oceans and vulcanoes while condensation and high temperature processes like fires, combustion and chemistry (168) are likely to produce small particles.

Atmospheric behaviour

The atmospheric behaviour of aerosols is mainly determined by the aerodynamic size of the particles. The residence time, which is a measure for the life time of the aerosol in the atmosphere depends mainly on this parameter (61). A rather long residence time leads to stable concentrations while a

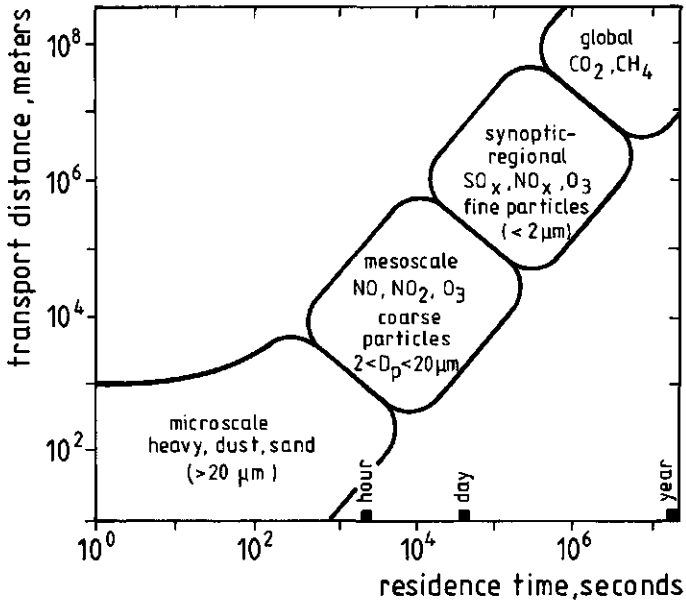

Figure 1: Indications of transport distances of particles as a function of residence times (taken from 70).

short residence time causes considerable variations. This effect is well-known for atmospheric gases and could be shown for atmospheric aerosols as well (72). Residence times for the smallest as well as for the largest particles are usually very short. Aitken particles (<0.01 μm) are subject to Brownian motion and consequently to coagulation. The largest particles are removed from the atmosphere because of their relatively high sedimentation velocity, a process called dry deposition. Particles of 0.1-1 μm are removed efficiently by a process called wet deposition (12, 146). Therefore, residence times of particles of 0.1-1 μm diameter are increased in higher atmospheric layers, due to a decreasing significance of wet deposition. Because of their long residence time particles of 0.1-1 μm diameter may be transported over thousands of kilometers (44, 70). Figure 1 gives an indication of transport distances as a function of residence time (70).

1.2 AIRBORNE PARTICLE BOUND ORGANICS AND MUTAGENICITY TESTING

The Salmonella/microsome assay was originally introduced by Ames (9,10) in 1971. It represents a rapid, cost effective and reliable in vitro screening method for mutagen detection. The assay is based on reversion of histidine-requiring auxotrophs to the wild type upon addition of mutagenic compounds. For mutagenesis screening, strains of Salmonella typhimurium can be used. The most commonly used strains for the detection of airborne mutagens are TA 100, causing base-pair substitutions and TA 98, causing frameshift mutations.

There are chemicals which are not reactive themselves but which can be transformed into reactive metabolites by enzymatic biotransformation processes in the liver and other organs. The addition of rat liver microsomal preparations (S9) to the assay approximates mammalian metabolism of pro-carcinogens and pro-mutagens. The Salmonella/microsome assay has been the major mutagenicity assay used for monitoring pure compounds as well as for complex environmental mixtures such as airborne particulate matter (60).

Salomone (138) analysed 30 PAH, of which only benzo(a)pyrene-quinone turned out to be a direct acting mutagen in the Ames assay; indirect activity was observed in 13 compounds and the remaining compounds did not show any activity. Teranishi et al. (162) found 7 PAH to be indirect acting mutagens. PAH, of which benzo(a)pyrene is an important representative, can be seen as a group of benzene-soluble compounds of which several are mutagens which require metabolic activation (165).

Polycyclic aromatic hydrocarbons, analysed in extracts of airborne particulate matter show indirect acting mutagenicity (51, 77, 99, 105, 107, 109, 138). Other organic constituents

of aerosols, such as nitroaromatics (53, 55, 111, 171, 172, 178), pyrene 3,4-dicarboxylic acid anhydride (135), dimethyl and monoethyl sulphate (85), 20-methylcholanthrene (108), 2- and 3-carbonhalogenated compounds (156) and polycyclic ketones and quinones (28) show mutagenic activity.

1.3 MUTAGENIC ACTIVITY OF AIRBORNE PARTICLES IN THE AMBIENT ATMOSPHERE

Sources of outdoor mutagens

Organic emissions from combustion processes are the major source of primary emissions of mutagenic compounds in the ambient atmosphere (74, 92). In urban atmospheres, the major source of mutagenic compounds is motor/vehicle exhaust (3). Most studies concerning automotive exhaust have concentrated on diesel emissions (16, 29, 30, 31, 42, 68, 69, 101, 135, 140, 171, 173), reflecting the relatively much higher particle emissions than in gasoline engine exhaust (93, 116, 125). Particles emitted from both diesel and gasoline engines produce direct as well as indirect mutagens, which are sensitive to TA 98 in particular.

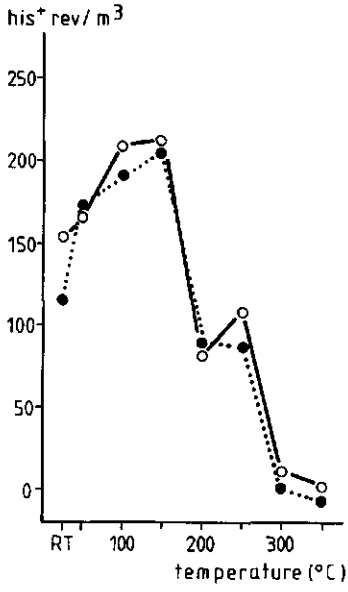

Figure 2: Mutagenic activity of coal fly ash; net His^+ revertants, ● - S9 and o + S9, as a function of combustion temperature (taken from 48).

Other sources of mutagenic compounds are emission of small residential wood burners (95, 133), coal combustion (11, 29, 56, 82, 83, 113, 143, 150), roofing tar (1, 121, 176), forest fires (170) and agricultural burning (29). Temperature, possibly in combination with other physical or chemical factors may affect the production of mutagens. Uncombusted diesel fuels (69), gasoline and unused motor oil (171) for example, were found to be non-mutagenic whereas used motor oil (171) and automotive exhaust particles were found to be mutagenic, which indicates that mutagens are products of, or concentrated by the combustion process. It was also reported that combustion conditions may dramatically alter the mutagenicity of emitted fly ash (83). Mutagenicity was observed for particulate matter,

collected during start-up and shut-down of the combustion system. These results and the results presented in figure 2 (taken from 48) indicate that there is a critical temperature range for mutagen production, above or below which there are no significant quantities of mutagens produced.
Contrary to primary emissions little is known about secondary emissions of mutagenic compounds (18), the chemical pathways in the atmosphere that convert organic compounds to mutagens are poorly understood. As more than 50% of the submicron particles is of a secondary nature and all kinds of atmospheric reactions may transform primary and secondary aerosols (43, 102) it is likely that secondary sources play an important role in the formation of mutagens, especially on particles between 0.1 and 1 μm diameter, which have long residence times (61).
Indications that atmospheric reactions have consequences for the mutagenic burden of air pollution were found by a number of scientists. Laboratory studies have shown that 1-nitropyrene and other direct acting mutagens are decomposed by irradiation with a wavelenght of 320-418 nm (154). Transformations of mutagenic organic compounds were observed in the presence of ozone (76, 126, 128) and nitrogen dioxide (50, 58, 62, 76). In outdoor chambers indirect mutagenicity of diesel emissions declines substantially, due to a reaction of PAH with NO_2, whereas simultaneous formation of nitro-PAH increases direct mutagenic activity in the Ames assay. It is probable that reactions observed in outdoor chambers also takes place in the ambient atmosphere. Indications of this phenomenon were found by comparing roof top samples with street level samples. Atmospheric reactions cause transformations of non-polar compounds in the primary emission at street level to more oxyginated mutagenic compounds after the time of transportation to roof top level (3, 91, 97, 106).

Levels of genotoxins outdoors

The outcome of the Salmonella/microsome assay studies provides clear evidence for the mutagenic potential of ambient airborne particulate matter. We may conclude that extracts of airborne particulate matter obtained in urban areas particularly cause frameshift mutations in TA 98 and do not require metabolic activation (33, 37, 127, 128, 159). Fractionation with respect to polarity yields fractions into which PAH are not partitioned, but which contain mutagenic activity (33, 37, 161, 165). This supports the conclusion that airborne particles contain mutagens other than PAH. In residential areas mutagenicity was found to a lesser extent (163, 175). Mutagenic potential of particulate matter in industrial areas was found to be comparable with extracts, obtained at urban locations (37, 130, 131, 145, 164, 165). Industrial activities may give a substantial contribution to

airborne particulate mutagenicity.
As opposed to urban and industrial locations mutagenicity at rural and background locations may be considerably less (3, 4, 7, 127, 154). Sometimes mutagenic activity could not be found (3, 4). However, in spite of the absence of sources direct and indirect mutagenicity may also be observed on rural locations, especially in the winter (7, 77, 127, 164). In urban samples a distinct diurnal pattern of mutagenicity with a peak in the morning (30, 75, 129) and one in the evening occurs (129). Morning and evening peaks, presented in figure 3 (taken from 129) appeared to be later than rush

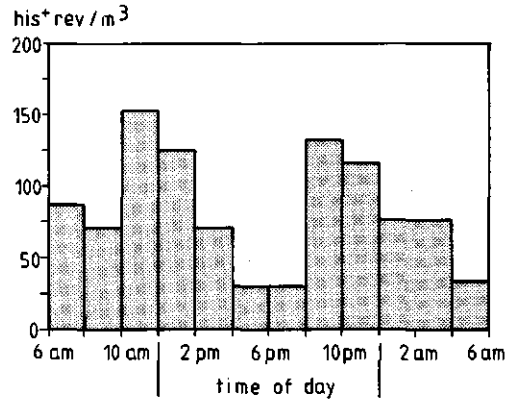

Figure 3: Diurnal variation of mutagenicity (+S9) of fine airborne particles collected in Rodeo, California, measured in the micro suspension method, a modification of the Ames/Salmonella assay (taken from 75).

hour. As the diurnal variation in lead concentrations was very similar to that of mutagenicity (and lead and indirect mutagenicity are strongly correlated), motor vehicles were suggested to be the major source of urban mutagens (129). However, the shift of the mutagenicity peak to the emission peak may be caused by the time needed for transformations to mutagenic compounds. Statistically significant correlations between mutagenicity and lead concentrations were also found in other studies, pointing to automotive exhaust as an important source of mutagens. Multivariate statistical analyses of air quality data in Detroit showed automobile exhaust to be accountable for 13% of the variance in mutagenicity (177).
In addition to diurnal variations, which are found daily, considerable variations in mutagenic activity of airborne particles were also observed from day to day. Mutagenic activity of particles, collected in Berlin, was found to be higher on workdays than in the weekend (77). A seasonal variation shown in fig 4 (taken from 3) could also be observed, with the highest average values in wintertime (3,

4, 7, 36, 38, 77, 88, 158). Those seasonal shifts may be due to wintertime space heating. Furthermore meteorologic parameters may be responsible for the remainder of the daily variations. Especially wind direction proved to be important (7). It was shown that extracts of airborne particulate matter, collected downwind from the industrial Rijnmond area in the Netherlands were significantly more mutagenic in Salmonella typhimurium strain TA 98 than those collected upwind (77, 130). Also with rural and background locations it was observed that the mutagenic burden varies with wind direction (3, 7, 67). As in rural or background locations primary sources are scarce it is suggested that the mutagenic potential of suspended matter does not originate from local sources but rather depends on large scale processes (7). Analyses of the trajectory path indicates that it is less important from which direction the air arrives at the sampler than from which area the air originates (44, 46, 139). With regard to the mutagenic potential of air pollutants few studies indicate the relevance of trajectory studies (4, 96).

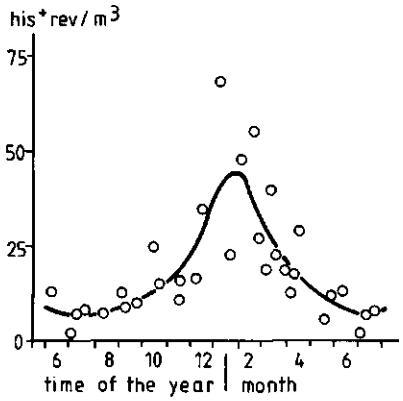

Figure 4: Mutagenic activity (TA 98, - S9) of airborne particulate matter collected during weekdays above the roof tops in the inner city of Stockholm. Each point represents the average of three to four consecutive 24-h samples. The sampling covers 28 periods between Februari 1979 and April 1981 (taken from 3).

Apart from a first step in the risk estimation mutagenicity testing of airborne particulate matter can be seen as a general air pollution parameter, expressing the level of particle bound organics. Comparison with other air pollutants and epidemiological studies has to show whether this parameter is useful. Statistically significant correlations between mutagenicity and lead concentrations were found (97, 129), while the relations with B(a)P concentrations appear to be rather variable. Some scientists report good correlations (33, 38, 50) while others report the opposite (165, 166). Differences of sampling procedure or location may account for this phenomenon. We may suppose that correlations, found between primary pollutants at urban or industrial locations are not found after transport and transformation of mutagens and their precursors. In this case correlations with commonly registered air pollutants may still be found. Correlations of mutagenicity and SO_2 (7, 105, 106, 177), NO_x (50, 177) and CO (75) are reported.

1.4 MUTAGENIC ACTIVITY OF INDOOR AIRBORNE PARTICLES

Indoor-outdoor relationship

Indoor air may be contaminated with airborne particles penetrating from outdoors, or produced indoors. Doors, windows, ventilation openings and cracks in walls permit entry of pollutants. More particles can enter if the air exchange rate increases (8, 13). The relationship between indoor and outdoor particulate matter has been studied by many people. An enclosed structure is "bathed" in an outdoor ambient atmosphere the quality of which varies in time and space in terms of mutagenic activity, as seen previously. The air enclosed by buildings is an extension of the surrounding environment. Therefore indoor air quality will respond to changes in outdoor air quality with a response rate depending on the permeability of the structure and the nature of particulate matter (180). Due to air filtration and adsorption there are significant differences in the chemical and physical nature of particulate matter collected indoors and outdoors. Usually indoor levels of particle pollution originating from outdoors are somewhat lower than the outdoor levels (8, 32, 40, 110).
Indoor-outdoor exchange times inferred for fine particle constituents are in accordance with those estimated for gas exchange, while coarse particle constituents exhibit time variability complicated with non-conservative behaviour such as removal by filtration and settling (112). Consequently the ratio indoor-outdoor is lower for compounds that occur in large particles, than for compounds that occur in small particles.
The main source of particulate matter indoors is cigarette smoking (13, 14, 17, 22, 24, 57, 122, 136, 142, 144). Table

TABLE II: Indoor concentration of suspended particles (average for 2 months) in relation to the number of smokers per house (taken from 13).

number of smokers	number of houses	av. conc. ug/m^3	range ug/m^3
0	4	55	20- 85
1	7	125	60-140
2	14	150	70-265
3	1	335	

II gives a relation between the number of smokers and the concentration of suspended particulate matter indoors (taken from 13). Wood combustion in stoves and open fire places may also give a substantial contribution to indoor levels of particulate matter (20, 21, 35, 52, 112, 137).

Sources of genotoxins in residences

As opposed to outdoor situations, data on the mutagenic activity of indoor air are scarce. Some data are available on the mutagenicity of extracts derived from airborne particles in working environments (107, 118, 155). Mutagenicity of indoor air from residences was only determined in 4 studies (5, 89, 94, 179).

Much more evidence exists on the sources of air pollutants inside homes than on the ultimate levels of genotoxins. Many authors reported on the production of mutagens by cigarette smoking (16, 39, 64, 78, 94, 103, 104, 176). Kier et al. (78) were the first to demonstrate the potential of cigarette smoke condensate to induce point-mutations. They showed that cigarette smoke condensate from nitrate treated cigarettes contained direct acting mutagens. All other mutagenic cigarette smoke condensates required metabolic activation. Although all mutagenic condensates caused frameshift mutations, only the cigarette smoke condensate of nitrate treated cigarettes also caused base-pair substitutions. The cigarette smoke condensates from high charcoal filter cigarettes turned out to be mutagenic , which indicates that such filters do not prevent the passage of certain mutagens into the lungs of smokers (78).

The concentration in sidestream smoke, compared to the concentration in mainstream differs for various smoke constituents. The ratio ranges from 0.7 to 6.2 for the particulate phase (64). It was estimated that sidestream smoke from one cigarette corresponds to 15,000 revertants (94).

Mutagenic activity was detected in organic extracts of wood combustion emissions (2, 5, 6, 87, 95, 133). The emission of mutagenic compounds comprises both compounds requiring metabolic activation and compounds mainly found in the polar fraction, which are active without exogenous activation (2,5). In most cases the addition of S9 increases mutagenic activity in frameshift as well as base pair strains (2, 5, 6, 71). The emission of mutagenic factors is strongly dependent on the construction of the wood stove. Poor isolation of the construction chamber results in low combustion temperatures and thus in relatively high emissions of mutagenic compounds (87, 95). Smoke condensates from marihuana cigarettes (19) and joss sticks (141) were also found to be mutagenic for TA 100 and TA 98. Metabolic activation was required in both cases. Emissions from residential oil furnaces showed a relatively low mutagenic activity (87).

An important source of mutagenic compounds that has to be taken into consideration is the processing of food, especially cooking. N-nitrosamines, polycyclic aromatic hydrocarbons and phenolic compounds, lipid polymerisation products, resulting from deepfat frying lipid oxidation, maillard browning and protein pyrolysis occur in a wide variety of heat processed food and are not limited only to

food cooked at very high temperature. However, in contrast to other sources of mutagens it should be noted that mutagenic activity appears to be considerably higher in foods processed at higher temperatures. The level of activity also varies with the source, but in general the highest activity is associated with proteinacous foods and the lowest with starchy foods (23, 34, 41, 45, 49, 54, 59, 66, 81, 117, 119, 120, 124, 152, 153, 157). Mutagenic activity was observed in strains sensitive to frameshift mutagens, in particular TA 98 and TA 1538 in the presence of metabolizing enzymes. In contrast to the findings of Felton et al. (4), that after normal cooking more than 20 times as much mutagenic material remained in the meat as was recovered from pan grease and vapors, other authors reported that considerable amounts volatilize during the cooking of food (114, 115, 134, 147). Smoke condensates obtained from broiling fish and beefsteak as well as smoke produced by the pyrolysis of proteins (114) showed mutagenic activity in Salmonella typhimurium TA 100 and TA 98 after metabolic activation. Figure 5 shows the results of Rappaport et al. (134), who found that the amount of mutagenic activity remaining in the meat was only 1-7% of the amount that was volatilized. This figure also shows the dependence of mutagenic activity on processing temperature. Volatilization of nitrosamines from foods was also reported (66, 147). As bacterial mutagenicity of processed foods may be considerable, volatilization of cooking products during processing may be regarded as a substantial source of indoor airborne mutagens.

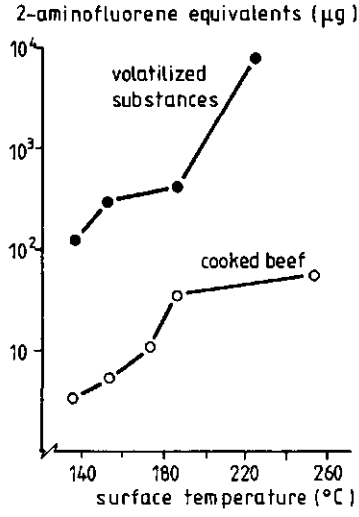

Figure 5: Amounts of basic mutagens, expressed as 2-aminofluorene equivalents (µg) produced at various cooking temperatures (taken from 134).

Levels of genotoxins in residences

Literature data on the resulting levels of bacterial genotoxins indoors are limited. A study comparing mutagenic activity in an office building to that in urban outdoor air showed that cigarette sidestream smoke represents an important source of airborne mutagenicity (94). The effect of wood burning in an open fire place on the mutagenic activity

of indoor air of a house could be considered as moderate, when comparing to those resulting from tobacco smoking in the room (5). Both studies show increased levels after addition of metabolizing systems, in particular in strain TA 98. Indoor mutagenicity mostly exceeded outdoor activity.
In a home with cooking as a major indoor source of airborne organic compounds mutagenic activity of particulate matter turned out to be lower than outdoors. In this study, however, information about the bacterial strain used is lacking (89). A serious drawback of the fourth indoor study (179) is the sample collection time of one month. Because of the length of the sampling period only median levels are observed, while extreme levels are disregarded. In a home with an unvented kerosene heater bacterial mutagenicity in TA 98 and TA 100 exceeded outdoor mutagenicity. In contrast to the other studies, addition of S9 resulted in a decreased number of mutants.

1.5 PHYSICAL FACTORS

Table III gives the fine mode (< 2.5 μm) and coarse mode (>2.5 μm) concentration for a number of non-polar organic compounds (taken from 174). First of all, the table shows that the total amount expressed in ng/m^3 of fine mode and coarse mode particle concentrations is the same in outdoor air, while indoors fine mode concentrations are higher. A comparison of the amounts of a given organic compound in the two modes clearly shows that due to surface to mass ratios the highest concentrations are found in the fine, respirable fraction, especially indoors. Consequently the concentration of respirable fraction associated organics are much higher indoors than outdoors. Other indications of the size of mutagenic particles are given by Hoegg (64), who found that particles of aged cigarette smoke do not exceed a size of 2 μm, which are therefore fully respirable. Furthermore the correspondence of polycyclic aromatic hydrocarbons with particle mass median diameter of about 0.5 μm was reported (22).
Several authors determined the effect of particle size on mutagenic activity (3, 29, 75, 86, 106, 151, 160, 165). Most of these studies were carried out by means of size

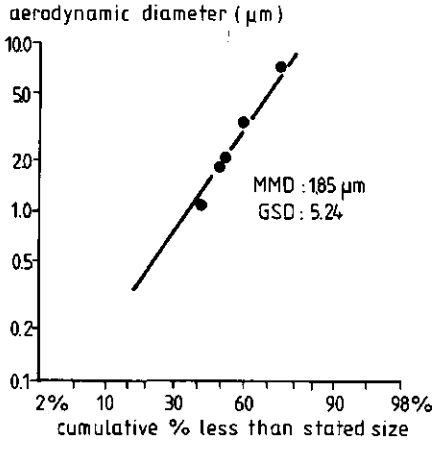

Figure 6: Size distribution of mutagenic particles, collected by the high volume Anderson sampler. Points shown are average values for the 5 sampling dates. MMD = Mass Median Diameter; GSD = Geometric Standard Deviation (taken from 151).

fractionation of urban air with cascade impactors. Although comparison of the results is not easily possible because different size fractions were studied it is quite clear that all studies show the same trend: the highest mutagenic activity is correlated with the finest fraction. The conclusion can be drawn that mutagenic compounds are almost exclusively located on particles smaller than 2.0 - 3.3 μm.

TABLE III: Fine mode and coarse mode concentrations for selected organic compounds both indoors (2/25/82 - 3/4/82) and outdoors (2/26/82 - 3/1/82) at Lubbock (taken from 175).

compound	fine ng/m^3	coarse ng/m^3	ratio fine/coarse
airborne particles			
outdoor	9070	9610	0.94
indoor	7060	2110	3.3
n-nonacosane			
outdoor	3.2	1.8	1.8
indoor	25.0	5.8	4.3
n-triacontane			
outdoor	0.4	0.2	2.3
indoor	17.0	1.0	17.0
n-hentriacontane			
outdoor	0.9	0.5	1.7
indoor	106.0	8.0	13.0
nictone			
outdoor	a	a	
indoor	0.9	<0.02	>45
dinonyl phtalate			
outdoor	a	a	
indoor	13.0	<0.05	>25
didecyl phtalate			
outdoor	a	a	
indoor	18.0	<0.05	>35
tris(butoxyethyl)phosphate			
outdoor	a	a	
indoor	24	<0.5	>45
bis(2-ethylhexylazelate			
outdoor	a	a	
indoor	8.0	<0.3	>25

Figure 6 shows the results of 5 size fractionated samples by the high volume Anderson sampler (75). The line represents data pooled from all 5 samples and the individual points shown are the mean values for the 50% cutoff diameter for each stage. From this figure it may be concluded that approximately 75% of the particles < 7.0 μm; 72% < 5.0 μm and 52% < 2.0 μm. At the same time the observation that mutagens are preferentially associated with fine particles may hide potential health hazards, since aerosol extracts which are not fractionated for size may present negative results, due

to the dominance of larger particles. A high percentage of large particles, a dust storm, for example, may completely obscure the mutagenic potential of particles not fractionated for size. For this reason mutagenic activity should be preferably expressed as revertants per m^3 of air sampled also because this approach seems to be more relevant with regard to the assessment of human exposure.

1.6. HUMAN EXPOSURE

Compared to exposure to pure gaseous pollutants, particulate matter represents a class of pollutants which requires a quite different interpretation. A reading of 2 $\mu g/m^3$ of particles for example, can cause very different toxic effects, due to the active constituents of the sample (73, 98). Moreover, even when the chemical composition of particles is identical, differences in particle size make toxicological risk assessment uncertain, since deposition at sensitive sites is dependent upon aerodynamic diameter. In comparison with other air pollutants aerosols have been given less priority (169). In order to achieve comprehensive control strategies for mutagenic particulate matter, much has to be done to clarify the relations with sources, precursors and other air pollutants. Exposure is measured by pollutant concentration available at the exchange boundaries of an organism during specified times. Atmospheric particulate matter may by inhalation, lead to deposition in certain regions of the respiratory tract. Dose is considered when the air pollutant crosses the boundary and reaches the lung (111). The deposition of particles in any pulmonary region is dependent upon:

TABLE IV. Average activity pattern of the Dutch population (> 12 years of age) (taken from 79).

	Time fraction hours/day	%
1. At home, indoors (e.g. sleeping, eating, housekeeping, television, newspaper)	17.6	73
2. Mainly indoors, not at home (e.g. work, study, sports, shopping)	5.4	23
3. Outdoors (e.g. recreation, gardening)	0.5	2
4. Miscellaneous	0.5	2
Total	24	100

1) exposure per unit diameter.
2) Breathing rate, a function of physical activities and respiratory functions.
3) Particle size and breathing mode (nasal, oral).
4) Duration of exposure.

The exposure unit is a product of concentration and time. Therefore, time budgets of people and pollutant concentrations are the data which have to be used to estimate population exposures to pollutants. In Table IV a summary of a time budget study in the Netherlands indicates that people spend about 73% of the time at home, while about 23% of the time is spent in other indoor locations (79). Although only 2% of the time is spent in outdoor locations research on air pollution has focussed mainly on the outdoor environment. Time budget studies in other western societies present the same pattern (13, 111, 180). As most of the time is spent in residences, it may be concluded that exposure will be determined to a large extent by the level of pollution inside houses.

A study done by Spengler et al. (98) indicates the significance of the level of respirable particles indoor for

TABLE V : Quantile descriptors of personal, indoor and outdoor respirable particle (RSP) concentrations (taken from 98).

		RSP Quantile mg/m^3						
RSP sample	N	95%	75%	50%	25%	5%	Mean	SD
Personal	(249)	113.0	48.5	34.0	25.5	17.0	43.9	32.9
Indoor	(266)	118.6	46.0	29.0	20.0	10.0	41.6	40.8
Outdoor	(71)	33.4	23.0	17.0	13.0	7.2	18.0	7.3

personal exposure. In this study 101 nonsmoking persons living in two cities carried personal monitors for a collection of 249 valid personal exposure samples. Each subject also had an identical sampler, collecting respirable particles simultaneously in the room that was used most often and outdoors. The results of these data collections are summarized in Table V (98). 95% of the personal exposures exceed median ambient exposure of 17 $\mu g/m^3$. The relative importance of indoor levels of pollution is clearly shown since the mean personal exposure (43.9 $\mu g/m^3$) is almost similar to the mean indoor exposure (41.6 $\mu g/m^3$).

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2 Mutagenic activity of outdoor airborne particulate matter related to meteorological and air pollution parameters

J.J. van Houdt, G.M. Alink and J.S.M. Boleij

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SUMMARY

Many studies indicate that aerosols in the ambient atmosphere may have mutagenic properties. Most of these investigations were conducted in industrial or urban areas or near specific sources. The purpose of our studies was to assess the variation in mutagenicity of airborne particulate matter using the Salmonella/microsome test, over a prolonged period of time.
In addition to 30 samples collected simultaneously in Wageningen (rural location) and at Terschelling (background location), a further 95 samples were collected in Wageningen. At both locations mutagenicity varied markedly with time. Our studies indicate that the level of mutagenicity follows a yearly cycle, with the highest activity being found in winter. In winters of different years prevalent mutagenicity differed considerably.
Mutagenicity was positively correlated between locations, pointing to large-scale processes and not to local sources. In the present study a relation between wind direction, air trajectories and mutagenicity was established. Differences within a short period of time could be explained by the trajectory of the sampled air mass.
Furthermore, mutagenic activity was correlated with commonly registered air pollution components; multiple regression shows that the air pollution parameters SO_2, NO_2, NO, CO and O_3 together account for 70% of the variation in direct mutagenicity and 80% of the variation in indirect mutagenicity. SO_2, NO_2, and SO_2, NO_2 and CO were significantly associated with the variation in direct and indirect mutagenicity.

INTRODUCTION

Epidemiological studies have shown the incidence of lung cancer to be higher in urban than in rural areas (Higginson and Jensen, 1977; Carnow, 1978; Doll, 1978). The 'urban factor' may be due to different smoking habits, occupational exposures and general air pollution (ICRP, 1966). Air

pollution components are present as gases or as particulate matter. Although gases may substantially contribute to the mutagenic potential (Claxton, 1985), this study only deals with mutagens related to particulate matter. Particulate matter in polluted air consists of a variable mixture of elemental carbon and inorganic oxides and salts on which organic components are adsorbed. The smallest particles (< 3 μm) are retained in the pulmonary region and exhibit the highest mutagenicity (Talcott and Harger, 1980; Möller et al, 1982; Sorensen et al, 1982; Kado et al, 1986). Since only 30-40% of the organic compounds on airborne particles have been indentified, the contribution of unidentified compounds to the toxicological risk may be significant. Therefore, the assessment of the overall mutagenic or carcinogenic activity of air samples may provide a more realistic basis for the evaluation of risks than testing individual compounds.

Short-term mutagenicity tests with a certain predictive value for carcinogenicity have been developed, and can be used to assess mutagenicity of extracts of complex mixtures. Using the sensitive microbiological assay developed by Ames (1971) many studies indicate that airborne particulate matter contains compounds which act as mutagens (Pitts et al, 1977; Tokiwa et al, 1977; Dehnen et al, 1977; Talcott and Wei, 1977). Most of these studies were conducted in urban or industrial areas. It is clearly shown that mutagens in the ambient air originate from various combustion processes, such as residential heating (Kubitschek and Venta, 1979; Kubitschek and Williams, 1980; Lee et al, 1980; Lewtas, 1981) motor vehicle exhaust (Huisingh et al, 1978; Wang et al, 1980; Ohnishi et al, 1980; Rappaport et al, 1980) and industrial sources (Dehnen et al, 1977; Fishbein, 1976; Tokiwa et al, 1977, 1980, 1983; De Raat et al, 1985).

In relatively few studies has the mutagenic potency of airborne particles collected at rural or background locations been presented (Pitts et al, 1977; Alfheim and Möller, 1979; Dehnen et al, 1981; Alink et al, 1983). In these studies the number of samples is limited, and the variations in mutagenicity of airborne particles at non-industrial locations are only indicated. During the last 6 years our institute has performed four studies on the mutagenic activity of airborne particles. Although the main purpose of these studies was not only the ambient atmosphere, outdoor samples were collected in all studies. Some results of these studies have already been published. Alink et al. (1983) described the mutagenic activity of airborne particulates at non-industrial locations; van Houdt et al. (1984) reported on the contribution of smoking and cooking to indoor mutagenicity in homes, and on the contribution of wood combustion in stoves and open fire places to indoor mutagenicity (van Houdt et al, 1986). A Total of 155 samples were collected at a rural and at a background location in the Netherlands.

Although previously correlations have been found between different sampling locations and between SO_2, wind direction

and mutagenic activity (Alink et al, 1983), additional data analysis of these results, with results of outdoor samples from other studies, may achieve a better understanding of the processes and factors which cause or are related to the variation of mutagenicity at non-industrial locations.
The mutagenicity data were correlated with air pollution parameters such as SO_2, NO_2, NO, CO and O_3 and meteorological parameters such as wind direction, wind speed, rainfall, temperature, global radiation, atmospheric pressure and air trajectories. Backward trajectories represent the estimated flow of the air mass, terminating at a site, at a certain arrival time.

MATERIALS AND METHODS

Sampling methodology

Sampling of particulate matter was performed at two locations 159 km apart (see Figure 5). The station at Terschelling, an island with no major industrial activities, is also used for background measurements of air pollution in the Netherlands. The sampling location in Wageningen was located at the meteorological station of the Agricultural University, which also acts as a station of the Royal Dutch Meteorological Institute. Wageningen is a small rural town (approximately 30,000 inhabitants) without industrial sources of pollution. Both measuring sites are far from traffic. From July 1979 to July 1980 samples were taken every fourth day in Wageningen and, simultaneously, from July 1979 to November 1979 at Terschelling (Alink et al, 1983). In the winter period of 1982/1983 and 1984/1985 weekly samples were collected in Wageningen as control samples for indoor mutagenicity (van Houdt et al, 1984, 1986). From August 1984 to December 1984 samples were collected over 2 h in Wageningen, in order to obtain extracts collected during the same meteorological conditions.
Two different sampling techniques were used: the standard high volume sampler and a low volume sampler (van Houdt et al, 1984). Differences in sampling methodology, which are summerized in Table 1, manifest themselves in the collection of large particles. Although these particles have hardly any relevance for the mutagenic potential of the samples, we must bear in mind that comparison of all extracts is simply not possible because of different sampling times. The variability and thus the absolute magnitude of mutagenicity is not only dependent on the concentration of mutagens, but also on integration time.
Gelman GF/A 20 x 24 filters were equilibrated and weighed before and after sampling at room temperature and 45% relative humidity. During sampling of particulate matter average levels of SO_2, NO_2, NO, NO_x, CO, and O_3, and temperature, wind direction, wind speed, relative humidity and rainfall

TABLE 1: Sampling characteristics of four sampling periods.

Sampling period	1979/80	1982/83	Aug.Nov.1984	1984/85
Sampling technique	High Vol	Low Vol	High Vol	Low Vol
Sample velocity (m^3/h)	100	10	300	10
Filter surface (m^2)	0.048	0.14	0.14	0.14
Linear velocity (m/s)	0.5	0.025	0.5	0.025
Sampling duration (h)	24	168	2	168
Sampling volume (m^3)	2400	1680	600	1680
Number of samples	W82 T30*	9	26	8
Storage temperature (°C)	4	-20	-196	-196
Sampling time (GMT)	0.00-0.00	12.00-12.00	11.00-13.00	12.00-12.00

* W = Wageningen T = Terschelling

were assessed for correlation with the mutagenicity data. 1000 mb (ground level) air mass back trajectories with positions given every 6 h during the 24 h track were calculated by the Royal Dutch Meteorological Institute. In total 80 trajectories corresponding to an arrival time of 12.00 GMT, terminating in De Bilt (35 km from Wageningen) were calculated. SO_2, NO_2, NO, CO and O_3 concentrations were obtained from the National Institute for Public Health.

Extraction

After weighing, the filters were extracted for 8 h with methanol in a Soxhlet apparatus. The use of methanol was based on preliminary results and literature data, which show methanol to be a suitable solvent (De Raat, 1983). The extracts were evaporated to dryness and the residue dissolved in dimethyl sulfoxide (DMSO). Extracts stored at 4° or -20°C were kept no longer than two weeks before analysis. Storage of the extracts over a prolonged period of time took place in liquid nitrogen.

Mutagenicity data

The extracts were tested in the Salmonella/microsome assay, according to the procedure described by Ames et al. (1975). The bacterial strain used was Salmonella typhimurium TA98, the most sensitive strain based on literature data and preliminary experiments. All experiments were performed with and without addition of 9,000 g liver supernatant, prepared from male Wistar rats, pretreated with Aroclor 1254. Four doses were tested per filter (max. 0.3 ml DMSO). Two experiments were performed in triplicate. Ethyl methane sulphonate and dimethylnitrosamine were used as positive controls in samples collected in 1979/1980. Benzo(a)pyrene and 4-nitroquinoline-oxide were used in the other samples. Alink et al. (1983) expressed mutagenicity as revertants per 30 m^3, calculated from two points of the dose-effect curve. In this paper mutagenic activity is expressed as rev/m^3, based on the linear regression of the dose-effect curve.

RESULTS

Variation of mutagenic activity of airborne particles

Figure 1 shows the number of direct revertants for samples collected simultaneously at Terschelling and in Wageningen. No differences between mutagenicity of particulate matter in Wageningen and Terschelling were found (Wilcoxon sign rank test, $p > 0.05$). Mutagenic activity at both locations was highly correlated ($r = 0.93$, $n = 27$, $p < 0.01$). On days when there was relatively high mutagenicity the values at Terschelling were generally higher than those in Wageningen. Mutagenic activity on days in September and October were significantly higher than those on days in July and August. This observation is supported by Figure 2, which presents mutagenic activity of airborne particles with and without S9 in August, September and November 1984.
The mutagenic activity of airborne particulate matter collected in Wageningen on 82 days over a 1-year period is presented in Figure 3. Although mutagenicity varied markedly from day to day, it was significantly higher in winter than in summer ($p < 0.01$). In addition to a variation within one

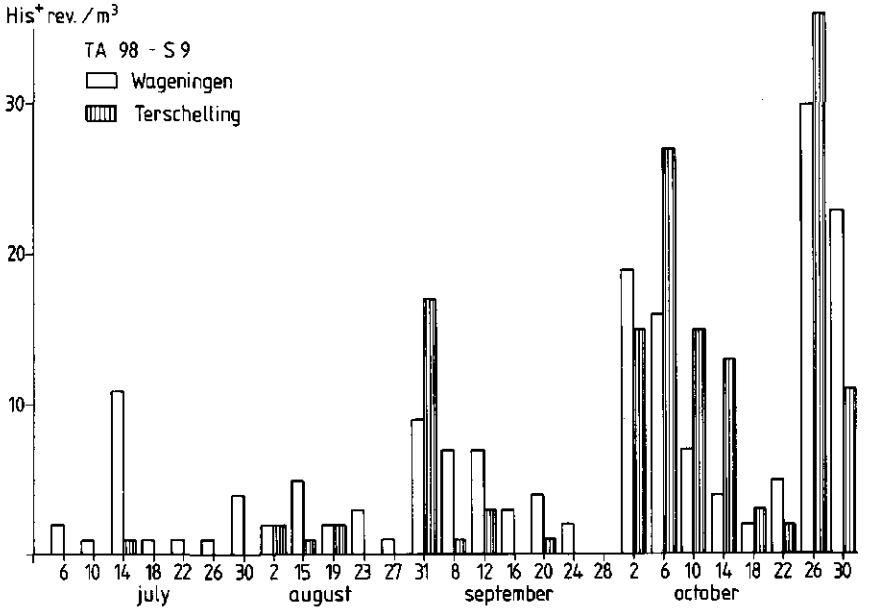

Figure 1: Mutagenic activity of extracts of particulate matter (rev/m^3) in Wageningen (rural location) and Terschelling (background location) in Salmonella typhimurium TA 98 (-S9) over a period of 4 months (July-October 1979).

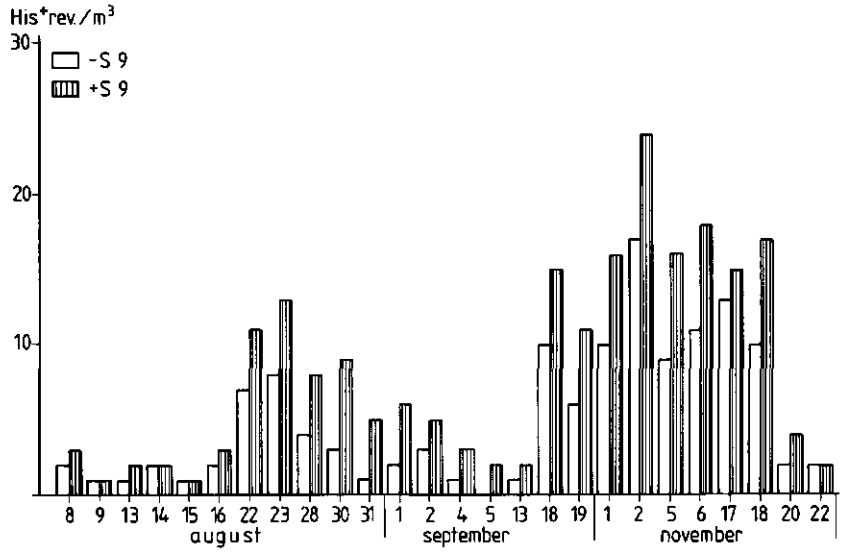

Figure 2: Mutagenic activity of extracts of particulate matter (rev/m^3) in Wageningen in Salmonella typhimurium TA 98 with and without S9 over a period of 3 months (August-September/November 1984).

period, a strong variation in mutagenic activity between periods is also found. The average direct mutagenicity of samples collected from October to March 1979/1980, 1982/1983 and 1984/1985 was 13, 4 and 51 revertants/m^3, respectively.

Effect of metabolic enzymes.

In addition to mutagenic activity, presented as a function of time, Figure 2 also shows the effect of adding liver S9 to the test plates. In all extracts collected from August to December 1984 a systematic increase was found. Similarly to the period August-November 1984 the mutagenic activity in the winter period 1982/1983 was significantly increased after adding liver S9.
In the sampling period 1979/1980 a significant decrease was found in the number of revertants after addition of S9, while in the winter of 1984/1985 systematic differences were not observed.

Relation with air pollution parameters

From Figure 3 it is clear that SO_2 concentrations are also higher in winter than in summer. Average SO_2 concentrations during a 24 h sampling period in 1979/1980 appeared to be

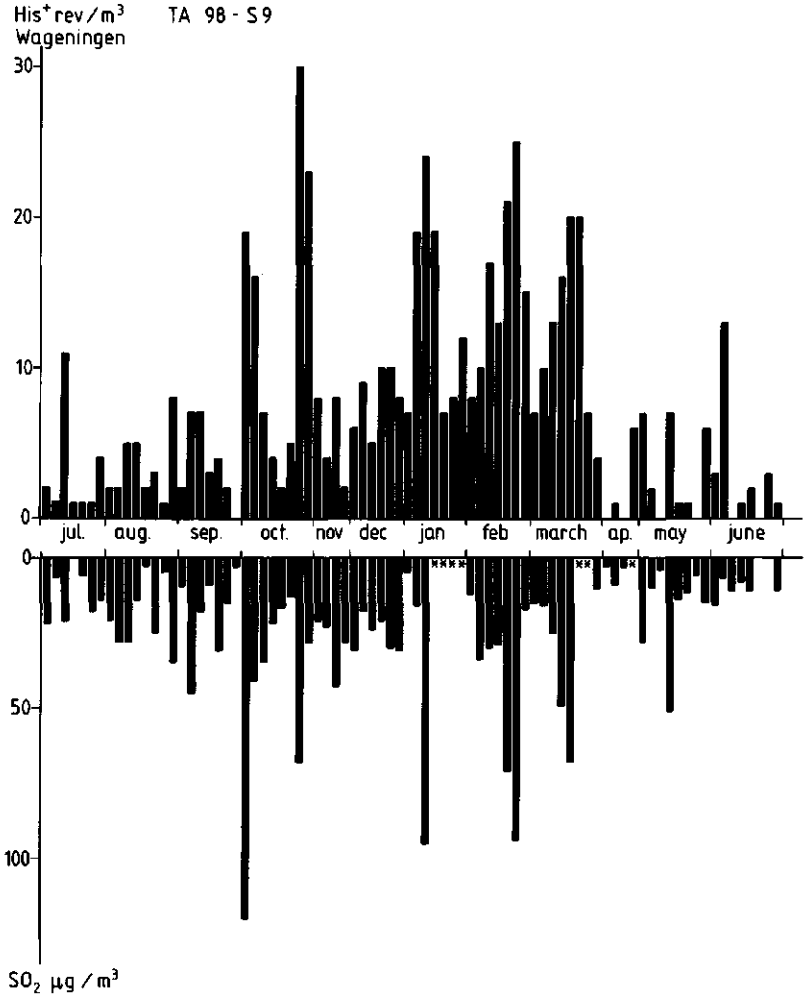

Figure 3: Mutagenic activity of airborne particulate matter (rev/m^3) collected in Wageningen in Salmonella typhimurium TA 98 (-S9) and SO_2 concentrations ($\mu g/m^3$) over a 1-year period (1979/1980). * = not analysed.

significantly correlated with mutagenic activity of airborne particles. Table 2 presents correlation coefficients between biological tests and air pollution parameters of samples collected in 1979/1980. It appears that not only are all correlations between mutagenicity and air pollution parameters significant, but those between air pollution parameters and between direct and indirect mutagenic activity are also significant. Table 3 shows SO_2, NO_2 and CO (only +S9) to be strongly associated.

TABLE 2. Matrix of correlation coefficients between pollution levels of SO_2, NO_2, NO, CO and O_3, temperature and mutagenic activity.

	rev/m^3 +S9	SO_2	NO_2	NO	O_3	CO	Temp.
rev/m^3 -S9	0.984	0.733	0.708	0.512	-0.617	0.638	-0.400
rev/m^3 +S9		0.701	0.678	0.703	-0.554	0.822	-0.450
SO_2			0.699	0.526	-0.501	0.582	-0.208*
NO_2				0.514	-0.560	0.638	-0.058*
NO					-0.411	0.836	-0.340*
O_3						-0.515	0.538
CO							-0.354*

n = 48 * not significant ($p > 0.05$)

Relation with meteorological parameters

Mutagenic activity and mean temperature are correlated, as shown in Table 2. Temperature appears to be correlated with mutagenicity, and not with other air pollution parameters. Parameters such as wind speed, rainfall, relative humidity, global radiation and atmospheric pressure did not give

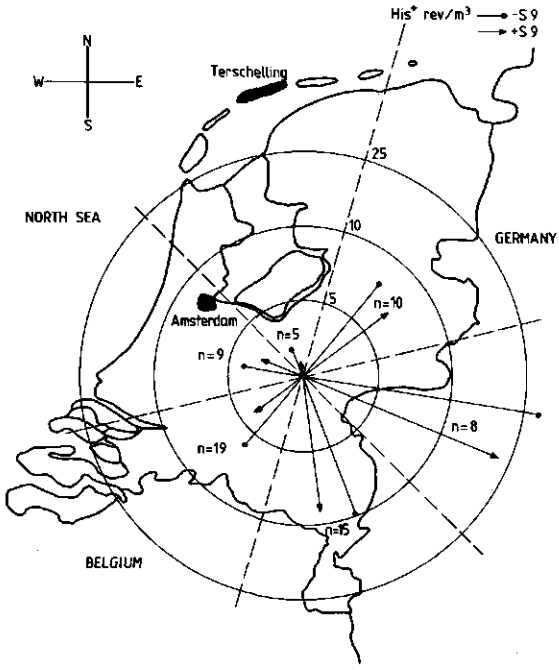

Figure 4: Mutagenicity of airborne particulate matter (rev/m^3) collected in Wageningen in Salmonella typhimurium TA 98 with (⟶) and without (⟶) S9 on days with different wind direction. Arrows represent mean wind direction in sectors of 60°.

TABLE 3. Multiple regression matrix of dependent variables, direct and indirect mutagenicity and independent variables, temperature and pollution levels of SO_2, NO_2, NO, CO and O_3.

Dependent variable	rev/m^3 -S9		Dependent variable	rev/m^3 +S9	
	β	p		β	p
Temp.	-0.247	0.043	Temp.	-0.262	0.011
NO_2	0.350	0.028	NO_2	0.300	0.079
SO_2	0.362	0.008	SO_2	0.250	0.024
NO	-0.124	0.444	NO	-0.009	0.949
O_3	-0.060	0.654	O_3	0.090	0.419
CO	0.189	0.304	CO	0.491	0.002
n = 48	R^2 = 0.706		n = 48	R^2 = 0.797	

significant correlations with mutagenicity.
Figure 4 shows mutagenic activity in 1979/1980 for various wind directions, in segments of 60°. Samples were only attributed to a segment if the wind persisted for at least 50% of the sampling time in that segment. Once more it is clear that the geometric mean of mutagenic activity after addition of S9 decreased in the period 1979/1980. This decrease is not correlated with wind direction. Air masses which originate from eastern or southern directions contain the highest concentrations of mutagenic compounds. A more exact indication of the origin of air is given by trajectories.

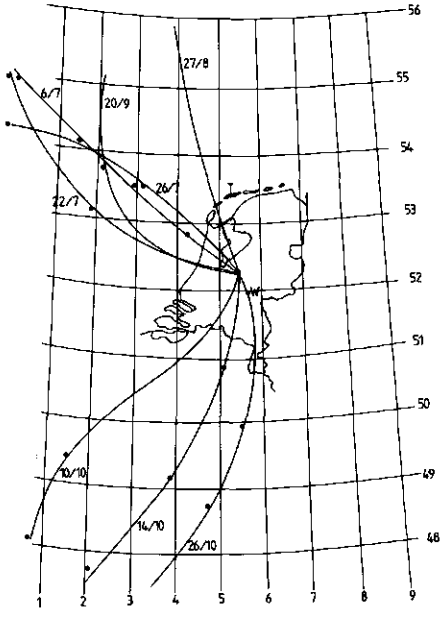

Figure 5: Trajectories (1200 GMT, 1000 mb, 24 h) on days when collection took place at Terschelling (background location) and in Wageningen (rural location).

Figure 5 shows the 1000 mb 24 h trajectories arriving at 12.00 in De Bilt on days presented in Figure 1 when particulate matter was sampled simultaneously in Wageningen and at Terschelling. Although it does not present the trajectories of the sampled air this figure gives an indication of the origin of the air collected in Wageningen and at Terschelling. Air masses originating from northern directions show weak mutagenic activity, which is slightly increased when sampled in Wageningen. Mutagenicity on 10, 14, and 26 October is caused by southerly winds. On these days mutagenic activity at

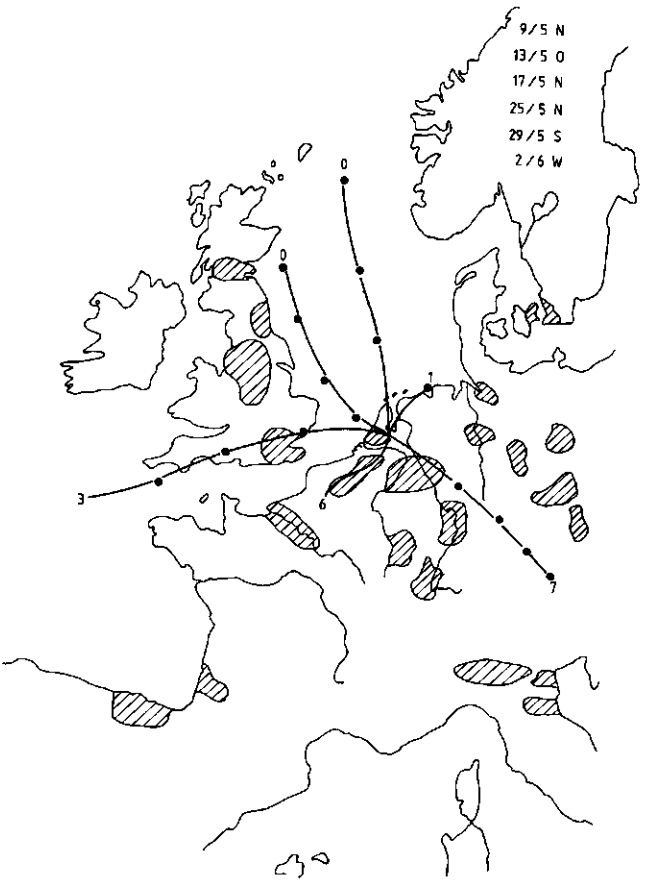

Figure 6: 12.00 GMT Trajectories (24 h, 1000 mb) and corresponding mutagenicity of airborne particles (rev/m^3) in Salmonella typhimurium TA 98 (-S9) on days in summer 1979 and 1980. Industrial areas are indicated.

Terschelling was higher than in Wageningen.
Figures 6 and 7 show direct mutagenic activity and trajectories of air masses of summer and winter days, respectively, of 1979/1980. Again the difference between summer and winter periods manifests itself. Actual differences between samples in the summer or winter period seem to correlate with the passage over industrial areas.

DISCUSSION

From this study it is clear that mutagens occur in the ambient atmosphere at non-industrial locations, as was

Figure 7: 12.00 GMT Trajectories (24 h, 1000 mb) and corresponding mutagenicity of airborne particles (rev/m³) in Salmonella typhimurium TA 98 (-S9) on days in winter 1979/1980. Industrial areas are indicated.

previously found by Alfheim and Möller (1979); Dehnen et al. (1981), and by our group (Alink et al, 1983). When mutagenicity was studied over a 1-year period in Wageningen (Figure 3) the variation of mutagenicity between different days over the entire period was considerable. Our studies indicate that mutagenicity is significantly higher in the winter period than in summer ($p < 0.01$). This is probably due to seasonal differences in generating sources such as combustion processes for space heating. This suggestion is supported by the negative correlation of temperature with mutagenic activity (Table 2). Differences between summer and winter have also been found at other locations by Alfheim and Möller (1979), Daisy et al. (1980), Möller and Alfheim (1980) and Dehnen et al. (1981).
Mutagenicity may vary markedly not only within a sampling

period, but also between sampling periods. Based on Figures 2 and 3 it seems likely that mutagenic activity follows a yearly cycle. However, this cycle is not the same every year. Average winter mutagenicity data (October-March) may show strong variations.
Table 2 and Figure 2 show that in the sampling periods 1979/1980 and 1984 a very strong correlation was found between direct and indirect mutagenicity ($p < 0.01$). The addition of metabolic enzymes gives variable results. Fluctuations from day to day are not found, but between certain periods of time the effect of S9 changes. These effects were neither correlated with season nor with meteorological or air pollution factors. The effect of liver microsomes on the mutagenic potential of airborne particles may be due to the changing composition of the extracts with time. In the first part of the sampling period of 1984/1985 an increase in the number of revertants was found and in the second part a decrease. This is supported by data obtained by Commoner et al. (1978), which show a changing action of liver S9 in the second part of a 1-year sampling period. The correlation ($p < 0.01$) of samples, collected simultaneously at different locations, suggests that the mutagenic potential of suspended matter originates not from local sources, but rather depends on a large-scale process. Alfheim and Möller (1979) suggested that mutagenicity at non-industrial locations is due to long-range transport of mutagenic particulate matter. This is supported by the observation that wind direction is of importance for the mutagenic burden. Commoner et al. (1978) previously made observations on the relationship between mutagenicity and wind direction. The long-range transport of air pollution in general in the Netherlands has already been documented. Van Egmond et al. (1978) demonstrated the influence of industrial areas in Belgium and Germany.
The origin of air masses can be studied by trajectorie studies. In this study, 24 h trajectories, arriving at 12.00 GMT in De Bilt, are used. Comparing mutagenic activity with those trajectories has limitations; the distance from De Bilt to Wageningen is a minor limitation; comparison of sampling duration (2 or 24 h) with air arriving at 12.00 GMT is a serious one. Because of the absence of easterly and westerly winds in the 1984 sampling period (2 h) a comparison is made for the period 1979/1980. In Figures 6 and 7 once again the summer/winter difference is clear. Actual differences within the summer or winter periods may be explained by the passage of the sampled air mass over industrial areas, particularly in Belgium and Germany. Although not representing the trajectories of sampled air masses in Wageningen and Terschelling, Figure 5 may give an indication of the origin of mutagenic compounds, and with that an answer to the question of why mutagenicity at Terschelling is sometimes higher than in Wageningen. Air originating from northern directions (marine) shows weak mutagenic activity at Terschelling. Because of sources in the northern part of the

Netherlands, a slight increase is found in mutagenic activity in Wageningen. Mutagenicity on 10, 14, and 26 October at Terschelling was higher than in Wageningen. This may be due to sources in Rijnmond (western part of the Netherlands) and the Ruhr area (German industrial area, south-east of Wageningen), which may be passed by trajectories arriving at Terschelling and not by the trajectory arriving in De Bilt/Wageningen. De Raat et al. (1985) also showed the contribution of sources in the Rijnmond area to the mutagenic burden of air masses passing Rijnmond.
Van Egmond et al. (1978) showed that long-range transport is important for SO_2 concentrations in the Netherlands; the question therefore arises whether correlations may be found between mutagenicity and commonly registrered air pollution parameters such as SO_2, NO_2, NO, NO_x, CO and O_3. Figure 3 shows that high levels of SO_2 correspond with high mutagenicity episodes. As shown in Table 2 all air pollution parameters appear to be strongly correlated with mutagenicity data. However, significant correlations are also found between air pollution parameters. Multiple regression shows that the air pollution parameters SO_2, NO_2, NO, CO and O_3 together account for 70% of the variation in direct mutagenicity and for 80% of the variation in indirect mutagenicity. SO_2 and NO_2 and SO_2, NO_2 and CO were significantly associated.
For these reasons it can be concluded that, as a general air pollution parameter, monitoring the mutagenic burden of aerosols does not contribute to our knowledge, obtained by monitoring SO_2, NO_2 and CO.
On the other hand the biological test is a pre-screening of environmental mixtures for mutagenic properties. This first step in the risk estimation is important, especially for mixtures from emissions of specific sources.
Furthermore, from time to time control measurements are necessary in order to evaluate possible changes in mutagenic properties.

ACKNOWLEDGEMENT

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3 Mutagenic activity of indoor airborne particles: contribution of smoking, food processing and outdoor sources

JJ van Houdt, WMF Jongen, GM Alink and JSM Boley

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SUMMARY

Indoor concentrations of total suspended particles often exceed outdoor concentrations. Although it is known that particulate matter may contain mutagenic compounds and that several sources in the home produce mutagens, virtually no data concerning the mutagenicity of indoor particulate matter are available.
In this study, experiments were carried out to determine the contribution of indoor and outdoor sources to the mutagenicity of indoor particles. Using six samplers, particles in kitchens, living rooms, and outdoors were collected simultaneously. Methanol extracts of the material obtained were tested in the Salmonella/microsome assay. An increase in mutagenic activity was shown in the presence of a metabolizing system in all indoor and outdoor samples but one. The data presented suggest that mutagenic components of indoor particulate matter are different from those found on outdoor particles. Indoor samples show a higher mutagenic activity after metabolic activation, while direct mutagenic activity of indoor particles was lower than that of outdoor particles. Furthermore, only indoor samples showed cytotoxic effects. Our findings suggest that, with respect to the mutagenic activity of particulate matter, cigarette smoke is the most important contaminant of indoor air. Kitchen samples also show mutagenic activity, probably as a result of volatilization of cooking products. No contribution of outdoor sources to mutagenicity of indoor particles was observed.

INTRODUCTION

Several authors reported on the mutagenic potential of suspended particulates in outdoor air (Pitts et al, 1977; Harnden, 1978; Tokiwa et al, 1983). Suspended particulates have been shown to be mutagenic both in an industrial setting (Pani et al, 1983; Stern, 1982) as well as in office

buildings (Löfroth et al, 1983). In view of the large fraction of time spent inside the home by people living in modern industrialized countries (Moschandreas, 1981), exposure to mutagenic compounds in breathing air might very well be determined predominantly by the concentration of mutagenic compounds people breathe inside their own homes.
Several potential indoor sources of contaminants, like cigarette smoking (Kier et al, 1974; Hutton and Hackney, 1975; Mizusaki et al, 1977a,b), volatile cooking products (Nagao et al, 1977; Spingarn and Weisburger, 1979; Rappaport et al, 1979; Spingarn et al, 1980), wood-burning stoves (Rudling et al, 1981), and burning joss sticks (Sato et al, 1980) have been shown to produce mutagenic compounds. Tobacco smoking seems to be one of the predominant sources.
Suspended particulate concentrations inside homes often exceed outdoor concentrations when smokers are present in the home (Spengler et al, 1980; Dockery and Spengler, 1981; Brunekreef and Boley, 1982; Girman et al, 1982). In the absence of smokers the ratio of indoor/outdoor concentration is usually less than one, owing to filtration and deposition of particles entering the home from outside (Alzona et al, 1979; Moschandreas et al, 1979; Cohen and Cohen, 1980; Boley and Brunekreef, 1982).
In homes, mutagenic activity of airborne particles has not been studied to date. The present study is an attempt to establish indoor/outdoor ratios for mutagenic activity of airborne particles.

MATERIALS AND METHODS

Sampling methodology

Samples of indoor particulate matter were collected in kitchens or living rooms of 39 houses of employees of the Agricultural University in Wageningen, The Netherlands, in October/November 1982 and Januari/Februari 1983. Wageningen is a small rural town (approximately 30,000 inhabitants) without important industrial sources of pollution. Simultaneously, outdoor particulate matter was collected at the local meteorological station, which is located away from pollution sources such as traffic. The samplers consisted of a centrifugal pump and a filter holder with 1-3 glass-fiber filters (Gelman GF/A 20 * 24 cm). Sampling was performed upside-down with a face velocity of 0.025 m/s, resulting in a sampling efficiency of about 94% for particles with an aerodynamic diameter of 7 μm (Ter Kuile, 1979). For both indoor and outdoor samples the same type of sampler, which sampled 10 m^3/hr, was used. Each week six samples were collected simultaneously. The sampling time was exactly one week, resulting in a sampled volume of 1,680 m^3. Before and after sampling, filters were conditioned at room temperature and relative humidity of 45 % for at least 24 hr

and weighed. The occupants of the houses were asked to register the number of cigarettes, cigars and pipes smoked; the amount of time spent cooking; and some habits like heating, ventilation, and the use of solvents. Five categories of locations were sampled: heavy smokers' living rooms (> 100 cig/week)(n=6), moderate smokers' living rooms 15 > cig/week < 100)(n=5), Nonsmokers' living rooms (< 15 cig/week)(n=5), kitchens of nonsmoking families (n=23), and outdoors (n=11).

Extraction

After sampling, the filters were extracted for 8 hr in a Soxhlet apparatus with 200 ml of methanol. The extracts were evaporated to dryness and the residues dissolved in dimethyl sulfoxide (DMSO).
The DMSO solution was stored at -20°C. The use of methanol is based on preliminary experiments on the extraction of indoor aerosols in which subsequent extraction of the methanol extracted filters with hexane and water resulted in nonmutagenic extracts. Literature data also show methanol to be a suitable solvent (de Raat, 1983). Extraction of unloaded filters resulted in nonmutagenic extracts.

Mutagenicity testing

The mutagenicity test with Salmonella typhimurium was performed essentially according to the procedure described by Ames et al (1975). As a minor modification, histidine and biotin were added to the bottom agar and not to the top agar. A 9,000 g (S9) liver supernatant was prepared from Aroclor 1254-induced male Wistar rats. The liver homogenate was stored in liquid nitrogen. In tests with metabolic activation, 50 µl of S9 was used with the necessary cofactors (Ames et al, 1975). The bacterial strain used was Salmonella typhimurium TA98, the most sensitive strain, based on preliminary results obtained with indoor extracts. Within 10 days after collection of the samples, two independent tests, both with and without liver S9, were carried out in triplicate.
Various amounts of extracts (maximum 0.3 ml of DMSO) together with the appropriate blanks and positive controls were tested. Five micrograms of benz(a)pyrene (B[a]P) per plate (+S9) and 0.5 µg of 4-nitroquinoline-oxide (NQO) (-S9) were used as positive controls . Test results were acceptable if the positive controls were within the range 350-550 revertants per plate for B(a)P and 350-600 for NQO.
In all results presented, the number of spontaneous revertants, which varied between 16 and 46 (-S9) and between 28 and 59 (+S9), are substracted. Addition of S9 mix always increased the number of spontaneous revertants. Mutagenic activity is expressed as revertants per m^3 of air, calculated from the slope of the linear part of the dose-effect curve.

RESULTS

Sampling data

The mean particle concentrations and their range for each sampling location and the results of the Salmonella/microsome assay are presented in Table I. The results show that

TABLE 1. Aerosol concentration ($\mu g/m^3$) and mutagenic activity in Salmonella typhimurium TA98 (rev/m^3) with and without addition of liver S9 for the various sampling locations.

Sampling location	Number	Concentration[a] ($\mu g/m^3$)	rev/m^3[a] -S9	rev/m^3[a] +S9
Outdoor	11	18 (6- 48)	5 (0-14)	7 (2-23)
Kitchen	23	37 (6- 83)	2 (0-23)	9 (2-30)
Living room (cig/wk < 15)	5	40 (18- 72)	2 (0- 4)	10 (0-29)
Living room (15 < cig/wk < 100)	5	50 (12- 96)	5 (2- 5)	17 (6-29)
Living room (cig/wk > 100)	6	210 (42-354)	8 (0-22)	90 (14-202)

[a] The number given represents the maen, the range observed is given in parenthesis.

indoor concentrations of total suspended particles exceed outdoor concentrations, and that cigarette smoking seems to be an important source. Concentrations in kitchens are comparable with those in nonsmokers' living rooms. Most living room samples were collected in October/November 1982, whereas most kitchen samples were collected in Januari/Februari 1983. Airborne particulate matter collected in kitchens, living rooms and outdoors contains compounds that are mutagenic in the Samonella/microsome assay. Most outdoor samples show a detectable direct activity, which is slightly increased after addition of liver S9. Indoor samples show a low or not detectable direct mutagenic activity. After addition of S9 a strong increase of the mutagenic activity was observed in all indoor samples but one. Consequently the ratio -S9/+S9 is much lower for indoor than for outdoor particles. The highest enzyme-mediated mutagenic activity is found in particulate matter from indoors.

Effect of smoking

Figure 1 presents a typical example of the effect of smoking on indoor mutagenic activity. The dose-effect curves of the extracts simultaneously collected in sample wk 1 are given. The figure clearly shows that in the outdoor samples only a slight mutagenic activity is detectable. The indoor samples

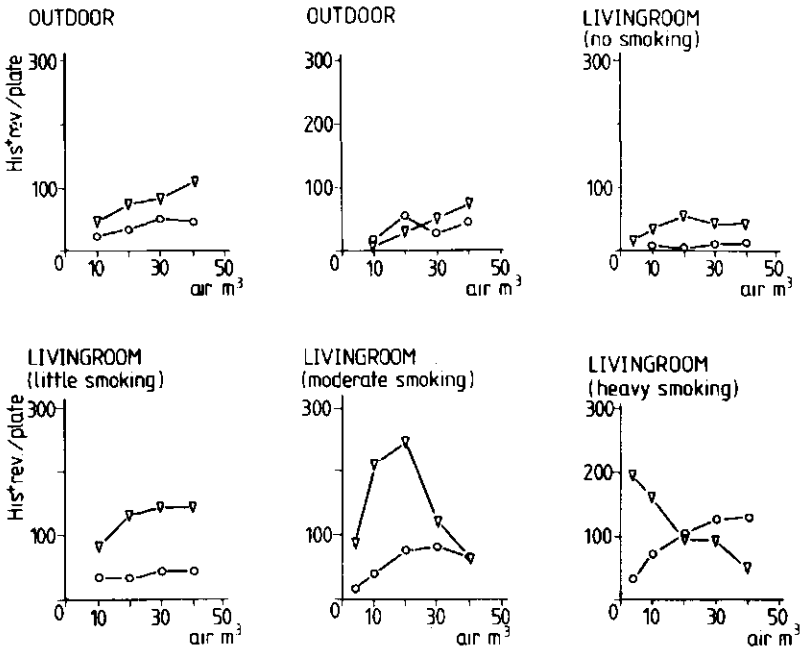

Figure 1. Mutagenic activity of extracts of airborne particulate matter, collected simultaneously in different types of living rooms, compared to outdoor mutagenicity. During collection of indoor samples respectively 0, 29, 90 and 160 cigarettes were smoked. Salmonella typhimurium TA98 with (-▽-) and without (-○-) liver S9 was used.

showed only a slight direct activity, while enzyme-mediated mutagenic effects for some samples were strong and related to the number of cigarettes smoked. The decrease in the number of revertants at higher doses after metabolic activation of samples collected in living rooms of smokers is caused by cytotoxic effects, as witnessed by a reduction of the bacterial lawn. The tested concentrations in the heavy smokers' living rooms were all cytotoxic; the optimum of the curve was probably not found, but is at least 195 rev/plate, corresponding to a mutagenic activity of 49 rev/m³. More examples of the influence of smoking are given in Figure 2. It shows the results obtained in three different living rooms of heavy smokers (> 100 cig/week), compared to the corresponding outdoor samples. Notwithstanding the degree of outdoor indirect activity, indoor enzyme-mediated levels are much higher than outdoors in all cases. Cytotoxic effects were observed at a relatively low dose in the presence, and, to a certain extent, in the absence of liver S9.

Kitchen effect

In Figure 3 an example of mutagenicity of kitchen samples is shown. Samples were collected at different times in two

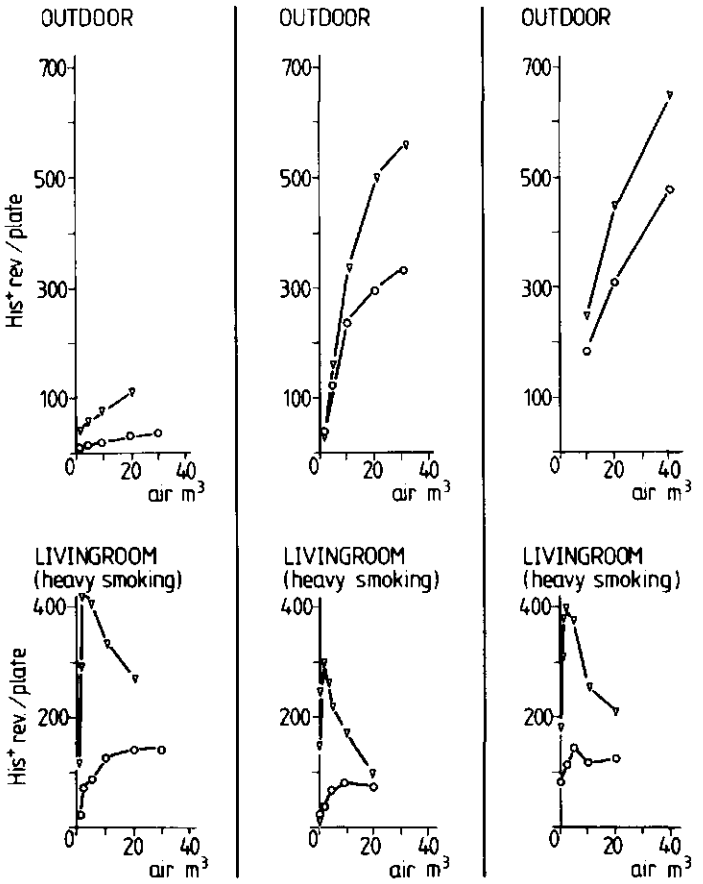

Figure 2. Mutagenic activity of airborne particulate matter, sampled in living rooms of heavy smokers, compared to simultaneously sampled outdoor particles. Salmonella typhimurium TA98 with (-▽-) and without (-○-) liver S9 was used as the tester organism.

kitchens that were separated from the living room. The corresponding outdoor sample was slightly mutagenic in one case, but much more active in the other. The kitchen samples showed minimal direct activity. However, after addition of liver S9 both kitchen samples showed a strong increase of mutagenic activity.

To answer the question of which sources are responsible for mutagenic activity of particulate matter in kitchens, samples were taken for 4 wk in kitchens of nonsmoking families. Figure 4 gives a typical example of samples collected simultaneously. In contrast to outdoor air, indoor kitchen samples showed minimal direct activity. The pronounced cytotoxic effects found in living rooms of smokers were not

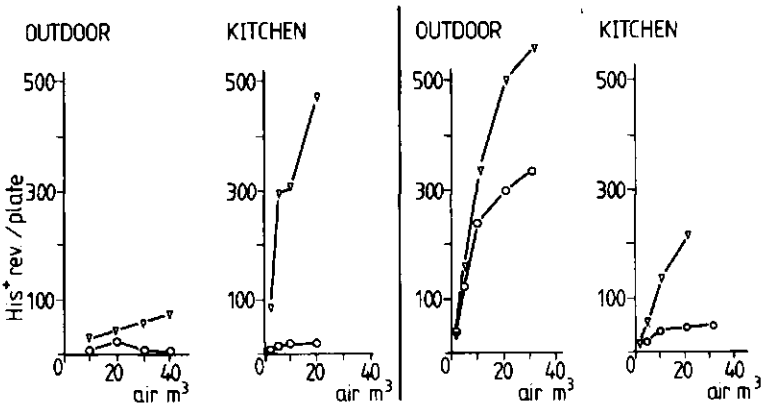

Figure 3. Mutagenicity of two kitchen samples, compared with simultaneously collected outdoor particles in Salmonella typhimurium TA98 with (-▽-) and without (-○-) liver S9.

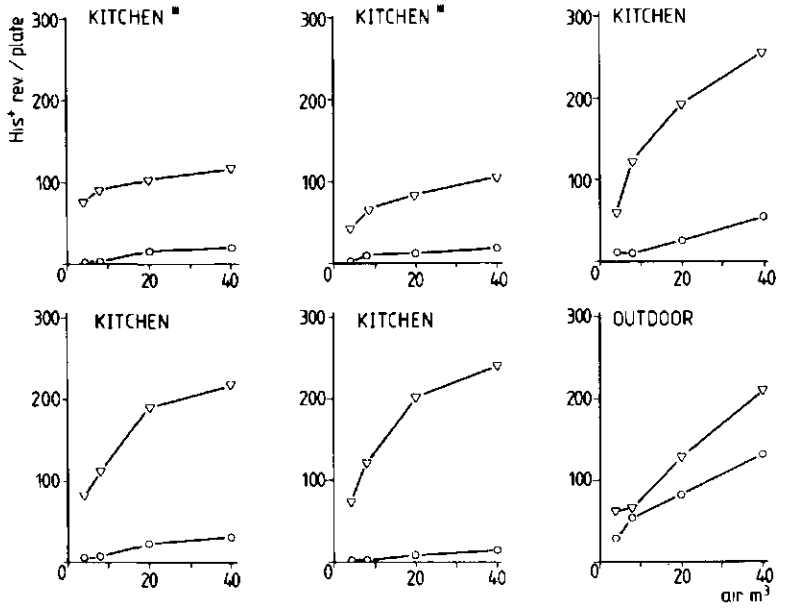

Figure 4. Mutagenicity in kitchens of nonsmoking families and the corresponding outdoor sample in Salmonella typhimurium TA98 with (-▽-) and without (-○-) liver S9. * Kitchen with ventilationhood.

observed. Two samples were collected in kitchens in which a ventilationhood was present. Its presence possibly explains the lower mutagenic activity observed for these samples.

Mutagenicity expressed as revertants/μg particulate matter

Mutagenic activity has, up until now, been expressed as revertants per cubic meter of air. Although this form of

expression is most suitable in terms of personal exposure, it might be interesting to look at the mutagenic response per mass unit of suspended particulate matter. Both particulate matter concentration and mutagenic activity are higher indoors than outdoors, especially in homes of smokers. The question arises as to whether variations in mutagenicity at different locations may be due to differences in particulate matter concentration. Table II gives the number of revertants

TABLE 2. Mean number of revertants in Salmonella typhimurium TA98 per 10 µg particulate matter for the various locations.

Sampling location	-S9	+S9
Outdoor	2.1	4.1
Kitchen	0.5	2.4
Living room (cig/wk < 15)	0.5	2.6
Living room (15 < cig/wk < 100)	0.6	3.2
Living room (cig/wk > 100)	0.4	5.5

per microgram suspended particulate matter. The table shows that indoor direct-mutagenic activity per mass unit of suspended particles is lower than outdoor activity, whereas the indirect mutagenicity per mass unit is comparable.

DISCUSSION AND CONCLUSIONS

As the particulate matter in this study was collected in winter, the outdoor values presented may exceed the annual mean values (Alink et al, 1983). This might also apply for mutagens produced indoors, because reduced ventilation in winter would lower the air-exchange rate. Also, the percentage of time spent indoors is higher in the winter period than in summer. In agreement with earlier results (Alink et al, 1983), a wide range in mutagenicity was observed for the outdoor samples in this study. Comparison of the mutagenic activity of particulate matter from indoor and outdoor samples indicates that the direct mutagenic activity of indoor suspended particulate matter in homes was lower than the direct mutagenic activity of outdoor suspended particulate matter sampled at the same time. Only when samples were taken in living rooms of heavy smokers, and when results were expressed as revertants per cubic meter, did the direct indoor mutagenicity exceed the outdoor mutagenicity. Indirect mutagenicity of indoor suspended particulate matter was equal to or higher than indirect mutagenicity of outdoor suspended particulate matter; indirect mutagenicity was high primarily in smokers' living rooms. This is in agreement with the results of Löfroth et al (1983), who found that smoking

caused an increase of the mutagenic response of suspended particulate matter inside an office building. In addition, cytotoxic effects were observed in smokers' living rooms. This is in agreement with observations of Kier et al (1974), who found cytotoxicity in cigarette smoke condensate. In extracts of kitchen air a very weak direct activity is observed, with an increase in the number of revertants after addition of liver S9. It is not likely that the indirect mutagenic activity in kitchens is produced by outdoor sources, because in that case direct indoor activity should also be stronger, and because some kitchen samples showed a much higher indirect mutagenic activity than outdoors. It is known that extracts of volatile components produced during cooking show mutagenic activity. A considerable amount of these components can be produced during cooking (Rappaport et al, 1979).
When the effect is expressed as number of revertants per mass unit, it is again obvious that indoor particles do not contain a large amount of direct-acting mutagens. Per mass unit, indirect mutagenicity was found to be comparable in all indoor locations as well as outdoors. However, a slight trend toward higher mutagenicity of suspended particulate matter (in number of rev/μg) was observed with increased smoking. The mutagenicity per mass unit also makes clear that the high level of enzyme-mediated activity in indoor samples cannot be attributed to the higher mutagenicity of the particulate matter itself, but to the high concentration of total suspended matter.
Various publications indicate that indoor air is contaminated by particles from outdoor origin (Alzona et al, 1979; Boley and Brunekreef, 1982; Moschandreas et al, 1979; Cohen and Cohen, 1980). However, in our study, outdoor particles do not appear to be the source of indoor mutagenic activity. Most outdoor samples show direct mutagenicity, while simultaneously sampled indoor particles do not show direct activity.
In this study the mutagenic activity of complex environmental mixtures was determined. Fractionation of the samples will be necessary to identify the responsible compounds or groups of compounds. Further evaluation of the observed bacterial mutagenicity in test systems with mammalian cells is necessary to assess whether extracts of airborne particulate matter represent a health risk to humans. Further work on the influence of other indoor sources like wood stoves and the contribution of various compounds is in progress.

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4 Mutagenic activity of indoor airborne particles: contribution of wood combustion and outdoor sources

JJ van Houdt, CMJ Daenen, JSM Boley and GM Alink

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SUMMARY

Wood combustion produces compounds that are mutagenic in the Salmonella/microsome assay.
As combustion products can be emitted in the home and the use of wood as a residential energy source is growing, an impact on human health might be of concern.
In this study experiments were carried out to determine the contribution of wood combustion in stoves and fire places to indoor mutagenic activity under normal living conditions. Airborne particles from living rooms which were heated by stoves, or by fire places, and from outdoors were collected simultaneously. In each room two samples were collected during two consecutive weeks: one week the room was heated by central heating, the other week by wood combustion. Sampling took place in a total of 24 homes. Methanol extracts of the samples were tested in the Salmonella/mammalian microsome assay.
Results show that mutagenic activity of outdoor air exceeds indoor mutagenicity. At the same time a correlation is found between indoor and outdoor mutagenicity, both with and without S9. However, a large difference is found between the ratio -S9/+S9 of indoor and outdoor mutagenic activity. Systematic differences in the ratio -S9/+S9 between control and experimental conditions are not observed.
The use of wood stoves caused an increase of indoor mutagenicity in 8 out of 12 homes. It could be concluded that the use of an open fire consistantly leads to an increase of mutagenic activity of indoor air. This increase was caused by wood combustion products.

INTRODUCTION

Reserach on mutagenic activity of total suspended particles has, until recently, been focused primarily on the outdoor environment (Pitts et al., 1977; Harnden, 1978; Tokiwa et al., 1983; Alink et al., 1983). People in western societies spend most of their time inside the home (Moschandreas, 1981; Boleij and Brunekreef; 1982, Yocom, 1982), where mutagenic

activity may differ from outdoors. From this point of view, exposure to mutagenic compounds in breathing air might very well be determined to a major extent by the level of genotoxins in the air inside homes.
Indoor air may be polluted by genotoxins produced indoors and, depending on particle size and air-exchange rate, by particles infiltrating from outdoors (Alzona et al., 1979; Cohen and Cohen, 1980; Dockery and Spengler, 1981). In addition, adsorption of organic compounds on the surface of indoor airborne particles takes place to a larger extent than on outdoor particles (Weschler, 1984).
Indoor sources like cigarette smoke (Kier et al., 1974; Hutton and Hackney, 1975; Mizusaki et al., 1977a,b), volatilization of cooking products (Nagao et al., 1977; Spingarn and Weisburger, 1979; Rappaport et al., 1979; Spingarn et al., 1980), wood combustion (Rudling et al., 1981; Hytönen et al., 1983; Ramdahl et al., 1984; Alfheim et al., 1984), and emissions of kerosene heaters (Yamanaka and Muruoka, 1984) have been shown to produce mutagenic compounds. As a consequence of cooking and especially smoking, indoor levels of genotoxic compounds can exceed outdoor levels (Löfroth et al., 1983; Van Houdt et al., 1984).
Wood combustion has been recognized as an important particle emission source: respirable emissions from residential wood combustion in the ambient atmosphere can easily exceed all other sources (Cooper, 1980).
An indication of a contribution of wood combustion in open fire places to mutagenic activity of indoor particulate matter was reported by Alfheim and Ramdahl (1984).
Since the use of wood as a residential energy source is increasing, concern about the emission of mutagens is caused. Emission of wood combustion products is dependent on the type of fire place, type of fuel and operating conditions (Butcher and Sorensen, 1979; Butcher and Ellenbecker, 1982; Muhlbaier Dasch, 1982).
Particles may be removed by deposition and ventilation requirements. As a consequence of these factors, a large variation in mutagenicity of particles within one home at different times, and between different homes can be expected. The purpose of this study is the determination of the contribution of wood combustion by wood stoves and open fire places to mutagenic activity of particulate matter in homes under normal living conditions.

MATERIALS AND METHODS

Sampling strategy

To make an adequate comparison between conditions with and without wood combustion a control and an experimental sample were collected in each home. During collection of the

experimental sample wood stoves were burning approx. 10 h/day; open fire places 4 h/day, different types of wood were used. Sometimes the control sample was collected before, sometimes after the experimental sample. Sampling time was one week, each week 8 identical samplers were collecting simultaneously in 8 homes.
The occupants of the houses were asked to behave similarly both weeks and not to take any special precautions. They registered some habits like ventilation, use of solvents and time of wood combustion.
Samples were collected in living rooms, separated from kitchens, in 24 homes of nonsmokers during the winter 1984/1985 in Wageningen, a small rural town, without any important industrial sources. Every week an outdoor control sample was collected simultaneously as described previously (Van Houdt et al., 1984).
In addition, two samples were collected in an uninhabited house and outdoors, in order to determine the influence of habitation. In Table 1 sampling data are presented.

TABLE 1. Sampling scheme: each home is identified by a letter.

			outdoor	indoor				
				wood stove		fire place		uninhabited
				Contr	Expt	Contr	Expt	
Period 1	week 1	1	ABCD			MNOP		
(21/11/84-06/12/84)	week 2	1			ABCD		MNOP	
Period 2	week 1	1	EFGH			QRST		
(06/12/84-20/12/84)	week 2	1			EFGH		QRST	
Period 3	week 1	1	IJ	KL		UV	WX	
(16/01/85-30/01/85)	week 2	1	KL	IJ		WX	UV	
Period 4	week 1	1						Y
(06/03/85-20/03/85)	week 2	1						Y

Sampling

Each sampler consisted of a centrifugal pump and a filterholder with 3 Andersen S935 glass fiber filters. Sampling was performed upside-down with a sampling volume of about 8 m^3/h. The resulting aspiration velocity was 1.6 cm/s, resulting in a sampling efficiency of 94% for particles of 5μm (ter Kuile, 1979).
In order not to disturb normal ventilation conditions and level of air pollution, sampling only took place in living rooms with air-exchange volumes of at least 80 m^3/h, so that the amount of air sampled was not more than 10% of the estimated air-exchange volume. Samplers were located at a

height of 1.50 to 2.00 m, at a distance of minimal 3 m from the fire place. After conditioning at room temperature and 45% relative humidity the filters were weighed before and after particle collection to determine the particle concentration.

Extraction

As preliminary experiments showed methanol to be the most suitable solvent, extraction and sample preparation took place as described in our first study on indoor mutagenicity (Van Houdt et al., 1984). DMSO extracts were stored in liquid nitrogen before use.

Mutagenicity testing

Mutagenicity testing with Salmonella typhimurium in the plate-incorporation assay was performed according to the procedure described by Ames and coworkers (1975). As minor modifications histidine and biotin were added to the bottom agar, not to the top agar. As metabolic activation 50 µl 9,000 g liver supernatant (S9) with necessary cofactors was used (Ames et al., 1975). The S9, obtained from 3 months old male Wistar rats, pretreated with Aroclor 1254, was stored in liquid nitrogen. Tester strain TA98 was used in all experiments.
Per extract, at least two independent experiments, each with and without S9 were performed. All experiments consisted of at least 4 doses of extract, blanks and the positive controls benzo(a)pyrene (5 µg) and 4-nitroquinoline-oxide (0.5 µg), in triplicate.
Test results were acceptable if the data were within the range 350-550 rev/plate for B(a)P and 350-600 for NQO. The number of spontaneous revertants, which may not exceed 70 revertants per plate were substracted from all results.
The level of direct and indirect mutagenic activity of each sample is expressed as revertants per m^3, calculated from the slope of the linear part of the dose-effect curve. The ratio -S9/+S9 gives expression to the mutagenic composition of the mixtures.

RESULTS

Some typical dose-effect curves obtained from extracts, collected in period 2 are depicted in Figure 1. Airborne particulate matter collected indoors, with and without wood combustion, and outdoors, contained compounds causing mutations in Salmonella typhimurium strain TA98. Addition of liver S9 increased mutagenic activity of indoor extracts. In contrast, no systematic change was found in the outdoor samples. In both weeks outdoor mutagenic activity reached the

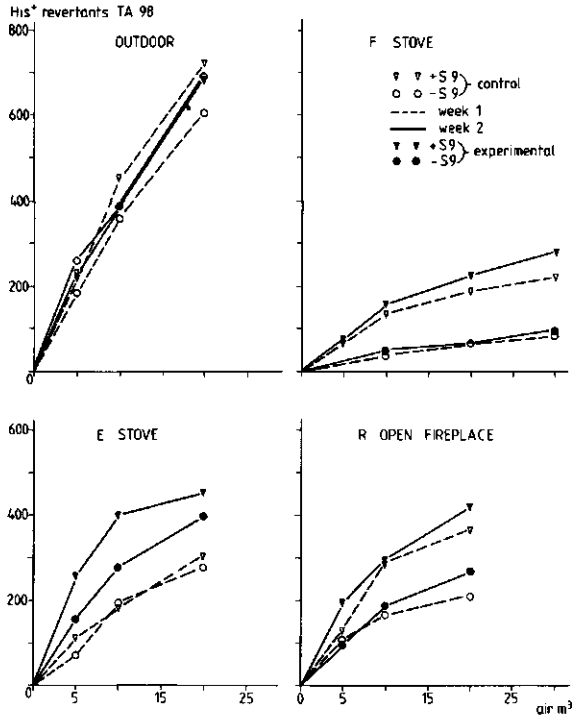

Figure 1. Mutagenic activity of extracts of airborne particulate matter, collected simultaneously during period 2 in living rooms without (control) and with (experimental) wood combustion, and outdoors. Salmonella typhimurium TA98 with (-▽-) and without (-○-) liver S9 was used.

same high level. However, in all homes mutagenicity of the experimental sample is increased.
In Figure 2 an example of different outdoor activities in week 1 and 2 (period 3) is presented. In week 1 sampling took place during a high pollution episode. Notwithstanding control or experimental conditions both indoor and outdoor activity is extremely high in week 1. The mutagenic composition of indoor and outdoor samples differs, as addition of liver S9 decreases outdoor, and increases indoor mutagenic activity. Mutagenic activity in week 2 (23/1 - 30/1) is low. Addition of liver S9 gives comparable results to week 1.
Table 2 presents mutagenic activity and the ratio -S9/+S9 of the outdoor samples and the median of the indoor samples for all sampling weeks. Outdoor direct and indirect mutagenic activities were higher than median indoor direct and indirect levels (Wilcoxon sign rank test $p < 0.01$). At the same time linear regression shows indoor and outdoor levels to be highly correlated ($r = 0.99$; $n = 24$; $p < 0.01$), both with and without S9. Table 2 also gives information about the way

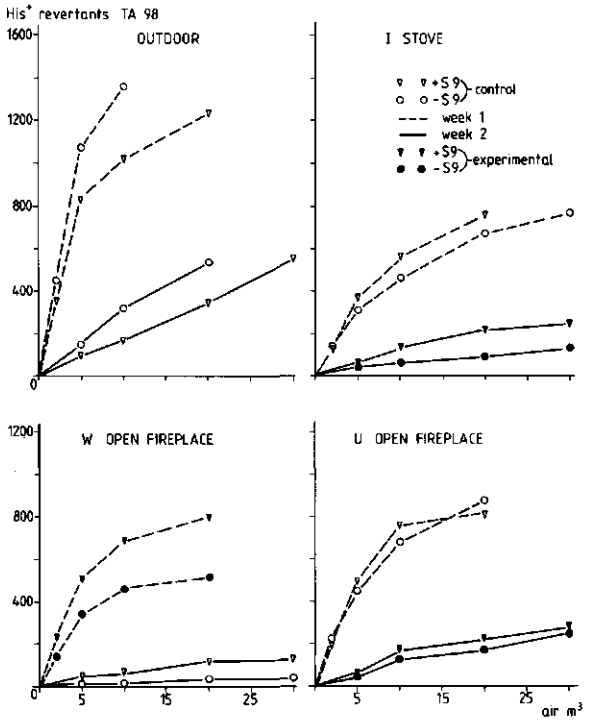

Figure 2. Mutagenic activity of extracts of airborne particulate matter, collected simultaneously during period 3 in living rooms without (control) and with (experimental) wood combustion, and outdoors. Salmonella typhimurium TA98 with (-▽-) and without (-○-) liver S9 was used.

liver S9 acts. The ratio -S9/+S9 is significantly higher outdoors (Wilcoxon sign rank test $p < 0.01$).
Table 3 gives mutagenic activities of particulate matter collected simultaneously outdoors and in an uninhabited home. Outdoor mutagenicity still exceeded indoor mutagenicity. In contrast to the results mentioned above the ratio -S9/+S9,

TABLE 2. Outdoor and median indoor mutagenic activity (rev/m^3) and ratio -S9/+S9 for all sampling weeks.

Sampling period/ week	-S9		+S9		-S9/+S9	
	Outdoor	Indoor	Outdoor	Indoor	Outdoor	Indoor
1.1	16	3	17	9	0.9	0.3
1.2	29	13	41	21	0.7	0.6
2.1	36	6	44	14	0.8	0.4
2.2	38	7	38	23	1.0	0.3
3.1	237	71	164	96	1.5	0.7
3.2	32	6	16	11	2.0	0.6

TABLE 3. Mutagenic activity (rev/m^3) of 4 samples, simultaneously sampled outdoor and indoor extracts. Indoor samples are collected in an uninhabited house.

Sampling period/ week	-S9		+S9		-S9/+S9	
	Outdoor	Indoor	Outdoor	Indoor	Outdoor	Indoor
4.1	10	5	7	3	1.4	1.7
4.2	10	5	5	3	2.1	1.7

however, was almost similar. Of all indoor samples only these two indoor samples showed a decrease after addition of liver S9.

Figure 2 and Table 2 make it clear that the initial goal of the sampling strategy, wood combustion to be the only difference between control and experimental conditions, was not realized. Assuming that in one home an outdoor effect, if there is one, is identical in the control and the experimental week, then the ratio indoor/outdoor mutagenic activity can be considered as mutagenic activity, adjusted for outdoor effects and the ratio indoor/outdoor -S9/+S9 as mutagenic composition, adjusted for outdoor effects.

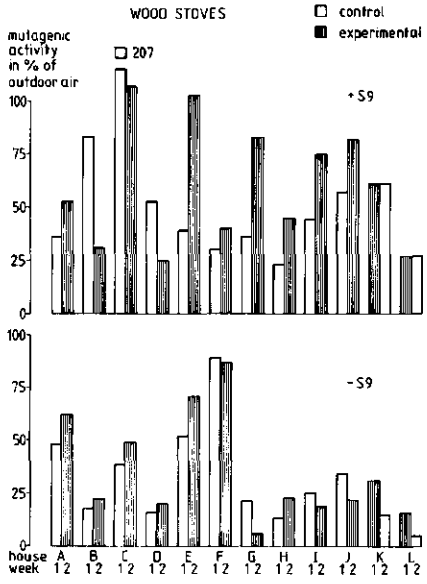

Figure 3. Mutagenic activity (+S9 and -S9) of control and experimental indoor samples in homes with wood stoves, expressed as percentage of outdoor mutagenicity.

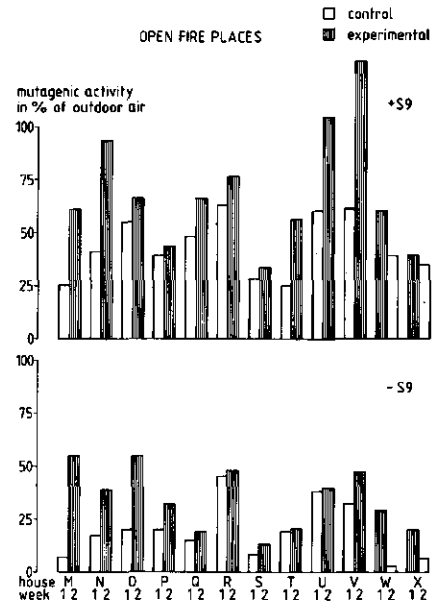

Figure 4. Mutagenic activity (+S9 and -S9) of control and experimental indoor samples in homes with open fire places, expressed as percentage of outdoor mutagenicity.

Figures 3 and 4 present the ratio indoor/outdoor mutagenic activity for corresponding control and experimental samples, collected in the same homes with wood stoves and open fire places. Just as in Table 2 it is conspicuous that the ratio of direct mutagenicity is lower than the ratio of indirect mutagenicity (Wilcoxon sign rank test $p < 0.01$).

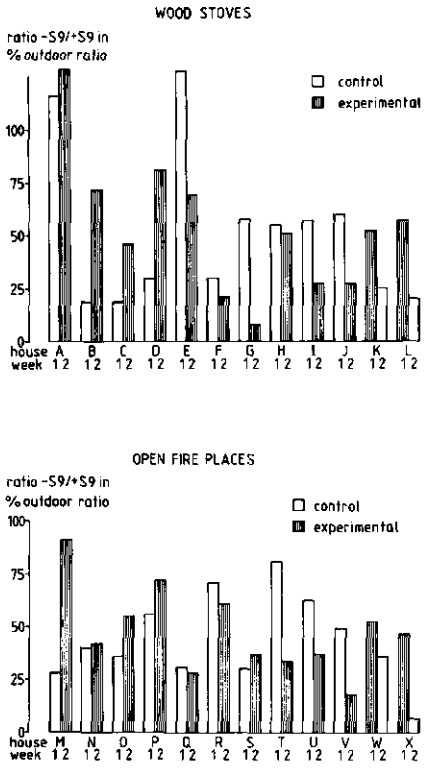

Figure 5. Ratio -S9/+S9 of all control and experimental indoor samples expressed as percentage of the outdoor ratio -S9/+S9.

Differences between mutagenic activity in control and experimental conditions are a consequence of wood combustion. When wood combustion takes place in wood stoves (Figure 3) mostly an increase in mutagenic activity is found. However, this difference is not significant (Wilcoxon sign rank test $p > 0.05$). Wood combustion in open fire places (Figure4) consistantly leads to an increase of direct, as well as indirect mutagenicity (Wilcoxon sign rank test $p < 0.01$).

Figure 5 gives the ratio -S9/+S9 for each sample in the control and the experimental week, in order to determine differences in composition of the mixture between control and experimental conditions. The ratio is expressed as percentage of outdoor ratio, to exclude possible outdoor influence.

Systematic differences between control and experimental conditions were not found (Wilcoxon sign rank test $p > 0.05$).

Mutagenicity expressed as revertants/μg particulate matter

Table 4 gives median levels of particulate matter in $\mu g/m^3$, mutagenic activity per unit mass and the resulting revertants per cubic meter.

Although mutagenic activity per cubic meter is most suitable in terms of personal exposure, mutagenic activity per unit mass might give some more information about the composition of the mutagenic burden. Mutagenic activity outdoors is higher than indoors. Table 4 shows that this is a result of

TABLE 4. Median weekly average levels of particulate matter ($\mu g/m^3$), mutagenic activity per unit mass (rev/μg) and mutagenic activity per m^3 for all sampling locations.

Sampling location	$\mu g/m^3$	rev/μg +S9	rev/μg -S9	rev/m^3 +S9	rev/m^3 -S9
Outdoor	66	0.54	0.50	39	34
Stove control	49	0.28	0.13	15	6
Stove experimental	48	0.44	0.21	16	8
Open fire place control	36	0.33	0.12	10	3
Open fire place experimental	59	0.45	0.26	25	14

higher particulate matter concentrations and mutagenicity per unit mass.
Once more it is clear that the organic composition of the indoor aerosol differs from outdoors: mutagenic activity is much lower, while the ratio -S9/+S9 is also different.

DISCUSSION AND CONCLUSIONS

In this study a wide range of mutagenic activity was observed for the outdoor samples, this is in agreement with previous studies (Alink et al., 1983; van Houdt et al., 1984). Due to meteorological conditions the level of mutagenic activity, however, was in contrast to those studies much higher. This high outdoor mutagenicity exceeds median indoor levels ($p < 0.01$), for which reason, besides wood combustion, infiltration of outdoor particles may also play an important role.
Outdoor mutagenic activity is highly correlated ($p < 0.01$) with indoor activity. However, in agreement with earlier studies (van Houdt et al., 1984) differences in composition of the extracts manifest themselves by a lower ratio -S9/+S9 ($p < 0.01$) and mild cytotoxic effects indoors. This phenomenon justifies the conclusion that penetration of outdoor particles may be one, but certainly not the only source of airborne mutagenic activity indoors.
Mutagenic activity, adjusted for outdoor effects as presented in Figures 3 and 4, shows that indirect activity exceeds direct mutagenicity. This observation supports the conclusions mentioned above, both direct and indirect acting mutagens are in equal concentrations located on the same fraction of outdoor particles (Table 4). A higher infiltration of only indirect acting mutagens therefore is not likely.
Figure 3 also shows that the use of wood stoves gives an increase of airborne mutagenicity in 8 of 12 homes

($p > 0.05$), while using open fire places (Figure 4) gives an increase in all homes ($p < 0.01$). Although the use of wood stoves is much more frequent than open fire places, it is very remarkable that the increase of airborne mutagenicity during wood combustion is only significant when open fire places are used. This itself is an important observation for winter 1984/1985.
The question arises whether the assumption that the air-exchange rate is equal in control and experimental conditions is correct. As a consequence of wood combustion the draught in the home may be increased. An increase of air-exchange rate may be accompanied by an enhanced penetration of outdoor particles. Besides emissions from the fire place this might very well determine the increeased mutagenicity in the experimental week.
As outdoor mutagenic activity is lower than indoors in most winters (Alfheim and Ramdahl, 1984; van Houdt et al., 1984; Yamanaka and Muruoka, 1984) an increase of air-exchange rates may lead to a decrease of airborne mutagenic activity indoors in those winters.
The contribution of enhanced penetration can be determined by the ratio -S9/+S9. The more the contribution of outdoor particles the higher the ratio -S9/+S9 indoors, this is clear from the results in the uninhabited house: a complete contribution of outdoor air results in almost equal ratios -S9/+S9 indoors and outdoors.
If penetration from outdoors is higher in experimental conditions, then the indoor ratio -S9/+S9 must be elevated in the experimental week. Figure 5 shows that in experimental conditions an increased contribution of outdoor air is not found ($p > 0.05$), pointing to wood combustion products as the major cause of increased mutagenic activity of indoor air.
Previous findings (van Houdt et al., 1984) showed that, with respect to mutagenic activity of indoor air, cigarette smoking was found to be a serious contaminant up to 250 rev/m^3, while kitchen samples also show mutagenic activity. When comparing these results with the findings presented in this study it is obvious that cigarette smoking is the predominant contaminant of homes with genotoxic compounds. As sometimes a 2-3 fold increase is found when wood combustion takes place, this may be a more important source than volatilization of cooking products.

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PART II

Biological fate of particle bound organic compounds

5 Introduction

5.1. RESPIRATORY TRACT DEPOSITION AND DEFENCE MECHANISMS

Deposition

A small part of airborne particulate matter may be deposited in the human respiratory tract (9). In figure 1, a scheme presents the possible pathways of airborne particulate matter, from emission or formation via transportation, deposition and clearance in the respiratory tract to effects. The most fundamental function of the respiratory tract is the exchange of oxygen and carbon dioxide between air and blood. Other functions are the tempering of inhaled air to body temperature, water vapor saturation and, of major concern for this thesis, lung defence (31). The relation between the concentration and characteristics of airborne particulate matter in breathing air to the resulting toxic doses and

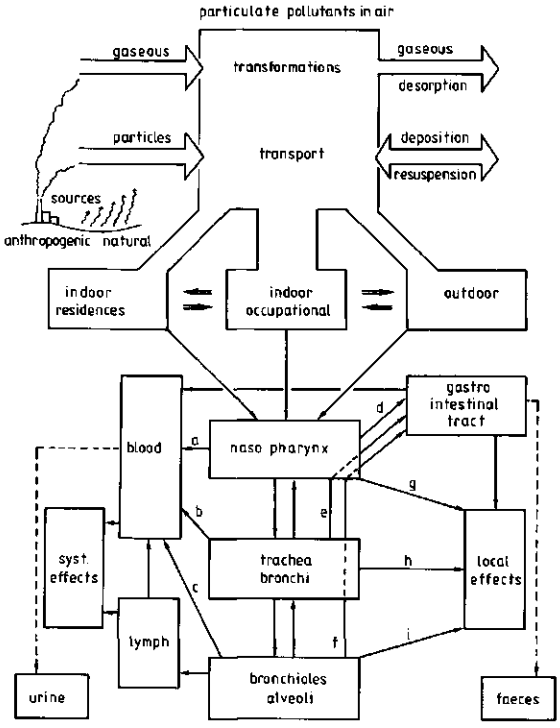

Figure 1: Routes of formation, exposition, deposition and clearance of airborne particulate matter.

potential hazards after their inhalation depends greatly on their patterns of deposition and the rates and pathways for their clearance from the deposition site (61, 89).
Deposition efficiency in each region depends on the aerodynamic properties of the particles, the anatomy of the bronchial tubes and the flow patterns. Particulate matter deposits in the various regions of the respiratory tract by varying physical mechanisms (34, 61, 62, 89). The three major mechanisms are impaction, sedimentation and diffusion.
Impaction of inhaled particles is the principal mechanism of large particle deposition in the upper region of the respiratory tract, affecting particles ranging from 3 to 20 µm in diameter. Each time the air changes direction by branching of airways the momentum of particles tends to keep them on their pre-established trajectories, which can cause them to impact on airway surfaces.
Sedimentation is one of the primary deposition mechanisms for particles ranging from 0.1 to 5 µm on places where the air velocity is low.
Deposition by diffusion or Brownian motion in the respiratory tract predominates for particles smaller than 0.2 µm. This Brownian motion, caused by the impact of gas molecules increases with decreasing particle size. Hence deposition by Brownian motion increases with decreasing particle size whereas deposition by impaction and sedimentation is enhanced with increasing particle size (61, 89). Figure 2 presents the major subdivisions of the respiratory tract, which differ markedly in size, function and response to deposited particles. Particles entering the body first of all pass through a web of nasal hairs. In this region the air is warmed, humified and partially depleted of particles with aerodynamic diameters > 1 µm by impaction and sedimentation.

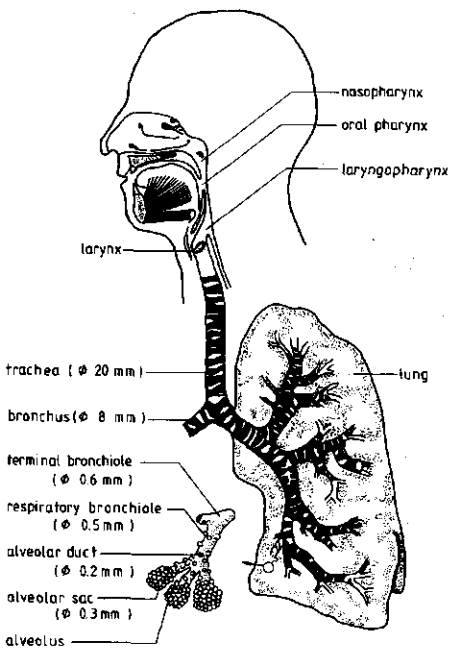

Figure 2: The human respiratory tract.

The aerosol particles next enter the nasopharyngeal region, characterized by stratified squamous epithelium, which extends from the oral pharynx to the larynx (89).
The tracheobronchial or conducting airways have the appearance of an inverted tree, the trachea being the trunk and the subdividing bronchi being the limbs. Following the subdivisions the airway diameter decreases, but because of

the increasing number of tubes the resulting air velocity also decreases (34, 61). Conducting airways have epithelium, consisting of ciliated cells, mucous producing goblet cells, intermediate and basal cells. Gas exchange takes place beyond the ciliated airways in the alveolar zone. The alveolar epithelium is very thin, it is composed largely by Type I and Type II cells. Soluble particles are believed to enter the blood within minutes (34, 61).
The anatomy of the airways is, by influencing respiratory and flow factors, of great importance for the particle deposition. Airway bifurcations are responsible for deflections of airstreams and thus for impaction. The carinas of the large airway bifurcations are the most likely deposition sites as a result of this mechanism. The anatomy of the distal tree is responsible for a decreasing air velocity, which makes sedimentation and diffusion in the smaller bronchi, bronchioles and alveolar spaces an important deposition mechanism (34). In the upper respiratory tract air velocity augments impaction but decreases sedimentation and diffusion by decreasing residence time.
In order to compute dosage of deposition, it is necessary to consider physical activities since the normal pattern of human activities may vary considerably. Breathing rate during the night will be 8 L/min, while during the day breathing rate will be about 20 L/min. Not only higher exposures at certain times, also different flow rates may affect regional deposition. Individual variations in airway anatomy may also affect deposition in several ways (21).
However, particle size is generally the most important factor in regional deposition (40, 44, 46, 61, 62, 89). A commonly used parameter is the aerodynamic diameter or mass median diameter, which incorporates both density and drag. The aerodynamic diameter is the most appropriate parameter for considering particle deposition by impaction and sedimentation, which generally accounts for the most of the deposition by mass. Diffusional displacement depends only on particle size, not on density or shape. For particles between 0.1 and 2.0 µm aerodynamic diameter, deposition in the conducting airways is generally very small in comparison with deposition in the alveolar regions. Thus for those particles total deposition approaches alveolar deposition. Total deposition as a function of particle size and respiratory parameters has been measured by many investigators. It should be noted that there are

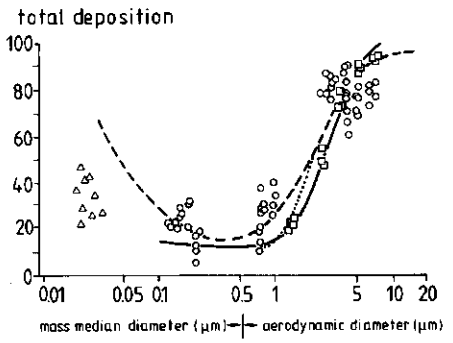

Figure 3: Total respiratory tract deposition as a function of particle size. Experimental data are from o Chan and Lippman, ▲ Swift et.al., ◘ Stahlhofen et al. The predictive models are those from — — — Yu, ---- Davies et al. and ——— Heyder et. al. (taken from 61).

considerable differences among the reported results. However, from figure 3 (61) it is clear that all appear to show the same trend with a minimum of deposition at 0.5 μm diameter. From this point increasing particle size gives rise to increasing deposition, whereas decreasing particle size also results in increasing deposition (61).

Clearance

Particle retention depends on particle deposition and clearance. Rates of clearance in the respiratory tract vary greatly from region to region. In the nasopharyngeal region and conducting airways in normal individuals, clearance is completed in less than one day. In the alveolar zone clearance proceeds by slower processes and may take days, months or years (9, 12, 21, 32, 44).
Clearance pathways consist of a set of processes involving the three principal routes of clearance i.e. blood, lymphatic circulation and gastrointestinal tract. The respiratory tract consists of about 40 different celltypes. Mucous glands, and epithelium serous and goblet cells which are found with varying density along the nasopharynx and the conducting airways are responsible for the production of mucous. Another cell type involved in the particle clearance of the upper respiratory tract is the ciliated cell. About 250 cilia are found on the luminal surface of each cell, they increase in length progressively with succeeding airway generations in the lung. Cilia beat about 1000 times per minute. The effective beat in the respiratory tract is always toward the pharynx.
Insoluble particles which deposit on the mucous lining of the ciliated airways - the fibrous nature of the mucous may help to entrap particles - are transported by the propelling action of the cilia towards the nasopharynx where they are generally swallowed. The mucous moves at approximately 5 mm/min along the trachea of healthy humans and at slower velocities in the distal airways. The total transit time of clearance from terminal bronchioles to the larynx varies from 2 to 24 h in healthy humans. Soluble particles may be dissolved in the mucous, clearance may take place much faster via the bronchial blood flow (13, 34, 61, 62, 89).
Beyond the ciliated airways, the alveoli are lined by a continuous layer of epithelial cells which are mostly Type I pneumocytes and Type II pneumocytes. These epithelial cells are covered by surfactant, which is absolutely essential for the maintance of normal pulmonary functions such as gas exchange (33). Without surfactant, which is a complex mixture of phospholipids, proteins and carbohydrates, the lungs would collapse (77). The free surface of the alveolar region contains a number of cells, mainly consisting of alveolar macrophages. Alveolar macrophages are mobile cells that

defend the lung against bacterial or viral infection and are responsible, by phagocytosis, for the collection and removal of particles, in particular in the alveolar region (12, 13). The initial step in this process involves movement of cells from the air sacs to the openings of the respiratory and terminal bronchioles. In this region a concentration of phagocytes, containing dust was observed, resulting in an accumulation of particles (61). How the movement of macrophages occurs is not known; respiratory movements and elastic recoil are important. Further passage may be aided by the flow of an alveolar surface film. Once on the bronchial epithelium the macrophages are transported by mucociliary movement (64).
The clearance of particulate matter by alveolar macrophages is a rather slow process, as phagocytosis may proceed until 6 hours after inhalation. Moreover, it is known that a substantial portion of the particles is retained over long periods of time (>100 days) in the lung (64). This makes absorption into alveolar/bronchiolar epithelium cells, interstitial spaces, blood and lymph possible.
The clearance pathways, as a major defence mechanism itself may be affected by chemical compounds. In man, the response to 'normal' smoking has been either an increase in tracheobronchial clearance or no effect. Impairment of mucociliary clearance in donkeys was observed at high levels of cigarette smoke. In vivo exposures of SO_2 at concentrations, measured in the ambient air are not likely to affect mucociliary clearance. At higher levels effects have been observed. The same was found for sulphur acid mist (62). Mucous production and mucociliary transport is enhanced by acute high levels of particles. Continuation of exposure to these high levels leads to bronchial mucous gland hypertrophy and goblet cell hyperplasia. As gland hypertrophy continues the mucociliary transport becomes inadequate in removing the excess secretions. This leads to an increase in residence time (62).

Adaptive responses of alveolar macrophages

The pulmonary macrophage system will respond to unusually heavy loads of inhaled particles by an adaptive outpouring of new cells into the alveolar spaces. Alveolar macrophages, like other mononuclear phagocytes, are derived from the bone marrow. A population of macrophage precursors within the pulmonary interstitium provides a reserve pool from which phagocytes may be recruited, depending on the number of particles and on their cytotoxicity (12, 50). The higher the cytotoxicity of the particles, the greater the number of phagocytizing cells recruited and the higher the contribution of neutrophilic leokocytes, which ingest only one or two particles, contrary to macrophages which may contain a large number of particles (50). A key role in the

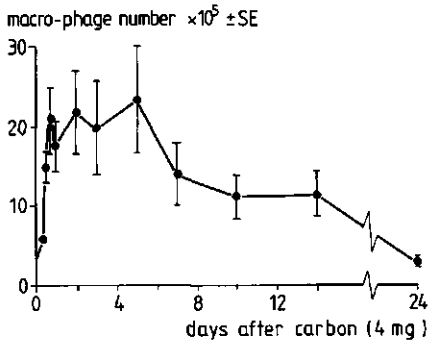

Figure 4: *The number of alveolar macrophages recovered from mouse lungs at intervals after carbon installation (from 12).*

recruitment of phagocytizing cells is played by macrophage breakdown products, which are liberated from damaged macrophages. In figure 4 (taken from 12) the induction of a massive response of alveolar macrophages as a result of pulmonary installation of 4 mg carbon (300 Å particles) into the lung of a mouse is presented. Because recruitment of phagocytizing cells takes some time penetration of particles or particle bound organics into the pulmonary interstitial tissue and lymph may take place. Thus at this time elimination from the lung will be less efficient (32). Induced stimulation of phagocytic recruitment can promote pulmonary dust clearance. However, hyperstimulation or stimulation against the background of a response of which the high intensity is already assumed by autocontrol can lead to an opposite effect, especially when exposure lasts for many years (50). Removal of an unusually large number of macrophages may be decreased, thus, in stead of elimination retention takes place. It was reported that macrophages from cigarette smokers had enhanced responsiveness to a chemotactic stimulant and an impaired responsiveness to macrophage migration (62). Moreover one must be aware that after heavy phagocytosis the macrophages may enter into a refractory period, at this time particle uptake will be suppressed for several hours (32).

Biotransformation in macrophages

Normal alveolar macrophages are rich in lysosomal enzymes, some of which are known to be involved in intracellular or extra cellular processing of foreign materials (32). Because this system is crucial in the functional response, any perturbation of the enzymatic mechanisms of these cells may have important consequences for the defence of the lung. Extracellular release of such lysosomal enzymes may occur as a result of cytotoxic damage or as a result of selective processes, without cell injury (32).
In vitro studies, in which alveolar macrophages of rabbit and rat were used as a metabolizing system in combination with V79 chinese hamster cells show that alveolar macrophages activate the premutagens benzo(a)pyrene (B(a)P), its 7,8-diol and 2-aminoanthracene (72, 76). Engulfment and incubation of

diesel particles in lung macrophages resulted in a considerable loss of mutagenic activity and significantly less 1-nitropyrene (55). Studies have also shown that macrophages from smokers have a significantly higher aryl hydrocarbon hydroxylase (AHH) activity than similar cells from nonsmokers (19, 32). This is important, since this enzyme system can convert polycyclic aromatic hydrocarbons into active carcinogens. Consequently the cytotoxic action of airborne particles may lead to the release of metabolizing enzymes and particles, both transformed or not transformed to harmless, toxic or carcinogenic intermediates.
Morphological alterations were observed as a result of addition of city smog to mouse alveolar macrophage cell line IC 21 (6) as well as asbestos in lung macrophage-like cell line P338 D1 (45). Dose dependent toxic efects were observed after addition of city smog in cell line IC 21 (81, 82). Coal fly ash was able to induce Sister Chromatid Exchanges (SCE) in cell line P338 D1 (2).
Cytotoxicity was observed in cultured rat alveolar macrophages by coal fly ash (30, 69) and by airborne metallic ions (17). Alterations in phagocytosis as a result of cytotoxicity of coal mine and mineral dusts were observed both in cell line IC 21 (80) and cultured rat alveolar macrophages (83, 93). Data on cytotoxicity of extracts of airborne particles, especially those collected indoors on rat alveolar macrophages are not available so far.
It may be concluded that any environmental chemical that can suppress the normal functioning of the host's defence, i.e. macrophage functioning, would probably increase the potential danger of such chemicals and may also increase the susceptibility of the toxic action of other chemicals.
Chemicals associated with particles, deposited on respiratory tract surfaces may have toxic effects either on their deposition sites or on other sites after dissolution into body fluids. They may also cause changes in airway size or fluid secretions which affect respiratory functions and/or clearance patterns (52). Clearance of deposited particles defends the organism towards harmful substances by reducing the residence time of particles on potentially sensitive epithelial surfaces. On the other hand clearance pathways may transport respired particles to or through sites which are more sensitive to adverse effects than the original deposition sites (52).

5.2. SOLUBILITY IN BODY FLUIDS

Chemical composition

Knowledge about the chemical composition of the mixture, guided by short-term bioassays may not only give indications

of the identity of the mutagenic components, but also provide information on the behaviour of the mixture in physiological fluids. In this way, crude fractions containing biological activity would be identified and given priority for efforts to characterize solubility in physiological fluids. The greater the similarity in chemical character, the better this prediction can be made. In recent years a lot of studies were conducted to evaluate the mutagenic activity of airborne particulate matter. As solvent systems mainly benzene, cyclohexane, pentane, dichloromethane, chloroform, acetone and methanol were used (3, 23, 25, 41, 58, 65, 66, 78, 79). The results show that solvents with different polarities are all able to extract mutagens from airborne particulate matter, which indicates that it is not likely that mutagenic activity is related to a homogeneous group of compounds.
Airborne particulate matter, collected in Antwerp was, after extraction with benzene, subjected to a separation into neutral, acidic and basic substances (18). Interpretation and comparison with reference data led to the identification of more than 100 compounds. In table I (taken from 18) a subdivision in different groups of compounds is given. In several studies airborne particulate matter, obtained from diesel emissions (4, 63, 64, 70), ambient air in residential areas (23, 25, 41, 42, 58, 65, 92) and ambient air in an industrial area (94) was investigated for mutagenic activity after chemical fractionation. From these studies it may be concluded that mutagenic activity was mainly observed for the organic acids and neutrals, PAH and oxigenated substances forming the neutral group. Complex mixtures obtained from

TABLE I: Identification of compounds in neutral, basic and acidic fractions of airborne particles, collected in a residential town (taken from 18).

Neutral	saturated aliphatic hydrocarbons
	polynuclear aromatic hydrocarbons (PAH)
	polar oxiginated substances
Acidic	homologue series of fatty acids
	aromatic carboxylic acids
Basic	nitrogen contaning PAH

cigarette smoke (4, 42, 51) and coke oven emissions (4, 36, 42) also show mutagenic activity in the basic fractions.
These studies show that the methods applied were capable of identifying broad chemical mutagenic classes within these mixtures. Chemical fractionation of the crude extract was both useful and necessary to accomplish adequate biotesting and chemical identification. So far, data on chemical fractionation of indoor air particles are not available. It may be concluded that organic compounds, responsible for mutagenic activity of airborne particles may differ in polarity and acidity to such an extent that a simple evaluation of their solubility in physiological fluids is not possible.

Physiological fluids

In order to investigate interactions between mutagens and physiological fluids, several studies on the solubility of airborne organics in serum were carried out. Serum soluble direct and indirect acting mutagens are associated with airborne particles collected from urban and non-urban air (71, 91). Because the maximum mutation frequency was obtained with a 2 h serum extract (fig. 5; taken from 71), mutagenic components seem to be rapidly solubilized in serum. However, from figure 6 (from 71) it is clear that this serum extract was less mutagenic for TA98 than the benzene extract. Takeda et al. (91) reported on the same phenomenon for indirect activity. Direct activity of serum and DMSO extracts were about the same. With regard to serum extractability of coal fly ash, both a 25 times greater mutagenic activity than cyclohexene extracts (20) and a strong decrease related to DMSO solubility were observed (57). For diesel particulate emissions organic solvents were more efficient in removing mutagens than physiological fluids (14).
The components soluble in serum are not likely to be easily water soluble inorganic or organic compounds, because only a slight induction of mutagenicity was obtained by saline extracts of the particles (71, 91). Not only saline, also

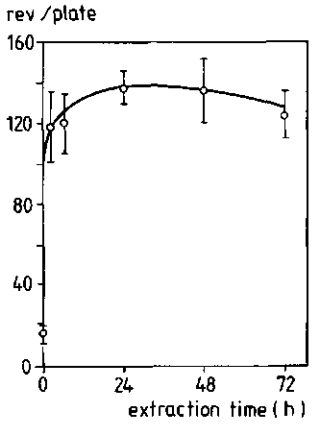

Figure 5: Extraction-time dependency of mutagenic activity in TA98 of serum extracts from airborne particles collected in an industrialized area (taken from 71).

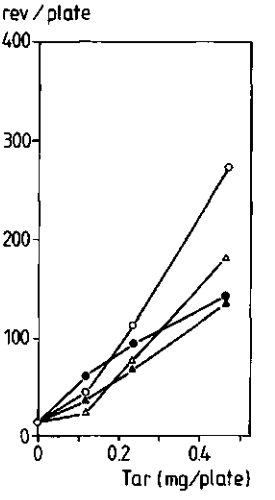

Figure 6: Influence of calf serum on the mutagenicity in TA98 of the benzene extract from airborne particulates. Closed symbols represent revertant counts in the absence of liver S9 mix. Open symbols indicate revertant counts in the presence of liver S9 mix. Circles show the counts without serum. Triangles show the counts with serum (taken from 71).

lung lavage fluid was less effective in removing mutagens when compared to serum and lung cytosol (54).
These findings indeed suggest that with regard to biological availability knowledge about mutagenic activity of airborne particles is only of relative importance for risk assessment. The difference in mutagenic potency of serum extracts and organic solvents suggests that (a) a biological fluid such as serum is less efficient in extracting mutagenic compounds from airborne particles or (b) serum has an antimutagenic activity. A reduction of mutagenic activity of serum seems to indicate that serum binds or inactivates the mutagenic components (71). Protein binding of the mutagenic components has been suggested to be responsible for the reduction of those mutagenic activities (53). Moreover, in vitro serum reduced the cytotoxic activity of diesel exhaust particle extracts to chinese hamster ovary (CHO)cells (59) and welding fume particles to bovine alveolar macrophages (97). Protective effects of fetal calf serum and bovine serum albumin (93) and calf serum and gluthathion (39) on toxicity of particulate matter was also observed. Silica particles coated with alveolar lining material proved to be much less cytotoxic to rat alveolar macrophages than uncoated silica (28).

Biomembranes

In several studies, both in vivo and in vitro, consideration was given to the bioavailability of PAH associated with carbonaceous particles. For example in soot, recovered from human lungs at autopsy, decreased amounts of B(a)P were observed, as compared to soot particles collected in the atmosphere (29). Evidence for a certain resorption in human beings was obtained by positive results in mutagenicity testing of urine of smokers, when it was compared to that of nonsmokers (16, 87).
An indication of resorption of inhaled airborne mutagens from cooking was obtained by a study by Decoufle and Staislawczyk (24) which shows an excess of lung and bladder cancer among cooks and kitchen workers. In contrast, exposure of rats to diesel exhaust did not result in mutagenic activity of urine (35). In a study by Buddingh et al. (15) elution of very small amounts of B(a)P (<0.005%) from carbon black into human plasma, swine serum, swine lung homogenate and swine lung washings was observed. The potential bioavailability of PAH, associated with wood stove particles, was also studied in an in vitro assay using phospholipid vesicles, composed of dimyristoyl phosphatidylcholine (DMPC). Application of this technique revealed that 25% of B(a)P and 68% of benzo(a)fluoranthene was eluted into DMPC vesicles (7).
Studies concerning deposition and biological fate of 1-nitropyrene showed that pure NP aerosol was cleared from

the respiratory tract by fast adsorption into blood and excreted in the urine, while NP associated with gallium oxide particles was cleared by both blood adsorption and mucociliary clearance, followed by ingestion and fecal excretion (90). In all studies mentioned, it was suggested that the composition of the carrier particles is of importance with respect to the lung clearance rate of chemicals present on these particles. In fact these data suggest that the rate limiting step in the bioavailability of particle associated chemicals may be the rate at which these chemicals are removed from carrier particles prior to lung clearance of the chemical and metabolites, particularly since inorganic 'carrier' particles clear very slowly (t 0.5 ≈ 70 d) (9).
The observation that lung retention of B(a)P and NP adsorbed onto diesel exhaust particles is greater than when adsorbed on gallium oxide particles suggests, that various physical and chemical interactions between the chemical and carrier particles may influence the rate at which the chemical is removed in vivo (32).
The data presented above suggest, that exposure to chemicals coated on organic or inorganic compounds results in a much higher lung burden than does exposure to pure aerosols.

5.3. BIOTRANSFORMATION

Biotransformation of xenobiotics

To some extent the drug metabolizing enzyme systems may be seen as a defence mechanism towards toxic compounds invading the body. If these compounds are lipophilic they are generally made more hydrophilic and less toxic by metabolic transformations, mainly oxidations or hydroxylations, and subsequent conjugations, to facilitate excretion. However, it has also been demonstrated for many chemicals that the biotransformation process may also lead to the formation of metabolites which are more toxic than the parent compound, a phenomenon which is called activation. Biotransformation processes occur in most organ systems , but the highest activity is generally observed in the liver. The extent of metabolism of compounds in other tissues is indicated by the following descending order: gastrointestinal tract > kidney > lung > skin (75).
The reactive intermediate metabolites of most known carcinogens are mutagens, and there is a substantial evidence that screening systems to detect mutagens can also serve to detect many potentially carcinogens(43). A lot of substances that are carcinogens in animal test systems are mutagenic in microbial test systems only if tissue homogenates are added to permit metabolic activation of the test compound. Most

microorganisms do not contain the mixed function oxidases and other enzyme systems involved in the activation of pre-mutagens and carcinogens (98). The opposite phenomenon, conversion from mutagenic active into non-mutagenic compounds, is also known to occur in vivo and inactivating enzymes for some reactive metabolites have been identified both in vivo and in vitro (31, 37). So far, relatively little attention has been given to the inactivation of direct-acting mutagens by biotransformation (31, 98).

The use of enzyme fractions in in vitro testing

The cytochrome P-450 system of the mammalian liver endoplasmatic reticulum consists of integral membrane hemoproteins, that catalyse the oxidative metabolism of a wide variety of both endogenous and exogenous compounds (72). An intensely studied property of several forms of liver microsomal cytochrome P-450 is their inducebility in rodents by certain xenobiotics, including drugs, pesticides, and polycyclic and polyhaloganated hydrocarbons. Several isoenzymes of liver microsomal P-450 have been purified and characterised. Various substances, like phenobarbital (PB), 3-methylcholanthrene (3 MC) and TCDD have been shown to be capable of inducing or augmenting the activity of individual enzymes (27, 47, 74, 85 ,96). Aroclor 1254, a mixture of polychlorinated byphenyls induces a variety of enzymes (72). Figure 7 (taken from 72) gives an indication of the time course of induction of cytochromes P-450 by Aroclor 1254.

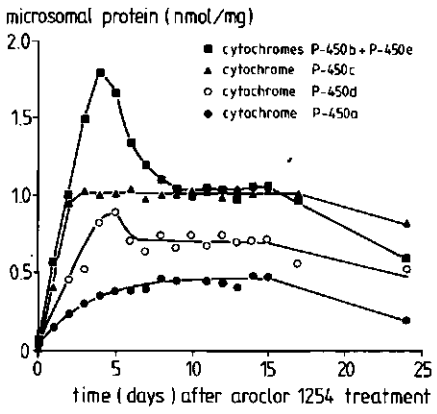

Figure 7: Temporal effects of Aroclor 1254 treatment of rats on liver microsomal cytochromes P-450 (taken from 72).

The mutagenicity of B(a)P, as well as cigarette smoke condensate and many other compounds were enhanced with Aroclor treatment (5, 27, 43, 68, 74, 98).
Mutagenicity studies on airborne particles are currently performed in many laboratories using liver preparations from Aroclor treated rats. Pretreatment takes place 5 days before sacrifice by i.p. injection of 500 mg/kg body weight (1).
The results obtained by this procedure may give a sensitive prediction of the mutagenic and carcinogenic potency of single compounds or mixtures.

Extrapolation

Although concentrations of PCB in the diet as low as 10 mg/kg are sufficient to produce measurable changes in the microsomal enzyme system (95) the question remains whether from in vitro testing with induced rat livers health hazards with regard to mutagenic activity of airborne particulates can reliably be predicted (a). There might be qualitative and quantitative differences between the biotranformation capacity of livers of various species, and that of human beings (species-specific differences) (b). There might be the same differences with regard to the induction of test animals and that of man under normal living conditions (induction-specific differences) (c). Since the lung, especially the bronchial epithelium is the target tissue for carcinogenic substances in airborne particles, it seems more appropriate to use lung homogenates than liver homogenates for the prediction of mutagenic hazards with regard to lung.

Species specific biotransformation

It has been found that quantitative as well as qualitative differences exists in the activity of biotransformation enzymes among species. Not only rat liver homogenates show metabolic activity towards 7,12 dimethylbenz(a)anthracene, benzo(a)pyrene and N-2-fluorenylacetamide, also liver homogenates of mice of six weeks old, especially after treatment of the test animals with TCDD (27). However, it was found that the biotransformation of B(a)P in rabbits decreased, despite the fact that P-450 activity significantly increased after pretreatment with Aroclor 1016 (96). Increases or decreases in the levels of cytochrome P-450 in rabbit liver may be caused by interactions of different xenobiotics (85). In hamster liver benzo(a)pyrene metabolism was not induced after treatment with 3-MC (74).
In a study concerning cytochrome P-450 content in seven animal species, the optimal substrate specificity proved to vary from one animal to another (88). In another study of seven species on mutagenic activity of B(a)P, cyclophosphamide, diethylnitrosamine, b-naphtylamine and o-aminoazotoluene, all known mutagens or carcinogens belonging to various chemical classes, the following conclusions were drawn: S9 fractions from untreated animals revealed distinct species specific differences in the metabolizing activity of the fractions and also differences from one substance to another. After Aroclor treatment all species produced positive effects with all substances. Mutagenic effects, provoked by various substances, when fractions from untreated animals were used, remained more or less the same or became more distinct. Species-specific differences were no longer discernible (68).

In a comparative study on the content of cytochrome P-450 in human beings and animal species, four substances (aminopyrene, benzphetamine, ethylmorphine and B(a)P) were shown to be less active in humans than in male rat, which is the most commonly used model. However, other animal species, such as the female Sprague-Dawley rat and the pig are much more similar to man (88).
Although the activity of cytochromes P-450 in man is generally lower than that of rat, this is by no means always the case. There are several important exceptions, including the N-hydroxylation of 2-acetamidofluorene (10). Selective induction of different forms of cytochrome P-450 can occur in human, for example by smoking (48, 49). However, the number of different classes of inducer in man is not yet known (10). For several xenobiotics similar activation rates in human and female rat liver microsomes were observed (56). With respect to B(a)P metabolism it was reported that the activity in human beings was only 15-20% of that, obtained with rodents (74). Cigarette smoke condensate was less mutagenic in the presence of human liver S9 than in the presence of rat liver homogenate (43), especially after Aroclor 1254 treatment (5). The opposite was observed with 2-aminoanthacene and, to a lesser extent with 2-aminofluorene (5).

Organ specific metabolism

Cytochrome P-450 dependent mono-oxygenase systems, which metabolize endogenous as well as foreign compounds are found in pulmonary tissues of mammals, including human beings. A form of cytochrome P-450 is localized in the non-ciliated bronchiolar epithelial cells (clara cells) of the small airways of rabbit lung (22, 84). The apparent high concentration in this cell type, when compared to liver may be an important determinant for the susceptibility of the lung to a number of toxic chemicals that undergo metabolic activation, although to a lesser extent metabolism was also found in alveolar type II cells (26, 73) and pulmonary alveolar macrophages (38). Morphological observations of characteristic types of cell specific injury in the lung may suggest a biochemical mechanism of toxicity for the particular chemical involved (11).
In the human lung cytochrome b 5 content corresponded to that of the rodent lung, whereas the apparent content of cytochrome P-450 was much lower (86). The content of certain P-450-isozymes in the rabbit lung may be increased (19, 60, 85) or decreased (85, 96) by xenobiotics. In rats, exposed to diesel exhaust pulmonary P-450 enzyme activity was not induced (35). It was reported that, compared to liver homogenates, homogenates of rat and human lung were relatively inactive with regard to activation of xenobiotics (43). From figure 8 (from 51), on the other hand it is clear

that both liver and lung homogenate show a high capacity of activating cigarette smoke condensate. Hamster and rat lung microsomal fractions metabolize B(a)P at 0.5% of the rate of liver homogenates, and the rate of formation was only increased by pretreatment with 3 MC. Metabolic activity of human lung was 40-60% as large as the activity obtained with rodent lung (74).
It was reported that with respect to 7,12-dimethyl benzo(a)anthracene pulmonary tissue of mice produced a mutagen-generating activity, that was five to ninefold above background (47). An overall positive correlation was found between monooxygenase activity of various mouse tissue preparations and the capacity to convert procarcinogens to mutagens in the Ames assay (27). Lung homogenates of male Fisher 344 rats were able to activate 1-nitropyrene.

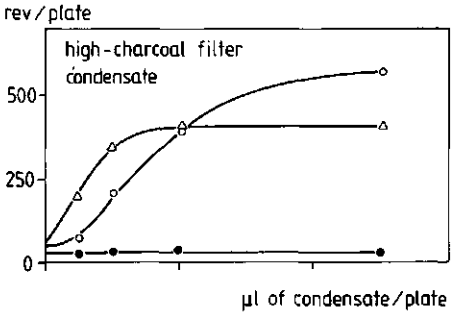

Figure 8: Mutagenic activity of high-charcoal filter cigarette smoke condensate towards TA1538 (taken from 51). ● = no S9 mix added; o = liver S9 mix; Δ = lung S9 mix.

Cigarette smoke condensate and N-nitrosopropylamines showed positive mutagenicity after addition of human lung S9 (67).

Mutagenicity testing in the Salmonella/microsome assay with liver homogenates gives an indication of the mutagenic potential of the tested chemical. As the lung is the target tissue for carcinogenic substances in airborne particles, the use of lung homogenates provides additional information on the toxicological implications of airborne particulate matter.
In view of the data available, which point to considerable organ and species specific biotransformation, the use of human lung homogenates provides better possibilities of adequate extrapolation than homogenates of other species.

The consideration presented in this introduction has largely influenced the design of the second part of this study. In the following chapters for instance, the release of mutagens in physiological fluids and the activation of extracts of airborne particulate matter by liver and lung homogenates of different species were studied.

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6 Toxicity of airborne particulate matter to rat alveolar macrophages: a comparative study of five extracts collected indoors and outdoors

J.J. van Houdt and I.M.C.M. Rietjens

[Toxicology in Vitro; in press]

SUMMARY

The toxicity of indoor and outdoor air samples to rat alveolar macrophages was determined by studying the phagocytic activity of these cells in vitro. Clean air samples did not affect phagocytosis at concentrations up to 60m^3/plate, but polluted outdoor air samples caused a dose dependent inhibition of the phagocytosis at concentrations varying from 6 to 60m^3/plate. Indoor air samples, especially when polluted with wood smoke affected phagocytosis at concentrations below 2m^3/plate.

INTRODUCTION

The air we breathe contains a wide variety of respirable particles that may contain pathogenic organisms, chemical toxicants, mutagens or carcinogens (Kraybill, 1983). To estimate the risk associated with inhaling harmful aerosols, information is needed about the toxic potential of airborne particles and their fate in the respiratory tract.
Mutagens are located mainly on the smallest particles (< 3 μm) (Talcott and Harger, 1980; Möller et al. 1982; Sorensen et al. 1982; Kado et al. 1986) and these may be deposited in the lower respiratory tract, where clearance is important to reduce their contact with the potentially sensitive epithelial surfaces. The free surface of the alveolar region contains a number of cells, mainly pulmonary macrophages, that are not connected with the epithelial lining, and these provide the initial defence of the lower respiratory airways against particles: through phagocytosis they ingest and remove foreign and endogenous particles from the alveoli and terminal bronchioli.
Rat alveolar macrophages are commonly used for in vitro studies on the toxicity of environmental chemicals because they are the primary target cells in the lung and can be

isolated relatively easily (Rietjens et al. 1985). One of the functions of alveolar macrophages that can be readily studied is phagocytic activity, which has been shown to be impaired after in vitro exposure of the cells to environmental chemicals (Seemayer and Manojlovic, 1980; Fisher et al. 1983; Tilkes and Beck, 1983; Gardner, 1984).
The aim of this study was to assess the toxicity of samples of airborne particulate matter to the phagocytic activity of rat alveolar macrophages in vitro. For this purpose five samples were collected that were typical of both indoor and outdoor environments (Van Houdt et al. 1984; 1986; 1987).

MATERIALS AND METHODS

Preparation of aerosol extracts

Sampling methodology. Sampling of airborne particulate matter was carried out in winter/summer 1986. Outdoor samples were collected using the standard high-volume technique at the meteorological station of the Agricultural University in Wageningen. Wageningen is a small rural town (approximately 30,000 inhabitants) with no important sources of pollution, and the sampling station was away from sources such as traffic. For indoor sampling a sampler consisting of a centrifugal pump and a filterholder for 3 filters (20 x 24 cm) was developed. In both sampling techniques Gelmann GF/A filters were used. Differences in sampling methology have been described previously (Van Houdt et al. 1987). In all, five samples were collected. Two were from heavily polluted living rooms; one polluted by smoking and one polluted by wood smoke from an open fire place. Three samples were obtained outdoors; one in winter when wind direction was easterly, and two in summer, when wind direction was northerly. In summer a second sample was taken using two filters, with the second filter representing clean air from which particles had already been removed.

Extraction. Filters were extracted for 8 h with methanol in a Soxhlet apparatus. The use of methanol was based on previous studies (Van Houdt et al. 1984; 1986; 1987). The extracts were evaporated to dryness and the residue was dissolved in dimethylsulfoxide (DMSO). The extracts were stored in liquid nitrogen.

Determination of phagocytosis

Cell culture. Alveolar macrophages were obtained from female

Wistar rats (weight about 200 g), as described by Mason et al. (1977), by the lavage of isolated perfused lungs. Approximately 10^6 cells were plated onto a petri dish (6 cm diameter). The cells were cultured in 2 ml Ham's F10 medium (Flow, Irvine, Scotland), supplemented with $NaHCO_3$ (1.2 g/l), 10% newborn calf serum (Gibco, Glasgow, Scotland), 50 IU/l of penicillin (Gist-Brocades, Delft, The Netherlands) and 50 µg/l streptomycin (Specia, Paris, France) at 37°C in a humified 5% CO_2 atmosphere. Cells were cultured for 3 days to allow attachment on the petridish.

Determination of phagocytic activity. Cells were exposed to at least five different concentrations of each extract for 2-h incubation periods. The highest concentation of DMSO did not exceed 1,5% of the total culture volume. Before exposure, cells were washed and fresh medium without serum was added. All experiments were carried out in duplicate. Phagocytic activity was determined after further incubating the cells for 1.5 h at 37°C with about 10^7 dead yeast cells coloured by boiling for 30 minutes in congo red (Fluka, Buchs, Switserland) with phosphate-buffered saline. Results are expressed as the percentage of 5 * 100 cells containing yeast (mean ± standard error). From these the dose causing a 50% reduction of phagocytosis (ID50) was determined.

RESULTS

Extracts of clean air at concentrations up to 60 m^3 /plate did not affect the phagocytic activity of rat macrophages (fig. 1). In contrast, extracts of outdoor airborne particulate matter caused a dose-dependent inhibition of phagocytosis at concentrations of 6-60 m^3 , while indoor air polluted by wood smoke and indoor air polluted by cigarette smoke appeared to be highly toxic at concentrations below 2 m^3 /plate. These differences were statistically significant ($p < 0.05$; n=5, unpaired student's t test).
From figure 1 the ID 50 values are 1.7, 2.8, 9.4 and 27.2 m^3/plate for indoor air polluted by wood smoke, indoor air polluted by cigarette smoke, outdoor air in winter and outdoor air in summer, respectively. The sample representing clean air did not inhibit phagocytic activity.

DISCUSSION AND CONCLUSIONS

Damage to alveolar macrophages by inhaled material may cause a release of particles and macrophage breakdown products. As a result clearance of particles may be decreased, which

facilitates the transepithelial passage of toxic material to the interstitium (Bowden, 1984; Katnelson and Privalova, 1984). Conversely, the recruitment of an unusually large number of cells to the free alveolar surface, due to the release of macrophage breakdown products, can promote lung clearance. However, this accumulation of cells may inhibit drainage causing dust to be retained (Katnelson and Privalova, 1984). Martin and Laughter (1976) reported that cigarette smokers had an impaired responsiveness to macrophage migration.

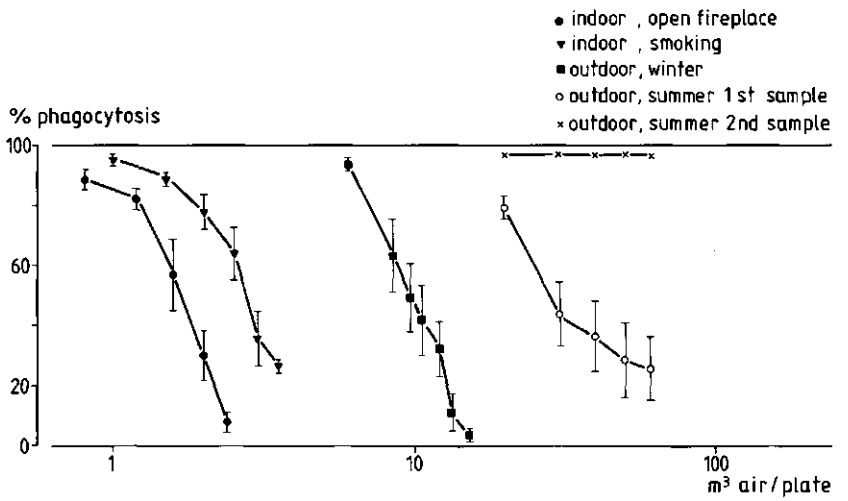

Figure 1. Toxicity of indoor and outdoor extracts of airborne particulate matter to rat alveolar macrophages in vitro, as determined by the phagocytic activity of the cells. At least five concentrations of each sample were tested. All experiments were carried out in duplicate. Results are expressed as the percentage of cells that contained yeast per count of 5 x 100 (mean ± standard error).

An additional risk may arise from biochemical activity by alveolar macrophages, which may be induced by environmental pollutants (Gardner, 1984): macrophages may metabolize pre-mutagens on airborne particles (Centrell, 1973; Harris et al. 1978) leading to the release of direct acting mutagenic metabolites in the alveolar spaces.
Although, in in vivo and in some in vitro studies macrophages are exposed to the particulate matter itself, in this study the cells are exposed to extracts, which may have the cytotoxic potency of the particles. Morphological alterations after exposure to environmental chemicals (Johnson and Davies, 1983; Gardner, 1984; Behrend et al. 1986) as well as alterations in phagocytosis (Seemayer and Manojlovic, 1980;

Fischer et al. 1983; Tilkes and Beck, 1983; White et al. 1983; Castronova et al. 1984; Gardner, 1984; Seemayer, 1985) have been reported.
Our results show a significant reduction of phagocytic activity in alveolar macrophages, after exposure to extracts of particulate matter, and the effect was greatest with indoor air polluted with wood smoke. Although more pronounced these results are in agreement with data obtained by mutagenicity testing (Van Houdt et al. 1984, 1986, 1987). Toxic effects were significantly greater with samples obtained in winter than with those obtained in summer, which may be because of seasonal differences in the operation of power-generating sources.

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7 The release of mutagens from airborne particles in the presence of physiological fluids

J.J. van Houdt, L.H.J. de Haan and G.M. Alink

[submitted for publication]

SUMMARY

Airborne particles collected indoors in residences and outdoors were extracted by Soxhlet extraction and sonication with methanol. In a comparative study in which mutagenic activity was evaluated in the Salmonella typhimurium reversion assay both soxhlet extraction and sonication proved to be suitable extraction methods.
The residue, obtained by sonication of loaded filters with methanol followed by evaporation to dryness (tar) was sonicated with newborn calf serum and lung lavage fluid from pigs. All serum extracts were mutagenic to Salmonella typhimurium TA98, and contained direct and indirect acting mutagens. However, mutagenic activity recovered by serum was only about half of the total mutagenic activity of the tar. The other part of the mutagenic activity remained in the tar. Lung lavage fluid was only able to remove 5-10% of direct acting mutagens from all samples. About 20% of indirect acting mutagens from indoor air were recovered in lung lavage fluid, while the lung lavage fluid extract from outdoor air did not show indirect mutagenic activity.
Mutagenic activity recovered by direct sonication of the filters with physiological fluids was comparable with the recovery obtained by sonication of the tar. However, after sonication of the filter with lung lavage fluid hardly any mutagenic activity remained on the filter, whereas after sonication of the tar a clear mutagenic activity was observed in the non-soluble residue.

INTRODUCTION

The utilization of short-term bioassays which detect mutagenic and possibly carcinogenic activities of environmental chemicals has increased dramatically in recent years. The wide acceptance of the mutagenesis bioassay using bacterial strain Salmonella typhimurium developed by Ames et al. (1975) has been a catalyst in the expanded growth of the

discipline of genetic toxicology. Because of the sensitivity and simplicity of this assay, it has been extensively used for the detection of mutagens in complex environmental samples.
Organic compounds which may have mutagenic potency have been detected and, in many instances quantified in emissions, products and by-products associated with combustion (Austin et al., 1985). Airborne particulate matter, collected outdoors (Pitts et al., 1977; Dehnen et al., 1985; van Houdt et al., 1987) and indoors in residences (van Houdt et al., 1984, 1986), proved to have mutagenic potential. Although mutagenic activity is located mainly on the smallest particles (Sorensen et al., 1982) the fate and target organs for these mutagens is still unknown.
Mutagenicity studies of environmental samples, using the Salmonella/microsome assay, involves sample collection, extraction and mutagenicity testing. Organic solvents are routinely used to remove mutagenic compounds from particulates, mostly using a Soxhlet extraction method. Although studies to evaluate the carcinogenicity of these organics are currently in progress, questions still remain as to whether or not resorption of these potentially hazardous components takes place.
Humans are exposed to particles and their associated mutagens primarily via inhalation. In the respiratory tract organic compounds may be eluted from particulate matter prior to clearance of the particles. Sites of elution are likely to be lipoidal regions such as membranes of cells lining the respiratory tract or the surfactant layer lining the alveoli. Thus a measure of potential bioavailability of mutagens would be elution from particles under conditions that resemble the environment that particles encounter in lungs.
The objective of this study was to evaluate the release of mutagens from airborne particles in the presence of an organic solvent, lung lavage fluid and serum, using the Ames assay as a test for mutagenicity. For this purpose three samples, typical for different air pollution exposure conditions as demonstrated in previous studies (Van Houdt et al., 1984, 1986, 1987) were collected and tested.

MATERIALS AND METHODS

Sample collection

Airborne particulate matter was collected in January 1987 in Wageningen, a small rural town with no important sources of pollution. For indoor sampling a sampler, consisting of a centrifugal pump and a filter holder for 3 filters (20 x 24 cm) was developed. Outdoor samples were collected using the high-volume technique. In both samplers Gelman GF/A filters

were used. Differences in sampling techniques have been described previously (Van Houdt et al., 1987).
In all, three samples were collected. Two were from heavily polluted rooms, one polluted by smoking and one polluted by wood smoke from an open fireplace. One sample was obtained outdoors at the roof of our institute, 25 m above street level. The indoor samples were composed of two 1-week samples, the outdoor sample of ten 24-h-samples.

Extraction

To obtain representative air samples, extraction and mutagenicity testing were performed with equal portions of each filter. For extraction two procedures were used: a Soxhlet extraction for 8 h and sonication for 30 min. Sonication was done with an ultrasonicator (50 Hz; Senior Instrument Corporation, Copiage, NY). Based on previous results, methanol was used as the organic solvent. The methanol extracts were filtered and evaporated to dryness (tar). For comparison of extraction methods these residues were dissolved in DMSO and tested. In order to compare solvent systems two procedures were used. Firstly, the tar, obtained by sonication with methanol and evaporation was partly dissolved in newborn calf serum (Gibco, Paisly, Scotland) and partly dissolved in lung lavage fluid from pigs, containing 89 mg phosphatydylcholine per ml saline (Tore Curestedt, Stockholm, Sweden). Physiological fluids and unsoluble tar were separated by filtration, dissolved in DMSO and tested.
Secondly, filters loaded in rooms with wood smoke were sonicated directly in serum or lung washing (12 mg/ml). Physiological fluids were separated from the filters by centrifugation at 9,000 g for 30 min and tested. The filter material was extracted again by sonication with methanol and tested as described above. The extracts were stored in liquid nitrogen.

Mutagenicity testing

The mutagenicity test with Salmonella typhimurium was performed essentially according to the procedure described by Ames et al. (1975). Minor modifications have been described elsewhere (Van Houdt et al., 1984). A 9,000 g liver supernatant was prepared from Aroclor 1254-induced male Wistar rats. The liver supernatant was stored in liquid nitrogen. In tests with metabolic activation 50 µl of S9 was used (Ames et al., 1975). Following previous studies Salmonella typhimurium TA98 was used as bacterial test

strain. All experiments consisted of at least three doses of extract, blanks and a positive control (5 µg of Benzo(a)pyrene) in triplicate. Test results were acceptable if positive controls were within the range 300-500 revertants/plate. Mutagenicity of each sample is expressed as revertants per m^3, calculated from the slope of the linear part of the dose-effect curve. The number of spontaneous revertants, which did not exceed 60 revertants/plate was substracted from all results.

RESULTS

Dose related curves for mutagenic activity in TA98 of methanol extracts from outdoor and indoor particulate samples were obtained both in the presence and in the absence of S9 mix. These results, depicted in figure 1 show that in the outdoor sample only a slight direct and indirect mutagenic activity is detectable. In contrast, indoor air samples show a strong mutagenic activity, especially after addition of Aroclor 1254-induced rat liver enzymes as can be seen by the high effective dose of outdoor air. Although Soxhlet extraction was slightly more effective in removing mutagens from airborne particulates, sonication may be regarded as an acceptable method for subsequent experiments.

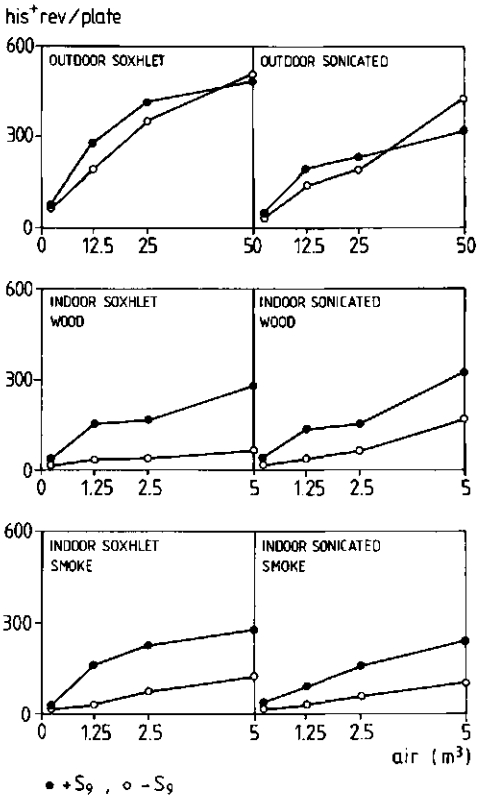

Figure 1 :Comparative mutagenic response of Salmonella typhimurium TA98 to extracts of outdoor and indoor particulate matter, prepared by soxhlet extraction and sonication (o = -S9 ; • = +S9)

Then tar, obtained from evaporation of methanol extracts was dissolved in physiological fluid. Further, the unsoluble residue was dissolved in DMSO. In figure 2 direct mutagenic activity of physiological fluids and DMSO extracts is expressed as a percentage of the total mutagenic burden of the air sample. The percentages were calculated from the slope of the dose-response curve. Mutagenic activity of indoor and outdoor air samples is

only partly soluble in physiological fluids: serum being more effective (43-63%) than lung washing (5-12%). A substantial part of mutagenic activity is observed in the DMSO extract, representing the residue, which is not soluble in physiological fluids.
The potency of releasing indirect acting mutagens from the tar by physiological fluids is presented in figure 3. This figure shows more or less the same pattern as was found in figure 2: serum was also more effective (44-47%) in removing indirect acting mutagens from the tar than lung lavage fluid. Differences between the behaviour of direct and indirect

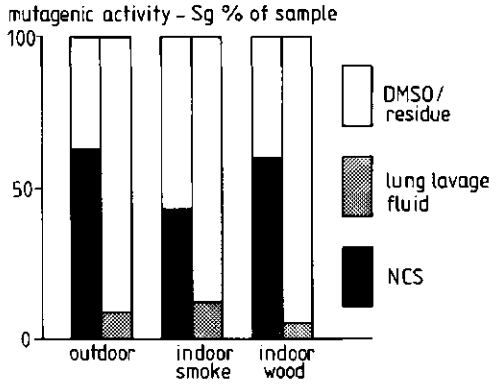

Figure 2 :Distribution in percentages of mutagenic activity of airborne particulate matter in various extracts. Salmonella Typhimurium TA98 without S9.

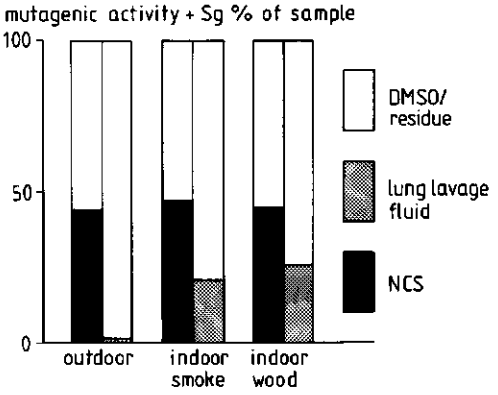

Figure 3 :Distribution in percentages of mutagenic activity of airborne particulate matter in various extracts. Salmonella Typhimurium TA98 with S9.

acting mutagens are only small: Indirect activity of outdoor air, extracted by lung lavage fluid was not observed. In contrast, for indoor air lung lavage fluid was more efficient in removing indirect acting mutagens than direct acting mutagens.

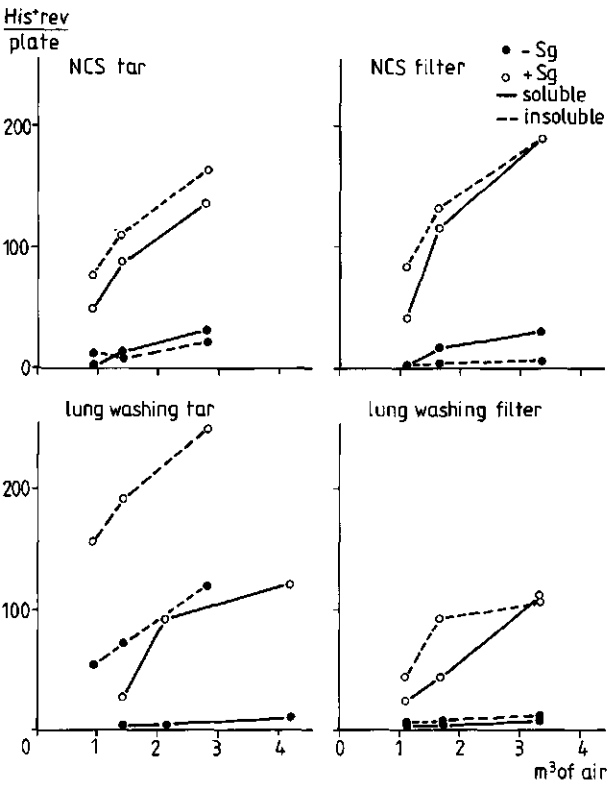

Figure 4 :Comparative mutagenic response of Salmonella typhimurium TA98 to extracts of airborne particles in rooms with wood combustion prepared by indirect (tar) and direct (filter) sonication with lung lavage fluid and newborn calf serum.

Figure 4 gives dose-related mutagenic activity of the indoor sample polluted with wood smoke in different circumstances. In the left part of the figure the extracts tested were obtained by dissolving the tar of the methanol extract (indirect method) whereas in the right part filters were directly sonicated with lung lavage fluid (12 mg phosphatydylcholine/ml saline) and newborn calf serum. Serum extracts show a clear dose dependent increase in mutagenic activity both in the presence and a weak effect in the absence of S9 mix. In lung lavage fluid only a slight effect

was detectable. A high mutagenic activity is observed in the DMSO extract, representing mutagenic activity of the residue, non-soluble in serum and lung lavage fluid. With respect to the mutagenic activity of physiological fluid extracts this figure shows that both extraction methods give comparable results. However, the total mutagenic activity recovered from the filter by subsequent extraction with lung lavage fluid and methanol is lower than when using the indirect extraction method.

DISCUSSION AND CONCLUSIONS

From the experiments in which extraction methods were compared it is clear that a moderate direct activity was observed for all extracts, whereas indirect activity, especially in the indoor extracts is strong. According to the results of previous studies conducted at our laboratory mutagenic activity of the extracts may be characterized as highly mutagenic indoor samples and a moderately mutagenic outdoor sample (Van Houdt et al., 1984, 1986, 1987). In contrast to results of Krishna et al. (1983) who found sonication the most efficient method for the extraction of ambient particles, the results of this study show soxhlet extraction to be slightly more efficient in removing mutagens from particulates sampled indoors and outdoors. However, because of the small differences between both methods, sonication may be regarded as a suitable extraction method.
The aim of this study was the determination of extraction potency of serum and lung lavage fluid. Soxhlet extraction which was commonly used in our previous studies is not suitable for physiological fluids because their stability may be affected by boiling. Therefore, for this purpose sonication of the filter is the method which mimics in vivo conditions best. Because of the small amount of lung lavage fluid available, only one particulate sample was sonicated directly in physiological fluids. This method was compared with the method of dissolving the tar, obtained by sonication with methanol and evaporation.
So far studies on the solubility of airborne particles in serum collected indoors and outdoors in rural areas are not performed. Investigations of interactions between mutagens and physiological fluids were carried out for diesel emissions, coal fly ash and airborne particulate matter, collected in urban areas. For diesel particulate emissions serum proved to be less efficient in removing mutagens than organic solvents (Brooks et al., 1979; King et al., 1981). This was also reported by Oshawa et al. (1983) for particles collected in urban air. Takeda et al. (1983) reported on the same phenomenon for indirect activity, however, in this study direct activity of serum and DMSO extracts was about the same. Regarding serum extractability of coal fly ash, both a

25 times greater mutagenic activity than cyclohexene extracts (Chrisp et al., 1978) and a strong decrease related to DMSO solubility were observed (Kubitschek and Venta, 1979).
Our results show that serum extracts of indoor and outdoor air have a lower mutagenic activity than methanol extracts. Indications were found that serum is more effective in removing direct acting mutagens from the tar than indirect acting mutagens. Mutagenic compounds from indoor air are not only soluble in newborn calf serum, also lung lavage fluid, although less efficient, is able to remove mutagens from the tar. In contrast to serum, in lung lavage fluid the percentage of dissolved indirect acting mutagens exceeds the percentage of direct mutagenicity. Indirect acting mutagens from outdoor air are almost completely insoluble in lung lavage fluid. The same phenomenon was found by King et al. (1981) for diesel particles.
Protective effects of serum on mutagenicity and cytotoxicity, probably due to protein binding, which were reported by several authors (King, 1980; Henderson et al., 1981; Li, 1981; Oshawa et al., 1983; Tilkes and Beck, 1983; White et al., 1983) were not found in our studies concerning sonication of the tar. Not only the serum extracts, also the DMSO extracts of the residue show for all samples a strong mutagenic activity, together accounting for about 100% of the total mutagenic activity. The only indication of protective effects has been found by direct sonication of the filters with lung lavage fluid. In this experiment only a small portion of the mutagenic compounds remained on the filter, whereas total mutagenic activity of the filter was not recovered in the lung lavage extract.
From these experiments it may be concluded that physiological fluids are not as efficient as organic solvents in removing mutagens from particles. Lung lavage fluid, containing a high concentration of phosphatidylcholine is only able to remove about 10% of the total mutagenic burden from airborne particles.
This may suggest that lung lavage fluid, surfactant, protects alveolar cells against properties of airborne particles. For risk evaluation of inhaled particulate matter, studies on the bioavailability of these mutagens have to be continued.

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8 Organ specific metabolic activation of five extracts of indoor and outdoor particulate matter

J.J. van Houdt, P.W.H.G. Coenen, G.M. Alink, J.S.M. Boleij and J.H. Koeman

[Archives of Toxicology 61: 213-217 (1988)]

SUMMARY

In this study liver and lung homogenates of untreated and Aroclor 1254-pretreated rats (Wistar) and mice (Swiss) were compared for their P-450 content and their capacity to activate extracts of airborne particulate matter sampled indoors and outdoors. Results show that in addition to liver, lung homogenates of rat (Wistar) and mouse (Swiss) are also able to activate extracts of airborne particulate matter in a comparative way. Uninduced liver and lung homogenates showed only minor differences in activation capacity in the metabolism of airborne particles. In contrast to liver homogenates, Aroclor 1254 pretreatment of test animals did not give strong induction of metabolic activation capacity of lung homogenates. P-450 content was observed in all liver and lung homogenates of mouse and rat and in human lung homogenates. The results obtained in this study suggest that the respiratory tract may be an important site for in vivo bioactivation of respirable particles.

Key words: mutagenicity, metabolic activation, lung, liver, airborne particulate matter, P-450.

INTRODUCTION

By inhaling airborne particles man is exposed to a variety of chemicals, including mutagens and carcinogens. In outdoor air mutagenicity of aerosols collected at different locations has been studied extensively by many authors (Pitts et al. 1977; Harnden 1978; Tokiwa et al. 1983; Alink et al. 1983; Van Houdt et al. 1987). The results of these studies show that mutagenic activity of airborne particulate matter in the Salmonella/microsome assay varies markedly. Addition of Aroclor 1254-induced rat liver microsomes gives variable results. Increases or decreases in the number of revertants, if found, are generally relatively small.

The indoor environment may be polluted by compounds, produced indoors or entering from outdoors. In contrast to outdoors, mutagenic activity of indoor particulate matter is predominantly enzyme mediated (Löfroth et al. 1983; Van Houdt et al. 1984; 1986). Indirect mutagenic activity of indoor particulate matter generally exceeds that of outdoor particles (Alfheim and Ramdahl 1984; Van Houdt et al. 1984; Yamanaka and Muruoka 1984).
As people in western societies spent most of their time inside the home (Moschandreas 1981; Boleij and Brunekreef 1982; Yocom 1982) exposure to mutagenic compounds might very well be determined to a large extent by the level of genotoxins inside homes. It may be concluded that exposure to indirect acting mutagens is quantitatively of greater concern than exposure to direct acting mutagens.
Since the lung is the target tissue for carcinogenic substances in airborne particles, it is not clear whether mutagenicity testing with liver extracts is a reliable prediction of mutagenic activity in the lung.
In order to estimate the risk from inhaled aerosols, knowledge about deposition in specific regions of the respiratory tract, physiological clearance from that region, solubility in lung surfactant and metabolic activation has to be obtained.
Mutagenic activity of particulate matter is mainly located on the smallest particles (<3 μm) (Talcott and Harger 1980; Möller et al. 1982; Sorensen et al. 1982; Kado et al. 1986). Deposition of particles in the lower respiratory tract may take place. The lung metabolizes a wide variety of xenobiotics and as mutagenic products may be formed (Hook and Bend 1975; Bond 1983; Mori et al. 1986), the question arises to what extent metabolic activation of particle bound organics may take place in the lung. Pulmonary cytochrome P-450 activity is mainly localized in the lower respiratory tract: in non-ciliated clara cells and to a less extent in ciliated epithelium cells, Type II cells and alveolar macrophages (Harris et al. 1978; Gardner 1984; Philpot and Smith 1984).
In this study a comparison was made between liver and lung homogenates for P-450 content and capacity to activate five extracts of particulate matter collected indoors and outdoors. Human lung homogenates were only involved in the comparison of P-450 content because of the small amounts of tissue available.

MATERIAL AND METHODS

Preparation of aerosol extracts

Sampling methodology and extraction of five samples which had already been used for cytotoxicity testing in rat alveolar

macrophages were performed according to the procedures described in the preceeding paper.
In summary, the following five extracts were prepared: indoor air polluted by wood combustion, indoor air polluted by smoking, outdoor air collected in winter with easterly wind direction, outdoor air, collected in summer with northerly wind direction and clean outdoor air.

Mutagenicity testing

Preparation of enzyme systems. Metabolic systems were prepared from livers and lungs of three-months-old male Wistar rats and six-weeks-old male Swiss mice. Half the animals were injected intraperitoneally with Aroclor 1254 (500 mg/kg) (Ames et al. 1975). After 5 days, the animals were anaesthetized with diethylether. The livers and lungs were perfused and removed aseptically, washed with sterile ice-cold KCl solution (0.15 M), minced and homogenized in 3 vol of KCl (3 ml/g liver or lung) with an Elvejhem potter apparatus at 1300 r.p.m. The lung homogenate was sonificated for 2 min with a sonifier B12 cell disruptor. The homogenates were centrifuged at 9,000 g for 20 min. The supernatant of each subcellular fraction was pooled and stored in liquid nitrogen. Human lung samples were obtained from operatively removed lung tissue on seven patients suffering from lung cancer. Microsomes were only prepared from healthy tissue, according to the procedure described above. In contrast with mouse and rat homogenates, human lung homogenates were not pooled.

Salmonella/microsome test. The experiments were performed using the standard plate assay, according to Ames et al. (1975), with minor modifications described elsewhere (Van Houdt et al. 1984).
In a preliminary experiment the extracts were diluted eight times in equal volumes. Each volume was tested once (dose extract effect-curve). Two independent tests were carried out per extract.
In the experiments described in this paper the optimal dose of each extract, together with blanks and positive controls were tested with four liver and four lung homogenates. Each homogenate was tested in concentrations of 5, 10, 20 and 40% in the S9 mix with cofactors in excess (twice the quantity described by Ames (1975)). Per plate this means an addition of 0.85, 1.7, 3.4 and 6.8 mg tissue (dose homogenate-effect curve). Two independent tests were carried out in duplicate. In the results presented, the spontaneous revertants are substracted. Tester strain TA 98 was used in all experiments. 2-Aminoanthracene, benzo(a)pyrene and 4-nitroquinoline oxide, mutagenic compounds, which may deal with airborne particulate matter, were used as positive controls. The metabolic

capacity of each enzyme preparation is expressed as metabolic factor, calculated from the slope of the rising part of the 'homogenate concentration-response curve' by linear regression.

Biochemical assays

Cytochrome P-450 content was determined by its CO-binding spectrum according to the method of Omura and Sato (1964). Absorption maxima of the Soret bands were determined by applying first derivative spectrometry. Protein concentrations were determined by the method of Lowry et al. (1951) with BSA as a standard.

RESULTS

The results of the experiments carried out in the preliminary tests are depicted in figure 1. Indoor samples showed only a

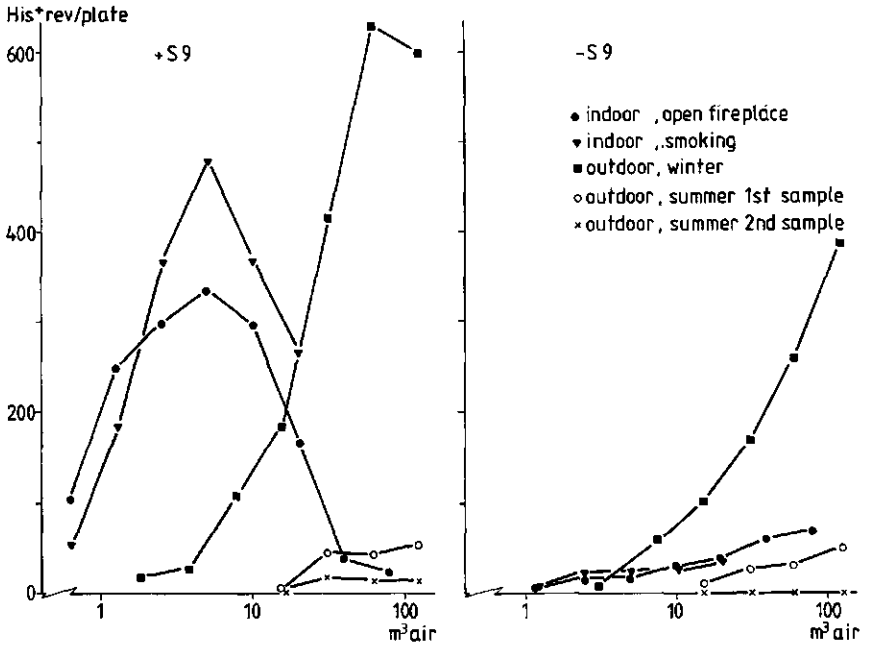

Figure 1. Mutagenic activity of extracts of airborne particulate matter in Salmonella Typhimurium TA 98, with and without Aroclor-induced liver S9.

slight direct activity, while enzyme-mediated mutagenic activity was strong. To a less extent this was also found in the outdoor sample collected in winter. Mutagenic activity of the extracts, expressed as revertants/m^3, calculated from the slope of the linear part of the dose effect curve is presented in table 1. Both with and without liver S9 outdoor samples collected in summer did not show any mutagenicity. The highest direct activity was found in outdoor air.

TABLE 1. Mutagenic activity (rev/m^3) of the extracts tested

Extract	-S9	+S9
outdoor clean	0	0
outdoor summer	0	0
outdoor winter	6	12
indoor wood combustion	3	69
indoor smoking	2	98

Based on the observations in the preliminary experiment the samples collected outdoors in summer were not tested in the metabolic activation studies. For indoor air a non-toxic dose of extract, corresponding to 5 m^3 was used as an obtimal dose in metabolic activation experiments, while for outdoor air collected in winter doses of 15 m^3 were used.

TABLE 2. Cytochrome P-450 content (in pmol/mg protein and pmol/ml S9) of various lung and liver tissue homogenates.

	P450 pmol/mg protein		P450/ml S9	
	Uninduced	Induced	Uninduced	Induced
Rat liver	159	1300	410	10300
Rat lung	27	14	150	110
Mouse liver	1112	1800	9340	10915
Mouse lung	65	504	370	2360
Human lung	15-43		80-120	

The cytochrome P-450 content in all tissue homogenates, including human lung was determined. The results presented in table 2 show that in lung and liver homogenates P-450 content differs markedly. After Aroclor 1254 induction cytochrome P-450 content is strongly increased in rat liver, mouse lung and mouse liver. In rat lung Aroclor induction gives a decrease of P-450 content. In the human lung homogenates P-450 content was comparable with uninduced rat lung: variations between 15 and 43 pmol/mg protein were found.

TABLE 3. Protein (mg), P-450 (pmol) content and metabolizing capacity (rev/plate) of 10% homogenate S9 mix of various liver and lung homogenates towards 2-amino-anthracene, benzo(a)pyrene and 4-nitroquinoline oxide. Salmonella typhimurium TA 98.

Homogenate	Protein mg	P-450 pmol	2-AA 2µg	NQO 0.5µg	B(a)P 5µg
-	-	-	12	288	16
Rat liver, uninduced	0.26	41	2050	46	200
Rat liver Aroclor	0.80	1030	3160	28	379
Rat lung uninduced	0.55	15	2400	111	170
Rat lung Aroclor	0.79	11	2600	54	240
Mouse liver uninduced	0.84	934	2580	14	238
Mouse liver Aroclor	0.61	1092	3280	47	310
Mouse lung uninduced	0.57	37	471	62	63
Mouse lung Aroclor	0.47	236	624	71	98

Table 3 shows the capacity of different enzyme preparations towards activation and deactivition of 2 µg 2-aminoanthracene (2-AA), 0.5 µg 4-nitroquinoline oxide (NQO) and 5 µg benz(a)pyrene (B(a)P). In this experiment only one dose of homogenate, including 1.7 mg tissue was added per plate. All homogenates show metabolic activity towards B(a)P and 2-AA. Aroclor 1254 induced and noninduced mouse lung homogenate was considerably less active than the other homogenates. NQO was deactivated by all homogenates, again lung homogenates of the mouse showed the lowest metabolic activity. In table 4 mutagenic activity of B(a)P, NQO and 2-AA are correlated with protein and P-450 content of the added homogenates. P-450 content appears to be correlated

TABLE 4: Matrix of correlation coefficients between activation and deactivation of B(a) P, 2-AA and NQO (rev/plate) and protein (mg) and P-450 content (pmol) of added homogenates.

	B(a)P	2-AA	NQO
protein	0.49*	0.15	0.42
P-450	0.74*	0.61	-0.65

* $p < 0.05$

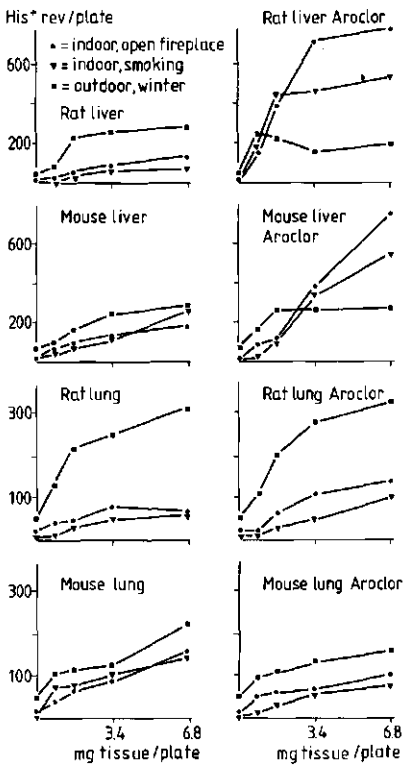

Figure 2. Mutagenic activity of extracts of airborne particulate matter in Salmonella Typhimurium TA 98 with different amounts of uninduced and Aroclor-induced liver and lung homogenates of rat (Wistar) and mouse (Swiss).

significantly with B(a)P activation ($p < 0.05$). Other statistically significant correlations were not observed.

Mutagenic activity of extracts of airborne particulate matter with liver and lung homogenates is presented in figure 2. Although the activating capacity of extracts of airborne particles by the lung homogenates measured in total number of revertants is less than that of the Aroclor induced liver homogenates, in all dose-effect curves of both tissue homogenates a high correlation ($c = 0.94$; $p < 0.05$) was found between the amount of added tissue and the number of revertants per plate. In figure 3 the results of figure 2 are expressed as the metabolic activation parameter, calculated from the slope of the linear regression curve. Induction by Aroclor 1254 gives a strong increase in the metabolic activation capacity of mouse and rat liver. Outdoor samples are, in contradiction to indoor samples relatively strongly activated by the rat tissue homogenates, while mouse tissue homogenates give a comparable activation of the indoor and outdoor extracts.

DISCUSSION AND CONCLUSIONS

In the preliminary experiments three extracts showed a relatively weak direct mutagenic activity, whereas indirect activity, especially in the indoor extracts, is strong. Two outdoor extracts did not show mutagenic properties.
According to the results of previous studies (Van Houdt et al. 1984, 1986, 1987) mutagenic activity of the extracts may be characterized as highly mutagenic indoor samples, one moderately mutagenic and two non-mutagenic outdoor samples.

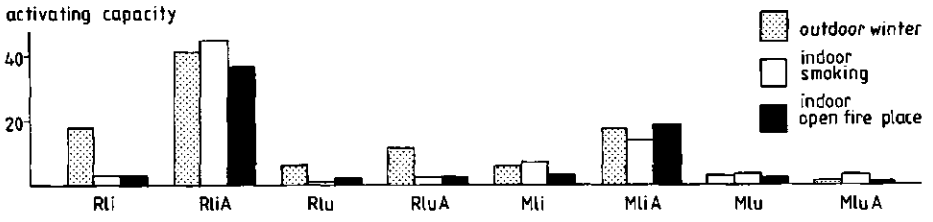

Figure 3. Mutagenic activating capacity of different liver and lung homogenates towards extracts of airborne particulate matter (R = rat, M = mouse, lu = lung, li = liver, A = Aroclor 1254-induced).

Mixed function oxidation is of primary importance in the elimination of lipid-soluble xenobiotics from the body. In table 2 cytochrome P-450 content in pmol/mg protein is presented for tissue homogenates of rat and mouse and for human lung homogenates. In uninduced liver and lung homogenates P-450 content is in the same order of magnitude. In contrast to lung homogenates, P-450 content in liver homogenates (Souhaily El Amri et al. 1986) is strongly enhanced by Aroclor induction. P-450 content in rat lung and its decrease after Aroclor induction are in agreement with data obtained by Liem et al. (1980), Serabjit-Sing (1983) and Ueng et al. (1985). Increase in P-450 content in mouse lung found after Aroclor induction was also found by Di Giovanni et al.(1979) and Mori et al. (1986), although less pronouced. In human lungs P-450 content varied between 15 and 43 pmol/mg protein. This is 1-4 times more than the results found by Prough et al. (1979). Philpot and Smith (1984) and Boobis and Davies (1984) reported on the alteration of P-450 isoenzymes by inductive, regressive and destructive effects of various exogenous chemicals. As human lung tissue in our study was obtained from humans suffering from lung cancer, variations in P-450 content, although measured in healthy tissue may occur. In this study liver and lung homogenates were compared for activation capacity of indirect acting mutagens. Table 3 gives an indication of the metabolizing capacity of liver and lung homogenates. The results are in agreement with data obtained by Hook and Bend (1975), Hutton and Hackney (1975), Juchau et al. (1979), Bond (1983) and Mori et al. (1986). The strongest activation of B(a)P was found after addition of Aroclor 1254-induced liver homogenates.
In agreement with our results, Juchau et al. (1979) also found considerably less activation by lung homogenates. Philpot and Smith (1984) reported preparations of rabbit lung to be a 20 - 30 fold more active than those from liver in the metabolism of 2-aminofluorene (2-AF) and 2-AA. Differences in activation capacity of B(a)P by liver and lung homogenates may be caused by the cytochrome P-450 content of the S9. In this study, the mutagenicity of three air samples was assessed by the Salmonella/microsome assay, using different lung and liver homogenates. From figure 2 it is clear that

Aroclor induced liver homogenates gave the strongest activation capacity, in contrast to other homogenates, which generally gave lower effects. Aroclor induction produces no increase in activation capacity of the lung homogenates. Comparison of the uninduced lung and liver homogenates shows that only minor differences in activation capacity are found. In contrast to mouse homogenates, a rather strong activation of outdoor air was observed after adding rat homogenates. However, a positive relation ($p < 0.05$) between amount of added tissue and number of revertants was found for all homogenates.
Differences in metabolizing capacity, presented in table 3 may be due to differences in P-450 content and protein content of the homogenates. Table 3 also gives the differences in P-450 content per ml S9. It may be concluded that there is no linear relation between metabolizing capacity of lung and liver homogenates and P-450 content. This is especially clear with regard to the induction of P-450 content in mouse lung and the lack of activation potential of mouse lung in mutagenicity assays. This may be due to a different compilation of isoenzymes of P-450, by which means substrate specificity differs (Hook and Bend 1975; Philpot and Smith (1984).
These results suggest that the respiratory tract may be an important site for in vivo bioactivation of inhaled particle-bound organic compounds, with metabolic activity patterns which differ from the liver. Therefore, it may be concluded that for a genotoxic risk evaluation of airborne particles the use of lung homogenates in addition to liver homogenates in in vitro mutagenicity testing may be useful.

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PART III

Summary and conclusions

9 Summary and conclusions

Introduction

Air pollution components are present as gases and as particulate matter. As particle deposition takes place in various parts of the respiratory system particulate matter may have other toxicological implications than gaseous pollutants, which all may penetrate in the lower part of the respiratory tract. In addition, suspended particulate matter represents a group of pollutants of variable physical as well as chemical composition. Therefore airborne particulate matter cannot be regarded as a single, pure pollutant.
This study deals with the mutagenic potency of airborne particulate matter bound organics and some of its toxicological implications.
The assessment of health hazards is not easily possible without knowledge of the chemical character of the particles. Risk assessment through a toxicological consideration of the individual constituents has serious drawbacks because of the large number of chemicals involved and the complexity of the mixture. Since only 30-40% of the organic compounds on airborne particles have been identified, the contribution of unidentified compounds to the toxicological risk may be significant. Therefore the assessment of the overall mutagenic or carcinogenic activity in air samples may provide a more realistic basis for the evaluation of the possible risks, than an evaluation on the basis of individual compounds.
The Salmonella/microsome assay has been a major assay used for monitoring the mutagenic potential of complex environmental mixtures such as airborne particulate matter. Results obtained with this test system may also be useful as a general air pollution parameter, representing particle bound extractable organics.
The Salmonella/microsome assay involves subsequently sample collection, extraction, exposure of the test strain and the quantitative assessment of the revertants. Extraction is carried out routinely with organic solvents, while generally liver homogenates are used to identify mutagens which require metabolic activation. In the first part of this study methanol and liver homogenates from Aroclor 1254-pretreated Wistar rats were used to study the occurrence of particle bound mutagens collected indoors and outdoors. In the second part of this study physiological fluids and lung homogenates, which are more representative for the environment particles encounter in lungs, are used as solvent and metabolizing system in the Salmonella/microsome assay. Also cytotoxicity of airborne particulate matter to rat alveolar macrophages was determined in order to study some toxicological

implications of inhalation of particle bound organic compounds.

Mutagenic activity of extracts of airborne particulate matter from outdoor and indoor environments

Chapter 1 provides a summary of literature data on physical and chemical characteristics of airborne particulate matter. The occurrence of mutagens in the environment and their sources are discussed.
Chapter 2 presents data on in vitro testing of airborne particles, collected outdoors. Much evidence exists on the mutagenicity of airborne particles at urban and industrial locations. This study shows that particulate matter at background (Terschelling) and rural locations (Wageningen) may also bear mutagenic compounds. In Wageningen the level of mutagenicity follows a yearly cycle, the highest activity being found in winter. Over the years considerable differences in mutagenicity were found. The correlation of mutagenicity of samples simultaneously collected on the same day at both locations and the relation between mutagenicity of air samples and wind direction and with air trajectories suggest that the mutagenic potential of suspended matter originates not from local sources but rather depends on large scale processes. This is supported by literature data which show that the mutagenic potential mainly occurs on the smallest particles which, as a result of their long residence time, may be transported over distances of thousands of kilometers.
The air, sampled at rural and background locations contains direct as well as indirect acting mutagens. The addition of liver microsomes gives variable effects on the level of mutagenic activity. Increases or decreases, if found are mostly relatively small. Furthermore, mutagenic activity was correlated with commomly measured air pollution parameters; multiple regression showed that SO_2, NO_2, NO, CO and O_3 together account for 70% of the variation in direct mutagenicity and 80% of the variation of indirect activity. SO_2 and NO_2, and SO_2, NO_2 and CO were significantly associated with the variation in direct and indirect activity respectively.
As variations in mutagenic activity can be explained to a large extent by commonly registered air pollutants, monitoring the mutagenic burden of aerosols does not contribute to our knowledge already obtained by monitoring SO_2, NO_x and CO.
Chapter 3 and chapter 4 deal with the comparison of particle bound mutagens collected indoors and outdoors. In chapter 3 the contribution of smoking and cooking and in chapter 4 the contribution of wood combustion to mutagenicity of indoor aerosols is established. The results show that not only extracts of outdoor particles, but also indoor particulate matter may contain mutagenic compounds. Comparison of the

mutagenic activity indoors and outdoors indicates that outdoor extracts show a direct mutagenic activity which is clearly detectable in nearly all samples, whereas indoor samples mostly show a low or undetectable direct activity. It is found that the indirect mutagenic activity is generally larger in indoor samples than in outdoor samples. Morever in indoor extracts cytotoxic effects are more pronounced.
One of the sources of indoor mutagens may be penetration of outdoor particles. In the winter 82/83 no contribution of outdoor sources to mutagenic activity indoors was observed, while in the winter 84/85 a correlation between the extremely high levels of outdoor mutagenicity and indoor mutagenic activity was found. However, the important differences in composition of simultaneously collected indoor and outdoor samples which manifest themselves by the ratio -S9/+S9 justifies the conclusion that penetration of outdoor particles may be one but certainly not the only source of mutagenic activity indoors. Mostly the contribution of outdoor sources to indoor mutagenicity is only small.
From our studies it is obvious that cigarette smoking is the predominant source of airborne genotoxicity in homes. Wood combustion appeared to be a second important factor producing genotoxic compounds as sometimes a 2-3 fold increase of mutagenic activity is found. Volatilization of cooking products represents a less important source of mutagens.
From these experiments it may be concluded that the removal of mutagens to the outdoor environment is not complete.

Exposure is a function of concentration and time. In The Netherlands people spend most of the time at home. As all indoor samples show a strong indirect mutagenic activity, it may be concluded that exposure to genotoxins will be determined to a large extent by the level of pollution inside homes, which implies that exposure to indirect acting mutagens is quantitatively of far greater concern than exposure to direct acting mutagens.

Biological availability of particle bound organic compounds

Chapter 5 reviews a number of studies which deal with some aspects of the biological fate of mutagenic compounds and their 'carrier' particles. Respirable airborne particles to which most potential mutagenic compounds, detected in the Ames assay, are adsorbed may deposit in various parts of the respiratory tract. One of the defence mechanisms with regard to possible harmful action of these particles is clearance, as it reduces residence time on potentially sensitive epithelial surfaces. Alveolar macrophages provide the initial defence of the lower respiratory tract towards particulate matter.
In chapter 6 results show a significant reduction of

phagocytic activity in rat alveolar macrophages in vitro after exposure to extracts of airborne particulate matter, and the effect was greatest with indoor air. Metabolizing enzymes, together with transformed or not transformed particles may be released in the alveolar spaces as a result of damage to alveolar macrophages.
In chapter 7 dissolution of particle bound organics into newborn calf serum and lung lavage fluid from pigs is described. Although from our results it is clear that physiological fluids, especially when obtained from lung lavage are less efficient in removing mutagens than organic solvents, the suggestion seems to be justified that a certain elution of environmental chemicals into body fluids takes place.
Chapter 8 deals with metabolic activation of extracts of indoor and outdoor particulate matter. Drug metabolizing enzyme systems may also be seen as a defence mechanism towards chemicals invading the body. These enzyme systems enable the organism not only to convert lipid soluble harmful drugs into harmless water soluble metabolites, but also more toxic or mutagenic metabolites may be formed. Our results show that in addition to liver, lung homogenates of rat (Wistar) and mouse (Swiss) are also able to activate extracts of airborne particulate matter in a comparable way. Uninduced liver and lung homogenates showed only minor differences in activation capacity in the metabolism of airborne particles. In contrast to liver homogenates, Aroclor 1254-pretreatment of test animals did not give a strong induction in metabolic activation capacity of lung homogenates.
In liver, almost all cells contribute to metabolic capacity, whereas in the lung metabolic capacity is almost exclusively located in the Clara cells, which is only one of the 40 different lung cell types. In certain parts of the lungs, especially in the terminal bronchioles, in which Clara cells are located, a rather high metabolic activation may take place. Therefore these results suggest that the respiratory system may be an important site for in vivo bioactivation of respirable particles.

10 Samenvatting en conclusies

Introduktie

Luchtverontreinigende komponenten kunnen in de atmosfeer voor komen als gassen of gebonden aan deeltjes. Door hun depositie op verschillende plaatsen in de luchtwegen kunnen aerosolen een andere toxicologische werking uitoefenen dan gassen, die allen in het alveolaire deel door kunnen dringen. Bovendien zijn aerosolen samengesteld uit een groep verbindingen met uiteenlopende fysische en chemische samenstelling. Om deze redenen kunnen aerosolen niet worden gezien als een eenduidige, enkelvoudige verontreiniging. Zonder kennis van de chemische samenstelling van de deeltjes is het voorspellen van toxicologische risico's een ongewisse zaak.
Dit onderzoek beschrijft de resultaten van een studie betreffende de mutageniteit van aan deeltjes gebonden organische verbindingen en enige aspecten betreffende hun toxicologische gevolgen.
Risico analyse gebaseerd op het analyseren van de afzonderlijke komponenten heeft belangrijke nadelen vanwege het grote aantal verbindingen en de complexiteit van het mengsel. Omdat slechts 30-40% van de aan deeltjes gebonden organische komponenten is geïdentificeerd kan de bijdrage van niet-geïdentificeerde komponenten aan het toxicologische risiko van groot belang zijn. Met het oog op de evaluatie van mogelijke risico's is om deze redenen het vaststellen van de mutageniteit van het gehele mengsel realistischer dan het beoordelen van afzonderlijke komponenten.
De Salmonella/microsoom test is de meest frequent gebruikte in vitro test voor het evalueren van de mutagene potentie van complexe mengsels. Bovendien kunnen resultaten van deze test van nut zijn als 'somparameter' voor in de lucht voorkomende organische komponenten.
Bij het voorspellen van de mutageniteit van aan aerosolen gebonden verbindingen door middel van de Ames test spelen monstername, extraktie, blootstelling van de bacteriestam en het tellen van het aantal mutanten een rol. Hierbij worden organische oplosmiddelen routinematig als oplosmiddel gebruikt, terwijl leverhomogenaten gebruikt worden om mutagenen, die metabole aktivering vereisen, te aktiveren. In deel I van deze studie zijn methanol en leverhomogenaat van met Aroclor 1254 voorbehandelde Wistar ratten gebruikt om het vóórkomen van mutagenen in binnen- en buitenlucht na te gaan. Teneinde enige toxicologische aspecten te bestuderen zijn in deel II van deze studie fysiologische vloeistoffen en longhomogenaten, die meer representatief zijn voor de omgeving, waar deeltjes in het lichaam binnenkomen, gebruikt als oplosmiddel en emzympreparaat. Verder is in deel II de

toxiciteit van aerosolextrakten op alveolaire macrofagen van de Wistar rat bestudeerd.

Mutagene aktiviteit van aerosolextrakten uit binnen- en buitenlucht

Hoofdstuk 1 bevat een samenvatting van de literatuur betreffende chemische en fysische eigenschappen van aerosolen. Het vóórkomen van aan aerosolen gebonden mutagene komponenten in binnen- en buitenlucht en de hiervoor van belang zijnde bronnen worden in ogenschouw genomen.
Hoofdstuk 2 bevat een weergave van de resultaten betreffende de mutageniteit van in de buitenlucht gemonsterde aerosolen. In de literatuur wordt veelvuldig melding gemaakt van het vóórkomen van mutagene aerosolen op stedelijke en industriele lokaties. Uit de resultaten blijkt dat ook aerosolen, verzameld op landelijke (Wageningen) en achtergrond lokaties (Terschelling) mutagene eigenschappen bezitten. In Wageningen blijkt de hoogte van de mutageniteit een jaarlijkse cyclus te volgen, waarbij in de winter de hoogste waarden worden waargenomen. Tussen verschillende jaren worden echter aanzienlijke verschillen gevonden. Het verband tussen gelijktijdig gemonsterde aerosolen op Terschelling en in Wageningen, en de relatie van mutageniteit van luchtmonsters met windrichting en luchttrajectoriën doen vermoeden dat de mutageniteit van deeltjes niet door lokale bronnen wordt veroorzaakt, maar dat grootschalig transport veeleer van belang is. Aanwijzingen hiervoor kunnen eveneens verkregen worden uit literatuurgegevens betreffende het vóórkomen van mutagenen aan kleine deeltjes, die vanwege hun lange verblijftijd over duizenden kilometers getransporteerd kunnen worden.
De lucht, verzameld op landelijke en achtergrondlokaties bevat zowel direkte als indirekte mutagenen. Toevoeging van leverhomogenaat geeft onvoorspelbare effecten op de hoogte van de mutageniteit. Een toename of afname, zo die al gevonden wordt, is doorgaans gering. Er is een verband aangetoond tussen mutageniteit en routinematig gemonsterde luchtverontreinigingsparameters. Uit meervoudige regressie bleek dat SO_2, NO_2, NO, CO en O_3 70% van de directe en 80% van de indirekte mutageniteit verklaren. De verbanden tussen SO_2, NO_2 en direkte mutageniteit en SO_2, NO_2 en CO en indirekte mutageniteit bleken significant. Hieruit mag worden geconcludeerd dat het in de tijd volgen van de mutageniteit van aerosolen nauwelijks bijdraagt aan de kennis die reeds wordt verkregen door continu waarnemen van SO_2, NO_x and CO.
In hoofdstuk 3 en 4 wordt de vergelijking tussen mutageniteit van binnen- en buitenlucht in beschouwing genomen. In hoofdstuk 3 wordt de bijdrage van roken en koken en in hoofstuk 4 wordt de bijdrage van houtverbranding aan binnenluchtmutageniteit vastgesteld. Niet alleen extrakten

verkregen uit buitenluchtaerosolen, ook die verkregen uit binnenlucht bevatten mutagene verbindingen. In tegenstelling tot vrijwel alle buitenluchtmonsters die een duidelijke direkte mutageniteit te zien geven is de direkte mutageniteit van binnenluchtaerosolen doorgaans laag. In binnenlucht extrakten is de indirekte mutageniteit in het algemeen hoger dan in buitenluchtextrakten. Bovendien zijn in binnenlucht extrakten de cytotoxische effecten meer uitgesproken.
Het binnendringen van buitenluchtaerosolen zou een van de mogelijke bronnen van binnenluchtmutagenen kunnen zijn. In de winter van 1982/1983 is geen bijdrage van buitenlucht aan binnenluchtmutageniteit waargenomen, wel is dit het geval in de winter van 1984/1985, toen de buitenlucht mutageniteit extreem hoog was. Echter, belangrijke verschillen in het karakter van de mutageniteit, tot uiting komend in de verhouding -S9/+S9 rechtvaardigen de conclusie dat buitenlucht in het algemeen slechts een geringe bijdrage aan de binnenluchtmutageniteit levert.
Uit de gegevens van hoofdstuk 3 en 4 blijkt dat het roken van tabak de overheersende bron van binnenlucht mutagenen is. Houtverbranding is eveneens een belangrijke bron, daar houtverbranding een twee tot drievoudige verhoging van de binnenluchtmutageniteit te zien kan geven. Vervluchtiging van kookprodukten lijkt een minder belangrijke bron.
Op grond van deze resultaten kan worden geconstateerd dat de afvoer van mutagenen naar de buitenlucht niet ideaal genoemd kan worden.

Blootstelling is een functie van concentratie en (expositie) tijdsduur. Daar men in Nederland het grootste deel van de tijd in de woning doorbrengt kan worden geconcludeerd dat de blootstelling aan genotoxische verbindingen voornamelijk door de hoogte van de verontreiniging binnenshuis bepaald wordt en dat daarmee blootstelling aan indirekte mutagenen kwantitatief van veel groter belang is dan blootstelling aan direkte mutagenen.

Biologische beschikbaarheid van aan aerosolen gebonden mutagenen

Respirabele aerosolen, waaraan de meeste, in de Amestest gedetecteerde mutagenen voorkomen kunnen op verschillende plaatsen van het ademhalingssysteem, waaronder de alveoli gedeponeerd worden. Een van de verdedigingsmechanismen tegen een mogelijk schadelijke werking van deze aerosolen is verwijdering, ofwel 'clearance', waardoor de verblijftijd van de deeltjes op potentieel gevoelige epitheel oppervlakken wordt gereduceerd. Alveolaire macrofagen vormen een belangrijk verwijderings mechanisme in de alveoli.
In hoofdstuk 6 is een significante afname van de fagocytose in alveolaire macrofagen van de rat <u>in vitro</u> waargenomen na

blootstelling aan extracten van aerosolen, met name die, die gemonsterd zijn in de binnenlucht. Als gevolg van een toxische werking kunnen enzymen, samen met al dan niet getransformeerde aerosolen vanuit de macrofagen vrijkomen in de alveolaire ruimten.
In hoofdstuk 7 is de oplosbaarheid van aan aerosolen gebonden deeltjes in fysiologische vloeistoffen nagegaan. Ofschoon uit de in hoofdstuk 7 verkregen resultaten duidelijk is dat fysiologische vloeistoffen, met name uitgespoeld longsurfactant minder effectief is in de verwijdering van mutagenen van binnen- en buitenluchtdeeltjes dan organische oplosmiddelen, lijkt de conclusie gerechtvaardigd dat een zekere oplossing van omgevingsmutagenen in lichaamsvloeistoffen plaats vindt.
In hoofdstuk 8 worden een aantal experimenten betreffende metabole aktivering van binnen- en buitenluchtextrakten weergegeven. Metaboliserende enzymsystemen kunnen in zekere zin ook als mechanisme voor de verdediging tegen deeltjes die het lichaam binnen komen opgevat worden. Deze enzymsystemen maken het het lichaam niet alleen mogelijk vetoplosbare schadelijke verbindingen om te zetten in niet schadelijke wateroplosbare metabolieten, eveneens kunnen door omzetting mutagene of anderszins schadelijke metabolieten gevormd worden. Uit de resultaten van hoofdstuk 8 blijkt dat niet alleen leverhomogenaten, maar ook longhomogenaten van rat (Wistar) en muis (Swiss) op een vergelijkbare manier in staat zijn luchtextrakten te aktiveren. Ongeïnduceerde long- en leverhomogenaten laten slechts marginale verschillen zien in de aktivering van luchtextrakten. De voorbehandeling van de proefdieren met Aroclor 1254 geeft, in tegenstelling tot leverhomogenaten geen sterke induktie van de metabole kapaciteit van longhomogenaten.
In de lever draagt vrijwel elke cel bij aan de metabole kapaciteit, terwijl in de long de metabole kapaciteit vrijwel uitsluitend gelokaliseerd is in de Clara cellen, een van de 40 verschillende longceltypen. In de terminale bronchioli, dat deel van de long waar de Clara cellen zich bevinden kan een relatief sterke metabole aktivering plaatsvinden. De verkregen resultaten wijzen er dan ook op dat het ademhalingssysteem een belangrijke plaats voor de metabole aktivering in vivo kan zijn.

Curriculum vitae

Hans van Houdt werd op 2 oktober 1955 te Scherpenisse geboren. Na in 1975 zijn diploma VWO te hebben behaald aan de Rijksscholengemeenschap te Bergen op Zoom, begon hij in datzelfde jaar zijn studie Milieuhygiëne aan de Landbouwhogeschool te Wageningen. Het ingenieursdiploma met als hoofdvakken Toxicologie en Luchthygiëne en -verontreiniging en als bijvak Gezondheidsleer behaalde hij in juni 1982. Van juni 1982 tot april 1987 was hij verbonden aan de vakgroepen Toxicologie en Luchthygiëne en -verontreiniging van de Landbouwhogeschool. Eerst in het kader van de wet gewetensbezwaren militaire dienst, later als wetenschappelijk ambtenaar.
Sinds april 1987 is hij werkzaam op de Hoofdafdeling Milieu van de Dienst Weg- en Waterbouwkunde van Rijkswaterstaat.