



Article Seasonal Variability and Risk Assessment of Atmospheric Polycyclic Aromatic Hydrocarbons and Hydroxylated Polycyclic Aromatic Hydrocarbons in Kanazawa, Japan

Masato Honda ^{1,*}^(D), Kazuichi Hayakawa ², Lulu Zhang ^{2,3}, Ning Tang ²^(D) and Hiroyuki Nakamura ⁴^(D)

- ¹ Botanical Garden, Institute of Nature and Environmental Technology, Kanazawa University, Kakuma-machi, Kanazawa 920-1192, Japan
- ² Institute of Nature and Environmental Technology, Kanazawa University, Kakuma-machi, Kanazawa 920-1192, Japan
- ³ School of Civil Engineering, Architecture and Environment, Hubei University of Technology, Wuhan 430068, China
- ⁴ Department of Hygiene and Public Health, Faculty of Medicine, Institute of Medical, Pharmaceutical and Health Sciences, Kanazawa University, Kanazawa 920-8640, Japan
- * Correspondence: mst-honda@se.kanazawa-u.ac.jp; Tel.: +81-076-264-5874

Abstract: Polycyclic aromatic hydrocarbons (PAHs) and hydroxylated PAHs (OH-PAHs) are ubiquitous atmospheric pollutants that are a concern because of their endocrine disrupting activities. In this study, seasonal air sampling was conducted in 2017 and 2018 in Kanazawa, Ishikawa Prefecture, Japan. The concentrations and seasonal variations of PAHs and OH-PAHs were analyzed, and health risks of individual congeners were evaluated based on their relative endocrine activity. The atmospheric concentrations of PAHs and OH-PAHs showed seasonal trends with higher concentrations in the winter (daily average ± standard deviation: 1.00 ± 0.26 ng/m³ for PAHs and 75.06 ± 23.38 pg/m³ for OH-PAHs) and lower concentrations in the summer (0.30 ± 0.09 ng/m³ for PAHs and 17.08 ± 4.83 pg/m³ for OH-PAHs). There were significant positive correlations between the concentrations of atmospheric PAHs and OH-PAHs. Additionally, the health risk from the endocrine disrupting potential of each OH-PAH was evaluated using relative estrogenic and antiestrogenic activities. OH-PAHs with four rings, such as OH-chrysenes and OH-benz[*a*]anthracene, had particularly high health risks. These results suggest that atmospheric OH-PAHs are a potential health risk for organisms and thus warrant further research.

Keywords: polycyclic aromatic hydrocarbon; hydroxylated polycyclic aromatic hydrocarbon; atmospheric pollution; health risk; endocrine disrupting chemical; seasonal variability

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a group of chemicals that contain two or more fused benzene rings without any functional group modifications. PAHs are generated through the incomplete combustion of organic matter such as wood, crop residues, and fossil fuels [1–3], and are released into the atmosphere through human activity. Therefore, PAHs are ubiquitous environmental pollutants, particularly in the atmosphere [2–6]. PAHs are of concern because of their mutagenicity, carcinogenicity and endocrine disrupting potential [7–12]. Because of its carcinogenicity, benzo[*a*]pyrene (one of major PAHs) is classified as a Group 1 carcinogen (carcinogenic to humans) by the International Agency for Research on Cancer. The United States Environmental Protection Agency has selected 16 representative PAHs, including those with between two and six rings, as priority compounds for environmental monitoring. Similar to other atmospheric combustion-related pollutants, PAHs show a distinct seasonal variability. The atmospheric concentrations of PAHs in the winter are generally higher than in the summer [4,13,14] because of the increased use of fuels for heating purposes.



Citation: Honda, M.; Hayakawa, K.; Zhang, L.; Tang, N.; Nakamura, H. Seasonal Variability and Risk Assessment of Atmospheric Polycyclic Aromatic Hydrocarbons and Hydroxylated Polycyclic Aromatic Hydrocarbons in Kanazawa, Japan. *Appl. Sci.* **2022**, *12*, 9469. https://doi.org/10.3390/ app12199469

Academic Editor: Eduardo Ferreira da Silva

Received: 31 August 2022 Accepted: 15 September 2022 Published: 21 September 2022

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). Interest in the health risks and atmospheric behavior of oxidized derivatives of PAHs is growing rapidly, and many studies have been conducted [15,16]. One group of PAH derivatives, hydroxylated PAHs (OH-PAHs), has been detected in the atmosphere [17–24]. OH-PAHs are oxidized derivatives of PAHs that are generated via combustion of organic matter such as tobacco, wood, and fossil fuel [17,21,25]. The OH-PAHs can also be generated via hydroxyl (OH) radical-initiated reactions in the atmosphere [26–29]. In a study in China, Lin et al. [22] found that biomass burning is the dominant source (79.6%) of atmospheric OH-PAHs during colder seasons when domestic heating appliances are used, whereas traffic emissions (59.6%) and secondary formation (21.7%) are the dominant sources in the warmer seasons.

OH-PAHs have high health risks, with their endocrine disrupting potential being of particular concern [8,12,20,30–32]. In a previous study, Hayakawa et al. [30] evaluated the relative estrogenic and antiestrogenic activities of 14 PAHs and 63 OH-PAHs. In their study, OH-PAHs with four rings, such as hydroxylated benz[a]anthracenes, hydroxylated chrysenes, and hydroxylated benzo[c]phenanthrene, showed strong estrogenic and antiestrogenic activity. Hydroxylated PAHs with four rings, especially hydroxylated chrysene, have a similar chemical structure to 17β -estradiol (estrogen). Consequently, several OH-PAHs can interact with the human estrogen receptor (hER). The hER includes ER α and $ER\beta$, which have similar binding affinities but different distributions in the tissues. It is believed that endocrine disrupting chemicals, such as OH-PAHs, bind to ERs and induce estrogenic and antiestrogenic activity [20,30]. Previous studies have revealed that OH-PAHs have much higher teratogenicity and developmental toxicity in fish compared to their parent PAHs [32,33]. While OH-PAHs are of toxicological concern, little is known about the seasonal variations in atmospheric OH-PAH concentrations [18,21]. In addition, a comprehensive toxicological risk assessment, especially on the estrogenic and antiestrogenic activities of atmospheric OH-PAHs towards humans, has not been conducted.

In this study, seasonal air sampling was conducted in 2017 and 2018 at Kanazawa, Ishikawa Prefecture, Japan. The concentrations and seasonal variations of airborne particlebound PAHs and OH-PAHs were analyzed. Human health risks of the individual PAHs and OH-PAHs were evaluated using their relative estrogenic and antiestrogenic activities. While human health risk from atmospheric pollution is a global concern, few studies focus on the environmental level and health risk of endocrine disrupting compounds such as OH-PAHs. This is the first study to evaluate the contributions of atmospheric OH-PAHs to endocrine disruption in humans.

2. Materials and Methods

2.1. Chemicals and Regents

A Supelco PAH standard mixture was purchased from Sigma–Aldrich (Waltham, MA, US) and contained the following nine target PAHs: fluoranthene (Flt), pyrene (Pyr), benz[*a*]anthracene (BaA), chrysene (Chr), benzo[*b*]fluoranthene (BbF), benzo[*k*]fluoranthene (BkF), benzo[*a*]pyrene (BaP), benzo[*g*,*h*,*i*]perylene (BgPe), and indeno[1,2,3-*cd*]pyrene (IDP). The following OH-PAH standards were purchased from Toronto Research Chemicals (Toronto, Canada): 1- and 2-hydroxylated naphthalenes (1OH- and 2OH-Nap); 1-, 2-, and 3-hydroxylated fluorenes (1OH-, 2OH-, and 3OH-Flu); 1-, 2-, 3-, 4-, 9-hydroxylated phenanthrenes (1OH-, 2OH-, 3OH-, 4OH-, and 9OH-Phe); 1-hydroxylated pyrene (1OH-Pyr); 2-, 3-, and 6-hydroxylated chrysenes (2OH-, 3OH-, and 6OH-Chr); 3-hydroxylated benz[*a*]anthracene (3OH-BaA); and 3-hydroxylated benzo[*a*]pyrene (3OH-BaP). The deuterated PAHs; Pyr-*d*₁₀ and BaP-*d*₁₂ were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). The deuterated OH-PAHs; 2OH-Nap-*d*₇, 2OH-Flu-*d*₉, 1OH- and 4OH-Phe-*d*₉, 3OH-BaA-*d*₁₁, 3OH-Chr-*d*₁₁, 1OH-Pyr-*d*₉, and 3OH-BaP-*d*₁₁ were purchased from Toronto Research Chemicals. All organic solvents, chemicals, and ultrapure water used in the experiments were analytical or special grade.

2.2. Air Sampling

Air sampling was conducted next to a main road in a residential area of Kanazawa, Japan, using a high-volume air sampler (HV-RW, Sibata Scientific Technology LTD., Tokyo, Japan) equipped with a quartz fiber filter with a cut-off for particulate matter with sizes less than 2.5 μ m (PM_{2.5}) (2500QAT-UP, Pallflex Products corporation, Putnam, CT, US) at a flow rate of 1000 L/min [2,34]. There were no obvious sources of combustion PM_{2.5} near the monitoring station except for traffic. Filter samples were collected daily (every 24 h) for 1 week in spring (24–30 April), summer (21–27 August), and autumn (6–12 November) in 2017, and winter (19–25 February) and spring (24–30 April) in 2018. The filters were preheated at 600 °C for 4 h before using to minimize background PAH contamination. The amounts of PM_{2.5} were calculated based on the difference of the filter weight before and after sampling. The filters were kept in a freezer at -30 °C until further analysis.

2.3. PAHs Analysis

The sample preparation and analytical methods for particle-bound PAHs are detailed in a previous study [2]. Briefly, the filters were cut into pieces and placed in separate flasks. An internal standard solution was added to each flask. Benzene/ethanol (3:1, v/v) was added as the extraction solvent and sonicated. The extraction was repeated twice. The extracts were filtered to remove solid materials and then washed with a NaOH solution (5%, w/v) and H₂SO₄ solution (20%, v/v). This was followed by washing with distilled water. An amount of 100 µL Dimethyl sulfoxide was added, and a rotary evaporator was used to concentrate the extracts to 100 µL. Ethanol (900 µL) was added, and the solutions were then filtered through a 0.45 µm pore size filter membrane. The filtrates were injected into separate vials and analyzed by high-performance liquid chromatography combined with fluorescence detection (Shimadzu Inc., Kyoto, Japan) using Inertsil ODS-P column (250 × 4.6 mm, 5 µm; GL Sciences Inc., Tokyo, Japan).

2.4. OH-PAHs Analysis

For preparation of the airborne particle-bound OH-PAHs, a previously established method was modified [21]. Briefly, the filters were cut into pieces and then placed in glass centrifuge tubes. An internal standard solution was added to each tube. Ethanol/pentane (1:1, v/v) was added for extraction, and the samples were sonicated. The supernatant was collected after centrifugation $(652 \times g)$. The extraction was repeated three times. The extracts were concentrated with N₂ gas to approximately 1 mL. Next, the concentrated samples were loaded onto Bond Elut NH₂ (Agilent, Santa Clara, CA, USA) that had been prewashed and conditioned with ethanol. The target compounds were eluted with ethanol/water (3:1, v/v). Each elute was filtered through a 0.21 µm pore size syringe filter. For sample analysis, we used a slight modification of an established method [35]. The target analytes were separated by high-performance liquid chromatography using a Kinetex F5 column (100 × 2.1 mm, 5 µm; Phenomenex, Torrance, CA, USA) and detected using tandem-mass spectrometry (LC/MS; Shimadzu Inc.).

2.5. Health Risk Assessment

To assess the endocrine disrupting risks posed by airborne particle-bound PAHs and OH-PAHs, the relative estrogenic and antiestrogenic activities and binding affinity to hER of each compound were calculated using the data reported in a previous study (Tables S1 and S2) [30]. In the previous study, the estrogenic and antiestrogenic activities were assessed using yeast (*Saccharomyces cerevisiae*) in a two-hybrid assay, and the binding affinity was assessed in a competition binding assay to hER α with 17 β -estradiol as a competitor. Relative estrogenic and antiestrogenic activities, and relative binding potential were calculated from atmospheric concentrations simply multiplied with reported relative effective potency of estrogenic activity (REP_E), relative effective potency of antiestrogenic activity (REP_{AE}) and relative binding affinity.

2.6. Quality Control and Quality Assurance

A method blank and a matrix-spiked sample were analyzed with each batch of 10 samples. Method blanks contained the target analyte at a concentration of less than the limit of detection (LOD) for PAHs, or between the LOD and 0.08 ng/mL for OH-PAHs. The recovery rates for the spiked samples were 85%–110% (PAHs) and 81%–100% (OH-PAHs). The instrumental LODs for the analytes were 9.1–83 pg/mL (PAHs) and 0.05–1.00 ng/mL (OH-PAHs). PAHs with two to three rings were excluded as target analytes because they had high vapor pressures and were difficult to quantify. Because of the difficulty of peak separation and identification by LC/MS, the following appeared as overlapping peaks: 2OH- and 3OH-Phe, 1OH- and 9OH-Phe, 2OH- and 3OH-Chr, and 6OH-Chr and 3OH-BaA.

2.7. Statistical Analysis

The concentrations of PAHs and OH-PAHs below the LOD were substituted with a value of LOD/2 for further statistical analysis. R software (version 4.0.2, R Development Core Team, Vienna, Austria) was used for statistical analysis with the significance level set at p < 0.05. The differences in PAHs and OH-PAHs concentrations among sampling seasons were examined using Tukey's test [36]. As a non-parametric statistical test, the correlations between total PAHs concentrations and total OH-PAHs concentrations were examined using Spearman's rank order correlation coefficient.

3. Results and Discussion

3.1. Atmospheric Concentrations of PAHs and OH-PAHs

The highest concentration of total PAHs was found in winter 2018 (daily average \pm standard deviation: 1.00 ± 0.26 ng/m³), followed by spring 2017 (0.86 ± 0.56 ng/m³), autumn 2017 $(0.66 \pm 0.23 \text{ ng/m}^3)$, spring 2018 $(0.55 \pm 0.30 \text{ ng/m}^3)$, and summer 2017 $(0.30 \pm 0.09 \text{ ng/m}^3)$ (Figures 1 and S1) [2,34]. The total PAH concentrations in spring 2017 and winter 2018 were significantly higher than those in summer 2017 (p = 0.02 and < 0.01, respectively). These results are consistent with previous reports of higher PAH concentrations with combustionderived PAH emissions in the winter and lower concentrations in the summer [4,13,14]. The concentrations of atmospheric PAHs in this study were similar to or lower than those in previous atmospheric surveys in Japan [14,30,37], China [38,39], and other Asian countries [40]. The current results were considered to reflect the regulation of emissions in Japan and neighboring countries [4]. Since 1966, the Japanese government has gradually strengthened regulations regarding the emissions of pollutants from new vehicles [41]. Similarly, the Chinese government has adopted a series of policies aimed at reducing emissions [42]. Pyr was the dominant PAH (23.7 \pm 5.5%), followed by Flt (19.6 \pm 5.3%), BbF (12.6 \pm 1.9%), and BgPe (12.1 \pm 2.8%). This trend was almost consistent throughout the year. Low molecular weight PAHs (4 rings PAHs) had higher percentages than high molecular weight PAHs (5 and 6 rings PAHs), which was considered to be caused by emission source and differences in the vapor pressures among PAHs.



Figure 1. Daily atmospheric concentrations of PAHs in April, August, and November 2017, and February and April 2018 in Kanazawa.

Similarly, the total OH-PAH concentration was highest in winter 2018 (75.06 \pm 23.38 pg/m³), followed by autumn 2017 (57.36 \pm 33.84 pg/m³), spring 2017 (49.81 \pm 24.12 pg/m³), spring $2018 (36.62 \pm 18.33 \text{ pg/m}^3)$ and summer $2017 (17.08 \pm 4.83 \text{ pg/m}^3)$ (Figures 2 and S2). The total OH-PAH concentration in autumn 2017 was significantly higher than in summer 2017 (p = 0.02), and in winter 2018 was significantly higher than those in summer 2017 and spring 2018 (p < 0.01 and p = 0.03, respectively). Similar to previous studies [22,43], the concentration of total OH-PAHs was approximately 6.9–24.8 times lower than that of the total PAHs (11.93–129.3 and 177.9–1757 pg/m³, respectively). On average, 2OH-Nap was the dominant PAH (20.1 \pm 11.7%), followed by 2/3OH-Phe (16.5 \pm 3.0%), 1OH-Flu (14.8 \pm 3.4%), and 2/3OH-Chr (12.0 \pm 2.9%). Atmospheric OH-PAHs (OH-Pyr and OH-Flu) were detected at lower concentrations (1OH-Pyr: 5.53 ± 4.22 and 2OH-Flu: 1.45 ± 1.12 pg/m³) to those previously reported for Nagasaki, Japan, in 1997–1998 (1OH-Pyr: 21.48 ± 14.97 and 2OH-Flu: 10.40 ± 10.71 pg/m³) [21]. The dominant component was 2OH-Nap in spring 2017/2018 (17.4%/22.8%) and summer 2017 (35.9%), but 2/3OH-Phe in autumn 2017 (19.6%) and winter 2018 (15.9%). This was probably caused by differences in the emission source [22,23] and atmospheric conditions [26,27]. Toriba et al. [24] reported more than 40 times lower concentration of 2OH-Nap compared to 2/3OH-Phe and these were considered to be emitted from exhaust gas. Moreover, Avagyan et al. [17] found the same levels of 2/3OH-Phe as 2OH-Nap from wood combustion. The current results may reflect these emission source differences. Compared to the results reported by Toriba et al. [24] for samples collected in Kanazawa in July 2013, the concentration of 2OH-Nap detected in the present study $(6.14 \pm 2.59 \text{ pg/m}^3 \text{ in August})$ was much higher ($20 \pm 16 \text{ fg/m}^3$ in July in previous study). Additionally, the proportion of 1OH-Pyr in the present study was much lower than in the study by Toriba et al. (8.9% in the present study, and 48% calculated from reported values in the previous study). These differences may reflect differences in the sampling locations between this study (roadside) and the previous study (rooftop of a building on a university campus). To the best of our knowledge, this is the first report on annual seasonal variations of multiple OH-PAHs in the atmosphere in Japan.



Figure 2. Daily atmospheric concentrations of OH-PAHs in April, August, and November 2017, and February and April 2018 in Kanazawa.

The ratio of total PAHs/total OH-PAHs did not show significant differences among the seasons (ratio: 11.5–14.4, p = 0.67–0.99 by Tukey's test), though these were weak differences between warmer seasons (spring and summer: 15.8–17.8) and colder seasons (autumn and winter: 13.4–13.7). These results are not consistent with those of Lin et al. [22], who reported clear seasonal differences with ratios of 33.3 in the warmer seasons (April–October) and 14.2 in the colder seasons (November–March) in Beijing, China. The samples in the present study were collected near a main road, and the major emission source was traffic. Additionally, compared with China, coal and biomass burning are rarely conducted in cities in Japan. Therefore, this inconsistency probably reflects differences in the emission sources of OH-PAHs.

The total PAH and total OH-PAH concentrations were significantly correlated (Figure 3, p < 0.01, r = 0.885). Additionally, there were significant correlations between Pyr and 1OH-Pyr (p < 0.01, r = 0.920) and BaA + Chr and OH-BaA + OH-Chrs (p < 0.01, r = 0.937). These results indicate that these two groups of chemicals have common emission sources. The OH-PAHs primarily originate as oxidized derivatives of PAHs through the combustion of organic matter, including fossil oil [17,21,25]. They can also form from PAHs after photochemical reactions in the atmosphere [26–29]. Both pathways likely caused the significant correlations between PAHs and OH-PAHs. Unlike the other PAHs and OH-PAHs, 3OH-BaP was not detected in any of the samples, even though BaP was detected in all analyzed samples ($0.04 \pm 0.02 \text{ ng/m}^3$). This indicated that 3OH-BaP was not contained in the emission source of BaP and/or was unlikely to form from BaP in the atmosphere.

To group and estimate emission sources, principal component analysis (PCA) and diagnostic ratio analysis were conducted. The results of the PCA for PAHs and OH-PAHs found some seasonal differences. For PAHs, spring, summer, autumn 2017 and spring 2018 formed a related group and winter 2018 was relatively separated (Figure S3). Similarly, for OH-PAHs 4 seasons formed a related group and the winter of 2018 was relatively separated (Figure S4). In both cases, winter 2018 was separated from summer, autumn 2017 and spring 2018, and spring 2017 was located between winter 2018 and other 3 seasons. These results likely indicate that these three seasons had similar contamination sources that differed from the winter 2018 samples. In addition, it may be that some abrupt event occurred in spring 2017, especially 29–30 April, such as the long-range transport from China [2]. The PAH diagnostic ratio analysis indicates clear seasonal differences, as well (Figure S5). The ratio of IDP and IDP+BgPe (IDP/(IDP+BgPe)) was used to assess the contribution difference between diesel vehicles and gasoline vehicles [34], and no clear difference was found with the five periods. However, combustion source estimation using the ratio of Flt

and Flt+Pyr (Flt/Flt+Pyr) [44] showed a remarkable difference, indicating that fossil fuel combustion was the dominant source in the spring and autumn, while petrogenic sources were dominant in the summer and organic matter combustion was the dominant source in the winter. In Japan, grass, wood, and related organic matter combustion (open burning) has been regulated [45]. The sampling point was located next to a main road in a residential area of Kanazawa city and there are low PAH and OH-PAHs emissions from residential areas. Hayakawa et al. [2] reported that the long-range transport of PM and PAHs from China has relatively high contributions to atmospheric PM and PAH contamination in Kanazawa. Therefore, the current results, which are consistent with the previous study, and seasonal variations in atmospheric PAH and OH-PAH concentrations were caused by local emissions and long-range transportation.



Figure 3. Correlations between the atmospheric concentrations of total PAHs and OH-PAHs.

In this study, the detected atmospheric PAHs and OH-PAHs were bound to airborne particles, such as PM_{2.5}. Atmospheric concentrations of PM_{2.5} were highest in spring 2017 $(21.7 \pm 12.1 \ \mu g/m^3)$, spring 2018 $(17.5 \pm 9.6 \ \mu g/m^3)$, winter 2018 $(14.2 \pm 2.6 \ \mu g/m^3)$, autumn 2017 (12.3 \pm 5.1 μ g/m³), and summer 2017 (10.6 \pm 4.3 μ g/m³) [2]. There were no significant seasonal variations (Tukey's test: p = 0.07-0.99). However, the total atmospheric PAHs and OH-PAHs concentrations were significantly correlated with atmospheric PM_{2.5} (p < 0.01, r = 0.496 for PAHs; and p = 0.02, r = 0.394 for OH-PAHs; Figure 4a,b). The observed significant correlation between atmospheric PAHs and PM_{2.5} concentrations is consistent with a previous study conducted in Japan [46]. The correlation between OH-PAHs and $PM_{2.5}$ was weaker than that between PAHs and $PM_{2.5}$. In this study, $PM_{2.5}$ was thought to be released mainly from vehicle exhaust with PAHs and OH-PAH adsorbed on the surface. The correlation weakness observed in the current study suggests that OH-PAHs are formed by secondary mechanisms after release into the atmosphere [27]. Additionally, the weakness of the correlation between the PAHs/OH-PAHs and PM_{2.5} also suggests the degradation and/or chemical change in target chemicals in the atmosphere and on the filter [47].



Figure 4. Correlation between the atmospheric concentrations of the target compounds and PM_{2.5}: (a) total PAHs versus PM_{2.5}, and (b) total OH-PAHs versus PM_{2.5}.

3.2. Health Risk Assessment

The relative health risks caused by the endocrine-disrupting activities of atmospheric PAHs and OH-PAHs were evaluated using the relative estrogenic and antiestrogenic activities and binding affinities to hER α , which were reported by Hayakawa et al. [30]. None of the PAHs showed any estrogenic and antiestrogenic activities in the previous study. Therefore, only the estrogenic and antiestrogenic activities of the OH-PAHs were analyzed in the present study.

The total estrogenic and total antiestrogenic activities showed general trends of higher levels in winter and lower levels in summer, as was the case for the atmospheric concentrations. Four-ring PAH derivatives, such as OH-Chrysene and OH-benz[*a*]anthracene, had particularly high health risks.

The calculated, relative, estrogenic activities of the atmospheric OH-PAHs are shown in Figure 5. The total estrogenic activity was highest in winter 2018 (1.58 ± 0.43), followed by autumn 2017 (1.16 ± 0.72), spring 2017 (1.12 ± 0.66), spring 2018 (0.70 ± 0.44), and summer 2017 (0.28 ± 0.12). The total estrogenic activity in summer was significantly lower than that in spring 2017 (p = 0.04), autumn 2017 (p = 0.03), and winter 2018 (p < 0.01), and that in winter 2018 was significantly higher than that in spring 2018 (p = 0.03). Because of the higher activity of the four-ring OH-PAHs [30], the activities of 2/3OH-Chr and 6OH-Chr/3OH-BaA were dominant in the total activity ($72.7 \pm 5.6\%$). In particular, 2/3OH-Chr showed high proportions in all samples ($53.6 \pm 12.9\%$). 2OH-Nap was dominant for the total concentration ($20.1 \pm 11.7\%$); however, it has low estrogenic activity (0%). The second most dominant component, in terms of total concentration, was 2/3OH-Phe ($16.5 \pm 3.0\%$), and it had a similar contribution to total estrogenic activity ($6.3 \pm 1.5\%$), although it appears consistently in all seasons.

The calculated, relative, antiestrogenic activities of atmospheric OH-PAHs are shown in Figure 6. Unlike with estrogenic activity, only OH-PAHs with four rings were antiestrogenic [30]. Therefore, only 2/3OH-Chr and 6OH-Chr/3OH-BaA had health risks (78.6 \pm 16.7% and 21.4 \pm 16.7%, respectively). Similar to estrogenic activity, the total antiestrogenic activity was highest in winter 2018 (1.02 \pm 0.27), followed by spring 2017 (0.72 \pm 0.41), autumn 2017 (0.71 \pm 0.40), spring 2018 (0.43 \pm 0.26), and summer 2017 (0.20 \pm 0.08). The differences in total antiestrogenic activity among the seasons was the same as that for the estrogenic activity. In addition, the total estrogenic activity and total antiestrogenic activity were significantly correlated (p < 0.01, r = 0.985).



Figure 5. Daily relative estrogenic activities of atmospheric OH-PAHs in April, August, and November 2017, and February and April 2018 in Kanazawa.



Figure 6. Daily relative antiestrogenic activities of atmospheric OH-PAHs in April, August, and November 2017, and February and April 2018 in Kanazawa.

Among the OH-PAHs, OH-chrysenes and OH-benz[*a*]anthracene had small contributions to the total concentration (9.8–26.1%). However, their high contributions to estrogenic activity (57.1–83.1%) and antiestrogenic activity (100%) suggest that they pose relatively high health risks for humans. The current results indicated that the health risks of endocrine disruption caused by atmospheric OH-PAHs are higher in the winter than in summer.

The binding potentials of PAHs and OH-PAHs to hER calculated from the reported binding affinities are shown in Figure 7. Several PAHs (BbF, BkF, BaP, BgPe, and IDP) and OH-PAHs (1/2OH-Nap, 1/3OH-Flu, 1/4/9OH-Phe, and 3OH-BaP) were considered to have no binding potential. Similar to the estrogenic and antiestrogenic activities, 2/3OH-Chr had the highest contribution to the total estimated binding potential ($26.1 \pm 5.5\%$), followed by 2/3OH-Phe ($19.5 \pm 4.2\%$). Unlike the estrogenic and antiestrogenic activities, Flt and Pyr had high contributions to the total, estimated, binding potential ($13.8 \pm 3.6\%$ and $13.8 \pm 4.5\%$, respectively), and 6OH-Chr/3OH-BaA had a low contribution to the total, estimated, binding potential ($5.8 \pm 4.5\%$). OH-PAHs contain a phenol group, which is considered an important functional group for binding with hER α , but not necessary for exhibiting antiestrogenic activity [30]. Previous research has suggested that the binding affinity to hER is not the only factor affecting the estrogenic and antiestrogenic activities.

The current results indicate that the high binding potentials of several atmospheric PAHs and OH-PAHs to the hER are not directly connected to their estrogenic and antiestrogenic activities. Instead, the results imply that the endocrine disruption risk with PAHs and OH-PAHs arises because of their relatively high atmospheric concentrations.



Figure 7. Daily relative binding potentials of atmospheric PAHs and OH-PAHs to the hER in April, August, and November 2017, and February and April 2018 in Kanazawa.

OH-PAHs are intermediate metabolites of PAHs in organisms [48], and are of concern because they are endocrine disrupting chemicals [30]. Consequently, exposure to PAHs in the environment could result in endocrine disruption caused by OH-PAHs. However, over time, the OH-PAHs are metabolized into conjugated forms, such as the glucuronic acid conjugated form, by detoxification enzymes in the liver [10,49] and excreted mainly via urine [35,50]. These conjugated forms can be transported through the vascular system, but they generally have higher water solubility and lower toxicity than the non-conjugated forms. Therefore, metabolized OH-PAHs may have low health risks. However, it is also possible that OH-PAHs in the atmosphere can be taken in via inhalation and directly enter the vascular system through alveoli. This means that OH-PAHs from the atmosphere could enter the body without being metabolized in the liver and then may cause endocrine disruption. These atmospheric PAHs and derivatives have high risks for lung cancer and respiratory disease [51–54]. Therefore, endocrine disruption caused by OH-PAHs can occur both indirectly through exposure to atmospheric PAHs, and directly through inhalation of atmospheric OH-PAHs. Wenger et al. [55] reported that OH-PAHs contributed to the in vitro estrogenicity of ambient particulate matter. Therefore, the monitoring of atmospheric OH-PAHs and the assessment of their estrogenic and antiestrogenic activities should be performed.

3.3. Research Limitations

In this study, all of the OH-PAHs evaluated in a previous study [30] were not analyzed. In future studies, hydroxylated benzo[*c*]phenanthrene should be analyzed. High molecular weight PAHs in the atmosphere are mainly distributed in the particle phase; however, low molecular weight PAHs are mainly distributed in the gas phase [56]. Therefore, monitoring of atmospheric PAHs and OH-PAHs should involve both the gas and particle phases. Additionally, atmospheric environmental conditions, such as wind direction, humidity, ozone, temperature, and sunlight, are important in the secondary formation of OH-PAHs from PAHs in the atmosphere. To assess the health risks of environmental pollutants via

11 of 14

inhalation, uptake factors are also important, in addition to their toxicities. Therefore, the uptake factors of PAHs and OH-PAHs, and especially differences among isomers, should be analyzed in future studies.

4. Conclusions

The concentrations of atmospheric PAHs and OH-PAHs showed strong correlations, with a seasonal increase in winter and a decrease in summer. Additionally, the health risk of each substance was evaluated in terms of estrogenic and antiestrogenic activities, with a focus on endocrine disruption. The current results indicated that the health risks of endocrine disruption caused by atmospheric OH-PAHs are higher in the winter than in the summer. Among the OH-PAHs, OH-chrysenes and OH-benz[*a*]anthracene had particularly high health risks. This is the first study to focus on the contribution of atmospheric OH-PAHs to endocrine disruption in humans. Further studies are needed for more target substances such as OH-benz[*c*]phenanthrene, which show strong antiestrogenic activity, so as to evaluate the uptake factors and the effects of environmental conditions on secondary formation.

Supplementary Materials: The following supporting information can be downloaded at https: //www.mdpi.com/article/10.3390/app12199469/s1: Figure S1: box plot of seasonal atmospheric concentrations of PAHs in Kanazawa; Figure S2: box plot of seasonal atmospheric concentrations of OH-PAHs in Kanazawa; Figure S3: PCA score plot in which the first two PCs for atmospheric PAHs; Figure S4: PCA score plot in which the first two PCs for atmospheric OH-PAHs; Figure S5: Diagnostic ratios calculated for atmospheric PAHs; Table S1: Estrogenic and antiestrogenic activities of OH-PAHs from a yeast two-hybrid assay conducted by Hayakawa et al., (2007); Table S2: Binding affinities of PAHs and OH-PAHs to hER conducted by Hayakawa et al., (2007). Reference [30] is cited in the supplementary materials.

Author Contributions: Conceptualization, K.H. and H.N.; methodology, M.H. and L.Z.; validation, K.H.; formal analysis, M.H.; investigation, L.Z. and N.T.; resources, K.H. and H.N.; writing—original draft preparation, M.H.; writing—review and editing, K.H., L.Z., N.T. and H.N.; supervision, K.H.; project administration, K.H. and H.N.; funding acquisition, K.H. and H.N. All authors have read and agreed to the published version of the manuscript.

Funding: This research was financially supported by Scientific Research (No. 17H06283) from Japan Society for the Promotion Science, the Environment Research, and the Environment Research and Technology Development Fund (Project Nos. 5-1951) of the Ministry of the Environment, Japan.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: We thank Hina Satone and staff of Food Industry Department, Niigata Agro-Food University for the analysis of OH-PAHs. We thank Edward G. Nagato, Faculty of Life and Environmental Sciences, Shimane University for review and editing. We thank Gabrielle David, from Edanz (https://jp.edanz.com/ac: accessed on 22 August 2022) for editing a draft of this manuscript. We thank the Kanazawa City Environment Division Environmental Policy Department for arranging the PM_{2.5} sampling site.

Conflicts of Interest: The authors declare no conflict of interest.

References

- 1. Abbas, I.; Badran, G.; Verdin, A.; Ledoux, F.; Roumié, M.; Courcot, D.; Garçon, G. Polycyclic aromatic hydrocarbon derivatives in airborne particulate matter: Sources, analysis and toxicity. *Environ. Chem. Lett.* **2018**, *16*, 439–475. [CrossRef]
- Hayakawa, K.; Tang, N.; Xing, W.; Oanh, P.K.; Hara, A.; Nakamura, H. Concentrations and Sources of Atmospheric PM, Polycyclic Aromatic Hydrocarbons and Nitropolycyclic Aromatic Hydrocarbons in Kanazawa, Japan. Atmosphere 2021, 12, 256. [CrossRef]
- 3. Kim, K.H.; Jahan, S.A.; Kabir, E.; Brown, R.J. A review of airborne polycyclic aromatic hydrocarbons (PAHs) and their human health effects. *Environ. Int.* 2013, *60*, 71–80. [CrossRef] [PubMed]

- Hayakawa, K.; Tang, N.; Nagato, E.G.; Toriba, A.; Sakai, S.; Kano, F.; Goto, S.; Endo, O.; Arashidani, K.I.; Kakimoto, H. Long term trends in atmospheric concentrations of polycyclic aromatic hydrocarbons and nitropolycyclic aromatic hydrocarbons: A study of Japanese cities from 1997 to 2014. *Environ. Pollut.* 2018, 233, 474–482. [CrossRef]
- Slezakova, K.; Pires, J.C.M.; Castro, D.; Alvim-Ferraz, M.C.M.; Delerue-Matos, C.; Morais, S.; Pereira, M.C. PAH air pollution at a Portuguese urban area: Carcinogenic risks and sources identification. *Environ. Sci. Pollut. Res.* 2013, 20, 3932–3945. [CrossRef]
- 6. Wang, G.; Kawamura, K.; Zhao, X.; Li, Q.; Dai, Z.; Niu, H. Identification, abundance and seasonal variation of anthropogenic organic aerosols from a mega-city in China. *Atmos. Environ.* **2007**, *41*, 407–416. [CrossRef]
- 7. Balcıoğlu, E.B. Potential effects of polycyclic aromatic hydrocarbons (PAHs) in marine foods on human health: A critical review. *Toxin Rev.* **2016**, *35*, 98–105. [CrossRef]
- 8. Honda, M.; Suzuki, N. Toxicities of Polycyclic Aromatic Hydrocarbons for Aquatic Animals. *Int. J. Environ. Res. Public Health* 2020, *17*, 1363. [CrossRef]
- 9. Lemieux, C.L.; Long, A.S.; Lambert, I.B.; Lundstedt, S.; Tysklind, M.; White, P.A. In vitro mammalian mutagenicity of complex polycyclic aromatic hydrocarbon mixtures in contaminated soils. *Environ. Sci. Technol.* **2015**, *49*, 1787–1796. [CrossRef]
- 10. Moorthy, B.; Chu, C.; Carlin, D.J. Polycyclic aromatic hydrocarbons: From metabolism to lung cancer. *Toxicol. Sci.* **2015**, 145, 5–15. [CrossRef]
- 11. Suzuki, N.; Ogiso, S.; Yachiguchi, K.; Kawabe, K.; Makino, F.; Toriba, A.; Kiyomoto, M.; Sekiguchi, T.; Tabuchi, Y.; Kondo, T.; et al. Monohydroxylated polycyclic aromatic hydrocarbons influence spicule formation in the early development of sea urchins (*Hemicentrotus pulcherrimus*). *Comp. Biochem. Physiol. Part C* 2015, *171*, 55–60. [CrossRef] [PubMed]
- Yadetie, F.; Zhang, X.; Hanna, E.M.; Aranguren-Abadía, L.; Eide, M.; Blaser, N.; Brun, M.; Jonassen, I.; Goksøyr, A.; Karlsen, O.A. RNA-Seq analysis of transcriptome responses in Atlantic cod (*Gadus morhua*) precision-cut liver slices exposed to benzo[*a*]pyrene and 17-ethynylestradiol. *Aquat. Toxicol.* 2018, 201, 174–186. [CrossRef] [PubMed]
- Prevedouros, K.; Brorström-Lundén, E.; Halsall, C.J.; Jones, K.C.; Lee, R.G.; Sweetman, A.J. Seasonal and long-term trends in atmospheric PAH concentrations: Evidence and implications. *Environ. Pollut.* 2004, 128, 17–27. [CrossRef] [PubMed]
- Yang, L.; Zhang, X.; Xing, W.; Zhou, Q.; Zhang, L.; Wu, Q.; Zhou, Z.; Chen, R.; Toriba, A.; Hayakawa, K.; et al. Yearly variation in characteristics and health risk of polycyclic aromatic hydrocarbons and nitro-PAHs in urban shanghai from 2010–2018. *J. Environ. Sci.* 2021, *99*, 72–79. [CrossRef]
- 15. Krzyszczak, A.; Czech, B. Occurrence and toxicity of polycyclic aromatic hydrocarbons derivatives in environmental matrices. *Sci. Total Environ.* **2021**, *788*, 147738. [CrossRef] [PubMed]
- Nowakowski, M.; Rykowska, L.; Wolski, R.; Andrzejewski, P. Polycyclic Aromatic Hydrocarbons (PAHs) and their Derivatives (O-PAHs, N-PAHs, OH-PAHs): Determination in Suspended Particulate Matter (SPM)—A Review. *Environ. Process.* 2021, 9, 2. [CrossRef]
- 17. Avagyan, R.; Nyström, R.; Lindgren, R.; Boman, C.; Westerholm, R. Particulate hydroxy-PAH emissions from a residential wood log stove using different fuels and burning conditions. *Atmos. Environ.* **2016**, *140*, 1–9. [CrossRef]
- 18. Barrado, A.I.; García, S.; Barrado, E.; Pérez, R.M. PM2.5-bound PAHs and hydroxy-PAHs in atmospheric aerosol samples: Correlations with season and with physical and chemical factors. *Atmos. Environ.* **2012**, *49*, 224–232. [CrossRef]
- Cochran, R.E.; Dongari, N.; Jeong, H.; Beránek, J.; Haddadi, S.; Shipp, J.; Kubátová, A. Determination of polycyclic aromatic hydrocarbons and their oxy-, nitro-, and hydroxy-oxidation products. *Anal. Chim. Acta* 2012, 740, 93–103. [CrossRef]
- Kamiya, M.; Toriba, A.; Onoda, Y.; Kizu, R.; Hayakawa, K. Evaluation of estrogenic activities of hydroxylated polycyclic aromatic hydrocarbons in cigarette smoke condensate. *Food Chem. Toxicol.* 2005, 43, 1017–1027. [CrossRef]
- Kishikawa, N.; Morita, S.; Wada, M.; Ohba, Y.; Nakashima, K.; Kuroda, N. Determination of hydroxylated polycyclic aromatic hydrocarbons in airborne particulates by high-performance liquid chromatography with fluorescence detection. *Anal. Sci.* 2004, 20, 129–132. [CrossRef] [PubMed]
- Lin, Y.; Ma, Y.; Qiu, X.; Li, R.; Fang, Y.; Wang, J.; Zhu, Y.; Hu, D. Sources, transformation, and health implications of PAHs and their nitrated, hydroxylated, and oxygenated derivatives in PM2.5 in Beijing. *J. Geophys. Res. Atmos.* 2015, 120, 7219–7228. [CrossRef]
- Ma, Y.; Cheng, Y.; Qiu, X.; Lin, Y.; Cao, J.; Hu, D. A quantitative assessment of source contributions to fine particulate matter (PM2.5)-bound polycyclic aromatic hydrocarbons (PAHs) and their nitrated and hydroxylated derivatives in Hong Kong. *Environ. Pollut.* 2016, 219, 742–749. [CrossRef] [PubMed]
- 24. Toriba, A.; Kasahara, C.; Bekki, K.; Samizo, M.; Tang, N.; Hayakawa, K. Quantification of hydroxylated polycyclic aromatic hydrocarbons in airborne particulate matter by GC/MS. *Bunseki Kagaku* 2019, *68*, 839–845. (In Japanese) [CrossRef]
- Simoneit, B.R.; Bi, X.; Oros, D.R.; Medeiros, P.M.; Sheng, G.; Fu, J. Phenols and hydroxy-PAHs (arylphenols) as tracers for coal smoke particulate matter: Source tests and ambient aerosol assessments. *Environ. Sci. Technol.* 2007, 41, 7294–7302. [CrossRef]
- 26. Atkinson, R.; Arey, J. Atmospheric chemistry of gas-phase polycyclic aromatic hydrocarbons: Formation of atmospheric mutagens. *Environ. Health Perspect.* **1994**, *102*, 117–126. [CrossRef]
- 27. Cochran, R.E.; Jeong, H.; Haddadi, S.; Derseh, R.F.; Gowan, A.; Beránek, J.; Kubátová, A. Identification of products formed during the heterogeneous nitration and ozonation of polycyclic aromatic hydrocarbons. *Atmos. Environ.* **2016**, *128*, 92–103. [CrossRef]
- 28. Gao, S.; Zhang, Y.; Meng, J.; Shu, J. Online investigations on ozonation products of pyrene and benz [*a*] anthracene particles with a vacuum ultraviolet photoionization aerosol time-of-flight mass spectrometer. *Atmos. Environ.* **2009**, *43*, 3319–3325. [CrossRef]

- 29. Miet, K.; Le Menach, K.; Flaud, P.M.; Budzinski, H.; Villenave, E. Heterogeneous reactions of ozone with pyrene, 1-hydroxypyrene and 1-nitropyrene adsorbed on particles. *Atmos. Environ.* **2009**, *43*, 3699–3707. [CrossRef]
- Hayakawa, K.; Onoda, Y.; Tachikawa, C.; Hosoi, S.; Yoshita, M.; Chung, S.W.; Kizu, R.; Toriba, A.; Kameda, T.; Tang, N. Estrogenic/antiestrogenic activities of polycyclic aromatic hydrocarbons and their monohydroxylated derivatives by yeast two-hybrid assay. J. Health Sci. 2007, 53, 562–570. [CrossRef]
- Hayakawa, K.; Onoda, Y.; Tachikawa, C.; Yoshita, M.; Toriba, A.; Kameda, T.; Tang, N. Interaction of hydroxylated polycyclic aromatic hydrocarbons to estrogen receptor. *Polycycl. Aromat. Compd.* 2008, 28, 382–391. [CrossRef]
- Suzuki, N.; Honda, M.; Sato, M.; Yoshitake, S.; Kawabe, K.; Tabuchi, Y.; Omote, T.; Sekiguchi, T.; Furusawa, Y.; Toriba, A.; et al. Hydroxylated benzo[*c*]phenanthrene metabolites cause osteoblast apoptosis and skeletal abnormalities in fish. *Ecotoxicol. Environ. Saf.* 2022, 234, 113401. [CrossRef] [PubMed]
- 33. Chen, K.; Tsutsumi, Y.; Yoshitake, S.; Qiu, X.; Xu, H.; Hashiguchi, Y.; Honda, M.; Tashiro, K.; Nakayama, K.; Hano, T.; et al. Alteration of development and gene expression induced by in ovo-nanoinjection of 3-hydroxybenzo [c] phenanthrene into Japanese medaka (*Oryzias latipes*) embryos. *Aqua. Toxicol.* 2017, *182*, 194–204. [CrossRef] [PubMed]
- 34. Xing, W.; Zhang, L.; Yang, L.; Zhou, Q.; Zhang, X.; Toriba, A.; Hayakawa, K.; Tang, N. Characteristics of PM2.5-bound polycyclic aromatic hydrocarbons and nitro-polycyclic aromatic hydrocarbons at a roadside air pollution monitoring station in Kanazawa, Japan. *Int. J. Environ. Res. Public Health* **2020**, *17*, 805. [CrossRef]
- Guo, Y.; Senthilkumar, K.; Alomirah, H.; Moon, H.B.; Minh, T.B.; Mohd, M.A.; Nakata, H.; Kannan, K. Concentrations and profiles of urinary polycyclic aromatic hydrocarbon metabolites (OH-PAHs) in several Asian countries. *Environ. Sci. Technol.* 2013, 47, 2932–2938. [CrossRef]
- 36. Ihaka, R.; Gentleman, R. R: A language for data analysis and graphics. J. Comp. Graph. Stat. 1996, 5, 299–314. [CrossRef]
- Kalisa, E.; Nagato, E.; Bizuru, E.; Lee, K.; Tang, N.; Pointing, S.; Hayakawa, K.; Archer, S.; Lacap-Bugler, D. Pollution characteristics and risk assessment of ambient PM_{2.5}-bound PAHs and NPAHs in typical Japanese and New Zealand cities and rural sites. *Atmos. Pollut. Res.* 2019, 10, 1396–1403. [CrossRef]
- 38. Hong, W.J.; Jia, H.; Yang, M.; Li, Y.F. Distribution, seasonal trends, and lung cancer risk of atmospheric polycyclic aromatic hydrocarbons in North China: A three-year case study in Dalian city. *Ecotoxicol. Environ. Saf.* **2020**, *196*, 110526. [CrossRef]
- 39. Yang, J.; Xu, W.; Cheng, H. Seasonal variations and sources of airborne polycyclic aromatic hydrocarbons (PAHs) in Chengdu, China. *Atmosphere* **2018**, *9*, 63. [CrossRef]
- Azimi-Yancheshmeh, R.; Moeinaddini, M.; Feiznia, S.; Riyahi-Bakhtiari, A.; Savabieasfahani, M.; van Hullebusch, E.D.; Lajayer, B.A. Seasonal and spatial variations in atmospheric PM2.5-bound PAHs in Karaj city, Iran: Sources, distributions, and health risks. *Sustain. Cities Soc.* 2021, 72, 103020. [CrossRef]
- 41. Ministry of Land Infrastructure Transport and Tourism. Regulation of Emission Gas from Cars. Available online: https://www.mlit.go.jp/jidosha_tk10_000001.html (accessed on 7 September 2022).
- Zheng, S.; Yi, H.; Li, H. The impacts of provincial energy and environmental policies on air pollution control in China. *Renew. Sustain. Energy Rev.* 2015, 49, 386–394. [CrossRef]
- Zhang, Y.; Chen, Y.; Li, R.; Chen, W.; Song, Y.; Hu, D.; Cai, Z. Determination of PM_{2.5}-bound polyaromatic hydrocarbons and their hydroxylated derivatives by atmospheric pressure gas chromatography-tandem mass spectrometry. *Talanta* 2019, 195, 757–763. [CrossRef] [PubMed]
- Tobiszewski, M.; Namieśnik, J. PAH diagnostic ratios for the identification of pollution emission sources. *Environ. Pollut.* 2012, 162, 110–119. [CrossRef] [PubMed]
- Ministry of the Environment. Waste Management and Public Cleansing Act. Available online: https://www.env.go.jp/recycle/ waste/laws.html (accessed on 7 September 2022).
- Pham, K.O.; Hara, A.; Zhao, J.; Suzuki, K.; Matsuki, A.; Inomata, Y.; Matsuzaki, H.; Odajima, H.; Hayakawa, K.; Nakamura, H. Different Transport Behaviors between Asian Dust and Polycyclic Aromatic Hydrocarbons in Urban Areas: Monitoring in Fukuoka and Kanazawa, Japan. *Appl. Sci.* 2022, *12*, 5404. [CrossRef]
- 47. Schauer, C.; Niessner, R.; Pöschl, U. Polycyclic aromatic hydrocarbons in urban air particulate matter: Decadal and seasonal trends, chemical degradation, and sampling artifacts. *Environ. Sci. Technol.* **2003**, *37*, 2861–2868. [CrossRef]
- Lotz, A.; Pesch, B.; Dettbarn, G.; Raulf, M.; Welge, P.; Rihs, H.P.; Breuer, D.; Gabriel, S.; Hahn, J.U.; Brüning, T.; et al. Metabolites of the PAH diol epoxide pathway and other urinary biomarkers of phenanthrene and pyrene in workers with and without exposure to bitumen fumes. *Int. Arch. Occup. Environ. Health* 2016, *89*, 1251–1267. [CrossRef]
- 49. Bekki, K.; Toriba, A.; Tang, N.; Kameda, T.; Takigami, H.; Suzuki, G.; Hayakawa, K. How do transport and metabolism affect on the biological effects of polycyclic aromatic hydrocarbons? *Yakugaku Zasshi* **2012**, *132*, 325–329. (In Japanese) [CrossRef]
- Lu, S.Y.; Li, Y.X.; Zhang, J.Q.; Zhang, T.; Liu, G.H.; Huang, M.Z.; Li, X.; Ruan, J.; Kannan, K.; Qiu, R.L. Associations between polycyclic aromatic hydrocarbon (PAH) exposure and oxidative stress in people living near e-waste recycling facilities in China. *Environ. Int.* 2016, 94, 161–169. [CrossRef]
- Higashi, T.; Kambayashi, Y.; Ohkura, N.; Fujimura, M.; Nakanishi, S.; Yoshizaki, T.; Saijoh, K.; Hayakawa, K.; Kobayashi, F.; Michigami, Y.; et al. Exacerbation of daily cough and allergic symptoms in adult patients with chronic cough by Asian dust: A hospital-based study in Kanazawa. *Atmos. Environ.* 2014, 97, 537–543. [CrossRef]

- 52. Hong, W.J.; Jia, H.; Ma, W.L.; Sinha, R.K.; Moon, H.B.; Nakata, H.; Minh, N.H.; Chi, K.H.; Li, W.L.; Kannan, K.; et al. Distribution, fate, inhalation exposure and lung cancer risk of atmospheric polycyclic aromatic hydrocarbons in some Asian countries. *Environ. Sci. Technol.* **2016**, *50*, 7163–7174. [CrossRef]
- Xia, Z.; Duan, X.; Tao, S.; Qiu, W.; Liu, D.; Wang, Y.; Wei, S.; Wang, B.; Jiang, Q.; Lu, B.; et al. Pollution level, inhalation exposure and lung cancer risk of ambient atmospheric polycyclic aromatic hydrocarbons (PAHs) in Taiyuan, China. *Environ. Pollut.* 2013, 173, 150–156. [CrossRef] [PubMed]
- 54. Zhang, L.; Morisaki, H.; Wei, Y.; Li, Z.; Yang, L.; Zhou, Q.; Zhang, X.; Xing, W.; Hu, M.; Shima, M.; et al. PM_{2.5}-bound polycyclic aromatic hydrocarbons inside and outside a primary school classroom in Beijing: Concentration, composition, and inhalation cancer risk. *Sci. Total Environ.* **2020**, *705*, 135840. [CrossRef] [PubMed]
- 55. Wenger, D.; Gerecke, A.C.; Heeb, N.V.; Schmid, P.; Hueglin, C.; Naegeli, H.; Zenobi, R. In vitro estrogenicity of ambient particulate matter: Contribution of hydroxylated polycyclic aromatic hydrocarbons. *J. Appl. Toxicol.* 2009, 29, 223–232. [CrossRef] [PubMed]
- 56. Esen, F.; Cindoruk, S.S.; Tasdemir, Y. Bulk deposition of polycyclic aromatic hydrocarbons (PAHs) in an industrial site of Turkey. *Environ. Pollut.* **2008**, 152, 461–467. [CrossRef]