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Review

Neurotoxicity Following Exposure to Chlorpyrifos

Mojtaba Ehsanifar [1, *], Alireza Esmaeili [2]

1. Department of Environmental Health Engineering, Torbat Jam Faculty of Medical Sciences, Torbat Jam, Iran; E-Mail: Ehsanifar@gmail.com
2. Department of Emergency Medicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran; E-Mail: AEsmaeili@gmail.com

* **Correspondence:** Mojtaba Ehsanifar; E-Mail: Ehsanifar@gmail.com

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Abstract

Neurotoxicity occurs when exposure to a biological, chemical, or physical agent, especially a neurotoxin, alters the normal activity of the nervous system in a way that results in permanent or reversible damage to neurons and nerve tissue and disrupts the functioning of the brain and nervous system. Chlorpyrifos is a broad-spectrum organophosphorus insecticide that has been used worldwide for more than 50 years and can damage the nervous system of insects by creating neurotoxicity. Epidemiological studies show that exposure to chlorpyrifos is associated with neurological disorders and cardiovascular diseases. Chlorpyrifos can also induce behavioral and developmental abnormalities, neurotoxicity, genotoxicity, hematologic malignancies, histopathological abnormalities, immunotoxicity, and oxidative stress. The mechanism of action of chlorpyrifos involves blocking the active sites of the acetylcholinesterase enzyme, which leads to adverse effects on the nervous system. The molecular mechanism of neuronal damage created in the nervous system is not fully understood. The present study deals with neurotoxicity caused by exposure to chlorpyrifos.

Keywords

Chlorpyrifos exposure; neuroinflammation; genotoxicity; neurotoxicity

1. Introduction

Chlorpyrifos (CPF) is a chlorinated organophosphate (OP) insecticide with the chemical formula $C_9H_{11}Cl_3NO_2PS$ and O,O-diethyl-O-(3,5,6-trichloro-2-pyridinyl)-phosphorothioate (IUPAC name) [1]. CPF is a white or colorless crystalline powder with low aqueous solubility and a mercaptan-like odor (thiol odor) that is available under various trade names, such as Dursban, Lorsban, Suscon, Equity, Empire20 and Whitmire PT270 [2] and it is easily soluble in benzene, corn oil, methanol, DMSO, acetone, xylene, Tween 20, and methylene chloride, among others [3]. CPF is a broadly used insecticide and one of the most well-known agricultural chemicals worldwide. It is found in many economically important crops, such as wheat, soybeans, corn, cereals, apples, peaches, grapes and citrus fruits, nuts and various ornamental plants and is used to protect against chewing varieties and sucking and mite pests. Additionally, CPF is notable for its domestic and domestic applications in developing and developed countries [4-6]. Although CPF is effective at pest control and thus improving crop yield, its toxicity is related to several physiological disorders, including neurological and endocrine disorders, and therefore poses risks to animal and human health [4] (Figure 1). The CPF's primary mechanism involves blocking AChE enzyme active sites and affecting the nervous system. After entering the body, CPF is metabolized to exons by oxidative desulfurization and then hydrolyzed to yield 3,5,6-TCP. Because CPF has a short persistence in the body, its metabolites DETP, TCP, and DEP act as biomarkers for CPF exposure in the urine or blood, and their measurement helps in determining in vivo CPF absorption extent [4]. The induced toxicity of CPF is attributed to CPF activity and CPF-OXT (its bioactive form), particularly in terms of AChE activity inhibition [7]; additionally, compared with CNS AChE, CPF-OXT has been found to impair AChE function, although it has inhibitory effects on plasma cholinesterase and erythrocyte cholinesterase (RBC-ChE) [7-9]. In addition, there are considerable findings that CPF can impact other neural processes and targets that are not directly associated with AChE [10]. In a study on animal models, when CPF was administered in combination with corn oil, the maximum absorption level of CPF was reported to be approximately 80% [11]. In addition, 50 mg/kg body weight oral CPF at a given dose resulted in maximal blood levels metabolites of TCP, DETP, DEP, and CPF within 3 h after dosing [5]. In a study, after 5 mg/kg body weight oral administration to pregnant rats, the maximum blood CPF concentration was reported to be 109 ng/g [12].

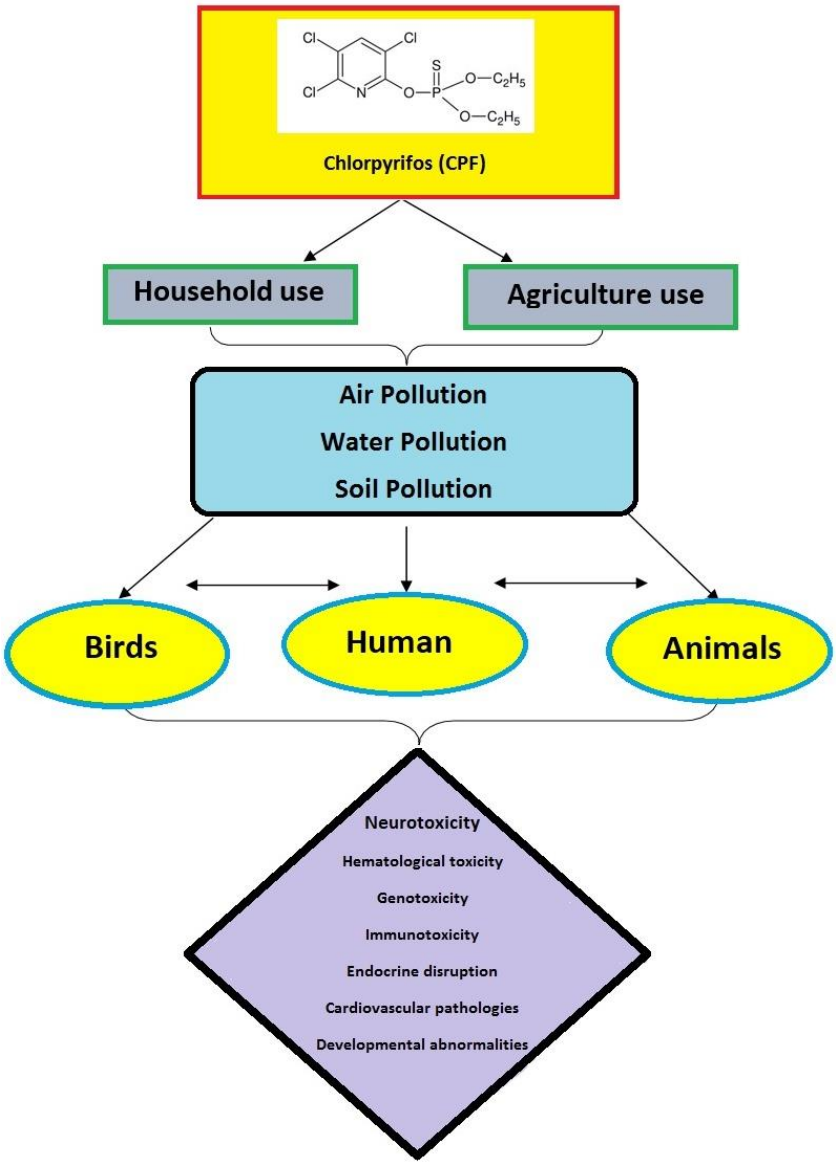

Figure 1 Toxicological effects of chlorpyrifos exposure.

Considering that TCP is a CPM metabolite that is present as a residue on vegetables and fruits at much higher (10- and 20-fold) concentrations than CPF, it is recognized as an insufficient biomarker for detecting exposure to CPF [5]. The insecticide CPM is a CPF structural analog widely used to treat stored grains and usually remains in staple foods. TCP is the major CPF metabolite excreted through urine and can increase the presence of CPF-related urinary metabolites. Oral LD_{50} of CPF varies among different animal species and has been reported to be 135 mg/kg in adult male rats [13]. The main route of CPF excretion after oral exposure in most mammals, including rats, is excretion through the urine [14]. During the test for acute CPF toxicity, the range of oral LD_{50} in rats was from 69 to 276 mg/kg. The highest tissue accumulation was observed in the kidney and liver, and less than 0.1 percent of the total oral dose in goats was observed in their milk [15]. Findings indicate that acute oral LD_{50} varies between 95 and 166 mg/kg body weight [16]. In dogs, the NOAEL was calculated to be 1.8 mg/kg/day [17]; in rats, it was 1 mg/kg/day [18], whereas in humans, it was 0.5 mg/kg [14]. CPF can bind to various proteins, such as plasma ALB. However, its highest accumulation has been detected in adipose tissue [19]. This study was conducted with the aim of emphasizing

more toxicological effects, especially the effect of neurotoxicity on humans, and the resulting damage to human health.

2. Methodology

A systematic search was conducted across multiple databases, including PubMed, Scopus, and Web of Science, using keywords such as chlorpyrifos neurotoxicity, oxidative stress markers, and pesticide neuroinflammation. The review includes studies published between 2015 and 2023, ensuring that the most current findings are represented. The inclusion criteria were focused on peer-reviewed articles that involved both animal and human models and specifically examined CPF's effects on oxidative stress and neuroinflammation. Studies unrelated to CPF, those involving other pesticide classes, or those lacking molecular insights were excluded. The selected studies were synthesized to assess consistent findings on CPF's ability to induce oxidative stress, including the production of reactive oxygen species (ROS), lipid peroxidation, and neuroinflammation. Special attention was given to studies addressing CPF's persistence in the environment and its potential for cumulative neurotoxicity through prolonged exposure.

3. CPF Persistence and Worldwide Prevalence

OP is more than 50% of all insecticides used worldwide, and CPF is one of the wide-spectrum used OP insecticides worldwide [20]. CPF is applied in agriculture and household pest control [9, 21]. CPF was registered in 1965 and entered the US market to control insects in homes, agricultural fields, and other environments, such as golf courses and against fleas, termites, bed bugs, aphids, and chinch bugs, and it was observed that it is very effective against common stem eaters, flea beetles, corn worms, cutworms, earthworms, grasshoppers, mosquitoes and ticks, etc. [4, 22]. The projected CAPs in the global market are estimated to be 5.5% between 2018 and 2023 [23]. CPF has a broad spectrum of effectiveness as a pesticide. Although mammalian bodies are less susceptible to CPF toxicity than insects are, CPF residues have been observed mostly in invertebrates, vertebrates and all living organisms [5]. During the 1960s, the use of CPF peaked, and between 1974 and 2005, approximately 278 cases of CPF poisoning in humans were reported in the USA [24]. This statistic was revealed in North America in 2000 due to a decrease in the use of CPF, as restrictions had been imposed [25]. Therefore, agricultural applications of CPF constitute 16% of the global consumption in the United States [5]. Due to its wide application, short shelf life, cost-effectiveness, low toxicity range, and high degree of efficacy, CPF has become a suitable choice for farmers worldwide and is still registered as a pesticide in most developed and developing countries; it is used in 50 different food products. However, its excessive use has harmed human and environmental health [26]. The CPF residues in underground water and soil reservoirs also indicate excessive and unwanted CPF use, and the resulting CPF, in addition to being present in edible food, can also infiltrate underground water supplies [27]. Studies conducted on bioaccumulation in water, snow, ice/air, vegetation and sediment samples from polar regions have shown the persistence of high CPF values under these conditions [1]. The residual CPF form can be determined by measuring the levels of DETP, TCP or DEP in blood or urine. Among CPF metabolites, CPF-OXN, the main active metabolite, is the most toxic to organisms [28, 29]. It has been reported that the persistence of CPF in aquatic ecosystems is more significant than that in soil due to its stability against hydrolysis. The persistence of CPF in soil has been shown to vary from a few days to 4 years, depending on environmental

factors such as soil texture. In addition, light degradation is minimized in shaded areas; thus, the half-life and CPF persistence are lower in colder, darker Arctic conditions than in tropical regions with many days of sufficient sunlight, and relatively higher temperatures are significantly greater. In addition, the persistence of these bacteria in soils with low pH was recorded to be greater than that in soils with high pH [1]. Expert studies by EPA in 2000 led to further elimination of domestic consumption of CPF due to its adverse health effects, such as restrictions imposed on the use of CPF in EU countries. Additionally, in 2011, the EPA conducted an initial comprehensive HHRA and revised it in 2014 and 2016; ultimately, in 2018, the EPA led to the US court ruling to ban CPF [5]. Despite that, CPF is still widely used in many countries due to its effectiveness and durability in residual form.

4. Ways of Exposure to CPF

4.1 CPF Absorption Following Ambient and Dietary Exposure

Animals and humans can be exposed to CPF through the ambient, and CPF can be generated through several routes, the main of which are dermal, oral, and respiratory [5, 30]. Affected individuals exhibit a higher degree of SCE, chromosomal aberrations and MN formation than nonexposed individuals. It is relatively common for farm workers, florists, fumigators or pesticide users to have occupational exposure or higher levels of workplace experience. The exposure duration, pesticide concentration, and use/nonuse of personal protective equipment can affect exposure outcomes. However, CPF exposure at trim levels is the primary nonoccupational exposure source [31].

Dietary sources are one of the major causes of nonoccupational CPF exposure in humans. Livestock and plants may be significant sources of human exposure to CPF [5]. Considering that CPF is commonly used in plant applications, pesticide residues, either as CPF or in other forms, such as the TCP form, have been detected in food [32]. This approach can inevitably expose animals and humans to traces of CPF. Therefore, the CPF-methyl presence in staple foods could explain 50% of the increase in urinary TCP of individuals previously exposed to CPF. The increase in the urinary TCP concentration can be attributed to CPF-methyl since most CPF-methyl reported in samples obtained from cereal products is due to TCP [5]. The solubility of CPF in water at 20-25°C is 0.7-2.0 mg/L, so it can permeate soil water when mixed with rain or irrigation water. Even if CPF enters water, it evaporates from the water's surface. However, after spraying, CPF increasingly binds to particles of soil and plant material, and very little CPF is washed and enters the groundwater. Generally, humans/animals may be exposed to CPF via inhalation, dermal, or oral routes; therefore, CPF absorption via these routes may vary and is primarily dose-dependent [33]. CPF administered in corn oil showed good absorption (approximately 80%) at a wide range of CPF dose levels [11]. The DETP, DEP, and TCP metabolites reached their maximum levels within 3 h after ingestion after CPF oral dose (50 mg/kg) in rats. In addition, in humans, a single oral dose of CPF results in the recovery of 70% of metabolites in urine with increased bioavailability [14] because some fractions can be passed through the bile and the feces or must have remained in the body as a partition of lipids. The compounds DEP, TCP, and DETP were well absorbed from the gut within 3 h [34], whereas in humans, 0.5-2 mg/kg CPF was only 20–35% absorbed [11]. In another study, the single-pass gastrointestinal perfusion method was used to determine the absorption rate of CPF, and the results showed that CPF is absorbed via the entire small and large intestines [35]. This difference in the CPF absorption rate may be due to the purity, source or different formulations used. Another study

studied the impacts of oral CPF administration for seven days in chickens (7-15 days). FCP at 5, 10, and 20 mg/kg oral doses significantly improved liver (31-46%), plasma (40-70%), and brain (43-69%) functions within 2 h after consumption by inducing cholinergic toxicity. Moreover, administering lower doses (2 and 4 mg/kg CPF) did not induce remarkable signs of toxicity, but a decrease in locomotor activity was recorded. In addition, a lack of activity was observed in the open-field behavioral pattern of chickens. These findings indicate that several days of CPF exposure can induce behavioral changes [36]. Intrauterine CPF exposure in humans has been investigated, and changes during gestation and low birth weight were observed in newborns [37]. The results also revealed a low sperm count, asthenozoospermia (poor sperm motility), and DNA changes in men. In addition, CPF traces were detected in cervical mucus and breast milk [38].

4.2 CPF Absorption Following Inhalation and Dermal Exposure

CPF absorption may occur through several routes such as inhalation of aerosols [39] through oral and dermal routes. Examination of human volunteer urinalysis (after CPF exposure) revealed that approximately 70% of CPF absorption occurs through the oral route, whereas less than 3% of CPF absorption occurs through the dermal route [14]. Since CPF sale for domestic use is prohibited in many countries, most exposure to inhaled CPF likely involves agricultural (occupational) or rural residential use. Although this concentration is not significant, it poses a serious risk of exposure to CPF in the community. Findings show that symptoms of exposure to CPF through inhalation are faster than those of dermal and oral absorption [5]. Therefore, most current studies on the effects of CPF exposure have focused only on occupational exposure to CPF in pesticide applicators and farm workers. It is generally thought that CPF is well absorbed into the lungs after exposure via the airway, although no measurements have proven this assumption directly [40]. Result studies on Sprague Dawley rats exposed to CPF at 3 to 5300 mg/m^3 concentrations, showed that 5300 mg/m^3 (a dose) for 4 h was lethal to 80% of the rats [5].

In general, skin absorption of CPF is not significantly different between inhalation and oral inhalation. After dermal exposure to CPF, only 1% of the TCP in human urine was recovered, whereas after the oral dose of CPF, approximately 70% of the TCP in urine was recovered. These essential findings indicated that CPF is rapidly absorbed after oral administration but is not well absorbed through the skin; one of the possible reasons may be keratinized layers' presence on the dermis. However, the duration of exposure and the exposed skin area are also effective [14]. Another study reported similar findings: 100% of CPF was absorbed orally, while just 1% of CPF metabolites were observed after dermal exposure [41]. Additionally, it has been shown that not all absorbed doses are excreted in the urine. The CPF half-life after dermal exposure is almost double that after oral exposure, both for the absorption and elimination phases, which is probably due to prolonged residence in the dermis layers or the lipophilicity of CPF [42].

5. CPF Toxicity

Restrictions and in some cases complete bans on the use of OP pesticides such as CPF have been established following numerous reports of health and safety concerns in many world regions [43]. CPF was introduced initially as a replacement for DDT in 1965; subsequently, CPF was introduced as part of the pattern whereby the banned chemical was replaced with another worse chemical [44]. However, due to widespread CPF use its toxic effects have been widely investigated in different

species. Exposure to CPF (0.7 µg/L) in Oncorhynchus kisutch (juvenile coho salmon) reportedly causes a 20% reduction in sensory function due to its neurotoxic effects [45]. Similarly, exposure to CPF caused neurotoxicity in two different Neotropical fish species, namely, Uruguayan tetra (Cheirodon interrupts) and ten-spotted livebearers (Cnesterodon decemmaculatus) [46]. Additionally, CPF toxicity has been observed in marine and terrestrial animals. For example, a 10-40 mg/kg CPF dose was reported as the "lethal dose" for cats [47]. The purported mechanisms of CPF toxicity are shown in Figure 2. Nervous system disorders, respiratory muscle defects, muscle weakness, and diarrhea have also been reported in dogs due to CPF exposure [48]. CPF has been observed to be toxic (LD_{50} = 8.41 mg/kg) to *Phasianus colchicus* (ring-necked pheasants) and harmful (LD_{50} = 5.62 mg/kg) to Quiscalus quiscula (common grackles). Furthermore, exposure to CPF caused severe toxicity in *Passer domesticus* (house sparrows), *Columba livia* (common pigeons), and Turdus migratorius (American robins) (LD_{50} = 10 mg/kg) [49, 50]. The range of CPF exposure in humans is usually determined through urine sample analysis of the TCP concentration. This method is the basis for deciding toxic substances' exposure range and presence [5]. Determining the range of exposure to CPF in humans is usually done through urine sample analysis of TCP concentration [2]. A clinical study found that CPF recovery in urine samples via TCP varied between 20 and 35% of the prescribed dose [11]. Therefore, these two possibilities suggest that either the CPF is retained by body adipose tissue or that the maximum fraction is not absorbed.

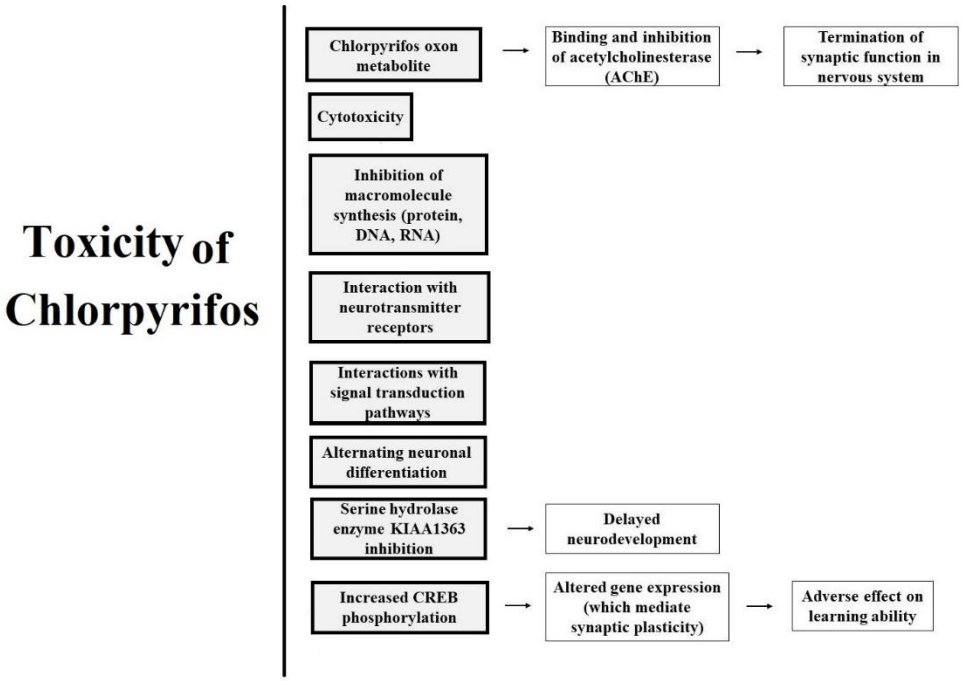

Figure 2 The purported mechanisms of CPF toxicity.

6. Neurotoxicity Following Exposure to CPF

Our previous findings showed that airborne PM could cross from BBB [51-56] and lead to behavioral alterations such as memory and learning disorders [53, 57] and depression and anxiety by causing changes in gene expression, oxidative stress, and neuroinflammation [58, 59]. Symptoms such as headache (cephalgia), numbness, dizziness, nausea and vomiting (vomiting) were observed in patients after accidental exposure, and an increase in CPF was observed after less than 1 hour,

showing rapid absorption and release of the toxic substance. This brain tissue distribution pattern may partially relate to the CPF lipophilic nature [60]. In addition, poisonous and lethal effects of CPF exposure have been observed in animal models. In one study, researchers observed CPF in blood samples from children's umbilical cords and reported a lack of working memory in these children after they reached the age of 7, so its effects were more visible in boys than in girls [61]. Several other studies have shown that the leading cause of disturbances related to the functional and structural integrity of the developing brain due to CPF and/or CPF-oxon exposure is impaired axonal transport and growth mediated by tubulin and similar structural proteins. These disturbances have been indicated to produce abnormal neural connectivity patterns and contribute to neurobehavioral alterations attributed to CPF exposure [43]. Additionally, based on several in vitro studies, CPF can induce neurodevelopmental toxicity by altering downstream signaling pathways involving neurotrophins and disrupting processes significantly regulated by these neurotrophins, neuronal repair, and neurite outgrowth [43, 62]. Other mechanisms underlying the effects of CPF include an imbalance in intracellular Ca^{2+} homeostasis, intensification of oxidative stress, increased signaling via inflammatory mediators such as cytokines and ILs, and protein kinases increased expression, including PKC and MAPK [43, 63, 64]. AChE-mediated enzyme inhibition is the primary mechanism of CPF-induced nervous system damage. It occurs mainly at chemical synapses and in postsynaptic neuromuscular bundles, especially in nerves and muscles, as well as in red blood cells. AChE is a cholinergic serine hydrolase enzyme that is considered to be a neurotransmitter, and by hydrolyzing acetylcholine into the choline and acetic acid, it prevents the dispersion of ACh and, thus, the activation of adjacent receptors [65, 66]. CPF inhibits plasma cholinesterase before brain cholinesterase is suppressed, and this inhibitory pathway is the most reasonable indicator of CPF toxicity [67]. However, in addition to inhibiting cholinesterase, various other mechanisms, such as interfering with cellular signaling components and changing oxidative stress parameters, are involved in the effects of CPF toxicity, which can cause reproductive defects, changes in the antioxidant gene expression and function, and weak estrogenic activity in vivo [68, 69]. In one study, pregnant rats were CPF exposed at doses of 0, 1, or 5 mg/kg on days 17 to 20 of pregnancy, after which the thyroid hormone levels of the in utero-exposed pups were tested. Although males were unaffected, female mice demonstrated the ability to forage in the unique foraging behavior assay maze. Also, CPF exposure caused a dose-dependent decrease in the thyroid hormone level in female mice, and exposure to CPF pesticide in utero caused sex-selective disruption of the thyroid gland and behavioral alterations and abnormalities [70]. In one study, researchers evaluated histopathological alterations in brain cells and associated behavioral changes following exposure to CPF (1.92 mg/L) for 4 days in respiratory catfish. The findings showed that fish whose brains were exposed to CPF had less motor activity, lost balance, and had fewer irregular movements than fish not exposed to CPF. Additionally, the results showed that brain cell exposure to CPF can cause a significant decrease in brain cell function, which leads to changes in brain activity, decreased movement and poor swimming performance [71].

7. Conclusion

CPF is a common OP pesticide used in agricultural and food products, residential homes and other workplaces to eliminate insect pests. Currently, the use of CPF has been banned in many countries due to its toxic effects such as oxidative stress, genotoxicity, neurotoxicity,

immunotoxicity, mutagenicity, cytotoxicity, etc. This study provides a brief description of the poisonous effects of CPF exposure and the possible mechanisms underlying its impact on neurotoxicology, for example, behavioral and developmental abnormalities, neurotoxicity, genotoxicity, hematologic malignancies, histopathological abnormalities, immunotoxicity, and oxidative stress. These surveys indicate that humans are significantly sensitive to dermal and oral CPF exposure, and some of the clinical manifestations include dystonia, cholinergic hyperstimulation symptoms, eye irritation, gear stiffness, Parkinson's disease, and respiratory distress. Thus, CPF's potential safety or toxicity can be resolved by appropriate quantitative tests using laboratory animals followed by human research on the known effects of OP pesticides. More studies and evaluations are needed to determine the health and environmental impact of using these toxins and fill the existing gaps.

Abbreviations

CPF	Chlorpyrifos
TCP	Trichloro-2-Pyridinol
AChE	Acetylcholinesterase
Ach	Acetylcholine
EPA	Environmental Protection Agency
DETP	Diethyl Thiophosphate
DEP	Diethyl Phosphate
CPM	Chlorpyrifos Methyl
NOAEL	No-Observed Adverse Effect Level
HHRA	Human Health Risk Assessment
DMSO	Dimethyl Sulfoxide
OP	Organophosphate
DDT	Dichlorodiphenyltrichloroethane
SCE	Sister Chromatid Exchange
MN	Micronuclei
USA	United States of America
PM	Particulate Matters
BBB	Blood-Brain Barrier
IL	Interleukin
PKC	Protein Kinase C

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Competing Interests

The authors declare that they have no competing interests.

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