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Pyrethroid Insecticides as the Mitochondrial Dysfunction Inducers

Celal Guven, Yusuf Sevgiler and Eylem Taskin

Additional information is available at the end of the chapter

Abstract

Pyrethroids are used to decrease vector-based health concerns and to increase field yield against agricultural pests. Their metabolism is a concern to disrupt a cell's homeostatic machinery via reactive oxygen species (ROS) production. They interact with lipid membranes to damage the fine balance between membrane lipids and membrane proteins, especially mitochondrial substrate transporters and electron carriers. Pyrethroids cause a shift in the metabolic energy production strategy, resulting in ROS production and intracellular lipid deposition. The change of open/closed conformation of some mitochondrial membrane proteins increases the vulnerability of mitochondria to Ca^{2+} ions. Membrane lipid fluidity change is also a concern because of permeability to the substrates and ions to produce energy and other substrates necessary for the cell. Pyrethroids can change the Ca^{2+} signaling and its interaction with ROS signals via disruption of the fine balance between endoplasmic reticulum and mitochondria. They can disrupt the mitochondrial DNA (mtDNA) via their hydrophobic nature or their ROS production capacity. In conclusion, mitochondria are the center of pyrethroid toxicity, and dysfunction of this organelle via pyrethroid toxicity plays an important role in the fate of cell. Their lipophilic and pro-oxidative nature together with Ca^{2+} homeostasis plays a synergistic role in this mitochondrial effect.

Keywords: pyrethroids, insecticides, mitochondria, calcium, reactive oxygen species, mtDNA

1. Introduction

A pesticide has been described as an agent applied to kill, repel, or mitigate industry-, public health- and/or agriculture-related pests. They can also be used as plant growth

regulator or nitrogen stabilizer. We use them to reduce the risk of decreased agricultural and industrial yield and prevent public health concerns such as vector-borne diseases, asthma and allergies, and microbial contamination (for more information: https://www.epa.gov/pesticides). Pesticides have entered into our lives more than 3000 years ago [1] and dried, ground Dalmatian pyrethrum flowers (contain natural pyrethrins) have been used against insect pests since ancient China. It has also been used in Europe more than 200 years ago against cockroaches, bedbugs, flies, and mosquitoes [2]. A pyrethrin-derived synthetic pyrethroid allethrin has been synthesized in 1949 and entered the market in 1952 to use against household pests [3]. To date, there are over 3500 pyrethroid-containing products registered [4].

The primary toxic effect of pyrethroids is on the voltage-gated sodium channels (VGSCs) like organochlorines such as DDT. The opening of these channels is extended by pyrethroid action and this causes the altered nerve function. According to their effect and chemical structure, pyrethroids divided into two types. Type I chemicals (allethrin, bifenthrin, bioresmethrin, permethrin, phenothrin, resmethrin, tefluthrin, and tetramethrin) do not contain a cyano group and they cause slowing in the closure of VGSCs. Therefore, the observed symptoms are tremors and seizures. On the contrary, Type II chemicals (cyfluthrin, cyhalothrin, cypermethrin, cyphenothrin, deltamethrin, fenpropathrin, fenvalerate, flucythrinate, flumethrin, fluvalinate, and tralomethrin) are the ones that are predominantly alpha-cyano-3-phenoxybenzyl alcohol esters and they cause a longer duration in the sodium current. The observed symptom is choreoathetosis accompanied by profuse salivation [5, 6]. Permethrin, a Type I pyrethroid, has not a disordering effect on polar head groups of phospholipids while it localizes within the hydrocarbon core [7]. Because of its cyano group, cypermethrin, a Type II, localizes preferentially in the hydrophilic/hydrophobic region of the lipid plasma membrane, shows greater permanence and more fluidic effect on the membrane compared to permethrin [8]. Therefore, the permanence of cypermethrin can be connected to the prolonged opening of sodium channels. This interaction could also be related to the more reduction of lipid-lipid interactions compared to Type Is; therefore, it decreases plasma membrane fluidity that is linked to the affected Na^+-K^+ ATPase activity to become the plasma membrane more permeable to the Na^+ cations [8]. Type I pyrethroids have a higher binding affinity to the protein of VGSCs [9] while they penetrate more easily into the cell. Although this is another issue for a review, the mutations observed on the VGSCs' protein produce more resistant individuals against pyrethroid intoxication (for more information, see Silva et al. [10]). There is also a discrimination between these types according to their effects on calcium and chloride channels [11]. **Table 1** shows the chemical structures of pyrethroids that are mostly discussed in the current chapter.

Long-term health effects of pesticides such as their developmental and reproductive, endocrine disruption, neurobehavioral, carcinogenic, and immunological ones besides their acute impact have been considered by many scientists and regulatory services such as WHO, FAO, USEPA, and ECHA for many years. Currently, we experience the pesticides via drinking water, soil, food, and air. Directly ingesting of pesticide products can be assessed as a suicide action, but millions of acute poisoning cases occur in every year worldwide [12]. Although

the pyrethroid insecticides are less persistent in the environment compared to organochlorines, they are highly lipophilic with their high octanol/water partition coefficient (K_{ow}) [13]. Therefore, dietary exposure to these compounds trigger the safety concerns [14]. Indoor application to control household pests is also another path for human exposure.
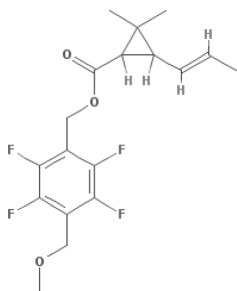
Significant pyrethroid residues have been found in drinking water, human breast milk, and cow milk in a sample location of South Africa where indoor residual spraying was applied for malaria control compared to a mountain population [15]. Malaria control or agricultural applications have caused pyrethroid accumulation such as cypermethrin, lambda-cyhalothrin, esfenvalerate/fenvalerate, and permethrin in breast milk from Brazil, Colombia, and Spain mothers [16]. However, the residues never exceeded the maximum daily intake levels. Babina et al. reported that more than one chemical and simultaneous exposure to organophosphate and pyrethroids was common in South Australian preschool children [17]. Barr et al. surveyed the U.S. population with 5046 samples between the period of 1999 and 2002 to detect pyrethroid residues in urine samples, and they concluded that pyrethroid exposure is widespread in the U.S. population and children probably have higher exposure risk compared to adolescents and adults [18]. Exposure to pyrethroids in the levels common in Canadian children's urine has been associated with parent-reported behavioral anomalies [19]. A sex-dependent attention-deficit/hyperactivity disorder has been found in U.S.
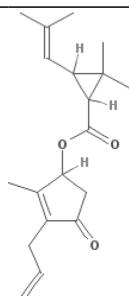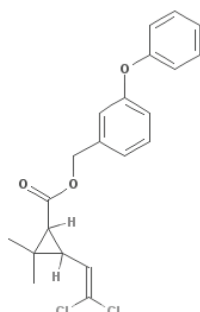
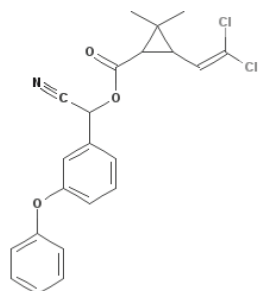| Allethrin[1] (Type I)		IUPAC name: (2-methyl-4-oxo-3-prop-2-enylcyclopent-2-en-1-yl) 2,2-dimethyl-3-(2-methylprop-1-enyl) cyclopropane-1-carboxylate CAS No: 584-79-2
Metofluthrin[2] (Type I)		IUPAC name: [2,3,5,6-tetrafluoro-4-(methoxymethyl) phenyl] methyl 2,2-dimethyl-3-[(E)-prop-1-enyl] cyclopropane-1-carboxylate CAS No: 240494-70-6
Permethrin[3] (Type I)		IUPAC name: (3-phenoxyphenyl) methyl 3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropane-1-carboxylate CAS No: 52645-53-1

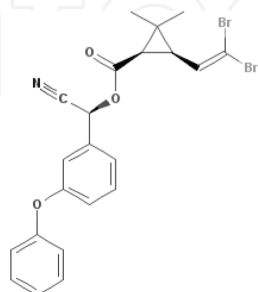|		
Cypermethrin[4] (Type II)		IUPAC name: [cyano-(3-phenoxyphenyl) methyl] 3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropane-1-carboxylate CAS No: 52315-07-8
Deltamethrin[5] (Type II)		IUPAC name: [(S)-cyano-(3-phenoxyphenyl) methyl] (1R,3R)-3-(2,2-dibromoethenyl)-2,2-dimethylcyclopropane-1-carboxylate CAS No: 52918-63-5

[1]National Center for Biotechnology Information. PubChem Compound Database; CID = 11,442, https://pubchem.ncbi.nlm.nih.gov/compound/11442 (accessed June 18, 2018).

[2]National Center for Biotechnology Information. PubChem Compound Database; CID = 5,282,227, https://pubchem.ncbi.nlm.nih.gov/compound/5282227 (accessed June 18, 2018).

[3]National Center for Biotechnology Information. PubChem Compound Database; CID = 40,326, https://pubchem.ncbi.nlm.nih.gov/compound/40326 (accessed June 18, 2018).

[4]National Center for Biotechnology Information. PubChem Compound Database; CID = 2912, https://pubchem.ncbi.nlm.nih.gov/compound/2912 (accessed June 18, 2018).

[5]National Center for Biotechnology Information. PubChem Compound Database; CID = 40,585, https://pubchem.ncbi.nlm.nih.gov/compound/40585 (accessed June 18, 2018).

Table 1. Chemical structures of the pyrethroids that are mostly discussed in the current chapter.

children associated with detectable levels of pyrethroid metabolites in the urine; therefore, abnormalities in the dopamine system that is more threatening for boys may be a result of growing use of pesticides, especially pyrethroids [20]. Urinary pyrethroid residues have been correlated with increased chronic heart disease in nonoccupational exposed Chinese people [21]. Occupational exposure to pyrethroids, for example, in the textile industry, is also an important issue throughout the world [22].

2. Reactive oxygen formation and its relation to the biotransformation of pyrethroids

Pyrethroids are the esters of acids like chrysanthemic acid, halo-substituted chrysanthemic acid, and 2-(4-chlorophenyl)-3-methyl butyric acid and alcohols like allethrolone and 3-phenoxybenzyl alcohol and they mostly contain more than one asymmetric carbon atom [3]. The stereoisomeric nature plays a significant role in the biotransformation of some pyrethroids like fenvalerate [23]. This can also contribute to their toxic effect. For example, different stereoisomeric forms of permethrin have caused the increase in intracellular reactive

oxygen species (ROS) and lipid peroxidation levels and decrease in superoxide dismutase (SOD) and catalase (CAT) activities in rat pheochromocytoma cells (PC12); but this effect is enantioselective, and the most effective stereoisomer is 1*R-trans*-permethrin [24].

Pyrethroid biotransformation in mammals including human consists oxidation, ester hydrolysis (both are called as Phase I reactions), and conjugation with endogenous molecules (Phase II reactions) [3, 25, 26]. Oxidation reactions are catalyzed by isoforms of cytochrome P450s (CYP450s), and ester bonds are hydrolyzed by carboxylesterase(s) [26].

The produced metabolites can be more potent endocrine disruptors than parent compound for humans [27]. Romero et al. found that CYP450-mediated oxidation products of deltamethrin (2'-OH and 4'-OH deltamethrin) are more toxic than the parent compound measured with cell viability, lipid peroxidation, and nitric oxide formation on human dopaminergic neuroblastoma SH-SY5Y cells [28]. Moreover, abnormal locomotor activity observed in prenatal deltamethrin exposure has been associated with increased expression of CYP450 enzymes in the offsprings of rats [29]. However, the pyrethroids are commonly used as a replacement of organophosphate and organochlorine insecticides because of their low mammalian toxicity at the first time of their popularity. The low toxicity has been attributed to their rapid metabolism in mammals [18]. For this reason, their metabolism considered as a detoxification because of rapid clearance from the body [25, 30]. Most of the metabolites are highly hydrophilic, and then rapidly excreted via urine and feces. Some of the metabolites from *R*-cyano-3-phenoxybenzyl alcohol derivative pyrethroids, however, shows incomplete excretion and have longer bioretention in skin and stomach [25, 26]. Moreover, some of the conjugation metabolites are lipophilic and participate in toxicity reactions [25]. The biotransformation to hydrophilic compounds may also be a source of their toxicity in mammals as described below.

A single dose of cypermethrin and/or fenvalerate has caused the increase in SOD and CAT activities and in lipid peroxidation levels in the erythrocytes of rats [31]. As specified, non-cyano (Type I)—cyano (Type II) discrimination can also be observed in oxidative stress-inducing potential of these chemicals. For example, permethrin (a Type I) disturbed the antioxidant defense more than cypermethrin (a Type II) in the erythrocytes of treated rats [8]. Because of its cyano group, cypermethrin shows longer permanence in the membrane, while permethrin can pass easily from this lipid bilayer with its lipophilic nature to reach more readily to cellular subcompartments such as endoplasmic reticulum (ER) membranes that contain CYP450s. Although the presence of α-cyano group decreases the hydrolysis rate of ester bond [32], this group decomposes to cyanides and aldehydes to produce free radicals [33]. Endogenously formed superoxide anion radical is dismutated to hydrogen peroxide (H_2O_2) spontaneously or a SOD-catalyzed reaction. The formed H_2O_2 is degraded to water via CAT in peroxisomes and/or glutathione peroxidases (GPx) in the cytosol, mitochondria, nucleus, and also in peroxisomes [34, 35]. Although the H_2O_2 is not assessed as a ROS, it can act as a substrate for hydroxyl radical formation via a metal (it is mostly iron) catalyzed reaction if it cannot convert to water efficiently. Hydroxyl radical is the strongest radical capable of oxidizing DNA, cellular membrane lipids, and proteins, and there is no effective agent to escape them in the cell [35]. The most important intracellular iron source is the active site of CYP450s because of their iron content in the catalytically active center [36–40].

Pro-oxidant nature of CYP450-mediated pyrethroid metabolism needs further clarification because of superoxide and H_2O_2 release from CYP450 enzymatic complex by CYP450-inducers [35–40]. Pro-oxidative toxicity of pyrethroids has been reported in mammalian studies. Raina et al. suggest that the induction of oxidative stress in dermal cypermethrin exposed rats should be related to its biotransformation via CYP450-catalysis [41]. Metofluthrin, a known carcinogenic agent at high doses, induces mainly CYP2B isoforms and increases oxidative stress via the increase of reduced glutathione (GSH) levels (a well-known cellular antioxidant molecule) in rats [42]. Without an induction of apoptosis, the authors conclude that the metofluthrin has reversible effects, and it may be noncarcinogenic for a human. On the contrary, deltamethrin and permethrin exposure has caused the induction of caspase 3/7 activities; therefore, it has been concluded that oxidative potentials of pyrethroids can trigger the apoptosis in human HepG2 cells and primary hepatocytes [43]. Deltamethrin and permethrin have also caused the stimulation of mRNA transcripts of CYP1A1, CYP3A4, and CYP2B6 isoforms and CYP3A4 protein levels. NADPH-dependent microsomal ROS formation has been observed in the liver of etofenprox exposed rats, and it has been concluded that observed lipid peroxidation and DNA oxidation in the liver should be related with CYP2B-induction by etofenprox exposure [34]. CYP450-mediated cytosolic and/or mitochondrial ROS formation [44, 45] might cause cell death [46], and we conclude that CYP450 activation via pyrethroid exposure might cause mitochondrial damage and cell death. Therefore, CYP450 inducers should be evaluated with this type of side effect.

Deltamethrin exposure has caused early ROS formation and subsequent decrease in GSH levels, Bcl-2 protein expression, and mitochondrial membrane potential and increase in Bax, p38 MAPK expressions, and caspase-3 activity in isolated splenocytes from mice [47]. Similar results have also been found in the brain of deltamethrin exposed rats [48]. The number of apoptotic cells has been decreased by N-acetylcysteine, a well-known antioxidant agent, while buthionine sulfoximine, a GSH depleting agent, worsened the effects [47]. Therefore, when redox balance favors the ROS formation, it could be the main curator of mitochondrial dysfunction and related cell death. Not only synthetic ones but also natural pyrethrins can cause ROS formation and related mitochondrial dysfunction and apoptosis in human hepatocarcinoma cell line HepG2 [49].

In fact, cells can die because of the ER stress-dependent pathways in pyrethroid intoxication. For example, Zhao et al. have suggested nonmitochondrial apoptotic pathway with an extracellular route [50]. According to their model, fenvalerate acts as an endocrine disruptor through the induction of apoptosis of mice germ cells. Fas/FasL-directed caspase-8 activation has caused the germ cell apoptosis without the change in Bcl-2, Bax, mitochondrial and cytosolic cytochrome c, and cleaved procaspase-9 levels.

Interestingly, ER and mitochondria have multiple contact sites called mitochondria-ER associated membranes with a characteristic set of proteins. From these domains, not only Ca^{2+} but also ROS-mediated signals may be transmitted to the mitochondria after ROS-based ER stress (for more details, see [51]). On these domains, inositol-1,4,5-triphosphate receptors interact with voltage-dependent anion channels (VDACs) on the outer membrane of mitochondria to transfer Ca^{2+}. As an important second messenger, Ca^{2+} interacts with other signaling systems

such as subtoxic levels of ROS. There is a fine balance between these two signaling systems and dysfunction in either of these systems can affect another one. Therefore, this situation is harmful or a signal for defense for a cell [52]. As stated in the review of Chirumbolo and Bjørklund [53], we believed that pyrethroids can exert their toxicity via the induction of ROS on ER membranes via CYP450 activity and uncontrolled Ca^{2+} release from ER stores (and/or intracellular flux), which are used to conduct a fine balance between the ER and mitochondria deciding the autophagy or apoptosis. In this sense, we try to explain the mitochondrial effects of pyrethroids considering their oxidative stress-inducing potential and Ca^{2+} homeostasis of the cell.

3. Cellular Ca^{2+} stores and pyrethroids

Cellular Ca^{2+} stores can be a target for pyrethroid action and pyrethroid-mediated intracellular Ca^{2+} load could be related to mitochondrial changes. For example, early life exposure to permethrin increased the intracellular Ca^{2+} influx in the heart of permethrin exposed rats [54]. Pyrethroids can activate the dose-dependent Ca^{2+}-influx in the tetrodotoxin-sensitive pathway (a specific inhibitor of VGSCs) with different potencies and efficacies in mouse primary cortical neurons [55]. However, the changes in Ca^{2+} dynamics could not always be dependent on VGSCs, at least for bifenthrin at nanomolar concentrations in mouse primary cortical neurons [56]. In fact, pyrethroids can modify voltage-gated Ca^{2+} channels at concentrations similar to VGSCs, and Type IIs are more potent to induce Ca^{2+} influx according to voltage- and patch-clamp electrophysiological and *in situ* functional studies [57]. High intracellular Ca^{2+} levels can cause damage to mitochondria [58, 59], and changes in intracellular Ca^{2+} levels via release from ER stores or via Ca^{2+} influx triggers the ROS formation and cell death [58, 60].

Deltamethrin can inactivate the VGSCs. Downregulation of gene transcripts of these proteins in deltamethrin exposed human SK-N-AS neuroblastoma cells has also been observed with an intracellular Ca^{2+} elevation and calpain activation-mediated pathway [61]. Therefore, this situation causes the ER stress-related nonmitochondrial apoptotic pathway in human SK-N-AS neuroblastoma cells by deltamethrin [62]. According to this model, deltamethrin-induced VGSC opening has been caused Ca^{2+} overload and activation of ER stress pathway engaging calpain and caspase-12 without an increase in cytosolic cytochrome c levels (an indicator for mitochondrial apoptotic pathway). In this way, resultant sodium influx via opening the VGSCs can activate the phosphatidylinositol turnover; the intermediates formed via this turnover will activate protein kinase C and the Ca^{2+} release from internal stores [63]. Deltamethrin can activate directly the protein kinase C enzyme at its very low dose [64]. According to the authors, "*deltamethrin has a direct-action site likely to be on protein kinase C, an inositol polyphosphates-independent Ca^{2+} triggering site (e.g., ryanodine receptor and ER stores), and/or phosphoprotein phosphatase.*" Interestingly, deltamethrin was able to increase the inositol 1,4,5-triphosphate levels in rat brain slices in the presence of neomycin or LiCl [64].

Cypermethrin and fenvalerate have rescued the tsBN7 (a temperature sensitive cell type) cells from apoptotic death with elevated temperature compared to cyclosporine A, a mitochondrial membrane permeability transition pore (mtPTP) inhibitor [65]. According to the authors, elevation in cytosolic Ca^{2+} is at the core of the formation of mtPTP, and these pyrethroids

could be effective via their disruptive effect on Ca^{2+} balance. Ca^{2+} overload only can contribute to the formation of mtPTP; however, oxidative stress measured with excessive ROS formation and Ca^{2+} overload has a synergistic role in the formation of this pore to stimulate mitochondrial apoptosis [66].

Voltage-gated Ca^{2+}-channel activation by allethrin has caused the mitochondrial cell death in rat Leydig cell tumor derived LC540 cells [67]. Allethrin exposure in these cell lines have resulted in the elevation of ROS, lipid peroxidation, intracellular Ca^{2+}, cleaved PARP levels (executed by caspase-1), increased p53 gene expression, fluctuated SOD, CAT, GPx enzyme activities, and decreased mitochondrial membrane potential, Bcl-2, and pro-caspase-3 protein levels. It has been concluded that mitochondrial apoptosis by allethrin could be an important factor in decreased male fertility [67]. Similarly, allethrin exposure has caused the significant decrease in mitochondrial membrane potential and subsequent release of cytochrome c to the cytosol in the human corneal epithelial cell line [68]. Pro-apoptotic Bax expression has been increased, while anti-apoptotic Bcl-2 decreased, resulting in caspase-3 activation. Therefore, allethrin can trigger the mitochondrial apoptotic pathway in human corneal epithelial cells; although, they have not correlated their results with Ca^{2+} signaling.

An interesting support to these findings has been obtained with an estrogen receptor α and β binding studies of pyrethroids [69]. The studied chemicals have weak (fenvalerate) or no (permethrin, deltamethrin, and bifenthrin) binding capacity to estrogen receptor α, while permethrin has shown high affinity binding to estrogen receptor β. Lower but still strong binding to this protein has been observed with deltamethrin and fenvalerate, while bifenthrin has no binding capacity to this receptor. In another study, cypermethrin and permethrin exposure have increased the estrogen receptor α and β mRNA levels in TM4 mouse Sertoli cells to adapt decreased spermatogenic potential under pyrethroid toxicity [70]. Estrogen receptor β plays a role in preventing the mitochondrial apoptotic pathway and its suppression causes Bax activation, cytochrome c release, caspase 3 activation, and PARP cleavage [71].

Dissipation of mitochondrial membrane potential is an important event of apoptotic and necrotic cell deaths. It was observed in deltamethrin exposed rat primary hepatocytes with subsequent elevation of ROS, while programmed necrosis has been measured in these cells [72]. A common cell death sign or toxic insult starts a common cell death progression; but the ATP presence determines the type of cell death, apoptosis or necrosis [66]. Pro-apoptotic potential via the mitochondrial pathway of pyrethroids has been reported in many studies [47, 49, 73]; however, necrosis can also be occurred because of the ATP demand as was seen in the kidney of permethrin exposed rats [74] or in the heart of cypermethrin exposed frogs (*Rana cameroni*) [75].

Anti-apoptotic protein Bcl-xL interacts with VDACs to transfer Ca^{2+} into the mitochondria [76]. A continuous supply of Ca^{2+} into mitochondria via this way is necessary to maintain mitochondrial bioenergetics because of pyruvate, 2-oxoglutarate, and the NAD^+-dependent isocitrate dehydrogenases, and three intramitochondrial tricarboxylic acid cycle (TCA) enzymes are stimulated by Ca^{2+} [77]. Anti-apoptotic members of Bcl-2 proteins (Bcl-2 itself, Bcl-xL, and Mcl-1) localized on the mitochondrial outer membrane and interact with the inositol-1,4,5-triphosphate receptors on the ER membrane to arrange the mitochondrial Ca^{2+} load during apoptotic signals and/or to enhance the mitochondrial metabolism for cellular resistance [76, 78].

Endoplasmic reticulum-mediated Ca^{2+} to mitochondria is necessary to adequate supply of reducing equivalents for oxidative phosphorylation because of enhanced phosphorylation of pyruvate dehydrogenase complex and activated AMPK (AMP-activated protein kinase) in the absence of this supply [79]. Giacomello et al. proposed a schema for anti- or pro-apoptotic proteins in ER-mediated Ca^{2+} supply to mitochondria [80]. Namely, Bax and other pro-apoptotic members of Bcl-2 family proteins enhance the ER Ca^{2+} load, and then mitochondria expose higher Ca^{2+} concentrations, mtPTP opens; while anti-apoptotic members of Bcl-2 cause the balanced Ca^{2+} concentration from ER stores; then apoptosis is inhibited, and the needed ATP levels are supplied enhancing the mitochondrial metabolism. According to Distelhorst and Bootman, under autophagy-promoting conditions, a mitochondrial Ca^{2+} transfer from ER protects the cells from death via adequate elimination of energy demands, while the excessive accumulation of Ca^{2+} via apoptosis-inducing chemicals and/or ROS triggers the irreversible apoptosis progression [81]. In fact, differential stimulation pathway of protein kinase C may result in the desensitization of inositol-1,4,5-triphosphate receptors via their phosphorylation by protein kinase C, which translocates to ER membranes in G-protein coupled protein subunit alpha s-cAMP pathway. In this way, desensitization of receptor to its ligand, inositol 1,4,5-triphosphate results in limited Ca^{2+} release from ER stores [82]. Enan and Matsumura have observed the translocation of protein kinase C from the cytosol to the membrane fraction in pyrethroid exposed rat brain synaptosomes [64]. Deltamethrin has caused the intracellular Ca^{2+} elevation, ROS formation, and mitochondrial apoptosis in HGB human glioblastoma cells; while these effects have been reversed by protein kinase C, ER Ca^{2+} pump, and inositol 1,4,5 formation inhibitors [83]. On the contrary, increased intracellular Ca^{2+} levels were not dependent on the phosphoinositide pathway in the effects of different pyrethroids in mouse primary neocortical neuron culture [55]. Therefore, tissue specificity and the dose-response curve of pyrethroid action on mitochondrial Ca^{2+} supply from ER and apoptosis induction should be further investigated.

4. Mitochondrial electron transport chain and energy production are affected by pyrethroid intoxication

Type I and type II pyrethroids could also be separated according to their toxic effects on different parts of the cell including mitochondria. Noncyano pyrethroid pyrethrin and permethrin increased the mitochondrial metabolic enzyme activities measured with the WST-1 method at low doses probably to support the bioenergetics needs of the cell in SH-SY5Y cells [84] while there is no or little effect on total ATP content. Mitochondrial enzyme activities and total ATP content have been decreased at higher doses. However, the most pronounced effect has been seen with an α-cyano compound cypermethrin starting with the low doses [84]. The same distinction could be done by their effect on human estrogen regulated breast cancer cell line (MCF-7). Coadministration of oestradiol has been potentiated the effects of these pyrethroids measured with total ATP and mitochondrial metabolic enzyme activities; but, the most pronounced effect has been observed in cypermethrin exposure, also [85].

According to the study of Gassner et al., permethrin and cyhalothrin caused the inhibition of complex I of electron transport chain in isolated rat liver mitochondria, and there are more than

40 regions of complex I as potential binding sites for pyrethroids because of their hydrophobic nature [86]. Inhibition of complex I may be related to ROS formation; but, it should be noted that complex I inhibitors can be divided into two groups as ROS producers and ROS production inhibitors [87]. Inhibition of complex I activity by permethrin has been caused a reduction in superoxide radical formation in striatum submitochondrial particles of rats [88]. Inhibition of succinate dehydrogenase activity, which has a role in TCA and in complex II, has been decreased after acute and subacute bifenthrin exposure in rat brain [89]. Deltamethrin has a major inhibition site between complexes II and III because of unaffected NADH dehydrogenase (complex I) and cytochrome c oxidase (complex IV) activities in the isolated rat liver mitochondrial preparation [90]. In this mitochondrial preparation, NADH oxidase, succinate oxidase, succinate dehydrogenase (complex II), NADH-cytochrome c reductase, and succinate cytochrome c reductase activities have been inhibited. Deltamethrin has also caused an inhibition of ADP-stimulated oxygen consumption and impaired the mitochondrial membrane potential [90].

A discrepancy has been found compared to the results presented by Braguini et al. [90]. Cytochrome c oxidase activity has decreased within different time series in deltamethrin-exposed rat brains *in vivo* [91]. In these *in vivo* mitochondrial preparations, deltamethrin has caused a decrease in mitochondrial cytochrome c levels, mitochondrial membrane permeability transition, and mitochondrial membrane potential. These changes can result in a mitochondrial apoptosis and may reveal the neurotoxic action of pyrethroids. However, succinate cytochrome c reductase activity has not changed, while cytochrome c oxidase activity increased in the liver of deltamethrin-intoxicated rats *in vivo* [92]. In these liver preparations, biotransformation enzymes of pyrethroids have also not changed. In addition to their ROS inducing by ER-bound CYP450 activities, pyrethroids can disturb the electron transfer on the transport chain and can cause the altered ATP levels and ROS formation to induce mitochondrial dysfunction and sequential death.

Metabolic shift determined by increased lactate levels are observed in tumor cells although they are grown in oxygenic cultures, and this can be a strategy to avoid oxidative stress and apoptosis induction [93]. Pyrethroid intoxication causes a metabolic shift through the oxidative phosphorylation to anaerobic glycolysis and altered lipid and protein metabolism *in vivo*. Several pyrethroids have decreased the hepatic protein levels, increased hepatic lactate dehydrogenase, blood and plasma urea levels in rats [94, 95]. Authors have concluded that pyrethroids are able to stimulate metabolic shift from oxidative phosphorylation to anaerobic glycolysis. A support for these observations has been obtained in the muscle and heart of cypermethrin exposed rats [96]. It has caused the decreased succinate dehydrogenase while increased glucose-6-phosphate dehydrogenase and lactate dehydrogenase activities reflecting the anaerobiosis. Decreased succinate dehydrogenase activity indicates the inadequate substrate supply for TCA [96]. A similar metabolic shift due to succinate dehydrogenase and malate dehydrogenase inhibition with increased lactate formation and lactate dehydrogenase activity has also been observed in cypermethrin-intoxicated fish *Labeo rohita* [97] or in fenvalerate-intoxicated fish *Oreochromis niloticus* [98].

Hepatic aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase activities, total lipids, phospholipids, free fatty acids, and cholesterol levels have increased, while glycogen and total protein levels decreased in beta-cyfluthrin exposed rats [95]. Aminotransferases produce oxaloacetate and pyruvate intermediates that are transported into the mitochondrial

matrix to maintain TCA or fatty acid production, and accumulation of oxaloacetate has caused the inhibition of malate dehydrogenase activity, a part of TCA of mitochondria in cypermethrin exposed rats [89].

Metabolomics approach is very effective to understand pyrethroid-induced metabolic changes. Reports show the metabolic shift to anaerobic fuel consumption and elevated fuel supply via gluconeogenesis to maintain energy levels in pyrethroid-induced stress conditions. For example, permethrin exposure increased urine lactate, acetate, 3-D-hydroxybutyrate, creatine, glycine, and formate while decreased citrate and 2-oxoglutarate levels in rats [99]. Elevated levels of urinary acetate and decreased TCA intermediates show the energy metabolism disorders. Similarly, Liang et al. reported that permethrin and deltamethrin exposure can cause the disturbance in energy metabolism via the enhanced rate of anaerobic glycolysis and fatty acid β-oxidation, and ketogenesis [100]. They found that these pyrethroids reduced the excretion of TCA intermediates and increased lactate, acetate, 3-D-hydroxybutyrate levels in treated rats. In another study, serum and urine metabolites levels have been changed by deltamethrin exposure, and then it was suggested that decreased utilization of pyruvate in TCA and consecutive anaerobiosis in exposed rats [101]. While a shift from aerobic respiration to anaerobiosis was also found in the brain of lambda-cyhalothrin exposed goldfish (*Carassius auratus*), a marked decrease has been observed in brain *N*-acetyl-aspartate levels, because of neuronal mitochondrial membrane damage via the ROS formation [102]. *N*-acetyl-aspartate is considered as a marker for mitochondrial dysfunction in neurons [103]. Higher levels of malate and alanine in cypermethrin exposed earthworms provide an argument for the increased gluconeogenesis and fueling the TCA for energy [104]. These effects have also been observed in the former studies [100–102]. However, as an opposite of these results, permethrin exposure has caused an increase in TCA intermediates and cellular fatty acids and a decrease in glutamate levels in rat neuroblastoma cell line B50 [105]. Increased fatty acid β-oxidation should be a response to permethrin toxicity in these cells.

Voltage-dependent anion channels located in the mitochondrial outer membrane is the only way to supply TCA intermediates from cytosol to mitochondria, and its closure causes a metabolic shift [106]. However, urea generation is also operated in the mitochondrial matrix, and it requires a bulk of substrates such as ornithine, citrulline, adenine nucleotides, respiratory substrates, and other metabolites across the mitochondrial outer membrane in/out of mitochondria, possibly via VDACs [107]. The mitochondrial outer membrane is rich in VDACs that opens in normal operated mitochondria and mitochondrial hexokinase bounds to VDAC to orchestrate respiration, glycolytic pathway, and other metabolic pathways such as the pentose phosphate shunt [108]. We think that pyrethroids can be effective on these mitochondrial membrane proteins via their substrate and/or membrane docking interaction(s), finally causing a metabolic shift in exposed cells together with their electron transfer disorder effect on transport chain.

5. Lipid metabolism is a target for pyrethroid-induced mitochondrial dysfunction

Reactive oxygen species reduces the oxygen consumption and decreases the fatty acid oxidation in adipocytes causing the lipid accumulation [109]. According to Chirumbolo and

Bjorklund, mitochondrial ROS formation and dysfunction could play a central role in the machinery of lipid accumulation via the interaction with AMPK and peroxisome proliferator-activated receptor (PPAR) pathways [110]. Stressed cells accumulate lipids and enhance the hypoxic stimulus, and this occurs via AMPK-signaling pathways.

Cypermethrin induces the *pyruvate kinase*, *glucose transporter*, *stearoyl-CoA desaturase-1*, *acyl-CoA oxidase*, and *carnitine palmitoyltransferase 1-α* mRNA levels in the liver of mice [111]. *PPAR-α* have also increased with increased pyruvate levels. In this study, hepatic free fatty acid transport genes have also been upregulated; then, cypermethrin is able to defect lipid metabolism and can cause the lipid accumulation (evidenced by increased lipid droplets in histologic sections) in this organ. An interesting situation is the overexpression of *stearoyl-CoA desaturase-1* gene because its activation is related to mitochondrial ROS generation, caspase-3 activation, and apoptotic cell death in the heart of rats fed with saturated fatty acid rich diet [112]. In this study, AMPK phosphorylation has been decreased with the overexpression of *stearoyl-CoA desaturase-1* gene. AMPK inactivation results in the activation of acetyl-CoA carboxylase. It increases malonyl CoA synthesis, and malonyl CoA reduces carnitine palmitoyltransferase activity to transport fatty acids into mitochondria for oxidation. Therefore, fatty acid oxidation is decelerated. It is known that mitochondrial fatty acid oxidation is an important ROS source [113]. However, mitochondria need fatty acids to maintain AMP/ATP ratio and to maintain its functions in physiological levels. Therefore, a subtle balance of fatty acid oxidation must be conducted. In this sense, PPAR (including all three forms) agonists upregulate the AMPK activity to mediate many physiological functions to protect cells from mitochondrial membrane potential change and ROS formation [114].

Carnitine palmitoyltransferase-1 and PPAR-α gene expressions have been upregulated by cypermethrin exposure in the liver of zebrafish (*Danio rerio*) with ROS activation [115]. The results reveal the importance of cypermethrin-induced oxidative stress on impaired fatty acid β-oxidation and mitochondrial dysfunction. PPAR-α is the most significant orchestrator of altered fatty acid metabolism in this process. Relation of pyrethroid-induced lipid accumulation and mitochondrial dysfunction has been conducted with some newer research. While Jin et al. [111] has not been found up or downregulated mRNA expression of *ppar-γ* with cypermethrin intoxication, Moustafa and Hussein [116] reported that lambda-cyhalothrin intoxication caused upregulation of *ppar-α* and *ppar-γ* transcripts in the liver of rats. Hepatic fat infiltration and periportal fatty changes have also been observed with an elevated ROS formation.

Cobalt chloride, a hypoxia mimetic agent, has caused downregulation of PPAR-γ, increased lipid accumulation, mitochondrial ROS production, and autophagy in mouse pre-adipocyte cells [117]. It is known that elevated levels of TNF-α can be found in dysfunctional neuronal cells. The high level exposure of TNF-α to mimic these cells has caused decreased PPAR-γ and AMPK proteins, ATP levels, and mitochondrial mass, while ROS levels and caspase-3 (an apoptotic executioner enzyme) increased in human neuronal stem cells [118]. Rosiglitazone, a PPAR-γ agonist, protected the cells from these adverse effects of TNF-α. Mitochondrial complex I activity has decreased in deltamethrin treated human dopaminergic neuroblastoma SH-SY5Y cells [73]. These cells had typical mitochondrial apoptotic signals. The authors revealed that the mitochondrial apoptosis was antagonized by PPARγ agonist rosiglitazone

resulting in the inhibited translocation of PTEN-induced putative kinase 1 (PINK1) to defend cells against ROS formation by dysfunctional mitochondria. In mitochondrial damage conditions, PINK1 accumulation in outer membrane results with a selective autophagy [119]. Therefore, PINK1-dependent mitophagy is responsible for maintaining a healthy mitochondrial population for undesired excessive ROS formation [120, 121]. Exposure to deltamethrin has caused the apoptotic and autophagic death in rat pheochromocytoma cell line PC12 [122]. Although the autophagy inhibitor, 3-methyladenine exacerbated the deltamethrin toxicity, pre-treatment with autophagy inducer rapamycin and antioxidant *N*-acetylcysteine have increased the cell viability via the prevention of apoptosis progression.

However, autophagy itself can be responsible for the ROS formation [123, 124]; therefore complex I-inhibition related cell death could be derived from PINK1-mediated mitophagy because of the inhibition of ROS formation and apoptosis via an antioxidant or PPAR-γ agonist treatment during mitochondrial autophagosome formation [124, 125]. Mitochondrial fusion can constitute a link between ROS formation and lipid accumulation. Downregulation of Mfn2 gene in human embryonic kidney cells 293 with siRNA caused triglyceride and ROS accumulation and decreased oxygen consumption [126]. Interestingly, impaired mitochondrial dynamics and dysfunctional autophagy can also be a cause *in vivo* triglyceride accumulation in aged rat tissues [126].

Shen et al. [127] reported that mouse pre-adipocyte cells showed increased fat accumulation via AMPK/PPAR-γ intersection by deltamethrin exposure. Phosphorylated AMPK/AMPK (pAMPK/AMPK) ratio has been decreased, while PPAR-γ protein levels increased in these deltamethrin exposed cells. Permethrin has also caused similar changes in these cells with the elevation of triglyceride levels and decrement of *carnitine palmitoyltransferase 1-α* mRNA levels [128]. In this study, permethrin exposure decreased protein kinase B (Akt) and increased its activated phosphorylated forms (at Ser473 and Thr308) in C2C12 myotubes in the presence of insulin. Therefore, permethrin alters lipid metabolism in adipocytes and impaired glucose metabolism in myotubes and then increases the obesity and type-2 diabetes progression risks in exposed individuals. Authors discussed that these changes are related to mitochondrial Ca^{2+} and ROS formation. In another study, pAMPK levels have been increased with increased autophagosome formation and abnormal autophagy in cypermethrin treated rats and SH-SY5Y neuroblastoma cells [129]. Authors indicated that increased phosphorylation of AMPK shows the decreased AMP/ATP ratio via mitochondrial dysfunction. Although the above authors revealed the mitochondrial dysfunction-related adipogenesis, Xiao et al. [130] have defined an intracellular Ca^{2+}- and ER stress-related adipogenesis in permethrin exposed mouse pre-adipocyte cells.

Solute carrier family 25 member 25 (Slc25a25) and *solute carrier family 2 member 1 (Slc2a1)* gene expressions have been affected by deltamethrin and cyfluthrin exposure in the cortical samples of rat brain *in vivo* with many other membrane proteins [131]. Slc25a25 serves as a solute carrier for adenine nucleotides in and from the mitochondrial inner membrane, while Slc2a1 is a major glucose transporter in the blood-brain barrier. These pyrethroids have also affected *pyruvate dehydrogenase kinase 4 (pdk4)* gene expression, which plays a role in glucose metabolism via inhibition of pyruvate dehydrogenase complex by phosphorylation.

Therefore, pyrethroids can be effective on cells at different levels of metabolism. In a similar manner, permethrin caused a significant elevation of *pdk4* and *phosphoenolpyruvate carboxylase (pepck)* gene transcripts in the muscle and liver of mice, respectively [132]. Permethrin exposure displayed similar results [133] that were seen in the study of Kim et al. [128]. In addition, phosphorylated Akt at Thr308 and glucose transporter 4 (glut4) protein levels have been decreased in the muscle; therefore, authors concluded that permethrin can alter the glucose and lipid metabolism via an AMPK-dependent pathway and produce insulin resistance and obesity risk in exposed groups. In contrast, insulin-stimulated Akt phosphorylation has been decreased by permethrin in pAMPK-independent and the ERK-dependent manner in C2C12 myotubes, and this mechanism could be a reason for insulin resistance development [134]. Therefore, the exact mechanism of lipid accumulation in different cell types may use different pathways; however, we believed that ER-mitochondria axis and their relation in Ca^{2+} and ROS signaling are the main curators of these effects of pyrethroids.

Affected lipid metabolism by pyrethroids has also been observed in other studies including fish and mammals [135, 136].

6. Mitochondrial membrane structures and dynamics in pyrethroid intoxication

Another effect of pyrethroids is on the structural integrity and dynamics of mitochondria observed in histopathological studies. *cis*-Permethrin has caused inner membrane disruption, and the cristae have been replaced with a denser matrix in Leydig cells of mice testis [137]. Therefore, hitching of cholesterol delivery diminished the pregnenolone formation, contributing to the endocrine disrupting function of pyrethroids. Mitochondrial swelling associated with ER cisternae has been observed in the liver of cypermethrin exposed rats [138]. Dilated round or ovoid mitochondria with short cristae and clear matrix have also been noticed while small damaged mitochondria containing electron dense inclusions occurred in different time series. A similar result has also been found from an amphibian study. According to Yilmaz et al. [139], severely damaged cristae and loss of mitochondrial matrix have been found in the cypermethrin exposed sciatic nerves of *Rana ridibunda*. Typical tubular appearance loss, perturbed fusion/fission equilibrium favoring the fission, and decreased mitochondrial membrane potential have been observed in tefluthrin, deltamethrin, bifenthrin, and α-cypermethrin exposed rat cerebral astrocytoma C6 cells with an increase in cell death ratio [140]. Obvious mitochondrial hypertrophy with distended membranes has been found in the liver of deltamethrin exposed rats [92]. Deltamethrin exposure resulted in irregular contours of mitochondria, tiny and few cristae, and cloudy matrix. Mitochondrial morphometry has also been affected by deltamethrin exposure. Therefore, mitochondria may be the most vulnerable organelle with its structure-function relationship for pyrethroids toxicity.

Pyrethroids can pass and interact with biological membranes because of their lipophilic nature [84]. As mitochondrion stand its membranous structures, mitochondrial membranes and other proteins in addition to the electron transport proteins are candidate structures for

pyrethroid action. For example, fenvalerate has not interacted with mitochondrial membrane proteins measured with intrinsic protein fluorescence, mainly by tryptophan fluorescence quenching in the isolated mitochondria from *Helicoverpa armigera* larvae (cotton bollworm) [141]. Because of its hydrophobic nature, deltamethrin has increased the mitochondrial membrane rigidity in the isolated rat liver mitochondrial preparation and this can cause the impaired transport of different ions between cytosol and mitochondrial matrix [90]. Permethrin has caused a decrease in mitochondrial membrane fluidity and this could be a reason for a bioenergetic crisis in the cell because of irregular energy transduction in striatum submitochondrial particles of rats [88]. Mitochondrial membrane fluidity at the hydrophilic-hydrophobic region of the bilayer has decreased, while fluidity in the hydrophobic core increased in the heart of 300-day old rats exposed the permethrin between 6 and 21 days of their life [142]. Moreover, decreased cholesterol levels in mitochondrial membranes have been observed while it increased in the plasma membrane of heart cells. Therefore, these observations and pro-oxidative properties of permethrin could cause the altered cardiac ultrastructure and function. This effect of permethrin has also been found in Leydig cells of mice testis as discussed above [137]. As an integral membrane protein, VDAC interacts with membrane cholesterol [143] and ATP synthesis, ATP/ADP exchange by adenine nucleotide translocator (ANT) at the inner membrane, ATP/ADP and metabolite exchange by VDAC can be affected by associated membrane composition [93].

Effects of pyrethroids are not limited to mitochondrial membranes because of fluidity decline in the hydrophobic core of cypermethrin exposed rat erythrocyte plasma membrane [144]. Similar fluidity decline has been observed in deltamethrin exposed common carp (*Cyprinus carpio*) erythrocyte plasma membranes [145]. Phosphatidylethanolamine, phosphoglyceride, phosphatidic acids, and cardiolipin levels were decreased, making the membrane more rigid and less permeable. Decreasing these components can cause oxidative stress and cell membrane ageing. Interestingly, cardiolipin is an exclusive component of the inner mitochondrial membrane, and it plays a significant role in governing the mitochondrial bioenergetics processes (interaction with respiratory chain proteins and substrate carriers) and dynamics [146]. Cardiolipin reduction has been observed via ROS-induced lipid peroxidation in nerve growth factor-deprived rat sympathetic neurons and this has caused the loss of mitochondrial density [147]. As a membranous structure, the same finding may be observed with mitochondrial preparations, but it is an issue for further studies. It has been concluded that high lipophilicity and pro-oxidative potential of pyrethroids can affect the biological membranes with their functional proteins to mediate the dysfunctional mitochondria.

While 18 kDa translocator protein (TSPO; formerly known as peripheral benzodiazepine receptor) ligands PK 11195 and Ro5-4864 are anti-apoptotic in the concentrations close to their TSPO affinity, they can also be pro-apoptotic agents at higher levels [148, 149]. It has been evidenced that pyrethroids can bind and interact with TSPO [150, 151], located on the mitochondrial outer membrane and participates to cholesterol transport as a cholesterol channel into mitochondria collectively with VDAC and ANT [152, 153]. Many type I and type II pyrethroids can bind this protein on rat brain membranes, while fluvalinate and fenvalerate have poor potency [154]. Furthermore, *cis*-permethrin has decreased the mRNA levels of *tspo* in mice testis [137]. In the study of Vadhana et al. [142], mitochondrial cholesterol levels have

been decreased, while cellular and plasma cholesterol levels increased in the heart of permethrin exposed rats. The pyrethroids may interact with TSPO protein with high affinity to affect its interaction with VDAC [93] to decrease cholesterol levels in mitochondria. Because mitochondrial function mostly depends on its membranous structures, a decrease in membranous and inner mitochondrial cholesterol levels could be effective on ROS production and abnormal autophagy as is exemplified above sections. Increased TSPO to VDAC ratio has been correlated with increased ROS production, decreased mitophagy, and accumulation of damaged mitochondria [155, 156]. Therefore, oxidative-stress inducing and apoptotic potential of pyrethroids could also be originated with this capability. TSPO attends to the ROS formation via mitochondrial membrane potential transition [148]. Produced ROS affect the bonding form of cytochrome c to cardiolipin through the tightly to loosely conformation and results in the release of it [157] to induce mitochondrial apoptotic pathway. Interestingly, in the events of VDAC closure and blockage of TSPO function cause a permeability increase of VDAC to Ca^{2+} and this can accelerate the mtPTP opening [158].

7. Mitochondrial DNA and pyrethroids

There are very few studies on the mitochondrial DNA (mtDNA) alterations induced by pyrethroids in vertebrates. According to the results of Wang and Zhao [159] study, mtDNA somatic mutation frequency has been increased in the lung tissue of pesticide exposed (including pyrethroids) fruit growers. They have concluded that the increased frequency of mtDNA mutations may result from ROS formation, and the frequency has somewhat like cancer patients' tissues. Because of the adjacency of mtDNA to possible ROS formation centers in mitochondria [160], pyrethroid-induced mtDNA mutations could be linked to their ROS inducing potentials. In cypermethrin exposed zebrafish larvae, ROS induction has been augmented, while *ogg1* (8-oxoguanine DNA glycosylase) mRNA levels decreased [161]. This gene is responsible for the excision of 8-oxoguanine bases occurred via ROS action on DNA. This enzyme has many alternative splicing variants, all of them are targeted to the mitochondria for localization (PUBMED Gene ID:4968; https://www.ncbi.nlm.nih.gov/gene/4968, last access: January 7, 2018). According to the study of Sampath et al. [162], $Ogg^{-/-}$ mice exhibited a preference to carbohydrate metabolism over fatty acid oxidation via downregulated key fatty acid oxidation genes' and TCA genes' mRNAs. Then, they are susceptible to adiposity and hepatic steatosis. Therefore, pyrethroids might able to change the cellular substrate metabolism, and mtDNA mutations are probably involved in this process.

Pyrethroids bifenthrin, cypermethrin, and deltamethrin have increased ρ-mutation frequency in *Saccharomyces cerevisiae* culture in a dose-dependent manner [163]. This type of mutation occurs mainly on mtDNA by large deletions [164], and mitochondrial protein synthesis and electron transport are blocked [163–165]. Interestingly, there are some studies related to the binding of pyrethroids to DNA macromolecule via different bonding mechanisms [166–169]. For example, permethrin can intercalate with DNA, and it is prone to bind G-C base pairs [167]. On the other hand, a complexation driven mechanism mainly by hydrogen-bond and van der Waals forces has been observed between DNA and tau-fluvalinate and fluvalinate molecules [169]. AT-rich sequences are more susceptible sites for this complexation.

We believed that pyrethroids can interact with mtDNA as seen in their electron transport complex bonding potential; therefore, can create mutations on mtDNA. However, further mechanistic research is needed.

8. Conclusion

In conclusion, pyrethroids can perform their toxic action via their oxidative potentials including unbalanced Ca^{2+} flux in/out of the organelles and cells. Mitochondria might be the most vulnerable organelle for pyrethroid toxicity. Pyrethroids probably can change the interaction of mitochondrion and ER to create an imbalance between the fine equilibrium of ROS and Ca^{2+} signals. This affects the form of cellular metabolic energy production, accumulation of lipids and other metabolites, and cell death type. Pyrethroids can also change the mitochondrial membrane structures to affect their ability for metabolism and ROS production capacity. These effects may be related to the endocrine disruption, diabetic, dopaminergic, and obesity-induction potential of pyrethroids that are observed in exposed individuals as exemplified in the above sections such as altered lipid metabolism and cholesterol delivery into the mitochondria. However, there are many gaps that must be solved, such as, interaction with mitochondrial membrane proteins, specific mutagenesis caused by pyrethroid molecule and mtDNA interaction, etc.

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