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# Distribution of pyrethroids and piperonyl butoxide in foods and feeds analysed with GC-MS/MS in 2022–2023

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A B S T R A C T

The production of pesticides in the world is increasing, newly synthesized ones are replacing old ones, so pyrethroids should replace organophosphorus pesticides. As the pests become resistant, other compounds are added to the formulations in addition to pesticides; the function of these added compounds is to enhance the effect of active substances by slowing down their degradation in the pests. A wide range of products, from vegetables, fruits, animal feed, fish feed, teas, spices and honey to cereals, was analysed in order to gain insight into the distribution of pesticides, with special reference to pyrethroids and piperonyl butoxide in the analysed samples. The technique of gas chromatography with tandem mass detection (GC-MS/MS) was used for these analyses.

## 1. Introduction

Pesticides are a very diverse group of toxic compounds that are used to reduce pest numbers. Pesticides include products that target viruses, bacteria, molluscs, birds and rodents. However, of major concern are products that target insects, plants and fungi, because they are so widely used across the expanse of agricultural lands, forests and residential areas (*Beasley, 2020; Poppenga et al., 2010*).

Pyrethrins are natural insecticides, which have been used for al least 2000 years, found in the flowers of the plant genus *Pyrethrum*. Pyrethrin, jasmolin and cinerin are representatives of active compounds in this group with insecticidal properties. Lack of stability of natural insecticides has led us to synthesize more stable compounds (less sensitive to hydrolysis and photodegradation), which are called pyrethroids (*Ensley, 2018*). Depending on whether they contain an α-cyano-3-phenoxybenzyl moiety, pyrethroids can be divided into two types, the first which does not contain this moiety: permethrin, allethrin, tetramethrin etc. and the second type which contains the α-cyano-3-phenoxybenzyl moiety: cyfluthrin, cypermethrin, deltamethrin etc. (*Lawrence et al., 1982*).

Mostly, research on pesticide impacts is based on the living world, and as the synergists are considered inactive components, their influence is marginalized. However, the influence of these components, which are found at levels several tens of times higher than the concentration of pesticides, is by no means negligible (*Tison et al., 2023*). Piperonyl butoxide (PBO) has been used as a pesticide synergist for more than 70 years. It was largely developed in the United States due to increased concern about the spread of insect-borne diseases (*Tozzi, 1999*).

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PBO is currently found in about 1700 products, where sometimes it is indicated as an active ingredient, but sometimes it is considered an inert ingredient and is not on the declaration. Commercial names are pybuthrin and butacide (*US EPA, 2005*).

Pyrethroids achieve their insecticidal properties by affecting the action potential of insect nerves by modifying the kinetics of ion channels (primarily voltage-gated sodium channels, secondary γ-aminobutyric acid (GABA)-gated chloride channels and voltage-gated calcium channels), whereby they become more permeable to sodium ions, which can lead to depolarization of nerves and loss of nerve function (*Solderlund, 2010*). PBO works to enhance pesticide efficacy by inhibiting detoxification induced by cytochrome P450 enzymes (CYPs). CYPs are a very big family of enzymes that have a prosthetic haem group. They play major roles in xenobiotic metabolism (drugs, pesticides and other chemicals), and almost 75% of ingested drugs will be processed by this family of enzymes. Beside xenobiotic metabolism, the metabolism of a very big, endogenous group of compounds (steroids, fat-soluble vitamins, fatty acids etc.) is not possible without CYPs (*Zhao et al., 2021*). PBO inhibits CYPs in two phases, firstly by binding to the active site and secondly by forming an irreversible complex with CYP's haem group. PBO also inhibits esterase and glucuronosyltransferase enzymes (*Snoeck et al. 2017*).

## 2. Materials and methods

All food and feed samples (see Table 1) were divided into several groups according to origin, water, sugar and fat content according to *SRPS EN 15662* (2018). All samples were routinely analysed from May 2022 to May 2023. Pesticides (209 compounds) were extracted with acetonitrile (≥ 99.9 % HPLC grade, Sigma-Aldrich, Chemie GmbH, Germany) and/or water (≥ 99.9 % HPLC grade, Sigma-Aldrich, Chemie GmbH, Germany) and then purified using the QuEChERS technique. Identification and quantification was done using gas chromatography with a triple quadrupole mass spectrometer (GCMS-TQ8050 NX, Shimadzu Corporation, Japan).

Sample preparation was based on the QuEChERS method developed by *Anastassiades et al.* (2003) and specified in *SRPS EN 15662* (2018). Homogenized sample (5 g) was weighed into a 50 mL centrifuge tube, internal standard PCB 52 (Lab Instrument srl, Italy) and 10 mL of acetonitrile or/and water were added, and the tube content was shaken vigorously for 1 minute. Extraction was done using 10 mL of acetonitrile (≥ 99.9 % HPLC grade, Sigma-Aldrich, Chemie GmbH, Germany) and QuEChERS Mix (4.0 g $MgSO_4$, 1 g NaCl, 1 g SCTD, 0.5 g SCDS; Phenomenex Inc., USA). Taking care not to form lumps, each sample was immediately shaken by hand for 1 minute followed by 15 min on a shaker (iRoll PR35, Neuation Technology Pvt. Ltd., India) and centrifuged (Sigma 2-16P, Sigma Laborzentrifugen GmbH, Germany) for 5 minutes at 3000 rpm. An aliquot of the extract was transferred to 15 mL centrifuge tube which contains dispersive SPE clean-up mixture (150 mg $MgSO_4$, 25 mg C18 sorbent, per mL of extract) (Phenomenex Inc., USA). The mixture was shaken by hand for 1 minute and centrifuged for 5 minutes at 3000 rpm. After centrifugation, the supernatant was transferred to a glass vial for gas-chromatography analysis. With each set of samples, a four-point matrix-matched series of calibration enriched samples (as well as a blank sample and a control enriched sample) were prepared so that final concentration were in range from 5 to 50 µg kg-1 (*Guidance SANTE, 2021*). All pesticide mixtures were bought from CPAchem Ltd, Bulgaria.

Chromatographic separation was done using a fused silica GC column (30 m × 0.25 µm ID and 0.25 df). All analytes were measured in MRM (multiple reaction monitoring) mode with one quantifier and at least one qualifier ion, with instrument setup according to the manufacturer (*Shimadzu application news*, 2022). Identification was done by comparing the retention times of the standards with the retention times of the peaks in the sample. The areas of the peaks in the standard and the control sample were determined and the yields calculated. Measured concentrations were corrected for the response factors of internal standards. Quantification was performed using Lab Solution data® processing software (Shimadzu Corporation, Japan).

Statistical analysis of experimental data was performed using software Minitab® 17.1.0 Statistical Software. Microsoft Office® Excel was used for graphic displays.

## 3. Results

The total number of analysed samples was 826, of which 145 were positive samples that contained at least one quantified pesticide (divided into the groups in Table 1). Detected pyrethroids were: bifenthrin (sum of all isomers), cypermethrin (sum of all isomers), deltamethrin (cis-deltamethrin), fenvalerate, λ-cyhalothrin (sum of all isomers), permethrin (sum of all isomers), τ-fluvalinate, tefluthrin and tetramethrin. All positive samples were in compliance with Serbia's national legislation (*Serbia*, 2022). The results for positive samples in 2022 and 2023 are presented in Table 1. Among the groups, vegetables and fruits had the highest numbers of pyrethroid-positive samples, 11 and 9, respectively. The group with the highest number of samples containing PBO were, in order, animal feed, fish feed and cereals and cereal products. Figure 1 shows the ratios of pyrethroids, PBO and other pesticides in the total number of positive samples according to sample groups.

Altogether, 17.6% of all samples analysed contained pesticides. Of the total number of samples, other pesticides accounted for 8.5%, PBO accounted for 6.2% and pyrethroids accounted for 2.9% of positive samples.

Table 2 shows the range of pyrethroids and PBO levels according to the sample groups. The only group of samples where almost all pyrethroids and PBO were detected and were quantifiable, with the exception of fenvalerate, permethrin and τ-fluvalinate, was vegetables. The groups of samples with the lowest levels of pyrethroid pesticides were

**Table 1.** Occurrence of pyrethroids and piperonyl butoxide in analysed groups of foods and feeds

| Sample group | Number of positive samples | | | |
|---|---|---|---|---|
| | Pyrethroids | PBO | Other pesticides | Total number of positive samples per group |
| **Vegetables** | 11 | 1 | 33 | 45 |
| **Fruits** | 9 | 0 | 21 | 30 |
| **Animal feed** | 1 | 23 | 5 | 29 |
| **Fish feed** | 0 | 12 | 1 | 13 |
| **Cereals and cereal products** | 2 | 9 | 2 | 13 |
| **Tea, spices** | 1 | 5 | 7 | 13 |
| **Honey** | 0 | 1 | 1 | 2 |
| **TOTAL** | 24 | 51 | 70 | 145 |

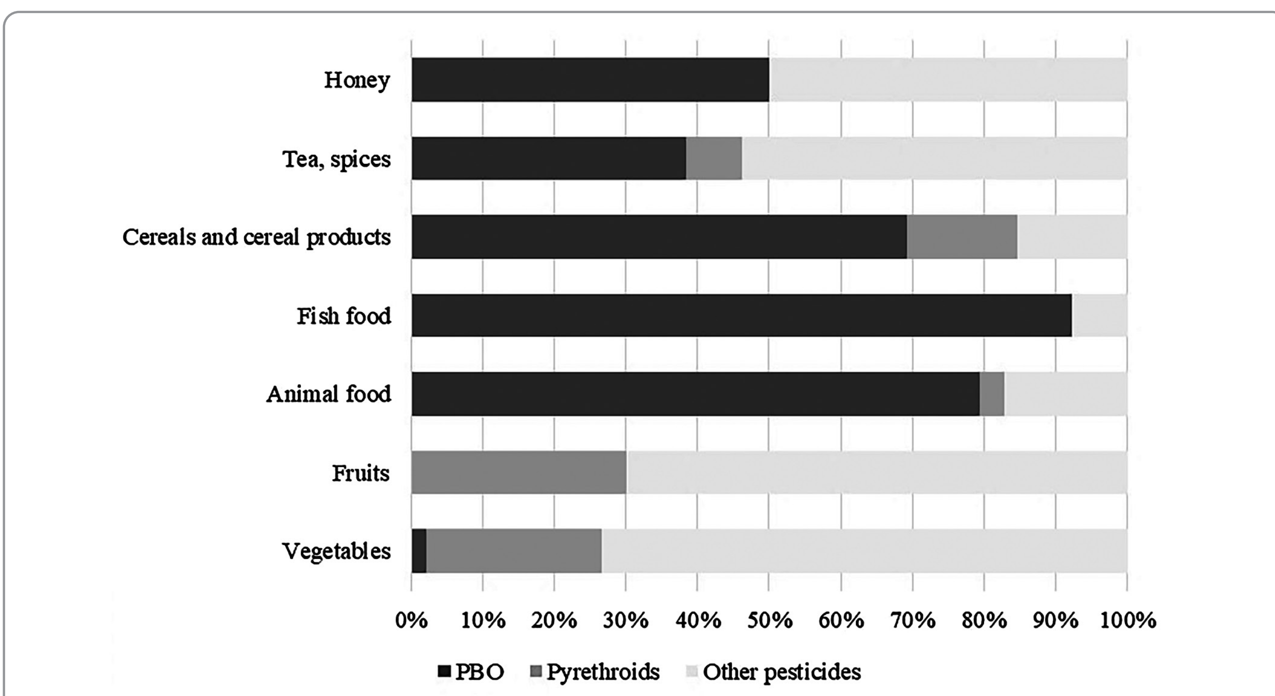


**Figure 1.** Ratios of piperonyl butoxide, pyrethroids and other pesticides among the pesticide-positive groups of foods and feeds

**Table 2.** Pyrethroid and piperonyl butoxide (PBO) levels (μg $kg^{-1}$) in grouped food and feed samples in 2022–2023

| Sample group | Bif | Cyp | Del | Fen | Cyh | Per | Flu | Tef | Tet | PBO |
|---|---|---|---|---|---|---|---|---|---|---|
| Vegetables | 9.2–30 | 6.1–1205 | 5.0–17 | / | 17 [a] | / | / | 6.2–15 | 9.1–11 | 30 [a] |
| Fruits | / | 5.3–190 | 5.0–19 | / | 18–23 | / | 464 [a] | / | / | / |
| Animal feed | / | 9.4[a] | 5.1–19 | / | / | 57–133 | / | / | / | 5.0–161 |
| Fish feed | / | / | / | / | / | 45–66 | / | / | / | 6.5–133 |
| Cereals and cereal products | / | / | 9.8–20 | / | / | / | / | 12 [a] | 8.3–11 | 5.7–378 |
| Tea, spices | / | 21–7920 | / | / | / | / | / | / | / | 6.0–152 |
| Honey | / | 5.3 [a] | / | 8.3 [a] | / | / | / | / | / | 32 [a] |

Bif – bifenthrin, Cyp – cypermethrin (sum of all isomers), Del – deltamethrin, Fen – fenvalerate, Cyh – *λ*-cyhalothrin, Per – permethrin, Flu – *τ*-fluvalinate, Tef – tefluthrin, Tet – tetramethrin,

[a] – one positive sample

fish feed and tea and spices. However, every sample of fish feed contained PBO. PBO was found in all sample groups except in fruits.

Cypermethrin with all of its isomers were quantified in all sample groups except in fish feed and cereals and cereal products. The highest quantified levels were for cypermethrin in spices and cypermethrin in vegetables, with levels of 7920 μg $kg^{-1}$ and 1200 μg $kg^{-1}$, respectively. Some pyrethroids were found at low frequencies in the foods and feeds, e.g., fenvalerate in one honey (8.3 μg $kg^{-1}$) and *τ*-fluvalinate, which was quantified in one fruit at a level of 464 μg $kg^{-1}$.

## 4. Conclusion

In the human body, pyrethroids are degraded in up to 24 hours, but when they are combined with PBO and when their degradation is stopped, that time is significantly longer. The activity of CYPs and other enzymes is reduced in the presence of PBO, and therefore, enzyme function is weakened and the release of toxins is reduced (*Singh et al., 2022*). This happens during acute contact with the pyrethroid-PBO combination, but as to what actually happens with chronic exposure to this combination over a period of several years, we remain unsure. We have barely scratched the surface of this problem. Some studies have shown that pyrethroid-PBO causes enlargement of the liver (*Hoberman et al., 2022*), promotes cancer formation (*Okamiya et al., 1998*), reduces foetus size and is toxic to male reproductive system (*Bae et al., 2021*), and is linked to neurodevelopment problems (*Horton et al. 2011*). From the obtained results, there is a high prevalence of low or relatively low concentrations of pesticides in the food chain, and this can affect the health of people and animals. A study that will include the combination of pyrethroid-PBO is certainly needed.

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