Determination of Organoiodine Antimicrobial Ingredients in Commercially Available Antimicrobial/Deodorant Agents

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(Received June 5, 2000; Accepted August 26, 2000)

In order to grasp the actual conditions of use of three kinds of organoiodine compounds, 3-iodo-2-propynylbutylcarbamate (IPBC), 1-bromo-3-ethoxycarbonyloxy-1,2-diiodo-1-propene (BECDIP), and 4-chlorophenyl-3-iodopropargylformal (CPIP), which are used as household antimicrobial/antifungal agents and wood preservatives, an analytical method using a gas chromatograph equipped with a flame ionization detector (FID-GC) and gas chromatography mass spectrometry (GC/MS) was employed. The calibration curve (peak area) by FID-GC showed good linearity in the range from 20 μg/ml to 100 μg/ml (correlation coefficient $R = 0.99$ or more). A purification method using a liquid–liquid partition by *n*-hexane/water and the cartridge column (florisil or alumina) was established, and the addition recovery exceeded 90% by this method. By employing this method, the analysis was carried out for 18 commercially available samples. As a result, IPBC was detected from 2 samples of aerosol type fungicides, BECDIP from 4 samples of wood preservatives, and CPIP from one sample of an aerosol type antifungal/insecticidal paint.

Key words —— organoiodine compound, household antimicrobial agent, gas chromatographic analysis, 3-iodo-2-propynylbutylcarbamate, 1-bromo-3-ethoxycarbonyloxy-1,2-diiodo-1-propene, 4-chlorophenyl-3-iodopropargylformal

INTRODUCTION

The following three kinds of organoiodine compounds are widely used as antimicrobial/antifungal agents: 3-iodo-2-propynyl butylcarbamate (IPBC), 1-bromo-3-ethoxycarbonyloxy-1,2-diiodo-1-propene (BECDIP), and 4-chlorophenyl-3-iodopropargylformal (CPIP).

IPBC is used as an antiseptic for wood and paint as well as in cosmetics.[1,2] In the "World Guide to Industrial Biocides" published by the Paint Research Association in 1995, 443 kinds of antifungal agents used by 72 paint manufacturers in 12 countries in the world are listed. It is reported that the analysis of the listed agents reveals that the most commonly used industrial antifungal agent is IPBC.[3] BECDIP is mainly used as wood preservative against termites,[4,5] while CPIP is used as an antifungal/antiseptic agent for fiber, leather, wood, and paint.[6,7] Although these compounds are thought to have low toxicity and are deemed to be practically safe, there are almost no studies reporting the toxicity test results except the data from the manufacturers of these compounds.[1,2,4–9] Since these compounds are used as fungicides and wood preservatives even in the home, there is a good possibility for people to be exposed to them in their own houses, by percutaneous absorption and respiratory tract absorption. For safety evaluation, it is necessary to carry out exposure tests based on the toxicity evaluation. However, the concentration of these compounds in drugs on the market is unknown to consumers. Therefore, it is necessary to establish an analytical method of these chemicals to investigate the actual conditions of their use in the home. Some studies have analyzed these pure compounds and their photolysis products to examine their photolysis properties.[10,11] Very few papers are available on the analytical methods of these compounds mixed with a large amount of other ingredients in antimicrobial/antifungal agents.[12] In this study, the analytical methods of these compounds mixed with other components in the commercially available products are investigated using a gas chromatograph equipped with a flame ionization detector (FID-GC) and gas chromatography mass spectrometry (GC/MS). The analytical meth-

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ods developed were then applied to the analysis of household antimicrobial/deodorant agents.

MATERIALS AND METHODS

Samples ——Eighteen samples, including 11 of commercially available aerosol type antimicrobial/antifungal drugs, 3 of paints, and 4 of wood preservatives against termites were collected for the test.

Reagents ——The following chemicals were used as standard substances for IPBC, BECDIP, and CPIP, respectively: Omacide IPBC (purity 99.7%) made by Nippon Oline Co., Ltd. (Tokyo, Japan), Sanpurasu (purity 99.05%) made by Sankyo Co., Ltd. (Tokyo, Japan), and IF1000S (purity 97.5%) made by Nagase Chemical Co., Ltd. (Hyogo, Japan).

A stock solution of IPBC was made by dissolving it in acetone up to the concentration of 1000 μg/ml. A series of working standard solutions was made by diluting it with *n*-hexane.

The stock solutions of BECDIP and CPIP were made by dissolving them with *n*-hexane up to the concentration of 1000 μg/ml. Working standards were made by diluting them with *n*-hexane when they were used.

Organic solvents such as *n*-hexane were those used for pesticide residue analysis done by Kanto Chemical Co., Ltd. (Tokyo, Japan). For florisil cartridge column and alumina cartridge column (Alumina N), Sep-Pak Plus, made by Waters Co., Ltd. (Massachusetts, U.S.A.), were used.

FID-GC Conditions ——Apparatus: 5890 Series II equipped with FID detector (Hewlett Packard Co., Ltd.), Automatic injector: 7673 type (Hewlett Packard Co., Ltd.), Data processor: Shimazu Chromatopac CR-5A (Kyoto, Japan), Column: Capillary Column DB-1 (0.53 mm i.d. × 15 m, film thickness 1.5 μm) made by J & W Scientific Co., Ltd. (Folsom, CA, U.S.A.), Carrier gas flow rate: He 10 ml/min, Make-up gas flow rate: He 20 ml/min, Hydrogen flow rate: 30 ml/min, Air flow rate: 500 ml/min, Column temperature: 80°C (1 min)–10°C/min–280°C (5 min), Injection inlet temperature: 250°C, Detector temperature: 280°C, Injection volume: 2 μl (whole volume injected).

GC/MS conditions——GC/MS apparatus: 5890 Series II equipped with MS detector (Hewlett Packard Co., Ltd.), Automatic injector: 7673 type (Hewlett Packard Co., Ltd.), Column: Capillary Column DB-5 (0.25 mm i.d. × 30 m, film thickness 0.1 μm) made by J & W Scientific (Folsom, CA, U.S.A.), Carrier gas flow rate: He 1 ml/min, Column temperature: 80°C (1 min)–8°C/min–280°C (5 min), Injection inlet temperature: 250°C, Detector temperature: 280°C, Injection volume: 2 μl Injection method: splitless, Ionization voltage: 70 eV (EI).

Preparation of Test Solutions——The sampling of aerosol type products was made by spraying the contents of the spray cans into an ice-cooled Erlenmeyer flask. The liquid sample was taken by weighing.

A sample of 0.2 g was taken in a 50 ml centrifuge tube each time. The ingredients were extracted in an *n*-hexane phase by shaking it for 5 min after adding 20 ml of purified water and 20 ml of *n*-hexane. The sample was centrifuged at 3000 r.p.m. for 5 min. The supernatant (*n*-hexane phase) was taken by 10 ml portions. After dehydration with anhydrous sodium sulfate, the solution was filtered using a G2 glass filter. After evaporating the solvent, the residue was dissolved with 2 ml of *n*-hexane for applying it to either a prepared florisil cartridge column (IPBC and BECDIP) or an alumina cartridge column (CPIP). After washing the column with 60 ml of *n*-hexane, each organoiodine compound was eluted with 20 ml of *n*-hexane containing 20% ethanol (IPBC and BECDIP), or with 40 ml of *n*-hexane containing 20% ethanol (CPIP). After adjusting the volume of the eluate with *n*-hexane, each compound was determined by means of FID-GC and confirmed by GC/MS.

RESULTS AND DISCUSSION

Investigation of Measurement Method by FID-GC and GC/MS

Three types of detection methods by GC:FID, ECD (electron capture detector), and GC/MS, were considered. Since high sensitivity was not required for the analysis of these compounds in this study, determination by FID-GC and confirmation by GC/MS were adopted. Investigating the operating conditions made it possible to measure these compounds by gas chromatography, as described above. IPBC was easily decomposed by adsorption in the GC at the detection level of ECD-GC. Though a previous paper showed that it was impossible to detect IPBC by ECD-GC,[13] in this study, retrieving the operating conditions made it possible to measure. Furthermore, these three compounds were qualitatively detectable by ECD-GC with high sensitivity of pg (ng/

ml). However, ECD-GC was not employed for the quantitative method in this study, because high sensitivity was not required.

Each 2 μl sample of standard solution (50 μg/ml) of IPBC, BECDIP, or CPIP was applied to GC.The variation coefficients of measurement results obtained from 5 repetitions were as follows: Peak area of IPBC, 2.42%; peak height of IPBC, 4.18%; peak area of BECDIP, 1.17%; peak height of BECDIP, 0.89%; peak area of CPIP, 0.89%; and peak height of CPIP, 0.73%. Good reproducibility was observed in all cases. Calibration curves were obtained in the range of 20–100 μg/ml from the peak area. In all cases, the correlation coefficient *R* was as high as better than 0.99, showing good linearity.

Removal Method of Coexisting Polar Substances by Liquid–Liquid Partition

IPBC is water insoluble but easily soluble in alcohol and glycol; it is not highly soluble in *n*-hexane. Drugs containing IPBC must be water-soluble solutions containing alcohol or the like. First, the liquid–liquid partition was investigated for the removal of polar substances. IPBC of 1000 μg or 100 μg each was added to a mixed solution of 20 ml of *n*-hexane, 4 ml of ethanol, and 16 ml of water. After shaking the mixture for 5 min, the partition of IPBC between organic and aqueous phases was examined. It was found that almost all (99%) of IPBC moved to the *n*-hexane phase, regardless of the original concentration. The *n*-hexane/water partition method was thus applied to the removal of polar substances in the sample solution.

BECDIP and CPIP are water insoluble but easily soluble in organic solvents such as *n*-hexane. The possibility of application of a liquid–liquid partition to the removal of polar substances was investigated. BECDIP or CPIP of 1000 μg or 100 μg each were added to a mixed solution of 20 ml of *n*-hexane and 20 ml of water. After 5 min of shaking, the residual content of BECDIP or CPIP in the *n*-hexane phase was measured. Almost 100% of both compounds remained in the *n*-hexane phase. This *n*-hexane/water partition method was also applied to the removal of polar substances in the sample.

Removal Method of Coexisting Non-Polar Substances by Cartridge Column

n-Hexane solutions containing 1000 μg or 100 μg of IPBC or BECDIP were applied to a prepared florisil cartridge column. The columns were washed with 60 ml of *n*-hexane. Neither IPBC nor BECDIP was eluted from the column. Then, 100 ml of *n*-hexane containing 20% ethanol was poured in 10 ml portions to obtain 10 fractions. Almost all of both compounds (IPBC, 98–100%; BECDIP, 99–100%) were eluted in the first and second fractions (20 ml). Neither IPBC nor BECDIP was detected in the third to tenth fractions. From the results, future elution from the column was made for both agents with 20 ml of eluant.

The product which contains IPBC seems to contain polar solvents such as methanol and acetone. Therefore, it also seems to contain the polar solvent in the *n*-hexane phase after liquid–liquid partition. When a similar operation was carried out using the *n*-hexane solution of IPBC which contained 5% acetone, 30% of IPBC was eluted in the first 20 ml of *n*-hexane eluate. So, in practical analysis, the organic solvent was evaporated after liquid–liquid partition, and the residue was dissolved in a 100% *n*-hexane solution before loading it on the column.

In the case of CPIP, washing the column with 60 ml of *n*-hexane resulted in the elution of CPIP by 53%. Next, when the column was washed with 100 ml of *n*-hexane containing 20% ethanol in 10 ml portions, 47% of CPIP was eluted in the first 20 ml. Therefore, the florisil cartridge column was not suitable for the pre-treatment of CPIP.

Therefore, for the pre-treatment of CPIP, the use of an alumina cartridge column was then examined. CIPC of 1000 μg or 100 μg was applied to a prepared alumina cartridge column. The column was washed with 60 ml of *n*-hexane. No CPIP was eluted from the column at all. Then 100 ml of *n*-hexane containing 20% ethanol was poured in 10 ml portons to obtain 10 fractions. Almost all of CPIP (94–95%) was eluted in the first and second fractions (20 ml). No CPIP was detected in the third to tenth fractions. It was decided that elution from the column would be made for CPIP with 40 ml of *n*-hexane containing 20% ethanol.

Addition–Recovery Experiments

To about 0.2 g of Sample No. 5 (Table 1), each 1000 μg of IPBC, BECDIP, and CPIP was added for analysis. Recoveries of IPBC, BECDIP and CPIP were 92, 94 and 95%, respectively.

Analytical Results of Samples

Eighteen samples of commercially available antimicrobial/deodorant products, including 11 samples of aerosol drugs, 3 of paints, and 4 of wood preservatives were analyzed. The analytical results

Table 1. Analytical Results of Marketing Antimicrobial/Deodorant Agents

Sample No.	Use	Physical form	Solubility	Conc. of organoiodine antimicrobial ingredients	Labelling of antimicrobial ingredients
1	Lotion for an air conditioner	Aerosol	Water soluble	N.D.	Antimicrobial agents
2	Antimicrobial/ deodorant agents	Aerosol	Water soluble	N.D.	Antimicrobial agents
3	Antimicrobial/ deodorant agents	Aerosol	Water soluble	N.D.	Antimicrobial agents
4	Lotion for an air conditioner	Aerosol	Water soluble	N.D.	Antimicrobial agents
5	Fungicides	Aerosol	Water soluble	N.D.	2-(4-triazolyl)-benzimidazol
6	Antimicrobial/ deodorant agents	Aerosol	Water soluble	N.D.	Imidazol antimicrobial agents, Bamboo extracts
7	Antimicrobial/ deodorant agents	Aerosol	Water soluble	N.D.	Antimicrobial/ deodorant agents
8	Fungicides	Aerosol	Oil soluble	N.D.	Fungicides
9	Fungicidal and insecticidal paint	Aerosol	Oil soluble	CPIP 880 μg/g	Antimicrobial agents, Insecticides, Fungicides
10	Fungicides	Aerosol	Water soluble	IPBC 2010 μg/g	IPBC, Fungicides
11	Fungicides	Aerosol	Water soluble	IPBC 750 μg/g	Organoiodine agents, Hiba oil
12	Fungicidal paint	Liquid	Water soluble	N.D.	Fungicides
13	Fungicidal paint	Liquid	Water soluble	N.D.	Fungicides
14	Wood preservative paint*	Liquid	Oil soluble	N.D.	Antimicrobial agents, Insecticides
15	Wood preservatives	Liquid	Oil soluble	BECDIP 12500 μg/g	Ethofenprox, S-421, BECDIP
16	Wood preservatives	Liquid	Oil soluble	BECDIP 3860 μg/g	Chlorpyrifos, BECDIP
17	Wood preservatives	Liquid	Oil soluble	BECDIP 10100 μg/g	Phoxim, S-421, BECDIP
18	Wood preservatives	Liquid	Oil soluble	BECDIP 7440 μg/g	Permethrin, BECDIP

IPBC: 3-iodo-2-propynyl butylcarbamate, BECDIP: 1-bromo-3-ethoxycarbonyloxy-1,2-diiodo-1-propene, CPIP: 4-chlorophenyl-3-iodopropargylformal. N.D.: Not detected. No. 14*: Dichlofluanide detected.

are shown in Table 1. As for the pretreatment method, the method for IPBC and BECDIP and that for CPIP were both employed. By FID-GC, chromatograms which had a retention time equal to that of IPBC were detected from 2 samples of aerosol drugs, while chromatograms which had a retention time equal to that of BECDIP came from 4 samples of wood preservatives, and the chromatogram which had a retention time equal to that of CPIP from one sample of aerosol drug was detected. These samples were confirmed by GC/MS. In Fig. 1, FID gas chromatograms of IPBC standard and Sample No. 10 are shown. Total ion chromatograms (TIC) and mass spectra of IPBC standard and Sample No. 10 are shown in Fig. 2. In Fig. 3, FID gas chromatograms of BECDIP standard and Sample No. 15 are shown. TIC and mass spectra of BECDIP standard and Sample No. 15 are shown in Fig. 4. In Fig. 5, FID gas chromatograms of CPIP standard and Sample No. 9 are shown. TIC and mass spectra of CPIP standard and Sample No. 9 are shown in Fig. 6.

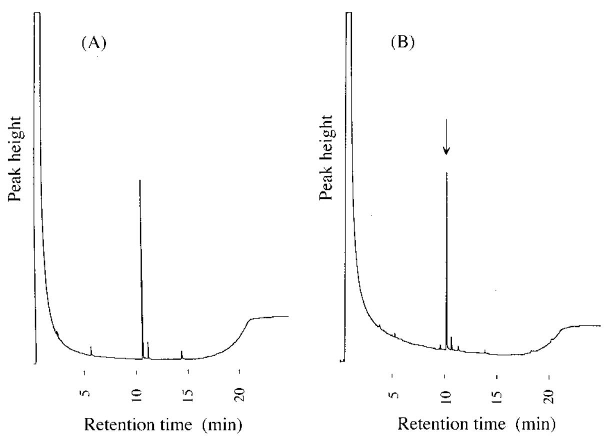

Fig. 1. FID Gas Chromatograms of IPBC Standard (100 ng) (A) and Commercially Available Fungicides (Sample No. 10) (B)
Operating conditions of FID-GC are given in the text.

Since these three organoiodine antimicrobial agents have been thought to be negligibly toxic and

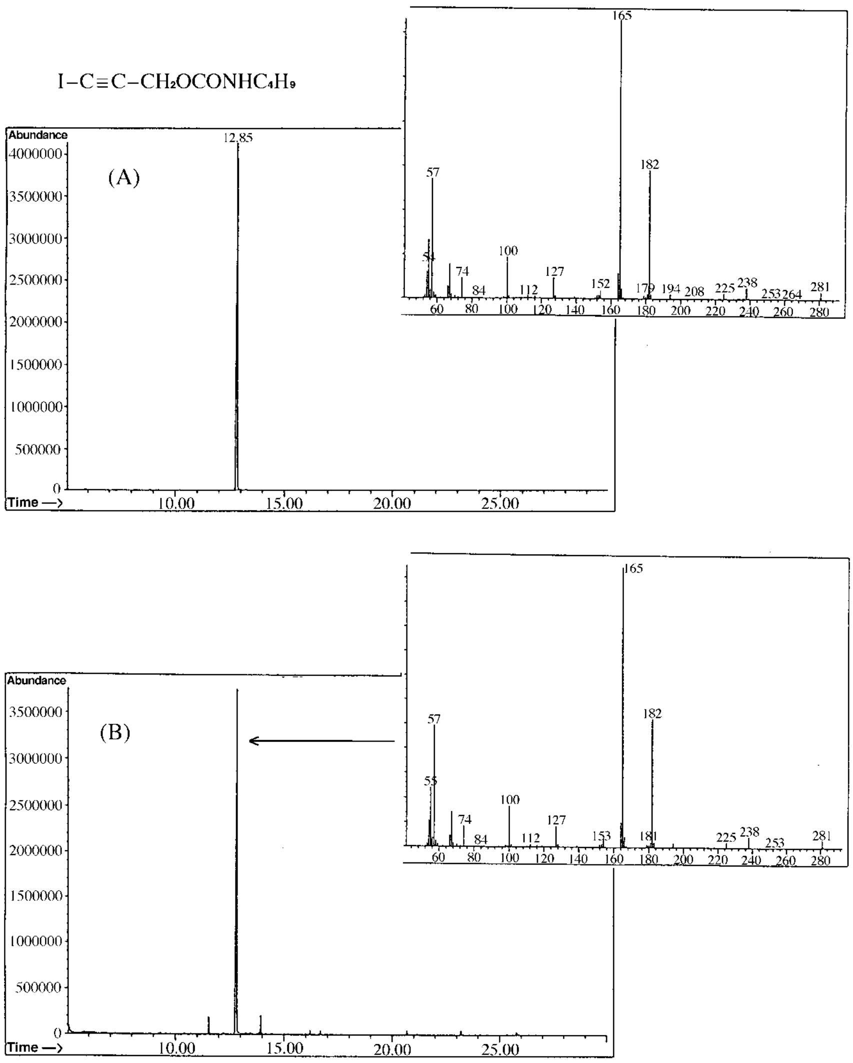

Fig. 2. Total Ion Chromatograms and Mass Spectra of IPBC Standard (A) and Commercially Available Fungicides (Sample No. 10) (B)
Operating conditions of GC/MS are given in the text.

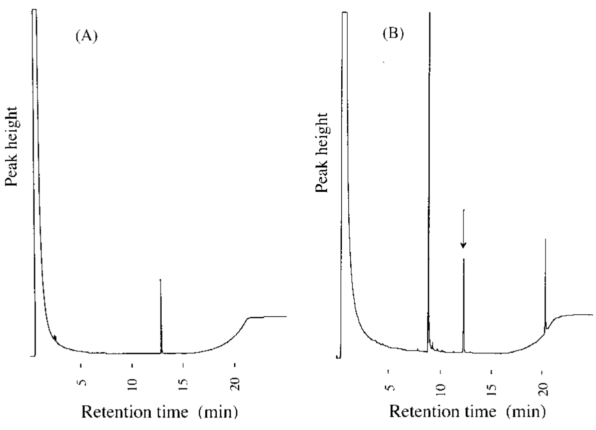

Fig. 3. FID Gas Chromatograms of BECDIP Standard (100 ng) (A) and Commercially Available Wood Preservatives (Sample No. 15) (B)
Operating conditions of FID-GC are the same as Fig. 1.

therefore safe compounds, they have been used as industrial fungicides, household antimicrobial agents and wood preservatives. However, there have been few studies reporting the toxicity of these compounds. Although some papers reported that both a skin sensitization test and teratogenesis test showed negative results,[1,2,8] Bryld, *et al.* showed that the repeated embrocation of IPBC on human skin brought about allergic symptoms in about 1% of subjects.[14] Concerning BECDIP and CPIP, there has been no paper reporting the appearance of skin sensitization and teratogenesis.[4–9] In a recent study on contact allergenicity with a guinea pig maximization test (GPMT),[15] these compounds all had sensitization potencies to skin, and a cross reaction took place among them.[16,17] Accordingly, it is necessary to review their toxicity by adding new test items, such as

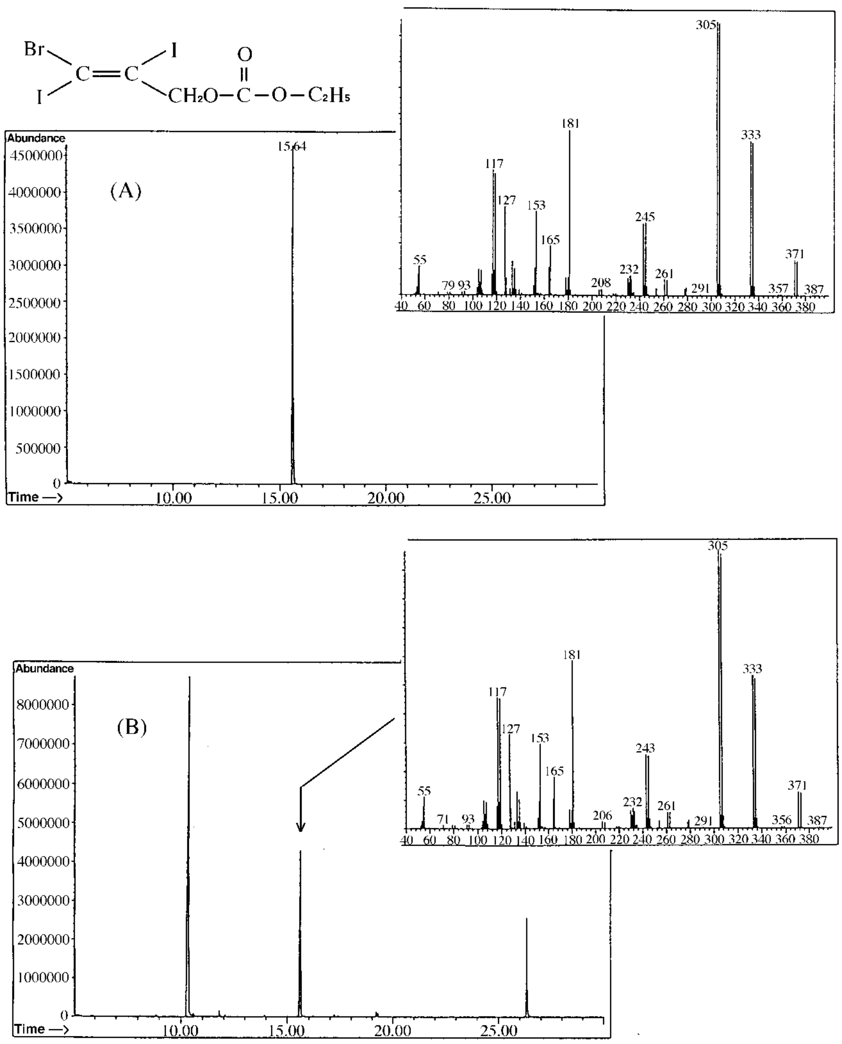

Fig. 4. Total Ion Chromatograms and Mass Spectra of BECDIP Standard (A) and Commercially Available Wood Preservatives (Sample No. 15) (B)

Operating conditions of GC/MS are the same as Fig. 2.

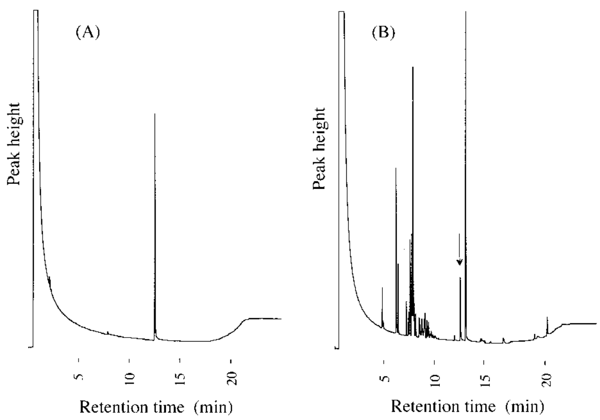

Fig. 5. FID Gas Chromatograms of CPIP Standard (100 ng) (A) and Commercially Available Fungicidal Paint (Sample No. 9) (B)

Operating conditions of FID-GC are the same as Fig. 1.

skin sensitization and teratogenesis.

For evaluating safety, an exposure test based on the toxicity evaluation is required. In this study, IPBC and CPIP were detected in commercially available household drugs. There is a possibility for people to be exposed (*via* skin or *via* the respiratory tract) to these antimicrobial agents. BECDIP was detected from business-use drugs for wood preservatives against termite, and is probably applied for household use as well. Besides, by contamination with BECDIP after application for termite control, human beings may be exposed to these agents and suffer health hazards such as allergic dermatitis and sick house syndrome. It is therefore necessary to consider guidelines for the usage of these drugs, taking into account the toxicity of the substances, exposure dose, and exposure conditions.However, most

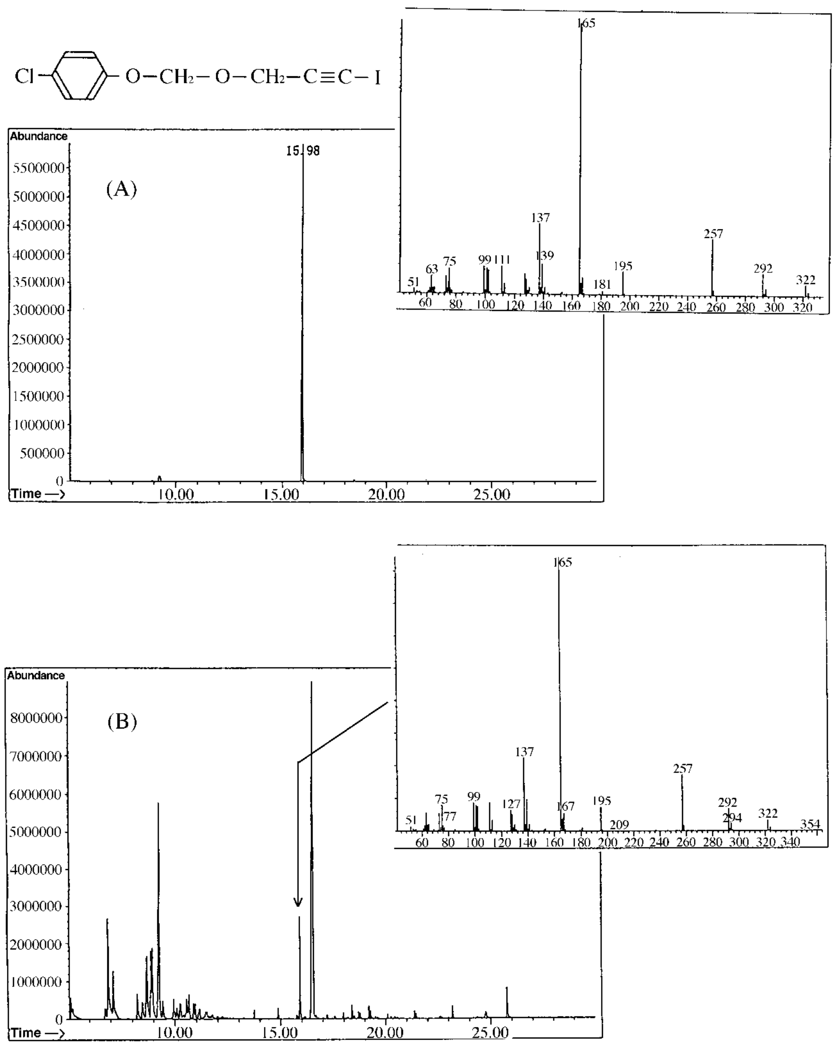

Fig. 6. Total Ion Chromatograms and Mass Spectra of CPIP Standard (A) and Commercially Available Fungicidal Paint (Sample No. 9) (B)

Operating conditions of GC/MS are the same as Fig. 2.

of the commercially available household drugs do not clearly indicate the names of agents and their concentrations. In this study, it was observed that most of the commercially available products merely noted that the drugs contained antimicrobial agents or antifungal agents. In the future, the names of compounds used in the drugs, as well as their content, should be listed for the purpose of giving drug information to consumers.

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