

EVALUATION OF MITOCHONDRIAL AND NEUROBEHAVIORAL DISORDERS IN BRAIN REGIONS OF OFFSPRING (F1, F2) AFTER GESTATING AND LACTATING FEMALE RATS EXPOSURE TO LOW-DOSE OF IMIDACLOPRID AND CYPERMETHRIN

Souhila Haddad¹, Zeyneb Chouit², Dounia Djellal², Salim Gasmi³, Messaoud Hachemi⁴, Mourad Hanfer⁵, Djamila Zama⁶, Mohamed Kebieche², Rachid Soulimani⁷

Address(es): Salim Gasmi & Mohamed Kebieche,

¹Laboratoire de Biomathématique, Biophysique, Biochimie et Scientométrie, University of Bejaia, Algeria.

²Faculty of natural and life sciences, LMAGECA and MBMP research laboratories, University of Batna2, 05078, Fesdis, Batna, Algeria.

³University of Larbi Tebessi, 12002, Tebessa, Algeria.

⁴University of Mohamed Seddik Ben Yahia, Jijel, Algeria.

⁵Veterinary department, University of Batna1, Algeria.

⁶University of Constantine, Constantine Algeria.

⁷Neurotoxicology, développement & Bioactivité, LCOMS, Campus Bridoux, Université de Lorraine, France.

*Corresponding author: salim.gasmi@univ-tebessa.dz & m.kebieche@univ-batna2.dz

<https://doi.org/10.55251/jmbfs.9541>

ARTICLE INFO

Received 15. 10. 2022

Revised 17. 1. 2023

Accepted 18. 1. 2023

Published xx.xx.201x

Regular article



ABSTRACT

The imidacloprid (IMID) and cypermethrin (CYP) pesticides are known to have neurotoxic effects and negative brain developmental consequences when used separately, but little is known about the consequences of using them as a mixture. That could be passed down from one generation to the next. In this context, we were interested in studying the effect of oral exposure of female rats during gestation and throughout the lactational period to real doses of IMID (1.2 mg/kg) and CYP (6.7 mg/kg) either alone or in a mixture on behavior and mitochondrial redox status. The first and second generation pups were followed from birth to juvenile age using a series of tests to assess reflex, coordination and general motor function, including surface righting, cliff aversion, negative geotaxis and the muscle strength test. At PND 21, cholinergic function, oxidative stress and mitochondrial integrity were assessed in the striatum and hippocampus. Our results showed that IMID and CYP, alone or in combination, induced decreased body weight gain, impaired neurobehavioral performance, and AChE inhibition. Oxidative stress markers including GSH level and SOD, CAT, GST and GPx activities showed a significant decrease. While, lipid peroxidation which was assessed by MDA assay, cytosolic calcium level, swelling and mitochondrial permeability recorded a significant increase. In conclusion, the disruption of mitochondrial redox homeostasis and the presence of neurobehavioral disorders even in the offspring of the F2 generation suggests that independent and combined exposure to IMID and CYP during a critical period of development has irreversible effects; long-lasting and persistent.

Keywords: Imidacloprid, Cypermethrin, Neurobehavior, Striatum, Hippocampus, Mitochondria, Oxidative stress, Rats

INTRODUCTION

The fetal period and early life are important stages when optimal brain development is assured. This is when the basic structure of the brain is built. This construction involves a series of processes that are particularly vulnerable to chemical stressors including pesticides. Impairment of this process could disrupt homeostasis, thereby increasing the risk of neurodegenerative disorders (Sunyer and Dadvand, 2019). Several studies have shown that exposure to pesticides is not only through inhalation, skin absorption or ingestion of contaminated food and water but also through the placenta and breast milk during critical periods of development (Dewailly et al., 2014). The use of pyrethroids and neonicotinoids has registered a significant increase due to their efficiencies and wide range of application; they are also less toxic to non-target organisms and efficiently degradable. These two families are considered green pesticides compared to organophosphates. However, the effect of their low concentrations on wildlife, especially bees, as well as humans, remains of concern (Zeljezic et al., 2017). The nervous system is the primary target of CYP, a pyrethroid that exerts a neurotoxic effect through voltage-gated sodium channels. Once this insecticide binds to the α subunit of the sodium channel, the latter remains in an open position, thus allowing a prolonged passage of sodium and a depolarization of the membrane and ends up disrupting neuronal transmission. Exposure to this pyrethroid also influences other channels such as voltage-gated calcium and potassium channels; it decreases dopamine and acetylcholine levels as it alters key enzymes involved in the synthesis and metabolism of neurotransmitters such as adenosine triphosphatases and AChE (Raszewski et al., 2016). In laboratory animals, intranasal exposure of mice to 5 and 20 mg/kg of CYP during gestation and lactation has been linked to mitochondrial dysfunction as a mechanism of developmental neurotoxicity (Laugeray et al., 2017). Another study conducted on

mice indicates that exposure to CYP results in the release of Ca^{2+} and free radicals leading to DNA damage and cell death (Maurya, et al., 2014).

Neonicotinoids such as IMID act by binding to the $\alpha 4$ and $\beta 2$ subunits of the nicotinic postsynaptic acetylcholine receptors (nAChRs) of mammals. The neurotoxicity of this class is manifested by the inhibition of acetylcholinesterase, thereby reducing the ability of this enzyme to break down acetylcholine in the synapses, which subsequently leads to continuous overstimulation of nerves and muscles. Prenatal exposure to its chemicals affects the formation of neural circuits in several regions of the brain that are involved in the regulation of depression, anxiety, memory and learning (Sano et al., 2016).

Osaka et al., 2016 reported the presence of metabolites of the neonicotinoid Thiamotoxam in the urine of young children. The increased resistance of pests to a single pesticide application has made it necessary to use a cocktail of these chemicals in order to increase its speed of action (Shittu et al., 2021). Therefore, the combined effect of this mixture can lead to a joint interaction between the chemicals thus altering the absorption, biotransformation, distribution and elimination of one on the other and subsequently causing the appearance of new metabolites which could be more dangerous in comparison with the basic preparation, which makes the question of the risk for ecology and human health even more serious (Aouey et al., 2017).

To our knowledge, the CYP and IMID cocktail has not been studied for its transgenerational neurotoxic effects in mammals. Therefore, the objective of this study was to evaluate the toxicity of these pesticides alone or in mixture by examining their effects on neuromotor development, mitochondrial integrity and oxidative damage in the striatum and hippocampus of pups of generations F1 and F2 born to mothers (F0) exposed during gestation and throughout the lactation period.

MATERIALS AND METHODS

Chemical products

Most of the chemicals used in this study were purchased from Sigma Aldrich, Germany. COMMANDO is the trade name of IMID, purchased from VAPCO, Jordan. CYRUX is the trade name of CYP, purchased from UPL Limited India. The doses were chosen by referring to the results of (Jallow et al. 2017) which found 1.2 mg/kg/d of IMID residues in cucumber and of (Skretteber et al. 2015) which found 6.7 mg/kg of CYP residue in peppermint. To convert the concentration of pesticides in cucumber and peppermint to a daily dose in experimental animal studies, we used a conversion factor which is 0.05 in rats (EFSA 2011).

Breeding and treatment of animals

Wistar albino rats weighing approximately 200g were provided by the Pasteur Institute in Algiers. Upon receipt, the animals were placed in cages for a two-week acclimatization period. They were maintained under standard temperature and humidity conditions, with a temperature of $22 \pm 2^\circ\text{C}$ and a humidity of approximately 60%, with a periodic cycle (light/dark) of 12 hours. The rats had free access to food and water. All experimental testing was performed in accordance with international guidelines for the care and use of laboratory animals. After two weeks of habituation to the animal house, the females were bred with males (2:1). The next day, the rats were examined by demonstrating the presence of spermatozoa in the vagina; which announces the first gestational day G1. After fertilization, 4 females (F0) were placed individually in cages. They were then divided into 4 exposure groups:

- Control group-receiving corn oil
- IMID group receiving 1.2 mg/kg of IMID
- CYP group receiving 6.7 mg/kg of CYP
- Mixture group receiving a mixture (IMID at 1.2 mg/kg/day and CYP 6.7 mg/kg/day).

In this study, IMID and CYP were dissolved in corn oil and administered orally from day 1 of gestation to day 21 of lactation. Adult females of the F1 generation (offspring of F0 mothers) were mated with males (not exposed to pesticides) to generate the F2 generation.

Sensory-motor development tests

At birth, eye opening was monitored and body weight was measured from day 1 to day 21 of postnatal age.

Evaluation of locomotor development

The turning reflex (surface righting)

From day 3 to day 5 of postnatal age, the rollover reflex was performed as described by Peiffer (2011). This test consists of placing the rat on its back on a horizontal board, the animal will then try to turn around by swinging to the right and to the left. In this test, the time it takes for the rat to get back on its four legs is timed.

Cliff dislike

From the 5th to the 7th day of postnatal age, Cliff aversion tests the young rat's reflex, strength and coordination. In this test, the rat's head and forelegs are positioned at the edge of a raised flat box. Two consecutive trials are carried out for each rat which will be scored by counting points and the time it takes to move away from the edge is timed (Peiffer, 2011).

Negative geotaxis

On days 7 to 9 of postnatal age, negative geotaxis is performed to assess motor coordination, counting the time it takes for a rat placed on a 45° slope with its head pointing down to turn 180° up (Feather-Schussler and Ferguson, 2016).

Suspension test (muscle strength)

From the 10th to the 13th day of postnatal age, front-limb Suspension test reflects muscle strength and coordination. It consists of hanging the rat's front legs on a metal wire stretched over a stable object, recording the total time of the fall (Albina et al., 2005).

Biochemical analysis

At the end of the lactational phase (PND21), the first and second generation rats were sacrificed by decapitation after deep ether anesthesia; the brains were

recovered and immediately washed with a cold phosphated saline solution (PBS), then dissected to separate the striatum and the hippocampus which are used to evaluate the effect of IMID and CYP alone or in mixture on the mitochondrial redox status and membrane integrity of this organ.

Separation of mitochondrial matrix from striatum and hippocampus.

The extraction of the mitochondrial fractions was carried out according to the method described by Sahu et al. (2014). Briefly, the hippocampus and the striatum are immersed and homogenized in a TSE buffer (10 mM tris, 250 mM sucrose, 0.1 mM EDTA, pH 7.2 at 4°C) to obtain a 10% homogeneous tissue. The homogenate recovered is centrifuged at 600 g/10 min in order to eliminate large cellular debris. The supernatant resulting from this centrifugation is recovered and then centrifuged at 10,000g/10min. The resulting supernatant is considered as the cytosolic fraction, and the pellet is resuspended in TS buffer (10mM tris, 250mM sucrose, PH 7.2) then centrifuged at 10,000g/10min. The pellet resulting from this last centrifugation is resuspended in the storage buffer (250 mM sucrose, 50 mM tris, PH 7.2) which will be used directly for the evaluation of the structural and functional integrity of the mitochondria; the rest is stored at -20°C for later analyses.

In order to burst the mitochondria and recover the mitochondrial matrix, we performed freeze-thaw combined with approximately 8 times homogenization; then, we performed a centrifugation at 10,000 g/10 min. The obtained supernatant was considered as the source of mitochondrial MDA, GSH, CAT, GST, GPx and SOD.

Determination of AChE activity and cytosolic calcium

AChE activity was estimated according to the procedure of Ellman et al. (1961), using acetylthiocholine as a substrate. 100 μl of cytosolic sample were added to 100 μl of DTNB (0.1 M, pH 8) + 1 ml of tris buffer (0.1 M, pH 7). Once the reaction was stable, 100 μl of acetylthiocholine substrate was added. Absorbance was monitored at 412 nm over 4 min for 20 min. AChE activity was expressed as IU/mg protein.

Calcium levels in the cytosol were measured following the recommendations of a commercial kit (SPINREACT, Spain).

Assessment of swelling and mitochondrial permeability

Swelling of mitochondria was assessed in the striatum and hippocampus using the method of Li et al. (2014) where the absorbance of mitochondria isolated from fresh tissue at 4°C is monitored at a wavelength of 540nm. The decrease in absorbance indicates swelling of the mitochondria, which is the result of loss of the mitochondrial permeability transition pore (MPTP). Mitochondrial permeability was evaluated according to the method designed by Kristal et al. (1996); this technique is based on the rate of passage of Ca^{++} followed by an increase in the size of the mitochondria detected every 30 s for 3 min at 540 nm.

Evaluation of markers of redox status in mitochondria of the striatum and hippocampus

Determination of glutathione and malondialdehyde acid levels

The glutathione concentration was measured by the method of Ellman (1959): 50 μl of trichloroacetic acid (TCA) (10%) + 50 μl of mitochondrial matrix are centrifuged at 1400 g/2 min. 50 μl of the supernatant are taken, to which 1 ml of phosphate buffer (PH=8) and 20 μl of DTNB (5,5'-dithio-bis-2-nitrobenzoic acid) (0.01M) are added. After 15 minutes of incubation, the reading of the optical density was carried out at 412 nm with respect to the reagent blank prepared under the same conditions. The evaluation of the end products of lipid peroxidation was carried out by the analysis of side products such as malondialdehyde (MDA) which is commonly measured by its reaction with thiobarbituric acid (TBA). The intense absorption of this complex occurs at a wavelength of 532nm. MDA levels were assessed using the method of Niehuis and Samuelson (1968).

Determination of CAT, SOD, GPx and GST activities

The enzymatic activity of catalase was determined according to the method of Clairbone (1985); this method is based on measuring the disappearance of hydrogen peroxide due to the activity of this enzyme. In a quartz cuvette, we put 1 mL of phosphate buffer (0.1 M, pH 7.4) + 0.950 mL of H_2O_2 (0.019 M) + 50 μl of the enzyme source (mitochondrial matrix). The reaction is monitored by recording the absorbance at 240 nm every 30 s for 3 minutes. The enzymatic activity is expressed in IU/mg of protein. The SOD activity was evaluated according to the method of Beauchamp and Fridovich (1971). The SOD activity was evaluated according to the method of Beauchamp and Fridovich (1971). Briefly, 50 μl of the enzyme source (mitochondrial matrix) were added to 2 ml of a reaction mixture which contains (sodium cyanide, NBT, EDTA, riboflavin and phosphate buffer at pH 7.8) in the presence of 100 μl of an electron donor such as methionine. The mixture is subjected to radiation from a 15 W lamp for 15 minutes. Absorbance

was measured at 560 nm. The GPx activity was carried out according to the procedure described by Flohe and Günzler (1984). GPx is the main enzyme that removes hydrogen peroxide. 0.2ml of sample (mitochondrial matrix) was added to a tube containing 0.4ml of GSH (0.1mM) and 0.2ml of phosphate buffer (0.067M, pH 7.8). The mixture is incubated in a water bath at 25° C. for 05 min. H₂O₂ (1.3mM) was added to initiate the reaction. After 10 min, 1% TCA (tri-chloroacetic acid) was added to stop the reaction and the mixture is put on ice for 30 min and centrifuged for 10 min at 3000 rpm. A volume of supernatant is placed in a cuvette to which Na₂HPO₄ (0.32M) + DNTB (1mM) have been added. Absorbance was measured at 412nm every 30 seconds for 05min. The GST activity was measured according to the method of Habig et al. (1974). The intensity of GST activity is directly proportional to the amount of 1-S-Glutathionyl 2-4dinitrobenzene formed from the conjugation between GST and CDNB (1-Chloro2,4-dinitrobenzene) which acts as a substrate. Absorbance was performed every 30s for 5min at 340 nm.

Statistical analysis

Data are presented as mean ± SD. In multiple comparisons, data were analyzed using a one-way ANOVA. Differences between mean values were considered significant at *p < 0.05; highly significant at **p < 0.01; and very highly significant at ***p < 0.001. The statistical study was carried out using Excel SPC software.

RESULTS

Sensory-motor development tests

Pup maturation was assessed by body weight gain from day 1 (PND1) to day 21 (PND21). Independent and combined exposure of dams (F0) to IMID and CYP significantly reduced weight gain of first and second generation pups (p ≤ 0.05 for IMID, p ≤ 0.01 for CYP and p ≤ 0.001 for MIX) (Fig 1).

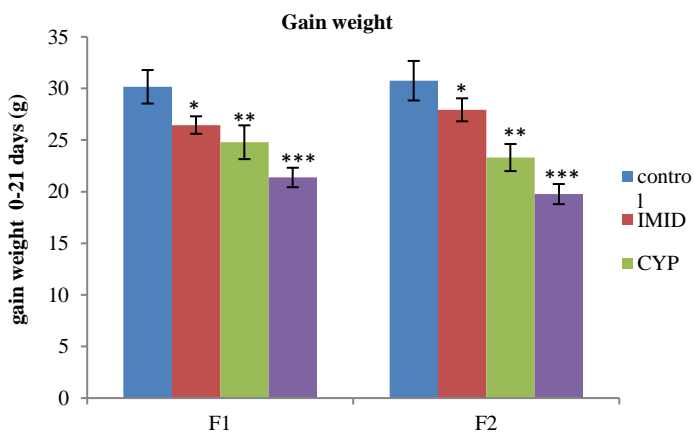


Figure 1 Effects of gestational and lactational exposure to IMID, CYP and their mixture on weight gain in neonates of the first and second generation. Values are means ± SD, (n= 10), * Significant difference versus control (p ≤ 0.05). ** Highly significant difference versus control (p ≤ 0.01). *** Very highly significant difference versus control (p ≤ 0.001).

Regarding eye opening, there were no significant changes in any of the treated groups compared to the control group (Fig 2).

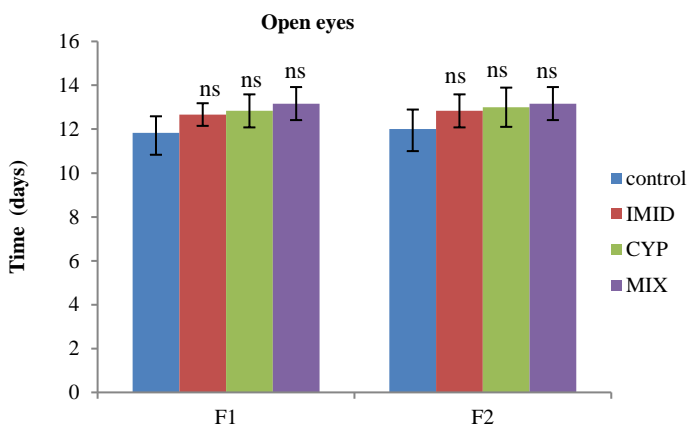


Figure 2 Effects of gestational and lactational exposure to IMID, CYP and their mixture on eyes opening in neonates of the first and second generation. Values are means ± SD, (n= 10), ns no significant difference (p > 0.05).

Evaluation of locomotor development

Neuromotor development was also studied by several tests such as surface righting, Cliff aversion, negative geotaxis and front-limb suspension.

The development of the ability to turn over (surface righting) was significantly impaired by IMID and CYP either alone or in combination. The mean time needed to turn around was higher in F1 and F2 generation pups born to treated mothers compared to the control (p ≤ 0.01 for IMID and CYP and p ≤ 0.001 for MIX) (Fig 3).

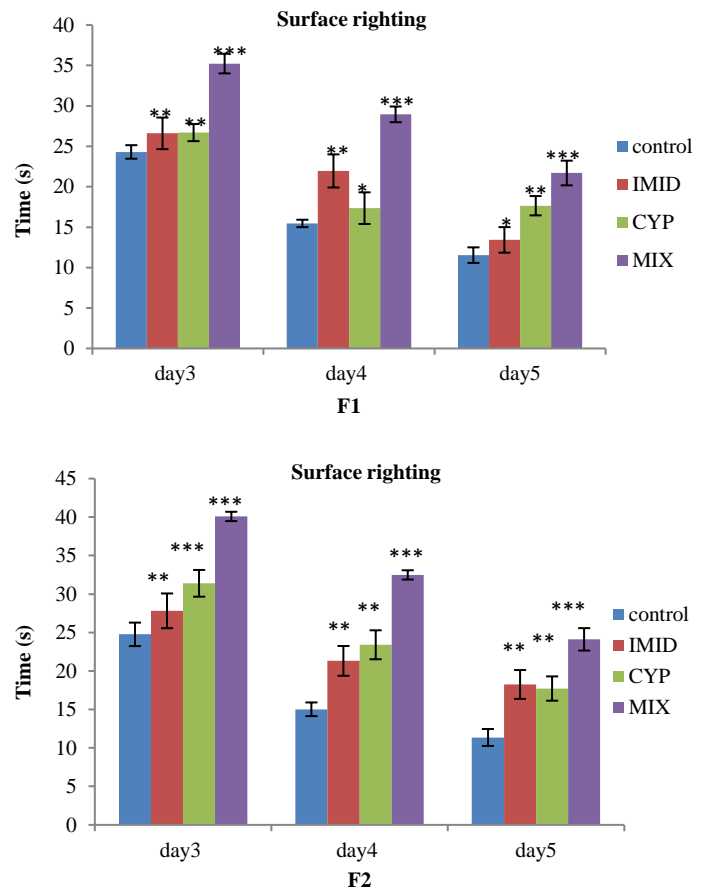
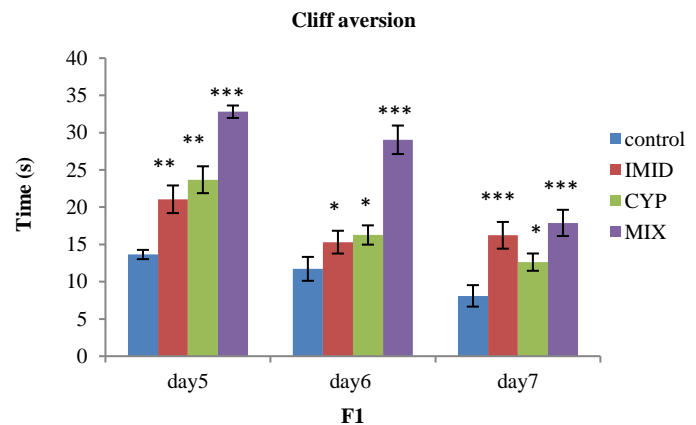


Figure 3 Effects of gestational and lactational exposure to IMID, CYP and their mixture on surface righting reflex in neonates of the first and second generation. Values are means ± SD, (n= 10), * Significant difference versus control (p ≤ 0.05). ** Highly significant difference versus control (p ≤ 0.01). *** Very highly significant difference versus control (p ≤ 0.001).

Cliff aversion in F1 and F2 offspring is shown in Figure 4. The pups born to the treated mothers took longer to move back on the platform during the three days of the test with a significant difference with the control, only in PND 5 the IMID did not exert any significant effect on the pups of the second generation.



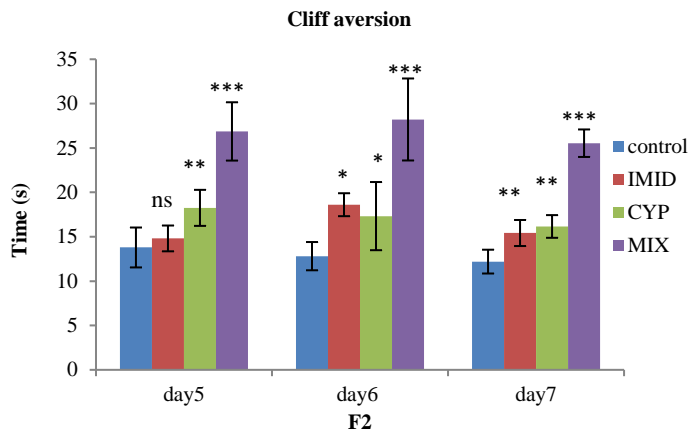


Figure 4 Effects of gestational and lactational exposure to IMID, CYP and their mixture on cliff aversion in neonates of the first and second generation. Values are means \pm SD, (n= 10), ns no significant difference (p > 0.05). * Significant difference versus control (p \leq 0.05). ** Highly significant difference versus control (p \leq 0.01). *** Very highly significant difference versus control (p \leq 0.001).

Pre- and postnatal exposure to IMID and CYP significantly impaired the ability of pups to exhibit reflex in the negative geotaxis test. However, in PND8 no significant difference was recorded in first and second-generation pups born to dams treated with CYP alone (Fig 5).

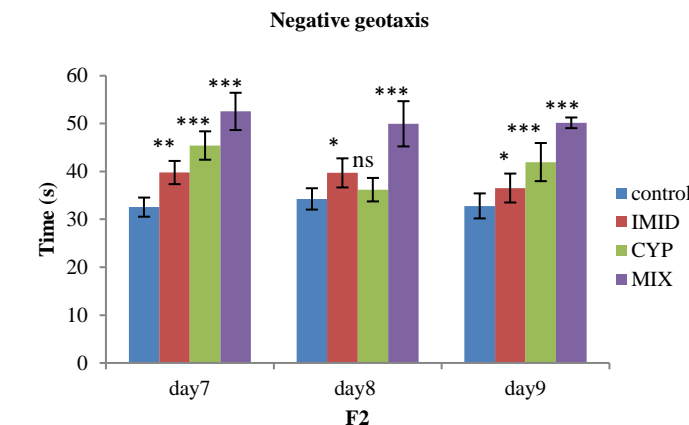
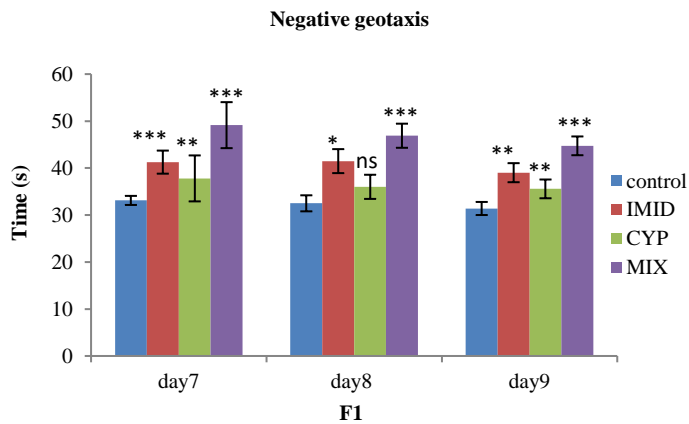


Figure 5 Effects of gestational and lactational exposure to IMID, CYP and their mixture on development of negative geotaxis reflex in neonates of the first and second generation. Values are means \pm SD, (n= 10), ns no significant difference (p > 0.05). * Significant difference versus control (p \leq 0.05). ** Highly significant difference versus control (p \leq 0.01). *** Very highly significant difference versus control (p \leq 0.001).

Coordination, development and motor strength were assessed by the front-limb suspension test. The results obtained showed that the fall latency was significantly affected, pups born to mothers treated with IMID and CYP either alone or in a mixture remained hanging from the wire longer than the controls (Fig 6).

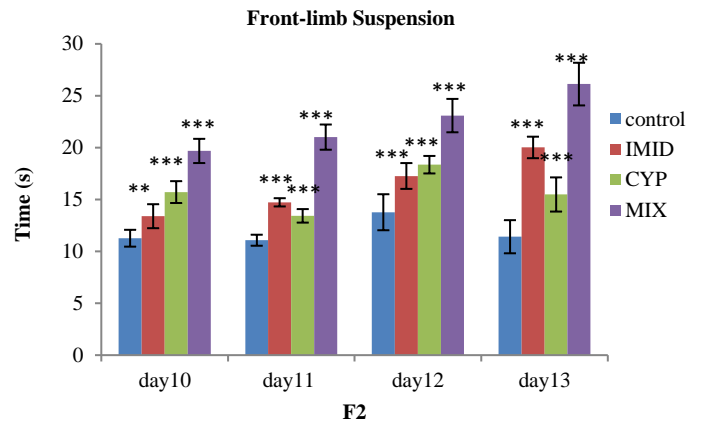
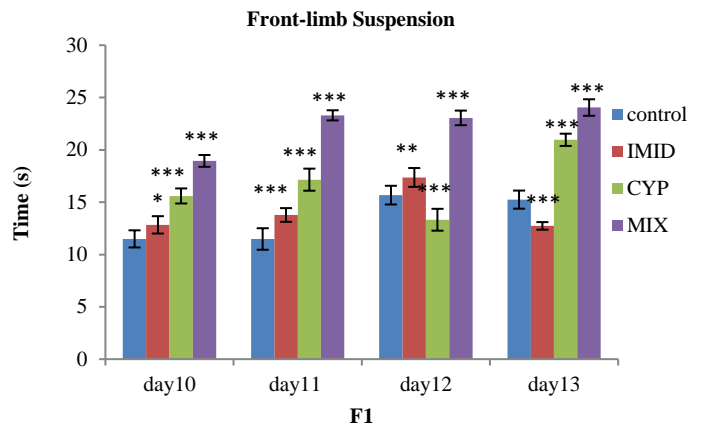
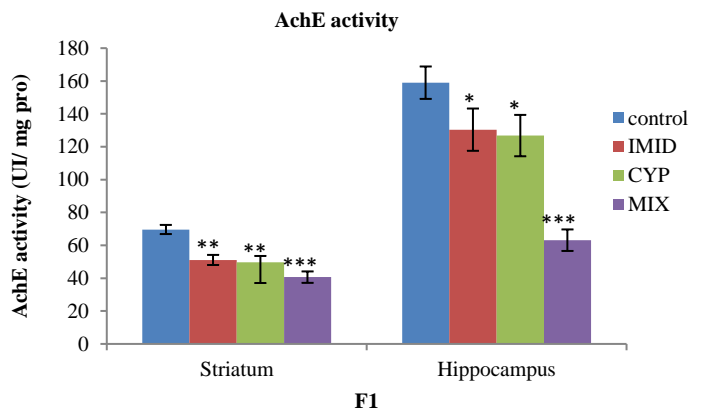


Figure 6 Effects of gestational and lactational exposure to IMID, CYP and their mixture on coordination, development and motor strength in neonates of the first and second generation. Values are means \pm SD, (n= 10). * Significant difference versus control (p \leq 0.05). ** Highly significant difference versus control (p \leq 0.01). *** Very highly significant difference versus control (p \leq 0.001).

Evaluation of AchE activity in pups of the F1 and F2 generation

Maternal exposure to IMID and CYP alone or in cocktail resulted in a significant decrease in AChE activity in the striatum and hippocampus of first and second generation juvenile pups (Fig 7).



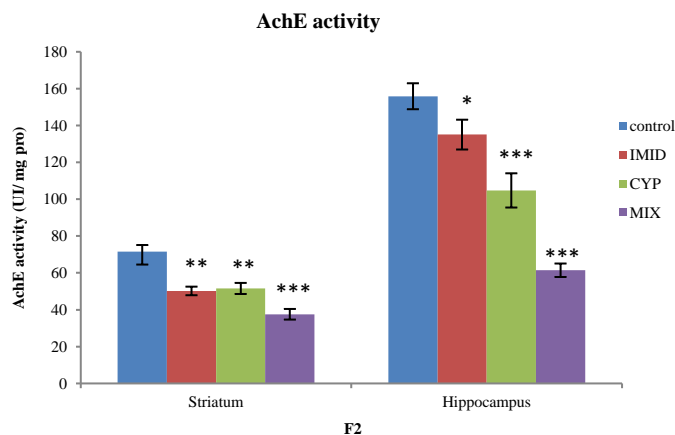


Figure 7 Effects of gestational and lactational exposure to IMID, CYP and their mixture on acetylcholinesterase activity in striatum, and hippocampus of rats of the first and second generation observed at 21 days of age. Values are means \pm SD, (n= 10). * Significant difference versus control (p \leq 0.05). ** Highly significant difference versus control (p \leq 0.01). *** Very highly significant difference versus control (p \leq 0.001).

Evaluation of mitochondrial swelling and membrane permeability

The results in Table 1, show a significant increase in mitochondrial swelling in the striatum and hippocampus of first and second generation pups born to dams treated during gestation and throughout lactation with IMID and of CYP, alone or in a mixture, compared to pups born to untreated mothers.

Table 1 Effect of gestational and lactational exposure to IMID, CYP and their mixture on mitochondrial swelling in striatum, and hippocampus of rats of the first and second generation observed at 21 days of age. Values are means \pm SD, (n= 10). ** Highly significant difference versus control (p \leq 0.01). *** Very highly significant difference versus control (p \leq 0.001).

	Mitochondrial swelling (optic density)			
	generations			
	F1		F2	
	Brains regions			
	Striatum	Hippocampus	Striatum	Hippocampus
Control	0.297 \pm 0.020	0.262 \pm 0.017	0.293 \pm 0.014	0.270 \pm 0.013
IMID	0.201 \pm 0.022**	0.185 \pm 0.012***	0.213 \pm 0.008***	0.179 \pm 0.017***
CYP	0.214 \pm 0.022**	0.170 \pm 0.015***	0.210 \pm 0.032***	0.192 \pm 0.021***
MIX	0.182 \pm 0.012***	0.118 \pm 0.014***	0.130 \pm 0.011***	0.098 \pm 0.006***

Maternal exposure to IMID and CYP, alone or in combination, significantly increased mitochondrial permeability in the striatum and hippocampus of first generation pups. This effect was transformed to the pups of the F2 generation born from the treated mothers even if there was no direct exposure of the F1 offspring (Tab 2).

The results of changes in calcium concentrations outside the mitochondria are shown in Figure 8. After pre- and postnatal exposure to IMID and CYP either alone or in combination, cytosolic calcium levels increased significantly in pups born to treated dams relative to those born to control dams.

Evaluation of mitochondrial redox status in the striatum and hippocampus of F1 and F2 generation pups

With respect to antioxidant potency, CAT, SOD, GST, GPx activity and GSH level in the striatum and hippocampus of pups born to IMID and CYP-treated mothers, either alone or in mixture, recorded values significantly lower than the control. For lipid peroxidation, our results showed a significant increase in MDA in pups born to treated mothers compared to those of the control (Table 3).

Table 3 Effect of gestational and lactational exposure to IMID, CYP and their mixture on antioxidant enzymes and malondialdehyde levels in striatum, and hippocampus of rats of the first generation observed at 21 days of age. Values are means \pm SD, (n= 10). * Significant difference versus control (p \leq 0.05). ** Highly significant difference versus control (p \leq 0.01). *** Very highly significant difference versus control (p \leq 0.001).

	Mitochondrial oxidative stress parameters							
	groups							
	Control		IMID		CYP		MIX	
	Stria	Hippo	Stria	Hippo	Stria	Hippo	Stria	Hippo
MDA (nmol/mg Pro)	1.707 \pm 0.10	1.51 \pm 0.24	2.681 \pm 0.36***	2.025 \pm 0.27**	2.13 \pm 0.09*	2.48 \pm 0.21***	3.27 \pm 0.34***	3.43 \pm 0.22***
GSH (mmol/mgPro)	0.411 \pm 0.03	0.417 \pm 0.03	0.356 \pm 0.03*	0.351 \pm 0.03*	0.33 \pm 0.02**	0.31 \pm 0.05***	0.21 \pm 0.03***	0.33 \pm 0.02**
GST (UI/mg Pro)	50.52 \pm 4.58	49.68 \pm 1.43	42.797 \pm 2.82**	38.498 \pm 1.41***	41.30 \pm 3.92**	39.11 \pm 1.89***	30.19 \pm 4.94***	27.01 \pm 2.76***
GPx (UI/mg Pro)	0.274 \pm 0.02	0.28 \pm 0.01	0.237 \pm 0.01**	0.254 \pm 0.01*	0.25 \pm 0.01*	0.24 \pm 0.02**	0.20 \pm 0.01***	0.24 \pm 0.02**
CAT (UI/mg Pro)	0.090 \pm 0.00	0.08 \pm 0.01	0.069 \pm 0.00**	0.064 \pm 0.013*	0.07 \pm 0.01*	0.05 \pm 0.00**	0.03 \pm 0.01***	0.03 \pm 0.01***
SOD (UI/mg Pro)	10.45 \pm 1.10	10.02 \pm 0.24	8.80 \pm 0.63**	8.12 \pm 0.84***	7.73 \pm 0.76***	8.46 \pm 0.74**	5.16 \pm 0.72***	5.12 \pm 0.94***

Stria : Striatum, Hippo : Hippocampus

Table 2 Effect of gestational and lactational exposure to IMID, CYP and their mixture on mitochondrial permeability in striatum, and hippocampus of rats of the first and second generation observed at 21 days of age. Values are means \pm SD, (n= 10). * Significant difference versus control (p \leq 0.05). ** Highly significant difference versus control (p \leq 0.01). *** Very highly significant difference versus control (p \leq 0.001).

	Mitochondrial permeability (Δ OD/ Δ t)			
	generations			
	F1		F2	
	Brains regions			
	Striatum	Hippocampus	Striatum	Hippocampus
Control	0.017 \pm 0.006	0.019 \pm 0.003	0.022 \pm 0.001	0.020 \pm 0.002
IMID	0.027 \pm 0.007*	0.031 \pm 0.003***	0.026 \pm 0.002**	0.030 \pm 0.002***
CYP	0.031 \pm 0.008**	0.037 \pm 0.005***	0.032 \pm 0.001***	0.037 \pm 0.001***
MIX	0.032 \pm 0.003**	0.040 \pm 0.005***	0.031 \pm 0.001***	0.041 \pm 0.002***

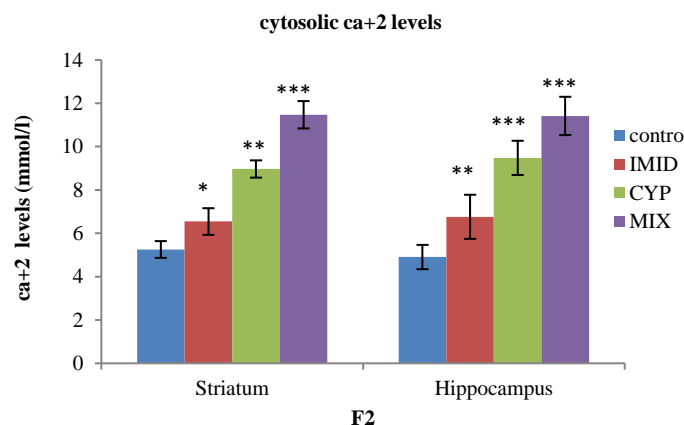
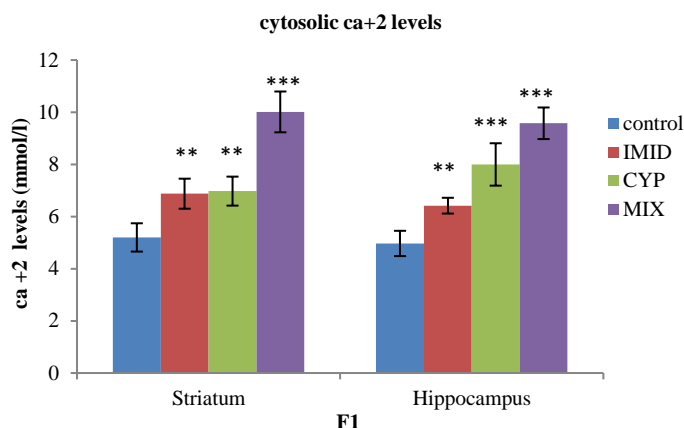


Figure 8 Effects of gestational and lactational exposure to IMID, CYP and their mixture on cytosolic ca²⁺ levels in striatum, and hippocampus of rats of the first and second generation observed at 21 days of age. Values are means \pm SD, (n= 10). * Significant difference versus control (p \leq 0.05). ** Highly significant difference versus control (p \leq 0.01). *** Very highly significant difference versus control (p \leq 0.001).

The results in Table.4 show the persistence of disruption of mitochondrial redox homeostasis in the F2 generation pups even though there was no direct exposure of the F1 offspring. The pups in the treated groups recorded a significant decrease (p \leq 0.001) in the activity of CAT, SOD, GST, GPx, the level of GSH and significantly higher values of MDA (p \leq 0.001).

Table 4 Effect of gestational and lactational exposure to IMID, CYP and their mixture on antioxidant enzymes and malondialdehyde levels in striatum, and hippocampus of rats of the second generation observed at 21 days of age. *Values are means ± SD, (n= 10).* * Significant difference versus control ($p \leq 0.05$). ** Highly significant difference versus control ($p \leq 0.01$). *** Very highly significant difference versus control ($p \leq 0.001$).

	Mitochondrial oxidative stress parameters							
	groups							
	Control		IMID		CYP		MIX	
	Stria	Hippo	Stria	Hippo	Stria	Hippo	Stria	Hippo
MDA (nmol/mg Pro)	1.653±0.17	1.686±0.26	2.970±0.39***	2.704±0.29***	3.111±0.43***	2.729±0.31***	4.536±0.66***	3.950±0.17***
GSH (mmol/mgPro)	0.418±0.01	0.409±0.01	0.304±0.03***	0.342±0.03**	0.292±0.04***	0.295±0.05***	0.197±0.02***	0.203±0.01***
GST (U/mg Pro)	49.87±1.51	49.53±1.39	40.24±1.48***	36.65±1.52***	40.57±1.76***	35.20±3.37***	24.52±1.86***	20.99±4.98***
GPx (U/mg Pro)	0.267±0.01	0.271±0.02	0.231±0.02**	0.231±0.02**	0.228±0.01***	0.232±0.01**	0.197±0.02***	0.227±0.01**
CAT (U/mg Pro)	0.087±0.00	0.080±0.01	0.06±0.01**	0.05±0.00**	0.05±0.009***	0.05±0.01***	0.029±0.01***	0.024±0.00***
SOD (U/mg Pro)	10.98±0.89	11.08±0.88	7.22±0.84***	6.01±0.96***	6.04±0.99***	5.51±0.72***	3.91±0.70***	4.11±0.74***

Stria : Striatum, Hippo : Hippocampus

DISCUSSION

The ubiquity of pesticides in the domestic and agricultural environment increases the likelihood of exposure for pregnant women and nursing mothers, putting their children at risk (Shittu et al., 2021). During fetal and childhood development, the environment can play an important role in the onset of many chronic diseases, including neurodevelopmental abnormalities. It has also been shown that these adverse effects can be transmitted to subsequent generations without the need for additional exposure (Blanc et al., 2020). The results of the present study demonstrated that exposure to a mixture of pesticides during a critical developmental period even at low doses that have been adapted for human consumption affects neuronal development and alters the mitochondrial redox status of 1st generation pups, these negative effects were also transmitted to the 2nd generation pups. It is well known that IMID, which is a member of the neonicotinoid family, acts in a similar way to nicotine by binding to postsynaptic nicotinic acetylcholine receptors (nAChRs). Hyperactivity of these receptors influences food intake and decreases body weight, which explains the reduction in weight gain in F1 and F2 offspring born to dams treated with IMID and CYP either alone or in combination. This reduction indicates reduced growth, which testifies to the vulnerability of developing rats to pesticides, this could be explained by the placental transfer of the compound from the mother to the fetus, this parameter is an important factor in determining developmental toxicity (Syed et al., 2016). Our results agree with those of Burke et al. 2018 and Elser et al. 2020 which showed weight reduction in the offspring of mice exposed during gestation and throughout lactation to 0.5 mg/kg IMID and 10 mg/kg CYP, respectively.

On the other hand, several studies have reported that exposure to pyrethroids and neonicotinoids affects neurobehavioral performance, hence the delay of the righting reflex (surface righting), disorders in the development of coordinated movement and the sense of movement. equilibrium that were recorded after pre and postnatal exposure to IMID and CYP either alone or in cocktails. This confirms the negative impact of these two pesticides on neuromotor development, motor functions and the vulnerability of the developing brain to xenobiotic insults (Syed et al., 2016). These neurobehavioral deficits could be the result of dysfunction in multiple areas of the central and peripheral nervous system; a recent study showed that fetal and lactational exposure to a neonicotinoid, clothianidin, inhibits the maturation of immature neurons in juvenile progenitor cells and the viability of adult progenitor cells (Maeda et al., 2021). Our results are in agreement with the work of Laugeray et al., 2017 who conducted a study in mice where females received 5 and 20 mg/kg/day of CYP throughout gestation and up to the 15th day. of lactation and those of Elser et al. 2020 who showed that maternal exposure to CYP influences the development of GABAergic neurons. The delay in the formation of these neurons can have a significant impact on their functions in the mature brain, which will subsequently cause neurodevelopmental problems in children. Moreover, a significant correlation between impaired memory performance, developmental/coordination problems and the application of ACh receptor antagonists has been demonstrated (Haense et al., 2012). It is now known that neonicotinoids and pyrethroids have the ability to inhibit AChE which leads to the accumulation of acetylcholine in synapses, subsequently causing persistent stimulation of cholinergic neurons (Arora et al., 2017). Overall, our results agree with those of Sinha et al. 2006 who tried to understand the mechanism of toxicity of a pyrethroid-based mosquito repellent at different stages of development of the central nervous system of rats and those of Liu et al., 2018 who confirm that thiamotoxam, another pesticide of the neonicotinoid family, has the ability to block the transfer of information between neurons and ultimately paralyze target organs. These results indicate that there is a link between cognitive delay in children and prenatal exposure to pesticides (Shelton., 2014; Gunier et al., 2017).

As the brain consumes a large amount of oxygen, contains high amounts of polyunsaturated fatty acids (PUFAs) and has low levels of antioxidant enzymes; which makes it very vulnerable to oxidative stress (Sharma et al., 2014), which is defined by an imbalance between the amounts of oxidant and antioxidant compounds, thus promoting the excessive generation of free radicals or slowing down their elimination (Bragante et al., 2022). Exposure of F0 generation rats to IMID and CYP, alone and in mixture, during gestation and throughout the lactation period resulted in increased cytosolic calcium levels in first generation rat pups. and the second generation. This dysregulation is due to the overload of the

mitochondria with calcium, which is known as an activating factor of several enzymes such as nucleases, proteases and phospholipases which will cause the oxidation of lipids and proteins of the mitochondrial membrane, thereby promoting the opening of the mitochondrial permeability transition pore (mPTP) (Panel et al., 2018). This mitochondrial depolarization should produce ATP depletion, followed by swelling and finally the release of cytochrome c which then promotes cell death signaling (Hosseini et al., 2013). Our results are in agreement with those of Muhammed et al. 2020 which prove that CYP has the ability to cause neurodegenerative disorders through mitochondrial dysfunction, caspase activation, genomic DNA damage, inhibition of AchE activity. Since neonicotinoids have the same structure as nicotine and also share agonist activity at nicotinic acetylcholine receptors (nAChRs), our results are consistent with those of Önal et al., 2004 which indicate that maternal exposure to nicotine alters the normal development of the different regions of the brain, in particular the CA1 of the hippocampus, by modifying its ultrastructure by the condensation of nuclear chromatin, the dilation of the rough endoplasmic reticulum and mitochondrial swelling.

As the brain consumes a large amount of oxygen, contains high amounts of polyunsaturated fatty acids (PUFAs) and has low levels of antioxidant enzymes; which makes it very vulnerable to oxidative stress (Sharma et al., 2014), which is defined by an imbalance between the amounts of oxidant and antioxidant compounds, thus promoting the excessive generation of free radicals or slowing down their elimination (Bragante et al., 2022). Exposure of F0 generation rats to IMID and CYP, alone and in mixture, during gestation and throughout the lactation period resulted in increased cytosolic calcium levels in first generation rat pups. and the second generation. This dysregulation is due to the overload of the mitochondria with calcium, which is known as an activating factor of several enzymes such as nucleases, proteases and phospholipases which will cause the oxidation of lipids and proteins of the mitochondrial membrane, thereby promoting the opening of the mitochondrial permeability transition pore (mPTP) (Panel et al., 2018). This mitochondrial depolarization should produce ATP depletion, followed by swelling and finally the release of cytochrome c which then promotes cell death signaling (Hosseini et al., 2013). Our results are in agreement with those of Muhammed et al. 2020 which prove that CYP has the ability to cause neurodegenerative disorders through mitochondrial dysfunction, caspase activation, genomic DNA damage, inhibition of AchE activity. Since neonicotinoids have the same structure as nicotine and also share agonist activity at nicotinic acetylcholine receptors (nAChRs), our results are consistent with those of Önal et al., 2004 which indicate that maternal exposure to nicotine alters the normal development of the different regions of the brain, in particular the CA1 of the hippocampus, by modifying its ultrastructure by the condensation of nuclear chromatin, the dilation of the rough endoplasmic reticulum and mitochondrial swelling.

According to our results, it seems that CYP is more neurotoxic than IMID. But in general, even as an insect-specific insecticide, IMID could alter the behavior and mitochondrial integrity of non-target species. Our results are in agreement with the work of Liu et al. (2018); which prove that pyrethroids like deltamethrin have a higher level of neurotoxicity compared to organophosphates and neonicotinoids due to their lipophilicity which allows them to accumulate in the cell membrane by disrupting its structure (Aouey et al. 2017). It is important to note that our results showed the persistence of neurobehavioral disorders and mitochondrial dysfunction even after cessation of exposure in F2 generation pups. A study that was conducted in Brazil, Colombia and Spain reported the presence of CYP residues in human breast milk indicating that IMID and CYP can be transferred from mother to offspring possibly through the placenta and through breast milk (Elser et al., 2020; Corcellas et al., 2012 ; Djellal et al., 2022 ; Gasmı et al., 2022). This proves that neonicotinoids and pyrethroids have transgenerational consequences that manifest later in life. This transmission could be explained by epigenetic mechanisms (Yuan et al., 2016). These results indicate that organisms may be subject to molecular changes accumulated over generations and/or that adaptive mechanisms may be able to reset their physiological state after a number of generations (Beck et al., 2017).

CONCLUSION

It can be concluded that maternal exposure to IMID and CYP, alone and in cocktails, during gestation and throughout the lactational period, even at low doses, affects not only the behavior and brain function of the generation F1, but these effects are also transformed to the next generation (F2) even though there was no direct exposure of the F1 offspring. This proves that independent and combined exposure to IMID and CYP during a critical period of development has long-lasting, even persistent, irreversible effects. From here, we can say that exposure to Imidacloprid and Cypermethrin pesticides causes several pathological effects at the level of the brain, which leads to its dysfunction, which was confirmed by previous experiences, especially with regard to behavior, memory and intelligence. This study also confirmed the hypothesis of transmission of these toxics through breastfeeding.

REFERENCES

- Albina, M. L., Bellés, M., Linares, V., Sánchez, D. J., & Domingo, J. L. (2005). Restraint stress does not enhance the uranium-induced developmental and behavioral effects in the offspring of uranium-exposed male rats. *Toxicology*, 215(1-2), 69-79. <https://doi.org/10.1016/j.tox.2005.06.027>
- Aouey, B., Derbali, M., Chtourou, Y., Bouchard, M., Khabir, A., & Fetoui, H. (2017). Pyrethroid insecticide lambda-cyhalothrin and its metabolites induce liver injury through the activation of oxidative stress and proinflammatory gene expression in rats following acute and subchronic exposure. *Environmental Science and Pollution Research*, 24(6), 5841-5856. <https://doi.org/10.1007/s11356-016-8323-4>
- Arora, S., Balotra, S., Pandey, G., & Kumar, A. (2017). Binary combinations of organophosphorus and synthetic pyrethroids are more potent acetylcholinesterase inhibitors than organophosphorus and carbamate mixtures: An in vitro assessment. *Toxicology Letters*, 268, 8-16. <https://doi.org/10.1016/j.toxlet.2016.12.009>
- Beauchamp C, Fridovich I (1971) Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. *Analytical biochemistry* 44(1): 276-287 [https://doi.org/10.1016/0003-2697\(71\)90370-8](https://doi.org/10.1016/0003-2697(71)90370-8)
- Beck, D., Sadler-Riggelman, I., Skinner, M.K., 2017. Generational comparisons (F1 versus F3) of vinclozolin induced epigenetic transgenerational inheritance of sperm differential DNA methylation regions (epimutations) using MeDIP-Seq. *Environmental Epigenetics* 3. <https://doi.org/10.1093/eep/dvx016>
- Blanc, M., Cormier, B., Hyötyläinen, T., Krauss, M., Scherbak, N., Cousin, X., & Keiter, S. H. (2020). Multi- and transgenerational effects following early-life exposure of zebrafish to permethrin and coumarin 47: Impact on growth, fertility, behavior and lipid metabolism. *Ecotoxicology and Environmental Safety*, 205, 111348. <https://doi.org/10.1016/j.ecoenv.2020.111348>
- Bragante, W., Sinhorin, V. D. G., Suguí, M. M., da Cunha, A. P. S., Dos Santos, W. B., & Sinhorin, A. P. (2022). In vivo mutagenic effects and oxidative stress parameters evaluation of cypermethrin and benzoate of emamectin and their mixtures in female mice. *Journal of Environmental Science and Health, Part B*, 57(3), 211-219. <https://doi.org/10.1080/03601234.2022.2045841>
- Burke, A. P., Niibori, Y., Terayama, H., Ito, M., Pidgeon, C., Arsenault, J., ... & Hampson, D. R. (2018). Mammalian susceptibility to a neonicotinoid insecticide after fetal and early postnatal exposure. *Scientific reports*, 8(1), 1-13. <https://doi.org/10.1038/s41598-018-35129-5>
- Claiborne A (1985) Catalase activity In: Greenwald RA (ed) *Handbook of methods for oxygen free radical research*. CRC Press, Boca Raton, pp 283-284. <https://doi.org/10.1093/clinchem/47.11.2075a>
- Corcellas, C., Feo, M.L., Torres, J.P., Malm, O., Ocampo-Duque, W., Eljarrat, E., Barceló, D., 2012. Pyrethroids in human breast milk: occurrence and nursing daily intake estimation. *Environ. Int.* 47, 17-22. <https://doi.org/10.1016/j.envint.2012.05.007>
- Dewailly, E., Forde, M., Robertson, L., Kaddar, N., Sidi, E. A. L., Côté, S., ... & Ayotte, P. (2014). Evaluation of pyrethroid exposures in pregnant women from 10 Caribbean countries. *Environment international*, 63, 201-206. <https://doi.org/10.1016/j.envint.2013.11.014>
- Djellal, D., Haddad, S., Gasmí, S., Chouit, Z., Kebieche, M., Hachemi, M., Hanfer, M., Ferhat, N., Bennoune, O., Fetoui, H., & Soulimani, R. (2022). Chronic thiacloprid exposure impairs cognitive function and triggers mitochondrial apoptosis pathway in rat striatum and hippocampus: neuropreventive effect of bitter apricot kernels extract (prunus armeniaca l.). *Journal of Microbiology, Biotechnology and Food Sciences*, 12(3), e9089. <https://doi.org/10.55251/jmbfs.9089>
- EFSA (European Food Safety Authority) (2011). Guidance on default assumptions used by the EFSA scientific panels and committee, and EFSA units in the absence of actual measured data: 1-30 <https://doi.org/10.2903/j.efsa.2012.2579>
- Ellman GL (1959) Tissue sulfhydryl groups. *Archives of biochemistry and biophysics* 82(1): 70-77 [https://doi.org/10.1016/0003-9861\(59\)90090-6](https://doi.org/10.1016/0003-9861(59)90090-6)
- Ellman GL, Courtney KD, Andres JrV, Featherstone RM (1961) A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochemical pharmacology* 7(2): 88-95 <https://linkinghub.elsevier.com/retrieve/pii/0006295261901459>
- Elser, B. A., Kayali, K., Dhakal, R., O'Hare, B., Wang, K., Lehmler, H. J., & Stevens, H. E. (2020). Combined maternal exposure to cypermethrin and stress affect embryonic brain and placental outcomes in mice. *Toxicological Sciences*, 175(2), 182-196. <https://doi.org/10.1093/toxsci/xfaa040>
- Farag, M. R., Alagawany, M., Moselhy, A. A., Said, E. N., Ismail, T. A., Di Cerbo, A., ... & Ahmed, M. M. (2022). The Neonicotinoid Thiacloprid Interferes with the Development, Brain Antioxidants, and Neurochemistry of Chicken Embryos and Alters the Hatching Behavior: Modulatory Potential of Phytochemicals. *Biology*, 11(1), 73. <https://doi.org/10.3390/biology11010073>
- Feather-Schussler, D. N., & Ferguson, T. S. (2016). A battery of motor tests in a neonatal mouse model of cerebral palsy. *JoVE (Journal of Visualized Experiments)*, (117), e53569. <https://doi.org/10.3791/53569>
- Flohe L, Günzler WA (1984) Assays of glutathione peroxidase. *Methods in enzymology* 105: 114-120 [https://doi.org/10.1016/S0076-6879\(84\)05015-1](https://doi.org/10.1016/S0076-6879(84)05015-1)
- Gasmí, S., Benaicha, B., Kebieche, M., Mennai, I. (2022). Preventive effects of citrullus colocynthis. L plant extract on deltamethrin pesticide induced pneumotoxicity in wister rats, *Journal of microbiology, biotechnology and food sciences: Vol. 11 No. 5* <https://doi.org/10.55251/jmbfs.5268>
- Guiner RB, Bradman A, Castorina R, Holland NT, Avery D, Harley KG, et al. Prenatal Residential Proximity to Agricultural Pesticide Use and IQ in 7-Year-Old Children. *Environ Res.* 2017; 158: 358-365. <https://doi.org/10.1016/j.envres.2017.06.036> <https://doi.org/10.1289/EHP504>
- Habig WH, Pabst MJ, Jakoby WB (1974) Glutathione S-transferases the first enzymatic step in mercapturic acid formation. *Journal of biological Chemistry* 249(22): 7130-7139. [https://doi.org/10.1016/s0021-9258\(19\)42083-8](https://doi.org/10.1016/s0021-9258(19)42083-8)
- Haense, C., Kalbe, E., Herholz, K., Hohmann, C., Neumaier, B., Kraus, R., & Heiss, W. D. (2012). Cholinergic system function and cognition in mild cognitive impairment. *Neurobiology of aging*, 33(5), 867-877. <https://doi.org/10.1016/j.neurobiolaging.2010.08.015>
- Hosseini, M. J., Naserzadeh, P., Salimi, A., & Pourahmad, J. (2013). Toxicity of cigarette smoke on isolated lung, heart, and brain mitochondria: induction of oxidative stress and cytochrome c release. *Toxicological & Environmental Chemistry*, 95(9), 1624-1637. <https://doi.org/10.1080/02772248.2014.884206>
- Jallow MF, Awadh DG, Albaho MS, Devi VY, Ahmad N (2017) Monitoring of pesticide residues in commonly used fruits and vegetables in Kuwait. *International journal of environmental research and public health* 14(8): 833 <https://doi.org/10.3390/ijerph14080833>
- Katić, A., Kašuba, V., Kopjar, N., Lovaković, B. T., Čermak, A. M. M., Mendaš, G., ... & Želježić, D. (2021). Effects of low-level imidacloprid oral exposure on cholinesterase activity, oxidative stress responses, and primary DNA damage in the blood and brain of male Wistar rats. *Chemico-Biological Interactions*, 338, 109287. <https://doi.org/10.1016/j.cbi.2020.109287>
- Kristal BS, Park BK, Yu BP (1996) 4-hydroxynonénal est un puissant inducteur de la transition de perméabilité mitochondriale. *J. Biol. Chem* 271 : 6033-6038 <https://doi.org/10.1093/emboj/cdg412>
- Laugeray, A., Herzine, A., Perche, O., Richard, O., Montecot-Dubourg, C., Menuet, A., ... & Mortaud, S. (2017). In utero and lactational exposure to low-doses of the pyrethroid insecticide cypermethrin leads to neurodevelopmental defects in male mice—An ethological and transcriptomic study. *PLoS one*, 12(10), e0184475. <https://doi.org/10.1371/journal.pone.0184475>
- Li, J., Yu W, Li XT, Qi SH, Li B (2014) The effects of propofol on mitochondrial dysfunction following focal cerebral ischemia-reperfusion in rats. *Neuropharmacology* 77: 358-368 <https://doi.org/10.1016/j.neuropharm.2013.08.029>
- Liu, X., Zhang, Q., Li, S., Mi, P., Chen, D., Zhao, X., & Feng, X. (2018). Developmental toxicity and neurotoxicity of synthetic organic insecticides in zebrafish (*Danio rerio*): A comparative study of deltamethrin, acephate, and thiamethoxam. *Chemosphere*, 199, 16-25. <https://doi.org/10.1016/j.chemosphere.2018.01.176>
- Maeda, M., Kitauchi, S., Hirano, T., Ikenaka, Y., Nishi, M., Shoda, A., & Hoshi, N. (2021). Fetal and lactational exposure of the no-observed-adverse-effect level (NOAEL) dose of the neonicotinoid pesticide clothianidin inhibits neurogenesis and induces different behavioral abnormalities at the developmental stages in male mice. *Journal of Veterinary Medical Science*, 20-0721. <https://doi.org/10.1292/jvms.20-0721>
- Maurya, S. K., Mishra, J., Tripathi, V. K., Sharma, R., & Siddiqui, M. H. (2014). Cypermethrin induces astrocyte damage: Role of aberrant Ca²⁺, ROS, JNK, P38, matrix metalloproteinase 2 and migration related reelin protein. *Pesticide biochemistry and physiology*, 111, 51-59. <https://doi.org/10.1016/j.pestbp.2014.03.005>
- Muhammed, R. E., El-Desouky, M. A., Abo-Seda, S. B., Nahas, A. A., Elhakim, H. K., & Alkhalaf, M. I. (2020). The protecting role of Moringa oleifera in cypermethrin-induced mitochondrial dysfunction and apoptotic events in rats brain. *Journal of King Saud University-Science*, 32(6), 2717-2722. <https://doi.org/10.1016/j.jksus.2020.06.006>
- Niehaus WG, Samuelson B (1968) Formation of malondialdehyde from and glucose 6-phosphate dehydrogenase from fermenting yeast and phospholipids arachidonate during microsomal lipid peroxidation. *Eur J Biochem* 6: 126-30
- Önal, A., Uysal, A., Ülker, S., Delen, Y., Yurtseven, M. E., & Evinç, A. (2004). Alterations of brain tissue in fetal rats exposed to nicotine in utero: possible

- involvement of nitric oxide and catecholamines. *Neurotoxicology and teratology*, 26(1), 103-112. <https://doi.org/10.1016/j.ntt.2003.08.005>
- Osaka, A., Ueyama, J., Kondo, T., Nomura, H., Sugiura, Y., Saito, I., ... & Kamijima, M. (2016). Exposure characterization of three major insecticide lines in urine of young children in Japan—neonicotinoids, organophosphates, and pyrethroids. *Environmental research*, 147, 89-96. <https://doi.org/10.1016/j.envres.2016.01.028>
- Panel M, Ghaleh B, Morin D (2018) Mitochondria and aging: A role for the mitochondrial transition pore? *Aging cell* 17(4): e12793 <https://doi.org/10.1111/ace1.12793>
- Peiffer, J. (2011). Étude de la neurotoxicité d'un Polluant Organique Persistant chez le rat: effets à court et à long terme de l'inhalation répétée de fluorène sur le développement sensori-moteur du jeune et le comportement à l'âge adulte (Doctoral dissertation, Institut National Polytechnique de Lorraine).
- Raszewski, G., Lemieszek, M. K., & Lukawski, K. (2016). Cytotoxicity induced by cypermethrin in human neuroblastoma cell line SH-SY5Y. *Annals of Agricultural and Environmental Medicine*, 23(1). <https://doi.org/10.5604/12321966.1196863>
- Sahu BD, Tatireddy S, Koneru M, Borkar RM, Kumar JM, Kuncha M, Srinivas R, Sunder SR, Sistla R (2014) Naringin ameliorates gentamicin-induced nephrotoxicity and associated mitochondrial dysfunction, apoptosis and inflammation in rats: possible mechanism of nephroprotection. *Toxicology and applied pharmacology* 277(1): 8-20 <https://doi.org/10.1016/j.taap.2014.02.022>
- Sano, K., Isobe, T., Yang, J., Win-Shwe, T. T., Yoshikane, M., Nakayama, S. F., ... & Maekawa, F. (2016). In utero and lactational exposure to acetamiprid induces abnormalities in socio-sexual and anxiety-related behaviors of male mice. *Frontiers in neuroscience*, 10, 228. <https://doi.org/10.3389/fnins.2016.00228>
- Scarpato, R., Testi, S., Colosimo, V., Crespo, C. G., Micheli, C., Azzara, A., ... & Ghirri, P. (2020). Role of oxidative stress, genome damage and DNA methylation as determinants of pathological conditions in the newborn: an overview from conception to early neonatal stage. *Mutation Research/Reviews in Mutation Research*, 783, 108295. <https://doi.org/10.1016/j.mrrev.2019.108295>
- Sharma, P., Firdous, S., & Singh, R. (2014). Neurotoxic effect of cypermethrin and protective role of resveratrol in Wistar rats. *International Journal of Nutrition, Pharmacology, Neurological Diseases*, 4(2), 104. <https://doi.org/10.4103/2231-0738.129598>
- Shelton JF, Geraghty EM, Tancredi DJ, Delwiche LD, Schmidt RJ, Ritz B, et al. Neurodevelopmental disorders and prenatal residential proximity to agricultural pesticides: the CHARGE study. *Environ Health Perspect*. 2014; 122: 1103-1109. <https://doi.org/10.1289/ehp.1307044>
- Shittu, M., Ambali, S. F., Ayo, J. O., Kawu, M. U., & Olatunji, A. O. (2021). Melatonin mitigates thyroid dysfunction induced by gestational and lactational exposure to mixture of chlorpyrifos and cypermethrin in male Wistar rats. *Comparative Clinical Pathology*, 30(3), 529-540. <https://doi.org/10.1007/s00580-021-03247-8>
- Sinha, C., Seth, K., Islam, F., Chaturvedi, R. K., Shukla, S., Mathur, N., ... & Agrawal, A. K. (2006). Behavioral and neurochemical effects induced by pyrethroid-based mosquito repellent exposure in rat offsprings during prenatal and early postnatal period. *Neurotoxicology and teratology*, 28(4), 472-481. <https://doi.org/10.1016/j.ntt.2006.03.005>
- Skretteberg, L. G., Lyrån, B., Holen, B., Jansson, A., Fohgelberg, P., Siivinen, K., ... & Jensen, B. H. (2015). Pesticide residues in food of plant origin from Southeast Asia—A Nordic project. *Food Control*, 51, 225-235. <https://doi.org/10.1016/j.foodcont.2014.11.008>
- Sunyer, J., & Dadvand, P. (2019). Pre-natal brain development as a target for urban air pollution. *Basic & clinical pharmacology & toxicology*, 125, 81-88. <https://doi.org/10.1111/bcpt.13226>
- Syed, F., John, P. J., & Soni, I. (2016). Neurodevelopmental consequences of gestational and lactational exposure to pyrethroids in rats. *Environmental toxicology*, 31(12), 1761-1770. <https://doi.org/10.1002/tox.22178>
- Yadav, A., Tandon, A., Seth, B., Goyal, S., Singh, S. J., Tiwari, S. K; Chaturvedi, R. K. (2020). Cypermethrin Impairs Hippocampal Neurogenesis and Cognitive Functions by Altering Neural Fate Decisions in the Rat Brain. *Molecular Neurobiology*. <https://doi.org/10.1007/s12035-020-02108-9>
- Yuan, T.F., Li, A., Sun, X., Ouyang, H., Campos, C., Rocha, N.B.F., Arias-Carrion, O., Machado, S., Hou, G. & So, K.F. (2016) Transgenerational Inheritance of Paternal Neurobehavioral Phenotypes: Stress, Addiction, Ageing and Metabolism. *Mol Neurobiol*, 53, 6367-6376. <https://doi.org/10.1523/ENEURO.0252-18.2018>
- Zeljezic, D., Vinkovic, B., Kasuba, V., Kopjar, N., Milic, M., & Mladinic, M. (2017). The effect of insecticides chlorpyrifos, α -cypermethrin and imidacloprid on primary DNA damage, TP 53 and c-Myc structural integrity by comet-FISH assay. *Chemosphere*, 182, 332-338. <https://doi.org/10.1016/j.chemosphere.2017.05.010>