

Long-Term Trends of Particulate Mutagenic Activity in the Atmosphere of Sapporo. 1. Determination of Mutagenic Activity by the Conventional Tester Strains TA98 and TA100 during an 18-Year Period (1974–1992)

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The Ames *Salmonella* mutagenicity bioassay using strains TA98 and TA100 of airborne particulates from Sapporo, Japan, was studied over an 18-year period starting in 1974. Measurements of mutagenicity and benzo[a]pyrene (BaP), as well as other polycyclic aromatic hydrocarbons (PAHs), were performed on organic extracts which were prepared from seasonal particulate composites of high-volume filter samples for each year. The average contribution of BaP to total indirect-acting mutagenic activity was 1.8% for strain TA98 and 3.5% for strain TA100. During the measurement period, BaP concentrations showed a large decline (to 25–20% their initial values), while indirect-acting mutagenic activity showed a moderate decline (to 66–50% their initial values) and direct-acting mutagenic activity did not show any clear increase or decrease. This clearly indicates that there was almost no change in the amount of exposure to mutagenic substances as a whole. A qualitative change was clearly seen in mutagens, being indicated by the continuous increase in the ratio of direct-acting to indirect-acting mutagenic activity (–S9/+S9 ratio) during the sampling period. Furthermore, these results suggest that, in the long term, BaP may not always be an appropriate indicator of the effect of air pollution on human health.

Introduction

A number of investigations have found that extracts of airborne particulates collected in urban environments can be carcinogenic (e.g., refs 1–3). Several hundred different substances that are either carcinogenic or suspected of being carcinogenic exist in the air with a wide range of concentrations (4, 5). The kinds and the composition of carcinogenic substances present in the air differ greatly depending on the source of pollution (6–8). Ideally, chemical analyses of all

the carcinogenic substances should be undertaken in order to determine their composite effect on health, but this is impractical.

Benzo[a]pyrene (BaP) has been used for many years as a carcinogenic indicator of air pollution, owing to its strong carcinogenic effect, its relative abundance in the air, and its strong correlation with other polycyclic aromatic hydrocarbons (PAHs) (9, 10). Several reports, however, have shown that the BaP concentration in the air greatly decreased under the shift of fuel from coal to oil during the 1960s and 1970s (11–14).

An assessment of the carcinogenicity (or genotoxicity) of all the air pollutants as a whole, rather than chemical analysis of each compound, would be more realistic for evaluating the effects on human health. At present, among the accepted methods, the Ames test (15, 16), which examines mutagenicity in *Salmonella* strains, is one of the best short-term bioassay methods for estimating mutagenic and potentially carcinogenic effects of complex environmental mixtures.

In this study, samples of airborne particulate matter collected from the air of a city located in the northernmost island of Japan over an 18-year period were examined for mutagenic activities and concentrations of BaP as well as other PAHs. No previous study has investigated variations in atmospheric mutagenic activity for a period of this length. Considering the cumulative effect of genotoxic hazards, a retrospective study will provide the basis for estimating the human health risks caused by airborne particulate matter. Moreover, long-term variations in characteristics of mutagenicity in the atmosphere are of interest, because a shift in fuel use patterns during this period, including decreased utilization of coal plus the increased use of diesel-powered vehicle, might contribute to compositional differences of airborne particulate matter.

Experimental Procedures

Study Site. The city of Sapporo, whose population in 1992 was 1.7 million and growing, is located in the cold northern region of Japan. It is the capital of Hokkaido Prefecture (the northernmost large island of Japan) with a local government and much commercial activity, but it is not heavily industrialized. While summer temperatures in Sapporo sometimes exceed 30 °C, the temperature in winter can drop to below –10 °C. Heating is used in home and office from mid-October through mid-April. Owing to this long period of heating, attention has been directed to the combustion of fossil fuels, as well as to vehicle exhaust.

Sample Collection. Samples of airborne particulate matter were collected approximately 10 m above ground level on the roof of our laboratory, which is situated in a residential area about 2 km northwest of the center of Sapporo. The building is surrounded by agricultural fields and a baseball field, and is situated approximately 150 m from a main road on which about 4500 vehicles pass per day. The site chosen for our study was considered to have an average level of air pollution for the city. High-volume (hi-vol) samplers were used to collect airborne particulate matter with an average flow rate of about 80 m³/h. A 24-h hi-vol sample was collected on a glass- or quartz-fiber filter every day for 5–15 continuous days per month throughout the sampling period (August 1974–February 1992). The total weight of the collected particulate matter was determined by weighing the filters before and after sampling following equilibration for 24 h at 20 °C. The filters were tightly wrapped with aluminum foil and stored at –20 °C until sample preparation, which was done after the 18-year sampling period.

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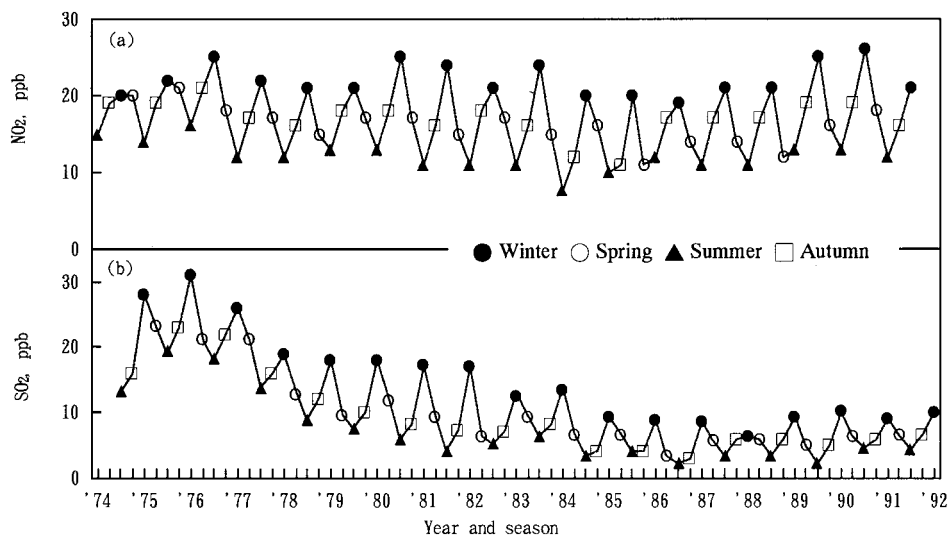


FIGURE 1. Long-term trends in seasonal concentration of (a)NO₂ and (b)SO₂.

Sample Preparation. Each filter sample, corresponding to 1500–2400 m³ of air, was divided into eight portions of identical size. Portions of samples were combined on a quarterly basis for each year. The seasonal composites included the following months: December, January, and February for winter; March, April, and May for spring; June, July, and August for summer; and September, October, and November for autumn. Thus, a total of 71 combined samples, consisting of 15–45 filters each, according to season, were obtained.

Each combined sample was extracted twice with 350 mL of dichloromethane, using ultrasonication for 20 min. Each extract was evaporated on a rotavapor and concentrated to a final volume of 20 mL. For the determination of gravimetric analysis of extracted organic matter, 0.5-mL aliquots were used in duplicate. An 8-mL aliquot was evaporated close to dryness and then the solvent was exchanged into dimethyl sulfoxide (DMSO) under a gentle stream of nitrogen. The DMSO solution was stored at –20 °C until mutagenicity testing and PAH analysis as described below.

Preparation of S9 Mix. Male Sprague–Dawley rats were administered with sodium phenobarbital and 5,6-benzoflavone. From these rats, liver supernatant containing microsomes was prepared with the necessary S9 and cofactors (S9 mix), which were purchased from Oriental Yeast Kogyo Co. (Tokyo, Japan). This S9 mix was used in the following activated mutagenicity testing (16).

Mutagenicity Testing. Mutagenicity of the samples was examined by the preincubation procedure of the Ames *Salmonella* mutagenicity assay (17). In the assay without metabolic activation (determination of direct mutagenicity; –S9 mix for short), 0.5 mL of phosphate-buffered saline (PBS, pH 7.4) and 0.1 mL of an overnight nutrient broth culture of the bacterial tester strain were added to each tube containing 0.1 mL of the test solution. In the assay with metabolic activation (determination of indirect mutagenicity; +S9 mix for short), 0.5 mL of S9 mix (1.14 mg protein) was added to the test solution instead of PBS. After preincubation at 37 °C for 20 min, the suspension was mixed with molten top agar and poured onto plates containing minimal medium. The plates were incubated at 37 °C for 48 h, and the number of revertants, i.e., histidine-independent colonies, were counted.

Each sample was twice assayed using four dose levels in duplicate for each level, and the response, expressed as revertants per cubic meter of air, was calculated from the linear part of the dose–response curve. Assays were performed with *Salmonella typhimurium* TA98 and TA100

(kindly supplied by Dr. B. N. Ames, University of California, Berkeley, CA). Positive controls were 2-(2-furyl)-3-(5-nitro-2-furyl) acrylamide for –S9 mix testing and BaP for +S9 mix testing.

PAH Analysis. Analyses of BaP and eight other PAHs were performed by high-pressure liquid chromatography (HPLC) with a fluorescence detector (18). The eight other PAHs measured were: pyrene, benzo[a]anthracene, chrysene, benzo[k]fluoranthene, benzo[e]pyrene, perylene, benzo[ghi]perylene, and coronene.

Results

The Concentrations of NO₂ and SO₂. At the particulate matter sampling site, NO₂ and SO₂, the most common air pollutants, have been continuously measured (19). The concentrations of NO₂ and SO₂ during the study period are shown in Figure 1. The NO₂ concentration showed marked seasonal fluctuations; high in winter, low in summer, and in between in autumn and spring. No long-term trend was evident, although there was a slight upward trend after the mid-1980s. The SO₂ concentration showed a clear decline from the mid-1970s to the mid-1980s, and after that, the concentration remained low with seasonal fluctuations. The average concentration remained around 10 ppb, even in winter.

The Concentration of BaP and the Eight Other PAHs in the Air. Seasonal and the 3-year average concentrations of BaP as well as 3-year average concentration of the eight other PAHs (2-year average for 1975–1976) are shown in Figure 2. The seasonal BaP concentrations were highest in winter, followed by autumn and spring, and lowest in summer for each year, except for 1980 when the concentration in autumn was greater than in winter. BaP showed a clear long-term downward trend repeating the seasonal fluctuations. The 3-year averages of the BaP concentration also showed a clear downward trend, decreasing by 83% from the average concentration of 4.87 ng/m³ for the period 1975–1976 to 0.81 ng/m³ for the period 1989–1991. The average annual BaP concentration over the whole period was 2.37 ng/m³, and the average seasonal concentrations were 4.58 in winter, 1.64 in spring, 0.54 in summer, and 1.81 in autumn. Thus, the average winter concentration was approximately eight times that of summer.

The seasonally summed concentration of the eight PAHs (excluding BaP) showed a strong correlation ($r = 0.99$) with the concentration of BaP. The 2- or 3-year average decreased by 76% during the period from 1975–1976 to 1989–1991.

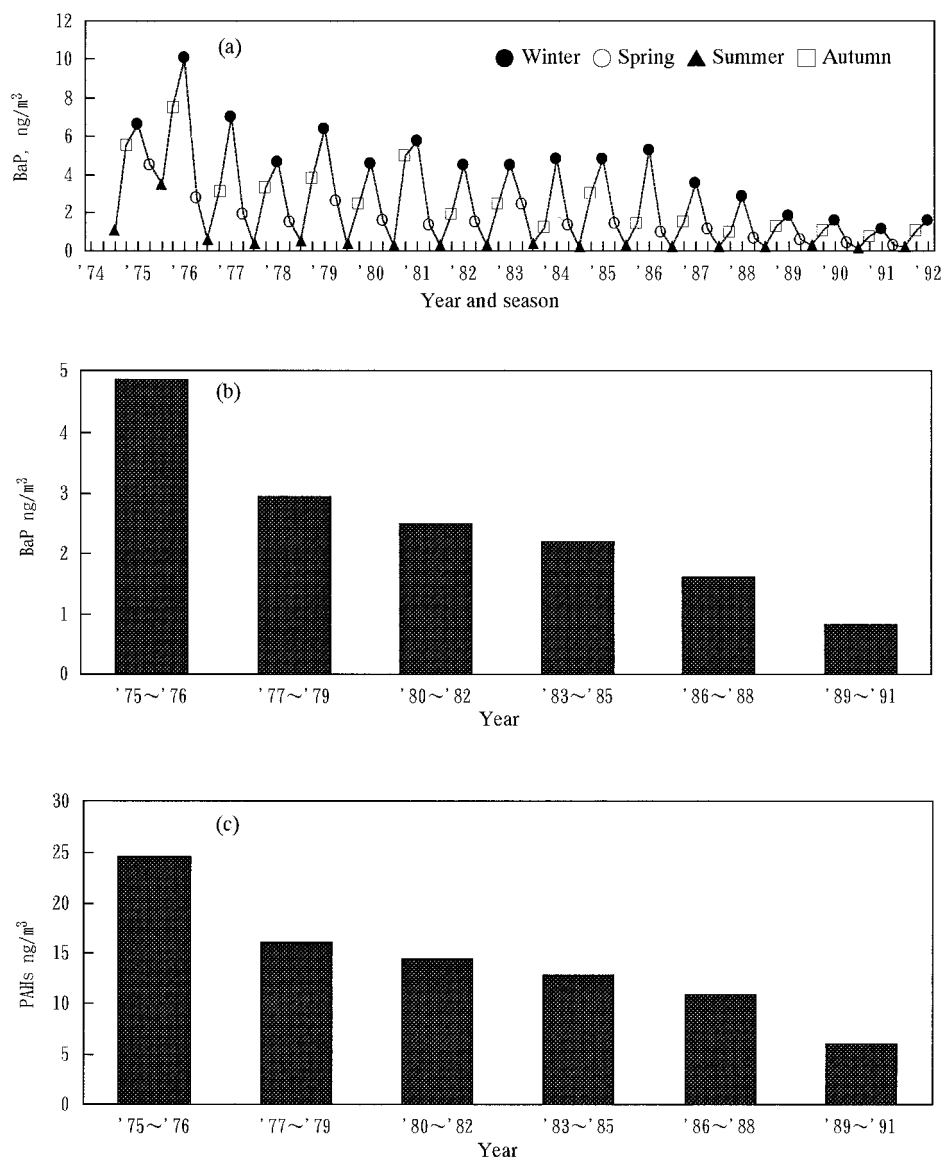


FIGURE 2. Long-term trends in (a) seasonal and (b) 3-year average (2-year average for 1975–1976) concentration of BaP, and (c) 3-year average (2-year average for 1975–1976) concentration of the eight other PAHs.

This downward trend in the concentration of PAHs was similar to that of BaP as shown in Figure 2.

Mutagenic Activity. Seasonal mutagenic activities expressed as revertants per cubic meter of air (rev/m³) are shown in Figure 3 for TA98 with or without S9 mix (\pm S9 mix) and TA100 (\pm S9 mix).

Mutagenic activities with the different test conditions show common seasonal fluctuations, which are similar to that observed above for the concentrations of NO₂, SO₂, and BaP. Long-term trends in the mutagenic activity included a reduction in the range of fluctuations in the last several years compared with slightly larger ranges during the mid-1980s.

We previously reported that the main factors causing the seasonal difference in mutagenic activity in cold regions are, in addition to fuel consumption for heating, meteorological factors such as temperature and atmospheric stability (20). Therefore, differences in yearly meteorological factors might have affected the mutagenic activity greatly in the same season of different years. To minimize the effect of the meteorological factors, we examined the mutagenic activity by 3-year moving averages, as shown in Figure 4. This figure shows that the mutagenic activity in TA98 (–S9 mix) remained

unchanged, while that in TA98 (+S9 mix) showed a moderate downward trend. Mutagenic activity in TA100 (–S9 mix) remained unchanged or decreased slightly, but that in TA100 (+S9 mix) showed a moderate downward trend. The rates of decline in the average level of mutagenic activity in the period 1989–1991 compared to 1975–1976 for the different test conditions were as follows: TA98 (–S9 mix), 0%; TA98 (+S9 mix), 57%; TA100 (–S9 mix), 16%; TA100 (+S9 mix), 43%.

Mean, minimum, and maximum values of mutagenic activities during the whole study period are shown in Table 1. In all cases, the maximum value was found in winter, and the minimum was found in summer, with the maximum value being 6–8 times greater than the minimum value.

Ratios of –S9/+S9 Mutagenic Activity. To evaluate qualitative differences in mutagenic substances in airborne particulate matter, we calculated the ratio of direct-acting to indirect-acting mutagenic activity (–S9/+S9 ratio). Three-year moving averages of the –S9/+S9 ratio in TA98 and TA100 are shown in Figure 5. For the –S9/+S9 ratio in TA98, a clear upward trend was seen and for the –S9/+S9 ratio in TA100, the long-term trend was slightly upward. The average –S9/+S9 ratio values for the periods 1974–1979, 1980–1985,

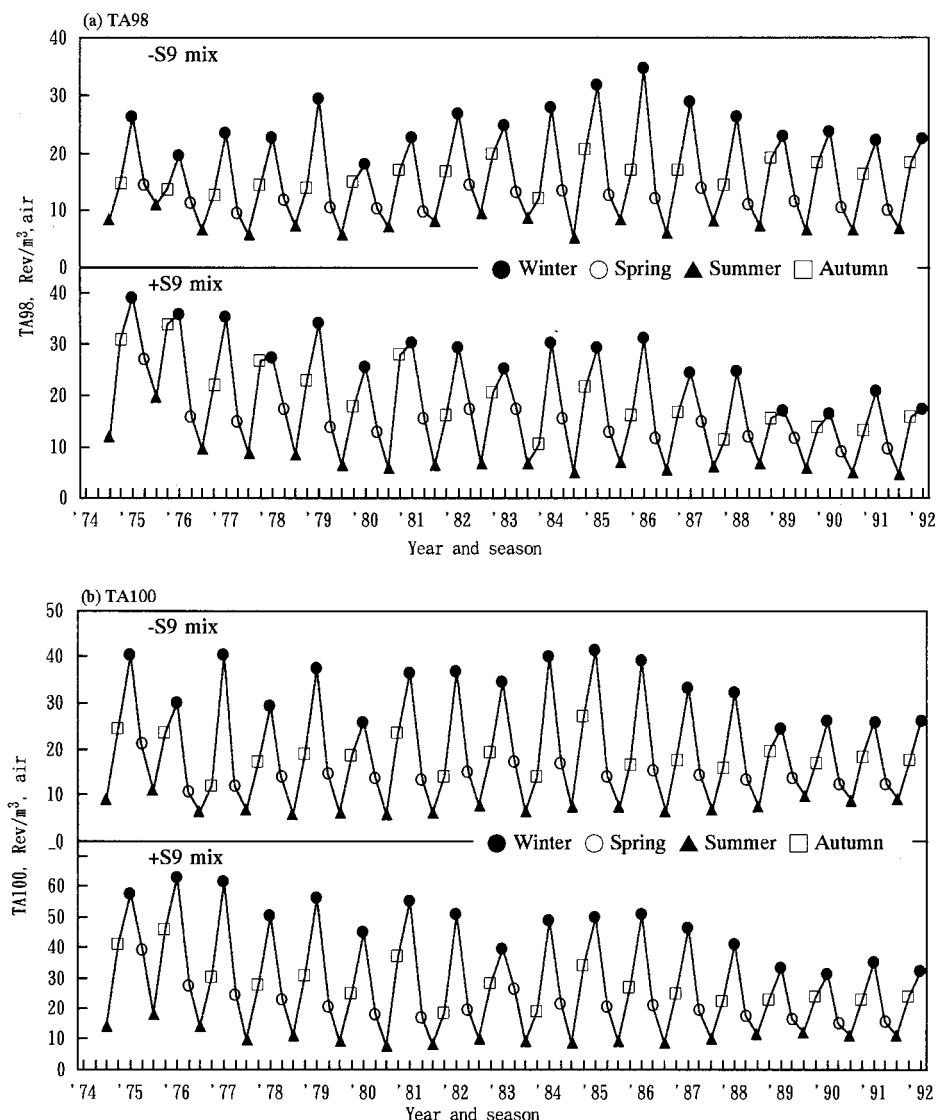


FIGURE 3. Long-term trends in seasonal mutagenic activity in (a) TA98 and (b) TA100.

and 1986–1992 were 0.74, 1.02, and 1.15 for TA98 and 0.63, 0.73, and 0.78 for TA100, respectively. Thus, the fact that the –S9/+S9 ratio showed a continuous increase throughout the study period suggests a change in the mutagenic composition of the air pollutants.

Contribution of BaP to Total Indirect Mutagenic Activity. The 3-year moving average values of the contribution of BaP to the total indirect mutagenic activity (in TA98 and TA100) are shown in Figure 6. These contributions are calculated by multiplying the mutagenic response of BaP by its concentration in the airborne sample divided with the mutagenic response of the airborne sample. A clear long-term downward trend was seen over the whole period for both TA98 and TA100 strains. This trend was especially prominent in the case of TA100.

Also, Table 2 shows the seasonal average values for the three periods (1974–1979, 1980–1985, and 1986–1992), and for the whole sampling period. The contribution showed a downward trend for both strains. The yearly average contribution values in the three periods were 2.5, 1.9, and 1.2% (TA98) and 4.9, 3.6, and 2.1% (TA100). The mean contribution for the whole period was 1.8% for TA98 and 3.5% for TA100. The maximum and minimum values of the contribution of BaP in all samples according to season were 4.6 and 0.57% for TA98 and 9.2 and 0.74% for TA100. The maximum value was 8 times that of the minimum value for

TA98 and 12 times that of the minimum value for TA100.

The fact that the average contribution for both strains was within a few percent implies that there was a large contribution of indirect-acting mutagens other than BaP. Also, the fact that the contribution of BaP to indirect-acting mutagens has been decreasing year by year suggests that the relative contributions from other indirect-acting mutagens are increasing.

Discussion

To evaluate genotoxic effects exerted by air pollutants, we should consider contact with airborne particulate matter in terms of long-term exposure. For the above reason, it is important to determine genotoxics levels over a long period. Although there have been many longitudinal studies on the mutagenicities of air pollutants (21–28), no study has been conducted over a long term, such as 18 years.

In this study, the mutagenicity of airborne particulate matter in Sapporo over an 18-year period was investigated. Since various chemical substances exist in the samples, we carried out mutagenic experiments for both strains TA98 and TA100 with and without S9 mix. In addition, concentrations of BaP as well as other PAHs were determined.

To ensure storage of samples was adequate, comparative testes were made for a 10 year period. During the storage

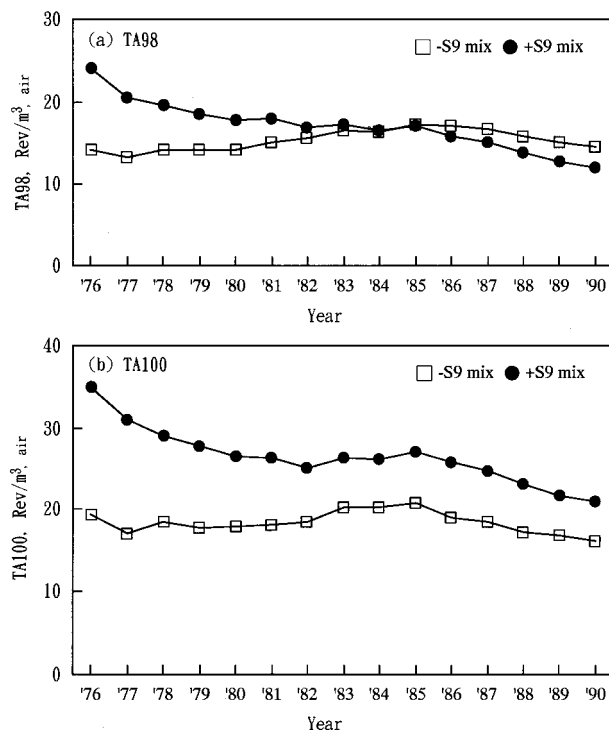


FIGURE 4. Three-year moving average mutagenic activity in (a) TA98 and (b) TA100.

TABLE 1. Seasonal Mutagenic Activity for the Entire Period (1974–1992)

Salmonella strains	rev/m ³ air				year		
	winter (av)	spring (av)	summer (av)	autumn (av)	(av)	(min)	(max)
TA98 – S9 mix	25	12	7.5	16	15	5.4	35
TA98 + S9 mix	28	15	7.7	20	18	4.9	39
TA100 – S9 mix	33	14	7.5	19	19	5.8	41
TA100 + S9 mix	47	21	11	28	27	7.7	63

of the filter samples from 1988 to 1998, no significant changes in mutagenic activity and BaP concentration could be detected. Thus, we presume that there has been little or no degradation of the stored samples (1974–present).

During the study period, several changes occurred. The number of vehicles in the Sapporo area increased (Figure 7), especially diesel-powered vehicles. The percentage of diesel-powered vehicles in Sapporo increased from less than 10% in 1975 to 32% in 1992. After the mid-1970s, stricter regulations on the sulfur content in fuel oil were imposed. Annual coal consumption in the Sapporo area, based on delivery records, showed a large downward trend after the mid-1970s (29; Figure 8). It is likely that these changes have influenced the above-mentioned long-term changes in air pollution.

The decreases in the concentrations of SO₂ most likely result from reduction of sulfur in the fuel and the decreased coal consumption. On the other hand, NO₂ concentration showed no change or even a slight upward trend. This is thought to be due to the increase in the number of vehicles, especially the increase in the number of diesel-powered vehicles. The diesel-powered vehicles have a high emission level of nitric oxides (NO_x). NO₂ is supposed to play a major role in the secondary formation of mutagens (see below).

The mutagenic activities of air particulates observed in our study are much higher than the background level at Cape Ochiishi Global Air Monitoring Station in northeast Hokkaido (30), indicating a contribution from human activities in

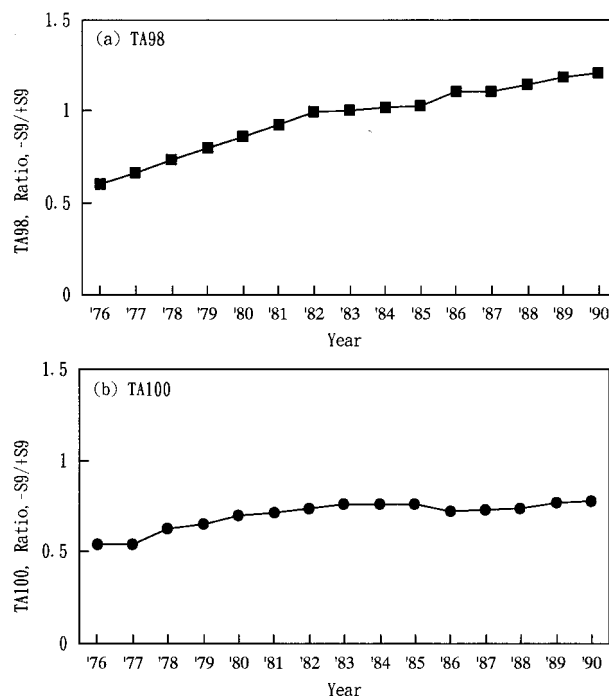


FIGURE 5. Three-year moving average ratios of direct mutagenic activity to indirect mutagenic activity (–S9/+S9 ratio) in (a) TA98 and (b) TA100.

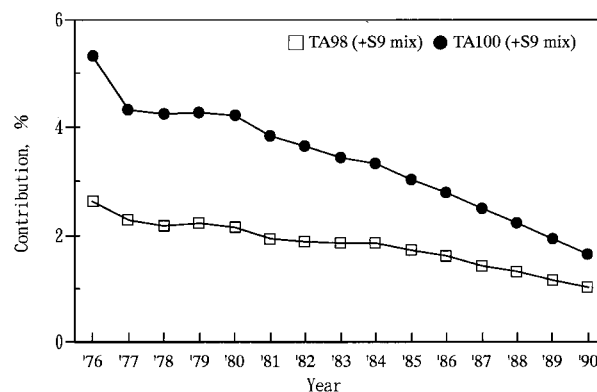


FIGURE 6. Three-year moving average contribution of BaP to the total indirect mutagenic activity.

Sapporo. The yearly mutagenic activity of airborne particulates in Kyoto, 20, 24, 32 and 33 rev/m³ (21), and in Tokyo, 19, 10, 19 and 14 rev/m³ (31) for TA98 (–S9 mix), TA98 (+S9 mix), TA100 (–S9 mix), and TA100 (+S9 mix), respectively, were reported. In a comparison of the mutagenic activity between these two large Japanese cities, the mutagenic activity in Sapporo possessed a moderate mutagenic potential. However, such comparisons need to be made carefully because of differences in bioassay methods, sources of pollutants, and meteorological and sampling conditions.

For both strains, mutagenicity in the presence of S9 mix showed a gentle downward trend (to 66–50% their initial values) and mutagenicity in the absence of S9 mix did not show any notable change. The following are supposed to be the main factors causing these long-term trends in mutagenic activity. Coal combustion produces a number of highly mutagenic compounds, such as PAHs, alkylated PAHs, nitro-PAHs, oxygenated PAHs, and nitrogen-heterocyclic compounds (32–35). Also produced are sulfur oxides (SO_x) and NO_x, which are thought to promote secondary formation of direct mutagens, such as nitro-aromatic compounds (36, 37). So the above-mentioned decrease in coal consumption is

TABLE 2. Seasonal Average Contribution of BaP to the Total Indirect Mutagenic Activity

period	contribution %									
	TA98(+S9 mix)					TA100(+S9 mix)				
	winter	spring	summer	autumn	year	winter	spring	summer	autumn	year
1974–1979	3.35	2.51	1.39	2.71	2.5	5.60	4.65	3.55	5.86	4.9
1980–1985	2.86	1.81	0.78	2.12	1.9	4.76	3.81	1.58	4.20	3.6
1986–1992	1.89	1.01	0.68	1.29	1.2	3.01	1.90	1.02	2.26	2.1
all	2.62	1.73	0.95	2.04	1.8	4.31	3.39	2.05	4.11	3.5

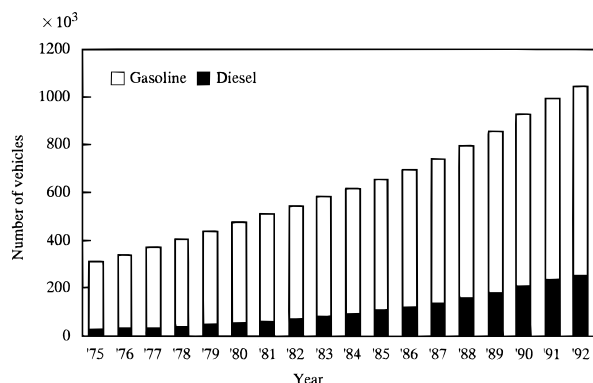


FIGURE 7. Numbers of vehicles in the Sapporo area, 1975–1992.

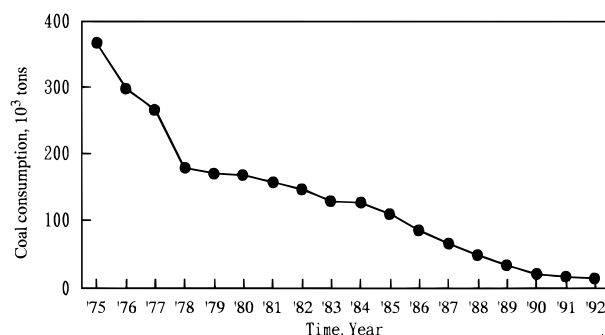


FIGURE 8. Coal consumption in the Sapporo area, 1975–1992.

likely to be largely responsible for the decrease in direct and indirect mutagenic activities in the air.

Diesel-powered vehicles emit nitro-aromatic compounds, such as 1-nitropyrene (1-NP) and dinitropyrenes (DNPs), which contribute significantly to the direct mutagenicity of diesel particle extracts (38, 39). Moreover, high concentrations of NO_x emitted from diesel-powered vehicles might accelerate the secondary formation of direct mutagens from NO_2 and PAHs (40, 41). In addition, it is reported that diesel emission particles have indirect mutagenic activity (42). Therefore, it is reasonable to assume that the increase in the number of diesel-powered vehicle has been responsible for a considerable proportion of the mutagenic activity in the air, especially the direct mutagenic activity.

Qualitative changes in mutagenic activities are more clearly shown by the ratio of mutagenic activities. The $-S9/+S9$ ratio clearly demonstrated an increase every year for both strains the TA98 and TA100 (Figure 5). This increase was especially prominent in TA98. A high concentration of PAHs, including BaP, is contained in particles emitted from coal combustion, and these PAHs are indirect mutagens which only show mutagenicity in the presence of S9 mix (32). We consider, therefore, that a decrease in the PAH concentration following the decrease in coal consumption in Sapporo, has led to a decrease in indirect mutagenic activity. It has been reported that the $-S9/+S9$ ratio (TA98) in airborne particulate matter in Beijing, where coal is used

as the main energy source for heating, cooking, and electricity, is as small as 0.17 (43). Therefore, this supports our conclusion that the decrease in coal consumption in Sapporo (Figure 8) is connected to the increase in the $-S9/+S9$ ratio.

While the $-S9/+S9$ ratio has been reported to be more than 1 in diesel emission particles (see below), smaller values have been reported for particles emitted from gasoline-powered vehicles [0.28 [TA98] (44), 0.31 [TA98] (45), 0.03 [TA98], and 0.02 [TA100] (46)]. It is thought that the indirect mutagenic activity in particle emissions from gasoline-powered vehicles is much larger than the direct mutagenic activity. Thus, it is thought that the mutagens in particle emissions from gasoline-powered vehicles in the 1970s, when vehicle exhaust control devices were incomplete, were strongly connected to the decline in the $-S9/+S9$ ratio. This assumption is supported by a report showing a $-S9/+S9$ ratio of approximately 0.3 in airborne particulate matter in Mexico, where exhaust control regulations are not strictly enforced (43).

As mentioned above, the increase in the number of diesel-powered vehicles is thought to have led to an increase in the concentration of nitro-aromatic compounds through both primary emission and secondary formations, and many of these compounds are direct mutagens which show mutagenic activity in the absence of S9 mix. There have been many studies on the $-S9/+S9$ ratio in diesel emission particles, all of which report a value of over 1 (44, 46, 47). Many of the direct mutagens in the extracts from airborne particulate matter are thought to be formed by atmospheric reactions with PAH in the presence of NO_x (48–50) which are emitted directly from diesel-powered vehicles. Thus, it is thought that the increase in the number of diesel-powered vehicles (Figure 7) is also connected to the increase in the $-S9/+S9$ ratio.

Therefore, it can be concluded that the long-term upward trend in the $-S9/+S9$ ratio is a result of a combination of factors, such as the decrease in coal consumption, emission controls on gasoline-powered vehicles, and the increase in the number of diesel-powered vehicles.

BaP has been widely used over the past 40 years as an indicator of lung cancer risk, because of its strong carcinogenicity and close correlation with the concentrations of other PAHs. We previously reported that the average annual BaP concentration in Sapporo in the period 1981 and 1983 had decreased by almost 90% compared with a level of 20–30 ng/m^3 in the late 1960s (51). In this study, we found a great decrease (by 80%) in 18 years. The decrease in the use of coal for heating homes and offices is considered to be the main cause of this long-term downward trend.

Downward trends in the BaP concentrations have also been observed in other cities in Japan (13, 14) as well as in other countries (11, 12, 22, 23).

To know the long-term trends in the potential mutagenicity/carcinogenicity of air pollutants based on the BaP concentration, it is necessary to calculate the contribution of BaP to mutagenic activity year by year over a long period. However, prior to this study, there have been no reports on the contribution of BaP over the long term.

The results of this study showed that the contribution of BaP to total indirect mutagenic activity decreased by 33–50% after 1974 to the end of the sampling period and that the maximum contribution rate of BaP was 10 times higher than the minimum value. These results suggest that mutagenicity cannot be estimated from the BaP concentration in samples collected over a long period, and that BaP is not a good indicator of mutagenicity/carcinogenicity in organic substances extracted from airborne particles. The fact that direct mutagenic activity did not decrease despite a decrease in the BaP concentration also supports the above results suggesting BaP is not a suitable indicator of mutagenicity/carcinogenicity. As the spectra and concentration range of air pollutants may also be changing with time, we should consider that BaP and mutagenicity/carcinogenicity are different parameters of air pollutants.

To summarize, although air pollution caused by substances such as SO₂ and BaP has decreased, the fact that the level of mutagenicity has remained relatively unchanged shows that genotoxic problems caused by air pollution have not really been improved. It is suggested that more attention should be paid to nitro-aromatic compounds which are emitted directly from diesel-powered vehicles and which are produced secondarily from NO_x in the atmosphere.

Acknowledgments

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Literature Cited

- (1) Wynder, E. L.; Hoffmann, D. *J. Air Pollut. Control Assoc.* **1965**, *15*, 155–159.
- (2) Asahina, S.; Andrea, J.; Carmel, A.; Arnold, E.; Bishop, Y.; Joshi, S.; Coffin, D.; Epstein, S. S. *Cancer Res.* **1972**, *32*, 2263–2268.
- (3) Epstein, S. S.; Fiyi, K.; Asahina, S. *Environ. Res.* **1979**, *19*, 163–176.
- (4) Sawicki, E. *Arch. Environ. Health* **1967**, *14*, 46–53.
- (5) Löfroth, G. *Short-Time Bioassay in the Analysis of Complex Environmental Mixtures II*; Plenum: New York, 1981; pp 319–336.
- (6) Heuper, W. C.; Kotin, P.; Tobor, E. C.; Payne, W. W.; Falk, H.; Sawicki, E. *Arch. Pathol.* **1962**, *74*, 89–116.
- (7) Holmberg, B.; Ahlberg, U. *Environ. Health Perspect.* **1983**, *47*, 1–30.
- (8) Daisey, J. M.; Morandi, M.; Wolff, G. T.; Lioy, P. J. *Atmos. Environ.* **1984**, *18*, 1411–1419.
- (9) Lioy, P. J.; Daisey, J. M. *J. Air Pollut. Control Assoc.* **1993**, *33*, 649–657.
- (10) Fukino, H.; Mimura, K.; Inoue, Y.; Yamane, Y. *Atmos. Environ.* **1984**, *18*, 983–988.
- (11) Faoro, R. B. *J. Air Pollut. Control Assoc.* **1975**, *25*, 638–640.
- (12) Commins, B. T.; Hampton, L. *Atmos. Environ.* **1976**, *10*, 561–562.
- (13) Kodama, Y. *J. Japan Soc. Air Pollut.* **1991**, *26*, 105–119.
- (14) Kawaraya, T. *Seikatsu Eisei* **1991**, *35*, 103–132.
- (15) Ames, B. N.; McCann, J.; Yamasaki, H. *Mutat. Res.* **1975**, *31*, 347–364.
- (16) Maron, D. M.; Ames, B. N. *Mutat. Res.* **1983**, *113*, 173–215.
- (17) Yahagi, T.; Nagao, M.; Seino, Y.; Matsushima, T.; Sugimura, T. *Mutat. Res.* **1977**, *48*, 121–130.

- (18) Matsumoto, Y.; Akiyama, M.; Sakai, S.; Kato, T. *Proc. 12th Symp. Environ. Monit.* **1997**, 7–10.
- (19) Japanese Standards Association, JIS B7952/7953, 1977.
- (20) Matsumoto, Y.; Nakajima, T.; Sakai, S.; Noguchi, I.; Akiyama, M. *J. Chem. Soc. Jpn.* **1991**, *6*, 837–844.
- (21) Ohe, T. *Nihon Kosyueisei Zasshi* **1982**, *28*, 261–272.
- (22) Seemayer, N. H.; Hadnagy, W.; Tomingas, R.; Manojlovic, N. J. *Aerosol Sci.* **1987**, *18*, 721–724.
- (23) Flessel, P.; Wang, Y. Y.; Chang, K. I.; Wesolowski, J. J.; Guirguis, G. N.; Kim, I. S. *J. Air Waste Manage. Assoc.* **1991**, *41*, 276–281.
- (24) Scarpato, R.; Di Marino, F.; Strano, A.; Curti, A.; Campagna, R.; Loprieno, N.; Barrai, I.; Barale, R. *Mutat. Res.* **1993**, *319*, 293–301.
- (25) Lee, H.; Su, S. Y.; Liu, K. S.; Chou, M. C. *Environ. Mol. Mutagen.* **1994**, *23*, 200–207.
- (26) Watts, R.; Lewtas, J.; Stevens, R.; Hartlage, T.; Pinto, J. *Intern. J. Environ. Anal. Chem.* **1994**, *56*, 271–287.
- (27) Sato, M. I. Z.; Valent, G. U.; Coimbra, C. A.; Coelho, M. C. L. S.; Sanchez, P. S.; Alonso, C. D.; Martins, M. T. *Mutat. Res.* **1995**, *335*, 317–330.
- (28) Rossi, C.; Poli, P.; Buschini, A.; Cassoni, F.; Cattani, S.; DeMunari, E. *Chemosphere* **1995**, *30*, 1829–1845.
- (29) Hokkaido Tsusan Kyoku. *Hokkaido ni okeru danbo yo sekitan torihiki daka no suii* [Hokkaido Bureau of International Trade and Industry; *Trend of coal consumption for heating in Hokkaido; in Japanese*]; 1993.
- (30) Alfhelm, I.; Möller, M. *Sci. Total Environ.* **1979**, *13*, 275–278.
- (31) Goto, S.; Kato, Y.; Orii, A.; Tanaka, K.; Hisamatsu, Y.; Matsushita, H. *J. Japan Soc. Air Pollut.* **1982**, *17*, 295–303.
- (32) Daisey, J. M.; Ming-xing, W.; Li-xin, R.; Wei-xiu, L. *Aerosol Sci. Technol.* **1983**, *2*, 407–415.
- (33) Li, A. P.; Clark, C. R.; Hanson, R. L.; Henderson, T. R.; Hobbos, C. H. *Environ. Mutagen.* **1983**, *5*, 263–272.
- (34) Moriske, H. J.; Rüdén, H. *Chemosphere* **1988**, *17*, 1167–1181.
- (35) Chuang, J. C. *Environ. Sci. Technol.* **1992**, *26*, 999–1004.
- (36) Fukino, H.; Mimura, S.; Inoue, K.; Yamane, Y. *Mutat. Res.* **1982**, *102*, 237–247.
- (37) Pitts, J. N., Jr. *Environ. Health Perspect.* **1983**, *47*, 115–140.
- (38) Rosenkranz, H. S. *Mutat. Res.* **1982**, *101*, 1–10.
- (39) Salmeen, I.; Durisin, A. M.; Prater, T. J.; Riley, T.; Schuetzle, D. *Mutat. Res.* **1982**, *104*, 17–23.
- (40) Zielinska, B.; Arey, J.; Atkinson, R.; Winer, A. M. *Atmos. Environ.* **1989**, *23*, 223–229.
- (41) Arey, J.; Harger, W. P.; Helmig, D.; Atkinson, R. *Mutat. Res.* **1992**, *281*, 67–76.
- (42) Nakajima, T.; Sakai, S.; Kawai, A.; Kobayashi, S. *Proceedings of the 33rd annual meeting of Japan Society of Air Pollution*, 1992; p 346.
- (43) Butler, J. P.; Kneip, T. J.; Mukai, F.; Daisey, J. M. *Environ. Sci. Res.* **1985**, *32*, 233–246.
- (44) Löfroth, G. *Environ. Int.* **1981**, *5*, 255–261.
- (45) Jones, E.; Riehold, M.; May, J. H.; Saje, A. *Mutat. Res.* **1985**, *155*, 35–40.
- (46) Crebelli, R.; Fuselli, S.; Conti, G.; Conti, L.; Carere, A. *Mutat. Res.* **1991**, *261*, 237–248.
- (47) Claxton, L. D. *Environ. Int.* **1981**, *5*, 389–391.
- (48) Pitts, J. N., Jr.; Cauwenberghe, K. A.; Grosjean, D.; Schmid, J. P.; Fitz, D. R. *Science* **1978**, *202*, 515–519.
- (49) Lindskog, A.; Brorström-Lundén, E.; Sjödin, A. *Environ. Int.* **1985**, *11*, 125–130.
- (50) Gundel, L. A.; Daisey, J. M.; de Carvalho, L. R. F.; Kado, N. Y.; Schuetzle, D. *Environ. Sci. Technol.* **1993**, *27*, 2112–2119.
- (51) Matsumoto, Y. *Proceedings of the 25th annual meeting of Japan Society of Air Pollution*, 1984; pp 173–174.

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