Environmental Pollution 243 (2018) 437–443

Contents lists available at ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol

Degradation of various insecticides in cooked eggs during *in vitro* human digestion☆

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ARTICLE INFO

Article history:
Received 1 May 2018
Received in revised form
24 August 2018
Accepted 4 September 2018
Available online 4 September 2018

Keywords:
Insecticide
Egg
Cooking
Enterobacteria
In vitro human digestion

ABSTRACT

The objective of this study was to determine the effects of cooking and *in vitro* human digestion on the changes of five insecticides—fipronil, bifenthrin, 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane (DDT), 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane (DDD), and 2,2-bis(*p*-chlorophenyl)-1,1-dichloroethylene (DDE)—in egg whites and yolks. Each insecticide was applied to egg whites and yolks at a concentration of 1000 μg/g. After cooking the egg whites and yolks, concentrations of bifenthrin, DDD, and DDE decreased ($P < 0.05$), whereas those of fipronil and DDT were unchanged ($P > 0.05$) in both egg whites and yolks. Next, an *in vitro* human digestion model that simulates all the steps of human digestion was employed. Until digestion in the small intestine, the concentrations of fipronil and DDT in the cooked egg whites and yolks were unchanged ($P > 0.05$), whereas those of bifenthrin, DDD, and DDE decreased ($P < 0.05$) at each digestion step. In the large intestinal digestion step with *Escherichia coli* and *Lactobacillus sakei* as enterobacteria, the concentrations of all the insecticides decreased ($P < 0.05$) in the cooked egg whites and yolks. Among the insecticides, bifenthrin showed the lowest concentration ($P < 0.05$). In conclusion, the use of bifenthrin as an insecticide would be comparatively less toxic than other insecticides in terms of environmental pollution and human health, because of its easy degradation.

1. Introduction

Recently, insecticides were found in chicken eggs supplied to Switzerland, Hong Kong, and 15 European Union countries in August 2017 (BBC News, 2017). A subsequent investigation was conducted as the use of fipronil in the production of human food is prohibited in Europe (Boffey and Connolly, 2017). In South Korea, insecticides containing fipronil and bifenthrin were found in eggs by the national authority and were reported in August 2017 (MARFA, 2017). As a result of this situation, consumers' anxiety about eggs has increased, and egg consumption has dropped markedly.

Various insecticides have been used in chicken farms to control chicken pests. Fipronil is a phenylpyrazole insecticide, and is classified by the World Health Organization as a moderately hazardous (class II) insecticide (WHO, 2011), whereas the maximum detection limit of fipronil in eggs is adjusted to 0.005 mg/kg by the European Union (EUR-Lex, 2005). Fipronil interferes with the function of γ-aminobutyric acid (GABA)-gated channels and causes severe neural excitation, excessive paralysis, and death at sufficient doses (Cole et al., 1993).

Bifenthrin is a pyrethroid insecticide that has been used against the imported red fire ant, which it exterminated by disturbing its nervous system. Although the use of bifenthrin is restricted in the United States, products containing low concentrations of bifenthrin have been approved for daily use (Toxipedia, 2011). The Environmental Protection Agency classified bifenthrin as a Category C compound, which is a possible human carcinogen (NPIC, 2011).

Organochlorine insecticides are chlorinated hydrocarbons that were mainly used from the 1940s–1960s in mosquito control and agriculture owing to their low acute mammalian toxicity, high efficacy against arthropods, and low cost. Representative compounds in this group include DDT, dieldrin, toxaphene, methoxychlor, and chlordane (Centers for Disease Control and Prevention, 2009). As they act as neurotoxins, many organochlorine insecticides have been prohibited worldwide. In addition, DDD (1,1,-dichloro-2,2-bis

☆ This paper has been recommended for acceptance by Charles Wong.

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https://doi.org/10.1016/j.envpol.2018.09.021
0269-7491/

[4-chlorophenyl]ethane) and its metabolite *p,p'*-DDE (1,1-dichloro-2,2-di[4-chlorophenyl]ethylene) are known to cause eggshell thinning in domestic hens (Holm et al., 2006).

Although DDT-group insecticides are banned in many high-income countries, many insecticides are still found in food products. Moreover, numerous researchers have studied the efficacy and toxicity of insecticides (Chung et al., 2018; Park et al., 2018; Loomis et al., 2015; Simon-Delso et al., 2015); however, the degradation of insecticides in cooked egg whites and yolks during *in vitro* human digestion and the effects of human enterobacteria on their concentrations have not been examined in detail. Therefore, the purposes of this study was to determine the effects of cooking egg whites and yolks at 100 °C followed by *in vitro* human digestion with enterobacteria (*Escherichia coli* and/or *Lactobacillus sakei*) on the concentrations of five insecticides: fipronil, bifenthrin, DDT, DDD, and DDE and to clarify the degradation mechanisms of five insecticides by several factors.

2. Materials and methods

2.1. Materials

Analytical grade acetone, hexane, and methanol were purchased from Fisher Scientific (Pittsburgh, PA, USA). Analytical grade 4,4′-DDD (99%), 4,4′-DDE (99%), α-amylase, mucin, urea, bovine serum albumin, uric acid, lipase, pancreatin, pepsin, and bile salt extract were purchased from Sigma-Aldrich (St Louis, MO, USA). Analytical grade calcium chloride, sodium bicarbonate, potassium chloride, and sodium chloride were purchased from Junsei Chemical (Tokyo, Japan). Luria–Bertani (LB) broth and de Man, Rogosa, and Sharpe (MRS) broth were purchased from Difco (Sparks, MD, USA). Analytical grade fipronil (97%) was purchased from Tokyo Chemical Industry (Tokyo, Japan). Analytical grade bifenthrin (99%) was obtained from Toronto Research Chemicals (Toronto, OA, Canada). Analytical grade 4,4′-DDT (99.26%) was purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany). Fresh eggs were kindly provided from a chicken farm in Gyeonggi-do (Join Co., Pyeong-Taek, Republic of Korea).

2.2. Methods

2.2.1. Contamination of insecticides in eggs and cooking

Five insecticides, fipronil, bifenthrin, DDT, DDD, and DDE, were respectively applied to egg whites and yolks at a concentration of 1000 μg/g. The concentration of five insecticides was decided based on the concentrations detected in the case of insecticides-containing eggs (MARFA, 2017) and the results of our preliminary study that we can analyze. Briefly, fipronil and bifenthrin were dissolved in absolute acetone (1:100 ratio) and the DDT group insecticides (DDT, DDD, and DDE) were dissolved in absolute hexane (1:100 ratio). The five dissolved insecticides were mixed with egg whites and yolks for 10 min and flushed with purified nitrogen gas to evaporate acetone and hexane. Egg whites and yolks containing the insecticides were placed in 50-mL tubes and stored at 15 °C for 24 h and then cooked using a water bath (WSB-30, Daihan Scientific Co., Wonju, Korea) at 100 °C for 10 min. The cooked egg whites and yolks were then chilled for 5 min with ice water. After cooling, the cooked egg whites and yolks were subjected to an *in vitro* human digestion procedure.

2.2.2. Preparation of digestive enzymes and juices for in vitro human digestion

A human *in vitro* digestion model (including enterobacteria) was used in the present study based on a previously described method with some modifications (Lee et al., 2016). The digestive enzymes and juices used in present study were modified from our previous studies (Hur et al., 2011; Lee et al., 2016; Kim and Hur, 2017). The compositions of the digestive enzymes and digestive juices are shown in Table 1. To simulate large intestinal digestion, 21 mL of a solution containing the enterobacteria *E. coli* and/or *L. sakei* was added to the digested mixture after the small intestinal digestion step (21 mL).

2.2.3. Preparation of enterobacteria for the large intestinal digestion

The protocol for preparing enterobacteria was modified from our previous studies (Lee et al., 2016; Kim and Hur, 2017). Liquid medium for *E. coli* was prepared by dissolving 25 g of LB broth powder, Miller (Difco, MD, USA) in 1 L of deionized-distilled water (DDW). Liquid medium for *L. sakei* was prepared by dissolving 55 g of lactobacilli and MRS broth powder (Difco) in 1 L of DDW. Each solution was sterilized using an autoclave (121 °C for 15 min) and then cooled down. *E. coli* and *L. sakei* stocks in the deep freezer (−70 °C) were thawed at room temperature (20–25 °C). Each of *E. coli* and *L. sakei* stocks (1%) was added to 10 mL of the sterilized broth. *E. coli* and *L. sakei* were activated by incubation at 37 °C for 12 h. Then, *E. coli* and *L. sakei* were incubated for 12 h at 37 °C again by adding 1 mL of each to 100 mL of sterilized liquid broth. The final number of *E. coli* and *L. sakei* was 9–10 log CFU/mL.

2.2.4. In vitro digestion procedure to analyze changes of various insecticides

The initial system contained 3 g each of egg white and yolk. For the mouth, stomach, small intestine, and large intestine digestion steps, 3 g of each sample was mixed with 3 mL of saliva solution, gastric juice, duodenal juice, bile juice, and liquid medium containing *E. coli* and/or *L. sakei*. Each mixture was stirred in a water bath at 150 rpm and 37 °C for 5 min, 2 h, 2 h, and 4 h for each digestion step, respectively. The dilution effect of adding digestive juice was considered for all the experimental steps.

2.3. Extraction and purification procedure of insecticides in egg whites and yolks

2.3.1. Extraction

The extraction and purification of five insecticides in egg whites and yolks were performed using the method of the Korean Food and Drug Administration (2017). Briefly, 5 g of each egg white and yolk was weighed and added to a 50-mL tube. Subsequently, 15 mL of acetonitrile containing 1% of acetic acid was added and vortexed for 1 min. Sodium acetate (1.5 g) and magnesium sulfate (6 g) were added and stirred in a water bath at 150 rpm and 25 °C for 10 min. After shaking, extracts were centrifuged at 4000 × *g* and 4 °C for 10 min.

2.3.2. Purification

The supernatant (6 mL) of centrifuged extracts was moved to 15 mL tubes and $MgSO_4$ (1200 mg), primary-secondary amine (400 mg) and graphitized carbon black (45 mg) were added. After 1 min of vortexing, the mixture was centrifuged at 4000 × *g* and 4 °C for 10 min. The supernatant (3 mL) was moved to 15 mL tubes and evaporated using nitrogen gas. The final volume was adjusted to 1 mL and filtered using a nylon membrane filter (0.2 μm). The purified filtrates were analyzed using high-performance liquid chromatography (HPLC).

2.4. Determination of the concentrations of various insecticides in egg whites and yolks

The concentrations of fipronil, bifenthrin, DDT, DDD, and DDE in