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Table 1. *H. pylori* infection induced-gastritis in MGs.

Group	Treatment	Effective No.	Stomach wet weight (g)	Inflammation score
A	Broth	15	0.647 ± 0.097	0
B	NIAN + Broth	22	0.631 ± 0.094	0
C	*H. pylori*	18	1.432 ± 0.445*	2.22 ± 0.43*
D	NIAN + *H. pylori*	26	1.483 ± 0.445*	2.38 ± 0.64*

*P<0.01 vs. group A and B; Values for results are expressed as averages ± SD.

Table 2. Incidence of glandular stomach adenocarcinoma in MGs.

Group	Treatment	Effective No.	No. of animals with glandular stomach adenocarcinoma (%)		
			Total	Well dif.	Moderately dif.
A	Broth	15	0 (0)	0 (0)	0 (0)
B	NIAN + Broth	22	0 (0)	0 (0)	0 (0)
C	*H. pylori*	18	0 (0)	0 (0)	0 (0)
D	NIAN + *H. pylori*	26	8 (31)*	7 (27)	1 (4)

Well dif., well differentiated adenocarcinoma; Moderately dif., moderately differentiated adenocarcinoma.

*$P<0.05$ vs. group A and C, and $P<0.01$ vs. group B.

Figure Legends

Figure 1. Chemical structure of NAIN and experimental protocol for the carcinogenicity study. (A) Chemical structure of NIAN. (B) Male six-week-old MGs were orally administered NIAN (100 mg/kg) in 50% DMSO (groups B and D) or 50% DMSO alone (groups A and C) two times a week for three weeks. One week after the final administration, the animals were inoculated with *H. pylori* (ATCC 43504) (groups C and D) or sterilized broth (groups A and B).

Figure 2. Autoradiograms of NIAN-DNA adducts in glandular stomach of MGs or calf thymus DNA treated with NIAN. Adducts were analyzed by ^{32}P-postlabeling method, as described in the Materials and Methods. DNA samples were isolated from glandular stomach of MGs (A) or calf thymus DNA (B) after treatment with NIAN. DNA samples were also prepared from glandular stomach of MGs without NIAN treatement (C). Arrowheads indicate adducts.

Figure 3. Macro- and microscopic views of gastritis in MGs infected or uninfected with *H. pylori*. (A) Normal gastric mucosa

in group A. (B) Severe infiltration of many inflammatory cells with development of heterophilic proliferative glands in group C; H&E staining x 40. Yellow boxes are shown at greater magnification below. x 200.

Figure 4. Histological findings of gastric adenocarcinoma in the animals treated with both NIAN and *H. pylori*. (A) Typical macrograph of a stomach. The yellow circle shows the suspected lesion of gastric cancer. (B) Well differentiated adenocarcinoma. (C) Moderately differentiated adenocarcinoma. (B,C) H&E staining x 400.

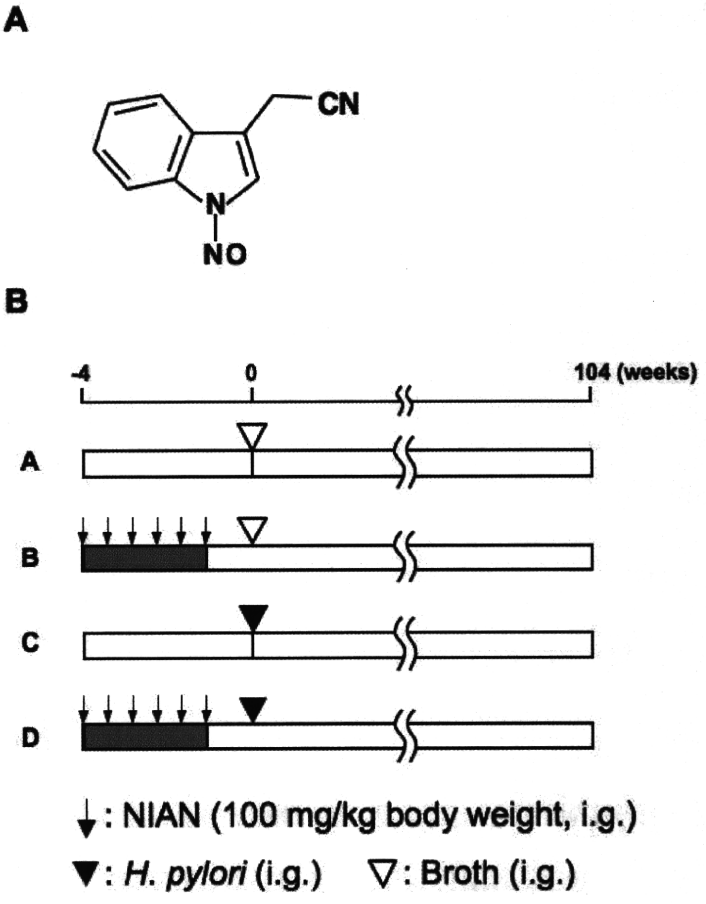
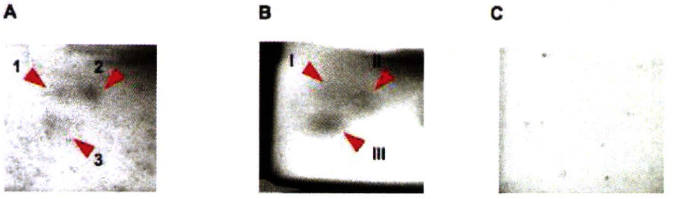


Figure 1

Figure 2



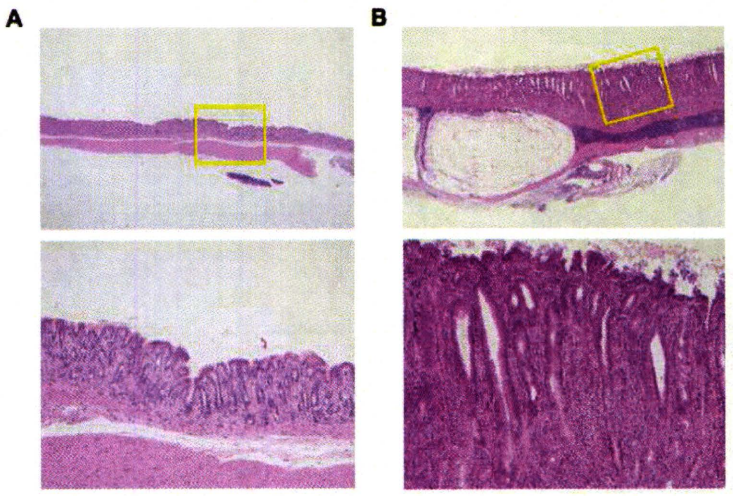


Figure 3

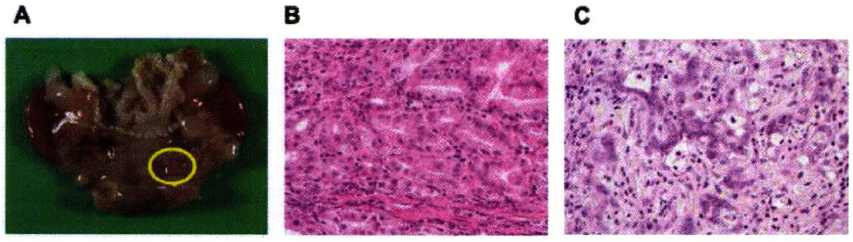


Figure 4

— **Regular Article** —

Determination of 3,6-Dinitrobenzo[*e*]pyrene in Tea Leaves as a Possible Exposure Source and in Human Hair as a Biomarker Using a Two-dimensional HPLC System

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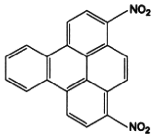
3,6-Dinitrobenzo[*e*]pyrene (DNBeP) is an extremely strong mutagen in *Salmonella* Typhimurium TA98 without a mammalian metabolic system (S9 mix). 3,6-DNBeP shows genotoxicity *in vitro* to mammalian cells, and produces DNA damage in the cells of several organs in mice in the comet assay. In a previous study, we developed an analytical method and clarified that 3,6-DNBeP widely exists in the environment, *i.e.*, surface soil and airborne particles, and that diesel engines and municipal incinerators are probable sources of 3,6-DNBeP. In this study, we improved the method of analyzing 3,6-DNBeP by combining one step of clean-up and fluorescence detection utilizing a two-dimensional HPLC system, and analyzed 3,6-DNBeP in tea leaves (n = 6), which is a possible exposure source of 3,6-DNBeP in our daily life, and in human hair samples (n = 8), as a possible biomarker of 3,6-DNBeP. 3,6-DNBeP was detected in all examined tea leaves and human hair as single peaks on the chromatograms, and was well purified by the HPLC system. 3,6-DNBeP was detected in the range of 8–1823 pg/g of tea leaves and the amount of 3,6-DNBeP in tea leaves differed depending on the growth site of the tea leaves. 3,6-DNBeP in human hair was detected in the range of 11–121 pg/g of hair and 86–1576 pg/mg of eumelanin. These results suggested that tea leaf is a possible source of exposure to 3,6-DNBeP and that 3,6-DNBeP detected in hair might reflect human exposure to 3,6-DNBeP.

Key words —— 3,6-dinitrobenzo[*e*]pyrene, two-dimensional HPLC system, human hair, tea leaf, biomarker, exposure

INTRODUCTION

We identified 3,6-dinitrobenzo[*e*]pyrene (DNBeP, Fig. 1) as a novel chemical in strongly mutagenic surface soil samples collected in general residential sites.[1,2] 3,6-DNBeP is an extremely strong mutagen toward bacteria and its potency was found to be comparable to that of 1,8-dinitropyrene, which is known as the most potent bacterial mutagen identified so far in the literature.[3] 3,6-DNBeP shows genotoxicity *in vitro* to mammalian cells, such as mutagenicity in *hprt* gene and induction of sister-chromatid exchange and micronucleus.[4] Furthermore, 3,6-DNBeP produces DNA damage in the cells of several organs in mice in the comet assay.[4] In previous studies, we developed a method of analyzing 3,6-DNBeP in surface soil and airborne particles using two steps of high-performance liquid chromatograph (HPLC) separation for clean-up and fluorescence detection,[5] and clarified that 3,6-DNBeP widely exists in surface soil and ambient air, and that diesel engines and municipal incinerators are its probable sources of 3,6-DNBeP.[6] In the present study, we



3,6-Dinitrobenzo[e]pyrene
(3,6-DNBeP)

Fig. 1. Structure of 3,6-DNBeP

3,6-DNBeP is an extremely strong bacterial mutagen, inducing 285000 revertants/nmol to *Salmonella* Typhimurium TA98 in the absence of S9 mix. 3,6-DNBeP shows genotoxicity *in vitro* to mammalian cells, and produces DNA damage in the cells of several organs in mice in the comet assay.

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improved the method of analyzing 3,6-DNBeP by combining one step of clean-up and fluorescence detection utilizing a two-dimensional HPLC system, and analyzed 3,6-DNBeP in tea leaves, which is a possible exposure source of 3,6-DNBeP in our daily life, and in human hair samples, as a possible biomarker of 3,6-DNBeP.

Tea is one of the most widely consumed beverages in the world. However, there have been reports on the detection of associated environmental contaminants, *e.g.*, polycyclic aromatic hydrocarbons (PAHs),[7–9] nitrated PAHs (NPAHs),[10] and dioxins[9,11] in tea leaves. Lin *et al.* reported that plants absorbed PAHs in air and surface soil via their leaves and roots, respectively, and accumulated PAHs in their leaves.[8] These reports suggest that environmental 3,6-DNBeP might be absorbed in tea plants, and might accumulate in their leaves such that 3,6-DNBeP in tea leaves could be one of the exposure sources.

Since 3,6-DNBeP was found to be widely distributed in surface soil and airborne particles,[6] humans are thought to be exposed to 3,6-DNBeP in daily life. Therefore, it is necessary to reveal the exposure level to 3,6-DNBeP. However there are no report on the exposure level to 3,6-DNBeP. PAHs are well-known environmental mutagens/carcinogens, which have been detected in surface soil and ambient air, and human hair has been utilized as a biological material to assess exposure levels to PAHs.[12] Moreover, drugs in hair have a long half-life compared with those in urine or blood, and hair samples are easily obtained. There is a possibility that 3,6-DNBeP in human hair is a biomarker of exposure to 3,6-DNBeP. To clarify whether hair is a promising material to monitor the level of exposure to 3,6-DNBeP in daily life, 3,6-DNBeP in human hair samples was quantified by the improved analytical method described in this study.

MATERIAL AND METHODS

Reagents —— 3,6-DNBeP (CAS 847862-64-0) was synthesized as described previously.[1] HPLC-grade acetonitrile and methanol were purchased from Nacalai Tesque (Kyoto, Japan). Sephadex LH-20 was purchased from Amersham Biosciences (Uppsala, Sweden). Silica gel (63–200 μm) was purchased from Merck (Darmstadt, Germany). All other reagents were of analytical grade.

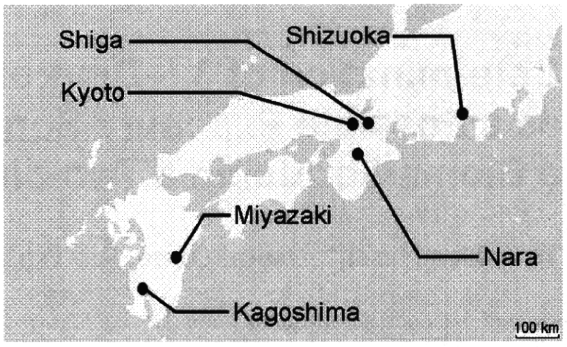


Fig. 2. Growth Sites of Tea Leaves
The tea leaves were grown in Kagoshima, Miyazaki, Nara, Kyoto, Shiga, and Shizuoka prefectures.

Sampling and Extraction of Tea Leaves —— Tea leaves, grown in Kagoshima, Miyazaki, Nara, Kyoto, Shiga, and Shizuoka prefectures in Japan (Fig. 2), were purchased at general markets. Ten g of each tea leaf was set in a Soxhlet extraction apparatus, and extracted using acetone/chloroform (1/1, v/v) for 24 hr. The extracts of the leaves were filtered through No. 5C filter papers and evaporated to dryness.

Clean-up of Extracts of Tea Leaves —— Organic extracts of tea leaves were dissolved in 10 ml of a mixture of methanol/chloroform (1/1, v/v) and 2.5 ml of the sample solution was applied to a glass column (400 × 20 mm I.D.) filled with Sephadex LH-20 in methanol/chloroform (1/1, v/v) as a mobile phase. Eluate was collected with 20 ml fractions. The elution volume of 100–120 ml was evaporated to dryness.

Organic extracts eluted in the elution volume of 100–120 ml from tea leaf samples were dissolved in 1 ml of chloroform completely and three aliquots were applied to three open columns (220 × 10 mm I.D.), which were filled with silica gel activated for 18 hr at 160°C and then deactivated with distilled water (7.4%, w/w). In order to remove interfering materials, the extracts were eluted with 20 ml of *n*-hexane, 20 ml of *n*-hexane/toluene (9/1, v/v), 20 ml of *n*-hexane/toluene (2/1, v/v), 20 ml of *n*-hexane/toluene (1/1, v/v), and 30 ml of toluene. 3,6-DNBeP was eluted in the toluene fraction. Toluene fractions were evaporated to dryness and the residues were dissolved in 0.5 ml of 70% acetonitrile. Then, 0.45 ml of these sample solutions was applied to a Cosmosil $5C_{18}$-MS-II (5 μm particle size, 250 × 4.6 mm I.D., Nacalai Tesque) for HPLC with 70% acetonitrile as a mobile phase at a flow rate of 0.7 ml/min. The eluates from

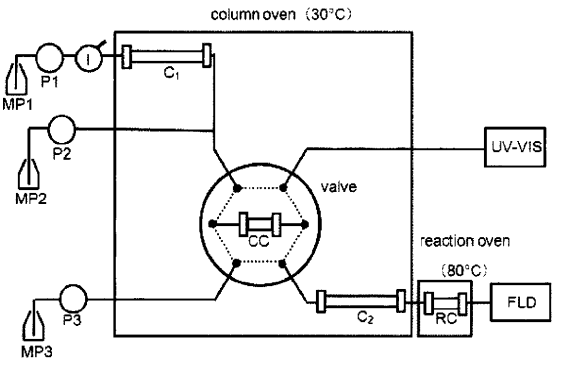
30.1 to 35.1 min were collected as 3,6-DNBeP fractions, since 3,6-DNBeP elutes at a retention time of 32.1 min. After evaporation, the 3,6-DNBeP fractions were dissolved in 0.5 ml of 85% ethanol, and 0.2 ml of the solution was applied on the two-dimensional HPLC system. HPLC procedures were carried out at 30°C. Eluates were monitored for UV absorption.

Sampling and Extraction of Hair Samples —— Hair samples used in this study were collected from 8 volunteers; 3 male smokers, 3 male non-smokers, and 2 female non-smokers with their informed consent following the Declaration of Helsinki and with approval from the Ethical Practices Committee of Kyoto Pharmaceutical University. Hair was obtained by general hair cutting; approximately 3–10 g of hair was collected. Hair samples were stored in a deep freezer at −80°C until extraction. Three g of hair was washed with 100 ml of 0.1% sodium dodecyl sulphate, 100 ml of dionized water four times, and once with 50 ml of ethanol using an ultrasonic apparatus for 5 min each time.[13,14] The washed hair samples were dried at room temperature. One hundred ml of 1N NaOH was added to the dried hair and this solution was heated at 100°C for 45 min in an oil bath. After standing to cool, the supernatant of the solution, centrifuged at 3000 rpm for 10 min, was filtered through Advantec Toyo No. 5C filter papers (Toyo Roshi Kaisha, Tokyo, Japan). The filtrates, neutralized to pH 7–9 with 6N HCl, were extracted by liquid-liquid extraction with 100 ml twice and 50 ml of ethylacetate and the extracts were evaporated to dryness.

Spectrophotometric characterization of melanin in hair samples was carried out.[13,15] Each hair sample (1 mg) or sepia melanin (1 mg) was dissolved in 1 ml of a mixture of Soluene-350 (PerkinElmer Life and Analytical Sciences, Boston, MA, U.S.A.) and water (9/1, v/v), followed by heating at 95°C for 45 min. Optical density was observed at 500 and 650 nm (A_{500} and A_{650}). A_{500} indicates the quantity of total melanin, and the ratio A_{650}/A_{500} equals the ratio of eumelanin to total melanin in hair samples.

Clean-up of 3,6-DNBeP in Hair Extracts —— Organic extracts from hair samples were purified by the silica gel column and Cosmosil $5C_{18}$-MS-II column continuously, as described above.

HPLC Analysis of 3,6-DNBeP in Tea Leaves and Hair Extracts —— 3,6-DNBeP fractions from tea leaves and hair extracts, which were prepared as described above, were dissolved in 0.5 ml of 85% ethanol and 0.2 ml of the solution was analyzed



Time	Valve position	P2
0–13.2 min	Position 1	0 ml/min
13.2–15.7 min	Position 0	4 ml/min
15.7–60 min	Position 1	0 ml/min

Fig. 3. Structure of the Two-dimensional HPLC System

The HPLC system is composed as described below. C1: Luna 5μ Phenyl-Hexyl (5 μm particle size, 250 × 4.6 mm I.D.), CC: Cosmosil $5C_{18}$-AR-II (5 μm particle size, 10 × 4.6 mm I.D.), C2: Wakosil PAHs (5 μm particle size, 250 × 4.6 mm I.D.), MP1: 95% methanol, MP2: ultra-pure water, MP3: 95% methanol, P1: 0.7 ml/min, P2: 0 ml/min (4 ml/min during t_R = 13.2–15.7 min), P3: 0.5 ml/min, RC: NP pak RL (< 150 μm particle size, 35 × 4.6 mm I.D.), UV-VIS: UV-VIS detector, FLD: fluorescence detector, I: injector.

using the two-dimensional HPLC system, which consisted of a Luna 5μ Phenyl-Hexyl (5 μm particle size, 250 × 4.6 mm I.D., Phenomenex, Torrance, CA, U.S.A., C1), Cosmosil $5C_{18}$-AR-II (5 μm particle size, 10 × 4.6 mm I.D., Nacalai Tesque, CC), and Wakosil PAHs column (5 μm particle size, 250 × 4.6 mm I.D., Wako Pure Chemical, Osaka, Japan, C2) as stationary phases and 95% methanol (MP1), ultra-pure water (MP2), and 95% methanol (MP3) as mobile phases (Fig. 3). At the starting condition, 95% methanol (MP1), ultra-pure water (MP2), and 95% methanol (MP3) were pumped at flow rates of 0.7 (P1), 0 (P2), and 0.5 (P3) ml/min, respectively. The flow rates of MP1 and MP3 were fixed during the analysis. The switch valve was set in position 1 at the starting condition. During 13.2–15.7 min, ultra-pure water (MP2) was pumped at a flow rate of 4 ml/min and the valve was switched to position 0. After 15.7 min, MP2 and the switch valve were returned to the starting condition. An NP pak RL reducer column (< 150 μm particle size, 35 × 4.6 mm I.D., Jasco, Tokyo, Japan,

RC) was set at 80°C. 3,6-DNBeP was reduced to 3,6-diaminobenzo[*e*]pyrene (DABeP) by on-line reduction with the NP pak RL column at 80°C to be detected using a fluorescence detector. The fluorescence detector monitored fluorescence intensity with excitation and emission wavelengths of 319 and 443 nm, respectively. A UV-VIS detector monitored the absorbance at 254 nm in order to confirm the elution time of 3,6-DNBeP from the Luna 5μ Phenyl-Hexyl column (C1).

Authentic 3,6-DNBeP as a standard was also dissolved in 85% ethanol injected at four doses into the column in order to draw calibration curves. The calibration curves were drawn with the peak heights of 3,6-DABeP on the chromatograms. HPLC procedure was carried out at 30°C.

Quality Assurance —— 3,6-DNBeP was quantified by the absolute calibration method. As described in previous studies,[5,6] the relative standard division (n = 5) of 3,6-DNBeP was less than 2%. When 3,6-DNBeP standard 0.2, 2, 20, and 200 pg was injected into the columns under the analytical conditions described above, the coefficient of variations was 0.064, 0.040, 0.006, and 0.022, respectively (each dose, n = 3). The calibration graph was obtained as $y = 111.46\,x$, and showed good linearity ($r^2 > 0.9999$) in the range of 0.2–200 pg. Quantification limit was 0.2 pg.

In order to calculate recovery rates, 3,6-DNBeP standard at 500 pg was applied on the Sephadex LH-20, silica gel, and Cosmosil 5C_{18}-MS-II columns. After eluates were evaporated to dryness, 3,6-DNBeP in the elutes was dissolved in 0.5 ml of 85% ethanol, and 0.2 ml of the solution was injected into the two-dimensional HPLC system. The peak heights of 3,6-DNBeP in the eluates were compared to those of corresponding 3,6-DNBeP standard. The recovery of 3,6-DNBeP on each column was sufficiently high at more than 98%. When 3,6-DNBeP standard was added to tea leaves and human hair extracts, and the extracts were purified as described above, the recoveries of 3,6-DNBeP standard in tea leaves and human hair extracts were 88% and 82%, respectively.

RESULTS

Analysis of 3,6-DNBeP with the Two-dimensional HPLC On-line Reduction by Fluorescence Detection

3,6-DNBeP was injected into the two-dimensional HPLC system (Fig. 3). 3,6-DNBeP, injected into the two-dimensional HPLC system, was separated from potential interfering materials in samples from tea leaves and human hair with the Luna 5μ Phenyl-Hexyl (C1), and eluted at the retention time of 14.2 min. At the starting condition, ultra-pure water (MP2) was not pumped with P2, and the switch valve was set at position 1. P2 started to pump at 4 ml/min 13.2 min after the injection of 3,6-DNBeP. At the same time, the switch valve was turned to position 0. 3,6-DNBeP, eluted from the Luna 5 μ Phenyl-Hexyl (C1), was trapped in the Cosmosil 5C_{18}-AR-II (CC) while ultra-pure water (MP2) was pumped. The switch valve returned to position 1 and P2 was turned off at the analysis time of 15.7 min. 3,6-DNBeP, trapped in the Cosmosil 5C_{18}-AR-II (CC), was eluted using 95% methanol (MP3) and separated with the Wakosil PAHs (C2). Eluted 3,6-DNBeP was reduced with the NP pak RL to 3,6-DABeP, and 3,6-DABeP was detected by the fluorescence with the FLD. A typical chromatogram of authentic 3,6-DNBeP is shown in Fig. 4A. 3,6-DNBeP was detected as 3,6-DABeP at a retention time of 32.2 min on the chromatograms.

Purification of 3,6-DNBeP in Tea Leaves and Hair Extracts

Tea leaves were grown at geographically different sites, namely, Kagoshima, Miyazaki, Nara, Kyoto, Shiga, and Shizuoka prefectures in Japan (Fig. 2). Since extracts of tea leaves include various chemicals, it was necessary to purify 3,6-DNBeP in the extracts. 3,6-DNBeP in tea leaf extracts was purified with the Sephadex LH-20 column, and 3,6-DNBeP was eluted in the elution volume 100–120 ml, with 100% recovery. The elution was applied on the silica gel columns with *n*-hexane and toluene as the mobile phases in a stepwise manner. 3,6-DNBeP was eluted in the toluene fraction and the recovery was 100%. The toluene fractions on the silica gel column were applied to the Cosmosil 5C_{18}-MS-II for HPLC. The retention time of 3,6-DNBeP on the Cosmosil 5C_{18}-MS-II with 70% acetonitrile as the mobile phase was 32.1 min and the recovery was 98%. Recovery on each purification step was quite high. 3,6-DNBeP fraction was applied to the two-dimensional HPLC system with on-line reduction and fluorescence detection.

The organic extracts from human hair were purified by the silica gel and Cosmosil 5C_{18}-MS-II columns, continuously, as described above.

Determination of 3,6-DNBeP Fractions from Leaves and Human Hair with the Two-dimensional HPLC

3,6-DNBeP fractions from tea leaves and human hair were injected into the two-dimensional HPLC system. 3,6-DNBeP from tea leaves and human hair was detected as 3,6-DABeP at a retention time of 32.2 min as shown in Fig. 4B and 4C, respectively. 3,6-DNBeP was detected in the analyzed tea leaf extracts in the range of 8–1823 pg/g of tea leaves (Table 1). The highest level of 3,6-DNBeP, 1823 pg/g of tea leaves, was detected in the leaves produced in Shizuoka prefecture.

In all hair samples were collected from the 8 volunteers including 3 smokers and 5 non-smokers. 3,6-DNBeP was detected in all hair samples in the range of 11–121 pg/g of hair and 86–1576 pg/mg of eumelanin (Table 2).

When blank-extract samples, which were purified in the same manners to both tea leaves and human hair, were applied to the two-dimensional HPLC system, no peaks of 3,6-DNBeP were observed in the chromatograms. When the entire process from extraction to analysis was repeated, the difference of quantification results was less than 8%.

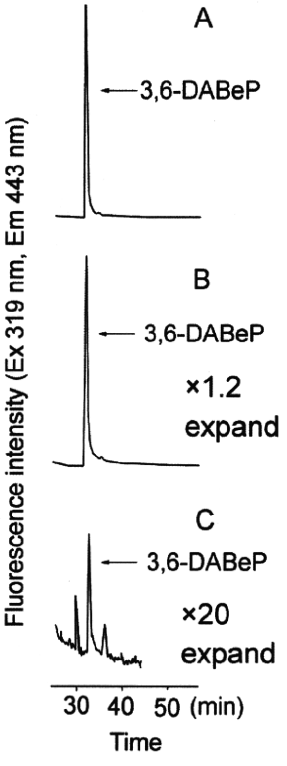


Fig. 4. Chromatograms of 3,6-DNBeP

Typical chromatograms of authentic 3,6-DNBeP (A), and 3,6-DNBeP in tea leaves (B) and human hair (C). 3,6-DNBeP was well purified using the silica gel and Cosmosil $5C_{18}$-MS-II column and the two-dimensional HPLC system. 3,6-DNBeP in the extracts was purified with the Luna 5μ Phenyl-Hexyl (C1), and continuously eluted from the Wakosil PAHs (C2) at the retention time of 32.2 min. 3,6-DNBeP was reduced to 3,6-DABeP by on-line reduction with the NP pak RL, and detected as 3,6-DABeP using the fluorescence detector. The peak heights of the chromatograms of authentic 3,6-DNBeP, and 3,6-DNBeP in tea leaves and human hair correspond to 200, 160, and 16 pg of 3,6-DNBeP, respectively.

Table 1. Amount of 3,6-DNBeP in Tea Leaves Purchased in General Markets

Sampling site	Amount (pg/g of tea leaves) MV ± S.D.
Shizuoka	1823 ± 1
Shiga	15 ± 0
Kyoto	8 ± 2
Nara	291 ± 21
Miyazaki	122 ± 17
Kagoshima	42 ± 8

Table 2. Amount of 3,6-DNBeP in Hair Collected from Smokers and Non-smokers, and from Males and Females

hair	Amount (pg/g of hair) MV ± S.D.	Amount (pg/mg of eumelanin) MV ± S.D.	Smoker or non-smoker	Sex
001	56 ± 10	777 ± 142	Smoker	male
002	31 ± 6	656 ± 132	Smoker	male
003	11 ± 1	86 ± 5	Smoker	male
004	32 ± 10	407 ± 122	non-smoker	female
005	121 ± 14	1576 ± 899	non-smoker	male
006	29 ± 15	145 ± 37	non-smoker	male
007	11 ± 10	252 ± 236	non-smoker	female
008	15 ± 11	201 ± 107	non-smoker	male

DISCUSSION

In a previous study, we developed a method of analyzing 3,6-DNBeP for environmental samples, *i.e.*, surface soil, airborne particles, and incinerator dust. In this method, 3,6-DNBeP in extracts from the samples was cleaned-up with a silica gel open column and two steps of HPLC separation, and 3,6-DNBeP was analyzed by HPLC using fluorescence detection of 3,6-DABeP after on-line reduction with a catalyst column. At each step of clean-up, eluates from the column, corresponding to 3,6-DNBeP, were treated manually, *i.e.*, evaporated to dryness, redissolved, and injected into the column in the next step. These handlings are laborious when many samples are analyzed and may cause a loss of samples and deviation of resulting values. In the two-dimensional HPLC system developed in this study, the second HPLC separation for clean-up and HPLC analysis using on-line reduction with the catalyst column were combined. Using this new system, we can decrease the number of handlings. The peaks in the chromatograms of 3,6-DNBeP in tea leaves and human hair indicated that it was well purified with the analytical method. When we applied surface soil and airborne particle extracts, which were purified using the silica gel and Cosmosil $5C_{18}$-MS-II columns continuously, to the two-dimensional HPLC system, 3,6-DNBeP in surface soil and airborne particles was also well purified, and detected as single peak on the chromatograms (data not shown). It is concluded that 3,6-DNBeP in complex mixtures of organic chemicals can be analyzed efficiently with the two-dimensional HPLC system employed in this study.

3,6-DNBeP was detected in all tea leaves in the range of 8–1823 pg/g of leaves. This is the first report on the detection of 3,6-DNBeP in tea leaves. The amount of 3,6-DNBeP detected in tea leaves differed depending on the growth site of tea leaves. As shown in Table 1, the amount of 3,6-DNBeP detected in tea leaves collected in Shizuoka prefecture was much higher than those of other sites. Lin *et al.* reported that plants absorbed PAHs in air and surface soil via their leaves and roots, respectively, and accumulated PAHs in their leaves.[8] 3,6-DNBeP in surface soil and air might be taken into leaves and 3,6-DNBeP detected in tea leaves might be influenced by the contamination levels of surface soil and air at growth sites of the tea leaves. However, the correlation between 3,6-DNBeP in tea leaves and that in surface soil and air around tea fields is still unknown. It is necessary to investigate the comprehensive distribution of 3,6-DNBeP not only in tea leaves but also in surface soil and airborne particles around tea fields.

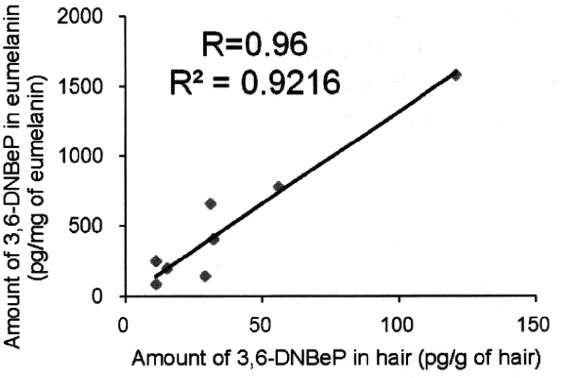


Fig. 5. Correlation between the Amount of 3,6-DNBeP per Gram of Hair and That per Milligram of Eumelanin

The coefficients of correlation and determination were quite high at 0.96 and 0.9216, respectively. The positive correlation between the amount of 3,6-DNBeP per gram of hair (*X* pivot) and that per milligram of eumelanin (*Y* pivot) can be observed in this correlation graph.

3,6-DNBeP was detected in all hair samples in the ranges of 11–121 pg/g of hair and 86–1576 pg/mg of eumelanin. This is the first report on the detection of NPAHs in hair. The correlation between the amount of 3,6-DNBeP per gram of hair (*X* pivot) and that per milligram of eumelanin (*Y* pivot) is shown in Fig. 5. The hair collected in this study did not include gray/white hair. The amounts of 3,6-DNBeP in hair shown per gram of hair and that per milligram of eumelanin showed a very strong correlation (r = 0.96), and this result suggested the possibility that 3,6-DNBeP binds to eumelanin in hair. In our previous study, we reported that 3,6-DNBeP widely exists in surface soil and airborne particles. Humans are thought to be exposed to 3,6-DNBeP via breathing in daily life. There is the possibility that 3,6-DNBeP detected in hair might reflect human exposure to 3,6-DNBeP, as reported for PAHs in hair. Since 3,6-DNBeP was detected in tea leaves, tea might be another souse of exposure to 3,6-DNBeP in our daily life.

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