

# Nitration of Pyrene in Metallic Oxides as Soil Components in the Presence of Indoor Air, Nitrogen Dioxide Gas, Nitrite Ion, or Nitrate Ion Under Xenon Irradiation

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The formation of nitrated pyrenes was investigated on nine metallic oxides as soil components with pyrene and various nitrogen sources under xenon lamp irradiation. The metallic oxides studied were CaO, Al<sub>2</sub>O<sub>3</sub>, MgO, Fe<sub>2</sub>O<sub>3</sub>, SiO<sub>2</sub> (quartz, silicic anhydride, and silica gel forms), and TiO<sub>2</sub> (rutile and anatase forms). Nitrated pyrene as a reaction product was extracted with benzene : ethanol (4 : 1, v/v) and analyzed by GC/MS selected ion monitoring (SIM). In the presence of indoor air, 1-nitropyrene (1-NP) was produced in all nine metallic oxides, and the yield of 1-NP was high on silicon dioxide (SiO<sub>2</sub>; silicic anhydride and silica gel forms) and titanium dioxide (TiO<sub>2</sub>; rutile and anatase forms). 2-Nitropyrene (2-NP) and 4-nitropyrene (4-NP) were produced in aluminum oxide (Al<sub>2</sub>O<sub>3</sub>), magnesium oxide (MgO), and TiO<sub>2</sub> (rutile form). In the presence of nitrogen dioxide gas (NO<sub>2</sub> gas), nitrite ion (NO<sub>2</sub><sup>-</sup>) or nitrate ion (NO<sub>3</sub><sup>-</sup>), 1-NP was produced in SiO<sub>2</sub> (silicic anhydride and silica gel forms), TiO<sub>2</sub> (rutile and anatase forms), Al<sub>2</sub>O<sub>3</sub>, and MgO. 2- and 4-NP were produced in most samples of Al<sub>2</sub>O<sub>3</sub> and MgO. Accordingly, NO<sub>2</sub> gas, NO<sub>2</sub><sup>-</sup>, and NO<sub>3</sub><sup>-</sup> were shown to be nitrogen sources for the formation of nitrated pyrenes in these metallic oxides. In addition, in the presence of these nitrogen sources, 2- and 4-NP were produced on metallic oxides as soil components. The yields and formation patterns of the three types of mononitrated pyrenes differed in the various metallic oxides, as well as with the type of nitrogen source (NO<sub>2</sub> gas, NO<sub>2</sub><sup>-</sup> or NO<sub>3</sub><sup>-</sup>). Hence, the nature of the photochemical reaction in the formation of the nitrated pyrenes differed depending on the type of metallic oxide and source of nitrogen. The yields of nitrated pyrenes depended on the amount of NO<sub>2</sub> in Al<sub>2</sub>O<sub>3</sub> and MgO and on the amount of NO<sub>3</sub><sup>-</sup> in Al<sub>2</sub>O<sub>3</sub> and SiO<sub>2</sub> (silicic anhydride form). In these experiments, dinitropyrenes were not detected in all cases.

**Key words** — 1-nitropyrene, 2-nitropyrene, 4-nitropyrene, nitration, xenon lamp irradiation, metallic oxide

## INTRODUCTION

Pyrene is a substance that does not show any mutagenicity in the Ames test. However, nitrated pyrenes have strong mutagenicity and carcinogenicity,<sup>1,2)</sup> and there are some reports tracing them in urban air.<sup>3–5)</sup> Recently, a sensitive method for the determination of nitrated pyrenes was developed, and the behavior of nitrated pyrene isomers in ambient air was reported.<sup>6)</sup>

Among the nitrated pyrenes (NP), 1- and 4-NP are emitted from various combustion sources, including diesel engine exhaust. Moreover, 1-, 2-, and 4-

NP are believed to be formed in the atmosphere by a vapor-phase reaction.<sup>7,8)</sup>

There have been some reports that 2-NP is produced in the atmosphere.<sup>8–10)</sup> However, details of the nitration of pyrene in the environment are not clear.

The formation of nitrated pyrenes in the environment increases the mutagenic and carcinogenic activity of the environment. Therefore, the study of nitration of pyrene in the environment seems to be very important in considering the environmental risk of chemicals. Pyrene exists mainly in coal tar, and is often detected in soil as well.<sup>11,12)</sup> Soil exists in air primarily as suspended particulate matter (SPM).<sup>13,14)</sup> It was previously reported that chlorinated pyrenes were produced from pyrene in soil and in the metallic oxides of soil,<sup>15–17)</sup> and the crude extract obtained from metallic oxides of soil showed strong mutagenicity.<sup>18)</sup> However, it has not yet been reported that

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pyrene is nitrated on the surface soil. Therefore, in this paper, to clarify the nitration of pyrene on various soil components, we have investigated the nitration of pyrene on some metallic oxides of soil components under xenon lamp irradiation.

## MATERIALS AND METHODS

**Materials** — The pyrene used was a standard reagent (99%) produced by GL Science, Ltd. 1-Nitropyrene (1-NP, 99%), 1,3-dinitropyrene (1,3-DNP, 99%), 1,6-dinitropyrene (1,6-DNP, 98%), and 1,8-dinitropyrene (1,8-DNP, 98%) were purchased from Aldrich Chemical Co. 3-Nitrofluoranthene (3-NF, 98%) was purchased from RK Chemical Co. 2- and 4-NP were synthesized by the methods of Hirayama,<sup>19)</sup> Bolton,<sup>20)</sup> and Ravin.<sup>21)</sup> These purities were confirmed by melting point measurement, elemental analysis and gas chromatography. The chemicals, whose purities were above 99%, were used for standard samples of GC/MS analysis. Sodium nitrite (special grade) and sodium nitrate (99.9%) were purchased from Wako Pure Chemical Industries Co., Ltd., and were recrystallized from ultrapure water. Nitrogen dioxide standard gas (1020 ppm) was purchased from Nihon Sanso Co., Ltd. High-purity argon gas (> 99.999%) was purchased from Taiyo Toyo Sanso Co., Ltd. Calcium oxide (CaO; < 9  $\mu\text{m}$ , 99.9%), aluminum oxide ( $\text{Al}_2\text{O}_3$ ;  $\alpha$ -alumina, 5  $\mu\text{m}$ ), magnesium oxide ( $\text{MgO}$ ; 0.2  $\mu\text{m}$ , 99.9%), ferric oxide ( $\text{Fe}_2\text{O}_3$ ; < 11  $\mu\text{m}$ , 99.9%), silicon dioxide [ $\text{SiO}_2$ ; silicic anhydride (12  $\mu\text{m}$ , 99.9%) and silica gel (Wakogel LC-5H, 5  $\mu\text{m}$ )], and titanium dioxide [ $\text{TiO}_2$ ; rutile (< 5  $\mu\text{m}$ , 99.9%) and anatase (< 5  $\mu\text{m}$ , 99.9%)] were purchased from Wako Pure Chemical Industries Co., Ltd.  $\text{SiO}_2$  (quartz, special grade, 35  $\mu\text{m}$ ) was purchased from Kanto Chemical Co., Ltd. Ultrapure water was obtained from a NANO pure column (Barnstead Co., Ltd.). The other reagents used were of analytical grade.

**Equipment** — The xenon lamp irradiation apparatus consisted of a WEL-45AX xenon long-life weather meter, with a rated electric power of 4.5 kW (Suga Test Instruments Co., Ltd). The spectrum of the xenon lamp began at 290 nm and was similar to the spectrum of sunlight. An MP 103T Mini Pump (Shibata Kagaku Co., Ltd.), an SGPU-22 air purifier (STEC, Inc.), and an SGGU-6000L standard gas generator (STEC, Inc.) were employed. The NO<sub>x</sub> measuring instrument was a Model 2201 NO<sub>x</sub> analyzer (Dylec, Inc.). Chromatography was performed

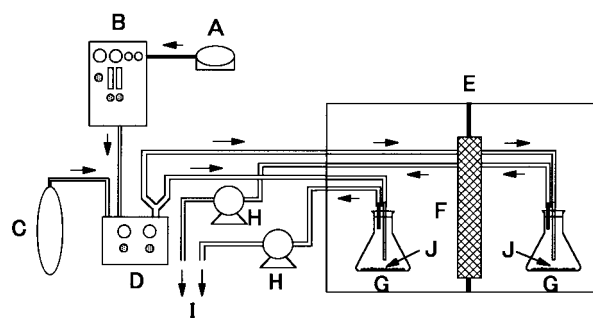


Fig. 1. Schematic Diagram of Xenon Irradiation Apparatus

(A) air compressor; (B) air purifier; (C) nitrogen dioxide gas cylinder; (D) standard gas generator; (E) xenon lamp irradiation apparatus; (F) xenon lamp; (G) quartz Erlenmeyer flask; (H) air pump; (I) exhaust; (J) sample.

with a Dionex Series 4500i ion chromatograph (Nihon Daionekusu Co., Ltd.), and GC/MS was carried out on an SX-102 instrument (JEOL Co., Ltd.). Other equipment was as described previously.<sup>22)</sup>

### Sample Preparation and Xenon Lamp Irradiation

**Indoor Air Exposure:** Ultrapure water was added to each of the nine metallic oxides, and the mixtures were scrupulously dried with a rotary evaporator and then kept in a desiccator in the dark.

A methanol solution of pyrene was added to each of the metallic oxides, and the methanol was removed in *vacuo* with a rotary evaporator. For each metallic oxide, 2 g of metallic oxide plus pyrene (500  $\mu\text{g}$ ) was placed in a laboratory dish having a diameter of 100 mm, and the sample was evenly spread out such that it made a circle having a diameter of about 80 mm.

Then, the dish was fixed in a sample frame located 250 mm from the xenon lamp and irradiated for 2 hr.

**NO<sub>2</sub> Gas Exposure:** For each metallic oxide, 2 g of metallic oxide treated with ultrapure water, as described in the above section, plus pyrene (500  $\mu\text{g}$ ) were placed in a quartz Erlenmeyer flask. The opening of the flask was hermetically sealed with a silicone plug. Two holes were drilled through the silicone plug, and glass tubes were inserted into each of the holes. The upper parts of the glass tubes were connected to silicone rubber tubes, and the tips of the silicone rubber tubes were connected to Teflon tubes. Nitrogen dioxide gas (0.2 ppm) diluted with pure air was passed through the samples at 1 l/min under xenon lamp irradiation for 2 hr. A schematic diagram of the xenon irradiation apparatus is shown in Fig. 1.

**Table 1.** Each Recovery and the Mean Recoveries of Mononitro- and Dinitro-Pyrenes

	Recovery (%) <sup>a)</sup>							
	1-NP	2-NP	4-NP	NPs (mean)	1,3-DNP	1,6-DNP	1,8-DNP	DNPs (mean)
CaO	92 ± 4	86 ± 2	82 ± 2	86.6	80 ± 1	81 ± 3	79 ± 1	80.0
Al <sub>2</sub> O <sub>3</sub>	81 ± 2	83 ± 4	83 ± 2	82.3	105 ± 1	102 ± 3	100 ± 6	100.6
MgO	77 ± 2	75 ± 3	75 ± 2	75.7	82 ± 1	79 ± 1	80 ± 3	80.3
Fe <sub>2</sub> O <sub>3</sub>	77 ± 2	73 ± 3	74 ± 3	74.7	73 ± 1	74 ± 2	75 ± 2	74.0
SiO <sub>2</sub> <sup>b)</sup>	86 ± 1	81 ± 2	81 ± 1	82.7	86 ± 2	87 ± 2	90 ± 2	87.7
SiO <sub>2</sub> <sup>c)</sup>	93 ± 6	91 ± 5	93 ± 5	92.3	86 ± 2	92 ± 4	98 ± 5	92.0
SiO <sub>2</sub> <sup>d)</sup>	84 ± 2	80 ± 3	82 ± 2	82.0	78 ± 3	79 ± 3	77 ± 2	78.0
TiO <sub>2</sub> <sup>e)</sup>	93 ± 3	82 ± 1	84 ± 3	86.3	78 ± 2	80 ± 3	74 ± 4	77.3
TiO <sub>2</sub> <sup>f)</sup>	86 ± 2	82 ± 2	84 ± 3	84.0	81 ± 1	82 ± 2	87 ± 3	83.3

a) mean ± S.D. (n = 3). b) quartz form. c) silicic anhydride form. d) silica gel form. e) rutile form. f) anatase form.

**Addition of NO<sub>2</sub> or NO<sub>3</sub> Ion:** Each metallic oxide was washed twice with ultrapure water, once with acetone, and then scrupulously dried with a rotary evaporator. Sodium nitrite, serving as the source of an NO<sub>2</sub> ion, or sodium nitrate, serving as the source of an NO<sub>3</sub> ion, was added to the metallic oxide and the mixture was scrupulously dried in an eggplant type flask. For each metallic oxide, 2 g of metallic oxide plus pyrene (500 µg) was placed in a 300 ml quartz Erlenmeyer flask, and the flask was hermetically sealed with the silicone rubber plug described above. After the air in the flask was exchanged with argon gas five times, the silicone rubber tubes connected to the glass tubes were closed. The sample was uniformly spread, fixed as described for the indoor air exposure experiment, and irradiated with a xenon lamp.

For the extraction recovery test, NPs (0.6 µg) and DNPs (1 µg) were added to the metallic oxides instead of pyrene.

**GC/MS Conditions:** After irradiation, the reaction products were extracted from the metallic oxides with 100 ml of a benzene:ethanol mixture (4 : 1, v/v), followed by centrifugal separation. The supernatant was concentrated to 2 ml. Nitrated pyrenes were determined by GC/MS (SIM) after the addition of 3-NF (200 ng) as an internal standard. The sample solution was injected into an MDN-5S fused-silica capillary column (10 m × 0.25 mm i.d.; film thickness, 0.25 µm; Supercio).

A 1 m nonpolar empty column was connected to the ion source side of the analytical column (MDN-5S). The tip of the empty column was inserted at about 1 mm into the ion source. The column oven temperature was initially kept at 60°C (1 min), then raised to 200°C at a rate of 30°C/min, and finally increased to 260°C at a rate of 10°C. The

insert tube was of the dual-taper type. Ions were monitored at *m/z* 217 and 247 for mononitropyrenes and at *m/z* 216 and 292 for dinitropyrenes.

## RESULTS AND DISCUSSION

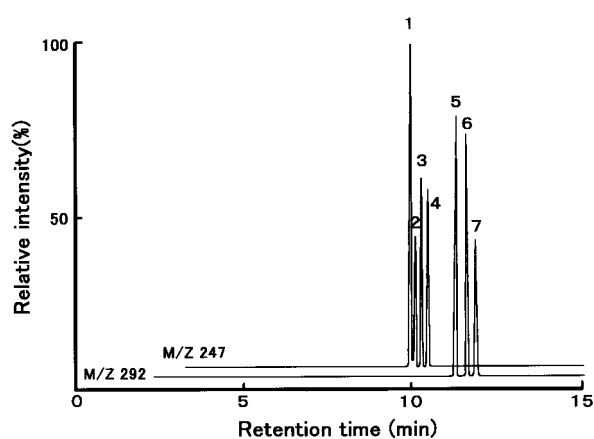
### Extraction Recoveries of Nitrated Pyrenes from Metallic Oxides

Each recovery of NP, DNP, and the average of these NP and DNP recoveries, are shown in Table 1. The recovery of NP and DNP was satisfactory and ranged from 73–93% and 73–105%, respectively. In the average recovery of NP, MgO and Fe<sub>2</sub>O<sub>3</sub> appeared in proportions of 75.7% and 74.7%, respectively. In the average recovery of DNPs, Fe<sub>2</sub>O<sub>3</sub>, SiO<sub>2</sub> (silica gel form) and TiO<sub>2</sub> (rutile form) appeared in portions of 74.0%, 78.0% and 77.3%, respectively. These metallic oxides may cause decomposition and/or absorption of the NPs and DNPs. When a long analytical column (30 m) was used, as described previously,<sup>22)</sup> the sensitivity was poor and the peaks were unstable. Therefore, the 30 m column was shortened to 10 m. Also, good sensitivity was obtained when the empty column was inserted at about 1 mm into the ion source. The reproducibility of the analysis value was verified with 3-NF as an internal standard.

As shown in Fig. 2, the 3-NF, NPs, and DNPs are well separated in SIM chromatograms of standard substances. The limits of the determination for the NPs and DNPs were 3 ng/g and 20 ng/g, respectively.

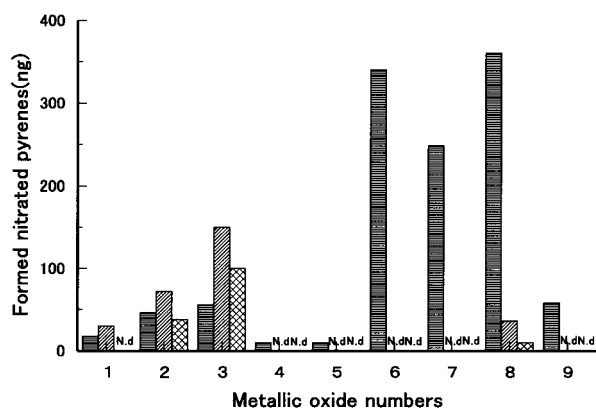
### Influence of Metallic Oxide on the Formation of Nitrated Pyrenes in the Presence of Indoor Air

During the experiments, the concentration of



**Fig. 2.** SIM Chromatogram of Nitrated Pyrenes

(1) 3-NF (100 pg); (2) 4-NP (50 pg); (3) 1-NP (50 pg); (4) 2-NP (30 pg); (5) 1,3-DNP (500 pg); (6) 1,6-DNP (500 pg); (7) 1,8-DNP (500 pg).

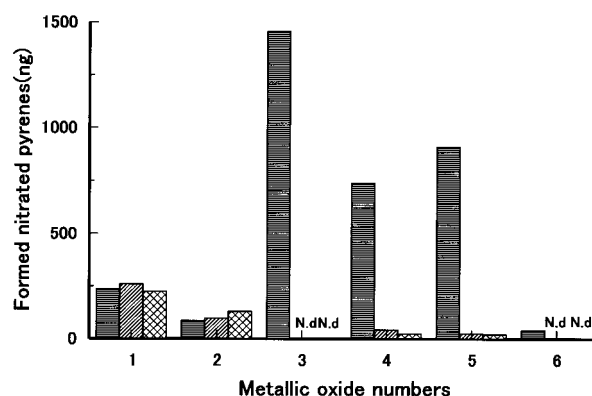


**Fig. 3.** Formation of Nitrated Pyrenes in Metallic Oxides in the Presence of Indoor Air with Xenon Lamp Irradiation

Shading patterns in each numbered group correspond to 1-NP, 2-NP, and 4-NP, from left to right, respectively. (1) CaO; (2) Al<sub>2</sub>O<sub>3</sub>; (3) MgO; (4) Fe<sub>2</sub>O<sub>3</sub>; (5) SiO<sub>2</sub> (quartz form); (6) SiO<sub>2</sub> (silicic anhydride form); (7) SiO<sub>2</sub> (silica gel form); (8) TiO<sub>2</sub> (rutile form); (9) TiO<sub>2</sub> (anatase form). Irradiation time, 2 hr; metallic oxide, 2 g; pyrene, 500 μg. Each column shows the average of two determinations.

NO<sub>2</sub> gas in the xenon irradiation apparatus was about 0.03 ppm.

The yields of nitrated pyrenes are shown in Fig. 3. 1-NP was detected in all nine metallic oxides in the range of 10–360 ng. Especially, it was found that 1-NP was produced in high quantities in three metallic oxides [SiO<sub>2</sub> (silicic anhydride 340 ng and silica gel forms 250 ng) and TiO<sub>2</sub> (rutile form) 360 ng]. 1-, 2-, and 4-NP were detected in yields of 46 ng, 72 ng and 38 ng in Al<sub>2</sub>O<sub>3</sub>, and 56 ng, 150 ng and 100 ng in MgO, respectively. Consequently, it was proven that the yields and formation patterns of



**Fig. 4.** Formation of Nitrated Pyrenes in Metallic Oxides in the Presence of Nitrogen Dioxide Gas with Xenon Lamp Irradiation

Shading patterns in each numbered group correspond to 1-NP, 2-NP, and 4-NP, from left to right, respectively. NO<sub>2</sub> gas, 0.2 ppm at a velocity of 1 liter/min. (1) Al<sub>2</sub>O<sub>3</sub>; (2) MgO; (3) SiO<sub>2</sub> (silicic anhydride form); (4) SiO<sub>2</sub> (silica gel form); (5) TiO<sub>2</sub> (rutile form); (6) TiO<sub>2</sub> (anatase form). Irradiation time, 2 hr; metallic oxide, 2 g; pyrene, 500 μg. Each column shows the average of two determinations.

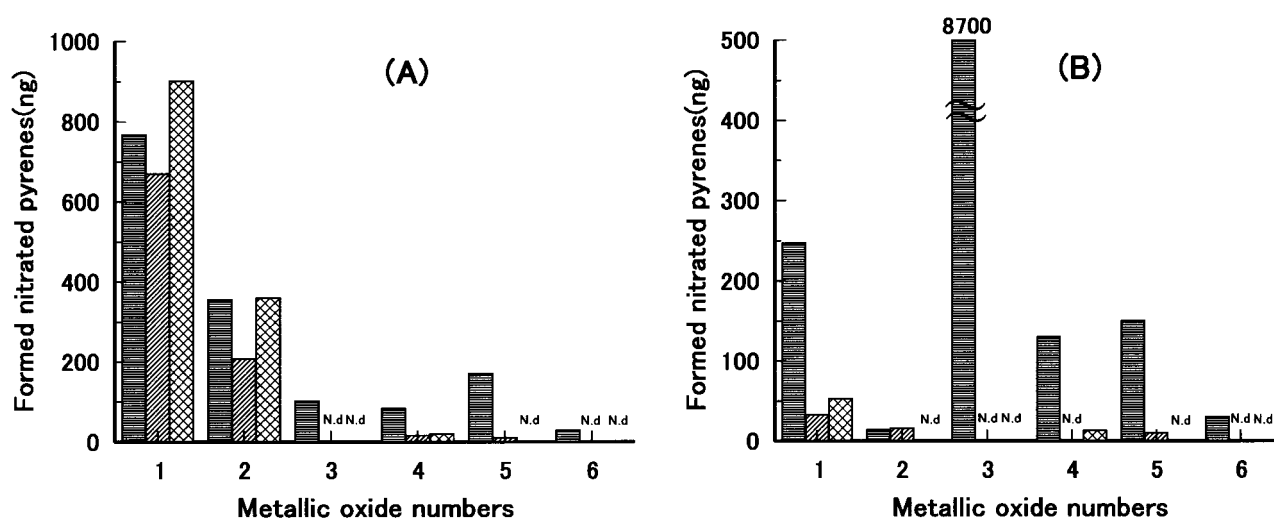
the three types of mononitrated pyrenes differed in the various metallic oxides. Therefore, we concluded that the nature of the photochemical reaction for the formation of the nitrated pyrenes differed in the various metallic oxides. On the other hand, nitrated pyrenes were not produced in samples left in the dark. No DNPs were detected in any of the samples.

### Influence of Metallic Oxide on the Formation of Nitrated Pyrenes in the Presence of NO<sub>2</sub> Gas

In the presence of indoor air, 1-, 2-, and 4-NP were produced in Al<sub>2</sub>O<sub>3</sub>, MgO, and TiO<sub>2</sub> (rutile form), and 1-NP was produced in high amounts in SiO<sub>2</sub> (silicic anhydride and silica gel forms) and TiO<sub>2</sub> (anatase form). Therefore, the following experiments were done using these six metallic oxides.

The concentration of NO<sub>x</sub> gas in pure air purified by the air cleaner was about 0.002 ppm, and all of the NO<sub>x</sub> was NO<sub>2</sub> gas.

The yields of nitrated pyrenes in these six metallic oxides are shown in Fig. 4. 1-NP was detected in all metallic oxides. Especially, 1-NP was produced in high amounts in three metallic oxides [SiO<sub>2</sub> (silicic anhydride and silica gel forms) and TiO<sub>2</sub> (rutile form) were detected in the amounts of 1500 ng, 740 ng and 910 ng respectively]. 1-, 2-, and 4-NP were produced in four metallic oxides [Al<sub>2</sub>O<sub>3</sub>, MgO, SiO<sub>2</sub> (silica gel), and TiO<sub>2</sub> (rutile)]. Clearly, the yields and formation patterns of mononitrated pyrenes differed in the various metallic oxides. The formation



**Fig. 5.** Formation of Nitrated Pyrenes in the Metallic Oxides in the Presence of Nitrite Ion (A) and Nitrate Ion (B) with Xenon Lamp Irradiation

Shading patterns in each numbered group correspond to 1-NP, 2-NP, and 4-NP, from left to right, respectively. Nitrite ion, 200  $\mu\text{g}$ . Nitrate ion, 200  $\mu\text{g}$ . (1) Al<sub>2</sub>O<sub>3</sub>; (2) MgO; (3) SiO<sub>2</sub> (silicic anhydride form); (4) SiO<sub>2</sub> (silica gel form); (5) TiO<sub>2</sub> (rutile form); (6) TiO<sub>2</sub> (anatase form). Irradiation time, 2 hr; metallic oxide, 2 g; pyrene, 500  $\mu\text{g}$ . Each column shows the average of two determinations.

patterns of nitrated pyrenes in the presence of NO<sub>2</sub> gas were similar to those in the presence of indoor air. Therefore, we considered NO<sub>2</sub> gas to be one of the nitrogen sources for the formation of nitrated pyrenes in the presence of indoor air.

Nitrated pyrenes were also produced from these metallic oxides in the presence of nitrogen monoxide gas (NO gas) (data not shown). However, since part of the NO gas is converted to NO<sub>2</sub> gas during the NO gas exposure, the individual contributions of the gases to the nitration of pyrene could not be judged. Therefore, the formation of nitrated pyrenes in the presence of NO gas is not described in this paper. In the presence of pure air containing about 0.002 ppm NO<sub>2</sub> gas, 1-NP was detected in all metallic oxides in yields of 6–80 ng.

### Influence of Metallic Oxide on the Formation of Nitrated Pyrenes in the Presence of NO<sub>2</sub> or NO<sub>3</sub> Ion

We were interested in determining whether NO<sub>2</sub><sup>-</sup> and NO<sub>3</sub><sup>-</sup> in the metallic oxides and NO<sub>2</sub> gas in pure air were nitrogen sources for the formation of NPs in these metallic oxides.

NO<sub>2</sub><sup>-</sup> and NO<sub>3</sub><sup>-</sup> were removed from the metallic oxides to eliminate any possibility of nitration of pyrene by these species. To remove water-soluble and water-insoluble nitrogen sources, the metallic oxides were washed with ultrapure water and acetone. NO<sub>2</sub><sup>-</sup> was not detected (< 0.5  $\mu\text{g/g}$ ) in any

sample.

Oxygen influences the nitration of polycyclic aromatic hydrocarbons.<sup>23)</sup> Therefore, to remove the effect of oxygen, the air in the Erlenmeyer flask was replaced with argon gas, which is heavier than air. The concentration of NO<sub>x</sub> in the argon gas was 0.003 ppm, and all of the NO<sub>x</sub> was NO<sub>2</sub> gas.

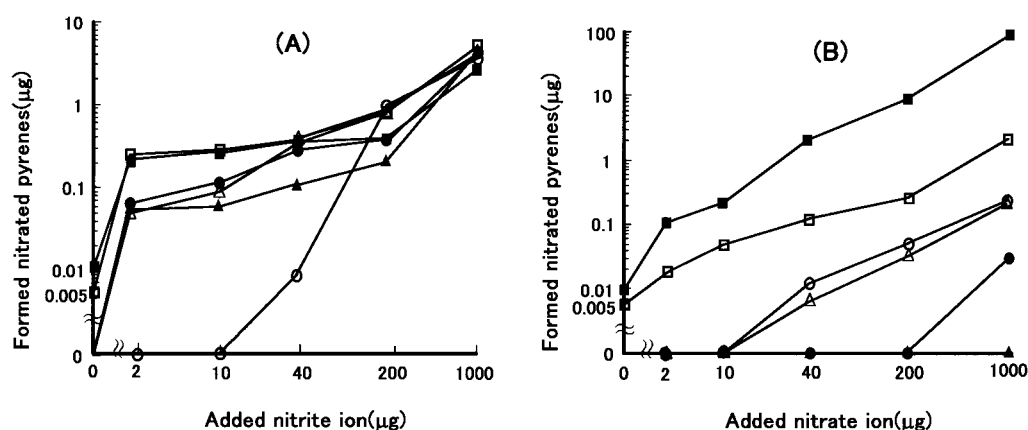
Moreover, after the recrystallization of sodium nitrite (the source of NO<sub>2</sub><sup>-</sup>), the amount of NO<sub>3</sub><sup>-</sup> in the NO<sub>2</sub><sup>-</sup> (200  $\mu\text{g}$ ) was 0.2  $\mu\text{g}$ .

Two grams of metallic oxide containing NO<sub>2</sub><sup>-</sup> (200  $\mu\text{g}$ ) and pyrene (500  $\mu\text{g}$ ) were irradiated with a xenon lamp for 2 hr in argon gas. 1-NP was detected in yields of 29–770 ng in all six metallic oxides [Fig. 5 (A)].

Especially, 1-NP was produced in high amounts in Al<sub>2</sub>O<sub>3</sub> and MgO.

Moreover, 2- and 4-NP were produced in high quantities in yields of 670 and 900 ng, respectively, in Al<sub>2</sub>O<sub>3</sub> and in yields of 200 and 360 ng, respectively, in MgO. Clearly, the yields and formation patterns of the three types of nitrated pyrenes differed in the various metallic oxides.

In the case of NO<sub>3</sub><sup>-</sup>, after recrystallization of sodium nitrate (the source of NO<sub>3</sub><sup>-</sup>), the amount of NO<sub>2</sub><sup>-</sup> in the NO<sub>3</sub><sup>-</sup> (200  $\mu\text{g}$ ) was below 0.02  $\mu\text{g}$ . Two grams of metallic oxide containing NO<sub>3</sub><sup>-</sup> (200  $\mu\text{g}$ ) and pyrene (500  $\mu\text{g}$ ) was irradiated with a xenon lamp for 2 hr. 1-NP was produced in yields of 14–8700 ng in all six metallic oxides [Fig. 5 (B)]. Especially,



**Fig. 6.** Effect of Added Nitrite Ion (A) and Nitrate Ion (B) on the Formation of Nitrated Pyrenes with Xenon Lamp Irradiation

(A): Al<sub>2</sub>O<sub>3</sub> (○, 1-NP; □, 2-NP; △, 4-NP); MgO (●, 1-NP; ■, 2-NP; ▲, 4-NP). (B): Al<sub>2</sub>O<sub>3</sub> (○, 1-NP; □, 2-NP; △, 4-NP); SiO<sub>2</sub> [silicic anhydride form (●, 1-NP; ■, 2-NP; ▲, 4-NP)]. Irradiation time, 2 hr; metallic oxide, 2 g; pyrene, 500 µg. Each marker shows the average of two determinations.

1-NP was produced in high amounts in SiO<sub>2</sub> (silicic anhydride form, 8700 ng). 1-, 2-, and 4-NP were produced in yields of 250 ng, 33 ng and 53 ng in Al<sub>2</sub>O<sub>3</sub>, respectively. On the other hand, 2- and 4-NP were hardly formed in the other five metallic oxides. Thus, the yields and formation patterns of the three types of mononitrated pyrenes differed in the various metallic oxides and also varied with the type of nitrogen source (NO<sub>2</sub><sup>-</sup> or NO<sub>3</sub><sup>-</sup>). Nitrated pyrenes were hardly formed in those metallic oxides left in the dark.

### Effect of NO<sub>2</sub> or NO<sub>3</sub> Ion Concentration on the Formation of Nitrated Pyrenes

Having found that nitrated pyrenes were detected in high amounts by using Al<sub>2</sub>O<sub>3</sub>, MgO in the presence of NO<sub>2</sub><sup>-</sup> and Al<sub>2</sub>O<sub>3</sub>, SiO<sub>2</sub> (silicic anhydride) in the presence of NO<sub>3</sub><sup>-</sup>, we performed the following experiments using these metallic oxides.

When NO<sub>2</sub><sup>-</sup> was added, each 2 g of metallic oxide containing pyrene (500 µg) and NO<sub>2</sub><sup>-</sup> (0, 2, 10, 40, 200 and 1000 µg) was irradiated with a xenon lamp for 2 hr. The relations between the amount of NO<sub>2</sub><sup>-</sup> added and the yields of nitrated pyrenes in the metallic oxides are shown in Fig. 6 (A). 1- and 2-NP were detected in yields of 260 and 52 ng in the presence of NO<sub>2</sub><sup>-</sup> (2 µg) in Al<sub>2</sub>O<sub>3</sub>, respectively, but 4-NP was not formed upon the addition of 2 and 10 µg of NO<sub>2</sub><sup>-</sup>. On the other hand, 1-, 2- and 4-NP were detected in yields of 220, 56, and 68 ng in the presence of NO<sub>2</sub><sup>-</sup> (2 µg) in MgO, respectively.

The yields of nitrated pyrenes increased with an increasing amount of added NO<sub>2</sub><sup>-</sup>, and the nitrated pyrenes were detected in yields of 2700 and 5100 ng

in Al<sub>2</sub>O<sub>3</sub> and MgO, when the amount of added NO<sub>2</sub><sup>-</sup> was 1000 µg.

Thus, the yields of nitrated pyrenes were dependent on the amount of NO<sub>2</sub><sup>-</sup> in the metallic oxides.

When NO<sub>3</sub><sup>-</sup> was added, each 2 g of metallic oxide containing pyrene (500 µg) and NO<sub>3</sub><sup>-</sup> (0, 2, 10, 40, 200 and 1000 µg) was irradiated with a xenon lamp for 2 hr. The dose relation curves between the amount of added NO<sub>3</sub><sup>-</sup> and the yields of nitrated pyrenes in the metallic oxides are shown in Fig. 6 (B). 1-NP was detected in yields of 15 and 100 ng in the presence of NO<sub>3</sub><sup>-</sup> (2 µg) in Al<sub>2</sub>O<sub>3</sub> and SiO<sub>2</sub> (silicic anhydride), respectively. The yields of nitrated pyrenes increased as the amount of added NO<sub>3</sub><sup>-</sup> increased. However, when up to 1000 µg of NO<sub>3</sub><sup>-</sup> was added to SiO<sub>2</sub> (2 g), 2-NP were not detected.

From the above results, in which 1000 µg NO<sub>3</sub><sup>-</sup> was added to 2 g of metallic oxides, it is estimated that almost all of the nitrate ions were converted to metallic salts such as Al(NO<sub>3</sub>)<sub>3</sub> and SiO<sub>2</sub> adduct, the complex form of which was not determined. It was concluded that the NO<sub>3</sub><sup>-</sup> ion, metallic nitrate salt and its adduct, but not NO<sub>x</sub> gas, contributed to the formation of 1-NP and the other nitropyrenes in these two metallic oxides under argon gas atmosphere.

In conclusion, NO<sub>2</sub> gas, NO<sub>2</sub><sup>-</sup>, and NO<sub>3</sub><sup>-</sup> were shown to be nitrogen sources for the formation of nitrated pyrenes on these metallic oxides under xenon lamp irradiation. 2-NP was known to be formed in the atmosphere by vapor-phase reaction. Therefore, the results were very interesting.

In the literature,<sup>1)</sup> 70.5 ng of 1-NP, 1.2 ng of 1,6- and 3.8 ng of 1,8-DNP per mg were found in diesel engine particulates, also, 0.082 ng of 1-NP, 0.028 ng

of 1,3-, 0.036 ng of 1,6- and 0.030 ng of 1,8-DNP per mg of crude extract were found in city gas collected by a filter paper method, followed by organic solvent extraction. However, three of the dinitropyrenes were not determined in any of our experiments.

NO<sub>2</sub> gas exists in the environment in the range of 0.03–0.06 ppm, and NO<sub>3</sub><sup>-</sup> exists everywhere in the soil at 100 ppm or less.<sup>24)</sup> It has been reported that NO<sub>3</sub><sup>-</sup> is included in suspended particulate matter at about 200 ppm.<sup>14)</sup> Therefore, we conclude that the nitration of pyrene on the surface of soil components by NO<sub>3</sub><sup>-</sup> is very important in considering the environmental risk of chemicals. Further experiments concerning the formation mechanisms of nitrated pyrenes on several soil components by xenon lamp irradiation should be conducted.

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