# Cypermethrin residues and diastereoselectivity in commercial and home-produced chicken eggs

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**Introduction**

Pyrethroids, including cypermethrin, are widely used on chicken egg farms for ectoparasite control and prevention. When applied topically to chickens, these compounds can be absorbed and mobilized to the eggs [1]. Pyrethroids are characterized by stereoisomerism: geometric (*cis* and *trans* diastereomers) and optical (*R* and *S* enantiomers). Authors cite diastereomeric accumulation pattern in different biological matrices [2,3,4]. Although pyrethroids are widely used in agricultural areas, there is little information about contamination in eggs. Study area is the largest poultry center located in Rio de Janeiro mountain region (Brazil). The place has great economic importance due to its intense agricultural activity. The aim of this research was to determine cypermethrin residues in eggs from commercial farms and from home producers. From the results, it was possible to compare cypermethrin diastereomeric pattern in eggs and in a commercial cypermethrin formulation used by local egg producers. This is the first study to include home-produced eggs in pyrethroid monitoring and it is also the first report about diastereomeric selectivity pattern in eggs produced for human consumption.

**Materials and methods**

The study included six commercial farms (F1 to F6) and three home producers (HP1 to HP3). Ten eggs were purchased at each site, totaling 90 samples. Each egg (yolk and white) was considered a sample. Matrix solid phase dispersion method was previously described [5], which consists in extracting 0.25 g lyophilized egg using 1.5 g of Florisil® as dispersant, 0.5 g of C18 as a lipophilic adsorbent, and eluting with 10 mL of acetonitrile. The lipid content was determined gravimetrically according to the method described [6]. GC-MS analysis were performed on NCI-SIM mode on a 7890A GC equipment coupled with a 5975C quadrupole mass spectrometer, both purchased from Agilent Technologies (Palo Alto, CA, U.S.A.). For quantification, a DB-5ms column (15m x 0.25 mm x 0.1 μm) was used. Diastereomer separation was carried out using a HP-5ms column (60 m x 0.25 mm x 0.25 μm). Quantification was based on matrix-matched calibration curve with six points between 5 $ng.mL^{-1}$ and 100 $ng.mL^{-1}$. Tetrachloro-m-xylene (100 $ng.mL^{-1}$) was used as an internal standard. Recovery tests were performed with organic eggs previously checked as cypermethrin free. Tests consisted in extraction of three blank samples and three spiked with 50 $ng.mL^{-1}$ of cypermethrin standard. Recoveries and coefficient of variation (CV) were: *cis* 1 (96.9% - 0.09), *trans* 1 (95.4% - 0.10) and *cis* 2 + *trans* 2 (101% 0.06). Limit of detection (LOD) was established with mean of ten analytical blanks plus three times standard deviation, while limit of quantification (LOQ) was calculated by the same mean of blanks plus ten times the deviation. The LOD and LOQ in $ng.mL^{-1}$ were respectively: *cis* 1 (0.04 and 0.09), *trans* 1 (0.02 and 0.04) and *cis* 2 + *trans* 2 (0.06 and 0.14). The ratio of area among diastereomers was calculated following a previously described study [2] and compared among five random samples of three sets of representative samples. Farm 1, where a cypermethrin commercial formula was used by producers, Farm 2 where there was no information about cypermethrin use and at Home producer 1. These sites presented 100% of the samples (eggs) with cypermethrin residues. Data normality was verified by Shapiro-Wilk test. Mann Whitney U test was applied for non-parametric distribution. For parametric distribution, the Student's T-test (t-test) was applied to verify the difference on diastereomeric abundance among samples from different farms. For all

statistical tests, the significance adopted level was 5% ($p < 0.05$). Microsoft Office Excel (2016)® and Graphpad Prism 5.0® statistical programs performed graphs and tests.

**Results and discussion**

Regarding the whole amount of samples (n=90), cypermethrin was quantified in 62 samples (69%) ranging from 0.29 – 6,408 $ng.g^{-1}$ (lipid weight – l.w.). Table 1 shows mean, standard deviation values, maximum and minimum concentration values ($ng.g^{-1}$ l.w.), and percentage of samples quantified (%n) for each sample points. According to the results, cypermethrin values were measured over the LOQ at all sampling points, with highest concentrations on Farm 1 (302 to 6,408 $ng.g^{-1}$ l.w.). Regarding cypermethrin residues among sample groups, the residues occurrence showed to be greater in domestic eggs (24 of 30 eggs - 80%) than in commercial ones (38 of 60 eggs - 63%).

**Table 1** Cypermethrin mean concentration, standard deviation, maximum, minimum values ($ng.g^{-1}$ l.w.) and percentage of positive samples (%n)

| Site | Mean | SD | Max. | Min. | %n |
|---|---|---|---|---|---|
| **Farm 1** | 2,324 | 2,117 | 6,408 | 302 | 100 |
| **Farm 2** | 17.9 | 10.2 | 33.7 | 1.28 | 100 |
| **Farm 3** | 0.34 | 0.72 | 1.90 | 1.50 | 20 |
| **Farm 4** | 0.68 | 0.61 | 1.42 | 0.29 | 70 |
| **Farm 5** | 2.27 | 1.89 | 5.80 | 1.00 | 80 |
| **Farm 6** | 0.14 | 0.46 | 1.44 | <LOD | 10 |
| **Home producer 1** | 11.1 | 6.18 | 26.0 | 3.03 | 100 |
| **Home producer 2** | 2.92 | 1.4 | 5.06 | 1.54 | 100 |
| **Home producer 3** | 2.75 | 4.07 | 11.8 | 3.96 | 40 |

SD: standard deviation; %n: percentage of quantified samples; <LOD: limit of detection.

Although commercial eggs showed a lower occurrence compared to samples from home-produced eggs, the highest residues concentrations were measured in commercial samples. Farm 6 was the site with the lowest residues occurrence, with only one sample. This farm was the only outside an agricultural area, situated in a recent urban sprawl; therefore, less exposed to contamination by agricultural pesticides. On the other hand, at Home producer 1, located in agricultural area with intense activity, producers reported that they did not use pesticides directly in chickens; however, 100% of the eggs had cypermethrin residues. In this site, there was pig farming and, in the surroundings, poultry production and other agricultural crops with regular use of pyrethroid insecticides. While this class of pesticides is widely used, we are aware of a single report about cypermethrin residues in commercial chicken eggs of 22 $ng.g^{-1}$ (wet weight – w.w.) [7]. In the present study, the extreme value, converted to wet weight, exceeded the previously concentration reported almost 30 times (656 $ng.g^{-1}$ w.w.). Moreover, all the eggs from Farm 1 presented values above the Maximum Residue Limit (MRL) of 10 $\mu g.kg^{-1}$ [8]. In this case, the wet weight mean values were approximately 23 times above the MRL (226 $ng.g^{-1}$ w.w.), with the minimum concentration reaching 3.5 times the MRL, and the maximum 66 times higher than the reference limit. On Farm 1, a commercial product with cypermethrin, specifically indicated for cattle, was topically administered to chickens, according to owners' reports.

Figure 2.A shows diastereomeric profile of cypermethrin commercial product topically applied to chickens, according to Farm 1 producers. In Figure 2.B, cypermethrin diastereomeric profile of one sample (egg) from the same farm can be observed.

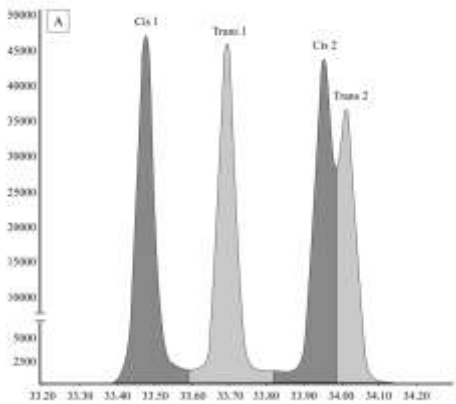


**Figure 2A** Diastereomeric cypermethrin profile in a commercial product used in Farm 1

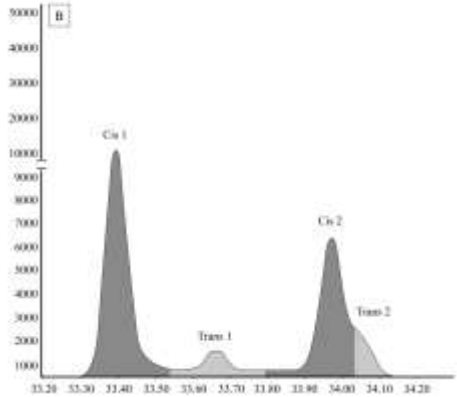


**Figure 2B** Diastereomeric cypermethrin profile in an egg sample from Farm 1

In figure 2A, a diastereomeric ratio characterized by racemic mixtures can be viewed: *cis* 1 (27%), *trans* 1 (26%) and *cis* 2 + *trans* 2 (47%). The same ratio *cis* / *trans* was observed in three analyzed products from different lots. In the chromatogram sample (Fig. 2B), the profile observed was: *cis* 1 (49%), *trans* 1 (6%) and *cis*2 + *trans* 2 (45%). Comparing chromatograms, a clear selectivity for *cis* 1 and *cis* 2 cypermethrin diastereomers can be seen. Regarding the other 61 positive samples for cypermethrin, the same pattern was observed, regardless diversity sources of exposure, as in samples of home-produced eggs. Figure 3 shows the percent abundance and standard deviation of cypermethrin diastereomers in egg samples from two commercial farms and from a home producer site.

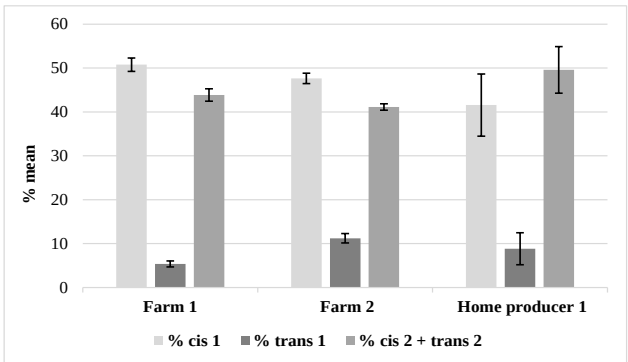


**Figure 3** Abundance (%) of cypermethrin diastereomers in chicken eggs from three egg producers

On Farm 1, chickens were exposed to cypermethrin commercial product (Fig. 1A). Farm 2 has a commercial production system similar to Farm 1, but in this case, it was not possible to obtain information about cypermethrin usage. In farm production, chickens are raised in suspended cages and fed with feed, which limits the sources of exposure mainly to possible pesticide residues in feed or by direct application to birds and in chicken houses treatment. The same *cis* diastereomer selectivity pattern was observed in samples from home producers, although it was possible to observe a greater *cis* 2 + *trans* 2 abundance. The variability observed in home samples might be due to a greater diversification of exposure sources, once these chickens live in direct contact with soil, being fed with a mixture of feed, food remains, forage grasses, and terrestrial invertebrates. However, in the three sampling points there was no statistical difference ($p < 0.05$) between selectivity patterns observed. Previous studies cite specificity in pyrethroids metabolism, where *trans* stereoisomers are hydrolyzed faster than *cis*, which oxidation is the main metabolic process [9] and may be a contributing factor to the selectivity observed. Cypermethrin *cis* diastereoselectivity in eggs, observed in this study, coincides with previous studies that reported the same *cis* selectivity in other biological samples, such as freshwater fish and mussel *Unio gibbus* [4,10]. A major factor of stereoisomeric selectivity in food samples, such as chicken eggs, is precisely the potential harm to consumer health, since a greater toxicity to mammals of *cis* stereoisomers was reported [11].

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