The Nature of the Mutagenicity and Carcinogenicity of Nitrated, Aromatic Compounds in the Environment

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Gaseous substances such as nitrogen dioxide (NO₂) and sulfur dioxide (SO₂) stimulate the process of nitration of polycyclic aromatic hydrocarbons, and the transformation products display a broad spectrum of mutagenicity, genotoxicity, and carcinogenicity.

Bacterial mutation by nitroarenes is specific. Tetracyclic nitroarenes are thought to be the most mutagenic compounds in the Salmonella test system, and some are carcinogenic in rats and mice. Furthermore, it was found that the mutational nitroarenes produced mostly DNA damage, which is subject to recombination repair in the rec assay system using Bacillus subtilis.

Nitroarenes in the environment seem to be ubiquitous; the majority of the compounds are emitted directly from diesel emissions, kerosene heaters, and gas and liquefied-gas burners or heaters. In nitroarenes induced during incomplete combustion, nitropyrene and nitrofluoranthene derivatives are the most important mutagens/carcinogens for determining the chronic toxicity of nitroarenes overall.

Introduction

Among nitrogen-substituted chemicals, monocyclic nitratated aromatic hydrocarbons (e.g., nitrotoluene) and heterocyclic nitrated chemicals (e.g., nitrofuran and furylfuramide) have been investigated by many workers. Nitroarenes and polycyclic aromatic hydrocarbons (PAHs) substituted with nitro moieties are widespread in the environment. Nitroarenes are thought to be normally genotoxic and mutagenic in bacteria and mammalian cells, and some of them are thought to be carcinogenic in animals.

NO₂, a cause of pollution outdoors and indoors, has been reported to be mutagenic in Salmonella typhi-murium and to induce chromosome aberration in rat lung cells (1,2), but a system of exposure of cells to NO₂ is not sufficiently established. NO₂ is much more reactive in vitro than nitric oxide (NO), and the nitro-substituted compounds are readily formed by reaction of the PAH with NO₂ under simulated atmospheric conditions (3,4). Transformation of some PAHs to nitro-PAHs has been shown to be a facile process (3), and thus, their reactants in the complex mixture are presumed to be more important than NO₂ for evaluating the chronic toxicity of these compounds.

Nitroarenes are detectable in various materials in the environment, e.g., automobile exhaust particulates, some species of carbon black and photocopies, ambient air particulates, kerosene heater particulates, and gas and liquefied-petroleum emission particulates. The presence of nitroarenes in the environment appears to result mainly from man's activities. The majority of nitroarenes display normal, direct-acting mutagenicity in the Salmonella microsome test system (5), and mutagenicity in mammalian cell cultures (6,7), chromosome aberrations in rat epithelial cells (8), and mitotic gene conversion in Saccharomyces cerevisiae (9) have been reported.

In this paper, the earlier findings on genotoxicity, mutagenicity, and carcinogenicity of nitroarenes are reviewed, and their distribution in the environment is speculated.

Mutagenicity of Nitroarenes in the Salmonella Microsome Test System

In regard to mutagenicity of nitroarenes, the reverse mutation test system that uses a set of mutants of S. typhi-murium is effective for determining the mutation frequency or potency of the compounds, but the response often differs according to whether the cells were in the logarithmic or stationary growth phase (10). Regarding the mutagenicity of a series of nitroarenes, the following properties were demonstrated in the present...
study: (1) Bicyclic compounds induce mainly mutations of the base substitution variety (TA1535, TA100), while nitroarenes possessing three or more fused rings induce primarily mutation of the frameshift type (TA1588, TA98). (2) Specific activity of a compound appears to be related to its optimal molecular size. The activity increases from the bicyclic to the tetracyclic ring system; thus, tetracyclic nitroarenes, e.g., nitrofluoranthenes (NFrs) and nitropyrenes (NPs), are the most mutagenic, as shown in Figure 1. Moreover, some polar compounds such as dinitropyrenes (DNPs) and dinitrofluoranthenes (DNFrs) show higher mutagenicity than the nonpolar ones (Figs. 1 and 2). (3) An increase in the extent of nitration is paralleled by an increase in mutagenicity (DNPs, DNFrs, and polynitrofluorenes). Tetranoitroarenes, however, exhibit greatly reduced activity. (4) Nitroarenes containing three or more rings induce primarily mutation of the frameshift type; i.e.,

they revert strains TA1538 and TA98, and the mutagenic specificity appears to be the result of intercalations between DNA base pairs (e.g., potential nitroarenes revert strain TA97 strongly). It is thought by some authors, however, that the mutagenic specificity of nitroarenes is different from that associated with the activity of agents that cause frameshift mutation, because the spectrum of activity in the Salmonella tester strains suggests that this is not due to simple intercalation but rather to adduct formation (11). On the other hand, it is recognized that the chemicals are dependent on the reduction of their nitro function to the corresponding hydroxylamine. In fact, bacterial mutants that have lost the enzyme that converts the nitro function (strain TA98NR) have been found to be unsusceptible to mutagens. Accordingly, many nitroarenes are detectable by the reduction in mutagenicity for expressing greatly decreased activity. In addition, some of the DNPs have been reported to induce mitotic gene conversion at the trp 5 and his 4 loci in Saccharomyces cerevisiae strain D1 (9), point mutation in cultured mouse lymphoma cells (12), and a mutagenic change in Chinese hamster V79 cells to obtain resistance (13). NP derivatives have also been tested for mutagenicity by using Chinese hamster lung fibroblasts (diphtheria toxin resistance) in the absence of microsome enzymes. In this system, NP and 1,3,6,8-tetranitropropane did not show mutagenicity, but other isomers were found to be mutagenic (14).

Nitroreductase activity against 1-NP in bacteria has been studied by Ohnishi et al. (15), Kinouchi et al. (16), and El-Bayoumy and Hecht (17) in detail. The nitroreductases extracted from some anaerobic bacteria, e.g., Bacillus fragilis, B. vulgatus, B. thetaiotaomicron, Bifidobacterium adolescentis, and Eubacterium lentum, which are major bacterial components of human feces, easily convert 1-NP to 1-aminopyrene. The ex-
tracts have been found to have high levels of nitroreductase for 1-NP, whereas extracts prepared from some aerobic bacteria have not shown certain activity. Generally, aromatic nitro compounds are reduced to nitro, N-hydroxyamino, and finally amino derivatives; the N-hydroxyamino intermediates are metabolically activated and approximate mutagen-carcinogens. Some of them are further acetylated for activation. In the in vitro metabolism of 1-NP, ring oxidation, in addition to nitroreduction, has been observed after treatment of 1-NP with rat liver S9 fraction (17).

The rec Assay of Nitroarenes in Bacillus subtilis

The kinds of damage induced in DNA by various chemical mutagens cover a wide spectrum and are subject to cellular repair of different types. In these detecting systems, the recombination repair test developed by Kada et al. (18) has been applied to chemicals of various types such as antibiotics, heavy metals, dyes, and food additives. As shown in Table 1, the nitroarenes that are mutagenic in the Salmonella microsome test system yielded positive results in the present rec assay system as well. These chemicals were inhibitory to the growth of both strains H17 (Rec-), and M45 (Rec-) or only M45, showing definitely larger inhibition zones with the latter strain depending on the dose of the chemical. It was suggested that NPs, NFrs, and 6-nitrochrysene at a low dose level effectively produce DNA damage which is subject to recombination repair (Table 1).

Genotoxic Effects in Cultured Mammalian Cells

Nitrated PAHs from biphenyl, fluorene, pyrene, chrysene, perylene, fluoranthene, and benzo[a]pyrene have been reported to induce a moderate increase in sister-chromatid exchanges (SCEs) in Chinese hamster ovary (CHO) cells and to induce DNA repair synthesis in cultured HeLa cells (6) and transformation of cultured normal human diploid fibroblasts (19). In the case of the SCE experiment, the addition of S9 preparation resulted in a large increase in SCEs induced by 1-NP, 1,8-DNP, 2-nitrofluorene, and 4-nitrobi phenyl (7). 1,6- and 1,8-DNPs have been shown to induce chromosome aberrations in an epithelial cell line (RL4) and have been shown to be potent as clastogenic agents in these cells (8).

Carcinogenicity of Nitropyrenes in Animals

Regarding carcinogenicity of nitroarenes, many long-term studies have been reported (20). 2-Nitrofluorene induced squamous-cell carcinoma in the forestomach when 1.62 nmole of the compound per kilogram of diet was fed to rats for 12 months (21). p-Nitrobenzene induced bladder tumors when mongrel dogs were fed 0.3 g of the compound three times a week (22). 5-Nitroacenaphthene has been used as an intermediate of a fluorescent whitening agent and a photochemical agent. Therefore, the carcinogenic action of this compound has attracted attention from the viewpoint of prevention of occupational cancer. It has been observed that this compound induces adenocarcinoma in the small intestine of rats when given to the animals orally (23).

Recently, 1-NP and 3-NFr injected SC were found to induce malignant fibrous histiocytomas in F344 rats (24). The finding for 1-NP was later changed to possibly negative, because the sample used in this test was found to be contaminated with 0.2 to 0.3% each of 1,3-, 1,6- and 1,8-DNP (25) (Table 2).

In addition, similar experiments on 1-NP have shown it to be nontumorigenic (26,27). In contrast, Hirose et al. (28) reported that highly purified 1-NP induced mammary tumors which developed at a site distant from the subcutis in rats, although malignant fibrous histiocytomas also were induced at the injection site. In addition, 1-NP has been reported to induce lung tumors in mice (29). Thus, the carcinogenicity of 1-NP is still questionable because of the impossibility of predicting the effect on humans based only on the information obtained so far.

DNPs have already been revealed to be potent mutagens/carcinogens. In Japan, Ohgaki and co-workers (24,25,30), Takayama et al. (31), and our research group (27) are carrying out long-term tests of the compounds in rats and mice (Tables 2 and 3).

In the subcutaneous injection experiments in rats and mice, 1,6- and 1,8-DNPs induced tumors that were determined histologically to be sarcomas or malignant fibrous histiocytomas at the local site (27,30), although in an experiment with BALB/c mice, 1,3-DNP and 2,7-dinitrofluorene did not induce subcutaneous tumors at the injection site (Table 3). The inducibility of tumors

| Nitroarene | µg/disk | Inhibition zone, mm |
|------------|---------|---------------------|
|            |         | H17 (Rec-), M45 (Rec-) |
| 1-Nitroanisole | 100  | 0, 3.3 |
| 2-Nitroanisole | 100  | 3.4, 10.4 |
| 1,3-Dinitroanisole | 100  | 1.6, 7.6 |
| 2,7-Dinitro-9-fluorene | 50  | 3.7, 2.1 |
| 2,4,7-Trinitro-9-fluorene | 100  | 9.3, 8.5 |
| 2-Nitrofluorene | 50  | 0, 2.6 |
| 2-Nitropyrene | 0.5  | 0, 9.7 |
| 3-Nitroanisole | 0.2  | 2.8, 25.0 |
| 8-Nitroanisole | 1.0  | 0, 7.2 |
| 3,7-Dinitrofluorene | 0.01 | 0, 6.5 |
| 3,9-Dinitrofluorene | 0.01 | 0, 5.3 |
| 1-Nitropyrene | 1.0  | 0, 4.5 |
| 1,6-Dinitrofluorene | 0.01 | 1.0, 3.0 |
| 1,8-Dinitrofluorene | 0.04 | 1.3, 4.5 |
| 1,3,6-Trinitrofluorene | 0.02 | 0.8, 2.6 |
| 1,3,6,8-Tetranitrofluorene | 2.0  | 0, 3.8 |

Table 1. Results of Bacillus subtilis rec-assay for nitroarenes.
Table 2. Carcinogenesis of nitropyrenes given to animals by various routes.*

| Nitropyrene | Animal      | Route | Dose           | Location of tumor          | Reference |
|------------|-------------|-------|----------------|---------------------------|-----------|
| 1-NP       | CD rat      | SC    | 100 μmole/kg of rat | Mammary gland (MFH)       | (28)      |
| 1-NP       | A/J mouse   | IP    | 0.71 mmole/kg   | Lung tumor                | (29)      |
| 1-NP       | Crl/CD-1 mouse | Painting | 1.0 mg/mouse  | No tumor                  | (26)      |
| 1-NP       | F344/DuCrj rat | SC    | 2 mg/mouse     | No tumor                  | (25)      |
| 1-NP       | BALB/c mouse | SC    | 2 mg/mouse     | No tumor                  | (27)      |
| 1,3-DNP    | F344/DuCrj rat | SC    | 4 mg/rat       | Subeutaneous (sarcoma)    | (21)      |
| 1,6-DNP    | 344/DuCrj rat | SC    | 4, 0.4, 0.04 mg/rat | Subeutaneous (sarcoma)    | (21)      |
| 1,6-DNP    | BALB/c mouse | SC    | 2 mg/mouse     | Subeutaneous (MFH)        | (27)      |
| 1,6-DNP    | Syrian golden hamster | SC | 13 mg | Lung tumor (adenocarcinoma) | (31) |
| 1,6-DNP    | F344/DuCrj rat | Lung | 0.15 mg | Squamous cell carcinoma (75%) | (32) |

*MFH, malignant fibrous histiocytoma; SC, subcutaneously, IP, intraperitoneally; II, intratracheal instillation.

Table 3. Tumorigenicity of nitroarenes by SC injection in BALB/c mice.

| Compound         | Incidence of tumors at the injection sites, %* | Dose, mg |
|------------------|-----------------------------------------------|---------|
| 1-NP             |                                               | 2       |
| 1,3-DNP          |                                               | 1       |
| 1,6-DNP          |                                               | 2       |
| 1,8-DNP          |                                               | 1       |
| 2,7-Dinitrofluorene |                                             | 2       |
| Benzo[a]pyrene   |                                               | 1       |
| 4-Nitroquinoline-N-oxide |                                         | 2       |

*Expressed as ratio of tumors at injection site to tumors at other sites.

in BALB/c mice might be due to the low level of production of the enzymes related to nitroarene metabolism. Tumor induction in rat lung by DNP's has been studied by Takayama et al. (31) and Maeda et al. (32), and results were positive in both experiments.

Sources of Nitroarenes

Inducibility of Nitroarenes in the Environment

It has been reported that benzo[a]pyrene and other PAHs undergo photochemical transformation when adsorbed onto a variety of support materials such as filters and soot particles (6). When benzo[a]pyrene and perylene were exposed to pollutant gases under simulated atmospheric conditions, directly active mutagens were formed, and the activity was attributed to nitro-substituted derivatives, which enhanced the mutagenicity in the Ames test system. In order to test this hypothesis, Pitts et al. (3) attempted to determine whether or not directly active mutagens were induced when benzo[a]pyrene and perylene were exposed to the pollutant gases O₃, NO₂, and peroxyacetyl nitrate (PAN) under simulated atmospheric conditions. The induced derivatives were benzo[a]pyrene-quinones and a few isomers of hydroxybenzo[a]pyrene, in addition to nitrobenzo[a]pyrenes. The main component of the substances directly active in the Ames test system, however, was identified as a mixture of 1-nitro-, 3-nitro-, and 6-nitro-benzo[a]pyrene, and when perylene was exposed to NO₂, 3-nitroperylene was induced.

In contrast, we exposed six kinds of PAHs to NO₂, SO₂, or HNO₃ (3,33) and found that mono nitro derivatives, all potential mutagens, were induced when PAHs of pyrene, phenanthrene, chrysene, fluorene, carbazole, and fluoranthene were exposed to NO₂ in an exposure apparatus. In addition, various products of PAHs have been found to be formed readily when PAHs were exposed to NO₂ under photochemical conditions (i.e., exposure experiments using an REFLamp, PRE-500 WB, as well as sunlight). Similarly, nitration with nitric acid has been observed to induce DNA repair synthesis in cultured HeLa cells in the absence of rat hepatic microsomes (6).

Nitroarenes in Diesel Exhaust Particulates

Mutagens in diesel emissions are almost all emitted as small particles that are capable of being inhaled into the alveoli. The results of our scanning electron microscopic analysis revealed that the particles collected from diesel exhaust are spheroidal, with a diameter of 0.01 to 0.3 μm, and about 70% of all of the particles are within the range of 0.01 to 0.05 μm and are the most highly mutagenic (34). In a further study, we found that the vapor-phase organics (or smaller size particles) in diesel emission were highly mutagenic for Salmonella strain TA98. For sampling the vapor-phase organics, a bottle with a capacity of 1 L was used and large particles were removed by collecting them on a membrane filter (0.22 μm). The organics capable of extraction with a solvent showed potent direct-acting mutagenicity for strain TA98, as shown in Table 4. These results suggest that the direct-acting mutagens were definitely emitted from the diesel engine and that they were not artificial prod-

Table 4. Mutagenicity of the vapor-phase organics and particulate-phase extracts in diesel emission.

|                        | Vapor-phase organics | Particulate-phase extracts |
|------------------------|----------------------|---------------------------|
| Benzene-methanol       | 5.85 mg              | 0.66 mg                   |
| Revertants per L (TA98)| 624                  | 466                       |
ucts; the sampling times for a bottle were as short as a few seconds, and the vapor-phase organics were immediately extracted with benzene-methanol. The extracts showed extraordinary mutagenicity from 2000 to 10,000 revertants/L for strain TA98, although this activity varied with the diesel engine used.

Accompanying the progress made in application of the Ames test, a number of NO₂-substituted PAHs have been detected in active fractions of diesel-emission particulates by using thin-layer chromatography (35), gas chromatography and mass spectrometry (36-40), and high-performance liquid chromatography (40-44). Substituted PAHs are thought to be induced during incomplete combustion of fuel in an engine; some of these compounds can be formed during sampling as described previously.

Most researchers state that the crude extract of diesel particulates mutates many more Salmonella tester strains in the absence of metabolic activation. In these extracts, toxic agents inducing the mutation directly have been reported to be responsible for the production of 3,4-dicarboxylic acid anhydride, one of a class of dicarboxylic acid anhydrides of PAH (42), 2-nitrofluorene (46), and 5-H-phenanthro[4,5-bcd]-pyran-5-one, 6-nitro-benzofalpyrene, 9-nitroanthracene, and 1-NP (47). It was considered, however, that a large percentage of the mutagenicity in the crude particulate extract is due to the nitroaromatic constituents, because more than 70 kinds of nitroaromatic compounds were identified. A number of researchers have estimated the mutagenic activity of diesel-emission extracts, and there is a consensus that the mutagenic activity is in the range of 8 to 15 revertants per μg of dichloromethane extract (35,39,44,46). The results of chemical analysis, as well as the microbial mutagenicity test, suggest that 1-NP and its isomers are the major mutagenic species, accounting for over 40% of the mutagenic activity present in diesel emission (11,33,43).

In our earlier studies, NP derivatives and 3-NF were found to contribute more than 48% of the total mutagenic activity of the crude extracts when they were analyzed by means of HPLC. DNOPs belong to a group of potential mutagens and have been shown to be principal mutagenic species in diesel-emission particulates (39,41). The demonstration of the presence of mutagens in the crude extract of diesel-emission particulates is illustrated in Figure 3a. The particulate matter collected was extracted with toluene-dichloromethane-methanol, and the neutral fraction of the crude extracts was subjected to chromatography after passing through a Sephadex LH20 column. Each eluted fraction was bioassayed by the Ames test system, and the active fractions were analyzed by means of HPLC for determining mutagens. As shown in Figure 3a, most of the activity was eluted in the effluents with retention times from 7 to 10 min, corresponding to the retention times of DNOPs. By using chemical procedures, the concentration of NP derivatives in diesel-emission particulates has also been studied by many investigators. The 1-NP content of diesel particulates was from 55.0 to 2030 ppm (43,44) and 55 ± 11 to 2030 ± 220 ppm (42) of the crude extract. Similarly, the amount of 1,3-, 1,6- and 1,8-DNP has been reported to be 0.5 to 5.2 ppm (39,44), and in our experiment, the quantities of 1,5- and 1,8-DNP were 1.2 and 3.4 ppm each in the crude extracts of diesel particulates (41).

The introduction of the diesel engine was accompanied by an increase in particulate emission. Based on U.S. Environmental Protection Agency estimates (48), the annual U.S. rate of emission particulates from light-duty diesel cars will account for 155,000 metric tons per year when the sale of diesel cars increases to the predicted 25% in the period 1985 to 1990 (48). In Japan, the number of diesel passenger cars has increased drastically since 1970.

Nitroaranes in Airborne Particulates

Various mutagens/carcinogens are found to be contained in airborne particulate matter that comes from stack and automobile emission. Many direct-acting mutagens have been detected in urban air particles (49-52), and all of them are nitro-substituted compounds and azaaranes. Also, there is a possibility of inducing nitroaranes in the atmosphere by nitration of PAHs with NO₂, NO₂, or PAN and by reaction of PAH with free radicals present in polluted atmospheres (5). Air pollution levels in Santiago, Chile, were found to be extremely high; the average concentration of particulate, NO₂, and SO₂ substances is 575 μg per m³, 0.018, and 0.01 ppm, respectively. Furthermore, the concentration of particulate matter was about three times that of an urban district in Japan, and the majority of the particulates were suspected to have come from diesel-engine exhaust.

To determine the characteristics of the direct-acting mutagens, part of a dichloromethane extract of each of five samples was spotted on a plate of silica gel and developed with toluene-hexane as solvent (51). The mutagenicity for strain TA98 in each fraction was found to be concentrated within the range of RF values from 0.3 to 0.5, corresponding to a similar RF value for DNOPs on a silica gel plate. The mutagenically active portion was found to be composed of 1-NP (0.06–0.15 μg per gram of particulate matter) and 1,6- and 1,8-DNP (about 0.2 μg per gram). Similarly, Gibson (52) reported that ambient particles collected in the Detroit area during the summer contained 0.2 to 0.6 ppm of 1-NP. The direct-acting mutagens are attributed mainly to automobile emission; in fact, based on the profile of mutagenicity that was investigated as a function of the time of day, the mutagen densities displayed pronounced maxima during the morning (6:00 a.m. to 9:00 a.m.) periods of high traffic density, with local maxima during the evening rush hour (50).

Nitroaranes in Indoor Pollutants

PAHs are known to be combustion by-products formed by burning of various organic substances (52),
and such chemicals as heterocyclic amines have been found to be produced by pyrolysis of amino acids, proteins, and proteinaceous food (54). Incomplete combustion of the fuel in kerosene heaters, gas burners, and liquefied petroleum burners often produce some pollutants, such as CO, CO₂, SO₂, formaldehyde, hydrocarbons, NO₂, and a variety of particles (55). Of these pollutants collected by the procedure of XAD-2 resin adsorption (56), the principal mutagens contained in particulates have been readily identified (57). Most of the mutagenicity was due to the activity of the DNP's (Fig. 3b–d), and their contribution to mutagenicity was about 20 to 40% in the crude extract. In the case of kerosene heater emission, however, benzo[a]pyrene, benzo[b]fluoranthene, and benzo[k]fluoranthene were present in only the small amounts of 0.31, 0.74, and 0.46 μg per g of the crude extract, respectively (Table 5).

In our earlier studies, the vapor-phase organics in city gas combustion were found to be mutagenic for Salmonella strain TA98. For sampling of the vapor-phase organics (including gaseous contaminants), a vacuum bottle with a capacity of 1 L was used. All of the particulates were removed by collecting them on a membrane filter (0.22 μm). The vapor-phase organics were collected above a gas range during the approximate complete combustion of the city gas and were extracted immediately with 10 mL of benzene-methanol by shaking for 10 min. The extracted material induced 341 revertants per μg of the extract, corresponding to 155 times the revertants induced by the particulate extracts described previously (2.2 revertants per μg). A large portion of the mutagenic activity was found to be due to 3,7- and 3,9-DNFr and 1,6- and 1,8-DNP, but no PAHs showing indirect-acting mutagenicity were detected (unpublished observations).

Table 5. Concentration of the identified mutagens in diesel, kerosene heater, city gas, and liquefied petroleum gas (LPG) emission particulates.

| Mutagen* | Diesel engineb | Kerosene heaterb | City gasc | LPGc |
|----------|----------------|------------------|-----------|------|
| 1-NP     | 70.5 (40)      | 0.082            | 0.10      |
| 1,3-DNP  | 0.53 ± 0.59 (57)| 0.028            | 0.23      |
| 1,6-DNP  | 3.25 ± 0.63 (57)| 0.086            | 2.14      |
| 1,8-DNP  | 3.4            | 0.030            | 1.64      |
| 8-NF     | 0.74           | 0.171            | 0.31      |
| B[a]P    | 0.06           | 16.11 (trace)    | 0.31      |
| B[a]A    | 26.3           | 59.98            | 0.24      |
| B[k]F    | 0.46           | 13.50            | 0.46      |
| B[b]F    | 0.74           | 27.16            | 0.74      |

*NP, nitropyrene; DNP, dinitropyrene; NF, nitrofluoranthene; B[a]P, benzo[a]pyrene; B[k]F, benzo[k]fluoranthene; B[b]F, benzo[b]fluoranthene.

b Nanogram per milligram of crude extract.

c Nanogram per milligram of particulates.
Consumption of Volatile, Kerosene, and Light Oil in Japan

The consumption of volatile, kerosene, and light oil in Japan is illustrated in Figure 4. In 1955, about 517,371 kL of kerosene were used; this amount gradually increased, and 23,124,219 kL were consumed in 1980. Similarly, 818,558 kL of light oil were used in 1955, and the consumption increased to 23,241,825 kL in 1980, 28 times the amount used in 1955. Lung cancer mortality in Japan has increased in parallel with the increase in consumption of these fuels. In particular, it is noticed that lung cancer mortality in women has been increasing since 1972. These exogenous factors present in the environment are important for determining whether or not the consumption of the fossil fuels is closely related to carcinogenesis or genotoxicity in humans.

Conclusion

The presence of NO₂, SO₂, O₃, and PAN in the atmosphere enhances the nitration of PAH, and the stable nitrosubstitutes formed are considered to be toxic agents that we cannot afford to overlook. Nitroarenes are the by-products of an incomplete combustion process; their presence in environmental material appears to result from man’s activities. With the exception of some carbon black and fly ash materials, the compounds have been detected mainly in automobile exhaust particulates. However, indoor pollutants that are emitted from kerosene heaters, gas burners, and liquefied-petroleum burners contain numerous nitroarenes possessing potential mutagenicity, as reported previously (57).

Among various nitroarenes, it should be noted that the DNP’s, which are powerful mutagens, are present in environmental material even though the amounts are small.

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