Formation of 1,3-, 2,4-, 1,2-, 1,6-, 1,8- and 1,7-Dinitropyrenes in Metallic Oxides as Soil Components in the Presence of Indoor Air with 1-Nitropyrene and Sodium Chloride Under Xenon Lamp Irradiation

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The reaction products obtained from the mixture of 1-nitropyrene (1-NP) and sodium chloride were investigated on three metallic oxides as soil components under xenon lamp irradiation in the presence of indoor air. These chemicals including 1-NP were extracted with benzene/ethanol (4/1, v/v) and analyzed by gas chromatography/mass spectrometry (GC/MS). The amounts of 1-NP gradually decreased as irradiation time elapsed on all three metallic oxides (TiO 2: titanium dioxide, anatase form; SiO 2: silicon dioxide, silicic anhydride form; and Al 2 O 3: aluminum oxide). In TiO 2, in particular, 1-NP showed a greater decrease than the other two metallic oxides for all irradiation times. Six types of dinitropyrenes (DNPs) were detected from the reaction products (1,3-, 2,4-, 1,2-, 1,6-, 1,8- and 1,7-DNP). In both TiO 2 and Al 2 O 3, all 6 DNPs were detected, while in SiO 2, 5 DNPs were confirmed except for 2,4-DNP. In TiO 2, 1,7-DNP was formed in especially rich amounts for all irradiation times in comparison with the other metallic oxides. We presumed that 4- and 2-NPs were generated from 1-NP by the photochemical reaction of OH radical, and that 2,4-, 1,2- and 1,7-DNPs were formed continuously by the nitration of these isomers. The formation of 2,4-, 1,2- and 1,7-DNPs was confirmed for the first time on the metallic oxides by the photochemical reaction system of the 1-NP-NOx-Cl ion. NOx in the indoor air was shown to be a source of nitrogen used in forming DNPs in these metallic oxides. The yields and formation patterns of DNPs differed in the 3 metallic oxides as the irradiation time was extended. Hence, the nature of the photochemical reaction in the formation of DNPs differed depending on the type of metallic oxide in question. In SiO 2, mutagenicity increased gradually up to 6 hr of irradiation when mutagenic potency showed 2.6 million revertants/sample, and then decreased to 1.5 million revertants/sample at 12 hr. In Al 2 O 3, mutagenicity increased gradually as irradiation time elapsed to a maximum of 1.64 million revertants/sample. In TiO 2, mutagenicity decreased rapidly to 60% at 10 min and thereafter decreased gradually to 22% at 12 hr.

Key words — nitropyrene, dinitropyrene, nitration, xenon lamp irradiation, metallic oxide, mutagenicity

INTRODUCTION

Many chemicals exist in the surface soil and in the urban air. As a result of irradiating sunlight, these chemicals react with each other and form other chemicals of significant toxicity. Pyrene is a substance that shows no mutagenicity in the Ames test; however, chlorinated pyrenes have moderately strong mutagenicity 1,2) and nitrated pyrenes have strong mutagenicity as well as carcinogenicity 3,4). Recent studies have traced these pyrenes in urban air.5–8) We have reported that chlorinated pyrenes are produced in soil 9,10) and in the metallic oxides of soil from pyrene with chloride ion, 11) and the crude extract obtained from the metallic oxides of soil showed strong mutagenicity. 12) Mono-nitrated pyrenes are produced from pyrene in the presence of indoor air, nitrogen dioxide gas, nitrite ion or nitrate ion under irradiation by sunlight or xenon lamp. 13) Chloride ion exists everywhere in the soil with a concentra-
19,20) Nitrogen dioxide gas exists in the environment in the range of 0.03–0.06 ppm, and nitrate ion exists everywhere in the soil at 100 ppm or less.16) Pyrene exists mainly in coal tar, and is often detected in soil as well.17,18) and soil exists in air primarily as suspended particulate matter (SPM).19,20)

It is believed that mononitrated pyrenes are decomposed or changed in the other chemicals in the environment over time. When this change or decomposition takes place, it is suggested that other very toxic chemicals are produced by reaction with nitrogen sources and chloride ion as described above, and that the formation of these chemicals increases the mutagenic and carcinogenic activity of the environment. Therefore, it is very important in considering the environmental risk of chemicals to study the decomposition or change of mono-nitrated pyrenes and the subsequent formation of other chemicals. In the present study, we investigated changes in 1-nitropyrene (1-NP) and the formation of the other chemicals on 3 metallic oxides [titanium dioxide (TiO₂; anatase form), silicon dioxide (SiO₂; silicic anhydride form) and aluminum oxide (Al₂O₃)]. For TiO₂ and SiO₂, it was previously reported that chlorinated pyrenes were detected from 9 sorts of metallic oxides as soil components with pyrene and sodium chloride in the presence of indoor air under xenon irradiation. During the experiments, it was observed especially that the color of the extracted solution had changed from colorless to yellow in the presence of SiO₂ (silicic anhydride form) and from yellow to light yellow in the presence of TiO₂ (anatase form) with the progress of xenon lamp irradiation time.10) From these observations, in both metallic oxides in particular, it was expected that nitro compounds as reaction products were formed rich amounts in comparison with the other metallic oxides. For Al₂O₃, 1-, 2- and 4-NP were produced in high quantities with pyrene and sodium chloride in the presence of nitrogen sources under xenon irradiation.13) Therefore, in this paper, we performed the experiments using these metallic oxides.

**MATERIALS AND METHODS**

**Materials** —— 1-NP (purity, > 98%) was purchased from Aldrich Chemical Co. (Milwaukee, WI, U.S.A.), 2,7-Dinitrofluorene (2,7-DNF, > 95%) was purchased from Tokyo Kasei Kogyo and was used as an internal standard for gas chromatography/mass spectrometry (GC/MS) analysis. 1,2- and 1,7-DNP were synthesized following the methods described by van den Braken-van Leersum, et al.21) and Bavin.22) 2,4-DNP was synthesized as described below. These purity values were confirmed by melting point measurement and gas chromatography. 1,2-, 2,4- and 1,7-DNPs, whose purities were above 98%, above 95%, and above 98%, respectively, were used for standard samples in GC/MS analysis. Sodium chloride was purchased from Wako Pure Chemical Industries Co. Ltd. (Tokyo, Japan), and was dried in an electric furnace at 550°C for 8 hr. Ethanol, methanol and benzene (for the analysis of grades of residual pesticides) were purchased from Wako. High purity argon gas (99.999%) was purchased from Taiyo Toyo Sanso Co. Ltd. (Tokyo, Japan), and titanium dioxide [TiO₂; anatase (< 5 µm, 99.9%)], silicon dioxide [SiO₂; silicic anhydride (12 µm, 99.9%)] and aluminum oxide (Al₂O₃; α-alumina, 5 µm) were purchased from Wako.

**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., Tokyo, Japan). The spectrum of the xenon lamp began at 290 nm and was similar to the spectrum of sunlight. GC/MS was carried out on an SX-102 instrument (JEOL Co. Ltd., Tokyo, Japan). Nuclear magnetic resonance (NMR) was carried out on a Valian XL-300 spectrometer (Valian Technologies Japan Ltd., Tokyo, Japan).

**Sample Preparation** —— Sodium chloride aqueous solution (5 mg/ml as chloride ion) was added to each metallic oxide, and the mixtures were scrupulously dried with a rotary evaporator and then kept in a desiccator in the dark. A methanol solution of 1-NP (200 µg/ml) was added to each metallic oxide, and the methanol was removed in vacuo with a rotary evaporator. For each metallic oxide, 2 g of metallic oxide including chloride ion (10 mg) and 1-NP (400 µg) was placed in a laboratory dish with a diameter of 100 mm, and the sample was evenly spread out such that it made a circle of about 80 mm in diameter. In the case of the sample for the blank test, for each metallic oxide, 2 g of metallic oxide including chloride ion (10 mg) and 1-NP (400 µg) was placed in a quartz Erlenmeyer flask. The opening of the flask was hermetically sealed with a sili-
cone 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 the glass tubes were closed. The sample was uniformly spread.

Xenon Lamp Irradiation ——— The dish and the quartz Erlenmeyer flask were fixed in a sample frame located at 250 mm from the xenon lamp and were then irradiated. After irradiation, the chemicals in the metallic oxides were extracted with 100 ml benzene/ethanol (4/1, v/v) and then subjected to centrifugal separation. The supernatant was concentrated to 2 ml. DNPs were measured by GC/MS (selected ion monitoring, SIM) after the addition of 2,7-DNF (1000 ng) as an internal standard. These samples were diluted 100 times. 1-NP in these samples was determined by GC/MS (SIM) and were concentrated at about 100-fold, and measurement of the 6 DNPs by GC/MS (electron impact, EI) was carried out.

GC/MS Conditions ——— 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; Sigma Aldrich Japan Co. Ltd. (Tokyo, Japan)]. The column oven temperature was initially maintained at 60°C (1 min); it was then raised to 220°C at a rate of 25°C/min, and finally increased to 270°C at a rate of 15°C/min. A dual taper insert tube was used. Ions were monitored at m/z 217 and 247 for 1-NP, and at m/z 216, 292 for DNPs.

Nitrination of 4-NP to Furnished 2,4-DNP ——— Three drops of fumic nitric acid were added dropwise to the solution of 80 mg of 4-NP in nitrobenzene (5 ml), and the reaction mixture was first heated to 60°C for 10 min, then poured into water and extracted with toluene. The solvent was evaporated in vacuo. The residues were applied to a silica gel column [ULTRON VX-SIL (normal phase) 250 mm × 20 mm, Shinwa Kako Co. Ltd., (Kyoto, Japan)] and eluted with n-hexane/ethyl acetate (9/1) to furnish four fraction, and major third eluted fraction was crystallized from ethanol/H₂O to give 2,4-DNP (20 mg) as pale yellow needles with an mp of 218°C. 1H-NMR and nuclear overhauser effect spectroscopy (NOESY) spectra were recorded for all solutions in dimethyl sulfoxide (DMSO)-d₆ operated at 300 MHz, and chemical shifts are reported in part per million using tetramethylsilane as an internal standard (Fig. 1).

Mutagenicity Test ——— The sample solution of 2 ml was filtered by Teflon filter and the crude extract was weighed after the solvent was completely volatilized in a nitrogen gas stream and was dissolved in DMSO. Mutagenicity assays were performed by the Ames method using Salmonella typhimurium (S. typhimurium) TA98 without S9 mix and were conducted in triplicate for each sample. The slope of dose–response (revertants/total extracts) was calculated by least-squares linear regression from the first linear portion of the dose–response curve. The mutagenic potency of each reaction time on several metallic samples is shown as revertants (rev.)/sample.

RESULTS AND DISCUSSION

Determination of 2,4-DNP (I)

I compound was formed by nitration of 4-NP. Figure 1 shows the 1H-NMR and NOESY spectra of I. The 3 aromatic singlet peaks at 8.89(2H) and 9.47(1H) ppm with their peaks assigned to the 1, 3 and 5 positions, as well as the double-doublet peaks (1H d.d-like j = 7.7Hz) at 8.79 and 8.87 ppm assigned to the 6 and 8 positions. Furthermore, the doublet peaks (1H d. j = 9.5 Hz) at 8.65 and 8.74 ppm assigned 9 and 10 aromatic protons, respectively, and triplet peaks (1H, t. j = 7.7 Hz) at 8.42 ppm are assignable to the 7 position. In the NOESY spectrum, the two areas marked a and b recognized NOE between positions 8 and 9, and positions 5 and 6, respectively. According to the results of high resolution GC/MS measurements, m/z of molecular ion of I was at 292.0492, and the calculated value of 2,4-DNP was 292.0484. The difference between I and 2,4-DNP was -0.8 mmu. Moreover, based on the results of GC/MS measurements, m/z (%) of I were at 292(M⁺, 40%), 262[(M-NO)⁺, 20%], 232 [(M-N₂O₂)⁺, 52%], 216[(M-N₂O₃)⁺, 51%], 200[(M-N₂O₄)⁺, 84%], and 188[(M-CN₂O₄)⁺, 100%]. Therefore, the structure of I compound has been established as 2,4-DNP.

Purification of 1-NP

When the standard 1-NP of several companies was measured by GC/MS, pyrene was detected as an impurity in all of them. In our previous work, mononitrated pyrenes including 2-NP were produced from pyrene in metallic oxides in the presence of...
indoor air or nitrate ion with xenon lamp irradiation. Additionally, it has been reported that DNPs are produced from 2-NP in the presence of HNO$_3$. Therefore, it was necessary to remove the pyrene impurity from the 1-NP. Standard 1-NP was dissolved in acetone at about 55°C and the supernatant was removed after cooling. The resulting concentration of pyrene as an impurity in 1-NP (200 ppm) was 0.05 ppm, and the concentrations of 1,3-, 1,6- and 1,8-DNP as an impurity of 1-NP (200 ppm) were below 0.01 ppm. Our experiments were conducted using the purified 1-NP.

**Relationship between the Decreasing Ratio of 1-NP and Xenon Lamp Irradiation Time**

Two g of metallic oxides which had been prepared to contain chloride ion (10 mg) and 1-NP (400 µg) was irradiated by xenon lamp for 0 min, 10 min, 30 min, 1 hr, 3 hr, 6 hr and 12 hr. The relationship between the irradiation time and the decreasing ratio obtained is shown in Fig. 2. The ratio of 1-NP was 33% in TiO$_2$, 4% in SiO$_2$ and 2% in Al$_2$O$_3$. After 10 min irradiation, after which the irradiation time was extended, up to final values of 95%, 63% and 37%, respectively, after 12 hr. We found that the decreasing ratio of 1-NP differed in the 3 metallic oxides. In TiO$_2$, the decreasing ratio of 1-NP was the highest at each exposure time, followed by SiO$_2$ and finally Al$_2$O$_3$.

**Confirmation of DNPs in the Metallic Oxides**

Two g of TiO$_2$ treated with chloride ion (10 mg)
and 1-NP (400 µg) was irradiated with the xenon lamp for 6 hr, and was then concentrated and analyzed by GC/MS(EI). Based on the GC/MS fragment patterns, the presence of 6 types of DNPs was confirmed. The mass spectra of the DNPs formed in the metallic oxides were consistent with the mass spectra of 1,3-, 2,4-, 1,2-, 1,6-, 1,8 and 1,7-DNP standard substances. The mass chromatograms of the 6 types of standard DNPs and the 6 types of DNPs formed in TiO₂ at m/z 292 are shown in Fig. 3.

Our results show that the retention time of the DNPs formed in TiO₂ was in complete agreement with that of standard DNPs; we therefore conclude that the chemicals formed in TiO₂ in the presence of indoor air with chloride ion and 1-NP under xenon lamp irradiation for 6 hr were indeed 6 types of DNPs (1,3-, 2,4-, 1,2-, 1,6-, 1,8- and 1,7-DNP).

The recovery of DNPs from metallic oxides was also studied (data not shown), and was satisfactory at over 80% for all samples. In addition to DNPs, the existence of chloronitropyrenes was also confirmed in these metallic oxides, however they are still under investigation.

**Relationship between the Yields of DNPs and Xenon Lamp Irradiation Time**

As described above, it was confirmed that DNPs are formed in the decreasing process of 1-NP and we examined the yields of DNPs formed in this process. Two g of each metallic oxide which had been prepared to contain chloride ion (10 mg) and 1-NP (400 µg) was irradiated with the xenon lamp for 0 min, 10 min, 30 min, 1 hr, 3 hr, 6 hr and 12 hr. The relationship between the irradiation time and the obtained yields of DNPs is shown in Fig. 4. The yields of 1,3-, 2,4-, 1,2- and 1,7-DNPs gradually increased in all 3 metallic oxides as the irradiation time extended up to 6 hr except for the yield of 2,4-DNP in SiO₂. After 12 hr, yields increased in TiO₂ and Al₂O₃ (1,3-DNP) and decreased for the other DNPs. 2,4-DNP was not detected in SiO₂. For each exposure time, the yields of 2,4-DNP and 1,7-DNP in TiO₂ were greater than those in SiO₂ and Al₂O₃.

The differences in the yield of 2,4-DNP in TiO₂ (92 ng) and in SiO₂ (0 ng), and that of 1,7-DNP in TiO₂ (180 ng) and in SiO₂ (6 ng) after 12 hr were particularly remarkable. In TiO₂, 1,7-DNP showed the greatest amount of all of the DNPs.

The yields of 1,6- and 1,8-DNP gradually increased in SiO₂ and Al₂O₃ as the irradiation time increased up to 6 hr, while in TiO₂, they reached maximum values at 1 hr and 10 min irradiation time, respectively, and then gradually decreased. In this case as well, all 6 kinds of DNPs were detected in all samples with the exception of 2,4-DNP in SiO₂.

Our results clearly show that the yields and formation patterns of DNPs differ in the various metallic oxides. Hence, we conclude that the nature of the photochemical reaction in the formation of DNPs likewise differs depending on the type of metallic oxide in question.

No DNPs were detected in any sample sealed in argon gas in the quartz Erlenmeyer flask. During the xenon irradiation experiments, the concentration of NOx within the xenon lamp apparatus and that in the ambient atmosphere was approximately 0.03 ppm in all cases; we therefore assume that the nitrogen source for the formation of DNPs in these metallic oxides was NOx in the atmosphere.

It has been reported that 1,3-, 1,6- and 1,8-DNP are produced in the atmosphere, however, there has been no report to date that 1,2-, 2,4- and 1,7-DNP are produced in the atmosphere. A previous report indicates that 1-NP is converted into a mixture of 1-hydroxypyrene and 1-hydroxy-2-nitropyrene under irradiation with light. Furthermore, there have been some reports that in the gas phase, 2-NP is formed by a two-step reaction mechanism in which an OH radical first attacks position 1 of pyrene and then NO₂ is added to position 2. Finally, other studies have shown that 1,2- and 1,7-DNP are formed when 2-NP is treated with concentrated nitric acid. Therefore, we assumed that an OH radical first attacks position 1 of pyrene in the process of change of 1-NP to 1-hydroxypyrene or in the next stage, and then NO₂ is added to position 2 with the result...
that 2-NP is formed. Furthermore, we assumed that 1,2- and 1,7-DNPs are generated from the 2-NP by NOx gas in the presence of indoor air under xenon lamp irradiation on the metallic oxide.

On the other hand, for 4-NP, it has been reported that an OH radical first attacks position 1 of pyrene, and then NO2 is added to position 4 in the gas phase.24) Therefore, in the present study, we assumed that the first OH radical attacks position 1 of pyrene by 1-NP photolysis, bringing about the formation of 4-NP. We also suggest that 2,4-DNP is generated from 4-NP by NOx gas in the presence of indoor air under xenon lamp irradiation on the metallic oxide. Fig. 5 shows the synthesis of the 6 DNPs from 1-, 2- and 4-NP by several nitration processes and mechanisms leading to the formation of these DNPs from 1-NP by photochemical reaction in ambient air.

In a previous paper, we reported that mononitropyrenes are formed under xenon lamp irradiation and in the presence of a nitrogen source from pyrene in metallic oxides.13) In the present report, therefore, it was very interesting that 1,2-, 2,4- and 1,7-DNPs were newly formed on the metallic oxides under xenon lamp irradiation during the decreas-

Fig. 4. Formation of DNPs in Metallic Oxides in the Presence of Indoor Air with 1-NP and Sodium Chloride under Xenon Lamp Irradiation

(1): 1,3-DNP; (2): 2,4-DNP; (3): 1,2-DNP; (4): 1,6-DNP; (5): 1,8-DNP; (6): 1,7-DNP. ○: TiO2; □: SiO2; △: Al2O3. Metallic oxide, 2 g; 1-NP, 400 µg; Chloride ion, 10 mg. Each Marker shows the average of two determinations.
ing process of 1-NP.

**Mutagenicity of the Photoreaction Products on the Three Metallic Oxides by Reaction Time**

1,3-, 1,6- and 1,8-DNPs show strong mutagenicity and carcinogenicity. It is thus anticipated that the 2,4-, 1,2- and 1,7-DNP newly confirmed in the present report should also show strong mutagenicity.

Nevertheless, in the present preliminary Salmonella test using TA98 strain, the mutagenic potencies of the 2,4-, 1,2- and 1,7-DNPs were 19126, 65 and 4790 rev./nmol, respectively without S9 mix, and their mutagenicity was not significantly stronger than that of the 1,3-, 1,6- and 1,8-DNPs. The results of the mutagenicity assay of the crude extract obtained from the photochemical reaction in TiO$_2$, SiO$_2$ and Al$_2$O$_3$ are shown in Fig. 6. The mutagenicity of the total extracts in TA98 without S9 mix were calculated for each exposure time, and are expressed net rev. per total extracts. In SiO$_2$, mutagenicity increased gradually up to 6 hr irradiation and the mutagenic potency was found to be 2.6 million rev./sample at that time; it then decreased to 1.5 million rev./sample at 12 hr. In Al$_2$O$_3$, mutagenicity increased gradually as irradiation time elapsed (1.644 million rev./sample), while in TiO$_2$, it decreased rapidly to 60% at 10 min and then further decreased gradually to 22% at 12 hr. In TiO$_2$, total mutagenic potency decreased as the irradiation time elapsed in spite of the increase of the yield of 1,8-DNP showed most strong mutagenicity (Fig. 4).

The mutagenic potencies of 1-NP and 1,8-DNP were 673 rev./nmol and 222000 rev./nmol (Fig. 5),
respectively. On the other hand, in TiO₂, after 10 min irradiation, the amounts of 1-NP and 1,8-DNP were 270 µg (Fig. 2) and 25 ng (Fig. 4), respectively. Therefore, in TiO₂, after 10 min irradiation, total mutagenic potency of 1-NP was rich in comparison with that of 1,8-DNP.

As the result, it was presumed that the decrease of the total mutagenicity potency in TiO₂ was caused by the decrease of the yields of 1-NP as the irradiation time elapsed.

Based on these results, it was estimated that the chemicals discharged in the environment changed in substances of strong toxicity due to their interaction with the environment, and we suggest that these chemicals thus increase the mutagenic and carcinogenic activity of the environment. Further study on the formation of chemicals in the environment is essential in examining environmental risk.

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