The stereoselective metabolic disruption of cypermethrin by a sub-acute study based on metabolomics

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Abstract

Due to the massive application of cypermethrins (CYPs) for pest control in China, the adverse effects on non-target organisms have aroused great attention. However, comparative studies between its different stereoisomers remain scarce, especially for metabolism perturbations. Herein, the rats were administered α-CYP, β-CYP and θ-CYP by gavage at doses of 8.5, 29.2 and 25.0 mg/kg, respectively, for 28 consecutive days. By blood examination, significant changes in liver and renal function parameters were observed in rats exposed to all three CYPs. The stereoisomeric selectivity in metabolism was assessed based on a metabolomic strategy via principal component analysis (PCA), orthogonal partial least squares discriminant analysis (OPLS-DA) and pathway analysis. The results demonstrated that amino acid and glycolipid metabolism were disrupted in all CYP groups. Among them, the most significant changes in the metabolic phenotype were observed in the θ-CYP group, with 56 differential metabolites enriched in 9 differential metabolic pathways. Perturbations in the alpha-linolenic acid metabolism associated with inflammation occurred only in the θ-CYP group of rats. At the same time, the endogenous metabolite trimethylamine oxide (TMAO), which is closely linked to the gut microbiota, was also significantly elevated in this group. Gender differences were evident in α- and θ-CYP-exposed rats, with perturbations in amino acid and glucose metabolism of greater concern in females and lipid metabolism of greater concern in males. Overall, β-CYP exhibited a lower risk of metabolic perturbations than α-CYP or θ-CYP, which helps to screen suitable agrochemical products for green agricultural development.

Introduction

Pyrethroid insecticides was one of the world’s top three extensively applied pesticides (Yoo et al. 2016), whose global market had exceeded US$3 billion in recent years (LI 2016). As a prototypical type II pyrethroid, cypermethrin (CYP) was applied for pest control in the field and in-home use as being relatively low toxicity to humans and mammals. In developing CYP products, highly efficient isomers isolated by chiral synthesis and catalytic isomerization were enriched in various commercial formulations to form different isomer pesticides, including α-CYP, β-CYP and θ-CYP. All of them are commonly used pyrethroid pesticides in China, of which β-CYP has the most registered products in China with 412 entries, followed by α-CYP with 77 entries (ICAMA). The frequent use of CYPs led to high detection rates in environmental media including soils (Deng et al. 2020, Yao, Lixian et al. 2010) and surface water (Paíga et al. 2021, Tang et al. 2018, Tsaboula et al. 2016). Consequently, the awareness of the ecotoxicological issues and health risks of these chemicals should be particularly noteworthy.

CYP isomers have been shown to exert a variety of toxic effects. β-CYP may cause reproduction toxicity (Lu et al. 2020). α-CYP induced significant oxidative stress states (Aksakal 2018, Hocine et al. 2016) and neurological disturbances (Organization 2006). The effects of α-CYP and θ-CYP on hippocampal CA3 neurons of rats were also investigated (Tian et al. 2008, Yu-Tao et al. 2009). However, none of these toxicity data was obtained in a parallel study that addresses the stereoisomer selectivity. According to our previous study, α-CYP, β-CYP and θ-CYP exhibited stereoselective endocrine-disrupting effects (Zhang et al. 2021). Endocrine disruption is an essential trigger for metabolic disorders considering that the
endocrine system regulates the body's metabolism and energy homeostasis through steroids (Khan et al. 2017, Ortiz-Villanueva et al. 2018). Therefore, as typical endocrine-disrupting chemicals (McKinlay et al. 2008), the role of CYP isomers in perturbing metabolic pathways deserves more attention. An imbalance in metabolic homeostasis is essential for the pathogenesis and progression of many disorders, such as metabolic syndromes pertaining to obesity, diabetes and hypertension (Alberti et al. 2006). Mammalian studies have demonstrated that CYP exposures lead to increased blood glucose and lipid levels in the serum of mice (Jin et al. 2015). Our preliminary study also showed that rats exposed to CYPs exhibited a distinct hyperlipemia phenotype (Zhang et al. 2023), a disorder of the body's lipid metabolism, as the increment of total triglyceride.

Blood is an essential mediator of the body's fluids, which contains various metabolites produced via metabolic pathways and linked to different organs and tissues (Kenéz et al. 2016). Thus, omics science like metabolomics is developing into sought-after analytical tools to compensate for the inability to probe for endogenous metabolites in blood biochemical tests. It offers novel insights into disease mechanisms, as the level of specific metabolites provides a direct functional readout of the physiological or pathological state (Patti et al. 2012).

In this study, we examined the blood biochemical indicators reflecting the hepatic function, renal function and oxidative stress in rats after exposure to α-, β- and θ-CYP, as well as the metabolic disturbances using ultra-performance liquid chromatography-quadrupole-time of flight mass spectrometry (UPLC-QTOF/MS)-based untargeted metabolomics approach. Multivariate statistical analyses like principal component analysis (PCA) and orthogonal partial least squares discriminant analysis (OPLS-DA) were used to discriminate the classification of groups and identify the altering endogenous metabolites in each group. The stereoisomer selectivity could be compared by enrichment of metabolic pathways. The results presented here may fill a gap in data on stereoselective metabolic disruption under exposure to CYPs and provide guidance for pesticide application in sustainable agricultural development.

**Materials And Methods**

**Chemicals and reagents**

Alpha-cypermethrin (CAS 67375-30-8), beta-cypermethrin (CAS 65731-84-2) and theta-cypermethrin (CAS 71697-59-1) with purities > 99% were obtained from Sunlida Biological Technology Co., Ltd. (Nanjing, China). Methanol (CAS 67-56-1), acetonitrile (CAS 75-05-8), ammonium acetate (CAS 631-61-8) and ammonium hydroxide (1336-21-6) were purchased from J&K Scientific Ltd. (Beijing, China) and were of LC-MS grade.

**Animal models**

Three-week-old Sprague–Dawley (SD) rats were purchased from Hangzhou Medical College and housed under specific pathogen-free (SPF) conditions. CYPs were dissolved in corn oil at approximately equal to 1/20 LD<sub>50</sub> (8.5, 29.2, 25.0 mg/kg/d) of each chemical and administered to rats by gavage once per day.
for 4 weeks. The control group was treated with corn oil alone at a dose of 2.5 mL/kg/d. On the endpoint, the SD rats were anaesthetized with 3% isoflurane for blood collection after fasting for 12 hours following the last administration. The plasma samples were stored at -80°C for subsequent blood biochemical and metabolomic analysis. All care and handling of the animals were approved by the Animal Experiments Committee of Hangzhou Medical College.

**Blood biochemical examination**

The levels of blood urea nitrogen (BUN), creatinine (Cr), glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), malondialdehyde (MDA), total protein (TP), albumin calibrator (ALB), aspartate aminotransferase (AST), alanine aminotransferase (ALT) and γ-glutamyl transferase (γ-GT) were determined by commercial ELISA kits (Nanjing Jiancheng Bioengineering Institute, China). The detailed manufacturer's instructions were followed. Absorbance values were measured using a DG5033A microplate reader (Nanjing Huadong Electronics Group Co., Ltd, China).

**Metabolomic analysis**

**Metabolites Extraction.** 100 µL of plasma sample was transferred to an EP tube, and 400 µL extract solution (acetonitrile: methanol = 1: 1) containing the isotopically-labelled internal standard mixture was added.

The mixture was sonicated for 10 min in a cooling bath followed by 30 sec of vortex and was incubated at -40 °C for 1 h. After centrifuging at 12000 rpm for 15 min at 4 °C, the supernatant was collected and dried in a vacuum. The procedure was repeated after adding 200 µL 50% acetonitrile. Finally, a total of 75 µL of supernatant was transferred to an LC injection vial. Pooled samples were prepared as quality control (QC) samples.

**UHPLC-MS instrumentation and data acquisition.** UHPLC analysis was performed on an ExionLC (AB Sciex) coupled to TripleTOF 5600 mass spectrometer (AB Sciex). Chromatographic separation was achieved on an Acquity UPLC BEH Amide column (2.1 * 100 mm, 1.7 µm, Waters) using continuous gradient elution described in the supplementary material (SI). The mobile phase consisted of 25 mmol/L ammonium acetate and 25 mmol/L ammonia hydroxide in water (phase A) and acetonitrile (phase B). The column temperature was kept at 25°C. The flow rate was set at 0.5 mL/min and the injection volume was 2 µL.

The MS/MS (MS2) spectra were acquired by the information-dependent acquisition (IDA) and converted from raw to mzXML format using ProteoWizard MSConvert V 3.0 and processed by R package XCMS (V 3.2) for retention time correction and peak detection and alignment. Metabolites were identified by matching to METLIN database (https://metlin.scripps.edu).

**Multivariate statistical analysis.**

Normalized data were analyzed using SIMCA V 14.1 software (Umetrics AB, Sweden). The data were first classified by unsupervised pattern recognition using principal component analysis (PCA). Orthogonal
partial least squares discriminant analysis (OPLS-DA) with a supervised model was then applied to comprehensively compare the metabolomic profiles that account for the separation between groups. The quality of models was assessed on $R^2$ and $Q^2$ scores by 200 times permutation tests. Potential biomarker metabolites were evaluated by the variable influence on projection (VIP) scores derived from the OPLS-DA, combined with fold change and P value. Pathway impact values (PIV) based on pathway topology analysis were used to find the relevant metabolic pathways of the above potential biomarkers using MetaboAnalyst 5.0.

**Statistical analysis**

The results of blood biochemical indicators are expressed as the mean ± standard error of mean (x ± SEM). One-way ANOVA followed by Tukey’s HSD post hoc test was performed in multiple group comparisons using SPSS (v20.0) with statistical significance set at $P < 0.05$.

**Results**

**Plasma biochemical indicators**

As shown in Fig. 1, BUN and Cr levels were increased in rats after exposure to all three CYPs, indicating that renal function is impaired. ALB and GLB are produced in the liver and are added together to form the total protein (TP), which reflects the hepatic protein synthesis. Although significant changes in ALB and GLB were observed in the $\alpha$-CYP and $\beta$-CYP groups, there was no change in TP. The liver enzymes aspartate aminotransferase (AST) and alanine aminotransferase (ALT) are considered sensitive indicators of the liver because they may leak into the bloodstream upon liver cell damage. In addition, $\gamma$-glutamyl transferase ($\gamma$-GT) liver enzyme was also measured to assess liver function. All three liver enzymes increased in rats after exposure to three CYPs, indicating that CYP exposure caused liver damage in rats. Both GSH-Px and SOD levels in the $\alpha$-CYP group showed a significant decrease. SOD is an antioxidant enzyme that scavenges reactive oxygen species from the cell, and GSH-Px is known to neutralize lipid hydroperoxides and free hydrogen peroxides. No significant changes in malondialdehyde (MDA) levels were observed.

**Data quality assurance**

Five QC samples were included to evaluate the stability and repeatability of the system. As shown in Fig.S1, the overlaid total ion chromatograms (TICs) proved a stable system performance. QC samples were closely distributed within the 2STD (two standard deviations) in Fig.S2, indicating the reliability of the analytical method and good quality of the data.

**Multivariate analysis of metabolites**

The overall changes in metabolic profiles were assessed by PCA. The rats of each group were clearly distinguished by gender on the magnitude of separation depicted as PC1 in ESI$^-$ mode (Fig. 2a). On PC2,
although samples were clustered by grouping category, there were still overlapping between different CYP groups. On the other hand, the samples were mixed and did not show good separation in ESI+ mode (Fig. 2b). Therefore, a supervised model is required for further analysis.

**Identification of differentially expressed metabolites in rats**

The OPLS-DA analysis was adopted to model the relationship between metabolite expression and sample classification groups. It has a stronger explanatory capability with an additional orthogonal operation, and variables in metabolites that are not correlated with categorical variables will be filtered out. The OPLS-DA score plots in ESI− mode are shown in Fig.S3. There were apparent differences between the control and experimental groups and the sex difference was so pronounced that the differentially expressed metabolites (DEMs) were able to be separated entirely between male and female rats. Significant inter-group differences were also exhibited in the ESI+ mode (Fig.S5), but no gender differences existed.

The numbers of DEMs detected in ESI- and ESI + modes are as follows (Table 1). A total of 38 endogenous DEMs were altered in the α-CYP group compared to the control group. Of these, 23 DEMs were significantly up-regulated, and the other 15 were significantly down-regulated. Regarding gender, 16 metabolites were found to show significant differences in female, and 6 in male rats (Table S1). The majority of the altered metabolites belonged to amino acids, fatty acids, bile acids and sugars derivatives. In the β-CYP group, 30 DEMs were detected compared to the control group. Among them, 8 metabolites were significantly up-regulated, and 22 were significantly down-regulated. A total of 9 differential metabolites were found to show significant changes in female rats, and 3 in males (Table S2). The upregulation of allocholic acid, cortisol, dihydrobrassicasterol and gluconic acid were observed in both female and male rats, indicating that the changes in these substances were not associated with gender. 56 altered metabolites were identified in the θ-CYP group, with half of the metabolites being up-regulated and half being down-regulated. On gender differences, there were 13 metabolites significantly altered in female rats and 16 in male rats (Table S3). The up-regulation of D-glucurono-6,3-lactone, pentadecanoic acid, cortisol, nervonic acid, allocholic acid and dihydrobrassicasterol, as well as the down-regulation of 2-methylbutyroylcarnitine were observed simultaneously in female and male rats. In general, DEMs differed significantly not only between groups but also by gender.
<table>
<thead>
<tr>
<th>Group</th>
<th>Total DEMs</th>
<th>Up</th>
<th>Down</th>
</tr>
</thead>
<tbody>
<tr>
<td>control vs α-CYP</td>
<td>38</td>
<td>23</td>
<td>15</td>
</tr>
<tr>
<td>control vs β-CYP</td>
<td>30</td>
<td>8</td>
<td>22</td>
</tr>
<tr>
<td>control vs θ-CYP</td>
<td>56</td>
<td>28</td>
<td>28</td>
</tr>
<tr>
<td>α-CYP vs β-CYP</td>
<td>19</td>
<td>6</td>
<td>13</td>
</tr>
<tr>
<td>α-CYP vs θ-CYP</td>
<td>42</td>
<td>26</td>
<td>16</td>
</tr>
<tr>
<td>β-CYP vs θ-CYP</td>
<td>40</td>
<td>29</td>
<td>11</td>
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There were also significant differences among the three experimental groups. A total of 19 DEMs were screened out between the α-CYP and β-CYP groups (Table S4), while the θ-CYP group showed greater variability when compared to both α-CYP and β-CYP groups, with more DEMs (≥ 40) screened (Table S5, S6). Multiple comparisons among four groups were also conducted based on the results of two-by-two comparisons. The screening of the inter-group DEMs is summarized in chord diagrams (Fig. 3, 4). Of the 26 DEMs detected in ESI⁻ mode, the top 3 abundant metabolites were cholic acid, hypogeic acid and myristic acid, while L-phenylalanine, L-leucine, and tetracosahexaenoic acid were the most abundant among the 34 DEMs detected in ESI⁺ mode.

**Perturbation analysis of metabolic pathways in rats**

The identified metabolites were mapped into specific metabolic pathways by integrating enrichment and pathway topology analysis. Figure 5 showed that three amino acid metabolic pathways, namely phenylalanine, tyrosine and tryptophan biosynthesis, phenylalanine metabolism and arginine biosynthesis were significantly disturbed under the exposure of all three CYPs. Compared to the β-CYP group, more metabolic pathways were perturbed in the α-CYP and θ-CYP groups. Arginine and proline metabolism, and lysine degradation were differential metabolic pathways in the α-CYP and θ-CYP groups, respectively. Apart from this, all CYPs caused a disturbance of the glycolipid metabolism pathways, including ascorbate and aldarate metabolism and glycerophospholipid metabolism. The other two glucose metabolic pathways including pentose and glucuronate interconversions and glycolysis/ gluconeogenesis were simultaneously perturbed in the α-CYP and θ-CYP groups. What's more, rats in the θ-CYP group had an additionally disrupted metabolic pathway, namely alpha-linolenic acid metabolism. It is known that several DEMs are characterized as gender-specific. Herein, the DEMs in female rats were mainly enriched in three amino acid metabolic pathways and two glucose metabolism-related pathways. In contrast, metabolites enriched in lipid metabolism and alpha-linolenic acid metabolism were differentially expressed in male rats.
When it comes to the differences among the three experimental groups, glycolysis/gluconeogenesis pathway remained as the differential metabolic pathway in β-CYP-exposed rats when compared to that of the α-CYP and θ-CYP groups. At the same time, glycerophospholipid metabolism differed between the α-CYP and β-CYP groups.

**Discussion**

Metabolomics offers a new perspective on the ecological health risks of pollutants. The sub-acute toxicity of bifenthrin and λ-cyhalothrin in mice has been investigated based on ¹H NMR and LC-MS/MS. The results showed that these two pyrethroids altered gut microbiota, lipid, nucleotide, tyrosine and energy metabolism (Miao et al. 2017). Similar studies on the metabolic damage caused by combined exposure to multiple pesticides have been carried out, while the different isomers of one particular pesticide lack relevant studies. Herein, three CYP stereoisomers commonly used in agricultural production in China were chosen to examine their effects on mammalian metabolic pathways using 3-week-old SD rats as model organisms. By pathway analysis, amino acid metabolism, glucose metabolism and lipid metabolism were disturbed in all CYPs-exposed rats, which also exhibited stereoisomeric selectivity.

Significant changes in various amino acids were observed. As reported, amino acids are crucial to regulating basic metabolism in the body (Sun et al. 2021). The levels of phenylalanine and citrulline were significantly decreased in all experimental groups. Phenylalanine is a precursor of tyrosine, both involved in synthesizing important neurotransmitters and hormones (Ruppert et al. 1992), which affect gluconeogenesis and lipid metabolism in the body. Therefore, the down-regulation of phenylalanine levels in each CYP group may disrupt normal metabolic functions. Citrulline is a sensitive indicator for evaluating intestinal barrier disruption (Piton & Capellier 2016). Its conversion from glutamine to arginine is achieved via the intestinal-renal axis (Curis et al. 2005, Vollmar & Menger 2011). Less citrulline was released into the bloodstream when exposed to CYPs, indicating that the rats’ intestine was damaged. This also corresponds to our previous study that cypermethrin remoulded gut homeostasis (Zhang et al. 2023). Although all three CYP exposures resulted in the disruption of arginine biosynthesis, arginine derivatives and a variety of other amino acids were most significantly altered in the θ-CYP group. In general, θ-CYP caused the most significant damage to the amino acid metabolic pathway in rats. On the other hand, there is a possibility that disruption of amino acid metabolism could affect liver protein synthesis (Cloarec et al. 2005) and trigger an inflammatory response. Correspondingly, albumin and globulin, indicators of hepatic protein production, were decreased in the α- and β-CYP groups and sensitive indicators of inflammation (e.g. leukocyte, lipopolysaccharide-binding protein) were changed in the θ-CYP group rats previously (Zhang et al. 2023). All of the blood physio-biochemical indicators corroborated the changing metabolic phenotype.

Ascorbic acid, also known as vitamin C, is an important antioxidant. The ability of erythrocytes to recycle ascorbic acid and the ability of ascorbic acid to protect cell membranes are potentially important mechanisms for preventing lipid peroxidative (May 1998). The oxidative damage induced by disruption of metabolic pathways, such as ascorbate and aldarate metabolism and pentose and glucuronate
interconversions (Salau et al. 2020), was also confirmed by blood biochemical parameters detected in rats with oxidative stress. Metabolites implicated in the pathogenesis of T2DM (type 2 diabetes mellitus) are thought to be closely linked to the pentose and glucuronate interconversions (Sun et al. 2014). Moreover, the levels of 2-Phospho-D-glyceric acid enriched in the glycolysis/gluconeogenesis pathway were significantly down-regulated in the α- and θ-CYP groups compared to the experimental and β-CYP groups. Glycolysis links carbohydrate metabolism (Guo et al. 2017). As a result, the exposure to α- and θ-CYP may be associated with the inhibition of metabolic carbon fluxes. The significant increase in blood glucose levels observed in the α-CYP group from our previous research (Zhang et al. 2023) suggests that the relative ratio of glycolysis to gluconeogenesis is reduced. This may be caused by a feedback mechanism of the TCA cycle, which can activate or inhibit glycolysis through changes in metabolite ratios (Weckmann et al. 2014). It has been suggested that glycolysis dysfunction is also involved in the pathology of T2DM (Dong et al. 2016). Therefore, changes in these sugars and derivatives impact normal physiological functions in rats.

Glycerophospholipids are the most abundant phospholipids in the body. In addition to forming biological membranes, they are involved in protein recognition and signalling by cell membranes (Liang et al. 2019). Interestingly, glycerophospholipid metabolism remained a differential metabolic pathway even when the α-CYP group was compared to the θ-CYP group, implying the selectivity of the CYP isomers in lipid metabolism perturbations. By measuring the levels of antioxidant indicators in blood, we found that GSH-Px, which is able to scavenge lipid peroxides induced by reactive oxygen species and -OH, also showed significant inter-group differences between α-CYP and θ-CYP, which validates the existence of stereoselective disturbance in glycerophospholipid metabolism.

The alpha-linolenic acid metabolism was perturbed only in the θ-CYP group. It's a precursor to arachidonic acid metabolism, which mediates disease by producing inflammatory factors (Sun et al. 2020). As a key metabolite in this pathway, alpha-linolenic acid was elevated in the θ-CYP group-rats and caused significant inflammation, which coincided with our former results. Besides, we found that trimethylamine N-oxide (TMAO) was significantly increased in the θ-CYP group compared to the control, α-CYP and β-CYP groups. TMAO is derived from the oxidation of trimethylamine (TMA), a breakdown product of choline by gut microbiota (al-Waiz et al. 1992, Dumas et al. 2006). Treating rats with a mixture of dichlorvos and deltamethrin caused an increase in TMAO (Wang et al. 2013), implying that pesticide exposure may disrupt the gut microbiota metabolism (Lukowicz et al. 2018, Wang et al. 2011). Also, the close association of TMAO with risk factors in cardiovascular disease (Li et al. 2021, Liu et al. 2021) has been proved. Taken together, an increase in TMAO plays a pivotal role in the microbiota-host interaction. It could be used as a biomarker for disturbances of gut microbiota metabolism resulting from θ-CYP exposure.

Gender differences were also shown in the present study. Perturbations in ascorbate and aldarate metabolism and pentose and glucuronate interconversions were evident in female rats exposed to α- and θ-CYP. In male rats, perturbations in glycerophospholipid metabolism were also pronounced within these two groups, whereas no sex differences were shown under β-CYP exposure. It is reported that sex
hormones, sex-specific molecular mechanisms and gender may influence glucose and lipid metabolisms (Gerdts & Regitz-Zagrosek 2019). A survey found that decreasing estrogen or increasing testosterone induces insulin resistance and pro-atherogenic lipid profiles (Regitz-Zagrosek et al. 2007). Our results confirm that gender is an influential factor in glycolipid metabolism perturbations. Furthermore, amino acid metabolism is also well known as sex-discriminating (Della Torre et al. 2018). The metabolic and reproductive functions are integrated by amino acid-dependent activation of the estrogen receptor (Della Torre et al. 2011), which explains the amino acid metabolic perturbations in female rats of the α- and θ-CYP group. Also, in alpha-linolenic acid metabolism that is evident in θ-CYP-exposed male rats, alphalinolenic acid is reportedly converted to eicosapentaenoic acid (EPA) and docosapentaenoic acid (DHA) in a sex-dependent manner (Burdge 2004, Burdge & Calder 2005). Therefore, our study further confirmed a sex-biased response in metabolic perturbations.

In conclusion, the present study investigated the perturbations of metabolism by three CYP isomers in sub-acute toxicity evaluation. Metabolomic profiling based on UPLC-QTOF/MS is valuable for pesticide toxicity assessment, which clearly showed that CYPs could cause significant changes in the plasma metabolites of rats and perturbated amino acid metabolism and glycolipid metabolism. Meanwhile, the significant stereoisomeric selectivity of three CYPs was also shown in metabolic perturbations, which needs to be taken into account in the development of pesticides.

**Declarations**

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**Ethics Approval**

Not applicable.

**Consent to Participate**

Not applicable.
Consent for Publish

All authors have read and agreed to the published version of the manuscript.

Authors Contributions

Quan Zhang: Supervision, Funding acquisition, Writing -Reviewing and Editing.

Sijia Gu: Project administration, Methodology, Software, Writing - Original Draft.

Jinping Gu: Methodology.

Cui Wang: Data curation.

Mengjie Chu: Validation.

Jing Li: Visualization.

Xunjie Mo: Formal analysis.

Competing Interests

The authors declare no competing interests.

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Availability of data and materials

Not Applicable.

References


16. ICAMA Pesticide registration http://www.chinapesticide.org.cn/


Figures
Figure 1

Concentrations of plasma biochemical indicators of SD rats (mean ± SEM). Abbreviations: GSH-Px, glutathione peroxidase; SOD, superoxide dismutase; MDA, malondialdehyde; TP, total protein; ALB, albumin; GLB, globulin; AST, aspartate aminotransferase; ALT, alanine aminotransferase; γ-GT, γ-glutamyl transferase; BUN, blood urea nitrogen; Cr, creatinine. Statistical
significance of the data was set as one asterisk representing $P<0.05$ and two asterisks representing $P<0.01$, three asterisks representing $P<0.001$.

Figure 2

PCA score plots of metabolites in (a) ESI- and (b) ESI+ mode.
Figure 3

Chord diagrams of the differential metabolite abundance (26 metabolites) among four groups in ESI⁻ mode.
Figure 4

Chord diagrams of differential metabolite abundance (the top 30 metabolites) among four groups in ESI+ mode.
Figure 5

Pathway analysis visualized by bubble diagrams. (a) control group vs. α-CYP group; (b) control group vs. β-CYP group; (c) control group vs. θ-CYP group; (d) α-CYP group vs β-CYP group;  
(e) α-CYP group vs. θ-CYP group;  
(f) β-CYP group vs. θ-CYP group.

Supplementary Files
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- 9.13SI.docx