HAL
archives-ouvertes.fr

Determination of cis-permethrin, trans-permethrin and associated metabolites in rat blood and organs by gas chromatography–ion trap mass spectrometry

Francois Lestremau, Marie-Emilie Willemin, Claudine Chatellier, Sophie Desmots, Céline Brochot

▶ To cite this version:

Francois Lestremau, Marie-Emilie Willemin, Claudine Chatellier, Sophie Desmots, Céline Brochot. Determination of cis-permethrin, trans-permethrin and associated metabolites in rat blood and organs by gas chromatography–ion trap mass spectrometry. Analytical and Bioanalytical Chemistry, Springer Verlag, 2014, 406 (14), pp.3477-3487. 10.1007/s00216-014-7774-z . ineris-01710209

HAL Id: ineris-01710209
https://hal-ineris.archives-ouvertes.fr/ineris-01710209

Submitted on 15 Feb 2018

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire HAL, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Determination of cis-, trans-permethrin and associated metabolites in rat blood and organs by gas chromatography ion trap mass spectrometry

F. Lestremau [1]*, M.-E. Willemin[2,3], C. Chatellier[1], S. Desmots[4], C. Brochot[2]

[1] Institut National de l'Environnement Industriel et des Risques (INERIS), Unité Innovation pour la Mesure (NOVA), Parc ALATA BP2, 60550 Verneuil en Halatte, France

[2] Institut National de l'Environnement Industriel et des Risques (INERIS), Unité Modèles pour l'Ecotoxicologie et la Toxicologie (METO), Parc ALATA BP2, 60550 Verneuil en Halatte, France

[3] Université de Technologie de Compiègne, CNRS UMR 7338, Laboratoire de Biomécanique et Bio ingénierie, France

[4] Institut National de l'Environnement Industriel et des Risques (INERIS), Unité Toxicologie Expérimentale (TOXI), Parc ALATA BP2, 60550 Verneuil en Halatte, France

* corresponding author

Abstract

An analytical method was developed to measure *cis*- and *trans*-permethrin in different biological rat matrices and fluids (whole blood, red blood cells, plasma, brain, liver, muscle, testes, kidneys, fat and faeces). The method was also suitable for the simultaneous quantification of their associated metabolites (*cis*- and *trans*-3-(2,2 dichlorovinyl)-2,2-dimethyl-(1-cyclopropane) carboxylic acid (DCCA) and 3-phenoxybenzoic acid (3-PBA)) in the blood (whole blood, red blood cells, plasma) and the liver.

The target analytes were derivatized in samples using a methanolic/hydrochloric acid solution and then extracted with toluene. The analysis was performed by gas chromatography, and detection using ion trap mass spectrometry in MS/MS mode. The selectivity obtained for complex matrices such as rat organs allowed for avoiding the use of a purification step for most of the investigated matrices. In the case of fat, where permethrin is suspected to accumulate, a dedicated purification step was developed. In fluids, the limits of quantification were established at a 50 ng/mL level for parent compounds and 3-PBA and 25 ng/mL for *cis*- and *trans*-DCCA. For solid matrices excluding fat, the limits of quantification were established, ranging from 50 ng/g for muscle to 100 ng/g for the brain and testes for both *cis* and *trans*-permethrin. The extraction recoveries ranged primarily between 80 and 120 % for the matrix tested. The stability of blood samples was tested through the addition of formic acid 1 % V/V. The developed methods were applied to a toxicokinetic study in adult rats. *Cis*-permethrin and the metabolites were detected in all corresponding matrices while *trans*-permethrin was only detected in the blood, plasma and faeces.

1. Introduction

Pyrethroid insecticides represent approximately one quarter of the worldwide insecticide market. They have been widely used in wood preservation, disinfection, mosquito control and also in wool carpets and textiles to prevent insect damage [1,2]. Pyrethroids are now detected in indoor and outdoor environments [3,4] as well as in humans [5]. Although reported to be less toxic to mammals than other pesticides, pyrethroids are suspected to induce neuro-toxicological and developmental alterations in mammals [6-10].

Permethrin (3-phenoxybenzyl (*1RS*,*3RS*;*1RS*,*3SR*)-*cis,trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclo-propanecarboxylate) is one of the most widely used pyrethroids. Permethrin is a chiral compound with two stereocentres in the cyclopropane ring (Figure 1) [11] and is usually produced as a mixture of the two stereoisomers (*cis*:*trans* with 40:60, 80:20 or 25:75 ratios). As neuro-toxicants, *cis*- and *trans*-permethrin both act on the nervous system of insects by interfering with sodium channels to disrupt neuronal activity [12]. In mammals, once absorbed, permethrin is rapidly distributed to various organs/tissues (liver, brain, adipose tissues...) and transformed into metabolites that are subsequently excreted in urine and faeces [13,14]. In rats, *cis*- and *trans*-permethrin are hydrolysed in the blood, small intestine and liver by an ester cleavage, due to the presence of carboxylesterases, to *cis*- and *trans*-3-(2,2Dichlorovinyl)-2,2-dimethyl-(1-cyclopropane), carboxylic acid (*cis*- and *trans*-DCCA) and 3-phenoxybenzoic alcohol (3-PBAlc) [11,15]. 3-PBAlc is then oxidized to form 3-phenoxybenzoic acid (3-PBA) in the liver by cytochromes P450 or deshydrogenases (Figure 1) [16,17]. Even if they share the same metabolic pathways, the *cis*-isomer is metabolized three times slower than the *trans*-isomer [18].

Several analytical strategies have been proposed to measure the permethrin different isomers and metabolites in biological matrices. Analyses have been performed using high performance liquid chromatography associated with an ultraviolet detector (HPLC-UV), in which *cis* and *trans* forms were separated [17,16]. The use of a special chiral column enabled a separation of the four enantiomers [*1R, 3R cis*], [*1S, 3S cis*], [*1R, 3S trans*], [*1S, 3R trans*] [6]. *Cis*- and *trans*-permethrin have also been monitored by liquid chromatography coupled with mass spectrometry (LC-MS/MS) to

improve sensitivity in comparison to HPLC-UV [19]. As permethrin is a non-polar and semi-volatile compound, it is more commonly analysed by gas chromatography (GC) coupled with flame ionization detection [20], electron capture detector [21] or mass spectrometer detection [22]. The metabolites *cis*- and *trans*-DCCA and 3-PBA are very polar substances and can be monitored by LC-MS/MS [23] since they are easily ionized in an electrospray ionization positive mode. They can also be analysed by GC using a derivatization step [24,21]. In this case, they have been primarily analysed by GC-MS [21] or GC coupled with high resolution mass spectrometry (HRMS) [25]. Permethrin has been previously monitored in blood or plasma and organs such as the brain, whereas metabolites have been primary measured in urine or plasma [14,21,13]. To our best knowledge, the metabolites and the permethrin compounds have not been monitored simultaneously in organs, but only in cellular fractions of organs (such as liver or intestine microsomes) exposed during *in vitro* experiments [16,17].

In this study, we aimed to develop an analytical method to monitor simultaneously *cis*-, *trans*-permethrin and metabolites (*cis*-, *trans*-DCCA and 3-PBA) in the biological fluids and organs of rats. According to the toxicokinetic properties and the target organs for toxicity of permethrin, the following fluids, organs or tissues were of interest: whole blood, red blood cell, plasma, fat, brain, testes, liver, kidney, muscle and faeces. Urine was not analysed in this study as analytical methods are already available for this matrix [26,21]. Despite the differences in physico-chemical properties and content of the investigated matrices, the extraction method was intended to be relatively simple and single step. With this objective, the selectivity of the detection was of utmost importance. Therefore, the analysis of these samples by GC/MS/MS ion trap detection was investigated and the experimental parameters optimized. The validated analytical method was applied to a toxicokinetic study in adult rats exposed to *cis*- and *trans*-permethrin separately.

2. Experimental

2.1 *Chemicals*

For analytical measurements, *cis*-permethrin (purity, 99 %) was purchased from Sigma-Aldrich (St Quentin Fallavier, France) and *trans*-permethrin (99 %) from Cluzeau Info Labo (Sainte-Foy-La-Grande, France). A mixture of the metabolites *cis*-3-(2,2-dichlorovinyl)-2,2-dimethyl-(1-cyclopropane) carboxylic acid (*cis*-DCCA) and *trans*-3-(2,2-dichlorovinyl)-2,2-dimethyl-(1-cyclopropane) carboxylic acid (*trans*-DCCA) (54:46 *cis*/*trans*) was acquired from Dr. Ehrenstorfer (Augsburg, Germany) and 3-phenoxybenzoic acid (3-PBA) (98 %) from Cambridge Isotope Laboratories (Andover, MA, USA). The internal standards *cis*-labelled-$^{13}C_6$-permethrin (98 %) (50 ng/µL in nonane), *trans*-labelled-$^{13}C_2$ DCCA (98 %) and $^{13}C_6$-3-PBA (98 %) (both 100 ng/µL in nonane) were acquired from Cambridge Isotope Laboratories (Andover, MA, USA). For the toxicokinetic study, *cis*-permethrin (purity, 99.4 %) was provided by ChemService (West Chester, PA, USA) and *trans*-permethrin (99 %) by Dr. Ehrenstorfer (Augsburg, Germany).

Hydrochloric acid was purchased from CarloErba (Val de Reuil, France) and methanol (MeOH), toluene, acetonitrile (ACN) and dichloromethane (DCM) from Merck (Darmstadt, Germany). SPE cartridges C18 were provided by JT Baker (Phillipsburg, NJ, USA) and Florisil by Grace (Epernon, France). Strata X-AW was from Phenomenex (Le Pecq, France).

2.2 *Preparation of internal standard solutions*

250 µL of stock solutions of *cis*-$^{13}C_6$-permethrin, $^{13}C_6$ 3-PBA and $^{13}C_2$-DCCA were reduced until dryness and then dissolved in 1 mL of MeOH to obtain a mixture with respective concentrations of 12.5, 25 and 25 ng/µL.

2.3 *Analytical system*

A Varian gas chromatograph 3800 (Les Ullis, France) coupled with a Varian ion trap mass spectrometer 4000 was used. Injections were performed using a split/splitless injector equipped with a quart wool liner and run in splitless mode at 250°C. The injection volume was 1 µL.

The GC column was a ZB-5MS (30 m×0.25 mm i.d x 1.0 µm film thickness) (Phenomenex, Le Pecq, France). It was used in pulse pressure at 16.8 psi for 1 min and then in constant flow at 1.3 mL/min. The temperature program started at 70°C for 1 min and then was ramped up by 10°C/min for 23 min to yield a final temperature plateau at 300°C for 10 min. The transfer line temperature was set at 250°C, the temperature of the manifold at 50°C, and the trap and source temperatures at 200°C and 220°C respectively.

The controls of the instrument and the data acquisition were performed using MS Workstation software (Varian).

2.4 *Animals*

Male Sprague Dawley (SD) rats (Janvier, Genet de lisle, France) were used to provide blank biological matrices and to characterize the toxicokinetics of *cis* and *trans*-permethrin. For the toxicokinetic study, each rat was housed in a cage with a 12 h light/12 h dark cycle at ambient temperature (22°C ± 2°C) and relative humidity (55 ± 15%). Food (Altromin for rat and mouse, Genestil, Royaucourt, France) and tap water were provided *ad libitum*.

2.5 *Preparation of biological samples*

Blood was collected in heparinized tubes after euthanasia of the rats with CO_2. To separate plasma and red blood cells (RBC), blood samples were centrifuged for 5 min at 6,100 rpm using the Centrifuge 5417R (Eppendorf, Le Pecq, France). 1% of formic acid was added to the blood after collection and to the plasma after centrifugation to inhibit the metabolism of *cis*- and *trans*-permethrin from carboxylesterases enzymes and to preserve the stability of the compounds [21]. All samples were frozen at -80°C. The liver, brain, testes, muscle, kidneys, fat and faeces were also immediately frozen at -80°C after collection. After thawing, organs and faeces were manually grinded with a mortar and a pestle.

2.6 *Extraction procedure*

2.6.1 Biological fluids (blood, red blood cells, plasma)

Before extraction, all fluids were thawed and maintained at 4°C. Depending on the sample volume available, 0.5-1 mL of fluids were spiked with 40 µL of the internal standard solutions and mixed by vortex for 1 min. 1 mL of MeOH and 1 mL of concentrated hydrochloric acid (10 M) were added and then stirred by vortex for 1 min. Samples were heated at 80°C for 20 min to produce a methyl ester derivatization of the metabolites (3-PBA-Me and *cis*- or *trans*-DCCA-Me) and the internal standards. The samples were left to cool for 15 min and then 2 mL of toluene was added. After 20 min of shaking, the samples were centrifuged for 15 min at 3,000 rpm. 1 µL of the supernatant was injected for GC-MS/MS analysis.

2.6.2 Organs (brain, muscle, testes, liver, kidney), fat and faeces

The extraction/derivatization method used for all organs (except fat) and faeces was similar to the one developed for the fluids. 40 µL of the internal stock solutions and 0.5 mL of MeOH were added to 1 g of each matrix. This mixture was then grinded with microbeads in Precellys (Bertin, Montigny le Bretonneux, France). 0.5 mL of MeOH and 1 mL of concentrated hydrochloric acid (10 M) were added to each sample then vortex mixing was applied for 1 min. After this step, the procedure was identical to the one described for the biological fluids (described in section 2.6.1), i.e. followed by a heating phase.

For fat, the extraction consisted in adding 40 µL of the internal stock solution to 0.5 g of fat mixed with 3 mL of ACN/DCM 75/25 (V/V). Vortex mixing was performed for 1 min and centrifugation at 3,000 rpm for 20 min at -10°C. After the reduction of 2 mL of the supernatant until dryness, the residue was dissolved in 2 mL of ACN and a purification step was applied.

Two methods were considered for purification. The first method, based on NF EN 1528 [27], consisted in purifying the solvent extract using C_{18} sorbent. 1 g of C_{18} was directly added to the ACN extract as dispersive solid phase extraction. An alternative purification method was evaluated as carried out by Rawn et *al*. [28] on fish samples using Strata X-AW sorbent. The same extraction process was used but in this case, the purification step was carried out with a blend of 100 mg of Strata X-AW + 150 mg of Na_2SO_4.

For both purification steps, the extract was then stirred by vortex for 1 min followed by a centrifugation at 3,000 rpm for 5 min. The supernatant was then extracted, reduced until dryness and dissolved in 1 mL of toluene. 1 µL of this solution was injected for GC-MS/MS analysis.

2.7 *Application to toxicokinetic studies*

The analytical method was applied to study the distribution of *cis*-permethrin, *trans*-permethrin and their metabolites in adult rats. Rats were separated into 2 groups exposed to 2 different doses, 5 and 20 mg/kg of bodyweight (BW). These two doses were tested to check the relevance of our analytical method for low and high doses of permethrin. The substance was dissolved in corn oil in a total volume of 1 mL/kg BW and was administered orally to the animals. Food and water were provided *ad libitum* 3 hours after the administration. Groups of two rats were euthanized by CO_2 at 2, 6 and 24 hours after administration. Fluids and organs were collected by the procedure described in section 2.5. The experimental protocol was approved by an internal ethics committee.

2.8 *Quality control tests*

For sample analysis, each sequence was controlled with several quality control solutions at respective concentrations of 100 ng/mL and 600 ng/mL. The control samples were analysed every 10 samples. When the measured concentrations of the control samples deviated from the expected concentrations beyond an 80 - 120% range, a new calibration curve was run.

3. Results and discussion

3.1 *Method development and performances*

Chromatographic separation

Gas chromatography was selected since it allowed both *cis*- and *trans*-isomers and their respective metabolites to be separated (a representative chromatogram is displayed in Figure 2). The method was developed based on Corrion et *al.* [24]. The retention times of permethrin compounds were around 27 min with a gap of 0.2 min between the *cis*- and *trans*-isomers, which was sufficient to achieve baseline resolution. Both derivatized *cis* and *trans* metabolites were also separated from each other and eluted before the parent compounds (12.2 and 12.5 min respectively). 3-PBA eluted at 18.6 min. The retention times are summarized in Table 1. The relative long retention times obtained for the permethrin enantiomers also enabled the target compounds to be better separated from the matrix interferences.

Choice of mass spectrometry parameters

An external source was used to reduce the rate of contamination from multiple sample injections. The detection parameters were optimized for each substance. The parent ion was first selected from the injection of a high concentrated standard. Then the fragmentation patterns were determined by applying various energies. Daughter ions were chosen from the ions that provided the maximum intensities for the various fragments. Examples of mass spectra obtained during the method optimization are presented in Figure 3. For GC-MS/MS detection, according to the Sanco/12495/2011 guide [29], a minimum of two transitions from one parent ion is required to confirm the identification of a substance. Therefore, two transitions, one for the quantification and one as the qualifier, were determined. The acquisition was segmented into windows, corresponding to transitions of target components to increase the acquisition rate, the sensitivity and selectivity of the detection.

The mass spectrometry parameters optimized for *cis*-, *trans*-permethrin and their derivatized metabolites are listed in Table 1.

Calibration procedure and linear range

The calibration standards were prepared following the extraction and derivation procedure used for fluids and organs (see section 2.6.1). As the method was intended to be applied to different biological matrices producing specific interferences, an isotopic labelled internal standard was added for each class of studied compound to take into account these phenomena. The calibration curves displayed good linearity in the 50 ng/mL to 2000 ng/mL range for *cis*- and *trans*-permethrin (R^2>0.99) and 3-PBA (R^2>0.99). For *cis*- and *trans*-DCCA, linearity ranged from 25 and 1000 ng/mL (R^2 at 0.98 for *cis*-DCCA and 0.99 for *trans*-DCCA).

The accuracy and precision of the analytical method were evaluated at the concentrations of 100 and 600 ng/mL for *cis*-, *trans*-permethrin, 3-PBA and the mixture of *cis*- and *trans*-DCCA. These concentrations were analysed every 10 samples per day to evaluate intraday accuracy and precision with a final number of samples between 10 and 12. Interday accuracy and precision were determined by comparing analyses performed over 3 different days (n between 32 and 36). Accuracy was the mean of the accuracy of each sample, calculated by (|measured concentration – added concentration|/added concentration) × 100. Precision was given by the standard deviation divided by the mean of the detected concentration. The results are reported in Table 2. The intraday comparisons provided results for all components in a range between 10-13 % for accuracy and 8-11% for precision. The interday comparisons provided accuracy values between 11 and 16% and precision between 7 and 19%.

Sample preparation and method performances for biological fluids (blood, red blood cells, plasma), organs and faeces.

To simplify the analytical protocol and take advantage of the selective MS/MS detection, a single sample preparation technique was developed for most of the target matrices and analytes. Thus, the method used by Corrion et *al.* [24] for the analysis of umbilical and maternal blood for pyrethroids (but not permethrin) and their metabolites was adapted for this study. The method proved to be compatible with all tested matrices except fat. Table 3 presents the recoveries determined for the extraction of the studied compounds in each matrix. The derivatization step did not influence the

recovery of the parent compounds. Therefore, the extraction procedure was satisfactory for all target analytes since all recovery factors were within the 65 - 132 % range. In light of these results, no correction was applied for the studied compounds. Representative chromatograms obtained after extraction of *cis*-permethrin and its metabolites in samples are shown in Figure 4.

Selection of extraction method for fat

Preliminary tests (results not reported) demonstrated that the extraction method used for organs and faeces did not provide satisfactory results for the analysis of fat. Indeed, the extracted lipids generated severe interferences during the chromatographic analysis. An extraction method based on the standard NF EN 1528 [27] was therefore selected. Metabolites were not quantified in fat, so the method was only optimized for *cis*- and *trans*-permethrin analysis.

Two methods of purification were considered (details for the extraction and purification steps are provided in the "experimental" section 2.6.2). The method was tested with samples containing no fat to verify the recovery step of the method and then with spiked fat samples. Purification with C_{18} provided recoveries at 109 % and 114 % without and with the matrix. Purification with Strata X-AW also provided satisfactory results as recoveries were respectively of 104% and 118% without and with the matrix.

The results obtained either with C_{18} or Strata X –AW sorbents yielded acceptable recoveries. Strata X-AW sorbent was chosen since the chromatograms obtained presented fewer interfering peaks than with the C_{18} sorbent.

A representative chromatogram for fat extraction is displayed in Figure 4.

Limits of quantification

The limits of quantification (LOQ) were determined as a signal-to-noise ratio of ten. The limits of quantification were established in each matrix by considering the results of the toxicokinetic experiments. In fluids, the LOQ were established at a 50 ng/mL level for parent compounds and 3-PBA and 25 ng/mL for *cis*- and *trans*-DCCA. For solid matrices excluding fat, the LOQ were established ranging from 50 ng/g for muscle to 100 ng/g for the brain and testes for both *cis* and *trans*-

permethrin. In fat, due to the purification step which produced a low level of noise, a LOQ of 50 ng/g was obtained.

The method and detection chosen generally provided enough sensitivity and selectivity to avoid developing any further concentration or purification steps for most matrices tested, which would have complicated the analytical process. However, to increase sensitivity, extracts could be concentrated by nitrogen evaporation to reduce the 1 mL of volume extract to 200 µL (or even further). The limits of quantification could then be reduced by a factor of 5. In order to prevent the accumulation of non-volatile interferences from the extract in the analytical system (injector, column or mass spectrometer parts), this pre-concentration process was only performed when needed, i.e. when no response was obtained with the standard protocol and a low amount of target compounds were expected, e.g. in testes.

3.2 *Application of permethrin analysis to TK studies in rats*

3.2.1 Formic acid to inhibit metabolic reactions in blood

Cis- and *trans*-permethrin are metabolized in the liver and intestines but also in the blood. The use of formic acid was tested to inhibit metabolic reactions after sampling. To that end, blank matrices were spiked with *cis*- and *trans*-permethrin. For samples treated with 1% of formic acid, the recoveries for *cis*- and *trans*-permethrin were 76 ± 6% and 85 ± 13% in blood, 67 ± 5% and 81 ± 7% in plasma, 63 ±12% and 84 ± 22% in RBC respectively. Without formic acid, the recoveries were at 50 ± 7% and 21 ± 6% in blood, 45 ± 3% and 1 ± 1% in plasma, 53 ± 16% and 28 ± 5% in RBC respectively. Lower recoveries were obtained without formic acid.

Similar results were observed with biological samples collected from 2 rats exposed to a dose of 5 mg/kg of bodyweight or a dose of 20 mg/kg of bodyweight. After an oral administration of a 5 mg/kg dose, the concentration of *trans*-permethrin was measured at 0.10 µg/mL in a blood sample with formic acid whereas no detection was observed in a blood sample without formic acid. For *cis*-permethrin, formic acid produced higher measured concentrations at 0.34 ± 0.11 µg/mL as compared

to 0.16 ± 0.04 µg/mL without. In plasma, 2 hours after a 20 mg/kg dose of *trans*- or *cis*-permethrin was administered, there was no detection of *trans*-permethrin without formic acid and only 0.60 +/- 0.50 µg/mL of *cis*-permethrin was determined. When formic acid was added to samples collected at the same time point, the measured concentrations were 0.41 µg/mL and 1.09 ± 1.14 µg/mL respectively. Leng *et al.* [21] also observed that the addition of formic acid improved storage stability of permethrin in plasma. These results are consistent with their conclusions and confirmed the need to add 1 % formic acid in blood samples to inhibit permethrin metabolism after sampling.

3.2.2 Toxicokinetic study

The analytical method was applied to blood, plasma, red blood cells and tissue samples collected in rats during the toxicokinetic study at an administration dose of 5 and 20 mg/kg. The kinetic profiles of the different compounds in blood and plasma with formic acid, red blood cells and tissues concentrations are presented in Figures 5, 6 and 7.

In fluids, the maximum concentration was observed between 2 and 6 hours after the administration of the parent compounds and the metabolites (Figure 5). *Trans*-permethrin was only detected at the time point 2 hours, whereas *cis*-permethrin was also detected at 6 hours. At equivalent doses, *cis*-permethrin was found in higher levels than *trans*-permethrin, suggesting that *trans*-permethrin has a higher metabolic rate, as has been observed in other studies [18]. This is also supported by higher blood concentrations of the metabolite 3-PBA, 7.37 ± 2.50 µg/mL at 6 h after the administration of 20 mg/kg of *trans*-permethrin, compared to 1.69 µg/mL in the same condition following a *cis*-permethrin administration. Moreover, the maximum concentrations of 3-PBA are higher than those of DCCA with 2.22 ± 0.57 µg/mL for 3-PBA and 0.21± 0.04 µg/mL for *trans*-DCCA in blood after 5 mg/kg of *trans*-permethrin. The concentrations of the different compounds are similar in blood and plasma whereas the concentrations in RBC are lower, especially for *cis*-permethrin and *trans*-permethrin metabolites.

Cis-permethrin was detected and quantified in all tissues at 2 and 6 hours after the administration of each dose, except in testes where it was determined only at the dose of 20 mg/kg of bodyweight (Figure 6). The maximum concentration was generally observed at time point 6 hours after

administration in the majority of the tissues (brain, testes, muscle, fat). For these matrices, *trans*-permethrin was detected only in fat, and this for both doses (Figure 7). In faeces, high levels of *cis*- and *trans*-permethrin were measured. On average, 25% of the administered dose of *trans*-permethrin was excreted via faeces and 38% for *cis*-permethrin.

Our data did not allow us to make a conclusion about the linearity of the kinetics of *cis*- and *trans*-permethrin under the dose regimen. For *cis*-permethrin, the ratios of the concentrations measured in fluids and organs for the low dose over those measured for the high dose ranged between 0.9 in fat to 3.5 in kidneys. However, these ratios should be taken with caution because of the low number of data points collected and the large inter-individual variability observed between the two rats in each group. The percentage of permethrin excreted in faeces was independent of the quantity administered for both isomers.

4. Conclusion

An analytical method for the simultaneous determination of *cis*- and *trans*-permethrin and their associated metabolites (*cis*-, *trans*-DCCA and 3-PBA) in several organs, fluids and faeces in rats was developed. Detection by ion trap spectrometry in MS/MS mode proved to provide sufficient sensitivity and selectivity to enable measurement in a real matrix with easy to use methods. The developed methods were successfully applied to samples collected in toxicokinetic studies. Our observations are consistent with existing knowledge of *cis*- and *trans*-permethrin toxicokinetics, *i.e.* substantial elimination in faeces, rapid elimination from tissues, accumulation of both *cis*- and *trans*-permethrin in fat due to their lipophilic properties (log K_{ow} = 6.5) and a higher clearance rate of *trans*-isomer compared to the *cis*-isomer.

Acknowledgements

This work was supported by the foundation of the University of Technology in Compiègne “La Fondation UTC pour l’Innovation” (Project ToxOnChip) and the French Ministry of Ecology and Sustainable Development.

References

1. Environmental Protection Agency (U.S. EPA) (2007) Permethrin & Resmethrin (Pyrethroids) Toxicity and Exposure Assessment for Children's Health (TEACH) Chemical Summary.

2. Wei BN, Mohan KR, Weisel CP (2012) Exposure of flight attendants to pyrethroid insecticides on commercial flights: Urinary metabolite levels and implications. International Journal of Hygiene and Environmental Health 215 (4):465-473.

3. Morgan MK, Sheldon LS, Croghan CW, Jones PA, Chuang JC, Wilson NK (2007) An observational study of 127 preschool children at their homes and daycare centers in Ohio: Environmental pathways to cis- and trans-permethrin exposure. Environmental Research 104 (2):266-274.

4. Feo ML, Eljarrat E, Barcelo D (2010) Determination of pyrethroid insecticides in environmental samples. Trac-Trends in Analytical Chemistry 29 (7):692-705.

5. Corcellas C, Feo ML, Torres JP, Malm O, Ocampo-Duque W, Eljarrat E, Barcelo D (2012) Pyrethroids in human breast milk: Occurrence and nursing daily intake estimation. Environment International 47 (0):17-22.

6. Jin YX, Liu JW, Wang LG, Chen RJ, Zhou C, Yang YF, Liu WP, Fu ZW (2012) Permethrin exposure during puberty has the potential to enantioselectively induce reproductive toxicity in mice. Environment International 42:144-151.

7. Meeker JD, Barr DB, Hauser R (2008) Human semen quality and sperm DNA damage in relation to urinary metabolites of pyrethroid insecticides. Human Reproduction 23 (8):1932-1940.

8. Wolansky MJ, Harrill JA (2008) Neurobehavioral toxicology of pyrethroid insecticides in adult animals: A critical review. Neurotoxicology and Teratology 30 (2):55-78.

9. Zhang SY, Ito Y, Yamanoshita O, Yanagiba Y, Kobayashi M, Taya K, Li C, Okamura A, Miyata M, Ueyama J, Lee CH, Kamijima M, Nakajima T (2007) Permethrin may disrupt testosterone

biosynthesis via mitochondrial membrane damage of leydig cells in adult male mouse. Endocrinology 148 (8):3941-3949.

10. Zhang S-Y, Ueyama J, Ito Y, Yanagiba Y, Okamura A, Kamijima M, Nakajima T (2008) Permethrin may induce adult male mouse reproductive toxicity due to cis isomer not trans isomer. Toxicology 248 (2-3):136-141.

11. Ross MK, Borazjani A, Edwards CC, Potter PM (2006) Hydrolytic metabolism of pyrethroids by human and other mammalian carboxylesterases. Biochemical Pharmacology 71 (5):657-669.

12. Soderlund DM, Clark JM, Sheets LP, Mullin LS, Piccirillo VJ, Sargent D, Stevens JT, Weiner ML (2002) Mechanisms of pyrethroid neurotoxicity: implications for cumulative risk assessment. Toxicology 171 (1):3-59.

13. Tornero-Velez R, Davis J, Scollon EJ, Starr JM, Setzer RW, Goldsmith M-R, Chang DT, Xue J, Zartarian V, DeVito MJ, Hughes MF (2012) A Pharmacokinetic Model of cis- and trans-Permethrin Disposition in Rats and Humans With Aggregate Exposure Application. Toxicological Sciences 130 (1):33-47.

14. Anadon A, Martinezlarranaga MR, Diaz MJ, Bringas P (1991) Toxicokinetics of Permethrin in the Rat. Toxicology and Applied Pharmacology 110 (1):1-8.

15. Crow JA, Borazjani A, Potter PM, Ross MK (2007) Hydrolysis of pyrethroids by human and rat tissues: Examination of intestinal, liver and serum carboxylesterases. Toxicology and Applied Pharmacology 221 (1):1-12.

16. Nakamura Y, Sugihara K, Sone T, Isobe M, Ohta S, Kitamura S (2007) The in vitro metabolism of a pyrethroid insecticide, permethrin, and its hydrolysis products in rats. Toxicology 235 (3):176-184.

17. Choi J, Rose RL, Hodgson E (2002) In vitro human metabolism of permethrin: the role of human alcohol and aldehyde dehydrogenases. Pesticide Biochemistry and Physiology 74 (3):117-128.

18. Scollon EJ, Starr JM, Godin SJ, DeVito MJ, Hughes MF (2009) In Vitro Metabolism of Pyrethroid Pesticides by Rat and Human Hepatic Microsomes and Cytochrome P450 Isoforms. Drug Metabolism and Disposition 37 (1):221-228.

19. Starr JM, Scollon EJ, Hughes MF, Ross DG, Graham SE, Crofton KM, Wolansky MJ, DeVito MJ, Tornero-Velez R (2012) Environmentally Relevant Mixtures in Cumulative Assessments: An Acute Study of Toxicokinetics and Effects on Motor Activity in Rats Exposed to a Mixture of Pyrethroids. Toxicological Sciences 130 (2):309-318.

20. Du JJ, Yan HY, She DD, Liu BM, Yang GL (2010) Simultaneous determination of cypermethrin and permethrin in pear juice by ultrasound-assisted dispersive liquid-liquid microextraction combined with gas chromatography. Talanta 82 (2):698-703.

21. Leng G, Kuhn KH, Idel H (1997) Biological monitoring of pyrethroids in blood and pyrethroid metabolites in urine: Applications and limitations. Science of the Total Environment 199 (1-2):173-181.

22. Schettgen T, Koch HM, Drexler H, Angerer J (2002) New gas chromatographic-mass spectrometric method for the determination of urinary pyrethroid metabolites in environmental medicine. Journal of Chromatography B-Analytical Technologies in the Biomedical and Life Sciences 778 (1-2):121-130.

23. Baker SE, Olsson AO, Barr DB (2003) Isotope dilution high-performance liquid chromatography-tandem mass Spectrometry method for quantifying urinary metabolites of synthetic pyrethroid insecticides. Archives of Environmental Contamination and Toxicology 46 (3):281-288.

24. Corrion ML, Ostrea EM, Bielawski DM, Posecion NC, Seagraves JJ (2005) Detection of prenatal exposure to several classes of environmental toxicants and their metabolites by gas chromatography-mass spectrometry in maternal and umbilical cord blood. Journal of Chromatography B-Analytical Technologies in the Biomedical and Life Sciences 822 (1-2):221-229.

25. Leng G, Gries W (2005) Simultaneous determination of pyrethroid and pyrethrin metabolites in human urine by gas chromatography-high resolution mass spectrometry. Journal of Chromatography B 814 (2):285-294.

26. Le Grand R, Dulaurent S, Gaulier JM, Saint-Marcoux F, Moesch C, Lachatre G (2012) Simultaneous determination of five synthetic pyrethroid metabolites in urine by liquid

chromatography-tandem mass spectrometry: Application to 39 persons without known exposure to pyrethroids. Toxicology Letters 210 (2):248-253.

27. Standard NF EN 1528- Fatty food. Determination of pesticides and polychlorinated biphenyls (PCBs). (1997).

28. Rawn DFK, Judge J, Roscoe V (2010) Application of the QuEChERS method for the analysis of pyrethrins and pyrethroids in fish tissues. Analytical and Bioanalytical Chemistry 397 (6):2525-2531.

29. EU Reference Laboratories (EURLs) (2012) Document No. SANCO/12495/2011: Method Validation & Quality Control Procedures for Pesticide Residues Analysis in Food & Feed.

List of tables

Table 1. Settings for mass spectrometry detection by tandem ion trap mode of target analytes.

Table 2. Intraday (n= 10 to 12) and interday (n = 32 to 36) accuracy and precision of the analytical method at 100 and 600 ng/mL of *cis*- and *trans*-permethrin, 3-PBA and the mixture of *cis*-DCCA and *trans*-DCCA in methanol.

Table 3. Recovery (%) after extraction of the different matrices spiked with 500 ng/mL of *cis*- and *trans*-permethrin, *cis*- and *trans*-DCCA and 3-PBA (n = 3).

Table 1: Settings for mass spectrometry detection by tandem ion trap mode of target analytes.

Compound	Retention time (min)	Acquisition segment (min)	Parent ion	Excitation storage level (m/z)	Excitation Amplitude (V)	Quantification ion	Qualifier ion
Cis-permethrin	26.98	25-34	183	60.5	48	168	165
Trans-permethrin	27.18	25-34	183	60.5	48	168	165
Me-*cis*-DCCA	12.22	10-15	187	61.8	1.05	151	123
Me-*trans*-DCCA	12.49	10-15	187	61.8	1.05	151	123
Me-3-PBA	18.59	15-25	228	75.3	43	196	195
Cis-$^{13}C_6$-permethrin	26.97	25-34	189	60.5	48	174	/
Me-*trans*-$^{13}C_2$-DCCA	12.35	10-15	190	61.8	1.05	154	/
Me-$^{13}C_6$-3-PBA	18.59	15-25	234	75.3	43	202	/

Settings are provided for the derivatized form of *cis*- and *trans*-DCCA, 3-PBA and their related internal standards. For *cis*- and *trans*-permethrin and *cis*-$^{13}C_6$-permethrin, Me-3-PBA and Me-$^{13}C_6$-3-PBA, the wave form type is high resonance (high voltage) and of low resonance for other analytes (low voltage).

Table 2: Intraday (n= 10 to 12) and interday (n = 32 to 36) accuracy and precision of the analytical method at 100 and 600 ng/mL of *cis*- and *trans*-permethrin, 3-PBA and the mixture of *cis*-DCCA and *trans*-DCCA in methanol.

Compounds	**Accuracy (%)**	**Precision (%)**
Intraday comparisons		
Cis-permethrin	12 ± 7	11 ± 6
Trans-permethrin	10 ± 6	8 ± 1
Cis-DCCA	13 ± 9	11 ± 5
Trans-DCCA	11 ± 11	10 ± 4
3-PBA	12 ± 10	9 ± 6
Interday comparisons		
Cis-permethrin	11 ± 8	7 ± 3
Trans-permethrin	15 ± 16	12 ± 3
Cis-DCCA	15 ± 10	12 ± 6
Trans-DCCA	16 ± 11	19 ± 9
3-PBA	11 ± 7	8 ± 3

Table 3: Recovery (%) after extraction of the different matrices (n = 3) spiked with 500 ng/mL of *cis*- and *trans*-permethrin, *cis*- and *trans*-DCCA and 3-PBA.

Compound	**Blood**	**Plasma**	**RBC**	**Liver**	**Faeces**	**Testes**	**Brain**	**Muscle**	**Kidney**	**Fat**
Cis-permethrin	88 ± 7	99 ± 1	90 ± 13	97 ± 15	65 ± 5	112 ± 10	105 ± 8	96 ± 9	98 ± 5	91 ± 6
Trans-permethrin	109 ± 7	99 ± 6	106 ± 18	109 ± 16	105 ± 11	131 ± 24	116 ± 49	121 ± 20	128 ± 3	102 ± 2
Cis-DCCA	85 ± 14	101 ± 5	113± 5	98 ± 24	/	/	/	/	/	/
Trans-DCCA	102 ± 2	111 ± 18	120 ± 15	132 ± 24	/	/	/	/	/	/
3-PBA	76 ± 5	79 ± 9	76 ± 4	71 ± 8	/	/	/	/	/	/

All values are expressed as mean ± SD. Recovery was calculated as the amount of compound in the different matrices compared to the expected concentration.

List of figures

Figure 1: Metabolic pathway of permethrin with the formation of 3-PBA and DCCA.

Figure 2: Chromatogram in SIM mode of standard solution for permethrin and derivatized metabolites (100 ng/ml each)

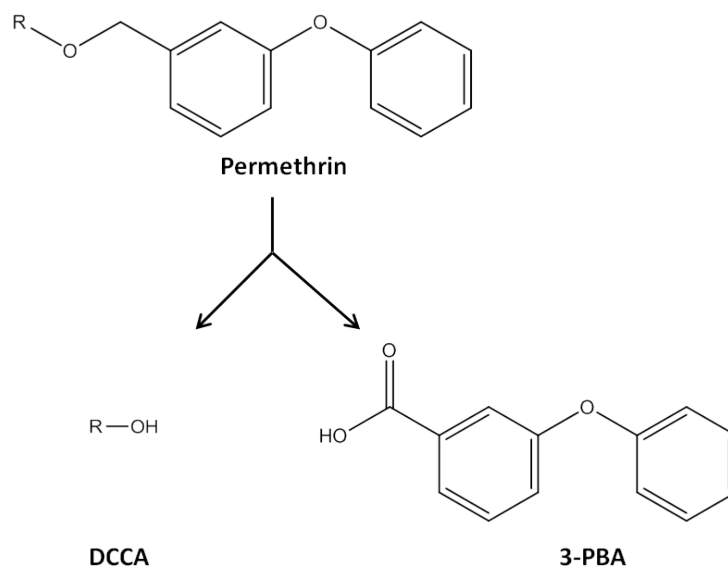
Figure 3: Examples of mass spectra obtained for target analytes: a) Full scan mass spectra for *cis*-permethrin, b) Full scan mass spectra for derivatized *trans*-DCCA, c) Daughter full scan spectra obtained for ion 183 of *cis*-permethrin, d) Daughter full scan spectra obtained for ion 187 of derivatized *cis*-DCCA.

Figure 4: Chromatogram obtained after extraction in plasma for a1) *cis*- and *trans*-DCCA, a2) 3-PBA a3) *cis*-permethrin and for *cis*-permethrin for b) muscle and c) fat.

Figure 5*: Cis*-permethrin (*cis*-P) (dot) and *trans*-permethrin (*trans*-P) (cross) concentrations and their metabolites 3-PBA (square), *cis*-DCCA (diamond) and *trans*-DCCA (triangle) concentration vs. time profiles in blood (A), plasma (B) and red blood cells (C) after an oral administration of 5 mg/kg (dashed line) and 20 mg/kg (solid line) of *cis*-permethrin or *trans*-permethrin. Mean values +/- SD for n = 2 at each time-point except for point associated with a star (n = 1).

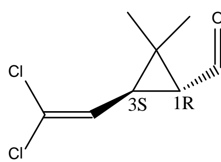
Figure 6: *Cis*-permethrin (*cis*-P) concentration vs. time profiles in liver (A), brain (B), muscle (C), kidney (D) and testes (E) after an oral administration of 5 mg/kg (dashed line) and 20 mg/kg (solid line) of *cis*-permethrin. Mean values +/- SD for n = 2 at each time-point except for point associated with a star (n = 1).

Figure 7: *Cis*-permethrin (*cis*-P) and *trans*-permethrin (*trans*-P) concentration vs. time profiles in fat after an oral administration of 5 mg/kg (dashed line) and 20 mg/kg (solid line) of *cis*- or *trans*-permethrin. Mean values +/- SD for n = 2.

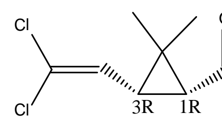


Where **R** may be

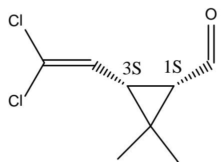
R_1 : [*1R,3R*] *cis*



R_3 : [*1R,3S*] *trans*



R_2 : [*1S,3S*] *cis*



R_4 : [*1S,3R*] *trans*

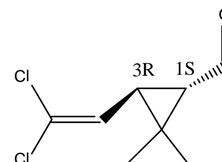


Figure 1: Metabolic pathway of permethrin with the formation of the metabolites 3-PBA and DCCA.

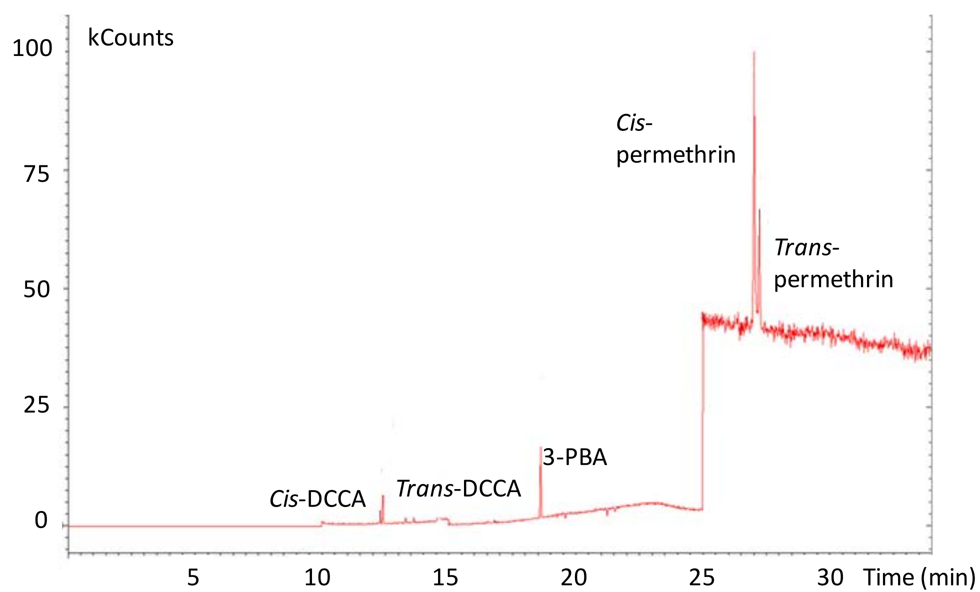


Figure 2: Chromatogram in SIM mode of standard solution for permethrin and derivatized metabolites (100 ng/ml each)

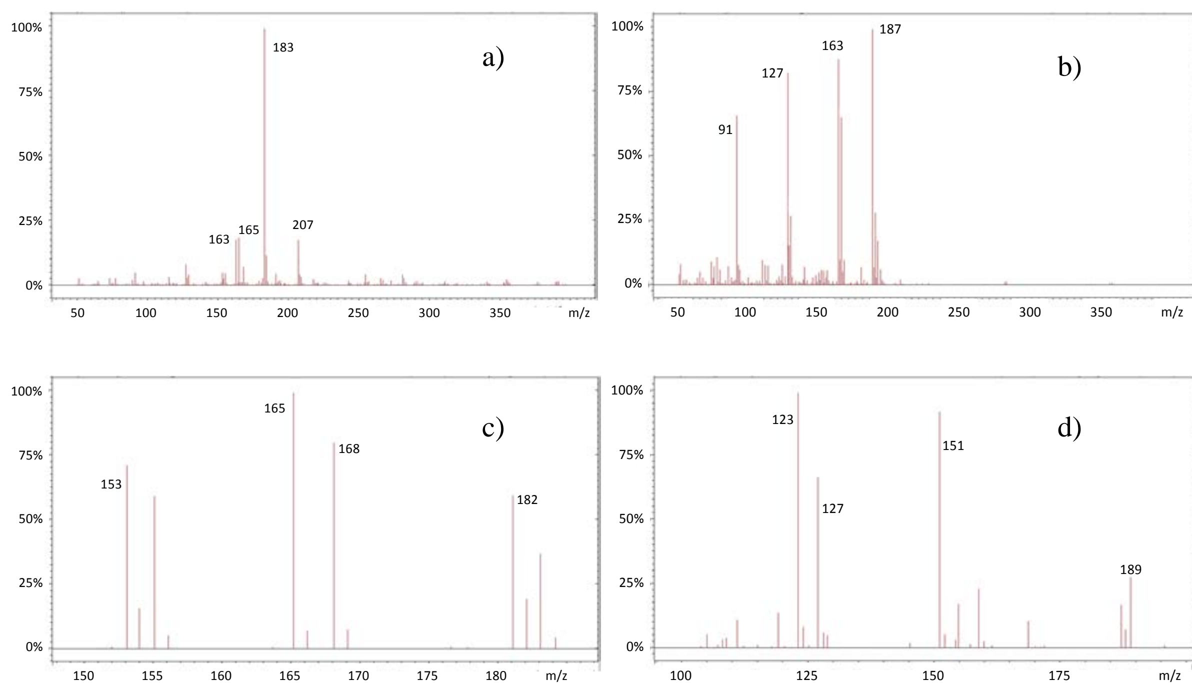


Figure 3: Examples of mass spectra obtained for target analytes: a) Full scan mass spectra for *cis*-permethrin, b) Full scan mass spectra for derivatized *trans*-DCCA, c) Daughter full scan spectra obtained for ion 183 of *cis*-permethrin, d) Daughter full scan spectra obtained for ion 187 of derivatized *cis*-DCCA.

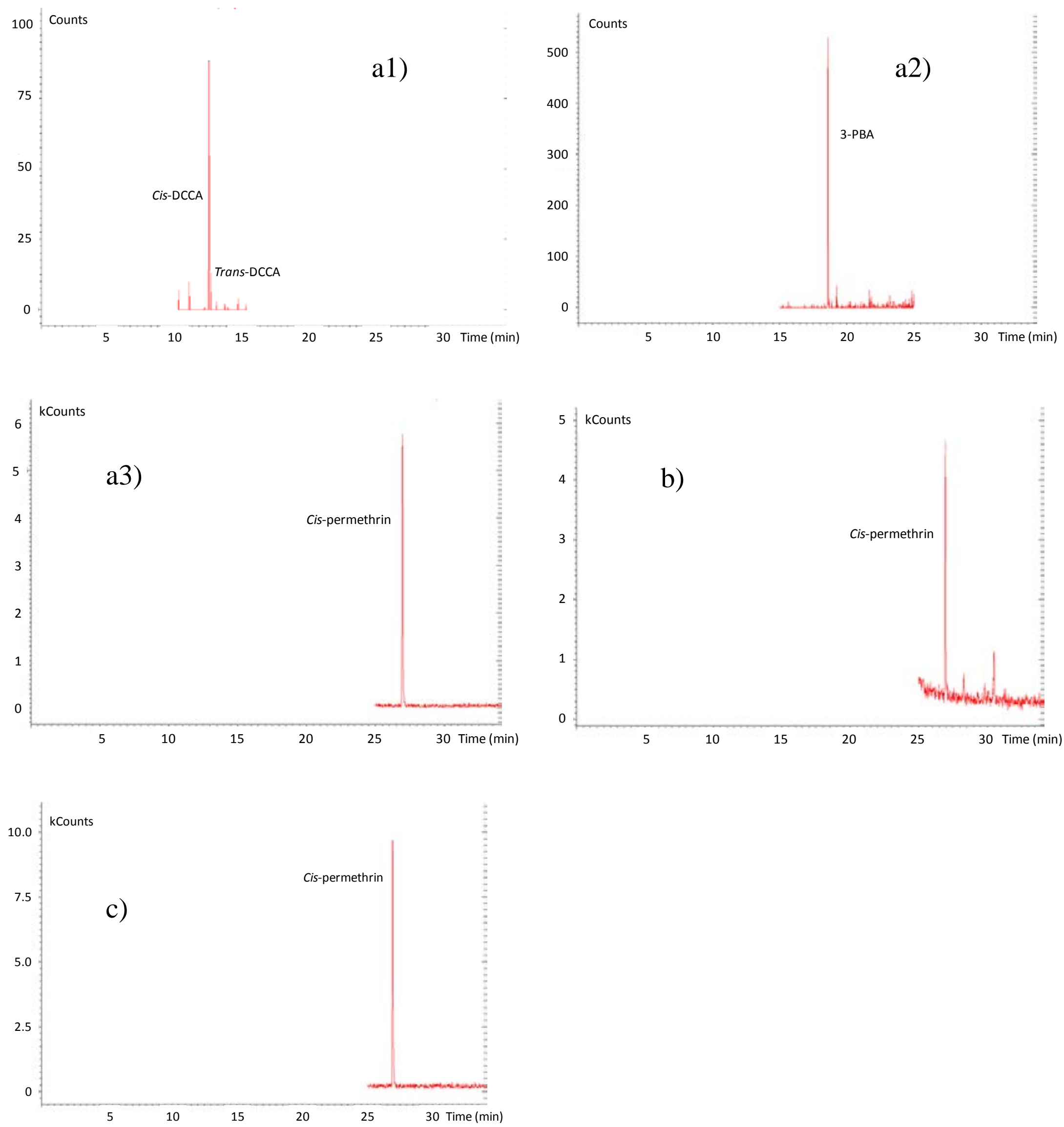


Figure 4: Chromatogram obtained after extraction in plasma for a1) *cis*- and *trans*-DCCA, a2) 3-PBA a3) *cis*-permethrin and for *cis*-permethrin for b) muscle and c) fat

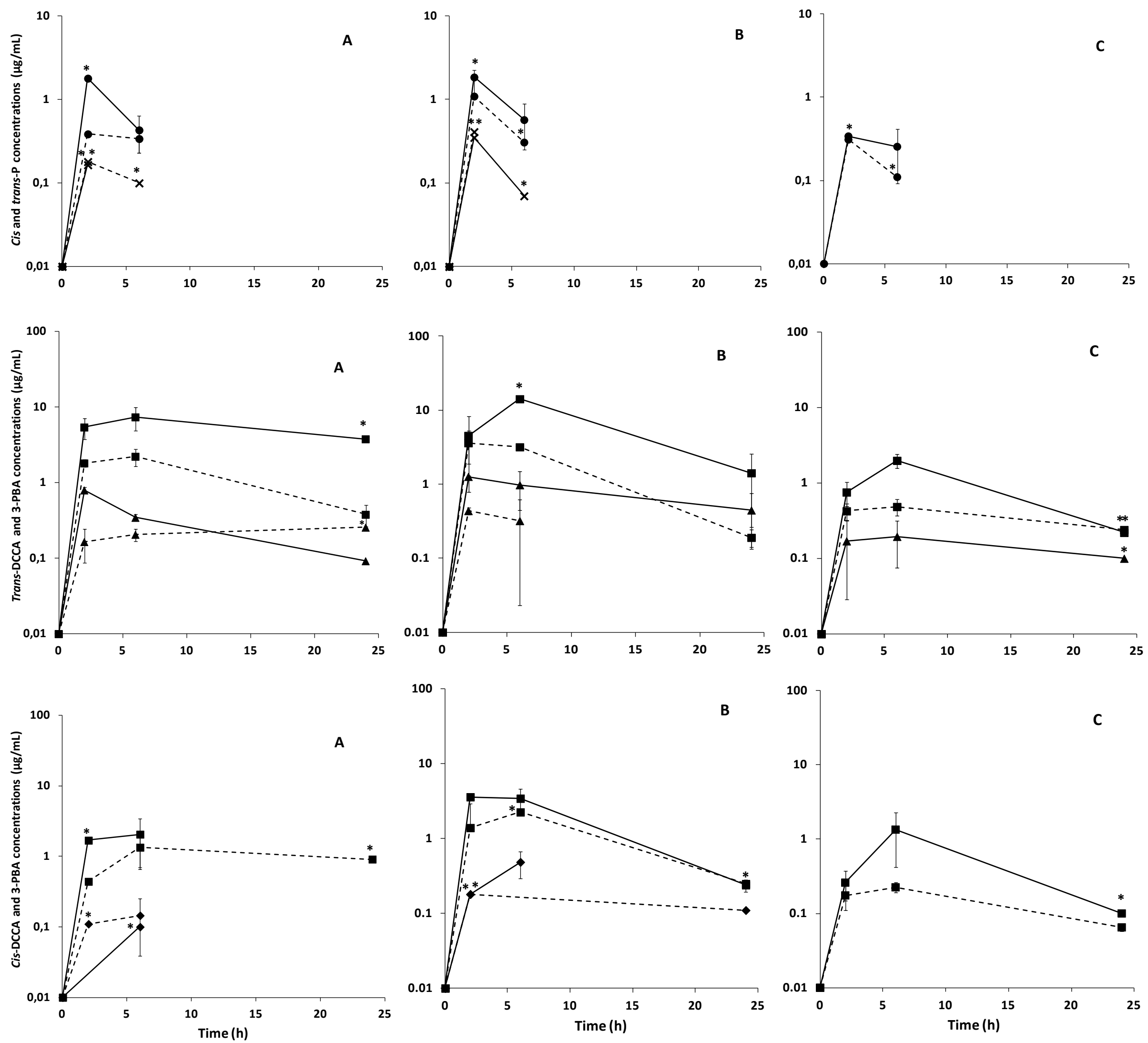


Figure 5*:* *Cis*-permethrin (*cis*-P) (dot) and *trans*-permethrin (*trans*-P) (cross) concentrations and their metabolites 3-PBA (square), *cis*-DCCA (diamond) and *trans*-DCCA (triangle) concentration vs. time profiles in blood (A), plasma (B) and red blood cells (C) after an oral administration of 5 mg/kg (dashed line) and 20 mg/kg (solid line) of *cis*-permethrin or *trans*-permethrin. Mean values ± SD for n = 2 at each time-point except for point associated with a star (n = 1).

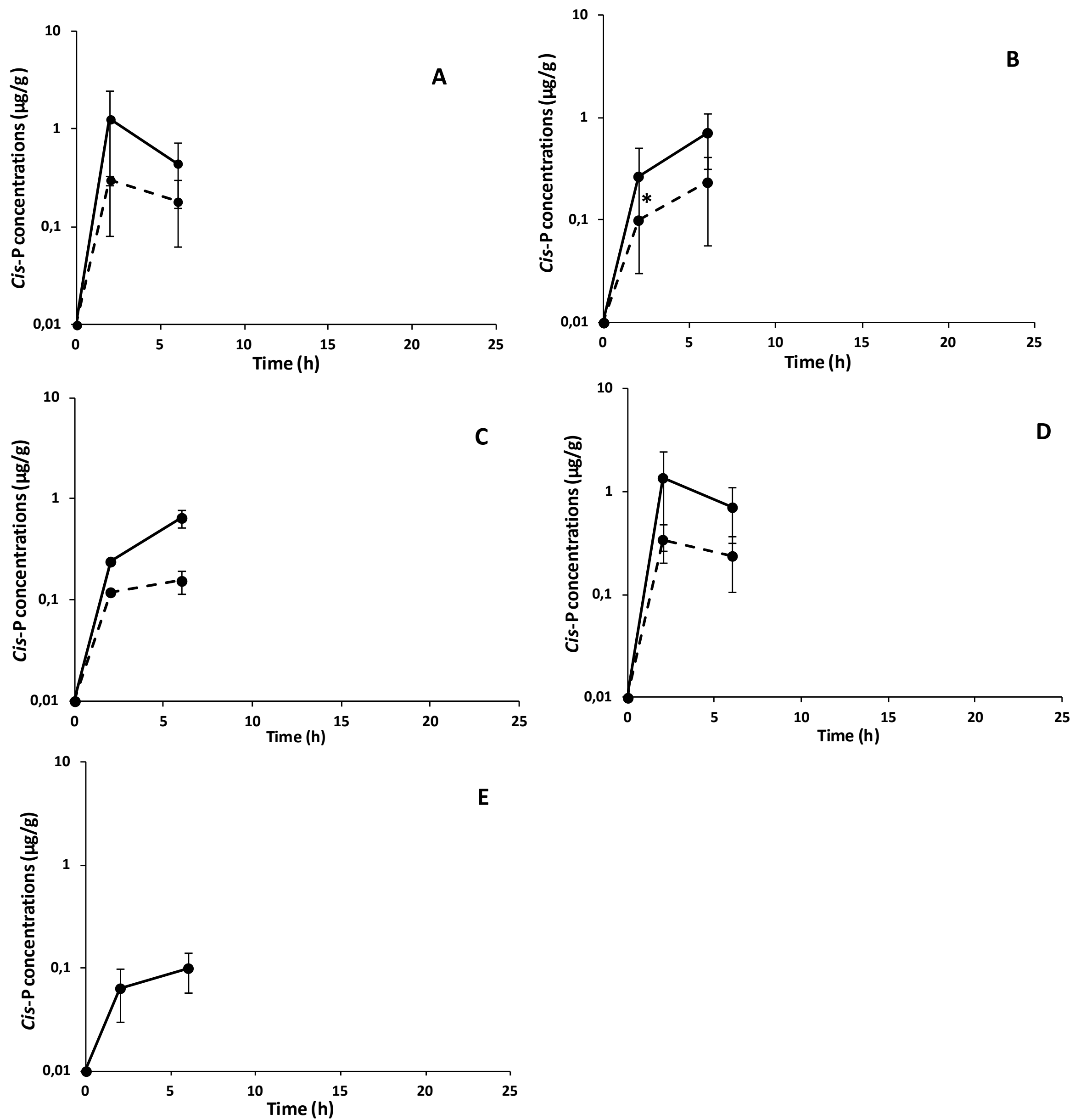


Figure 6: *Cis*-permethrin (*cis*-P) concentration vs. time profiles in liver (A), brain (B), muscle (C), kidney (D) and testes (E) after an oral administration of 5 mg/kg (dashed line) and 20 mg/kg (solid line) of *cis*-permethrin. Mean values ± SD for n = 2 at each time-point except for point associated with a star (n = 1).

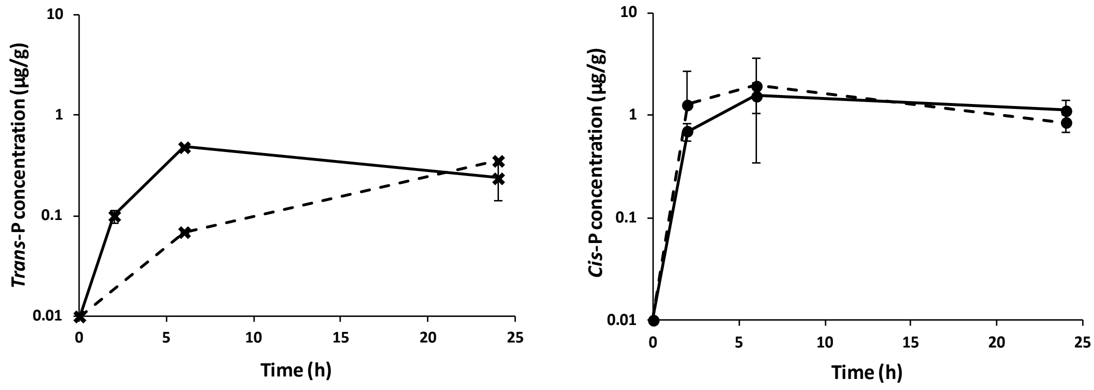


Figure 7: *Cis*-permethrin (*cis*-P) and *trans*-permethrin (*trans*-P) concentration vs. time profiles in fat after an oral administration of 5 mg/kg (dashed line) and 20 mg/kg (solid line) of *cis*- or *trans*-permethrin. Mean values +/- SD for n = 2 at each time-point.