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The Effects Of Repeated Low-Dose Exposure To Chlorpyrifos On Cognitive Functions And Potential Non-Cholinergic Mechanisms

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Abstract

Background: Cognitive impairment in animal models has been linked to exposure to chlorpyrifos, an organophosphate pesticide that is widely used worldwide.

Aim: This study's objective was to perform an analysis of the findings from animal models to provide external validation of chlorpyrifos neurotoxic effect. In addition, to consider any potential non-cholinergic mechanisms.

Methods The relationship between chlorpyrifos exposure and cognitive function in animal models was examined in original articles that were found through an online literature search of databases like PubMed, EBSCO, and Google Scholar. Including and excluding criteria were used to choose the articles. Results: Eighteen articles were found in the database, but only 11 met the requirements for inclusion, so these studies were included in the analysis. Most studies on repeated low-dose Chlorpyrifos exposure showed cognitive impairment; however, this effect is unrelated to cholinesterase inhibition.Discussion and conclusion: Collectively, the included studies emphasized that CPF-induced cognitive impairments by chronic low environmental exposures and highlighted various noncholinergic mechanisms, including mitochondrial dysfunction, disturbed brain lipid metabolism, oxidative stress, neuroinflammation, and glutamate-induced excitotoxicity. Hence, the need to keep investigating into non-cholinergic mechanisms of CPF (low dose) is still required in an attempt to help a vulnerable group who are most liable to exposure through new strategies.

***Keywords*:** Chlorpyrifos, cognitive impairment , low dose, non-cholinergic mechanism

1. Introduction

A broad-spectrum organophosphorus (OP) with a high soil adsorption coefficient and contamination (1), chlorpyrifos (CPF) is moderately toxic to humans. All over the world, it replaces extremely toxic Ops in both agricultural and nonagricultural settings. Their affordability, ease of use, and effectiveness against insects, unfortunately, are what drive their use (2). CPF deposits have been identified in human breast milk, urine, and blood after repeated exposure to it through water, agricultural food products, and other foods. They have more human exposure than is deemed safe (3). Due to the harmful effects on children's developing nervous systems, there is an international movement to limit its use (1).

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However, the risk of exposure having a long-term negative impact on human health continues due to residues in food and the environment. Exposures to low levels of OP over an extended period of time that are insufficient to produce acute intoxication symptoms have grown to be a major concern (4,5). An answer is needed to whether exposure to a low level is detrimental since chronic exposures to low levels probably pose a much greater risk to the public than acute poisoning does. They have previously been linked to adult neurological deficits. As a result of OP toxicity, cognitive deficits, depression, anxiety, and personality issues have been reported (6,7). Executive function, speed of processing, visual perceptual skill, and working memory/attention, all of these demonstrated significant associations with impaired neurobehavioral function. The development of cognitive impairment has been linked to exposure to chlorpyrifos in animal models, though the evidence is mixed.

Epidemiology studies frequently evaluate chemical mixture exposure, which makes it difficult to quantify the effects of single compounds as CPF. Different animal models, however, enable the assessment of CPF-related effects comparable to those seen in humans. To provide external validation of its neurotoxic effect, a systematic review of the findings from animal models must be conducted. The symptoms of CPF neurotoxicity are linked to raised brain anticholinesterase activity, ROS, as well as nitrogen species, and nitric oxide levels (8). In a prior study, cognitive impairment was observed at a CPF dose without any impact on brain acetylcholinesterase (AchE) (9). Later, noncholinergic mechanisms have been suggested to play a role in the CPF effect on cognition. Even with low dose CPF, inflammatory responses were seen in the hippocampus after prenatal and postnatal treatment (10–12). Rodents exposed to low doses of CPF showed neurotoxic effects that were more obvious, unrelated to AchE inhibition, however, the fundamental mechanisms are still not well comprehended. Thus, in the present work, we engaged in pooled estimates for the effect of chlorpyrifos exposure on cognitive function and memory in animal model. Additionally, the postulated non cholinergic mechanisms were assessed together with their supporting evidence.

1.1. The search method

By using the following search terms (("Chlorpyrifos"[Mesh]) AND "Memory Disorders"[Mesh]) AND "Learning Disabilities"[Mesh] OR ("Chlorpyrifos"[Mesh]) AND "Neurobehavioral Manifestations"[Mesh]), we were able to locate all publications describing the neurotoxic effect of CPF exposure in different animal species on the cognitive function. Two people independently used different search methods on the databases. Additional publications were found by searching for pertinent articles. Two reviewers independently screened abstracts to determine which ones met our inclusion criteria, and any discrepancies were then discussed with a third party.

1.2. Criteria for inclusion and exclusion

Studies involving learning, cue memory, working memory, and spatial memory in a group of animals exposed to chlorpyrifos were contrasted with a control group receiving an appropriate vehicle. Researches with other neurobehavioral symptoms as outcomes, observational studies, case reports, and acute poisoning effects were all disregarded (Table 1).

1.3. Data extraction

Two researchers extracted the following data from each included study: recipient animal; intervention data (time, administration route, and number of injections); and cognitive outcome evaluations. Morris Water Maze (MWM), radial arm maze, fear conditioned avoidance, open field, and novel object recognition tests were used to evaluate cognitive outcomes. Due to their frequent use in experimental studies, these tests were chosen. We took specifics about the various study characteristics from each publication. The data were treated as an independent study when a single study reported results from multiple experiments. The final data were the ones used in cases when serial neurobehavioral tests' measurements were done (Table 2).

1.4. Methodological quality of the study

The Collaborative Approach to Meta-Analysis and Review of Animal Data from Experimental Studies

(CAMARADES), with some minor modifications, was used to assess the quality of individual studies. There were 10 items on the checklist. Each quality criterion received one point. Two reviewers independently evaluated the studies' study-quality ratings. Animals should be assigned to various treatment groups in a random manner in order to prevent biases. Randomization is a crucial component of experimental design. There should be a clear explanation of the randomization process. randomized blocking or completely random groups in experiments. They regarded the designs as being accurate. (13).

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2. Results

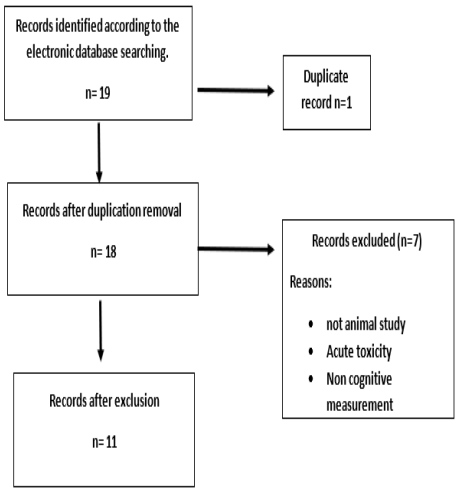
2.1. Study inclusion

A total of 18 publications were identified, of which 11 met our inclusion criteria (Figure 1). In Table 2, characteristics of these studies are shown. According to the modified CAMARADES checklist, the median quality score for the 11 included studies was good (median 70%, with scores ranging from 60% to 80%) No study received a score of either 0 or 10. Six studies reported blinded outcome assessments (Table 3).

2.2. Outcomes: behavioral experiments

In order to evaluate CPS' precocious neurotoxic hazards, CPF either in utero, during gestation and lactation, or postnatal exposure were the ones included. Cognitive function (e.g., working memory, spatial memory, clue memory, learning), anxiety and depression were generally assessed during early postweaning, adolescence, or adulthood. All the included studies employed doses that lower than that expected to inhibit brain AChE. Unfortunately, there are few reliable assessments of AChE in the brain together with neurobehavioral effects. Routes of administration were included oral either by gavage or through dietary supplementation and cutaneous administration, in an attempt to mimic exposure in humans. Table 2 presented a summary of all behavioral tests performed for the cognitive dysfunction.

Fig. 1. Diagram of the review's eligible studies that were evaluated and included.



3. Discussion

Despite the fact that pesticides have generally increased agricultural productivity, their widespread use has severely polluted the environment and put human health in danger. It is widely used due to its broad spectrum of effective and affordable activity and is frequently chosen to replace persistent organochlorine compounds (24). Some studies have concurred CPF's neurotoxic impact, yet there is currently a paucity of knowledge concerning its linkage with neurodegenerative disorders.

Table 1

Inclusion/exclusion criteria for studies in this review article.

Inclusion criteria	**Exclusion criteria**
• **Stu**dies with Chlorpyrifos being the only or one of the components of pesticides. • Effects of repeated prolonged exposure • Neurobehavioral outcomes involve Learning and memory. • **Assessment of cognitive functions was done via multiple testings including; open field, 14-unit T-maze, Morris water maze, Novel object, Fear conditioning, Radial arm WM.**	• Other organophosphates • Effect of Acute poisoning • Human cohort studies • Neuro-behavioral outcome Measures aside from learning and memory. • Physiological and/or biochemical outcomes only were measured. • Control group was not used. • Case reports • Non-English language papers

Table 2

Included studies outline:.

study	Animal, age	Dose, route	Duration of therapy	Outcome, timing	mechanism
(14)	Male Wistar rats (PND60)	18.0 mg/kg every other day sc	30 d	radial arm maze Morris water maze 50 days after the last exposure	none
(15)	Male Wistar rats	18 mg/kg/d sc	14 days	Y-maze novel target recognition 24 h after the last dose of CPF	Oxidative stress induced modulation in GSK3β-Nrf2 signaling pathway
(12)	Neonatal Sprague Dawley rats (17–20 g) PND 11–14	2.5 mg/kg/d	60 days	Morris water maze postnatal day 40-44 and 70-74	Astrocyte activation, with elevated TLR4, p-NF-κB p65 and HMGB1 expression
(16)	Wistar rats	1 mg/kg/ d oral	from GD 1 until the weaning of pups (PND 21)	Male and female Offspring fear conditioning PND 34	None
(17)	Pregnant Hartley guinea pigs GD 53–55	25 mg/kg sc	10 consecutive days	non-associative memory assessed in open fields. MWM spatial memory	Non cholinergic (AchE not inhibited)
(18)	Male Wister rats PND90.	5mg/kg/day dietary	6 months	MWM Seven months after the cessation of CPF dietary treatment	None
(19)	mice carrying apoE2, apoE3 or apoE4. 4 months	2 mg/kg/day Dietary	13 weeks	Barnes maze task.	A role apoE genotype to the severity of the toxicity
(20)	Pregnant guinea pigs GD 50	20 mg/kg/d, s.c	10 days	Morris Water Maze (MWM) at PND 40–45	None
(21)	Male albino Wistar rats	0, 5.0 and 10.0 mg/kg/d intragastric	4 weeks	Morris water maze	mild inhibition effects on cholinesterase activity, non-cholinergic
(22)	Pregnant mice GD 9-18	1, 3, 5, 10 or 20 mg/kg/day sc	Weaned PND25	Morris water maze PN75	None
(23)	Sprague-Dawley rats PND1–4	1 mg/kg/d sc	Weaned PND21	radial arm maze PND64	none

Table 3

The CAMARADES quality items

Study	1	2	3	4	5	6	7	8	9	10	Quality score (√items)	Quality score (%)
(14)	√	√	no	?	no	?	√	√	√	√	6	60%
(15)	√	√	no	√	no	?	√	√	√	√	7	70%
(12)	√	√	no	?	√	?	√	√	√	√	7	70%
(16)	√	√	no	√	√	?	√	√	√	√	8	80%
(17)	√	√	no	?	√	?	√	√	√	√	7	70%
(18)	√	√	no	√	√	?	√	√	√	√	8	80%
(19)	√	√	no	?	√	?	√	√	√	√	7	70%
(20)	√	√	no	√	√	?	√	√	√	√	8	80%
(21)	√	√	no	?	√	?	√	√	√	√	7	70%
(22)	√	√	no	√	√	?	√	√	√	√	8	80%
(23)	√	√	no	√	√	?	√	√	√	√	8	80%s

√ denotes fulfilling the criteria, no denotes not fulfilling the criteria? denotes no enough data to fulfill the criteria. (1) peer-reviewed publication; (2) randomization in treatment groups; (3) dose response evaluation; (4) blind evaluation of behavioral measurements; (5) physiological assessment e.g., body temperature; (6) sample size calculation and sufficient power; (7) animal welfare regulations' commitment; (8) No anesthetics with pronounced neuroprotective effects (e.g., ketamine); (9) conflict of interest declaration; (10) utilizing an appropriate animal model.

Thus, the current study was created to assess the evidence of neurotoxicity caused by low dose chlorpyrifos by critically analyzing the published article that discusses daily exposure. An effort was done to mimic the repetitive nature of the exposure, by which it allows for a gradual, persistent absorption of CPF into blood, simulating human cutaneous exposures being the most common route. (12). Daily CPF doses were set to beneath that resulting in overt acute toxicity symptoms, simulating occupational human exposure. All the included studies employed doses that are lower than that expected to inhibit brain AChE. Unfortunately, brain AChE measurement with neurobehavioral consequences is lacking.

3.1. The possible underlying mechanisms of chlorpyrifos induced neurotoxicity:

The traditional theory of chlorpyrifos neurotoxicity posits that the molecule is converted by the liver to the chlorpyrifos oxon, which results in an irreversible inhibition of acetylcholinesterase, that breaks down the widely present acetylcholine (ACh), is thought to be the established target for OP insecticides. Higher OP exposure levels cause the cholinergic system to become hyperactive and accumulate ACh, which disrupts normal physiological processes. Interestingly, previous literature stated that CPF and other OPs at doses that are insufficient to affect brain ChE and with no resultant cholinergic system hyperactivity, still impacted behavioural and chemical consequences (25, 26). Therefore, the toxicological effects of low doses CPF involve a currently unidentified non-cholinergic mechanism of action.

The late and persistent effects seen after chronic OPs repeated exposure have been theorized to be caused by a variety of non-cholinesterase targets (27).

As first reported by Richards et al., 2000, a number of promising targets have been implicated the chronic CPF's post-exposure hazards. (28). ChE is less likely than acyl peptide hydrolase enzyme to be inhibited by CPF (29). The fact that acyl peptide hydrolase is involved in the degradation of oxidatively damaged proteins makes a role in chronic toxicity possible. Small peptides are broken down by the enzyme into N-acetylated amino acids in the brain, liver, and red blood cells. It plays no part in the acute toxicity of OP exposure (30).

Endocannabinoid system is an additional noncholinergic target that raises concerns about the developmental toxicity of CPF insecticides. This system is a group of neuromodulatory lipids, which is responsible for a number of physiological processes,

including learning, pain perception, and synaptic plasticity. Due to their lipophilic nature, the two main endocannabinoids, anandamide and 2-arachidonoylglycerol are produced and released when needed in response to increases in calcium intracellularly. Endocannabinoids have physiological effects in the brain that depend on the area of the brain, the second messenger system used, and the type of synapse involved. These physiological effects are primarily mediated by cannabinoid receptor 1 (CB1) binding. Following CB1 receptor activation, activation, glycerol-ester acylhydrolase and fatty acid amide hydrolase are crucial enzymes in endocannabinoids' breakdown. (31).

Another suggested non cholinergic mechanism is neuroinflammation that might contribute to the neurotoxic effects of CPF (32). Repeated low-level chlorpyrifos exposure has also been associated with increased inflammatory responses in cultured astrocytes and upregulation of inflammatory cytokines, which significantly impairs spatial memory (33). Acute elevations in ROS' generation could be evoked by CPF per se and not CPF Oxon. Ending CPF's exposure, announces the end of this sequel, probably via defense systems that mitigate the deleterious effect. (34).

Additionally, lipid peroxidation in the developing brain was mentioned. Repeated low-level exposure to chlorpyrifos has been demonstrated to cause neuronal apoptosis in adult rats by raising mitochondrial calcium levels and oxidative stress (35).

Numerous neurodegenerative disorders are largely influenced by problems with mitochondrial function, being responsible for cellular energy via oxidative phosphorylation system (36,37). Recently, CPF-related neurobehavioral complications have been linked to Complex I (a component of oxidative phosphorylation) inhibition (38). Hence, the elimination of damaged mitochondria may contribute to the neurodegenerative changes. The best-known signaling mechanism for mitophagy, which is the selective destruction of mitochondria by autophagy, is regulated by "phosphatase and tensin homolog"-induced putative kinase 1 (PINK1) and ubiquitin ligase (Parkin). CPF-induced apoptosis would be reduced when PINK1/Parkin-mediated mitophagy is activated, denoting increased mitophagy may provide a therapeutic approach for neurological conditions brought on by CPF. (39).

According to previous studies, CPF exposure led to mitochondrial dysfunction and excessive ROS production in PC12 cells, which led to the death of dopaminergic neuronal components (40). This suggests that excessive ROS production and mitochondrial damage may be key factors in CPF-induced neuroapoptosis involving dopaminergic neurons. Moreover, it inhibits gene and protein expression of MAO with more effect on dopaminergic pathway. (41).

CPF raises the level of glutamate extracellularly, however the exact mechanism is still unknown. Antagonists of either NMDA or AMPA glutamate receptors reduced CPF hazardous sequalae, indicating that both types of receptors were implicated in the neuronal death. (42).

Covalent binding of CPF to tubulin is another potential mechanism. Microtubules are considered as pillars in axonal transport. In vitro studies have drawn attention to organophosphates reacting with tubulin disrupting microtubules' structure. (43) .

Serine lipase inhibition may play a role in alterations of FFA in the brain following low dose CPF exposure. The amount of FFA in the brain is regulated by a large number of serine hydrolases that disrupt ester bonds connecting fatty acids to its backbone, glycerol. It was shown that CPF-oxon inhibits serine hydrolases similarly to how AChE does (44,45). The distribution of FFAs and other lipid-derived molecules in the brain may change due to CPF's inhibition of hydrolases and lipases, which could lead to transcriptional changes brought on by FFAs. The serine lipases Fatty Acid Amide Hydrolase (FAAH) and Monoacylglycerol Lipase (MAGL) are both extremely sensitive to different OP oxons. The lipidome of the mouse brain is considerably altered by FAAH or MAGL inhibition, with different brain areas being affected to differing degrees. (46).

Uncertain initiating events are thought to be responsible for the transcriptomic alterations brought on by CPF exposure. However, advances in lipidomic methodologies have shown that novel free fatty acids (FFAs) derived from lipids in organelle membranes or plasma can significantly affect the regulation of transcription (47). Through PPARs and NF-B, free fatty acids can directly influence gene transcription by binding to nuclear receptors in a way that is similar to how hydrophobic hormones do (48,49).

The expression of genes critical for neural signaling and cognitive function can change in response to each of these signaling pathways. FFA can have indirect effects on transcription by altering G-protein-coupled receptors and the protein kinase C (PKC) signaling pathway. FFAs can also have an impact on mRNA turnover rate and protein abundance (50). Cellular differentiation, growth, signaling, and metabolism can all be affected by accumulated FFA.

4. Conclusion

The included studies highlighted that chronic low environmental exposures of CPF induces cognitive impairments and emphasized a number of noncholinergic mechanisms, including mitochondrial dysfunction, abnormal brain lipid metabolism, oxidative stress, neuroinflammation, and glutamate-induced excitotoxicity.

Integrative research utilizing animal models and proper testing for the involved mechanisms would presumingly result in development of better-quality treatments for CPF toxicity.

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