Refined Exposure Assessment for Three Active Ingredients of Humidifier Disinfectants

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Abstract
Exposure assessment for three major active ingredients used for humidifier disinfectants, polyhexamethylene guanidine (PHMG), oligo(2-(2-ethoxy)ethoxyethyl guanidinium chloride (PGH), and 5-chloro-2-methylisothiazol-3(2H)-one/2-methylisothiazol-3(2H)-one (CMIT/MIT) mixture, was conducted in a bedroom using an air sampler for a refined risk assessment. The experimental site was selected to reflect consumer exposure conditions. Aerosols formed by a humidifier were sampled during 8 hr at 7.5 L/min. Absorbed PHMG and PGH by the sampler were quantified using a spectrophotometric method, and high performance liquid chromatography-ultraviolet detection was used for CMIT/MIT. Three exposure scenarios were assumed for adding humidifier disinfectants to the humidifier water at 1, 2, and 10 times the volume recommended by the product suppliers, and the humidifier was on at its maximum rate of producing aerosols in order to consider reasonable worst-cases. The sampled mass of PHMG and PGH ranged 200 to 2,800 µg, and 140 to 1,900 µg, respectively, under different exposure conditions, whereas the absorbed mass of CMIT/MIT was barely detected at the detection limit of 0.11/0.29 mg/L, only at 10 times the recommended level. The resulting risk quotients for PHMG and PGH ranged 1,400 to 20,000, and 1,000 to 13,000, indicating that health risks could be significant. For CMIT/MIT mixture, risk quotients were much smaller than estimated by assuming that they are conservative in the indoor environment, probably due to oxidative reactions. The refined exposure assessment presented here may provide a useful tool for assessing risks posed by active ingredients in spray-type biocidal products.

Keywords: Air sampler, chloromethyl/methyl isothiazolinone (CMIT/MIT), Indoor air, polyhexmethylene biguanidine (PHMG), Risk assessment

1. Introduction
In 2011, the Korea Centers for Disease Control and Prevention reported that an unidentified fatal lung disease was likely to be caused by chemical disinfectants used with household humidifiers [1, 2]. The causative active ingredients were identified as polyhexamethylene guanidine (PHMG) and oligo(2-(2-ethoxy)ethoxyethyl guanidinium chloride (PGH), based on the epidemiological studies and in vivo histopathological readings, after instillation of those active ingredients to rats [3]. Chloromethyl/methyl isothiazolinone (CMIT/MIT) evaluated at the same time was not likely to cause fatal lung disease [3].

Although epidemiological evidences have revealed that those polymeric chemical disinfectants could be fatal when inhaled, quantitative risk assessment for the inhalation of those chemicals has not been conducted, except for a screening-level health risk assessment [4]. Lee et al. [4] assumed that the active ingredients of humidifier disinfectants are inert and homogeneously distributed in a bedroom (i.e., no chemicals react, precipitate, or were removed by other pathways). The resulting health risk quotients were calculated at 2,500, 10,500, and 9.41 for PHMG, PGH, and CMIT/MIT, respectively. These values at the screening level risk assessment are very high, indicating potentially significant health concerns, and requiring a refined risk assessment. Uncertainties with risk quotient at the screening-level lie in uncertainties with the predicted exposure concentration using simple steady-state modeling, as well as the reliability of the “read across” method used to predict the long-term toxicity data for the derivation of the reference concentrations.

In this study, we intended to measure the realistic exposure concentration, based on the human exposure scenarios under normal and excessive use conditions. Products of humidifier
disinfectants containing three active ingredients (PHMG, PGH, and CMIT/MIT) were added to the humidifier water at the level recommended by the product suppliers and released to a room using a household humidifier. Aerosols containing active ingredients were sampled at a pumping rate close to the average breathing rate for Koreans, to estimate the human intake rate according to the exposure scenarios. Refined health risk quotients were estimated, based on the measured intake rates.

2. Materials and Methods

2.1. Active Ingredients and Chemicals

Three active ingredients of the humidifier disinfectants used in this study were PHMG, PGH, and CMIT/MIT. Chemical structures of all three ingredients are shown in Fig. 1. Aqueous solutions of PHMG-phosphate (25% w/w) and PGH (25% w/w) were kindly provided by SK Chemical Industries, Inc. Analytical grade 5-chloro-2-methyl-4-isothiazolin-3-one (CMIT, 98%) was purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany), and analytical grade 2-methyl-4-isothiazolin-3-one (MIT, 98%) was purchased from Sigma-Aldrich (St Louis, MO, USA). Three commercial humidifier disinfectant products containing three active ingredients each were purchased from the market, before they were recalled after November, 2011.

Eosin Y solution (0.5% w/v), glycine (98.5%), magnesium nitrate (Mg(NO₃)₂, 99%) and magnesium chloride (MgCl₂, 99%) were purchased from Sigma-Aldrich. Hydrochloric acid (35%) was purchased from Dae-Jung (Siheung, Korea). Methanol (high performance liquid chromatography [HPLC] grade) was purchased from Burdick & Jackson (Ulsan, Korea).

2.2. Sampling Site, Generation of Humidifier Aerosols, and Aerosol Sampling

A bedroom (area, 21 m²; volume, 47 m³) was rented for the experiments. A humidifier that uses a water boiling system combined with ultrasonic mist generation was used for the production of mists. This type of the humidifier was similar to those humidifiers used by patients who suffered the unidentified lung disease. The water tank size was 4.8 L and approximately 4 L evaporated during the 8 hr sampling period.

Air containing humidified aerosols was sampled using a custom-made air sampler, consisting of two serial 250 mL impingers containing 100 mL of aqueous solution and a constant flow sample pump (The QuickTake 30; SKC Inc., Eighty Four, PA, USA) (Fig. 2) [5]. Distilled water was used as the sampling medium for PHMG and PGH aerosols, and aqueous solution containing 2.5 g/L Mg(NO₃)₂ and 0.5 g/L MgCl₂ was used for sampling CMIT/MIT, because PHMG and PGH are cationic polymers, and CMIT/MIT have high water solubility. Preliminary studies using a sampler with four serial impingers showed that two impingers were sufficient for quantifying the disinfectants in the sampled aerosols, since the trapped amount from the third impinger was below the detection limit. Air was sampled at the rate of 7.5 L/min, the mean breathing rate for Koreans at rest [6] with all windows and doors closed. The room was ventilated by opening all windows and doors, between independent exposure measurements, to minimize any potential carry-over effects from the previous measurement.

2.3. Instrumental Analyses of Active Ingredients

2.3.1. Spectrophotometric determination of PHMG and PGH

Quantitative analysis of polymeric active ingredients (PHMG-phosphate and PGH) containing guanidine group was conducted, using the color-changing reaction of guanidine with tetrabromofluorescein (Eosin Y) [7, 8]. Glycine buffer solution at pH 3.6 was prepared by adding 50 mL of 0.1 M glycine solution, and 2.5 mL of 0.2 M hydrochloric acid to 100 mL aqueous solution. Analytical standards were prepared at 1, 2, 4, 6, and 8 mg/L for PHMG-phosphate and PGH, for spectrophotometric determination of their concentration in the sampling solution. In case the sampled concentration exceeded the range of analytical standards, the solution was diluted appropriately before the analysis. Sample solution (10 mL) taken from the impingers was mixed with 10 mL of glycine buffer solution (pH 3.6) and 1.0 mL of 0.05% (w/v) Eosin Y solution. The mixture was vortexed briefly, and left for 5–10 min at room temperature for color development. Then, the absorbance of the mixture was measured.