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Research Article

Characterization of Polyhexamethylene Guanidine Oligomers in Solutions and Aerosols Emitted during Humidifier Use

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The behavior of polyhexamethylene guanidine (PHMG), the causative agent of many humidifier-induced lung diseases, is not well known because of its various oligomer structures and analytical difficulties. The aim of this study was to identify different PHMG oligomer types both in solution and aerosols and to estimate the airborne concentration of oligomers during humidifier use. Three products containing PHMG as the main component were diluted to the manufacturer's recommended concentration (6.5 ppm) or the worst-case concentration (65 ppm or 125 ppm). Samples were qualitatively and quantitatively analyzed with liquid chromatography-quadrupole time-of-flight (LC-qToF) mass spectrometry in the diluted solution and in the air at 0.5 m and 1 m. The LC-qToF data were processed using UNIFI software to characterize the PHMG structure. For all products in both the humidifier solution and air, the linear type was predominant over the branched/cyclic structure, but each product had different characteristics. The linear structure in the Oxy product, the main product of lung diseases, accounted for 90.6%, while that of the Scunder and BOC Sciences' products accounted for 78.6% and 75.8%, respectively. The concentration of the oligomer in air for the Oxy product was estimated to be 35.89 and 390.96 $\mu g/m^3$ at 6.5 and 65 ppm, respectively. Most of the oligomers in the solution were found in air at a short distance (0.5 m), with a negligible concentration beyond 1 m. Oligomers with 1–7 monomer units were identified in the humidifier solution, whereas mainly monomers, dimers, and trimers were identified in the air. The results of this study will facilitate further investigations of the mechanisms of lung disease by identifying the behaviors and forms of PHMG in the air, along with previously revealed toxicity results.

1. Introduction

From 1994 to 2011, humidifier disinfectant products containing polyhexamethylene guanidine (PHMG) have been widely used in Korea to prevent microbial contamination due to microbial growth in ultrasonic humidifier reservoirs [1–4]. Despite an outbreak of severe lung damage due to inhalation of aerosols containing humidifier disinfectants, products containing the disinfectant PHMG remained available on the market and widely sold. Since recognizing that these products have accounted for the majority of fatalities, the sale of PHMG was prohibited by the Korean government since 2011.

The negatively charged surfaces of bacterial cells are stabilized by the presence of Mg^{2+} and Ca^{2+} ions. Oligoguanidines, which are used as disinfectants, displace Mg^{2+} and Ca^{2+}, resulting in bacterial death caused by cellular leakage and loss of essential cellular components [5–7]. Therefore, guanidine-based chemicals have been widely used as biocides. For example, PHMG phosphate (PHMG-P; CAS No. 89697-78-9) has been used in wet wipes and cleaning supplies, owing to its strong bacteriostatic action. However, there was relatively low inhalation toxicity information available for PHMG-P before a large number of patients appeared [8–10]. PHMG is a highly soluble guanidine-

based polymer that is synthesized from hexamethylene and guanidine salts. The structure of PHMG can be linear (types A–C), branched, or cyclic (types D–G) [11, 12].

Several epidemiological, toxicological, and analytical studies on guanidine-based chemicals have been conducted to date. However, most of these studies have focused on PHMG raw materials with only a few studies analyzing airborne PHMG. Since PHMG is a polymer containing various oligomers, thorough chemical characterization remains challenging because of the presence of complex PHMG oligomer mixtures with different structures [5]. PHMG polymers are mainly analyzed using matrix-assisted laser desorption/ionization-time-of-flight mass spectrometry, high-performance liquid chromatography (HPLC), and liquid chromatography-mass spectrometry (LC-MS) [13–15]. However, because there is no oligomer standard solution for each subtype, previous studies have focused on identifying various oligomers in the stock solution of PHMG. Despite epidemiological investigations on the health effects of PHMG-containing humidifier disinfectants and the analysis of PHMG concentrations in products and aerosols, different subtypes of PHMG in solutions and aerosols remain unidentified.

To fill this gap, in this study, liquid chromatography-time-of-flight mass spectrometry (LC-qToF-MS) was used for more accurate measurements of the mass value of parent molecular ions, which are typically analyzed by LC-MS, and the exact mass value of the fragment ions was determined. Furthermore, a software program was developed to accurately identify targeted materials, and the analysis data were processed to improve the accuracy of PHMG oligomer classification. This study can thus help to identify different PHMG oligomer types and contents in both products and aerosols, enabling estimation of the airborne concentration of oligomers during humidifier use.

2. Materials and Methods

2.1. Preparation of PHMG. The experiment was conducted using one product and two solutions containing PHMG.

Among humidifier disinfectant products containing PHMG used in Korea, Oxy products (brand name Oxysaksak, Oxy, Korea) were the most widely used, which have since been legally discontinued [16, 17]. Since no legal safety data sheet was generated during the sale of Oxy products, we obtained information on the raw material from the data submitted to the government when registering the product, as reported previously [16]. In brief, the Oxy solution product contained approximately 1,300 ppm of PHMG which is the average concentration among Oxy products assessed in previous studies, 14 ppm of hexamethylenediamine, 55 ppm of sodium chloride, and lavender fragrance [2, 16]. We also tested BOC solution (BOC Sciences, USA), comprising approximately 25% of PHMG with the remaining components including water. Finally, we assessed the Scunder product (Scunder, China), also comprising approximately 25% of PHMG, with less than 0.5% ash content, and the remaining ingredient being water.

Although the manufacturer's recommended dilution ratio for the Oxy product was 200 : 1, users had reported that they typically used the product by diluting up to approximately 20 : 1. Therefore, in this study, 200 : 1 was assumed to be the normal dilution ratio, and 20 : 1 was set as the worst-case scenario [16, 18].

The exact PHMG concentration in each product was unknown, and no standard PHMG solution with a known concentration is available on the market. Therefore, the approximate concentration suggested by the manufacturer was considered as the nominal maximum concentration. The concentration of the Oxy product was calculated to be approximately 6.5 ppm at 200 : 1 dilution and 65 ppm at 20 : 1 dilution. The dilution factors for BOC and Scunder products were set to produce similar concentrations by diluting to approximately 40,000 : 1 and 4,000 : 1 for 6.25 ppm and 62.5 ppm, respectively. In addition, dilutions of BOC and Scunder products were prepared at two times the worst-case scenario (125 ppm). This high-concentration experiment was not conducted for the Oxy product because this product is no longer sold, and thus, the available quantity was not sufficient. Distilled water for the dilution of PHMG in all experiments was produced using a commercial purification system (Milli-Q; Merck Millipore, Germany).

2.2. Experimental Setup and Measurements. The same sterile exposure chamber described in previous humidifier disinfectant studies was used in the present experiment [2, 19]. In brief, the study was conducted in a 40.3 m^3 sterile room of class 1,000. The ultrasonic humidifier (H-U977, Ohsung, Korea) used for the experiment had a 6.5 L tank containing the spray liquid and a design commonly used in household humidifiers. The humidifier had a spray-operated output with a rate of approximately 320 mL/h, and the spray volume was set to the maximum value in this study.

A previous study was conducted at a flow rate of 1 L/min based on the National Institute for Occupational Safety and Health (NIOSH) 5521 method [20]. However, because of the low concentration of airborne PHMG, in this study, we used a 30 mL impinger equipped with a pump (GilAir; Sensidyne, USA), operating at 2 L/min. Ten milliliters of distilled water (Milli-Q; Merck Millipore, Germany) was used as the adsorbing liquid for the impinger. To confirm the loss in the impinger due to the high-flow rate sampling, the preliminary sampling was conducted at a distance of 0.5 m with the impinger in series, and the humidifier was operated at a high concentration of 125 ppm, which was the highest concentration for Scunder and BOC. A total of six samples were analyzed, and the measurements were repeated thrice for each product; not all samples were detected in the third impinger. Based on the average ratios of all oligomers, 84% of PHMG was found at the first impinger, and the remaining PHMG was found at the second impinger. Therefore, we used the total amount of PHMG to estimate its concentration in the air. The limit of detection of each oligomer was measured seven times at a concentration of approximately 10 ppm and was calculated as three times the standard deviation. The sampling zone was set to 0.5 m and 1 m, and samples were collected thrice while the humidifier was operated for 8 h as shown in Figure S1.

2.3. Analysis of PHMG. The conditions for the LC-qTOF analysis are shown in Table S1. The Waters ACQUITY UPLC I-Class system and Masslynx ver. 4.1 software were used. Chromatographic separation was performed on an ACQUITY UPLC BEH C18 2.1 × 100 mm (1.7 μm) column (Waters, USA), and the column temperature was set at 40°C. The total run time was 15 min, and the sample injection volume was 10 μL. All chemicals were of HPLC grade, and their purity was greater than 99.9%. A binary mobile phase system composed of 0.1% trifluoroacetic acid (Lot No. STBF4960V; Sigma-Aldrich, St. Louis, MO, USA) in distilled water (A) and 100% acetonitrile (Lot No. SHBH4463V, Sigma-Aldrich) (B) at a flow rate of 0.4 mL/min was used. The gradient elution program is shown in Table S1. The temperature of all samples was maintained at 10°C.

A SYNAPT G2-Si HDMS mass spectrometry system was used for electrospray ionization and run in positive-ion mode (+H). The mass analyzer scanned over a mass range of 100–1500 Da in a full scan. The data were acquired in continuum mode. The dual-dynamic collision energy was 25–45 V.

Five standard solutions of PHMG in the three products (Oxy, Scunder, and BOC) were individually prepared at concentrations of 1, 5, 10, 50, and 100 ppm, respectively. Oligomer peaks were identified using an internal standard (FC-8302, FUTURECHEM, Korea). The guanidine carbon in the internal standard was replaced with a carbon isotope with a mass of 13. A 10 ppm internal standard solution was added to all oligomer standards, and each oligomer solution had a retention time within 0.5 min of the time at which the internal standard was detected.

2.4. Data Processing with UNIFI Software. Postacquisition data processing was performed using the UNIFI software (v.1.8.2, Waters, USA). This program considers the estimated peak intensity, molecular weight, and molecular structure of the sample and presents the information in the form of a Molfile.

This UNIFI software is a kind of informatics platform that acquires analysis data, performs data mining, searches libraries, and compiles the results [7]. Since PHMG does not have a library, our research team created a library (Molfile) by drawing the structural formula for each structure using the molecular formula, molecular weight, and basic structure form. Next, the chromatogram of each sample solution was matched with the constructed Molfile library to determine the state of each oligomer. Since PHMG exists in various oligomer forms during the polymerization process, it is difficult to accurately determine it through the above process alone. Therefore, the analyst checked each peak individually using UNIFI software and checked the molecular weight, retention time, and fragment pattern to determine the oligomer state. This procedure is illustrated in Figure S2 and Figure S3.

There are seven types of PHMG oligomers: linear structures (types A–C) and cyclic or branched structures (types D–G), as shown in Figure S4 [7]. For each PHMG type, as the monomer unit increases, the molecule increases to a dimer, trimer, and so on. Therefore, polymers with different molecular structures and masses may be present in the same PHMG sample. Table S2 shows the molecular structure and molecular weight according to the type of PHMG and the increase in the number of monomer units when the ion mode was positive (+H).

Here, we refer to each oligomer by a combination of the type and number of monomers. For example, a dimer of type A is referred to as A2. Therefore, the intensities of all oligomer types were compared according to the molecular structure (Figure S4) and mass (Table S2) information. Each Molfile PHMG oligomer was created using Chem3D (PerkinElmer, USA). For UNIFI program analysis, we set certain criteria for locating the corresponding oligomers. First, we identified oligomer peaks using the Masslynx program (PerkinElmer). Second, oligomer peaks were detected above the background concentration. Finally, all standards were confirmed to have similar high and low fragments, and the oligomer peaks and their intensities were identified based on the standards.

3. Results

3.1. Characteristics of Raw Materials in PHMG Products and Solutions. Figure 1 shows the chromatogram of the standard solution with the highest concentration (100 ppm) for each product. Linear types were the main products identified (types A–C), although branched or cyclic types were also rarely identified in some products (types D–F). Each product had a unique PHMG profile. Specifically, in the Oxy product, type A was dominant, followed by similar patterns for types B and C. By contrast, in the Scunder solution, type C was dominant, followed by types A and B. Type C was also clearly dominant in the BOC solution, with slight presence of types A and E appeared slightly, and type B was barely detected.

The oligomers in PHMG present in each product ranged from monomers to polymers with up to seven monomer units, mainly ranging from dimers to oligomers with six monomers. Figure 2 shows the intensities of several of the main oligomer types in each product based on the results of the UNIFI analysis. The histogram of each oligomer represents the peak intensities of all standard solutions. First, the dimer peaks of both types A and C had the highest intensities among all products. In all products, type B mainly comprised monomers, followed by dimers and trimers. Types B7 and C7 were not detected at any product concentration. For the BOC products, only types B1 and B2 were detected above standard 4 (50 ppm). Second, among the polymers with branched or cyclic structures (types D, E, F, and G), types D1 (monomer) and D2 (dimer) were only detected in the Oxy product and not in the Scunder and BOC products. Type E2 (dimer) was detected in all products, and type E3 (trimer) was detected only in the Scunder and BOC products.

The relative peak intensity of each oligomer in each product was different. Figure 3 shows an example of the relative intensity based on a 100 ppm solution. The linear structure (types A, B, and C) accounted for 90.6% of the Oxy product, but 78.6% and 75.8% of the Scunder and BOC product, respectively, with the remaining represented by

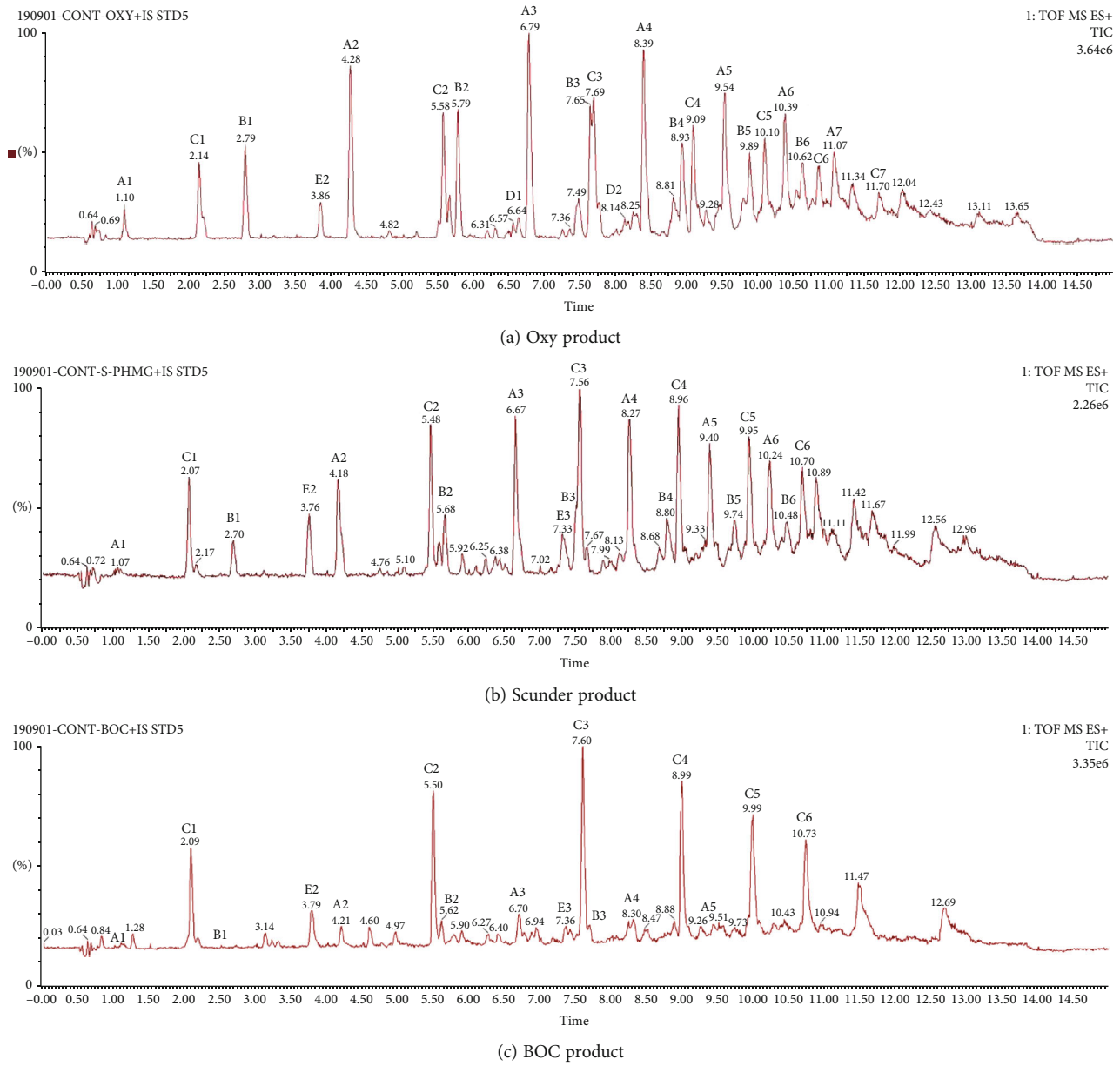
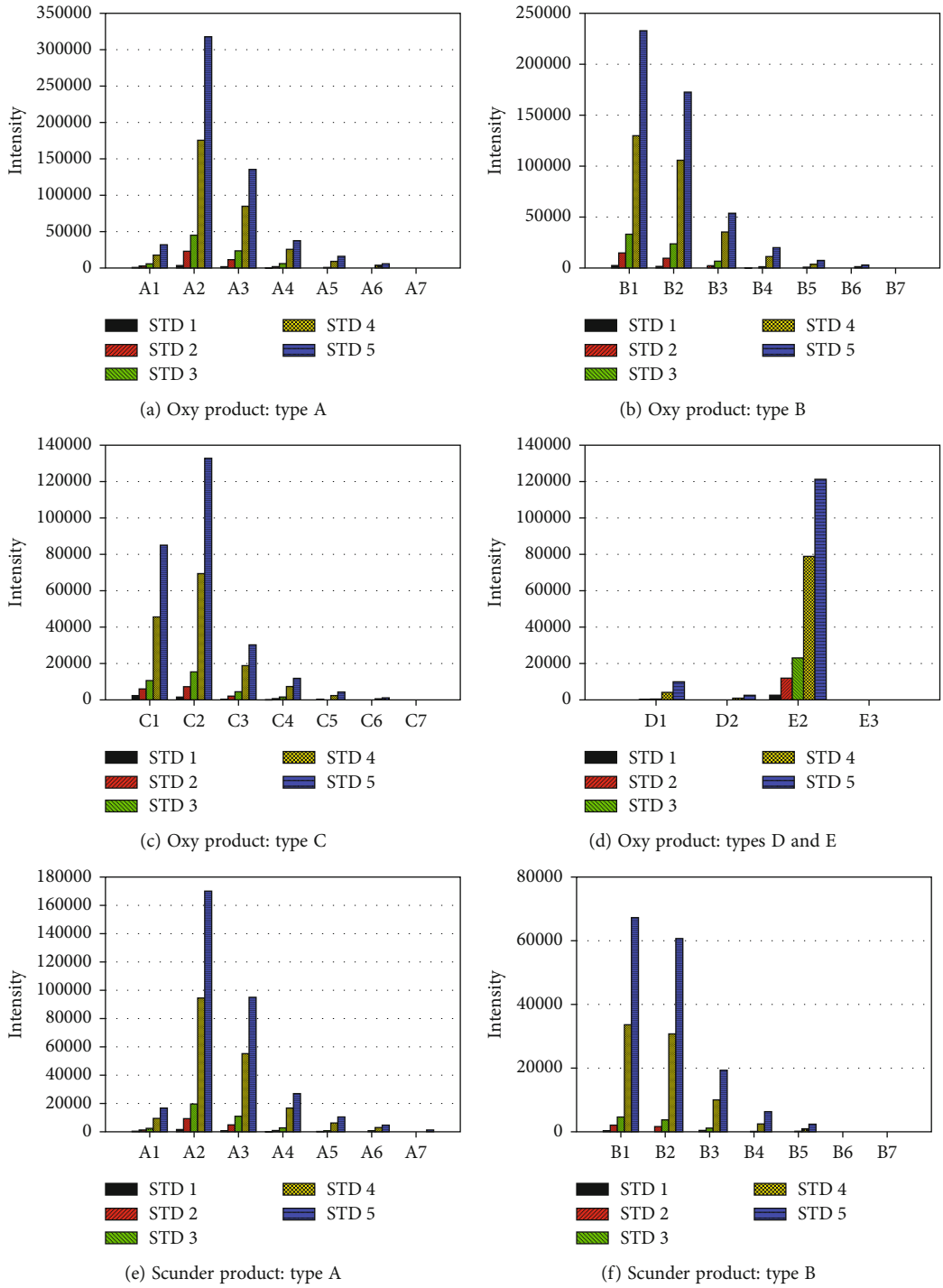


Figure 1: Chromatogram of three PHMG products based on standard solution (100 ppm). Types A, B, and C have a linear structure, whereas types D and E have a branched or cyclic structure. The cyclic F structure was not detected. The numbers after the type letter indicate the monomer unit; for example, A2 stands for the linear structure (Figure S4) with two monomer units (dimer).

branched (type D) or cyclic (type E) structures. In the Oxy product, type A (38%) and type B (34%) structures were most abundant (72%), whereas type A (33%) and type C (30%) structures were most abundant in the Scunder product. However, the most abundant components of the BOC product were types C (69%) and E (24%), accounting for approximately 93% of the total.

The proportions of each oligomer were as follows: A2 (22.2%), B1 (16.2%), and B2 (12.2%) for the Oxy product; A2 (17.7%), E2 (17.0%), and C2 (15.4%) for the Scunder product; and C2 (34.6%), C1 (23.7%), and E2 (20.8%) for the BOC product. Therefore, even the same PHMG product could have different oligomer components.

3.2. Estimated Concentration of PHMG Sample Collected from the Air. Since there is no available standard solution or known concentration of each PHMG oligomer type, we assumed that the PHMG concentration would be proportional to the intensity, regardless of the oligomer type in the product, to estimate the airborne PHMG concentration. For example, if the instrument intensity of 100 ppm PHMG is 10,000 and the intensity of type A1 is 1,000, it was assumed that the PHMG concentration of type A1 is 10 ppm in the sampled solution. The airborne concentration of each PHMG was then calculated by dividing by the sampled air volume. Table 1 summarizes the airborne PHMG concentrations of the three products at 0.5 m and 1 m away



(a) Oxy product: type A

(b) Oxy product: type B

(c) Oxy product: type C

(d) Oxy product: types D and E

(e) Scunder product: type A

(f) Scunder product: type B

Figure 2: Continued.

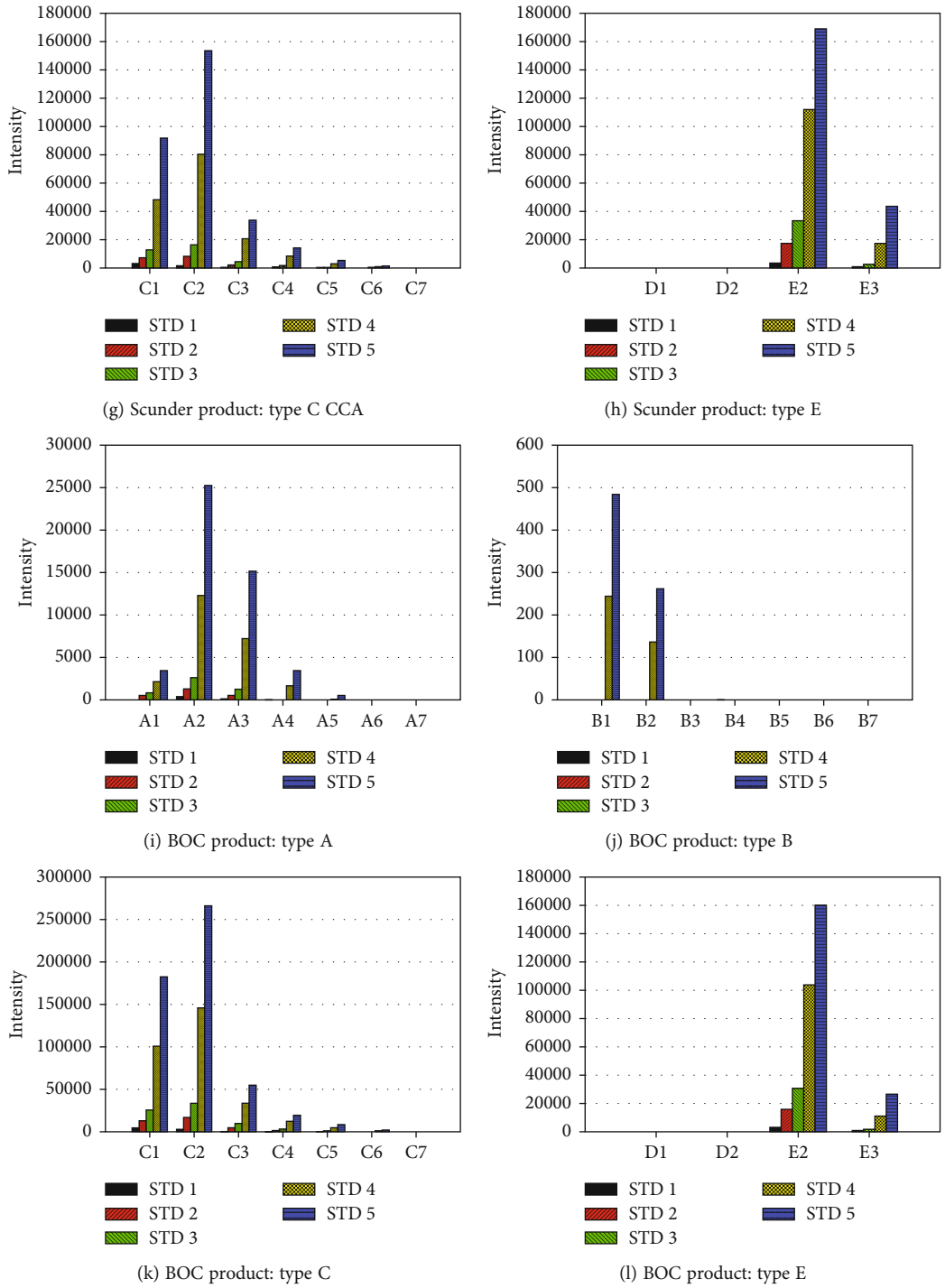


Figure 2: Comparison of peak intensities based on the PHMG standard solution of each product. Types A, B, and C indicate the linear structure, whereas types D and E indicate the branched and cyclic structure, respectively (Figure S4). There were relatively more E structures than D structures in Oxy product (d), and there was no D structure in Scunder product (h) and BOC product (l). STD1: 1 ppm; STD 2: 5 ppm; STD 3: 10 ppm; STD 4: 50 ppm; STD 5: 100 ppm.

from the humidifier. Tables S3 and S4 show the same calculations for the Scunder and BOC products, respectively.

The following characteristics were evident from these data. First, when a low concentration of humidifier disinfectant (i.e., 6.5 or 6.25 ppm PHMG solution of Oxy product) is used, many PHMGs could be detected in the air, and only a few PHMGs were detected at low concentrations in the Scunder and BOC products. Second, many PHMG oligomers were detected when the concentration was high (62.5 ppm) at a distance of 0.5 m from the humidifier disinfectant, but oligomers were hardly detected at a distance of 1 m, and only some components of types C and A were

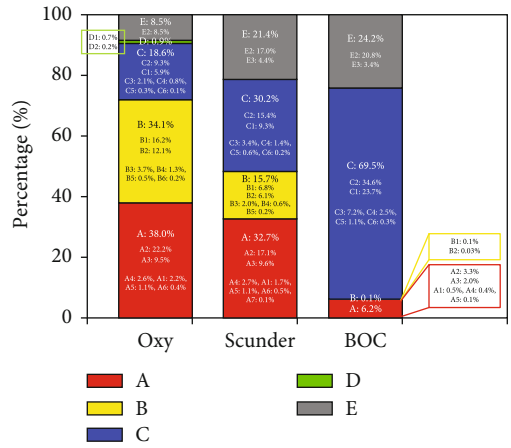


FIGURE 3: Relative intensities of PHMG types and oligomer components in a 100 ppm PHMG solution. A–E represent the PHMG structure, and 1–7 indicate the number of monomer units (also see Table S2 and Figure S4).

detected. Third, the higher the concentration of PHMG solution in the humidifier, the higher the concentration of PHMG in the air. For example, when the PHMG concentration of the solution in the Oxy product was increased by 10 fold from 6.5 ppm to 65 ppm, the total PHMG concentration detected in the air also increased by approximately 10.9 times from 35.9 ± 12.8 ppm to 391.0 ± 205.5 ppm. As the spraying concentration increased from 62.5 ppm to 125 ppm for the Scunder and BOC products, the airborne concentration increased by 2.2 and 2.4 times, respectively. However, the increases in the individual components were different. For example, when the concentration of the solution in the Oxy product was increased by 10 times, type A1 increased 4 times, and type A2 increased 12 times. This trend was similar for the other products as well.

Fourth, the detection and concentration of each oligomer of PHMG (Table 1) were highly correlated with the peak of each oligomer (Figure 1). For example, when a 65 ppm solution of Oxy product was used, most of the types A, B, and C oligomers were detected in PHMG in air, as shown in Table 1, which was similar to the peaks shown in Figure 1. In addition, the concentrations of oligomers of types A2, A3, and A4 in the air were high, and their peaks were also large in Figure 1. Similar trends were observed for the other products.

Finally, most of the PHMGs in the air had a linear structure rather than branched or cyclic structures, and most of them existed as monomers, dimers, and trimers. Type E2 was detected most frequently and at a relatively high concentration in the cyclic structure. Some PHMGs had four or five monomer units, but no oligomers were larger.

4. Discussion

The aim of this study was to characterize different types and contents of PHMG oligomers in products and aerosols and to estimate the airborne PHMG concentration during humidifier use. We analyzed each oligomer in the solution of the three PHMG products, and their compositional profiles were found to differ depending on the product, although most of the identified structures were linear rather than branched or cyclic, as shown in Figure 1. The Oxy product, which has been the greatest contributor to health problems caused by humidifier disinfectants in Korea, contained types A, B, and C, which are all linear structures and are the most common, along with the type E cyclic structure showing a small peak. The Scunder and BOC products showed different profiles. Specifically, the main structures in the Scunder product were types C and A, and type B existed as a small peak, whereas type C was very dominant in the BOC product, and types A and E were identified as small peaks. Furthermore, in all products, there were structures with 1–7 monomer units, but no larger oligomers were detected.

The structures and concentrations of oligomers in the air of the PHMG component were proportional to those in the product solution. This was confirmed by simultaneously observing the airborne PHMG concentration (see Tables 1, S3, and S4), the solution chromatogram (Figure 1), and the intensity of each standard solution (Figure 2). In the Oxy product, monomers, dimers, and trimers of types A, B, and C were all linear structures and mainly found both in solution and in air. Additionally, type E2, which is a dimer of a cyclic structure, was identified. Similar to the Oxy product, the Scunder and BOC products contained monomers, dimers, and trimers that were mainly found in both solution and air. Type B structures were identified in the Scunder product and in the air, along with a small amount confirmed in the BOC product that was not detected in the air. There were differences in the structure and concentration of each product in the air.

Comparing Figure 1, which shows the distribution of PHMG in solution, and Table 1, Table S3, and Table S4, which show PHMG in the air by distance, it is clear that relatively small molecules are easily released into the air and have a higher concentration at 0.5 m than at 1 m. If particulates emitted from the PHMG solution into the air exhibit dimensions exceeding 0.1 micrometers, their descent will adhere to Stokes' law, resulting in rapid settling over short distances. However, as reported in previous papers (7, 16, and 22), the size of PHMG in the air is mostly nanoparticles smaller than 0.1 um, making it difficult to explain using Stokes' law. Stokes' law is conventionally employed in the context of particles possessing diameters significantly surpassing the mean free path of the constituent molecules within the air medium. Its accuracy diminishes notably for particles of minute dimensions, particularly when approaching scales akin to molecular dimensions. At very small sizes, other factors such as Brownian motion and thermal effects become more significant, and its applicability to particles smaller than 0.1 um may be limited [21].

In addition, the humidifier's driving force has a greater effect on short distances, and when it reaches about 1 m, the driving force will decrease significantly, and the spray angle will also become large, leading to a sharp decrease in concentration. In an epidemiological study on lung injury

Table 1: Estimated airborne PHMG concentration by distance ($\mu g/m^3$) when using the Oxy product solution containing 6.5 ppm and 65 ppm of PHMG.

			Concentration in the humidifier solution and measurement distance from the humidifier (mean (SD))			
			6.5 ppm PHMG solution		65 ppm PHMG solution	
			0.5 m	1 m	0.5 m	1 m
Linear structures	Type A	A1	2.67 (0.51)	—	10.69 (4.41)	0.31 (0.39)
		A2	6.18 (3.30)	—	76.73 (40.44)	—
		A3	1.72 (1.54)	—	39.90 (20.66)	—
		A4	0.04 (0.07)	—	10.91 (6.25)	—
		A5	—	—	3.78 (2.76)	—
	Type B	B1	2.09 (1.89	—	53.35 (29.75)	—
		B2	0.79 (0.80)	—	33.38 (18.46)	—
		B3	—	—	14.36 (9.31)	—
		B4	—	—	3.78 (2.96)	—
	Type C	C1	12.64 (1.58)	—	40.14 (14.73)	3.98 (1.46)
		C2	3.83 (1.34)	—	39.99 (21.16)	—
		C3	0.44 (0.35)	—	10.90 (6.19)	—
		C4	0.003 (0.003)	—	3.50 (2.45)	—
Branched or cyclic structures	Type D	D1	0.01 (0.01)	—	1.73 (1.22)	—
		D2	—	—	0.55 (0.44)	—
	Type E	E2	5.47 (1.50)	—	47.28 (24.42)	0.19 (0.33)
		E3	—	—	—	—
Total			35.89 (12.83)	—	390.96(205.50)	4.48 (2.18)

caused by humidifiers, humidifiers were mainly used in children's bedrooms. The odds ratio was 13.2 when the distance between the bed and the humidifier was less than 0.5 m compared to when the distance between the bed and the humidifier was 1 m or more and 2.7 when it was between 0.5 and 1 m, which indicates that the concentration was much higher at close distance [22].

As shown in Figure 1, the presence state of oligomers in solution is different depending on each product. This may be because the oligomer polymerization conditions are different for each manufacturer. It has been reported that as polymerization conditions such as temperature, vacuum, and time increase, the molecular weight increases, that is, the number of polymer units increases [7]. It has been reported that the sterilizing power increases as the molecular weight increases, that is, when the molecular weight of the polymer is over 640 g/mol. As shown in Figure 1, various polymers exist in this product. As shown in Figure 1, there are more polymers (e.g., A2 or higher polymers) than monomers (e.g., A1) of each structure. The cyclic structure is created by changing the linear structure when the temperature and time of the polymerization reaction are increased, but there is no need for the manufacturer to do this. Therefore, as shown in Figure 3, this may be the reason why there are many linear structures A, B, and C, and the cyclic structure E is small. As shown in Figures 1 and 3, although the cyclic structure is smaller than the linear types, there are small peaks and contents of E types among the cyclic structures, which is similar to the polymerization reaction results in other papers [7]. In another paper that investigated the PHMG content in solutions of various humidifier disinfectants, the main structures found were linear structures A, C, and B, and among cyclic structures, E structure was contained in a small amount, which was consistent with the results of this study [23]. In this study, we confirmed that different PHMG products have different oligomer components. Linear types A–C were detected at the highest levels in all products, accounting for approximately 91% in Oxy, 79% in Scunder, and 76% in BOC. Type B was almost undetectable, and type C accounted for 68% of the total BOC product. Type D, a branched or cyclic structure, was detected only in the Oxy products. Type E2 was detected at the highest level for type E, amounting to approximately 20.8% in BOC and 8.5% in Oxy (Figure 3). Among the branched and cyclic structures (types D, E, F, and G in Figure S4), types D1 (monomer) and D2 (dimer) were only detected in the Oxy product, but not in the Scunder and BOC products. Type E2 (dimer) was detected in all products, and type E3 (trimer) existed in both the Scunder and BOC products. Our results regarding the components of PHMG are consistent with those of previous studies. Hwang et al. reported that linear structures (types A–C) were the most abundant, and type C oligomers were more abundant than type B oligomers [24].

Although it has been approximately 10 years since the humidifier disinfectant accident, few studies have characterized the components of airborne PHMG. Several studies have focused on PHMG oligomer analysis, and one study has analyzed the concentration of airborne PHMG. However, the exact structure of the PHMG oligomers could not

be identified [20]. The results for the dilution of 200 : 1 in other study [20] were similar to the estimated concentrations obtained in the present study [20]. When using the Oxy product, the total airborne concentration was 35.89 $\mu g/m^3$ in our study (Table 1) at 0.5 m and was estimated to be 30.5 $\mu g/m^3$ in the previous study [20]. Furthermore, the airborne concentration increased linearly with the dilution ratio in previous studies, and a similar pattern was also observed in the present study (Tables 1, S2, and S3).

The toxicity of PHMG is a subject of concern. When PHMG is aerosolized and inhaled, it harms the lungs, causing death of the cells lining the bronchioles and widespread damage to alveoli, along with bronchiolitis obliterans, an often fatal form of nonreversible obstructive lung disease in which the bronchiole are compressed and narrowed by fibrosis (scar tissue) and/or inflammation [10, 11, 13]. Disinfectants were used in humidifiers to inactivate microorganisms. Not only the antibacterial activity but also the toxicity to the human body will vary depending on the structure of PHMG. However, no research has been conducted on the antibacterial activity or toxicity of each structure of PHMG because it is difficult to obtain a standard for a single structure. In one study, PHMG complexes produced according to polymerization conditions were found to have a medium-sized PHMG (e.g., 640 g/mol) rather than a molecular weight that was too small (e.g., 516 g/mol) or too large (e.g., 956 g/mol), which proved to have great sterilizing power at high concentration [7]. In an in vivo inhalation test on PHMG of nanosized particles (93.35 ± 1.73 nm) in animals and an in vitro toxicity test on human cell lines, it was found that small airborne particles cause pulmonary inflammatory and fibrotic responses [25]. In another study, various brands of PHMG solutions that caused an outbreak of humidifier disinfectant lung injuries (HDLI) in Korea were investigated, and the monomer unit was found to be within the range of two to four with a molecular weight of 441.0–678.0 g/mol [17].

As shown in Figure 1 of this study, 2 to 4 monomer units were the most common in the solution, and although it is not exactly proportional as shown in Table S2, the molecular weight also somewhat matches the range mentioned above depending on the structure of PHMG. A higher percentage of low-molecular particles in a solution might increase the possibility of these particles reaching the alveoli, thereby enhancing the potential for greater health effects.

The toxicity of a substance is multifaceted. Factors such as concentration, duration of exposure, structure, exposure route, and interaction with other chemicals can also contribute to the overall toxicity. The toxic effects of each PHMG ingredient require further study in the future.

The detection limit can be determined by analyzing the lowest concentration (e.g., lowest calibration curve concentration) multiple times and using its variation [19, 26]. However, in this study, the intermediate concentration (10 ppm) was used instead of the lowest concentration (1 ppm) because oligomers of PHMG of various structures were not detected at the 1 ppm concentration. That is, at the lowest concentration, all oligomers were not detected and were detected at the 10 ppm level.

LC-qTOF is a highly sensitive and specific technique that can identify all compounds in a complete matrix and achieve accurate quantitation with excellent dynamic range, without detector saturation and experimental flexibility. It is a powerful tool for PHMG analysis and is preferred over other detection methods for its superior performance [13, 15, 26–29]. We also used the UNIFI program for data processing to improve the data quality, because the intensity can be determined based on the mass and structure of the target materials [27–30]. The UNIFI program is useful in the absence of a standard for each type of monomer. PHMG is a complex oligomer, as described in Introduction and Results. This study thus improved the accuracy of the detected chromatogram using the UNIFI program to analyze the complex structure of PHMG and each oligomer type for the first time.

Although this study provides useful novel information on the form and concentration of the humidifier disinfectant PHMG in the air that has become a major social problem in Korea, it has several limitations. First, there is no official standardized sampling or analytical method for PHMG. In this study, impinger sampling was conducted using several sampling methods, including filter and adsorbent sampling. This sampling method is a modification of the NIOSH 5521 method that was used in a previous study [20], but it is not a validated method. However, to increase the collection efficiency of this method, we used two or three (in preliminary experiments) impingers connected in series and confirmed that the sample was not detected in the third impinger. Second, since standards for each oligomer required for calculating the concentrations of PHMG in solution and air were not available, it was assumed that all concentrations, regardless of the oligomer type, had the same intensity. Therefore, the estimation of the concentration of each oligomer was dependent on the intensity in the LC-qToF chromatogram. However, the methodology adopted in this study to evaluate the air behavior of products containing various oligomers can logically be considered to be the most appropriate approach based on current knowledge.

In this study, the distance between the humidifier and the sampler was selected by considering the worst-case scenario of severe exposure among the distances investigated in previous studies [31, 32]. When sleeping at home, the humidifier was usually placed at the head of the bed. In a previous study, victims exposed to humidifier disinfectants were investigated at distances of <0.5 m, 0.5~1 m, >1 m, 1-2 m, <2 m, etc. The cases of <0.5 m and 0.5~1 m were found to be about 30% to 70% [29, 30]. However, since victims occurred at longer distances than this, exposure at various distances and conditions should be studied further in the future.

In summary, we analyzed PHMG products manufactured in different countries and characterized their oligomers. Furthermore, we analyzed airborne PHMG oligomers using a humidifier under the conditions of the actual humidifier disinfectant accident. The PHMG products by different manufacturers contained different types of structures. Oxy products were found to be responsible for the lung diseases emerging in Korea, and Scunder products have linear structures A, B, and C, whereas the B-type structure is

rarely found in BOC products. Among the three products, there was a structure with 1–7 monomer units, although mainly monomers, dimers, and trimers were present in the air. Among the cyclic structures, the dimer of the E structure (E2) was predominant in both the product and the air.

Data Availability

All data that support the findings of this study are available in the supporting information (see Supporting Information). The more detailed datasets of the current study are available from the corresponding author upon reasonable request.

Disclosure

The funders played no roles in study design; in the collection, analysis, and interpretation of data; in the writing of the report; and in the decision to submit the article for publication. The experimental results of this manuscript are part of the first author's doctoral dissertation, but the interpretation and discussion of the results have changed a lot during the submission process to this journal. The original copy can be found at the following link, where the doctoral dissertation is stored: https://snu-primo.hosted.exlibrisgroup.com/permalink/f/1l6eo7m/82SNU_INST21770834800002591.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Sunju Kim (first author) was responsible for the methodology, experiment, data analysis, and original draft preparation (https://orcid.org/0000-0001-9997-0776). Chungsik Yoon (corresponding author) was responsible for the conceptualization, supervision, discussion, and writing, reviewing, and editing (https://orcid.org/0000-0001-7822-0079).

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Supplementary Materials

The sampling diagram in the clean room during humidifier use is shown in Figure S1. The conditions of LC-qToF for the analysis of PHMG are presented in Table S1. The molecular formulas and weights of the various PHMG oligomers are listed in Table S2. Figure S2 shows an example of A2 analysis of the Oxy product using the UNIFI program. An example of oligomer chromatogram in PMHG standard solution and chromatogram in Oxy product and confirmation of A2 peak among them using UNIFI software program is presented in Figure S3. Table S3 and Table S4 show the concentration of PHMG in the air by distance when the Scunder and BOC products are used, respectively. The structural formulas of PHMG linear, branched, or cyclic types are available in Figure S4. *(Supplementary Materials)*

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