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Biomarkers for monitoring profluthrin exposure: Urinary excretion kinetics of profluthrin metabolites in rats

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ABSTRACT

Recently, a pyrethroid profluthrin [(2,3,5,6-tetrafluoro-4-methylphenyl)methyl 2,2-dimethyl-3-(prop-1-enyl)cyclopropane-1-carboxylate] is widely used as mothproof repellents in indoors. The urinary excretion kinetics of its metabolites was examined in rats to search for urinary metabolites suitable as biomarkers of profluthrin exposure in the general population. A single dose (26–400 mg/kg body weight) of profluthrin was administered intraperitoneally to the rats, and then their urine was collected periodically. Four major profluthrin metabolites, 4-methyl-2,3,5,6-tetrafluorobenzyl alcohol (CH3-FB-Al), 4-hydroxymethyl-2,3,5,6-tetrafluorobenzyl alcohol, 4-methyl-2,3,5,6-tetrafluorobenzoic acid and 2,2-dimethyl-3-(1-propenyl)-cyclopropanecarboxylic acid (MCA) were determined in the urine samples by gas chromatography/mass spectrometry. The kinetic evaluation for each metabolite was achieved by moment analysis of the urinary excretion rate *versus* time curve. The urinary excretion amounts of the three metabolites, expect for MCA, were estimated to be proportional to the amounts of absorbed profluthrin over a wide exposure range. Urinary CH3-FB-Al was considered to be an optimal biomarker for monitoring of profluthrin.

1. Introduction

Indoor air quality is very important for our health because we spend much time indoors, for example, at home. Chemicals in indoor air have been known for some time to cause sick building syndrome and multiple chemical sensitivity (Meggs and Cleveland, 1993; Norback et al., 1995). Many insecticides have been found in indoor air in previous studies (Bouvier et al., 2006; Whyatt et al., 2007; Yoshida et al., 2007). In recent years, the use of synthetic pyrethroids derived from chrysanthemic acid is increasing replacing more toxic insecticides such as organochlorine and organophosphorus insecticides (Barro et al., 2006). Over the last few years, pyrethroids containing tetrafluorobenzene nucleus in their chemical structures such as profluthrin [(2,3,5,6-tetrafluoro-4-methylphenyl)methyl 2,2-dimethyl-3-(prop-1-enyl)cyclopropane-1-carboxylate] (Fig. 1), transfluthrin [(2,3,5,6-tetrafluorophenyl)methyl 3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropane-1-carboxylate] and metofluthrin [(2,3,5,6-tetrafluoro-4-(methoxymethyl) phenyl)methyl 2,2-dimethyl-3-(prop-1-enyl)cyclopropane-1-carboxylate], which have high vaporization properties and

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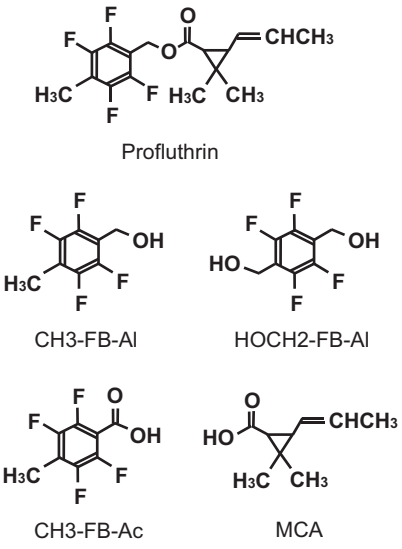

Fig. 1 – Chemical structures of profluthrin and four urinary metabolites targeted in the present study. Abbreviations: CH3-FB-Al, 4-methyl-2,3,5,6-tetrafluorobenzyl alcohol; HOCH2-FB-Al, 4-hydroxymethyl-2,3,5,6-tetrafluorobenzyl alcohol; CH3-FB-Ac, 4-methyl-2,3,5,6-tetrafluorobenzoic acid; MCA, 2,2-dimethyl-3-(1-propenyl)-cyclopropanecarboxylic acid.

high insecticidal activities in comparison with conventional pyrethroids, are being widely used in indoors to control household pests in Japan.

Profluthrin is used as mothproof repellents, mainly for clothes. Profluthrin was detected from indoor air in houses where mothproof repellents containing this compound were used experimentally (Yoshida, 2009), though there have hardly been any reports on indoor air pollution by profluthrin. However, profluthrin has been known to have adverse effects on the nervous system and liver according to results from subacute and chronic toxicity studies in mammals (Ujihara et al., 2010). Exposed rats had tremors, clonic convulsions, increased liver weight and diffuse hepatocellular hypertrophy. Furthermore, profluthrin had been suggested to have effects on protein and lipid metabolism. Therefore, knowing the amount of the compound absorbed by the body is important for evaluating its adverse effects on humans in indoor environments.

Generally, the amounts of hazardous substances absorbed are monitored by measuring their metabolites excreted *via* the urine. In order to do this, the urinary metabolites that can serve as markers for the absorption amounts of the parent compound must be selected from the metabolites. There have been many reports on monitoring of human exposure to conventional pyrethroids, such as cyfluthrin, permethrin, phenothrin, cyphenothrin, cypermethrin and deltamethrin, by measuring their urinary metabolites. However, the types of metabolites targeted are limited to 3-phenoxybenzoic acid, 4-fluoro-3-phenoxybenzoic acid, 3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylic acid, 3-(2,2-dibromovinyl)-2,2-dimethylcyclopropanecarboxylic acid and chrysanthemumdicarboxylic acid (Couture et al., 2009; Fortin et al., 2008; Heudorf and Angerer, 2001; Qi et al., 2012; Schettgen et al., 2002a,b; Wielgomas and Piskunowicz, 2013; Wielgomas et al., 2013). There have been no reports on the human metabolism of profluthrin, and knowledge about its urinary metabolites in animals is also limited. Ujihara et al. (2010) investigated the metabolism of profluthrin in rats using ^{14}C-marked profluthrin. The ^{14}C concentration in the plasma reached maximum at 6–8 h after oral administration (1 mg/kg) of the compound. Profluthrin underwent metabolic reactions such as ester hydrolysis, oxidation and glucuronic acid conjugation. Most of the dosage was excreted from the body within two days of administration. The remaining percentage of profluthrin in the body at 7 days after administration was 0.3% or less of the dosage, and it was therefore assumed to have a low residual property in the tissues of the rats. However, no detailed information was published about the types of each urinary metabolite nor about the relationships between doses and the amounts of the metabolites. Namely, the biological markers for evaluating absorption amounts of profluthrin have not been established for humans or animals. Previous research from our laboratory has identified four metabolites, 4-methyl-2,3,5,6-tetrafluorobenzyl alcohol (CH3-FB-Al), 4-hydroxymethyl-2,3,5,6-tetrafluorobenzyl alcohol (HOCH2-FB-Al), 4-methyl-2,3,5,6-tetrafluorobenzoic acid (CH3-FB-Ac) and 2,2-dimethyl-3-(1-propenyl)-cyclopropanecarboxylic acid (MCA), shown in Fig. 1, in urine samples of rats dosed intraperitoneally with profluthrin (Yoshida, 2012). Most of the absorbed profluthrin was considered to be metabolized prior to excretion *via* the urine because none of the parent pyrethroid was found in the urine of the rats. Most of the metabolites produced were suggested to be excreted mainly as conjugates in the urine. We also developed an analytical method to measure the urinary metabolites accurately and precisely by gas chromatography/mass spectrometry (GC/MS) (Yoshida, 2013).

The present study describes the kinetic evaluation of urinary excretion of a fluorine-containing pyrethroid profluthrin in rats using moment analysis of the urinary excretion rate of its metabolites *versus* time curves. A biomarker for monitoring profluthrin exposure is discussed based on the urinary excretion kinetics.

2. Materials and methods

2.1. Chemicals

CH3-FB-Ac and HOCH2-FB-Al were purchased from Tokyo Kasei Kogyo (Tokyo, Japan), and CH3-FB-Al and MCA, which were not available as reagents, were gifts from Sumitomo Chemical (Tokyo, Japan) as authentic standards to quantify the urinary metabolites of profluthrin. 4-Methoxy-2,3,5,6-tetrafluorobenzoic acid and 4-methoxy-2,3,5,6-tetrafluorobenzyl alcohol as internal standards were obtained from ART-CHEM (Berlin, Germany) and AK Scientific (CA, USA), respectively. *N*-(*tert*-Butyldimethylsilyl)-*N*-methyltrifluoroacetamide from Tokyo Kasei Kogyo and *N*-trimethylsilylimidazole and trimethylchlorosilane from Wako Pure Chemical (Osaka, Japan) were used as derivatizing reagents for the metabolites and standards. Sulfatase from *Helix pomatia*, Type H-1, lyophilized powder (activity: