Environmental Health Perspectives
Vol. 47, pp. 227-238, 1983

Bioassay of Extracts of Ambient Particulate Matter

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Organic extracts from airborne particles collected at various sites in Scandinavia have been tested for mutagenicity in the Ames Salmonella/microsome assay. Extracts from particles in the respirable size fraction (diameter less than 3 μm) were mutagenic with and without metabolic activation. The mutagenic activity varied from day to day, mainly due to variations in meteorological parameters, especially wind speed and atmospheric stability. A seasonal variation could also be observed, with the highest average values in winter time.

Samples collected in urban areas were considerably more mutagenic than samples from background areas. The results suggest that exhaust from motor vehicles are the most important source of mutagenic particles in urban areas.

Comparison of roof top samples with street level samples indicated that atmospheric reactions cause transformation of nonpolar compounds in the primary emission to more oxygenated mutagenic compounds. It is, however, not known to which degree this causes an overall increase of the mutagenic activity.

The mutagenic activity of emissions from stationary combustion sources have also been studied, and residential heating by burning solid fuels in small combustion units have been shown to be a major contributor to mutagens in the environment.

Introduction

About 40 years ago it was demonstrated that subcutaneous injections of benzene extracts of airborne particulate matter caused tumor formation in mice (*1*). Several studies performed in the next 20 years demonstrated that the number of tumors produced in animals treated with extracts of airborne particles was considerably higher than what would be expected from the sample's content of polycyclic aromatic hydrocarbons (PAH) (*2–4*).

Hueper and co-workers (*3*) fractionated the particle extracts before administration to experimental animals. They found tumorigenic activity to be associated mainly with the neutral and the oxygenated fraction. The acidic fraction was found to contain substances with tumor-promoting effect. This finding was supported by some *in vitro* experiments performed by Gordon and co-workers (*5*). They found that not only benzene extracts, but also more polar compounds, soluble in methanol, but insoluble in benzene, could induce cell transformation in rat and mice fibroblast cultures.

About five years ago, organic extracts of airborne particulates were shown to cause mutagenic activity when assayed by the Ames Salmonella/microsome assay (*6–9*). The mutagenic activity was shown to be most pronounced in the absence of liver microsomes, whereas conventional PAH compounds need metabolic conversion to give a positive response in the Salmonella test system. Several studies have shown that only a small part of the observed mutagenic activity in extracts of particles can be caused by such PAH compounds (*9–12*).

The Ames Salmonella assay has proven to be a very useful tool for investigation of the potential carcinogenic air pollutants. Parameters that are important for the interpretation of the results from such testing, such as methods for sampling and extraction and the influence of meteorological and topographical conditions, has recently been reviewed by Chrisp and Fisher (*13*). Results obtained with the Ames Salmonella test and other short-term tests

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for mutagenicity on air samples have been reviewed by Hughes et al. (*14*).

The present paper gives a summary of results obtained in the last five years from ongoing studies on the mutagenic activity of extracts of particulate matter in ambient air in Scandinavia.

Materials and Methods

Sampling

The samples were collected with high-volume samplers on glass fiber filters (Gelman, type AE or Stora Kopparberg Special Produkter, Sweden). A description of the various sampling sites is given in Table 1. For some of the studies, meteorological parameters and the concentration of several organic and inorganic pollutants were measured simultaneously. In some cases size fractionation was performed using a Sierra high volume cascade impactor mounted in front of the filter stage. All Gelman filters were prewashed with acetone. The filters were conditioned and weighed in a few cases, but in most of the studies the filters were kept frozen until extraction was performed, a procedure that prohibited weighing of the samples.

Extraction of Samples

The filters were Soxhlet-extracted for 16 hr with acetone. This solvent was chosen among several since acetone extraction gave the highest yield of mutagenic activity (*15*). The extracts were carefully concentrated to near dryness and then redissolved in dimethyl sulfoxide (DMSO).

Chemical Fractionation

Acetone extracts of airborne particulates were fractionated according to their polarity by two different procedures. With the first method, the extract was fractionated into an acidic, a basic and several neutral fractions as described previously (*16,17*). The other procedure implies a separation into four fractions of increasing polarity on a μ-Porasil silica gel column (Waters Associates) using a Waters HPLC system, according to the method described by Schuetzle et al. (*18*). An aliquot of the sample was transferred to 100 μL dichloromethane (DCM) and injected onto the column using 5% DCM in *n*-hexane as an eluent. After 10 min isocratic elution, a linear gradient of 5% DCM/min was applied. The solvent was held at 100% DCM for 10 min, and was then switched to 100% acetonitrile for at least 15 min. The column was reconditioned by flushing with DCM for 5 min and 5% DCM in *n*-hexane for 15 min.

Analysis of PAH

PAH compounds were analyzed by splitless injection on a Carlo-Erba Fractovap 2101 AC gas chro-

Table 1. Sampling sites.

	Site	Sampling area
Sweden		
S-1	Stockholm	Roof top of a 10-story building in the northern part of the inner city
S-2	Upplands Väsby	Roof top of a 2-story house in a suburban community, 22 km NW of Stockholm; space heating in the area is mainly by electricity
S-3	Solna	Street level; S side of a W-E street; about 17000 vehicles per weekday
S-4	Solna	Roof top of a 5-story building located 300 m ESE of S-3
S-5	Solna	Street level, N side of a WSW-ENE street; about 27000 vehicles per weekday
S-6	Solna	Roof top of a 15-story building located 600 m ESE of S-5
Norway		
N-1	Oslo-St. Olavs plass	Roof top of a 10-story building in the inner city of Oslo
N-2	Oslo-Sagene brannstasjon	In a fire station tower, corresponding to the height of a 4-story building, urban area about 3 km north of N-1
N-3	Oslo-Rådhusgata	Street level (2 m above the ground) N side of a street canyon with heavy traffic, approximately 30,000 vehicles per weekday
N-4	Oslo-Rådhusgata	Roof level. Roof top of an 8-story building. The sampler was located right above the sampler at N-3, about 10 m from the edge of the roof
N-5	Birkenes	A background station in southern Norway, approximately 25 km from the coast; the sampling site is far from any local pollution sources and about 3 m above ground level
N-6	Hummelfjell	A background station in the mountains in eastern Norway about 20 km SW of Røros; the station is situated 1539 m above the sea level; the nearest road is 5 km away
N-7	Industrial site	A residential area, about 7 km from an area with heavy chemical industry; samples were taken only with wind directions from the industrial area
N-8	Background	A sampling site about 30 km NW of N-7; no other contamination sources in the vicinity; Samples were taken only when the wind came from N.

matograph with a glass capillary column and flame ionization detector after a clean up by the procedure of Grimmer and Böhnke (*19*) as modified by Bjørseth (*20*).

Mutagenicity Assay

Mutagenicity was determined by the Salmonella plate incorporation method with bacterial cultures fully grown overnight as described by Ames et al. (*21*). A mixture of 2 mL of molten top agar, 0.1 mL bacterial culture, an aliquot of the sample and, if used, 0.5 mL S9 mix was poured onto minimal medium plates. The plates were incubated at 37°C for 48 hr, after which the number of revertants, i.e., histidine-independent colonies, were counted. Each sample has been assayed at several occasions using several dose levels, and the response, expressed as revertants per cubic meter of air, was calculated from the linear or almost linear part of the dose-response curve.

Assays were performed with the tester strains TA 98 and TA 100 obtained from B. N. Ames (University of California, Berkeley, CA) and the nitroreductase-deficient strains TA 98 NR, TA 98/1,8 DNP_6 and TA 100 NR obtained from H. S. Rosenkranz (Case Western Reserve University, Cleveland, OH). The microsome-containing rat liver supernatant (S9) was prepared from Aroclor 1254-induced male Sprague-Dawley or Wistar rats and was used with the necessary cofactors (S9 mix). The amount of S9 used was generally 20 or 50 μL per plate.

Intercomparison

Extraction and mutagenicity assay have been intercalibrated between our two laboratories. The 24-hr samples of particulate matter were collected for 4 days at the roof top level in Stockholm with two simultaneously operating samplers. One filter from each day was extracted and assayed in each laboratory. Some of the final results are given in Table 2. The intercomparison shows that both laboratories detect similar mutagenic responses with the strain TA 98 but that there is a discrepancy with the strain TA 100. This difference in TA 100 is also present in tests with a reference sample of 1-nitropyrene. The reason for this difference is presently being investigated.

Results and Discussion

Extracts of ambient particulate matter are generally mutagenic in several Salmonella tester strains in the absence of mammalian activation systems. This shows that the extracts contain compounds which are directly acting mutagens and/or are converted to ultimate mutagens by bacterial metabolism. Addition of a mammalian activation system such as the microsome-containing S9 may increase, decrease or not change the mutagenic response of such extracts. The effect of S9 is dependent on the relative amounts of compounds requiring mammalian activation in order to show mutagenic effects and compounds which are inactivated by the mammalian system. The degree of activation/deactivation is also dependent on the amount of S9 added.

Seasonal Variation

The mutagenicity of extracts of samples collected at the roof top size in the inner city of Stockholm has been evaluated since 1978 covering 28 periods

Table 2. Intercomparison of test results in the Ames/Salmonella assay between two laboratories SI and SU.[a]

	Determined by SI				Determined by SU			
	TA 98		TA 100		TA 98		TA 100	
Sample	–S9	+S9[b]	–S9	+S9	–S9	+S9[b]	–S9	+S9
Revertants/m^3 air								
81 03 30	12	↓[c]	11	↓	18	↓	20	↓
81 03 31	24	↓	14	↓	22	↓	55	↓
81 04 01	20	↓	12	↓	21	↓	25	↓
81 04 02	16	↓	13	↓	20	↓	33	↓
Average	18	–	12	–	20	–	33	–
Revertants/plate								
100 ng 1-nitropyrene[d]	315	–	–	–	442	–	–	–
1 μg 1-nitropyrene	–	–	646	–	–	–	1268	–
2.5 μg benzo(a)pyrene	–	393	–	670	–	350	–	668

[a]SI = Central Institute for Industrial Research; SU = University of Stockholm.
[b]50 μL S9 per plate.
[c]Addition of S9 decreases the response.
[d]1-Nitropyrene from Koch-Light Ltd, England, sold under the name of "3-nitropyrene"; contains trace amounts of other nitro compounds.

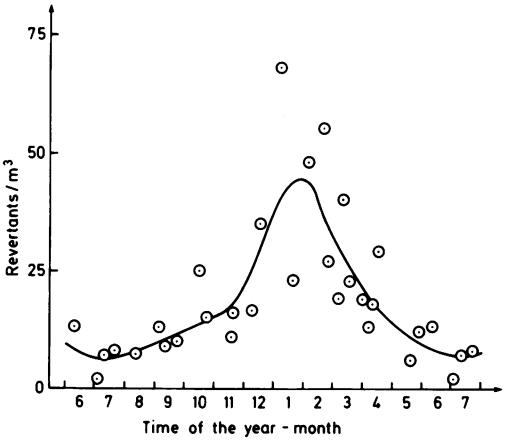

FIGURE 1. Mutagenic activity with TA 98 in the absence of S9 of extracts of particulate matter collected during weekdays above the roof tops in the inner city of Stockholm. Each point represents the average of three to four consecutive 24-hr samples. The sampling cover 28 periods between February 1979 and April 1981.

of 3 or 4 consecutive days. Results to date are presented in Figure 1, which gives the average mutagenic activity of each period in TA 98 in the absence of S9 as a function of the time of the year when the samples were collected. The average mutagenic activity of all periods is about 20 revertants/m^3. The results indicate that the mutagenic activity is highest during the winter months. Similar results were obtained in Oslo when sampling was performed over a period of 3 months from February to April, 1978. Only nonpolar compounds were extracted and tested, but the average value in February was two to three times that in April (*12*). Daisey et al. have also found a higher mutagenic activity in New York City during the winter than during the summer, attributing the increase to fuel oil combustion for heating (*22*). However, the occasional higher mutagenic activity during the winter was probably not due solely to heating as indicated by a great variation between daytime and nighttime samples (*15*).

Simultaneously sampling at the roof top level at the suburban site 2 km NNW of Stockholm gave extracts with mutagenic activities somewhat lower than those detected in the inner city (Table 3). There is a good rank correlation between the two sampling sites (S-1 and S-2), the rank correlation coefficient being 0.93 for TA 98 ($p > 0.99$), showing that air pollution as detected by mutagenicity in the Salmonella system may be of a regional importance.

Daily Variations

The mutagenic activity of airborne particulates collected at a specific site was found to vary considerably from day to day as illustrated in Figure 2. When samples were collected at several sites in the same area, but not too close to a specific source, e.g., motor vehicle exhaust, this day-to-day variation was usually greater than the variation in samples from one site to another (*12,15,16,23*).

The variation in mutagenic activities of samples collected at the same site seems to be caused by changing meteorological conditions. For winter samples the most important factors appeared to be atmospheric stability and wind speed .

Figure 2 illustrates a typical feature of samples collected in Oslo. Roof top samples exhibit nearly

Table 3. Mutagenic activity in the absence of S9 of extracts of particulate matter collected at roof top level in the inner city of Stockholm (S-1) and simultaneously in a suburban area 22 km NW of Stockholm (S-2)

	Revertants/m^3 air			
	Stockholm		Suburban	
Sampling period	TA 98	TA 100	TA 98	TA 100
Dec. 20-22, 1978	36	37	27	37
Feb. 19-23, 1979	55	24	30	14
Apr. 9-12, 1979	18	11	12	7
May 28-June 1, 1979	12	9	7	5
July 2-6, 1979	2.2	1.5	0.9	0.6
Sept. 3-7, 1979	13	13	4.4	3.0
Oct. 15-19, 1979	25	14	7	3.3
Dec. 17-21, 1979	35	13	22	2.6
Feb. 4-8, 1980	48	52	28	29
Apr. 14-18, 1980	29	52	21	32
June 9-13, 1980	13	15	6	10
July 21-25, 1980	8	7	2.9	4.1
Sept. 22-26, 1980	10	18	6	12
Dec. 8-12, 1980	16	20	6	6
Jan. 19-23, 1981	23	46	12	32

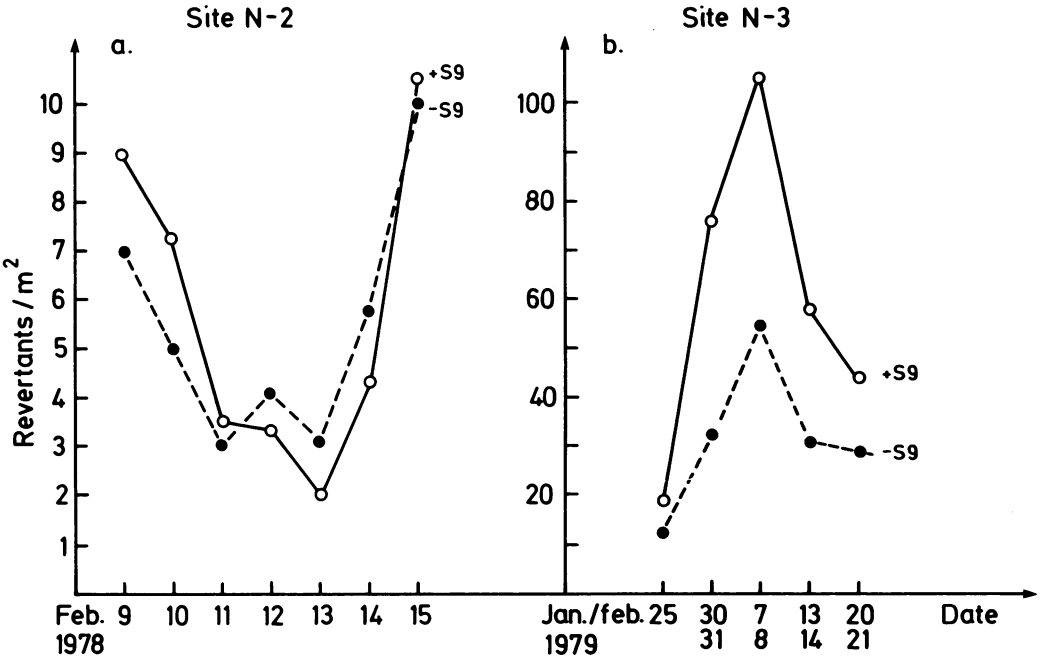

FIGURE 2. Day-to-day variations in mutagenic activity of extracts of airborne samples collected at the same site: (a) Particles collected at roof top, sampling site N-2, during February 1978, 24-hr consecutive samples, extracted with cyclohexane; (b) particles collected at street level in a street canyon with dense traffic, sampling site N-3, for two consecutive days (not nights). One sample was collected each week during January and February 1979. The filters were extracted with acetone.

the same mutagenic activity with and without S9, whereas samples collected at street level always showed enhanced mutagenic activity when S9 mix was added (*16*).

Mutagenic Activities in Urban Air Compared to Background Levels

Studies of the mutagenic activity in extracts of airborne particles have mainly been performed in samples from urban and industrial areas. One of the first American studies that was reported included samples from a nonurban site (*6*). None of these samples showed mutagenic activity.

As already mentioned, the mutagenicity in samples from a suburb of Stockholm was significantly lower than in samples from the inner city.

In Norway, some samples have been collected simultaneously in urban/industrial areas and at background sites. The results are summarized in Table 4. Samples from the real background sites showed very low or no detectable mutagenic activity. Site N-5, Birkenes, is considerably influenced by long-range transported air pollution. It has been shown that the mutagenicity at this site varies with the origin of the sampled air masses, but mutagenic activity higher than 1 revertant/m^3 has never been detected (*24*). The yield of mutagenic compounds is

Table 4. Mutagenic activity in airborne particles collected in urban/industrial areas compared to background levels.[a]

Sampling site	Sampling time	Mutagenic activity (TA 98) rev/m^3 (range)[b] +S9	Mutagenic activity (TA 98) rev/m^3 (range)[b] -S9	Solvent for extration
Oslo, N-1, N-2; urban	Feb. 81	5 (2-11)	5 (2-12)	Cyclohexane
Birkenes, N-5; background	Feb. 81	0.2 (n.d. -0.7)	0.2 (n.d. -0.6)	"
Hummelfjell N-6; background	Feb. 81	n.d. (< 0.1)	n.d. (< 0.1)	"
Industrial area N-7[c]	Winter 81	7 (2-13)	8 (2-16)	Acetone
Background N-8	Winter 81	n.d. (< 1)	n.d. (< 1)	"

[a]The values given are the mean of about five samples from each of the background sites and of 23 samples from roop top sites in Oslo.
[b]n.d. = not detected (detection limits).
[c]Very little contribution from vehicle exhaust.

lower in cyclohexane extracts than in acetone, so the two sets of samples should not be compared.

Particle Size Fractionation

It is well documented that most of the organic compounds in urban airborne particulate matter are adsorbed to small, respirable particles (*25*). This has also been shown to be the case for mutagenic compounds (*15,26*). The results obtained by Löfroth (*15*) are given in Table 5 (sampling 1 and 2). As revealed by the table, the sum of the mutagenic response of the fractionated samples was less than that of the simultaneously collected unfractionated samples. A similar low recovery was also obtained during a third sampling period with high mutagenic activity. The low recovery has subsequently been shown to be caused by extractable compounds in the glass fiber impactor substrates supplied by the impactor manufacturer. These compounds seem to interfere with the expression of the mutagenic effect in the absence of mammalian enzyme activity. In sampling 4 (Table 5) glass fiber impactor substrates were made of the same material as used for the unfractionated samples. In this case, the recovery of mutagenic activity from the fractionated sample was complete as compared to the simultaneously collected unfractionated sample.

Contribution from Motor Vehicle Exhaust to the Mutagenic Activity of Airborne Particles

One of the main purposes for studying mutagenic activity of air pollution is to identify the most important sources for mutagenic compounds. In order to get an indication of the contribution from motor vehicle exhaust to the mutagenic activity of ambient air, the mutagenicity of samples collected simultaneously at street level and at roof top level have been compared both in Stockholm and in Oslo.

It has previously been indicated that an average mutagenic activity of about 20 revertants/m^3 in the Stockholm area is 10–100 times higher than what can be accounted for by known emissions from motor vehicles (*15*), and it was suggested that this discrepancy may be due to atmospheric transformations to higher mutagenic activity during the residence time in the atmosphere.

A preliminary investigation with simultaneous street and roof top sampling was performed in the city of Södertälje in the spring of 1980 (*27*). The results showed that the activity at the roof top level above the fourth floor was about 70% of the activity at the street level in a city street with about 10,000 vehicles per day. This result led to further studies in which simultaneous sampling at street and roof top levels were performed in the city of Solna (a part of the Stockholm area, bordering the city of Stockholm) in conjunction with an air quality study arranged by the Solna Environment and Health Board and executed by Studsvik Energiteknik AB during the winter of 1981. Samples were collected for 24 hr during consecutive days at two different street/roof top sites (S-3 to S-6). After a preliminary mutagenicity screening, samples from three consecutive days were combined to one sample which was further assayed for mutagenicity and also analyzed for PAH.

The results are given in Table 6, together with

Table 5. Mutagenic activity in the absence of S9 of extracts of size-fractionated particulate matter collected in the inner city of Stockholm (S-1) compared with the mutagenic activity of samples collected simultaneously without size fractionation.

Sample	Particle size μm[a]	Mutagenicity (TA 98) revertants/m^3 Sampling period 1 (Oct. 22-26 1979)[b]	Sampling period 2 (Nov. 19-23 1979)[b]	Sampling period 3 (Jan. 8-11 1980)[b]	Sampling period 4 (Apr. 6-10 1981)[c]
Impactor stage 1	7.2	0.3	0.2	0.7	0.6
2	3.0	0.5	0.3	1.0	0.6
3	1.5	0.5	0.4	1.2	0.9
4	1.0	0.7	0.6	1.6	1.3
5	0.5	1.4	1.0	2.4	1.7
Back-up filters		6.2	5.4	20	8.6
Sum of impactor stages 1-5 and back-up filters		10	8	27	14
Reconstituted sample: impactor stages 1-5		—	—	—	5.0
Reconstituted sample: impactor stages 1-5 and and back-up filters		—	7	—	13
Filters without impactors		15	16	68	13

[a]Particle sizes are those given by the manufacturer at 50% collection efficiency for spherical particles with unit mass density.
[b]Impactor substrates from the manufacturer of the impactor plates.
[c]Impactor substrates prepared from glass fiber filter (Stora Kopparberg Specialprodukter, Sweden).

Table 6. Comparison between mutagenic activity and PAH concentration in extracts of particulate matter collected at street and roof top levels in the Stockholm area.

	Solna, 2/17-20/81		Solna, 3/2-5/81	
	Street, S-3	Rooftop, S-4	Street, S-5	Rooftop, S-6
Mutagenicity, revertants/m^3				
TA 98 –S9	21	13	16	15
TA 98 +S9[a]	14	7	11	9
TA 98 NR –S9	14	6	12	10
TA 98/1, $8DNP_6$	7	3	7	7
TA 100 –S9	23	16	25	34
TA 100 +S9[a]	21	11	17	20
TA 100 NR –S9	12	7	14	19
PAH, ng/m^3[b]	24	11	21	16
Benzo(a)pyrene, ng/m^3	0.86	0.44	0.64	0.55
Pb, μg/m^3[c]	0.52	–	0.39	–
CO, mg/m^3[c]	1.0	–	0.4	–
NO_x, μg/m^3[c]	64	–	53	–
NO, μg/m^3[c]	24	–	6	–
Wind direction[d]	~ NNE		~ NW - NE	
Wind speed, m/sec (range)[d]	3.9 (2-6)		2.2 (0-4.5)	
Temperature, °C (range)	–3 (–6 + 2)		–4 (–8 + 0)	

[a]20 μL per plate of liver S9 from Aroclor 1254-induced rats.
[b]Sum of 22 PAH components in the range phenanthrene-coronene.
[c]Calculated from data reported by Studsvik Energiteknik AB.
[d]Calculated from meteorological data obtained from an airport station located 3-4 km from the sampling sites.

other parameters measured during the sampling periods. During the first sampling period at the Solna sites S-3 and S-4, the mutagenic activity as well as the concentration of PAH were about twice as high at street level compared to roof top level. The density of the street was about 17,000 vehicles per day, and the wind direction was from the street towards the sampler. During the second sampling period at the Solna sites S-5 and S-6, the street and roof top activities and PAH concentrations were nearly the same, indicating a low contribution from the local traffic. This result seems reasonable since the street level sampling was performed at the windward side of the street.

In the Norwegian study, samples were collected at street level (sampling site N-3), and at the roof of an eight-story building (sampling site N-4) in Oslo's most trafficked street. Samples were collected in Feburary 1979 and in August 1981. In the winter study several meteorological and air quality parameters were measured simultaneously. The results have been published in detail elsewhere (*16*) and are summarized in Table 7. The results showed that the mutagenicity in daytime street level samples were 4–9 times as high as in the corresponding roof top samples as measured with S9. The activity measured without metabolic activation was one-half to one-third of the activity with S9 in street level samples, whereas roof top samples showed rather similar activities with and without S9.

A comparison of day samples with night samples showed that the mutagenicity at night was reduced to 15–35% of the daytime activity. A corresponding reduction was observed in the average traffic intensity from 18,000 vehicles/hr during the day to 425 vehicles/hr at night. The PAH concentrations showed a similar reduction from day to night, but the variation between street level and roof top level samples was much less pronounced than the corresponding mutagenic activity.

The concentration of lead on the particles was taken as an indicator of the dilution of exhaust particles from street level to roof top level. Roof level concentrations of lead were 10–20% of the street level concentrations. Roof top values for mutagenic activity in the range 5–25% of the street level activities, indicate that vehicle exhausts are the main source of mutagenic compounds at both sampling stations. However, the qualitative difference in the mutagenic response between street level and roof top level samples as well as the low day to night variation in roof top samples suggests that some of the primary emitted compounds have been transformed to other components before reaching roof top level.

To evaluate the degree of transformation, a similar study was repeated in summer when traffic was believed to be the only local source of mutagens in the area. The results are summarized in the lower part of Table 7, but will be discussed in detail elsewhere (*28*).

The results were consistent with the winter study. The mutagenic activity in 24-hr samples from roof top were 20% of the corresponding activity of street

Table 7. Comparison of mutagenic activity and PAH concentrations in extracts of particulate matter collected at street level (N-3) and rooftop level (N-4) in a street with heavy traffic in Oslo.

	Mutagenicity in TA 98, rev/m^3				PAH, ng/m^3			
	+S9		–S9		Σ PAH[a]		BaP	
Sampling period	Street	Rooftop	Street	Rooftop	Street	Rooftop	Street	Rooftop
Winter								
Jan. 26, days, 7 A.M. - 9 P.M.	180	34	45	41				
Jan. 30, 31, days, 7 A.M. - 9 P.M.	76	17	33	11	385	144	17.5	8.0
Feb. 7, 8, days, 7 A.M. - 9 P.M.	105	12	55	14				
Jan. 30-31, Jan. 31 - Feb. 1, nights	28	12	10	9	79	79	4.4	5.6
Summer								
Sept. 3, 4, days, 7 A.M. - 9 P.M.	28	4	18	3				
Sept. 2-3, 3-4, nights, 9 P.M. - 7 A.M.	15	8	8	4				
Average of 8 days and nights, Aug.-Sept.	37	7	21	6				
Average of 3 days and nights (included in the eight samples above)	49	9	37	8	65	14	2.5	0.4

[a]Sum of 22 compounds.

level samples on an average, whereas the concentration of lead on the particles from roof level was only 10% of the street level concentrations. As for winter samples, the ratio between the activity measured with and without metabolic activation was considerably higher in street level samples than in roof top samples. This supports the hypothesis that some of the mutagens detected at roof top are due to atmospheric reactions and indicate that transformation reactions mainly result in the direct mutagens.

In agreement with the assumption that traffic was the only source of mutagens in the area, we found a better correlation of the mutagenicity with the PAH concentrations at street level and roof top level in the summer study than in the winter study.

A discrepancy may seem to exist between the ratio of street to roof top level mutagenicity in Oslo and in the Stockholm area. However, this difference may be explained in terms of the location of the sampling sites. In Oslo the sampling was performed at a narrow inner city street canyon, whereas the sampling sites in the Stockholm area were located at streets with good possibilities for ventilation. The explanation is supported by the observation that the ratio of street to roof top level mutagenicity seemed to be influenced by the wind direction in the Stockholm samples, but not in the Oslo samples. Furthermore, the Oslo samples were collected over a longer period of time, and whereas the average values for mutagenicity were five times as high at street level as at roof top, some of the samples from single days with very little wind (not given in the table) showed such ratios to be down to 1.2.

The studies reported from both cities indicate that the mutagenic activity of particulate matter in urban areas with mainly oil-based space heating, such as Oslo and Stockholm, originates largely from motor vehicles. They also indicate that the mutagenicity is not only due to compounds emitted from the vehicles, but also to compounds formed in atmospheric transformations. However, further investigations involving both mutagenicity assays and chemical analysis are needed to confirm this hypothesis.

Chemical Fractionation

Evaluation of the environmental impact of mutagenic compounds present in urban air requires an identification of the most active mutagens. The chemical characteristics of the mutagens present on airborne particles were investigated by chemical fractionation of some of the samples followed by mutagenicity testing of the separate fractions and of reconstituted samples. Extracts from four consecutive filters collected at sampling site N-3 in February 1979 were pooled and fractionated in an acidic, a basic and 5 neutral fractions. The results have been reported in detail elsewhere (*16*). Only 30% of the mutagenic activity measured without metabolic activation was recovered after fractionation. The recovery increased to 48% when S9 was added. Most of the mutagenicity was found in the aromatic fraction followed by the acidic and the oxygenated fractions. Similar results have been obtained with samples from Stockholm (S-1) (*17*).

A similar experiment has been performed with summer samples from the same Norwegian sampling sites, street level (N-3) and roof top level (N-4). The distribution of mutagenic activity in the summer samples resembled the winter samples, and no qualitative difference could be observed

between street samples and roof top samples. The recovery of mutagenic activity was about the same as for the winter sample. Testing of reconstituted samples increased the recovery of mutagenicity only slightly as compared to the sum of test results for separate fractions.

The low recovery of mutagenic activity upon fractionation was probably due to the presence of relatively unstable components which were degraded when exposed to strong acids or bases. The possibility that such labile compounds are released from car exhaust and are degraded in the atmosphere may be part of the explanation for the qualitative difference observed between street level and roof top level samples.

To avoid degradation of mutagens by the chemical treatment, the summer samples were also fractionated using a more gentle procedure, i.e., by use of silica gel column HPLC as described in Methods. The results are summarized in Table 8.

The recovery of mutagenic activity was nearly complete when this fractionation procedure was used. For the street level sample, the sum of the mutagenic activity with S9 in the individual fractions was higher than the activity of the unfractionated or reconstituted sample. This indicates that the fractionation may have resulted in the separation of antagonistic compounds or, more likely, that the activity in the individual fractions increases due to a more optimal concentration of S9 present. For the roof top samples, the observed differences in the sum and the activity of the reconstituted samples were within the uncertainty of the test results.

Street level samples as well as roof top samples had most of the mutagenic activity without metabolic activation in the most polar fraction, 47 and 68% respectively. Furthermore, the ratio between the activity of street level and roof top samples varied for the separate fractions, indicating that some of the mutagens present at street level have been transformed to more polar compounds when they reach roof top levels. (Alfheim, in preparation).

Mutagenic Components

The presence of PAH in ambient particulate matter has been known for a long time. The application of short-term bioassay as the Salmonella mutagenicity test has, however, unequivocally shown that extracts of ambient particulate matter as well as combustion emissions contain organic mutagens and potential carcinogens which are not conventional PAH. For instance, the benzo(a)pyrene present in street level samples from Oslo (N-3) could account for less than 4% of the mutagenicity of the samples (*16*).

Although it is conceivable that some derivatives of aliphatic hydrocarbons such as chloro, bromo, nitro and oxo derivatives of alkanes and alkenes may elicit a mutagenic response, the main interest so far has concentrated on derivatives of polycyclic aromatics. Table 9 gives a schematic description of the behavior of mutagenic polycyclic aromatics in the Salmonella test, i.e., whether they are mutagenic in the absence or presence of mammalian activation systems.

GC/MS analysis of samples from street level in Oslo (N-3) (Alfheim et al., unpublished results) has shown that compounds of nearly all the chemical classes listed were present. The contribution of mutagenicity from the various classes are, however, not known so far.

A major interest has focused on nitroarenes, as the presence of such compounds might partly or fully explain a mutagenic response in the absence of mammalian activation. The presence of nitroarenes

Table 8. Mutagenicity of separate fractions of increasing polarity from samples collected at site N-3 and N-4, August 1981, fractionated by use of silica column HPLC.

		Revertants/m^3 air in TA 98[a]			
		Street level		Rooftop level	
Fraction no.	Type of compound	+S9	-S9	+S9	-S9
Unfractionated sample		16	14	2.4	3.1
1	Nonpolar compounds, alkanes, PAH, alkylated PAH and S-heterocyclic PAH	2.1 (13)	<0.8 (<7)	0.2 (7)	n.d.[b]
2	Nitro-substituted PAH	4.7 (28)	1.9 (17)	0.6 (21)	0.3 (12)
3	Ketones, aldehydes, quinones, phenols	3.2 (19)	3.2 (28)	0.3 (11)	0.5 (20)
4	Most polar compounds *N*-heterocyclic PAH, acid	6.3 (38)	5.3 (47)	1.7 (61)	1.7 (68)
Sum		16.8 (100)	11.2 (100)	2.8 (100)	2.5 (100)
Reconstituted sample		14.5	16.2	3.2	2.6

[a]Numbers in parentheses represent percent recovery in each fraction.
[b]n.d. = not detected.

Table 9. Salmonella mutagenicity requirement.

Class of compounds	Mammalian activation	
	Not required	Required
PAH	–	+
Heterocyclic (N,S) PAH	–	+
Aminoarenes	–	+
Nitroarenes	+	+
Nitrosoarenes	+	?
Arene epoxides	+	–
Arene phenols	+	+
Arene quinones	+	?
Arene ketones	+	?
Arene dicarboxylic anhydride	+	–

in extracts of ambient particulate matter is evident from results of mutagenicity assays with nitroreductase deficient strains such as those given in Table 6. The failure to detect analytically simple nitroarenes in concentrations sufficient to explain the mutagenic response indicates that responsible compounds have a more complex structure; it may be suggested that oxygenated nitroarenes are likely candidates. Nitroarenes that require mammalian activation in order to show mutagenicity are also known (*29*).

Little is known about the carcinogenicity of the mutagenic nitroarenes. Some of them, such as 2-nitrofluorene, have been shown to cause cancer in experimental animals, but most of these compounds have not yet been tested on animals.

Emission of Mutagens

Motor vehicle exhaust has been shown to be responsible for most of the mutagenic activity in street level samples from urban areas. However, testing of roof top samples from various places has shown that there are several other sources contributing to the mutagenicity associated with airborne particles. Emissions from several sources, for instance diesel engines, residential heating systems and industrial boilers, have been tested in several countries. The results have shown that emissions of mutagens from all these sources will vary considerably from one equipment to another, depending on the construction of the combustion unit, the fuel, the climate and the mode of operation. This implies that data cannot easily be transferred from one part of the world to another. Table 10 lists data from emission sources that have been tested in Scandanavia.

Conclusions

Ambient particulate matter contains a multitude of organic compounds among which are many mutagens and potential carcinogens which act by a genotoxic mechanism. With the exception of conventional PAH and heterocyclic PAH, most of these mutagens have not been identified, although they may be responsible for a major part of the mutagenic response detected by the Salmonella mutagenicity test.

Mutagens in airborne particulate matter originate mainly from combustion emissions. It is not yet known under which conditions atmospheric reactions occurring during the residence time in the air can increase or decrease the mutagenic potential of emitted compounds.

In the absence of complete information about the chemical composition and about the genotoxic poten-

Table 10. Emission of mutagens from various sources in Scandinavia.

Fuel	Combustion unit	Mutagenicity, rev./g fuel[a]	Ref.
Wood	Residential stoves, Sweden	300–3400	(*30*)
Wood	Residential stoves, Norway	600–35000	(*31*)
Wood	Boiler, 35 kW	1800–3800	(*30, 32*)
Wood chips	Boiler, 35 kW	9–3700	(*30, 32*)
Wood chips	Boiler, 1.8 MW	250	Rudling and Löfroth (unpublished)
Wood chips	Boiler, 30 MW	0–100	(*33*)
Peat	Boiler, 1.8 MW	100	Rudling and Löfroth (unpublished)
Peat	Boiler, 100 MW	< 4	(*33*)
Coal	Boiler, fluidized bed,[b] 5 MW	1400	(*34*)
Coal	Boilers, 25-270 MW	0–2	(*34*)
Coal	Power plant, 600 MW	0–2	(*34*)
Oil	Boilers, 10-500 MW	0–20	(*34*)
Diesel	Motor vehicle	3000	(*17*)
Gasoline	"	200	(*17*)

[a]The numbers are given as revertants/g fuel as tested with TA 98. The highest mutagenic response obtained with or without mammalian activation is reported.

[b]The cleaning system did not function properly.

tial for man, prudence should be to keep the emission and the ambient level of mutagenicity as low as possible whenever there is a choice between different energy and transportation strategies. Our results have shown that whereas mutagenicity can be found to correlate with the presence of certain compounds, like PAH, at a specific site, no such correlation can be found from sampling site to sampling site. Research projects aiming at the identification of the most important mutagens in ambient air are in progress all over the world.

However, until more information is available, we do not recommend use of one or a few compounds, like benzo(a)pyrene, as risk indicators, but suggest that mutagenicity may be the best indicator of mutagenic and potential carcinogenic potency of airborne particles.

Financial support from the Royal Norwegian Council for Scientific and Industrial Research, the National Swedish Environment Protection Board and the Nordic Council of Ministers through the joint Nordic project MIL-2 is acknowledged.

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