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Pyrethroid insecticides in wild bird eggs from a World Heritage Listed Park: A case study in Doñana National Park (Spain)☆

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ABSTRACT

Recent studies demonstrated that the common pyrethroid insecticides are present in aquatic biota tissues. In this study, 123 samples of unhatched eggs of 16 wild bird species collected from 2010 to 2012 in Doñana National and Natural Park were analysed to determine 13 pyrethroids. This study represents the first time that pyrethroids are detected in tissues of terrestrial biota, 93% of these samples being positive to those pollutants. Levels of total pyrethroids ranged from not detected to 324 ng g^{-1} lw. The samples were characterized by stable isotope analysis. Species with diets based on anthropogenic food showed higher levels of pyrethroids and lower values of $\delta^{15}N$. Finally, we characterized the isomers of pyrethroids and discerned some isomeric- and enantiomeric-specific accumulations. In particular, tetramethrin and cyhalothrin showed an enantiomeric-selective accumulation of one enantiomer, highlighting the need to assess toxicological effects of each enantiomer separately to be able to make a correct risk assessment of pyrethroids in birds.

1. Introduction

Pyrethroids are semisynthetic insecticides very common worldwide. They derive from the natural pyrethrins and are related to chrysanthemic acid. Given their chemical structure, they are chiral molecules with 2 or 4 enantiomeric pairs depending on their number of chiral centres (2 or 3). Their use is extensive in several different fields such as agronomics, veterinarian and domestic use. They are even used in public health against human parasites like lice, scabies or mosquito vectors of some diseases (Barr et al., 2010).

The increased usage of these pesticides is due to their properties and the current regulation of other organochlorine and organophosphate insecticides which have been banned in several countries around the world. One of these properties is their environmental persistence, generally lower than 90 days (UH, 2011). Their toxicity is another important issue. Aquatic ecosystems seem to be especially sensitive to these compounds (Corcellas et al., 2015a; Weston et al., 2014). However, pyrethroids have demonstrated low acute toxicity in mammals and birds, in part because of their easy metabolization (Demoute, 1989; Scollon et al., 2009).

However, recent studies have shown that the abuse of pyrethroids could make them ubiquitous in environment (Xue et al., 2005; Feo et al., 2010a; Kuivila et al., 2012; Weston and Lydy, 2010). Pyrethroids have been detected in biota tissues of fish (Corcellas et al., 2015a), dolphins (Alonso et al., 2012, 2015) and even humans (Corcellas et al., 2012; Sharma et al., 2014; Ostrea et al., 2009). Moreover, maternal transfer of pyrethroids by both gestational and lactation pathways has been observed in mammals (Alonso et al., 2012, 2015). The exposure of pyrethroids in wild birds near farmlands was even determined (Bro et al., 2015). Additionally, some authors, described disruptions of the endocrine system and depression of male fertility (Cinzia et al., 2013; Zhang et al., 2008; Jin et al., 2011) in rats and mice at non-acute toxic doses of pyrethroids. Besides, these studies showed different toxicological behaviour depending on the specific isomer of each pyrethroid, remarking the importance of taking into account the isomerism in risk assessment studies.

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In bird species toxic doses and effects have also been described (Khan et al., 2012). For example, the lethal dose (LD_{50}) of cyfluthrin in most of the studied bird species is in the order of thousands of mg kg^{-1}. Only canaries seemed to be especially sensible to this pyrethroid with LD_{50} from 68 to 274 mg kg^{-1} (EPA, 2014). Moreover, even when direct toxicity for birds is low, indirect environmental effects of their residues are not negligible. For instance, they could indirectly affect insectivorous birds in their behaviour and physiology, as well as through decline in their prey base (Pendleton and Baldwin, 2007).

There are few studies that have evaluated pyrethroid residues in avian eggs. Furthermore, these studies have been conducted on poultry so that their results are not environmentally representative (dell'Oro et al., 2014; dos Reis Souza et al., 2013). Some studies with wild grey partridges analysed eggs and carcasses of these birds with multiresidue methods and they found low amounts of lambda-cyhalothrin (Bro et al., 2016), tefluthrin, cyfluthrin and cypermethrin (Millot et al., 2015). The aim of our work was to evaluate for the first time the occurrence of pyrethroids in unborn eggs from wild birds of a National Park and surrounding areas in order to evaluate the presence of pyrethroid in wild fauna. That meant the quantitative determination of 13 common pyrethroids (*cis*-bifenthrin, cyfluthrin, cypermethrin, λ-cyhalothrin, deltamethrin, fenvalerate, fenpropathrin, fluvalinate, permethrin, phenothrin, resmethrin, tetramethrin and tralomethrin) in egg samples, the isomeric and enantiomeric characterization of these residues, and the relation of all these data with the determination of $\delta^{13}C$ and $\delta^{15}N$ in the same samples, in order to assess the influence of trophic level and food sources in pyrethroid content.

2. Materials and methods

2.1. Sampling

Doñana (National Park and Natural Park) is a nature reserve located in southwestern Spain and inscribed as a World Heritage Site, a Ramsar Site, a Biosphere Reserve and a European Community Special Protection Area. More than 360 bird species are estimated to live or breed inside its limits (UNESCO). In fact, its location is in the middle of the East Atlantic Flyway and it is considered a key site of this migratory route.

In consecutive campaigns in 2010, 2011 and 2012, we collected 14, 22 and 87 eggs that had failed to hatch, respectively. All eggs came from different nests. The 123 eggs belonged to 16 different species from several trophic levels and seven Orders (Table 1). However, when statistical analyses needed it, we grouped these samples in few categories. In order to test for variations in pyrethroid concentrations by ecological role, we grouped species by their taxonomical Order, such as Accipitriformes and Falconiformes (diurnal raptors), or Ciconiiformes (herons, storks and ibises). This allowed us to group together species with broadly similar ecological requirements. However, the Order Strigiformes (i.e. owls) was composed by one only sample and was pooled with the Order Falconiformes given their similar predatory trophic level.

Egg samples were collected opportunistically during nest checks and chick ringing operations, so that the number of samples per species depended on local abundance in the three study years. All the eggs were frozen and sent to the laboratory in individual and protected containers. Egg samples were measured (larger diameter) and broken. The egg content was weighted, homogenized and freeze dried. Lyophilized samples were weighted and homogenized again and stored at −20 °C until analysis.

2.2. Analytical methodologies

Sample treatment was adapted from previously developed methodologies for biota samples (Corcellas et al., 2015a; Feo et al., 2011). Briefly, an exact amount (0.2–0.3 g) of freeze-dried sample was spiked overnight with 2.5 ng and 1.25 ng of d_6-*trans*-permethrin and d_6-*trans*-cypermethrin, respectively. Extraction procedure was carried out twice with 20 mL of hexane:dichloromethane 2:1 and assisted by ultrasound for 15 min. All solvent was dried by a N_2 stream. A following tandem SPE cleaned up (basic alumina and C18 cartridges, 30 mL acetonitrile as eluent) was carried out. The eluent was evaporated under N_2 and the sample reconstituted in 100 μL of ethyl acetate. Analyses were performed on an Agilent Technologies 7890 A coupled to a 7000 A GC–MS Triple Quad. The selected mass spectrometry (MS) mode was negative chemical ionization with ammonium as reagent gas. The columns chosen were a DB5-ms (Agilent Technologies, Santa Clara, CA, USA) (15 m × 0.25 mm × 0.1 μm) for the quantitative analysis and a BGB-172 (BGB Analytik, Switzerland) (30 m × 0.25 mm × 0.25 μm) for the enantiomeric determination. Details of chromatographic conditions and MS-MS parameters to both achiral and chiral analyses are reported elsewhere (Corcellas et al., 2015b).

In parallel, lipid content was determined gravimetrically. An equivalent extraction procedure used for pyrethroid determinations was applied to 1 g of sample. Then, solvent extract was removed under a N_2 stream and the resulting residue was weighted.

2.3. Standards and reagents

Analytical standards were purchased from Dr. Ehrenstorfer (Augsburg, Germany). d_6-*trans*-permethrin and d_6-*trans*-cypermethrin were chosen as surrogate standards. Standard solutions were prepared in ethyl acetate ("for gas chromatography" quality from Merck, Darmstadt, Germany). Calibration curves were prepared at different concentrations ranging between 0.4 and 150 ng mL^{-1}. The rest of organic solvents were obtained from J.T. Baker "for use in HPLC" quality (Deventer, The Netherlands). Solid phase extraction (SPE) cartridges (C18, 2 g 15 mL^{-1}) were obtained from Isolute Biotage (Uppsala, Sweden) and cartridges of basic alumina (5 g 25 mL^{-1}) from Interchim (Montluçon, France).

2.4. Quality assurance/control

For each batch of 12 samples, one methodological blank was carried out. Levels of blanks were subtracted to all corresponding samples in case the blank signal was higher than 1% of the sample signal. Linearity in the selected range of concentration was verified obtaining correlation coefficients higher than 0.98 for all analytes. The mean recovery was 79%, being 53% the lower value, obtained for deltamethrin. Limits of detection (LOD) ranged from 0.03 to 0.46 ng g^{-1} lipid weigh (lw) and limits of quantification (LOQ) from 0.10 to 1.54 ng g^{-1} lw.

2.5. Isomeric and enantiomeric analyses

After quantitative analysis, representative samples of species were selected in order to be analysed with the chiral column. This method allowed discerning the isomeric proportion of bifenthrin, cyhalothrin, cyfluthrin, cypermethrin, permethrin and tetramethrin. In our previous work we described the peak assignation and the correspondences between both analyses (Corcellas et al., 2015b). Enantiomeric factors (EFs) for each enantiomeric pair were calculated with Eq. (1).