# Evaluation of the Relationship between Exposure to 1-Nitropyrene and Oxidative Stress and Genetic Damage Marker in Humans

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I'm going to take the next path in my life.

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## Abstract

### Evaluation of the Relationship between Exposure to 1-Nitropyrene and Oxidative Stress and Genetic Damage marker in Humans

1-Nitropyrene (1-NP), a specific nitro-polycyclic aromatic hydrocarbons (nitro-PAHs), is a significant marker for diesel exhaust exposure, which is associated with increased risks of cardiovascular diseases, respiratory diseases, even lung cancer. The study's goal is to find out how much 1-NP is in the air in different places and times of the year and to see if there is a link between these levels and metabolites, signs of oxidative stress, and genetic damage in people.

The research involved 43 adults from Cheongju, South Korea, who were exposed to diesel exhaust particles (DEPs) near industrial complexes, incinerators, and clean rural areas. Air and biological samples were collected to once in winter and once in summer, respectively. measure 1-NP atmospheric concentration and its metabolites in urine. And oxidative stress markers in urine and genetic damage marker in sputum.

Results showed significant seasonal and regional variations in 1-NP concentrations. Winter had higher concentrations of 1-NP than summer, and regional variations were observed during the winter season. The oxidative stress marker, malondialdehyde (MDA), was also higher in summer than winter. A positive correlation was found between air 1-NP concentrations and the metabolite 6-hydroxy-1-nitropyrene (6-OHNP), while a weak negative correlation was observed between 1-NP and MDA. Additionally, 1-N-

acetylamino-pyrene (1-NAAP) showed a weak positive correlation with both MDA and 8-hydroxydeoxyguanosine (8-OHdG) oxidative stress indicators. This study identified significant seasonal variations in air 1-NP concentrations. A correlation was established between 1-NP and its main metabolite, 6-OHNP. However, no clear relationship was found between atmospheric 1-NP levels and markers of oxidative stress or genetic damage. Conversely, a relationship was observed between 1-NAAP concentrations and the oxidative stress markers MDA and 8-OHdG. These results underscore the necessity for further research into the human metabolism of 1-NP to elucidate its pathways of toxicity in humans.

Key words: 1-nitropyrene, 6-hydroxy-1-nitropyrene, n-acetyl-1-aminopyrene, oxidative stress, single nucleotide polymorphism

# I. Introduction

## Background

1-Nitropyrene, nitro-polycyclic aromatic hydrocarbons (nitro-PAH), has been suggested as a marker for Diesel exhaust particles (DEPs). It is crucial to emphasize that the creation of 1-NP is not primarily influenced by atmospheric photochemical processes [1,2]. The metabolic pathway of 1-NP is as follows (Figure 1) [3,4].

1-NP is hypothesized to induce genetic mutations and chromosomal aberrations [5,6]. Moreover, the IARC has categorized 1-NP as a Group 2A substance [7]. As 1-NP is predominantly generated within diesel engines, exposure to 1-NP is further associated with an augmentation of oxidative stress, inflammation, and endothelial dysfunction, which collectively contribute to an elevated risk of cardiovascular disease, respiratory disease, allergic sensitization, asthma, and even lung cancer [8-10].

Although it is recognized that no one analyte can accurately capture the intricate and changing makeup of diesel particulate matter in every situation, utilizing 1-NP as an indication has the potential to yield an accurate DE exposure assessment. Additionally, this method might provide a more realistic depiction of the carcinogenic qualities of diesel exhaust [11]. Quantification of urinary concentrations of 1-NP metabolites, encompassing 6-hydroxy-1-nitropyrene (6-OHNP), 1-aminopyrene (1-AP), and N-acetyl-1-aminopyrene (1-NAAP), constitutes a significant methodology for evaluating environmental exposure to 1-NP and DEPs.

Consequently, the atmospheric concentration of 1-nitropyrene tends to be higher during winter in Korea [12,13]. Therefore, the composition ratio of nitropyrene is likely

to vary based on the pollution source. 1-AP is correlation with 1-NP exposure in air [14]. However, detailed studies on the concentrations and composition ratios of nitro-PAHs in South Korea's atmosphere are scarce, particularly those analyzing variations by region or pollution source. Essentially, there is limited data on the extent of exposure of Koreans to nitro-PHAs.

Mitochondria are vulnerable to damage because they lack protective histones and have a diminished capacity for DNA repair. To compensate for this damage, they replicate mitochondrial DNA (mtDNA), thereby increasing the cell's mitochondrial DNA copy number (mtDNAcn) [15,16]. An analysis of mt DNA copy number offers a comprehensive evaluation of the toxic impacts of environmental pollutants. This includes the effects of unidentified substances and potential interactions among various contaminants [17]. A correlation of mtDNAcn and oxygen consumption rate demonstrated [18], Research in both humans and animals has verified that elevated absolute levels of wild type mtDNA genomes can mitigate the negative effects of pathogenic mtDNA mutations [19]. The potential of PAHs to inflict damage is particularly heightened in mtDNA, exhibiting an affinity for mtDNA that is 40 to 90 times higher than for nuclear DNA [20,21]. The metabolic activation of PAHs produces ROS capable of disrupting electron transport chains and causing damage to mtDNA. This process also contributes to substantially increased levels of oxidized guanine in mtDNA [22,23].

Even these facts, inhalation of airborne particulate matter can expose individuals to harmful PAHs such as B[a]P and 1-NP. The metabolism of these PAHs can generate ROS, which in turn can lead to changes in mtDNA copy number. 1-NP is notably the most studied compound within the nitroPAHs. Information regarding its metabolites and metabolic pathways is established. However, research delineating the

concentration thresholds at which human toxicity arises and the subsequent duration for post-exposure effects remains limited.

Based on the above, this study intends to measure 1-NP atmospheric concentrations across predicted variable regions and seasons. The objective is to methodically assess the relationship between these concentrations and the presence of metabolites, oxidative stress markers, and genetic damage in humans.

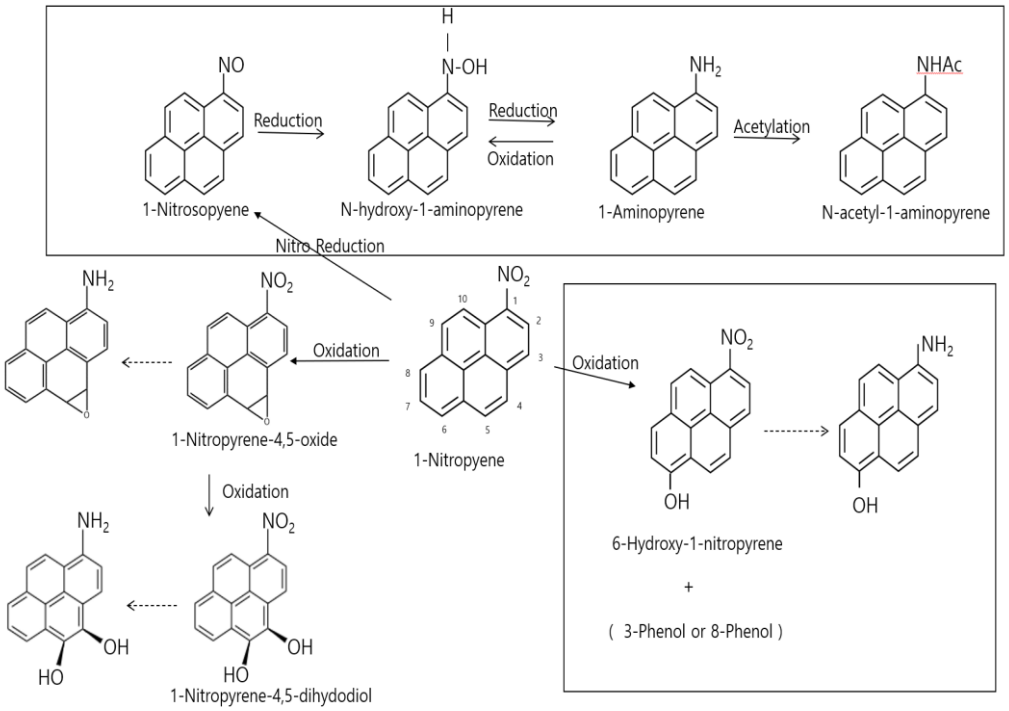


Figure 1. The main pathways of the metabolism of 1-NP *in vivo*

1-NP is metabolized in vivo [3,4]. The body in human metabolizes over 90% of the 1-NP by oxidation, resulting in the formation of 6-OHNP, 8-OHNP, and 3-OHNP. Less than 10% of the 1-NP is metabolized through nitrogen conversion, leading to the production of 1-AP or 1-NAAP [24,25].

# II. Objectives

To ascertain the correlation between ambient concentrations and urinary biomarkers known to metabolize 1-NP, the present study will measure its concentrations in the air and the respective biomarkers in exposed individuals' urine. Additionally, the study seeks to evaluate the correlation between air exposure, metabolites, oxidative stress, and the extent of genetic damage (Figure 2).

The detailed objectives of this study are as follows:

(1) To assess the concentrations of 1-NP in the air and its relationship with urinary biomarkers of exposure. And investigate whether the concentration of 1-NP varies depending on the area or season.
(2) To assess the exposure levels of 1-NP and measure the oxidative stress marker to determine their correlation.
(3) To assess the exposure levels of 1-NPe and evaluate the extent of genetic damage to determine their correlation.
(4) Upon a comprehensive analysis of the results, the research will validate the determinants influencing exposure to 1-NP and analyze the optimal metrics, coupled with the appropriate timing for sample collection.

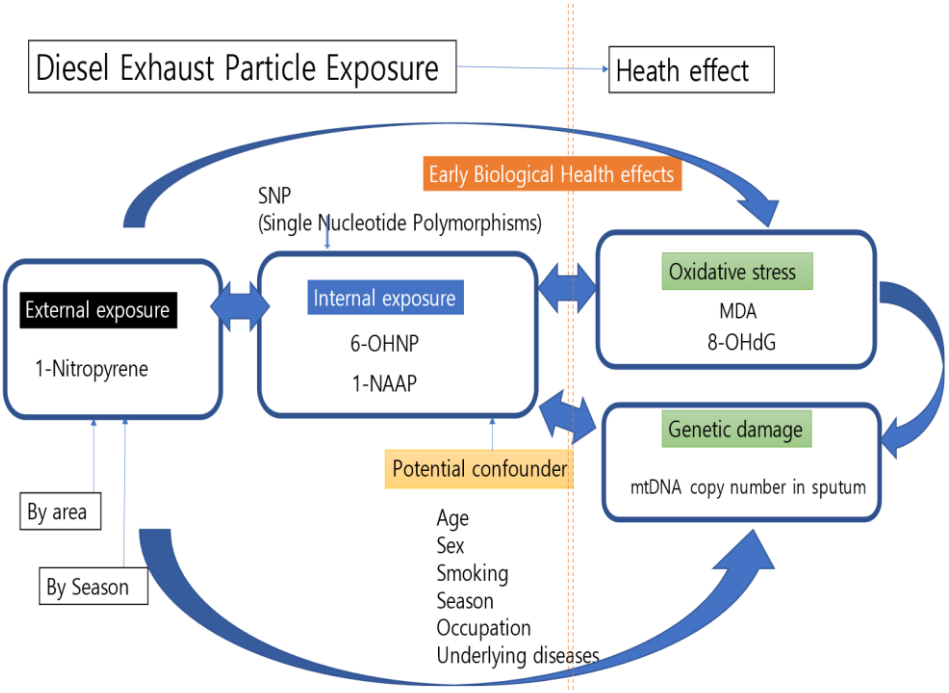


Figure 2. Relationship between 1-NP exposure and its metabolites and oxidative stress and genetic damage

# III. Materials and Methods

## 1. Study Participants

This study comprised a sample of 43 adult individuals residing in the vicinity of the industrial complex in Cheongju, which includes a trash incinerator, as well as in suburban regions. A qualitative methodology was employed to collect data on demographic characteristics, smoking behaviors, and daytime activity. This was done by distributing a questionnaire. Each participant submitted their initial morning urine sample, which was then followed by air sampling. Participants were given a comprehensive explanation of the study and gave written consent to continue with data collection. This work underwent review by the Institutional Review Board of Chungbuk National University, with approval numbers CBNU-201708-SDBR-0075 and CBNU202010-HRBR-0163.

The techniques used for atmospheric analysis of 1-NP, analysis of 1-NP metabolism in urine, analysis of oxidative stress markers, and analysis of mtDNAcn are the same as those described in the author's prior publications [26,27].

## 2. Measurement of Atmospheric 1-NP Concentration

Air and biological material were sampled during both the summer and winter seasons, specifically from November 2020 to August 2021. Sampling seasons were classified as winter (November to February) and summer (June to August). During both the summer and winter seasons, atmospheric air was collected using a personal air sampler (Apex Standard, SN0376420 Casella CEL, Bedford, UK) on two separate occasions.

A 37 mm polytetrafluoroethylene (PTFE) filter with a particle size of 2 μm, specifically the Teflo brand from Pall Corporation in Ann Arbor, MI, USA, was linked to a personal air sampler. The sampler was then fastened to the subject's collar and operated for a duration of 24 hours, drawing in air at a rate of around 3 liters per minute.

Following the sample process, the filters were extracted and transferred into a flask, where 2 mL of dichloromethane was subsequently introduced. The flask underwent vortex mixing for a duration of 1 hour and was thereafter subjected to ultrasonic extraction for a period of 30 minutes. The extract was thoroughly dehydrated and subsequently reconstituted in 300 μL of acetonitrile. A 100 μL portion of the solution was put into a two-dimensional high-performance liquid chromatography (HPLC) system equipped with a fluorescence detector (FD) to measure the amount of 1-NP [28]. The sample that was injected underwent elution using a clean-up column (Cosmosil, 5NPE, 150 × 4.6 mm i.d., 5 μm, Nacalai Tesque, Kyoto, Japan) together with a guard column (10 × 4.6 mm i.d.).

The conversion of 1-NP to 1-AP was achieved by using a reduction column (NPpak-RS, 10 × 4.6 mm i.d., Jasco, Tokyo, Japan) working at a temperature of 80 ℃. The clean-up and reduction columns were operated using a mobile phase composed of a mixture of ethanol and acetate buffer (pH 5.5) in a ratio of 95:5 (v/v). The mobile phase flowed at a rate of 0.2 mL/min. A fraction of 1-AP that was separated from the reduction column using the mobile phase was mixed with 30 mM ascorbic acid, flowing at a rate of 1.6 mL/min, and subsequently collected on the concentration column (Spheri-5 RP-18, 30 × 4.6 mm i.d., 5 μm, Perkin Elmer, MA, USA).

The concentrated fraction was subjected to chromatographic separation using

two Inertsil ODS-P columns (250 × 4.6 mm i.d., 5 μm, GL Sciences, Tokyo, Japan) together with their guard column (10 × 4.6 mm i.d.) in a sequential manner. All columns, with the exception of the reduction column, were kept at a temperature of 20 ℃. The separation columns were subjected to a controlled gradient elution using a 10 mM imidazole solution with a pH of 7.6.

Ultimately, the isolated substances were identified by use of the dual-channel FD. The excitation and emission wavelengths for 1-AP were 260/420 nm and 254/425 nm, respectively. The quantities of 1-NP adsorbed on the filter were measured using High Performance Liquid Chromatography (HPLC) with a fluorescence detector, following the reduction of 1-NP and 2-NP to 1-AP and 2-AP, respectively. The reference material peaks of 1-aminopyrene and 2-aminopyrene were successfully separated using a dual-channel fluorescence detector. Additionally, the peaks of these two substances were also detected in the actual sample (Figure 3).

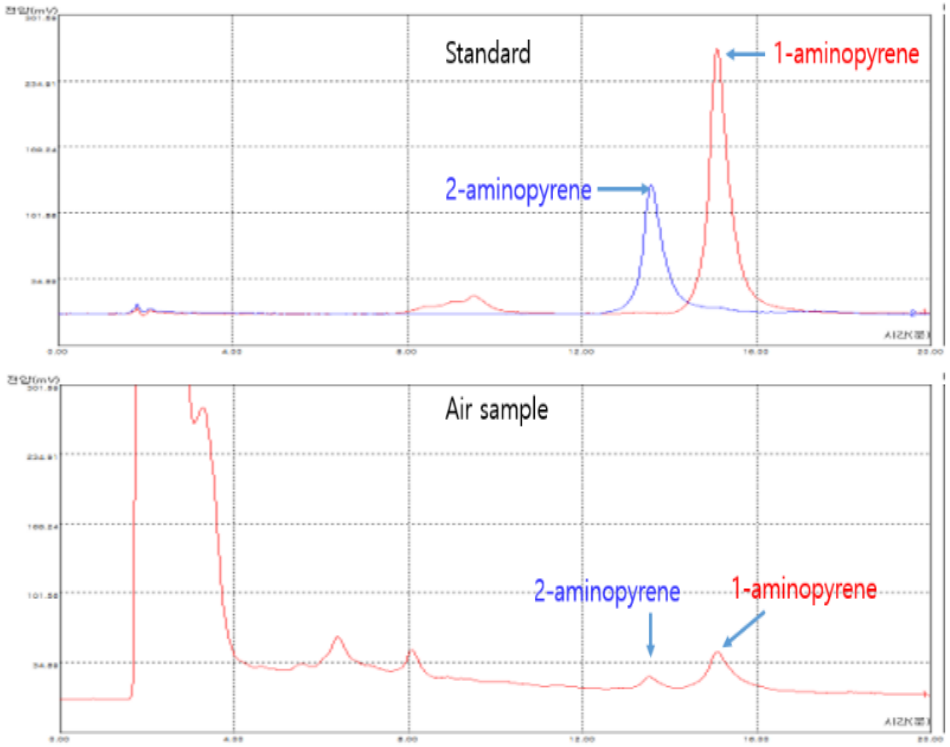


Figure 3. Air sample chromatograms of 1-aminopyrene (1-AP)

## 3. Measurement of the Metabolites of 1-NP in Urine

In order to evaluate the extent of exposure to 1-NP, the study analyzed the urine concentration of 1-NP metabolites, specifically 6-OHNP and 1-NAAP. Regarding the measurements of 6-OHNP and 1-NAAP concentrations. The eluent was subjected to a nitro-reduction process by putting it through the online reduction column (NPpak-RS). The amounts of 6-Hydroxy-1-aminopyrene were determined using the identical HPLC equipment and column. The excitation/emission wavelength of the fluorescence detector (FD) was 285 nm/428 nm. The limit of quantification for the concentration of 6-OHNP, as determined by this approach (Figure 4), was 10.8 pg/L.

At ambient temperature, a volume of 200 μL of acetic anhydride was introduced into the eluent that had undergone nitro-reduction by passing it via an online reduction column. The reaction required a duration of one hour to reach completion. Following the extraction process using 1 mL of dichloromethane, the resulting extract was subjected to drying using N2 gas. Following dissolution in 200 μL of 50% methanol, the solution was passed through a 13 mm polytetrafluoroethylene syringe filter (PTFE-H, with a pore size of 0.2 μm, manufactured by Hyundai Micro in Seoul, Republic of Korea) into a vial. The excitation and emission wavelengths of the FD were 273 nm and 385 nm, respectively [29]. The method used to determine the concentration of 6-OHNP had a quantitation limit of 4.6 pg/L. Since the study employed an amidification approach to convert 1-AP to 1-NAAP, the measured concentration of 1-NAAP in this study represents the combined concentrations of both 1-AP and 1-NAAP. The urinary concentrations of those substances were corrected by the urinary creatinine concentration.

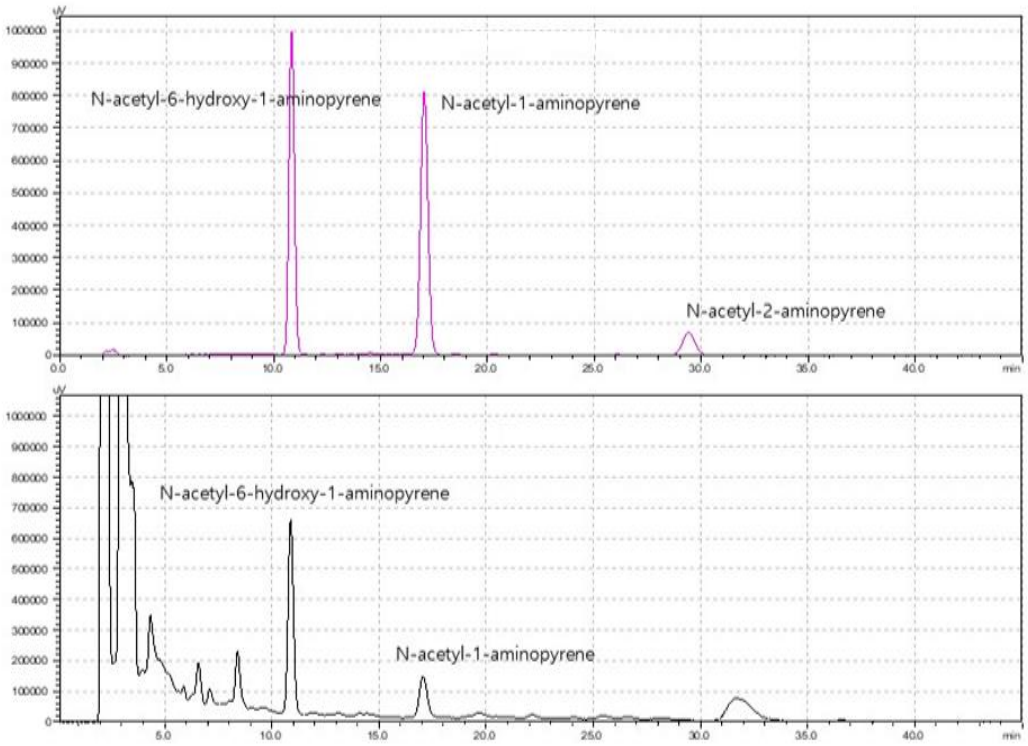


Figure 4. Urine sample chromatograms of n-acetyl-1-aminopyrene (1-NAAP)

## 4. Measurement of the Oxidative Stress Markers

### 4.1. 8-Hydroxydeoxyguanosine (8-OHdG) Urinary Concentration Measurement

The concentration of 8-OHdG in urine was determined using an ELISA kit (New 8-OHdG Check, JaICA, Fukuroi, Japan) following the instructions provided by the manufacturer. The measurement method can be succinctly described as follows: a) The urine samples and standards were introduced to a microtiter plate coated with 8-OHdG; b) Following a wash, an enzyme-labeled secondary antibody was added to the plate; c) Any unbound HRP-conjugated secondary antibody was removed through washing, and the substrate solution was added; and d) Lastly, after the reaction was stopped, the absorbance was measured at 450 nm.

### 4.2. Malondialdehyde (MDA) Urinary Concentration Measurement

The measurement of MDA levels was conducted using a microplate reader [30] at three distinct wavelengths: fluorescence (λ-ex 530 nm and λ-ex 550 nm); λ-ex 515 nm and λ-ex 553 nm; and absorbance (532 nm). The MDA concentration was determined utilizing the subsequent equation: The MDA level (μM) can be calculated using the equation: MDA level (μM) = −0.282 + 1.830 × (the MDA level measured at the fluorescence wavelengths of λ-ex 530 nm and λ-em 550 nm, μM) -0.685 multiplied by the MDA level recorded at the fluorescence wavelengths of λ-ex 515 nm and λ-em 553 nm, in units of μM, plus 0.035 multiplied by the MDA level measured at the absorbance wavelength of 532 nm, in units of μM.

## 5. Determination of the Copy Number of Mitochondrial DNA in Sputum

The DNA was isolated from 200 mL of sputum using the QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany), following the directions provided by the manufacturer.

The mtDNAcn was measured using a quantitative polymerase chain reaction (PCR) technique known as real-time PCR, as outlined in the study conducted by Wu et al. (2019) [31]. A DNA template with a mass of one hundred nanograms was used. The study employed gene-specific primers for the mitochondria: ND1 forward primer (5'-cctagccgtttactcaatcct-3'), ND1 reverse primer (5'-tgatggctagggtgacttcat-3'), ND2 forward primer (5'-aacccgtcatctactctaccatct-3'), and ND2 reverse primer (5'-gcttctgtggaacgagggtttatt-3'). Furthermore, the study incorporated 18s rRNA primers as a point of reference. These primers comprise an 18s rRNA forward primer (5'-cttagagggacaagtggcgttc-3') and an 18s rRNA reverse primer (5'-cgctgagccagtcagtgtag-3') [26].

The PCR reaction mixture was prepared by combining 50 microliters of Accupower 2X GreenStar qPCR Master Mix and DEPC-DW, all sourced from Bioneer in Daejeon, Korea. The real-time PCR was performed using the CFX Opus Real-Time PCR Systems manufactured by Bio-Rad, based in Hercules, CA, USA. The PCR cycling settings were established according to the following specifications: The protocol began with an initial denaturation phase at 95 °C for 5 minutes. This was followed by 40 cycles of denaturation at 95 °C for 15 seconds, annealing at 54 °C for 20 seconds, and extension at 72 °C for 20 seconds. The program concluded with a final denaturation step at 95 °C for 15 seconds.

The CT values for both mtDNA and 18s rRNA were derived from the real-time PCR software. The subsequent calculation for ND1 mtDNA and ND2 mtDNA levels relative to 18s rRNA DNA was conducted using the designated formula:

$\Delta C_T$ = (18s rRNA $C_T$ – mtDNA $C_T$)

Relative mitochondrial DNA content = $2 \times 2^{\Delta C_T}$

The relative content of ND1 mtDNA and ND2 mtDNA was calculated, and the mean value was used as the mtDNAcn value [32].

## 6. Statistical Analysis

To mitigate the impact of outliers on statistical analysis results, any measures that deviated from the mean by more than three standard deviations were replaced with values equal to the mean + three standard deviations.

The mean value difference between the two groups was assessed using an unpaired t-test. Additionally, Mood's median test was employed to investigate the seasonal regional differences in 1-NP concentrations in air, 6-OHNP in urine, 1-NAAP, 8-OHdG, and MDA measurement based on the difference in median value. The one-way analysis of variance (ANOVA) was employed to compare the mean values across the three groups, while the Mood's test was utilized to examine the median values.

A regression analysis was performed to determine the correlation between the level of 1-NP exposure in the air and the concentrations of its metabolites in urine. Additionally, the investigation aimed to investigate the relationship between urinary 8-OHdG and MDA concentrations, as well as mtDNAcn in sputum.

A general linear model was employed to statistically examine the impact of 1-NP exposure on the concentrations of 8-OHdG and MDA in urine, as well as the mtDNAcn in sputum. This analysis accounted for age, sex, smoking habit, and levels of other exposure markers.

The statistical analysis was conducted using SAS On Demand, a software platform developed by SAS Institute in Cary, NC, USA. A p-value below 0.05 was taken as evidence of statistical significance, indicating a high probability that the detected correlations were not due to chance.

# IV. Results

## 1. 1-NP Exposure

### 1) 1-NP Concentration in Air

The study has 43 participants with an average age of 63.67 years, suggesting a primarily elderly demographic. Out of the entire group, 18 individuals (41.9%) are males, while 25 individuals (58.1%) are females, with a slightly larger number of female participation. Among these individuals, 27 self-identify as non-smokers, whereas 7 (16.2%) assert that they are currently smokers. From a geographical standpoint, 18 individuals (41.9%) reside in pristine rural environments, 9 individuals (21.0%) live in areas characterized by industrial complexes, and 16 individuals (37.2%) live in close proximity to incineration plants (Table 1). The analysis of indoor air samples, as outlined in Table 2, indicates that the average concentration of 1-Nitropyrene (1-NP) is 0.869 pg/m³, with a standard deviation of 0.662 pg/m³ (Table 2). Significantly, there is a pattern of seasonal modulation, where concentrations reach their highest point during the winter months (p-value < 0.000).

Although spatial analysis did not indicate noticeable variations in average concentrations among different places, both the mean and median values clearly demonstrate a considerable seasonal discrepancy throughout the winter. The phenomenon of increased winter concentrations is consistently observed in all regions.

Localized analysis reveals a higher average concentration in the vicinity of incinerators, although the difference from the overall average and middle values is not statistically significant. Nevertheless, the winter season once again emphasizes notable variances that are peculiar to some areas. In contrast, during the summer

season, there are minimal variations in area, possibly because of the significantly lower average values. The observations are further supported by a thorough unpaired t-test, which takes into account both seasonal and area-based parameters. This analysis yields statistically significant results, as shown in Table 2.

### 2) 1-NP Metabolites in Urine

This study examines the level of human exposure to 1-NP by evaluating its metabolites, 6-OHNP and 1-NAAP, in urine. The average concentration of 6-OHNP in urine is 2.931 ng/g creatinine, with a standard deviation of 4.963 ng/g creatinine and a median of 1.280 ng/g creatinine (Table 3). Winter has higher seasonal concentrations in comparison to summer, which is a statistically significant finding. Although there are no notable changes in average values between the places, winter concentrations exhibit large variances, especially between pristine rural regions and industrial complexes.

The average concentration of 1-NAAP is 0.253 ng/g creatinine, with a measure of variability of 0.341 ng/g creatinine and a middle value of 0.190 ng/g creatinine (Table 4). There are no notable variations observed based on seasons or regions. Nevertheless, even taking into account both criteria, there are noticeable variances between incinerators and clean rural regions, with median values indicating substantial variations based on seasonality and regionality.

After examining the impact of both seasonal and location-specific factors, it is evident that there is a consistent tendency of increased concentrations during the summer in all places. The study determined that there were statistically significant seasonal fluctuations in the median values. Substantial disparities were noted between pristine rural regions and industrialized complex regions, as well as between pristine

rural regions and places with incinerators. Regarding average values, a substantial distinction was found between unpolluted rural regions and places with incinerators, although no noteworthy disparities were noted in comparisons between other areas. In winter, the vicinity surrounding the incinerator exhibited elevated mean and median values, but the remaining two sectors displayed higher mean and median values during the summer.

## 2. Oxidative Stress Markers and Genetic Damage Marker

### 1) Oxidative Stress Markers in Urine

In order to assess the extent of oxidative stress in humans, the levels of 8-OHdG in urine, which serves as a measure of gene damage, and urinary MDA, which indicates lipid damage, were quantified. The average concentration of 8-OHdG in urine is 1.287 ng/g creatinine, with a measure of how much the values vary from this average being 0.779 ng/g creatinine, and the middle value being 1.119 ng/g creatinine. The information is presented in Table 5. The seasonal variations between the pristine natural area and the industrial complex area are statistically significant. The average value observed during the summer season is greater than the value observed during the winter season.

The average concentration of MDA in urine is 2.147 ng/g creatinine, with a measure of how much the values vary from the average of 1.282 ng/g creatinine, and a value that represents the middle of the data set at 1.863 ng/g creatinine. The following information is shown in Table 6. The variation in MDA concentration across seasons is statistically significant. The average MDA value is greater during the summer compared to the winter, indicating statistically significant variations. In

winter, there are statistically significant variations between different areas. When analyzing the data, taking into account both seasonal and geographical factors, it is shown that both the clean natural environment and the industrial complex area have significant statistical value in terms of both mean and median comparisons.

### 2) Mitochondrial DNA Copy Number in Sputum

To assess human genetic damage, the mitochondrial DNA copy counts in sputum were quantified. The link between exposure to air and the degree of DNA damage in blood and sputum was determined by assessing the extent of mitochondrial DNA damage, which is a typical effect of PAHs. The findings are shown in Table 7 and no significant seasonal variations were seen from a statistical standpoint.

## 3. Relationship between Exposure to 1-NP and the Levels of Oxidative Stress Markers and Genetic Damage Marker

Table 8 presents the results of the correlation analysis conducted to examine the relationship between the levels of 1-NP in the air, 6-OHNP in urine, 1-NAAP in urine, 8-OHdG in urine, MDA in urine, and mtDNAcn in sputum. The data was subjected to correlation analysis, which was conducted separately for overall and seasonal analyses. The content of 1-NP in the air and 6-OHNP in urine showed statistically significant differences in both general and winter periods. A statistically significant weak negative connection was observed between 1-NP and MDA. Additionally, there was a modest positive connection observed between metabolite 1-NAAP and the oxidative stress markers MDA and 8-OHdG, which was statistically significant. Statistical significance was seen only in the link between 1-NP air concentration and 6-OHNP

during the winter season, as indicated by the analysis results.

Table 9 examined smoking status, cumulative smoking amount, ambient 1-NP concentration, and 1-NP metabolites as potential explanatory variables for oxidative stress and genetic damage markers in a generalized linear model. The findings demonstrated that both smoking status and 1-NAAP had a substantial impact on 8-OHdG levels during winter. Additionally, smoking amount, smoking status, and 1-NAAP seemed to influence MDA levels during summer. Nevertheless, no noteworthy factors were detected in connection with mtDNAcn in sputum.

Table 1. General characteristics of the study participants

| Variables | | N (%) | Mean ± S D. |
|---|---|---|---|
| Age | | 43 (100%) | 63.67 ± 12.2 yr |
| Sex | Male | 18 (41.9%) | |
| | Female | 25 (58.1%) | |
| Smoking habit | Never smokers | 27 (62.8%) | |
| | Ex-smokers | 9 (20.9%) | |
| | Current smokers | 7 (16.2%) | |
| Residential area | Rural area | 18 (41.9%) | |
| | Industrial complex area | 9 (21.0%) | |
| | Incinerator area | 16 (37.2%) | |

Table 2. Concentration of 1-NP in air by area and season (in pg/m$^3$)

| | | Total | | | | Winter [a] | Summer [b] | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Diff | p-value | | | Diff | p-value |
| All area | N | 86 | | | | 43 | 43 | b-a | |
| | Mean(SD) | 0.869(0.662) | | | | 1.314(0.548) | 0.424(0.427) | -0.890 | 0.000* |
| | Median | 0.678 | | | | 1.195 | 0.240 | -0.955 | 0.000* |
| Clean rural area [1] | N | 36 | 2-1 | | | 18 | 18 | | |
| | Mean(SD) | 0.733(0.521) | | 0.207 | 0.213 | 1.022(0.418) | 0.444(0.456) | -0.578 | 0.000* |
| | Median | 0.636 | | 0.271 | 0.567 | 1.046 | 0.324 | -0.723 | 0.000* |
| Industrial complex area [2] | N | 18 | 3-1 | | | 9 | 9 | | |
| | Mean(SD) | 0.940(0.584) | | 0.248 | 0.149 | 1.359(0.462) | 0.521(0.342) | -0.838 | 0.000* |
| | Median | 0.907 | | 0.300 | 0.233 | 1.536 | 0.570 | -0.966 | 0.000* |
| Incinerator area [3] | N | 32 | 3-2 | | | 16 | 16 | | |
| | Mean(SD) | 0.981(0.820) | | 0.041 | 0.839 | 1.616(0.573) | 0.346(0.448) | -1.270 | 0.000* |
| | Median | 0.936 | | 0.029 | 0.834 | 1.697 | 0.229 | -1.468 | 0.000* |
| p-value (mean) | | 0.27 | | | | 0.004* | 0.607 | | |
| p-value (median) | | 0.427 | | | | 0.014* | 0.185 | | |

Note: Note: difference in 1-NP concentration in air by area or season is tested in terms of mean and median values, and associated p-values are provided in the table. Test of difference in means (medians) between two groups is based on unpaired t-test (Mood's median test), and test of difference in means (medians) among three groups is based on one-way anova (Mood's median test).

Table 3. Concentration of 6-OHNP in urine by area and season (in ng/g creatinine)

| | | Total | | | | Winter [a] | Summer [b] | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Diff | p-value | | | Diff | p-value |
| All area | N | 86 | | | | 43 | 43 | b-a | |
| | Mean(SD) | 2.931(4.963) | | | | 4.184(6.476) | 1.679(2.166) | -2.505 | 0.020* |
| | Median | 1.280 | | | | 2.535 | 0.705 | -1.830 | 0.001* |
| Clean rural area [1] | N | 36 | 2-1 | | | 18 | 18 | | |
| | Mean(SD) | 2.519(2.846) | | 0.256 | 0.729 | 3.598(2.950) | 1.440(2.348) | -2.157 | 0.000* |
| | Median | 1.244 | | 0.696 | 0.086 | 2.899 | 0.667 | -2.233 | 0.009* |
| Industrial complex area [2] | N | 18 | 3-1 | | | 9 | 9 | | |
| | Mean(SD) | 2.775(2.384) | | 0.964 | 0.493 | 3.121(2.009) | 2.430(2.788) | -0.691 | 0.000* |
| | Median | 1.940 | | -0.3 | 0.335 | 2.535 | 0.669 | -1.866 | 0.169 |
| Incinerator area [3] | N | 32 | 3-2 | | | 16 | 16 | | |
| | Mean(SD) | 3.483(7.398) | | 0.708 | 0.622 | 5.441(10.131) | 1.525(1.519) | -3.917 | 0.000* |
| | Median | 0.944 | | -0.996 | 0.080 | 1.468 | 0.871 | -0.597 | 0.486 |
| p-value (mean) | | 0.723 | | | | 0.62 | 0.512 | | |
| p-value (median) | | 0.214 | | | | 0.761 | 0.761 | | |

Note: Note: difference in 6-OHNP concentration in urine by area or season is tested in terms of mean and median values, and associated p-values are provided in the table. Test of difference in means (medians) between two groups is based on unpaired t-test (Mood's median test), and test of difference in means (medians) among three groups is based on one-way anova (Mood's median test).

Table 4. Concentration of 1-NAAP in urine by area and season (in ng/g creatinine)

| | | Total | | | | Winter [a] | Summer [b] | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Diff | p-value | | | Diff | p-value |
| All area | N | 86 | | | | 43 | 43 | b-a | |
| | Mean(SD) | 0.253(0.341) | | | | 0.200(0.186) | 0.305(0.443) | 0.105 | 0.158 |
| | Median | 0.190 | | | | 0.149 | 0.215 | 0.066 | 0.018 |
| Clean rural area [1] | N | 36 | 2-1 | | | 18 | 18 | | |
| | Mean(SD) | 0.275(0.491) | | -0.038 | 0.661 | 0.151(0.089) | 0.398(0.675) | 0.247 | 0.141 |
| | Median | 0.175 | | 0.058 | 0.253 | 0.131 | 0.206 | 0.074 | 0.001* |
| Industrial complex area [2] | N | 18 | 3-1 | | | 9 | 9 | | |
| | Mean(SD) | 0.237(0.111) | | -0.038 | 0.673 | 0.176(0.063) | 0.297(0.118) | 0.120 | 0.019* |
| | Median | 0.233 | | 0.0 | 1.000 | 0.182 | 0.280 | 0.098 | 0.022 |
| Incinerator area [3] | N | 32 | 3-2 | | | 16 | 16 | | |
| | Mean(SD) | 0.237(0.207) | | 0.000 | 1.000 | 0.269(0.279) | 0.205(0.070) | -0.064 | 0.384 |
| | Median | 0.180 | | -0.053 | 0.560 | 0.215 | 0.165 | -0.050 | 0.486 |
| p-value (mean) | | 0.882 | | | | 0.169 | 0.455 | | |
| p-value (median) | | 0.517 | | | | 0.549 | 0.024* | | |

Note: Note: difference in 1-NAAPconcentration in urine by area or season is tested in terms of mean and median values, and associated p-values are provided in the table. Test of difference in means (medians) between two groups is based on unpaired t-test (Mood's median test), and test of difference in means (medians) among three groups is based on one-way anova (Mood's median test).

Table 5. Concentration of 8-OHdG in urine by area and season (in ng/g creatinine)

| | | Total | | | | Winter [a] | Summer [b] | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Diff | p-value | | | Diff | p-value |
| All area | N | 86 | | | | 43 | 43 | b-a | |
| | Mean(SD) | 1.287(0.779) | | | | 1.178(0.670) | 1.396(0.845) | 0.218 | 0.197 |
| | Median | 1.119 | | | | 1.017 | 1.154 | 0.137 | 0.284 |
| Clean rural area [1] | N | 36 | 2-1 | | | 18 | 18 | | |
| | Mean(SD) | 1.297(0.765) | | -0.002 | 0.993 | 1.044(0.620) | 1.549(0.827) | 0.505 | 0.046* |
| | Median | 1.119 | | 0.207 | 0.567 | 0.934 | 1.640 | 0.706 | 0.049* |
| Industrial complex area [2] | N | 18 | 3-1 | | | 9 | 9 | | |
| | Mean(SD) | 1.294(0.903) | | -0.024 | 0.898 | 1.288(1.031) | 1.301(0.817) | 0.013 | 0.977 |
| | Median | 0.912 | | 0.1 | 0.630 | 0.940 | 0.884 | -0.056 | 0.647 |
| Incinerator area [3] | N | 32 | 3-2 | | | 16 | 16 | | |
| | Mean(SD) | 1.273(0.746) | | -0.021 | 0.933 | 1.268(0.576) | 1.278(0.904) | 0.009 | 0.972 |
| | Median | 1.178 | | 0.266 | 0.560 | 1.274 | 1.127 | -0.148 | 0.486 |
| p-value (mean) | | 0.992 | | | | 0.574 | 0.611 | | |
| p-value (median) | | 0.842 | | | | 0.761 | 0.761 | | |

Note: Note: difference in 8-OHdG concentration in urine by area or season is tested in terms of mean and median values, and associated p-values are provided in the table. Test of difference in means (medians) between two groups is based on unpaired t-test (Mood's median test), and test of difference in means (medians) among three groups is based on one-way anova (Mood's median test).

Table 6. Concentration of MDA in urine by area and season ( in ng/g creatinine)

| | | Total | | | | Winter [a] | Summer [b] | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Diff | p-value | | | Diff | p-value |
| All area | N | 86 | | | | 43 | 43 | b-a | |
| | Mean(SD) | 2.147(1.282) | | | | 1.546(0.779) | 2.748(1.407) | 1.202 | 0.000* |
| | Median | 1.863 | | | | 1.572 | 2.493 | 0.921 | 0.001* |
| Clean rural area [1] | N | 36 | 2-1 | | | 18 | 18 | | |
| | Mean(SD) | 2.437(1.355) | | -0.600 | 0.172 | 1.637(0.836) | 3.236 | 1.599 | 0.000* |
| | Median | 2.257 | | -0.885 | 0.022 | 1.724 | 3.490 | 1.765 | 0.001* |
| Industrial complex area [2] | N | 18 | 3-1 | | | 9 | 9 | | |
| | Mean(SD) | 1.837(1.545) | | -0.442 | 0.125 | 1.044(0.651) | 2.630(1.798) | 1.586 | 0.032* |
| | Median | 1.372 | | 0.5 | 0.630 | 1.049 | 1.833 | 0.784 | 0.169 |
| Incinerator area [3] | N | 32 | 3-2 | | | 16 | 16 | | |
| | Mean(SD) | 1.995(0.974) | | 0.158 | 0.699 | 1.725(0.698) | 2.265(1.149) | 0.540 | 0.121 |
| | Median | 1.738 | | 0.366 | 0.243 | 1.577 | 2.366 | 0.789 | 0.486 |
| p-value (mean) | | 0.189 | | | | 0.087 | 0.127 | | |
| p-value (median) | | 0.072 | | | | 0.027* | 0.024* | | |

Note: Note: difference in MDA concentration in urine by area or season is tested in terms of mean and median values, and associated p-values are provided in the table. Test of difference in means (medians) between two groups is based on unpaired t-test (Mood's median test), and test of difference in means (medians) among three groups is based on on one-way anova(Mood's median test).

Table 7. Copy number of mitochondrial DNA tandem repeats

| Season | Area | mtDNAcn in sputum (N=43) Mean(SD) |
|---|---|---|
| Winter | | 6.091(9.896) |
| | Clean rural area | 5.280(10.288) |
| | Industriral complex area | 10.562(13.780) |
| | Incinerator area | 4.489(6.120) |
| Summer | | 4.254(5.794) |
| | Clean rural area | 1.676(1.125) |
| | Industriral complex area | 5.132(6.022) |
| | Incinerator area | 6.661(7.654) |

Table 8. Pearson's correlation coefficient 1-NP in air, with 1-NP metabolites and oxidative stress markers and genetic damage marker

| | 1-NP | 6-OHNP | 1-NAAP | MDA | 8-OHdG | mtDNAcn in sputum |
|---|---|---|---|---|---|---|
| PanelA : Total N(86) | | | | | | |
| 1-NP | 1 | | | | | |
| 6-OHNP | 0.3236*** | 1 | | | | |
| 1-NAAP | -0.1657 | -0.0964 | 1 | | | |
| MDA | -0.2135* | -0.0732 | 0.2563** | 1 | | |
| 8-OHdG | -0.0897 | -0.0649 | 0.2209** | 0.1776 | 1 | |
| mtDNAcn in sputum | 0.0618 | -0.031 | -0.1225 | -0.0776 | -0.0753 | 1 |
| PanelB: Winter N(43) | | | | | | |
| 1-NP | 1 | | | | | |
| 6-OHNP | 0.3158** | 1 | | | | |
| 1-NAAP | -0.0508 | -0.1299 | 1 | | | |
| MDA | 0.1272 | 0.2501 | 0.1439 | 1 | | |
| 8-OHdG | -0.0106 | -0.1269 | 0.2166 | -0.0292 | 1 | |
| mtDNAcn in sputum | 0.0702 | -0.0772 | -0.2106 | -0.007 | -0.0137 | 1 |
| Panel C: Summer N(43) | | | | | | |
| 1-NP | 1 | | | | | |
| 6-OHNP | -0.1173 | 1 | | | | |
| 1-NAAP | -0.1218 | -0.0416 | 1 | | | |
| MDA | 0.21 | -0.2177 | 0.2278 | 1 | | |
| 8-OHdG | 0.0266 | 0.1894 | 0.2115 | 0.2026 | 1 | |
| mtDNAcn in sputum | -0.2204 | 0.0057 | -0.0783 | -0.0552 | -0.1354 | 1 |

*p-value <0.05 ** p-value <0.01 ***p-value<0.001

Table 9. Generalized linear model for 8-OHdG and MDA and mtDNAcn in Sputum

| Variables | Season | 8-OHdG in urine (μg/g Creatinine ) | | MDA in Urine (μM/g Creatinine) | | mtDNAcn in sputum | |
|---|---|---|---|---|---|---|---|
| | | β | p-value | β | p-value | β | p-value |
| Current smoker (no=0, yes=1) | Winter | 0.462 | 0.241 | -0.215 | 0.670 | -1.251 | 0.773 |
| | Summer | 0.392 | 0.309 | 1.759 | 0.001*** | -0.800 | 0.85 |
| Cumulative smoking amount (Pack year) | Winter | 0.001 | 0.0438** | 0.001 | 0.393 | 0.000 | 0.959 |
| | Summer | 0.000 | 0.573 | -0.002 | 0.002*** | 0.001 | 0.852 |
| 1-NP in air (pg/m3) | Winter | 0.112 | 0.626 | 0.139 | 0.636 | 2.085 | 0.413 |
| | Summer | 0.167 | 0.576 | 0.197 | 0.607 | -3.273 | 0.324 |
| 6-OHNP in urine (ng/g creatinine) | Winter | -0.027 | 0.238 | 0.007 | 0.820 | -0.149 | 0.553 |
| | Summer | 0.093 | 0.104 | -0.111 | 0.132 | -0.054 | 0.931 |
| 1-NAAP in urine (ng/g creatinine) | Winter | 1.135 | 0.089* | 0.739 | 0.384 | -11.588 | 0.116 |
| | Summer | 0.470 | 0.088* | 0.773 | 0.030** | -1.401 | 0.643 |

*p-value <0.1 ** p-value <0.05 ***p-value<0.01

# V. Discussions

## 1. 1-NP Concentration in Air and 1-NP Metabolites in Urine

The seasonal and regional variability of atmospheric 1-NP concentrations were investigated. Notably, the average concentration of 1-NP in the atmosphere during winter was significantly higher than in summer (winter 1.314 ± 0.548 pg/$m^3$, summer 0.424 ± 0.427 pg/$m^3$), a finding that was statistically highly significant. Furthermore, significant regional differences in 1-NP concentrations were observed during the winter season. This aligns with previous studies that found higher atmospheric concentrations of 1-NP in winter, correlating with increased fossil fuel usage [12,13].

The average concentrations of 1-NP were 0.849 and standard deviation is 0.662 by area was highest in the incinerator area, with an average of 0.987 pg/$m^3$ and a standard deviation of 0.820 pg/$m^3$. while the clean rural area had the lowest average concentration of 0.733 pg/$m^3$. This presents the same results in line with previous studies conducted on 1-NP concentrations between regions. The distribution of past measurements of 1-NP in outdoor air are as follows (Figure. 5).

The 1-NP concentrations in this study are significantly lower than previous studies and its occupational exposure [33]. In addition, previous studies on indoor air measurements have been scarce. The results from this study were generally lower compared to previous research data [7]. In this study, the daily exposure of participants was measured for 24 hours using a personal sampler, and although the situation was controlled for possible exposure to diesel combustion gases, there is a limitation that the measurements were not taken in a laboratory setting. Nevertheless, clear seasonal and regional differences were observed in the three areas near Cheongju support past

research suggesting that 1-NP is strongly influenced by fossil fuel usage exposure sources [7,24].

The average concentration of 6-OHNP was found to be 2.941 ng/g creatinine ± 4.963 ng/g creatinine, with higher levels observed in winter (4.184 ± 6.476 ng/g creatinine) compared to summer (0.424 ± 0.427 ng/g creatinine). This elevation correlates with increased atmospheric 1-Nitropyrene 1-NP concentrations during winter. Correlation analysis also confirmed a significant association between 1-NP and 6-OHNP. However, no such relationship was apparent in the summer, which could be attributed to the lower absolute values and a consequent lack of statistical significance.

Conversely, 1-NAAP concentrations were higher in the summer with an average of 2.53 ng/g creatinine ± 0.341 ng/g creatinine, compared to winter (0.200 ± 0.186 ng/g creatinine) and summer (0.305 ± 0.443 ng/g creatinine). Whilst no significant seasonal differences were observed in average values for 1-NAAP, median value comparisons did reveal significant differences. There is no theoretical basis for higher 1-NAAP levels in the summer, suggesting a need for further research. Regional differences were noted between the incinerator area and the clean rural area.

This pattern of 1-NP metabolites concentrations in the body, where absorbed 1-NP is predominantly metabolized to 6-OHNP, 8-OHNP, and 3-OHNP through oxidation processes, and less than 10% is metabolized to 1-AP or 1-NAAP, can be explained by previous studies [24,25].

## 2. Relationship between 1-NP Exposure to 1-NP and the Levels of Oxidative Stress Markers and Genetic Damage Marker

In the assessment of oxidative stress indices, urinary 8-OHdG and MDA demonstrated no correlation with atmospheric 1-NP concentrations. Both markers showed a tendency to be higher in the summer, with MDA exhibiting a statistically significant seasonal variation (winter: 1.546 ± 0.779; summer: 2.748 ± 1.407, $p < 0.000$). This observation is consistent with previous research suggesting that high temperatures and dehydration may augment oxidative damage, and antioxidant mechanisms may be upregulated in response to increased oxidative stress in warmer environments [34]. The mtDNAcn of a respirator exposed to 1-NP through direct absorption did not exhibit any link with the concentration or metabolic substance of the 1-NP area. Considering the absence of an association with smoking, it is reasonable to question the inclusion of mtDNA in the subject. Further research indicates that modifications to the technique of object selection are necessary. Additional research is required to ascertain whether mtDNAcn in the bloodstream is a viable indicator of genetic harm caused by exposure to 1-NP.

Benzo[a]pyrene, a notable compound among PAHs, is widely known to have neurobehavioral effects on both adults and children, often due to environmental and occupational exposures [35,36]. Once absorbed into the body, BaP triggers the activation of cytochrome P450 1A1, resulting in its conversion to benzo[a]pyrene-7,8-diol-9,10-epoxide (BPDE) [37]. This process subsequently causes an overproduction of reactive oxygen species (ROS) during its metabolic breakdown [38].

Correlation analysis indicated a weak association between oxidative stress markers and 1-NAAP, but not with atmospheric 1-NP or 6-OHNP. There have been

previous studies showing that 1-NP causes oxidative stress [39,40]. This finding supports the hypothesis that the metabolic processing of 1-NP may be determined by genetic polymorphisms in metabolic enzymes [41]. Even with high exposure to 1-NP, oxidative stress could occur in specific metabolic pathways, implicating genetic metabolic enzymes in the determination process.

The limitation of this study is first, that the 1-NP concentration in the general public is very low and requires sufficient consideration as to whether it can be correlated with statistical analysis. Second, MDAs are known to have a very short half-life [42] and may be influenced by individual underlying diseases, age, diet, and stress, other than environmental factors by 1-NP [43], but the study did not strictly control other variables. Finally, there are experimental errors that may occur during atmospheric measurement, urine sample analysis. A new guideline was introduced in GUM with the concept of measurement uncertainty, uncertainties of chemical analysis [44]. In this study, no calculation of the measurement uncertainty was made. A more accurate correlation can be seen if statistical analysis is carried out after the calculation of the uncertainty for future measurements and sample analysis.

However, the significance of this study lies in its novel approach of using a personal sampler to collect and analyze 1-NP among the general population, and in being one of the first to examine its metabolite, 1-NAAP, in relation to human oxidative stress and genetic damage markers. This study's results regarding the lack of correlation between atmospheric 1-NP concentrations and oxidative stress or genetic damage, underlines the need for sophisticated research designs that can identify and control confounding variables.

Further studies are also needed to explore whether 1-NAAP is a meaningful

biomarker for the human health effects of 1-NP exposure. Whilst the small number of study participants may introduce potential errors in statistical analysis, the research established seasonal and regional differences in atmospheric 1-NP levels and suggested that metabolic processes might be a strong determinant of human health effects than atmospheric concentrations.

## VI. Conclusion

This study identified significant seasonal variations in air 1-NP concentrations. A correlation was established between 1-NP and its main metabolite, 6-OHNP, but no clear relationship was found between atmospheric 1-NP levels and markers of oxidative stress or genetic damage. Conversely, a relationship was observed between 1-NAAP concentrations and oxidative stress markers MDA and 8-OHdG, indicating the need for further research. These results underscore the necessity for further research into the human metabolism of 1-NP to elucidate its pathways of toxicity in humans.

Key words: 1-nitropyrene, 6-hydroxy-1-nitropyrene, n-acetyl-1-aminopyrene, oxidative stress, single nucleotide polymorphism

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# 국문 초록

## 1-Nitropyrene 노출과 인체 내 산화적 스트레스 및 유전 손상 표지자 간의 관련성 평가

1-나이트로피렌(1-NP)은 니트로-다환 방향족 탄화수소(nitro-PAHs)로, 디젤 배기 가스 노출의 중요한 지표이며, 이는 심혈관 질환, 호흡기 질환, 심지어 폐암의 증가된 위험과 관련이 있다. 이 연구의 목적은 계절별 지역별 공기 중 1-NP의 노출정도를 확인하고, 1-NP 대사산물, 산화 스트레스 표지자, 유전적 손상 간의 연관성을 알아보고자 한다.

대한민국 청주시 인근에 거주하는 43명의 성인을 대상으로 연구를 실시하였다, 이들은 산업단지, 소각장, 그리고 청정한 농촌 지역 근처에서 디젤 배기 입자(DEPs)에 노출되었다. 겨울과 여름 각각 한 번씩 대기 중 1-NP 농도와 소변 내 대사산물, 소변 내 산화스트레스 표지자, 객담의 미토콘드리아 DNA 복제 수를 확인하여 유전 손상 정도를 확인하였다.

결과는 1-NP 농도의 계절적 및 지역적 변동이 유의하게 나타났다. 겨울에는 여름보다 1-NP 농도가 높았으며, 계절적 변화는 겨울철에 관찰되었다. 산화 스트레스 표지자인 마론디알데하이드 (MDA)은 여름보다 겨울에 더 높았다. 공기 중 1-NP 농도와 대사산물인 6-하이드록시-1-나이트로피렌(6-OHNP) 사이에는 양의 상관관계가 발견되었으며, 1-NP와 MDA 사이에는 약한 음의 상관관계가 관찰되었다. 또한, 1-N-아세틸아미노-피렌(1-NAAP)은 MDA와 8-하이드록시디옥시구아노신(8-OHdG) 산화 스트레스 지표와 약한 양의 상관관계를 보였다. 이 연구는 공기 중 1-NP 농도의 계절적 변동이 유의하다는 것을 확인하였다. 1-NP와 주요 대사산물인 6-OHNP 간의 상관관계가

겨울철에 확인되었다. 그러나, 대기 중 1-NP 수준과 산화 스트레스 표지자, 유전적 손상 표지자 간의 명확한 관계는 발견되지 않았다. 그러나 1-NAAP 농도와 산화 스트레스 마커인 MDA 및 8-OHdG 사이의 관계가 관찰되었다. 이는 1-NP 노출에 의한 인체 독성이 1-NP 대사 경로의 단일염기다형성이 관여하는지에 대한 후속연구의 필요성을 보여주는 결과이다.

핵심어: 1-nitropyrene, 6-hydroxy-1-nitropyrene, n-acetyl-1-aminopyrene, 산화적 스트레스, 단일염기다형성