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# Long term biochemical changes in offspring of rats fed diet containing alpha-cypermethrin

Hafeda Ghorzi [a], Hafida Merzouk [a,*], Leila Hocine [a], Sid Ahmed Merzouk [b]

[a] Laboratory of Physiology and Biochemistry of Nutrition, Department of Biology, Faculty of Natural and Life Sciences, Earth and Universe, University Abou-Bekr Belkaïd, Tlemcen 13000, Algeria
[b] Department of Technical Sciences, Faculty of Engineering, University Abou-Bekr Belkaïd, Tlemcen 13000, Algeria

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ABSTRACT

To investigate the possible developmental programming, we analyzed the effects of maternal and postnatal low dose alpha-cypermethrin exposure on metabolic and redox parameters in the offspring. Postnatal changes in plasma biochemical parameters and plasma and tissue oxidative stress markers were determined in offspring of dams fed standard chow or diet containing alpha cypermethrin at 1.50 mg/kg/day during gestation and lactation, weaned on to standard chow or on treated diet until adulthood (5 months).

Our results showed that exposure to alpha cypermethrin induced a significant reduction in body weight, food intake and metabolic alterations such as an increase in plasma glucose, triglyceride, urea, creatinine and AST levels in both postnatal and prenatal/postnatal treated female and male rats. This increase was more pronounced in prenatal/postnatal exposed rats. Alpha-cypermethrin exposure resulted in an imbalance of oxidant/antioxidant status, marked by high levels of carbonyl proteins and MDA, and low levels of antioxidants in erythrocytes, liver and kidney of both male and female offspring. Offspring of exposed dams have pre-existing oxidative stress that was accentuated with postnatal pesticide exposure.

In conclusion, maternal alpha-cypermethrin exposure affected metabolism leading to permanent changes in biochemical parameters, enzyme activities and redox markers in the offspring. These abnormalities in offspring were worsened under postnatal pesticide exposure from weaning to adulthood.



## 1. Introduction

As a new class of agricultural insecticides, synthetic pyrethroids are widely used to control insect pests [1]. Synthetic pyrethroids are modified derivatives of pyrethins, natural substances obtained from flowers of pyrethrum species. Concerning to their high bio-efficacy at low concentrations, enhanced photo-stability and relatively low mammalian and avian toxicity, pyrethroid insecticides are widely used in agriculture, domestic and veterinary applications than other insecticides, particularly organochlorine, organophosphate and carbamate insecticides [2].

Synthetic pyrethroids occupy an important position among commonly applied pesticides [3]. The main effects of these substances are essentially on sodium and chloride channels, they modify the gating characteristics of voltage-sensitive sodium channels to delay their closure [4,5]. However, other mechanisms have been also proposed for pyrethroid activity, such as their ability to antagonize γ-aminobutyric (GABA)-mediated inhibition, to modulate nicotinic cholinergic transmission and to markedly enhance adrenaline and noradrenaline release [6–9].

Among pyrethroids, Alpha-cypermethrin (α-CYP) is one of the most widely used insecticides [10]. The technical grade cypermethrin (CYP) is a racemic mixture of eight isomers (four Cis and four Trans isomers). Two stereoisomers are termed alpha-isomer of CYP, which is believed to be the most active isomer, and is known as α-CYP which is extensively used as an ectoparasiticide in animals, and as insecticide in crop production and public health program [11].

Along with the wide use of pesticides in the world, awareness of their health impacts is rapidly growing. There is evidence on the relation between pesticide exposure and elevated rate of chronic diseases such as different types of cancers, diabetes, neurodegenerative disorders like Parkinson, Alzheimer, and amyotrophic lateral sclerosis (ALS), birth defects, and reproductive disorders. There is also circumstantial evidence on the association of pesticide exposure with some other chronic diseases like respiratory problems, particularly asthma and chronic obstructive pulmonary disease (COPD), cardiovascular disease such as atherosclerosis and coronary artery disease, chronic nephropathies, autoimmune diseases like systemic lupus erythematous and rheumatoid arthritis, chronic fatigue syndrome, and aging [12,13].

* Corresponding author.
*E-mail address:* hafida.merzouk@mail.univ-tlemcen.dz (H. Merzouk).

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Intensive use of these compounds is a public health problem because of their potential adverse effects [14]. Pesticide exposure does not only affect pesticide user farmers, but the general population is also exposed to low concentrations through foodstuffs and the environment. Pesticide exposure can occur *via* inhalation, ingestion, dermal contact, or across the placenta. Biomarkers currently exist for some pesticides in blood serum, semen, ovarian follicular fluid, amniotic fluid, umbilical cord blood, breast milk, meconium and urine [15].

Several studies were conducted on pesticide toxicity and effects on human health. The link between alpha-cypermethrin and metabolic disorders and oxidative stress was reported by many authors [3,10, 11]. However, the implications of pesticide residues for human health are not yet fully documented. Free radicals play an important role in pesticide toxicity. Pesticides may induce oxidative stress, leading to free radical generation and alterations in antioxidants and in the scavenging enzyme system [16,17].

Factors such as age, sex, nutritional status, lifestyle and genetic variability can modify the effects of pesticides in children, who are particularly susceptible to these compounds. The health risks derived from toxic agent exposure are strongly influenced by genetics as a result of the variability of the genes that code for metabolizing enzymes [18–20]. Several studies have shown that exposure to pesticides during fetal development is associated with behavioral and biochemical alterations in offspring [21–23]. Human exposure begins during early prenatal and continues during the breast-feeding neonatal periods, which are critical stages for the development and differentiation of sensitive body organs and systems. In fact, these chemicals cross the placenta to the fetus and are secreted into breast milk [24,25]. Indeed, the developmental origins hypothesis proposes that the pathogenesis of many chronic diseases begins not in adulthood, but during early development. Several recent reports including some from our laboratory suggest that maternal nutritional state in the early postnatal phase are important in promoting metabolic abnormalities in offspring [26,27].

In our laboratory, a previous study investigated the effects of alpha-cypermethrin exposure on biochemical and redox parameters in pregnant rats and their newborns [28]. The findings confirmed that maternal exposure to low doses of alpha-cypermethrin (at admissible daily intake) resulted in metabolic disorders and oxidative stress during pregnancy. These abnormalities were also observed in newborns [28].

To extend these previous results, the aim of this present study was to investigate the effect of prenatal and postnatal chronic low dose exposure of alpha-cypermethrin on offspring male and female Wistar rats, until adulthood. We performed this study to establish if maternal exposure to alpha-CYP during gestation and lactation affected offspring metabolism and redox status in adulthood. This study was performed on offspring males and females separately in order to identify any gender differences for maternal alpha-CYP exposure during gestation and lactation. The results of this study should increase our understanding on the long-term effects of prenatal and postnatal low dose exposure of alpha-cypermethrin and the physiological changes that occur in offspring of exposed female rats. This work might provide important new insights into the mechanisms of developmental programming.

## 2. Materials and methods

### 2.1. Animals and experimental protocol

Adult Wistar rats were obtained from Animal Resource Centre (Pasteur, Algeria). Experiments were performed on adult virgin female rats Wistar weighting about 100 ± 20 g at the beginning. Adult male rats were used for fertilization. All rats were kept under controlled conditions. They were housed in wood-chip bedded plastic cages with free access to food and water with a twelve hour day and night cycle and at ambient temperature of 20–25 °C and humidity (60% ± 5%). They were given pellet food and drinking water *ad libitum*. The experimental protocol met the national guidelines on the proper care and use of animals in the laboratory research. The control group (n = 8) was exposed to standard laboratory chow (386 kJ/100 g; composed of 20% of energy as protein, 60% of energy as carbohydrate and 20% of energy as lipids), while the experimental group (n = 8) was administered the standard laboratory chow treated by alpha-cypermethrin at 1.5 mg/-kg body weight/day dose. This corresponded to the no observed effect level (NOEL) dose of the drug [29].

To prepare experimental diet, appropriate concentrations of alpha-cypermethrin were dissolved in corn oil (1 mL) and added to control diet (15 g/rat) according to the rat weight. Each diet was analyzed for alpha-cypermethrin concentration using high-performance liquid chromatography (HPLC) to assure that the proper dose of alpha-cypermethrin was delivered. The different diets were consumed by the rats one month before and during the entire gestation (21 days). The rats received 15 g of each diet every day in the morning at 8H, and 25 g in the afternoon. For the experimental group, 15 g of diet containing alpha-cypermethrin were given in the morning and 25 g without the insecticide in the afternoon. This step allowed us to deliver the proper dose to the rats.

Female rats were monitored throughout the 3-week gestation period and housed individually before delivery. The dams were fed the same diet continuously for the entire gestation and lactation periods. Weaning occurred on day 21 of lactation and male and female offspring were separated. After weaning, control group was divided to two groups: the first one (control group) in which offspring received standard chow like their moms; the second group (experimental group 1) with offspring receiving insecticide treated chow diet at weaning. The third group (experimental group 2) contained offspring of insecticide treated mothers receiving treated chow diet at the same mother's insecticide dose. All offspring maintained this diet *ad libitum* until they reached adulthood (5 months). This protocol yielded six groups: male offspring of standard chow fed dams weaned on to standard chow (n = 8), female offspring of standard chow fed dams weaned on to standard chow (n = 8), male offspring of standard chow fed dams weaned on to insecticide treated chow (n = 8), female offspring of standard chow fed dams weaned on to insecticide treated chow (n = 8), male offspring of insecticide treated chow fed dams weaned on to insecticide treated chow (n = 8), and female offspring of insecticide treated chow fed dams weaned on to insecticide treated chow (n = 8). The weight and food consumption of each animal were measured daily.

The study was conducted in accordance with the national guidelines for the care and use of laboratory animals. All the experimental protocols were approved by the ethical committee of the experimental animal care at Tlemcen University.

At the end of the experimental period, and after overnight fasting, rats were anaesthetized with intraperitoneal injection of sodium pentobarbital (60 mg/kg of body weight). The blood was drawn from the abdominal aorta into heparinized tubes, and plasma was used for biochemical determinations. After removal of plasma, erythrocytes were washed and were lysed with ice-cold distilled water (1/4) and stored at 4 °C for 15 min. The cell debris was removed by centrifugation (2000 g for 15 min). Erythrocyte lysates were assayed for catalase activity, glutathione, malondialdehyde and carbonyl protein contents. Tissues (liver, kidneys) were collected, and immediately placed on dry ice. An aliquot of each tissue was homogenized in 10 volumes of ice-cold 10 mmol/l phosphate-buffered saline (pH 7.4) containing 1.15% KCL. The homogenate was subjected to a 6000*g* centrifugation at 4 °C for 15 min. The supernatant fractions were collected and used for redox marker determinations.

### 2.2. Chemical analyses

Plasma glucose, total protein, creatinine, urea and triglyceride and total cholesterol concentrations were determined using colorimetric enzymatic assays (kits from BioAssay Systems, Hayward, CA) with an interassay CV in the range of 1.5–3%.