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# *In-vitro* bioaccessibility of five pyrethroids after human ingestion and the corresponding gastrointestinal digestion parameters: A contribution for human exposure assessments

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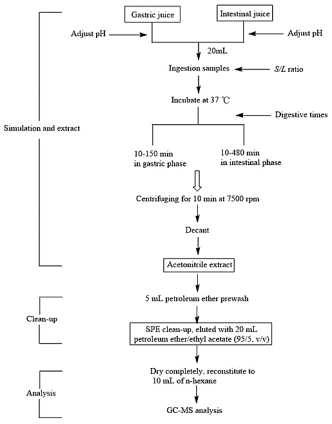
## HIGHLIGHTS

- *In-vitro* bioaccessibility of five pyrethroids applied on apples were measured and corresponding analytical methods.
- Both pH value and *S/L* ratio were the main factors affecting the bioaccessibility.
- Digestive times could be weak to delay weak the absorption efficiency.
- The extent to which compounds desorb accounts for less than 10 percent under normal conditions.
- Bioaccessibility of pesticides was a non-ignorable factor in human health risk assessment.

## GRAPHICAL ABSTRACT

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## ABSTRACT

Bioaccessibility is a crucial parameter in assessing the absorption of contaminants during the human digestive process, but few studies have involved the differences in the bioaccessibilities of pesticides. To investigate the mode of using the *in vitro* bioaccessibility to refine estimates of dietary exposure to pesticide residues, this study measured the bioaccessibilities of five pyrethroids in apples, and then, it modelled physicochemical predictors (gastrointestinal pH, digestive times, and the solid-liquid (*S/L*) ratio) of the bioaccessibilities of pyrethroids. Apple samples of gastric and intestinal phase digestive juices were obtained from an *in vitro* simulated digestion model. Our survey of *in vitro* digestion models found that the bioaccessibilities ranged from 4.42% to 31.22% and 10.58%–35.63% in the gastric and intestinal phases, respectively. A sharp trend similar to a normal distribution was observed between the bioaccessibilities and pH values. The bioaccessibility reached its highest value at a pH of 1.91 in the simulated gastric juice and did not significantly change with an increase of the digestive time. A significant negative correlation occurred between the bioaccessibility and *S/L* ratio, which followed a logarithmic equation. The correlation coefficients ($R^2$) ranged from 0.9259 to 0.9831 and 0.9077 to 0.9960 in the simulated gastric and intestinal juice, respectively, suggested that both the pH value and *S/L* ratio

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were the main factors affecting the bioaccessibility. Furthermore, a combination of the acceptable daily intake (ADI) and bioaccessibility for human exposure assessments indicated the implication that traditional risk assessment using ADI may seriously overestimate the actual risk.



## 1. Introduction

As one of the most frequently consumed food groups, the consumption of fruits and vegetables in China constitutes over 30% of the consumer diet (Chen et al., 2011). Fruits and vegetables are commonly consumed raw or semi-processed (Lemos et al., 2016; He et al., 2006), resulting in a higher dietary exposure to pesticide residue levels (Al-Shamary et al., 2016; Melnyk et al., 2011) and a cumulative effect of pesticides in the individual (Jensen et al. 2013, 2015). A widespread focus on controlling and assessing the safety of fruits and vegetables has been conducted in recent years (Zentai et al., 2016; Fang et al., 2015).

In current risk assessment, the most widely used method for the evaluation of pesticide exposure is based on the exposure calculation of the acceptable daily intake (ADI) (Evans et al., 2015; Oomen et al., 2003), which was calculated using the ingestion of individual residue levels. This exposure assessment assumes that the maximum amount of a compound in the ingested food has been absorbed by the human gastrointestinal tract (Juhasz et al., 2016). However, this approach overlooks the extent to which compounds are solubilized from the food matrix after gastrointestinal digestion and become bioavailable. Bioaccessibility is a crucial step for allowing contaminants to be bioavailable during the digestion process (Sotomayor-Gerding et al., 2016; Shen et al., 2016). The differing physicochemical properties of contaminants result in quantitative differences in their bioaccessibilities (Starr et al., 2016). Thus, beyond the determination of total concentrations, studies of pesticide risk assessment should also take into account the bioaccessibility.

As an important determinant in assessing the absorption of contaminants, the bioaccessibility has been commonly used to evaluate the total contents of heavy metals (Zhu et al., 2016; Gu et al., 2016) and other contaminants, e.g., soils and dusts (Liang et al., 2016; Bi et al., 2015), but few report the oral bioaccessibilities of pesticides during the human digestive process and the bioaccessible fractions. Without considering the oral bioavailabilities of pesticides released from the liquid or food matrices, the risk assessment of pesticides can be overestimated (Prokop et al., 2016; Denyes et al., 2016).

There are five commonly used *in vitro* digestion models that have been developed as a short period, simple, cheap, and reproducible tool to investigate the bioaccessibilities of contaminants. They include the Deutsches Institut für Normunge.V. (DIN) (Li et al., 2014), Rijksinstituut voor Volksgezondheid en Milieu (RIVM) (Walraven et al., 2015), Physiologically Based Extraction Test (PBET) (Safruk et al., 2015), Simulator of Human Intestinal Microbial Ecosystem (SHIME) (Laird et al., 2009), and TNO Intestinal model (TIM) (Rocha et al., 2013). These methods can be used to determine how the bioaccessibility is affected by several factors, including the physical-chemical parameters of the human digestive process (gastrointestinal pH, digestion times, and solid-liquid (*S*/*L*) ratio) (Peixoto et al., 2016; Garcia-Sartal et al., 2011; Moreda-Pineiro et al., 2011) and the presence of dietary components, such as cellulose, tannin and phytate (Espert et al., 2016; Peixoto et al., 2016). However, most of the reports generally only set one condition for each parameter of those methods (Peixoto et al., 2016), without considering how variations in the parameters just mentioned may affect the bioaccessibility of a compound. In addition, the metabolism of contaminants in the human intestinal system is also a non-negligible factor (Van de Wiele et al., 2004). With a distinct difference in comparison with other static or dynamic methods mentioned, the SHIME method can stimulate the colonic environment (Laird et al., 2007), which more accurately reflects the bioavailabilities of contaminants after consumption because the SHIME model has a wider application in measuring the bioaccessibilities of contaminants and their metabolic processes *in vivo*.

The objective of this study was to quantify the bioaccessibilities of pesticides and investigate related parameters in human gastrointestinal digestion after ingestion. Five pyrethroids applied on apples, including lambda-cyhalothrin, beta cypermethrin, fenpropathrin, fenvalerate and deltamethrin, were the compounds studied in a series of *in vitro* investigations, and we have studied the effects using an *in vitro* simulated digestion model (SHIME method). In addition, the combination of the ADI value and bioaccessibility for human exposure assessments provided a more realistic estimate of potential risk.

## 2. Materials and methods

### 2.1. Chemicals and samples

Reagents of analytical grade, including acetonitrile, petroleum ether and ethyl acetate, were purchased from the Xilong Chemical Co., Ltd. (Swatow, China) as well as soluble starch (98.8%) and pancreatin. Arabinogalactan, pectin from apples and xylan were obtained from the Meifeng Chemical Industry Co., Ltd. (Sichuan, China) as well as hydrochloric acid, sodium bicarbonate, anhydrous sodium sulphate and sodium chloride. Yeast and peptone were purchased from the Beijing Aoboxing Biotechnology Co., Ltd. (Beijing, China). Mucin, cysteine, glucose, pepsin, purified ox bile dihydrate and gastric acid were obtained from the Shanghai Source Poly Biological Technology Co., Ltd. (Shanghai, China). A Florisil solid-phase extraction (SPE) column (1000 mg 6 $mL^{-1}$) was purchased from Agela Technologies (Tianjin, China). All deionized water used for this study was prepared in a CSR-1-20 ultra-high purity water (UHP) system (Beijing Ace Tektronix Technology Development Co., Ltd., China) with 18.2 MΩ resistance and purified on site.

Pesticides, including lambda-cyhalothrin (99.2%), beta cypermethrin (99.2%), fenpropathrin (99.2%), fenvalerate (99.0%) and deltamethrin (98.4%), were used as internal standards for the correct quantification in gas chromatography (GC) (Agilent 7890B, Agilent Technologies Inc., California, USA). They were obtained from the National Pesticide Quality Supervision and Inspection Centre (Beijing, China), diluted in n-hexane (HPLC grade, Tedia Company, Inc., Ohio, USA), and stored at 4 °C until use.

The apple samples from local markets in the city of Hefei (China) were collected and used in the bioaccessibility assays. All samples were homogenized and stored at −20 °C.

### 2.2. In vitro digestion model based on human SHIME

An adapted static SHIME model was established to simulate the gastric and intestinal phases of the human gastrointestinal digestion process, as described by Yu et al. (2016) (Yu et al., 2016). The