

# Unintentional Generation of Chlorinated Polycyclic Aromatic Hydrocarbons during Cooking

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## Introduction

Some of polycyclic aromatic hydrocarbons (PAHs) are carcinogens and/or mutagens. For example, benzo[*a*]pyrene (BaP) is classified as Group 1 by International Agency for Research on Cancer (IARC) <sup>1</sup>. In addition, PAHs have aryl hydrocarbon receptor (AhR)-mediated activities, which increases the risk of oncogenic action. On the other hand, chlorinated PAHs (CIPAHs), which are compounds whose one to several hydrogen atoms are substituted to chlorine atoms, have attracted attention in recent years. Some CIPAHs have more AhR activities than their parent PAHs <sup>2</sup>. Ohura et al. (2007) <sup>3</sup> assessed AhR activities as an agonist of 3–5 ring CIPAHs and their corresponding parent compounds by lacZ receptor gene assays using yeast YCM3 cells and showed that AhR activities are increased by increasing the number of chlorine atoms substituted.

PAHs and CIPAHs are unintentionally generated by incomplete combustion of organic substances, thus they are considered to be mainly emitted from waste incinerators and e-waste recycling sites so far. Given that cooking processes satisfy the conditions to generate PAHs and CIPAHs (e.g., sufficient organic substances, heat, and chlorine source), they can be generated during cooking processes such as grilling and charcoal-broiling foods. In fact, burning foods (e.g., pork, beef, chicken, and fish) over direct flame resulted in the unintentional generation of various PAHs, and PAHs were detected from not only cooked foods, but also from cooking exhaust at high concentration. Although CIPAHs are likely to be generated by the reaction of PAHs with chlorine atoms during cooking, the occurrence of CIPAHs in cooked foods and cooking exhausts are still missing.

In this study, PAHs and CIPAHs in cooked foods and cooking exhausts were measured. As target cooked foods, a pork, beef, and chicken barbecued in outdoor and a pacific saury grilled in indoor, which is typical fish consumed in Japan, were selected in this study. In addition, PAHs and CIPAHs in cooking exhausts generated during cooking the pacific saury in accordance with typical Japanese cooking procedure were also measured.

## Materials and methods

### *Sample collection for meats and cooking exhaust samples*

A pork, beef, chicken, and pacific saury were purchased in Japanese market in 2017. A pork, beef, and chicken were barbecued by a barbecue hibachi in outdoor in accordance with typical cooking procedure. After grilling the meats, the surfaces of them were sampled. A pacific saury was grilled in a Japanese typical fish oven in an indoor kitchen by stove burner in accordance with typical Japanese cooking procedure. After grilling the fish, the skin of fish was collected. As a reference, raw each meat was also sampled.

During the cooking the fish, the cooking exhaust was also sampled at the exhaust outlet of fish oven by a pump (2 L min<sup>-1</sup>). Gaseous- and particle-PAHs and CIPAHs in the cooking exhaust were trapped by a PUF/Amberlite XAD-2/PUF cartridge. The cartridge was connected with a funnel (15 cm diameter) and kept off 15 cm from the outlet.

### *Extraction and clean-up procedures for meat samples*

The raw and cooked meat samples were brayed with anhydrous sodium sulfate. Then, PAHs and CIPAHs in these samples were extracted by the Soxhlet extraction method with 250 mL of dichloromethane after spiking recovery standards. The extract was purified and fractionated by using an activated carbon column connected with a 2% KOH silica gel column. After washing the connected column with 10% dichloromethane/hexane, the silica gel column was removed, and the activated carbon column was reversed. Then, the target compounds (PAHs and CIPAHs) were eluted from the activated carbon column with 120 mL of toluene. The toluene fraction was spiked with internal standards and concentrated to 100  $\mu$ L for the analysis by a gas chromatograph with high resolution mass spectrometer (HRGC-HRMS).

### *Extraction and clean-up procedures for cooking exhaust sample*

The cooking exhaust sample corrected by the cartridge was extracted by the Soxhlet extraction method with 250 mL of dichloromethane after spiking the recovery standards. The extract was purified by using the 2% KOH

silica gel column, and then the target compounds were eluted with 120 mL of 10% dichloromethane/hexane. The purified extract was spiked with the internal standards and concentrated to 100  $\mu\text{L}$  for the HRGC-HRMS analysis.

#### HRGC-HRMS analysis

The concentrations of PAHs and CIPAHs in the samples were determined by the HRGC-HRMS. Gas chromatographic separation was accompanied by a 60-m BPX-DXN fused silica capillary column (0.25 mm internal diameter). Two microliters of the aliquot were injected in splitless mode at 280°C. The temperature of the column oven was kept at 130°C for 1 min, raised with a rate of 5°C min<sup>-1</sup> to 250°C, raised with 10°C min<sup>-1</sup> to 320°C, and held for 18 min. The HRMS was operated in an electron-impact selected ion monitoring (SIM) mode at resolution  $R > 10,000$  (10% valley). Peaks were identified by comparison of the retention times of samples to those of standards if the signal-to-noise ( $S/N$ ) ratio was  $> 3$  and were quantified if target/qualifier ion ratio were within 15% of the theoretical values.

#### Estimation procedure of BaP equivalent concentration

The BaP equivalent concentrations ( $BaP_{eq}$ ) of individual CIPAH were calculated by multiplying their concentration with their toxicity equivalency factors relative to BaP ( $REP_{BaP}$ ). The values of  $REP_{BaP}$  for CIPAHs were obtained from a literature<sup>4</sup>.

## Results and discussion

### Occurrence of PAHs/CIPAHs in raw and cooked meat samples

The concentrations of PAHs in the cooked pork, beef, and chicken and raw and cooked fishes are shown in Figure 1A. The total concentrations of PAHs (sum of concentrations of each PAH) in the raw and cooked fish (skin) were 2.6, 6.6 (cooked without salt), and 30 ng g<sup>-1</sup> (cooked with salt), respectively. Cooking significantly increased the total PAH concentration, especially in the case of cooking with salt (more than 10 times).

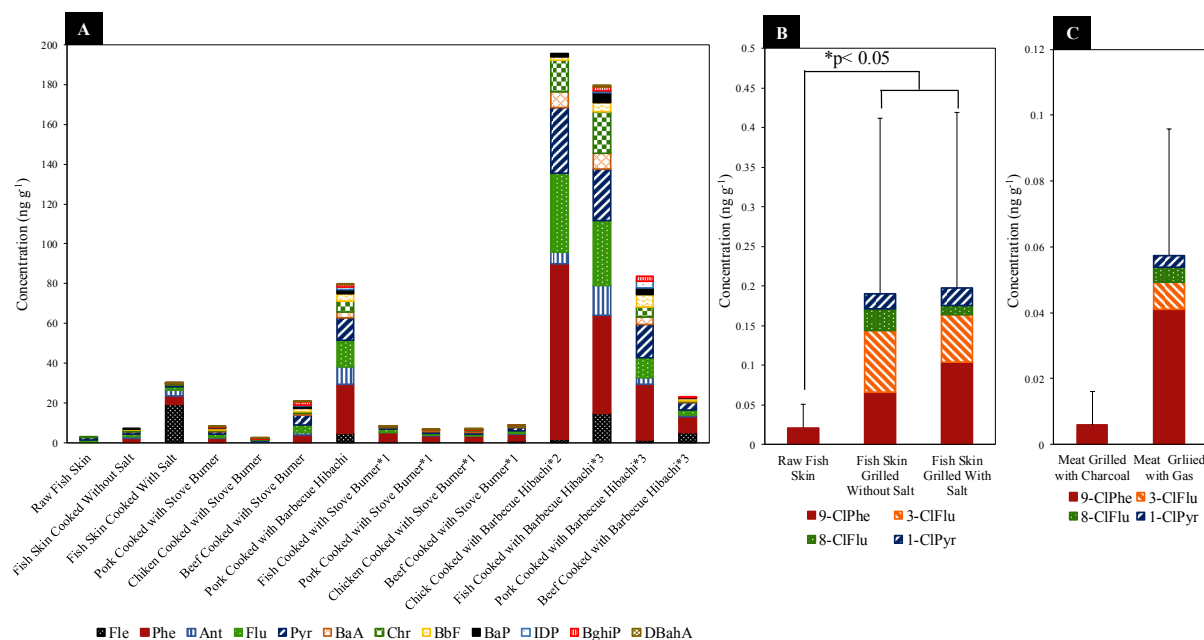
The concentrations of PAHs in the cooked fish ranged 0.92–93 ng g<sup>-1</sup>. Fluorene (Fle) (<3.8–78 ng g<sup>-1</sup>), Phenanthrene (Phe) (<2.4–19 ng g<sup>-1</sup>), Fluoranthene (Flu) (<0.61–6.5 ng g<sup>-1</sup>), and Pyrene (Pyr) (<0.27–2.4 ng g<sup>-1</sup>) were detected at relatively high concentration. On the other hand, the concentrations of PAHs in the cooked meats (pork, beef, and chicken) ranged in 2.0–120 ng g<sup>-1</sup>. Fle (<0.54–13 ng g<sup>-1</sup>), Phe (2.3–38 ng g<sup>-1</sup>), Flu (<0.45–22 ng g<sup>-1</sup>), and Pyr (0.51–18 ng g<sup>-1</sup>) were detected at relatively high concentration. These concentrations were comparable to those reported in a previous study<sup>5</sup>.

The concentrations of CIPAHs in the raw and cooked fishes are shown in Figure 1B. While only 9-monochlorinated Phe (9-CIPhe) was detected from the raw fish (0.021 ng g<sup>-1</sup>), several CIPAHs, such as 9-CIPhe (0.087 ng g<sup>-1</sup>), 3-monochlorinated Flu (3-CIFlu) (0.059 ng g<sup>-1</sup>), 8-monochlorinated Flu (8-CIFlu) (0.015 ng g<sup>-1</sup>), and 1-monochlorinated Pyr (1-CIPyr) (0.019 ng g<sup>-1</sup>), were detected in the cooked fish. A significant difference between the total concentrations of CIPAHs in the raw and cooked fishes was observed ( $p < 0.05$ ). This result revealed that CIPAHs could be unintentionally generated during cooking. The compositions of parent PAHs of CIPAHs in the cooked fish were similar to those of PAHs detected (except for Fle). This could be attributed to the formation mechanism of CIPAHs. According to our previous study, CIPAHs are likely to be produced by the reaction of parent PAHs with chlorine atoms<sup>6</sup>.

Effects of salt on the concentrations of CIPAHs in the cooked fish are shown in Figure 1B. The concentrations of CIPAHs in the fishes cooked without and with salt (1 g-salt/100 g-fish) were 0.19 and 0.20 ng g<sup>-1</sup>, respectively, and they were comparable. According to a literature<sup>7</sup>, a pacific saury naturally includes sodium chloride (0.3 g-salt/100 g-fish). Therefore, salt naturally included in the fish could be sufficient as a chlorine source to generate CIPAHs.

The concentrations of CIPAHs in the cooked meats (pork, beef, and chicken) are shown in Figure 1C. While 9-CIPhe was detected from the meats cooked by the barbecue hibachi (0.0059 ng g<sup>-1</sup>), several CIPAHs, i.e., 9-CIPhe (0.041 ng g<sup>-1</sup>), 3-CIFlu (0.082 ng g<sup>-1</sup>), 8-CIFlu (0.045 ng g<sup>-1</sup>), and 1-CIPyr (0.036 ng g<sup>-1</sup>) were detected in the meats cooked by the stove burner. While the concentrations of CIPAHs in the meats cooked with the barbecue hibachi and stove burner were 0.010 and 0.039 ng g<sup>-1</sup>, respectively, those of PAHs were 80 and 10 ng g<sup>-1</sup>, respectively. The concentrations of CIPAHs in the meats cooked with the stove burner were significantly higher than those cooked with the barbecue hibachi. Interestingly, the tendencies of the changes in the concentrations of PAHs and CIPAHs were opposite. These results revealed that the stove burner could more efficiently generate CIPAHs than the barbecue hibachi. This could be due to the difference of the reaction temperature. While the meats were continuously heated with the direct flame in the case of cooking with the

stove burner, far-infrared heating was dominant in the case of cooking with the barbecue hibachi. Therefore, the reaction temperature (the temperature at the surface of fish) in the case of cooking with the barbecue hibachi, could be lower than that with the stove burner. The reaction temperature in the case of cooking with the stove burner could be more suitable to generate CIPAHs.



**Figure 1 Comparisons among concentrations of PAHs in cooked pork, beef, and chicken and raw and cooked fishes (A); Effects of cooking (B) and heat source (C) on concentrations of CIPAHs.**

<sup>1</sup>Perello et al. (2009)<sup>8</sup>; <sup>2</sup>Kao et al. (2014)<sup>9</sup>; <sup>3</sup>Viegas et al. (2012)<sup>5</sup>

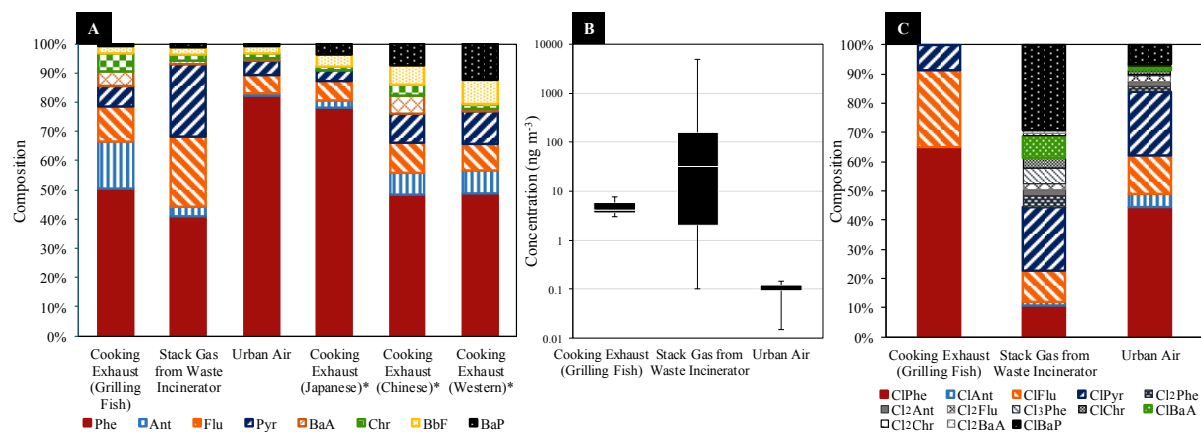
#### Occurrence of PAHs/CIPAHs in cooking exhaust

The total concentration of PAHs in the cooking exhaust during cooking the fish was 4600 ng m<sup>-3</sup>. Low molecular PAHs (Flu: 2500 ng m<sup>-3</sup>, Phe: 1100 ng m<sup>-3</sup>, Ant: 340 ng m<sup>-3</sup>) were obtained at high concentrations. Benz[*a*]anthracene (BaA) (110 ng m<sup>-3</sup>), chrysene (Chr) (130 ng m<sup>-3</sup>), benzo[*b*]fluoranthene (BbF) (47 ng m<sup>-3</sup>), and BaP (21 ng m<sup>-3</sup>) were also detected. The compositions of PAHs in the cooking exhausts and the stack gas from waste incinerators and the urban air as a reference are shown in Figure 2A. The compositions of PAHs were varied with cooking methods (Japanese, Chinese, and Western). Abdullahi et al. (2013)<sup>10</sup> reported that the temperature at cooking can affect the generation of PAHs. While the cooking at the high temperature results in the productions of PAHs with higher molecular weight, the cooking at low temperature is likely to generate PAHs with lower molecular weight.

Figure 2B shows the concentrations of CIPAHs in the cooking exhaust and the stack gas from waste incinerators and the urban air as a reference. The total concentration of CIPAHs in the cooking exhaust was 24 ng m<sup>-3</sup>. 9-CIPhe (16 ng m<sup>-3</sup>), 3-CIFlu (4.2 ng m<sup>-3</sup>), 8-CIFlu (2.0 ng m<sup>-3</sup>), and 1-CIPyr (2.1 ng m<sup>-3</sup>) were detected. According to a literature, the concentrations of CIPAHs in stack gases from waste incinerators ranged 0.1–5100 ng m<sup>-3</sup><sup>11</sup>. Although the stack gas from waste incinerator is considered as one of major CIPAHs emission sources, the total concentration of CIPAHs in the cooking exhaust was comparable to that of the stack gas. In addition, it was 350 times higher than the total concentration of CIPAHs in the ambient air in Japan<sup>12</sup>.

Figure 2C shows the compositions of CIPAHs in the cooking exhaust, stack gas, and urban air. Monochlorinated CIPAHs were dominant in both of the cooked fish and its cooking exhaust. Three- to four-ring CIPAHs (CIPhe, CIFlu, and CIPyr) were also detected from the cooking exhaust. CIPhe, CIFlu, and CIPyr accounted for 65%, 26% and 8.8% of the total CIPAHs ( $\sum$ CIPAHs), respectively. Although di-chlorinated CIPAHs, di-chlorinated anthracene (Cl<sub>2</sub>Ant), Cl<sub>2</sub>Phe, and Cl<sub>2</sub>Flu, were detected in the stack gas from the waste

incinerator and the urban air, they were not detected in the cooking exhaust. In general, the temperature at typical cooking ranges 46.3–252.6 °C<sup>8</sup>, which is lower than that at the combustion in waste incinerators (furnace temperature: 850–950 °C)<sup>12</sup>. This could be a reason for the inconsistency of the compositions in the cooking exhaust, the stack gas, and the urban air.



**Figure 2** Compositions of PAHs in cooking exhausts, stack gas from waste incinerators, and urban air (A); Concentrations (B) and compositions (C) of CIPAHs.

\*Li et al. (2003)<sup>13</sup>

#### BaP equivalent concentrations of PAHs/CIPAHs in cooked fish and its cooking exhaust

The sum of BaP equivalent concentration of PAHs (Ant, Flu, Pyr, BaA, Chr, BbF, BaP, Indeno[1,2,3-cd]pyrene (IDP), Benzo[ghi]perylene (BghiP) and Dibenz[a,h]anthracene (DBahA)) and CIPAHs (9-CIPhe, 3-CIFlu, 8-CIFlu and 1-CIPyr) in the cooked fish were 0.71 and 0.016 ng- *BaP*<sub>eq</sub> g<sup>-1</sup>, respectively. On the other hand, those in the cooking exhaust was 2.0 and 140 ng- *BaP*<sub>eq</sub> m<sup>-3</sup>, respectively. This results revealed that the BaP equivalent concentration of PAHs was much higher than that of CIPAHs. It should be noticed that CIPAHs have many isomers and many of them were not able to be analyzed due to the lack of relevant analytical standards. Therefore, these BaP equivalent concentrations of CIPAHs calculated in this study could be underestimated.

#### **Acknowledgements:**

This study was supported by a Grant-in-Aid for Scientific Research (KAKENHI) from the Japan Society for the Promotion of Science (grant number JP16H05891)

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