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Review

A comprehensive review on chlorpyrifos toxicity with special reference to endocrine disruption: Evidence of mechanisms, exposures and mitigation strategies

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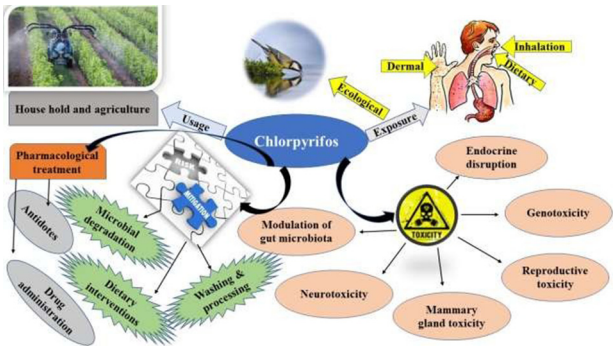
HIGHLIGHTS

- Chlorpyrifos (CPF) is an extensively used insecticide for fruits and vegetables.
- CPF contributes towards the air, water, and land pollution.
- Dietary sources are the foremost cause of non-occupational CPF exposure in humans.
- A number of CPF intoxication incidents have been reported around the world.
- CPF and its environmental degradation products are associated with endocrine disruption.

GRAPHICAL ABSTRACT



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ABSTRACT

Chlorpyrifos (CPF) is a broad-spectrum chlorinated organophosphate (OP) pesticide used for the control of a variety of insects and pathogens in crops, fruits, vegetables, as well as households, and various other locations. The toxicity of CPF has been associated with neurological dysfunctions, endocrine disruption, and cardiovascular diseases (CVDs). It can also induce developmental and behavioral anomalies, hematological malignancies, genotoxicity, histopathological aberrations, immunotoxicity, and oxidative stress as evidenced by animal modeling. Moreover, eye irritation and dermatological defects are also reported due to CPF toxicity. The mechanism of action of CPF involves blocking the active sites of the enzyme, acetylcholinesterase (AChE), thereby producing adverse nervous system effects. Although CPF has low persistence in the body, its active metabolites, 3,5,6-trichloro-2-pyridinol (TCP), and chlorpyrifos-oxon (CPO) are comparatively more persistent, albeit equally toxic, and thus produce serious health complications. The present review has been compiled taking into account the work related to CPF toxicity and provides a brief compilation of CPF-induced defects in animals and humans, emphasizing the abnormalities leading to endocrine disruption, neurotoxicity, reproductive carcinogenesis, and disruptive mammary gland functionality. Moreover, the clinical signs and symptoms associated with the CPF exposure along with the possible pharmacological treatment are reported in this treatise. Additionally, the effect of food processing methods in reducing CPF residues from different agricultural commodities and dietary interventions to curtail the toxicity of CPF has also been discussed.

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Contents

1. Introduction

Chlorpyrifos (CPF) is one of the most recognized agrochemicals worldwide. A pervasively-used insecticide on many economically significant crop plants, such as corn, soybean, wheat, apple, grapes, peach, and citrus fruit trees, grains, nuts, and various ornamental plants for protection against a wide variety of chewing and sucking pests and mites, CPF is also significant for its household and domestic applications in both the developed as well as the developing countries (EPA, 2000; Eaton et al., 2008; Chen et al., 2012). CPF was first registered and introduced into the American market in 1965 for controlling insects in households, agricultural fields, and various non-agricultural settings such as golf course fairways, and was found to be highly effective against termites, fleas, aphids, billbugs, chinch bugs, common stalk borers, cutworms, flea beetles, corn borers, earthworms, ticks, grasshoppers and mosquitoes, etc. (EPA, 2000; ATSDR, 1997).

CPF, despite being effective in controlling pests and consequently, improving crop yields, has been shown to pose human and animal health risks, as its toxicity has been linked with several physiological and lifestyle-related disorders including neurological, endocrine disruption, hematological and reproductive abnormalities (EPA, 2000). The primary mechanism by which CPF affects the nervous system is by blocking the active sites of the enzyme, acetylcholinesterase (AChE). After ingestion, CPF is metabolized by oxidative desulfuration to oxon and then undergoes hydrolysis to yield 3,5,6-trichloro-2-pyridinol (TCP). Since CPF has a low persistence in the body, its metabolites, TCP, diethylthiophosphate (DETP), or diethylphosphate (DEP), function as biomarkers for exposure of CPF in blood or urine, and their measurement can aid in determining the extent of *in vivo* absorption of CPF (EPA, 2000).

CPF-induced toxicity has been attributed to the activity of CPF (and its bioactivated form, CPF-oxon) particularly in terms of the inhibition of AChE activity (Shaffo et al., 2018), and in turn, has been shown to hamper AChE function, albeit the inhibition activity appears to be more significant in the case of the erythrocyte cholinesterase (RBC-ChE) and plasma cholinesterase, as compared to the CNS AChE (Kondakala et al., 2017; Howell 3rd et al., 2018; Shaffo et al., 2018). Moreover, there is significant evidence that CPF-oxon may have effects on various other neuronal targets and processes not directly associated with AChE (Gao et al., 2017). Timchalk et al. (2002) reported that the maximum absorption level of CPF in animal models, when administered in combination with corn oil, was around 80%. Moreover, the oral administration of CPF at the rate of 50 mg/kg body weight manifested in peak blood levels of DEP, DETP, and TCP, the CPF metabolites, within 3 h of dosing (Eaton et al., 2008). Furthermore, Mattsson et al. (2000) in their study with pregnant rats, reported the peak blood levels of CPF at 109 ng/g after oral administration at the rate of 5 mg/kg of body weight.

Timchalk et al. (2007) following oral administration of CPF, DEP, TCP, or DETP to a group of rats at the doses of 140 μmol/kg body weight, reported that the metabolites demonstrated near-optimal absorption, and the peak blood levels were achieved between 1 and 3 h post-dosage. In another experiment, a single-pass intestinal perfusion method was used, for testing the assimilation of CPF by the small intestine in the bodies of rats, and it was discovered that absorption occurred over the entirety of the small intestine and that it can be deduced that almost 99% of the administered CPF was absorbed (Eaton et al., 2008). The analysis of TCP levels in urine samples of 322 human males visiting an infertility clinic revealed a dose-dependent decrease in estradiol levels with increased TCP detected in urine (Meeker et al., 2008). However, according to various studies, TCP is considered as an inadequate biomarker for CPF exposure recognition, because it is also a metabolite of chlorpyrifos-methyl (CPM), occurring in residual forms on fruits and vegetables, in far greater concentrations (between 10 and 20 times) as compared to CPF (Eaton et al., 2008). CPM as an insecticide is a structural analog of

CPF and is extensively used for the treatment of stored grains, and rather more commonly persists in food staples. The major metabolite of CPF is TCP, which is excreted in the urine and can enhance the presence of urinary CPF metabolites.

The oral LD_{50} for CPF has been reported at 135 mg/kg in male Wistar rats. However, this value varies across different animal species (Goel et al., 2007). Following oral exposure, the principal route of CPF excretion in most mammals is through urine (Nolan et al., 1984), including rats. The peak tissue accumulation has been shown in the liver and kidney, and <0.1% of the total oral dose was detected in the case of the goats, in milk. During the test of acute CPF toxicity, oral LD_{50} in rats varied between 69 and 276 mg/kg (Gaines, 1969). However, according to more recent literature, the acute oral LD_{50} ranges between 66 and 195 mg/kg b.w. (Kopjar et al., 2018). When animals were treated with sub-chronic doses, the most prevalent effect noted was cholinergic signs with cholinesterase activity. In dogs, the no-observed-adverse-effect level (NOAEL) was recorded at 1.8 mg/kg/day (Dow, 1964), in rats 1 mg/kg/day (Szabo et al., 1988), while in the case of humans, it was calculated at 0.5 mg/kg (Nolan et al., 1984).

The highest accumulation of CPF has been recorded in the adipose tissues and may also occur in combination with various proteins such as plasma albumin. After an oral CPF dose in rats, it was found that CPF accumulation in the adipose tissue, as compared to other tissues of the body, was higher (Bakke et al., 1976). In 2000, the studies by experts at the US Environmental Protection Agency (EPA) led to the withdrawal of most of the household usage of CPF owing to its adverse effects on health, as well as restrictions introduced regarding CPF application in the European Union countries (Eaton et al., 2008). EPA also conducted a comprehensive preliminary human health risk assessment (HHRA) in 2011, revising it in 2014 and 2016, and ultimately led to a US court order for EPA to ban CPF in 2018. However, it is still used on a large scale in developing countries due to its persistence in residual form, and efficacy. The objective of this publication is to highlight the toxicological, and in particular, the endocrine-disruptive effects in humans, and the resulting damage to human health in the developing countries (Fig. 1).

2. Physical and chemical properties

CPF is a broad-spectrum chlorinated organophosphate (OP) insecticide, with the IUPAC name, *O*,*O*-diethyl-*O*-(3,5,6-trichloro-2-pyridinyl)-phosphorothioate, and chemical formula, $C_9H_{11}Cl_3NO_2PS$ (Watts, 2012). CPF is commercially available under various trade names, most notably, Dursban, Lorsban, Equity, Suscon, Empire20, and Whitmire PT270. Belonging to a class of organic compounds called aryl thiophosphates, the structure of CPF contains the thiophosphoric acid (or its derivative) as a functional group. A colorless to white crystalline powder, with a mercaptan-like (thiol) odor, CPF has low aqueous solubility (Rathod and Garg, 2017). However, it is readily soluble in corn oil, benzene, dimethyl sulfoxide (DMSO), methanol, acetone, xylene, methylene chloride, and Tween 20, among others (Smith et al., 2009). Table 1 lists some salient chemical and biological properties of CPF.

3. Worldwide prevalence

Organophosphate insecticides account for >50% of the total insecticide use worldwide, and CPF is among one of the most widely utilized

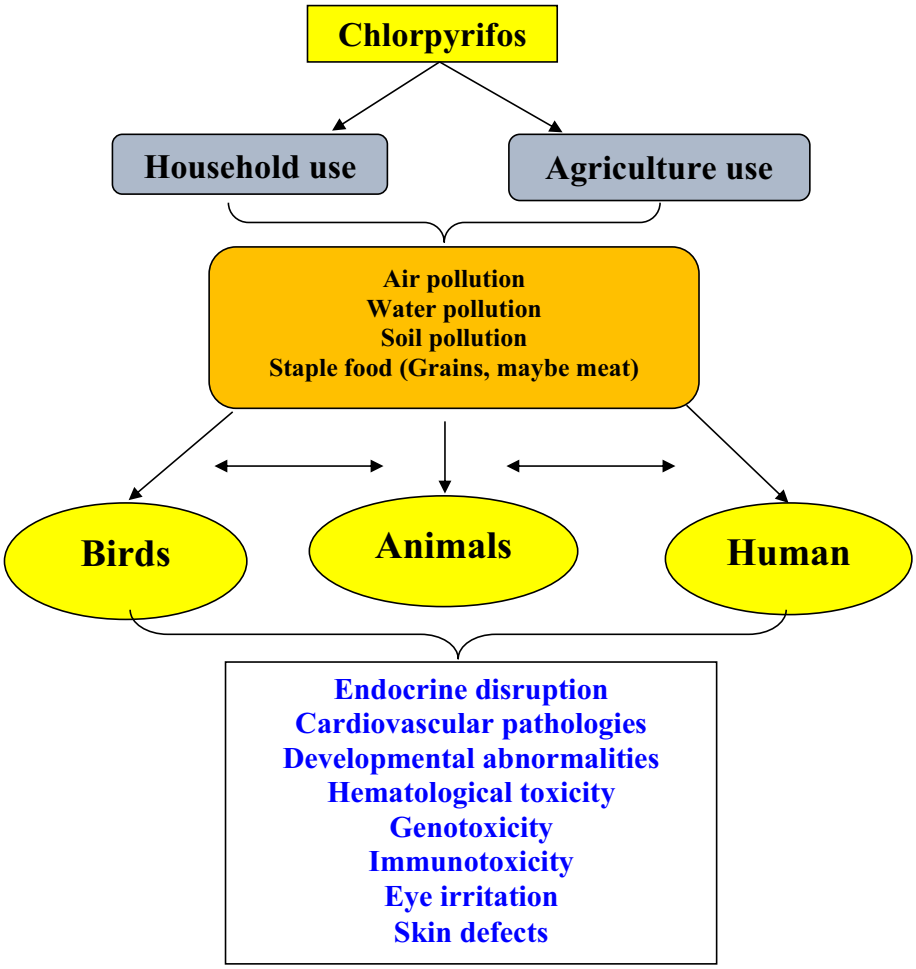


Fig. 1. Environmental and toxicological effects of CPF on humans, birds, and animals.

OP insecticides globally for agricultural applications and domestic pest control (Sharma and Chadha, 2016; Howell 3rd et al., 2018). According to Yang et al. (2020), the worldwide market related to OPs is estimated to have a projected compound annual growth rate (CAGR) of 5.5% between the years 2018–2023. CPF, owing to its wide-ranging applicability, low persistence, cost-efficiency, a high degree of effectiveness, and low range of toxicity, became the insecticide of choice for farmers globally, and is still used in most of the developed and developing countries as a registered product on >50 different food crops. However, indiscriminate, and excessive application practices have resulted in detrimental effects on the environment (Sharma and Chadha, 2016). The discovery of the CPF residues in the soil and groundwater reservoirs of Pakistan also indicates excessive and unsolicited use of CPF and has led to the conclusion that CPF, apart from being present in the edible foodstuff, can also leach into the groundwater reserves (Ahmed et al., 2011).

4. Persistence

Bioaccumulation studies conducted on ice, snow, water/sediment microcosms, air, and vegetation samples from arctic regions indicate a high persistence of CPF under these conditions (Watts, 2012). The residual forms of CPF can be determined through measurements of blood or urine levels of TCP, DETP, or DEP. Among the metabolites of CPF, CPF-oxon, being the major reactive metabolite, is comparatively more toxic for organisms (Wang et al., 2010; Supreeth and Raju, 2017). The persistence of CPF has been reported to be greater in aquatic ecosystems, because of stability to hydrolysis, as compared to that in soils. Soil stability of CPF has been shown to vary, ranging from a few days to up to 4 years, and is dependent upon the soil texture, as well as other environmental factors. Moreover, according to Watts (2012), photodegradation is minimized in shady areas, and as a consequence, the half-life and consequently persistence of CPF, are significantly greater in the colder, darker conditions of the Arctic, as compared to that of tropics with numerous days of ample sunlight and comparatively higher temperatures. Furthermore, persistence was recorded to be greater in soils with low pH (Fig. 2).

5. Exposure

CPF, as a pesticide, has broadband effectivity. The residues have been detected in almost all living organisms, from vertebrates to invertebrates; although the mammalian body has proven to be less prone to CPF toxicity as compared to insects (Eaton et al., 2008). CPF usage peaked during the 1960s, and subsequently, between the years 1974 and 2005, there were around 278 recorded incidents of CPF intoxication in humans (EPA, 2011). These statistics manifested in the form of a reduction in CPF usage in North America in 2000, as restrictions were introduced (Saulsbury et al., 2009). However, 16% of the global CPF use for agricultural applications was reported in the United States (Eaton et al., 2008). Whyatt et al. (2004) investigated the *in utero* exposure of CPF in humans, whereby low birth weight (LBW) and variations in the duration of gestation were observed in the newborn babies. California EPA (2008) reports also revealed DNA alterations, asthenozoospermia (poor sperm motility), and low sperm count in males. Moreover, traces of CPF were discovered in breastmilk, as well as cervical mucus.

5.1. Types of exposure

5.1.1. Environmental exposure

Humans and animals can be exposed to CPF through the environment, and it may transpire through several pathways, the main being the oral, dermal, and inhalation routes (Eaton et al., 2008; Uchendu et al., 2012). The individuals affected, exhibit a greater degree of chromosomal mutations, sister-chromatid exchanges (SCE), and formation of micronuclei (MN) as compared to those unexposed. It is fairly common that floriculturists, agricultural farm workers, fumigators, or pesticide applicators (spraying various pesticides at any given time and are, therefore, highly likely to getting exposed to multiple types of agrochemical mixtures), experience higher occupational, or workplace exposure levels. Bolognesi (2003) concludes that the duration of exposure, and the concentration of pesticide, as well as the use/nonuse of personal protective equipment (PPE) also influences the exposure results. Nevertheless, it is the exposure to CPF at trace levels that constitutes the primary source of incidences of non-occupational exposure.

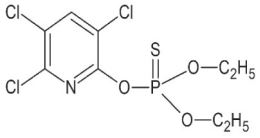
5.1.2. Dietary exposure and absorption of CPF

As CPF is commonly used for crop applications, the pesticide persists in the form of residues on foodstuff, either in undissociated form or in residual forms such as TCP (Han et al., 2009). This, inevitably, can expose humans and animals to traces of CPF that are present on wholegrain food or appear in various processed food products. Dietary sources are thought to be the foremost cause of non-occupational CPF exposure in humans. In addition to plants, livestock can also serve as a major source of CPF exposure for humans. The presence of CPF-methyl in food staples can, therefore, explain the 50% increase in the urinary TCP of already CPF-exposed individuals. Eaton et al. (2008) hypothesized that this increase in urinary TCP could be attributed to CPF-methyl and that most of the CPF-methyl reported on the samples from grain products was in effect, TCP. CPF can also leach into soil water when mixed with irrigation or rainwater since its solubility in water is 0.7 to 2.0 mg/L at 20 to 25 °C. However, after spraying, it binds increasingly to the soil particles and plant materials, and very little CPF enters the water to leach underground. Even if CPF does enter the water, it will volatilize from the water surface.

As mentioned earlier, in general, animals/humans can be exposed to CPF through oral, dermal, and inhalation routes, and the absorption of CPF through these routes, therefore, varies, and primarily depends upon dose. For research purposes, rats and mice were used to categorize the toxicological behavior of CPF and to demonstrate the associated mechanisms. Along with that, few studies were accomplished in farm animals to investigate the residual properties of CPF in milk and meat. CPF administered in corn oil (Sandhu et al., 2013) demonstrated good absorption (approximately 80%) over a range of dose levels (Timchalk et al., 2002). After a 50 mg/kg oral dose of CPF in rats, the metabolites (DEP, DETP, and TCP) achieved their peak levels within 3 h postdosing. Similarly, a single oral dose to humans produced 70% of CPF metabolite recovery in urine (Nolan et al., 1984) with its higher bioavailability because some fractions must have passed through bile and feces or retained in the body as lipid partitioning. When rats were dosed with TCP, DEP, and DETP, these compounds were well absorbed through the intestine (Timchalk et al., 2007) within 3 h, while, in humans, 0.5 to 2 mg/kg CPF was absorbed only up to 20–35% as reported by Timchalk et al. (2002). This difference in the two studies could be due to the source or purity of CPF or different formulations. In another experiment conducted by Cook and Shenoy (2003), a single-pass gastrointestinal perfusion method was used to ascertain the uptake of CPF, with the results indicating that CPF is very well absorbed through the whole of the small and large intestine.

In another investigation, Al-Badrany and Mohammad (2007) studied the effect of oral administration of CPF for seven days using chick modeling (7–15 days). The results revealed that CPF with oral dose rates of 5 mg/kg, 10 mg/kg, and 20 mg/kg considerably reduced plasma (40–70%), liver (31–46%), and brain (43–69%) functionality within 2 h of ingestion by inducing cholinergic toxicities. It was also observed that lower doses of CPF (2 mg/kg, 4 mg/kg) did not induce significant toxicity signs, however, decreased locomotor activity was recorded. Additionally, hypoactivity was noticed in the open-field behavioral paradigm of chicks. The findings strongly suggest that repeated oral exposure of CPF for several days can cause behavioral changes and the results can help conduct further studies elaborating neurobehavioral changes as a result of CPF-induced toxicity.

Table 1
Important physicochemical properties of CPF.

Name/property/hazard	Description	Reference
Common name	Chlorpyrifos	
IUPAC name	*O,O*-diethyl-*O*-(3,5,6-trichloro-2-pyridinyl)-phosphorothioate	Watts (2012); EPA (1992)
Trade names	Dursban, Lorsban, Empire, Equity.	Watts (2012); EPA (1992)
Chemical structure		
Chemical formula	$C_9H_{11}Cl_3NO_3PS$	Eaton et al. (2008); Watts (2012)
Molecular weight	350.575 g/mol	Watts (2012)
Physical state	White crystalline solid	Watts (2012)
Melting point	41.5–42.5 °C	Watts (2012)
Boiling point	Decomposition at 160 °C	Eaton et al. (2008)
Water solubility	0.0014 g/L at 25 °C (less soluble)	Christensen et al. (2009)
Solvents used	Corn oil, dimethylsulfoxide, saline/Tween 20, acetone, methylene chloride, xylene, etc.	Smith et al. (2009); EPA (2011)

(continued on next page)

Table 1 (*continued*)

Name/property/hazard	Description	Reference
NOAEL for short-term acute exposure	0.5 mg/kg	EPA (1992)
NOAEL for long-term chronic exposure	1 mg/kg	EPA (1992)
Vapor pressure	1.87×10^{-5} mm Hg at 25 °C	EPA (2011)
Field half-life	About 60 days	EPA (2011)
Potential metabolites	3,5,6-Trichloro-2-pyridinol (TCP), chlorpyrifos-oxon (CPO)	EPA (2011); Watts (2012)

5.1.3. Dermal exposure and absorption of CPF

Dermal absorption, from the viewpoint of CPF exposure, is generally not perceived as significant when compared to the oral, or inhalation routes. However, dermal exposure is an important pathway of chemical toxicity. An important experiment conducted by Nolan et al. (1984) explained that after an oral dose of CPF, 70% of the TCP was recovered from urine while only 1% of TCP was present in human urine after dermal exposure. This was an important finding that explains "CPF is not very well absorbed through dermal route but is rapidly assimilated once ingested". The possible reason could be the presence of keratinized layers on the dermis. However, it also depends upon the area of skin exposed, and the duration of exposure.

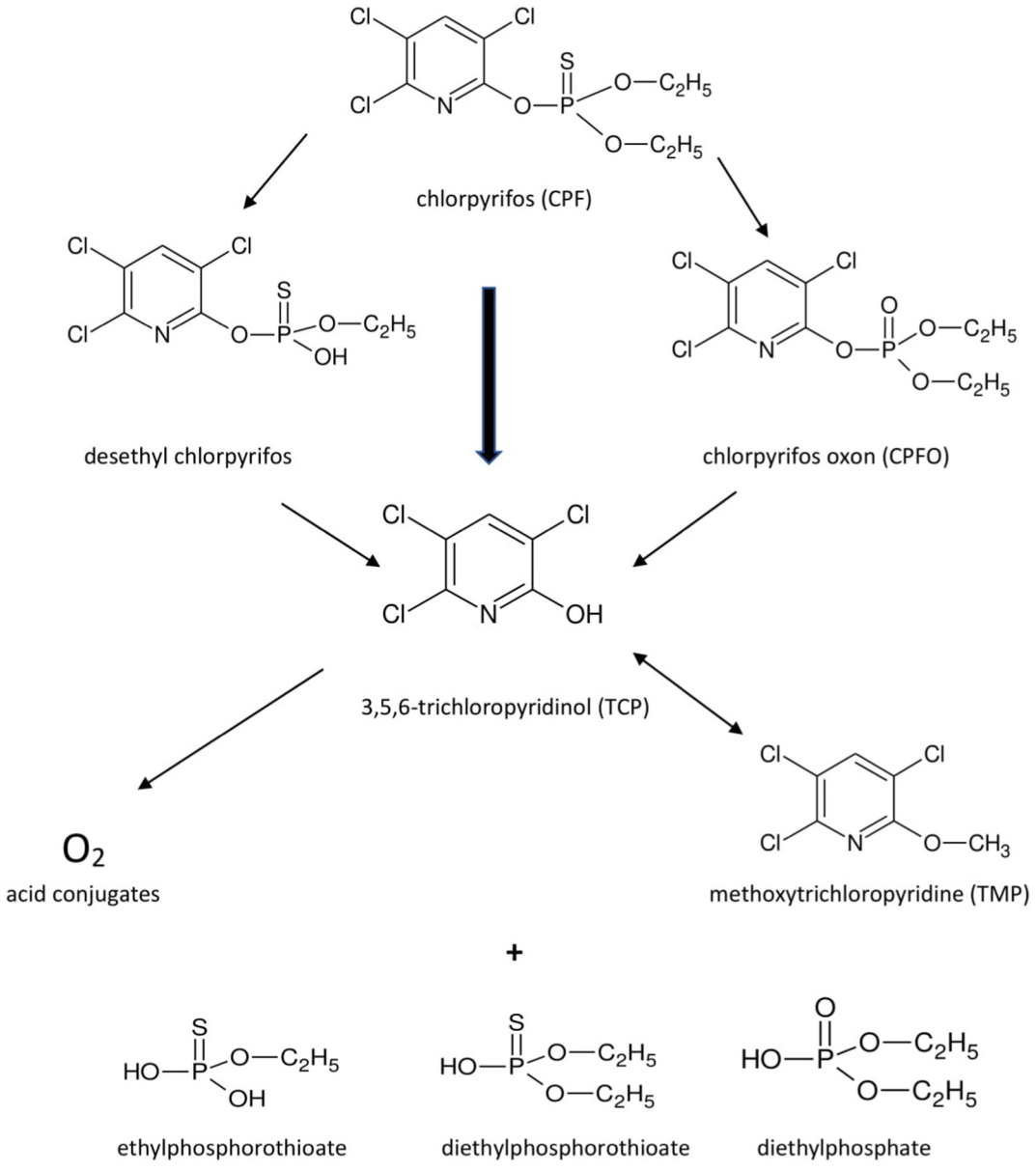


Fig. 2. Pathways for environmental degradation of CPF.
(Adapted from Aponso (2001) and Solomon et al. (2014).)

Another study conducted by Griffin et al. (1999) reported similar results that 1% metabolites of CPF were present after dermal exposure while 100% of CPF was absorbed through the oral route. It is fairly evident that the dermal application of CPF has about twice the half-life as compared to the oral dose both for the absorption-associated phase of the time course, as well as the elimination phase probably due to lipophilicity, or is retained in the dermis layers. However, it has also been established that all the absorbed dose will not be excreted in the urine. Additionally, Meuling et al. (2005) discussed that after application of 5 or 15 mg of CPF on arms of volunteers revealed that the non-absorbed fraction was 42–67% of the applied dose, while 4.3 to 5 mg was excreted in urine as TCP.

5.1.4. Inhalation exposure and absorption of CPF

CPF absorption can occur through multiple routes, including the oral and dermal pathways, whereby urinalysis studies of human volunteers (after experiencing exposure to CPF) indicate an approximate 70% oral absorption, and <3% dermal absorption rates (Nolan et al., 1984). The manifestation of exposure symptoms, however, was more rapid in the case of inhalation as compared to that of oral and dermal absorption. However, since the sale of CPF for household use has been banned in many countries, it is highly probable that most of the inhalation exposure of CPF involves either the agricultural use (occupational) or rural residential, as a result of the drift, although the concentrations are not significant to produce serious community exposure risk (Eaton et al., 2008). Most of the current studies, therefore, are focused only on the occupational exposure of CPF in farm workers, and pesticide applicators.

According to Geer et al. (2004), after the exposure via airborne route, CPF is generally thought to have good absorption in the lungs, although no direct measurements have substantiated this presumption. Short-term research studies in Sprague Dawley (SD) rats regarding CPF exposure have involved the concentrations ranging between 3 and 5300 mg/m^3, with a dose of 5300 mg/m^3 for 4 h proving lethal for 80% of the study cohort (Eaton et al., 2008).

6. Hazards/toxicity of CPF

Organophosphate pesticides have been vastly used for protecting a wide variety of economically significant agricultural crops. However, reports of various safety and health concerns have led to restrictions, as well as in some instances, complete bans on their usage in many regions of the world. Among OPs, CPF has been reported as the most widely employed pesticide for agricultural and non-agricultural purposes worldwide (Burke et al., 2017). CPF was initially introduced as an alternative to the organochlorine insecticide DDT (Dichlorodiphenyltrichloroethane) in 1965, later becoming a part of the pattern, sometimes called as “regrettable substitution”, whereby a banned chemical substance is substituted with another chemical potentially as harmful, or in some instances, even worse (Mughal et al., 2018). The widespread application of CPF, however, led to increased research into its toxicity mechanisms, and greater regulatory scrutiny.

The greater viability of CPF as a pesticide of choice can be attributed to its several advantageous properties when compared to other OPs. The claimed benefits of CPF over other OP pesticides include its broad-spectrum pest control, cost-effectiveness (being a comparatively cheaper alternative), relatively short persistence, tank-mix flexibility (ease of tailoring the formulations as per the requirement), as well as the flexibility to be used in multiple delivery systems, high efficacy, rapid knockdown, less disruptive to the several beneficial insect populations, flexible application methods and timing options, convenience in handling, reliability as a rotation partner for the management of insect resistance, high crop safety, moderate mammalian toxicity, and a robust technical support database (Nelson and Schneider, 2016). The availability of a considerably significant body of research concerning the safety of application, application limits, and mechanisms of toxicity of CPF, as compared to other OP pesticides, essentially implies that the harmful effects of its use can be mitigated in a far more effective manner.

Some researchers have compared the safety and toxicity levels of CPF with other pesticides. In this regard, Kumar et al. (2010) studied the toxicity of different pesticides in the freshwater shrimp (*Paratya australiensis* Kemp) and reported that alpha-cypermethrin induced the highest levels of chronic toxicity in shrimp followed by CPF, carbaryl, dimethoate, fenarimol, and diuron. In another investigation, Wang et al. (2020) compared the toxicities of two OP pesticides (CPF, and acephate), and a triazole fungicide (tetraconazole) against nine pyrethroid pesticides in honey bees (*Apis mellifera* L.), and concluded that OP pesticides were more toxic as compared to the pyrethroid pesticides. Hence, both types of studies are available on the comparative toxic effects of CPF and therefore, comprehensive research is needed to explore the toxicities of other pesticides for a concrete conclusion. However, based on the evidence that CPF can induce numerous types of toxicities as a result of both acute and chronic exposures, the proceeding section summarizes the literature describing the toxic effects of CPF.

OP insecticides produce toxicity with the aid of their activated metabolites by inhibiting the activity of the enzyme AChE in the brain, and peripheral nervous system (PNS), and by altering the activity of serum paraoxonase-1 (PON1) which can be used as an effective biomarker to evaluate the OP-induced toxicity (Dardiotis et al., 2019). As discussed earlier, CPF mostly accumulates in the adipose tissue, often in combination with plasma albumin (Eaton et al., 2008), and it has been increasingly associated with hepatic and immunological modulation, genotoxicity, embryotoxicity, teratogenicity, and neurobehavioral changes (Yin et al., 2009). Apart from the AChE inhibition pathway, CPF can induce toxicity through other mechanisms as well, such as: Affecting the synthesis of macromolecules (DNA, RNA, proteins); Interfering with receptors for neurotransmitters; Interacting with different enzymes; Impeding the pathways for signal transduction; Impairment of the differentiation of neurons; Producing reactive oxygen species culminating in oxidative stress; Obstructing other neurochemical functions, such as the release/uptake of neurotransmitters (Eaton et al., 2008; Saulsbury et al., 2009). Generalized mechanisms of CPF toxicity have been presented in Fig. 3.

The important metabolites of CPF such as TCP, DEP and DETP are generally thought to have no significant toxicological effects however, this conclusion might be based on the limited evidence as several studies report the toxicity of CPF metabolites. For example, CPF-oxon causes irreversible AChE inhibition by binding the active site of the enzyme (Eaton et al., 2008). Likewise, TCP toxicity has also been reported through several investigations with oral LD_{50} value of 800 mg/kg in rats and dermal LD_{50} of higher than 2000 mg/kg in rabbits (Hanley et al., 1998, 2000). Additionally, the main target organs for the subacute TCP toxicity after four weeks of dietary exposure are kidneys and liver. The reported dose level for adverse renal and hepatic effects is 120 mg/kg-day and greater (Dow, 2002; Hanley et al., 2000). The toxicity levels of various CPF metabolites have been presented in Table 2.

The toxic effects of CPF have been widely explored across various species. It has been reported that aquatic species are being highly affected due to the environmental exposure of CPF. Studies have revealed that CPF-induced toxicity caused significant variations in AChE activity in aquatic fish species including bluegill sunfish (*Lepomis macrochirus*), and fathead minnow (*Pimephales promelas*), as well as the aquatic midge (*Chironomus tentans*) (Mehler et al., 2008). Another study concluded that CPF exposure (24-h, LC_{50} of 0.7 μg/L) caused decreased feeding rate in giant freshwater prawns (*Macrobrachium rosenbergii*) (Satapornvanit et al., 2009). Additionally, CPF exposure (0.7 μg/L) in juvenile Coho salmon (*Oncorhynchus kisutch*) caused a 20% reduction in the sensory function, which can be attributed to its neurotoxic effects (Sandahl et al., 2004). Likewise, CPF (0.084–8.4 nl/L) also induced neurotoxicity in two different species of Neotropical fish i.e., the ten spotted live-bearer (*Cnesterodon decemmaculatus*), and Uruguay tetra (*Cheirodon interruptus*) (Bonifacio et al., 2017).

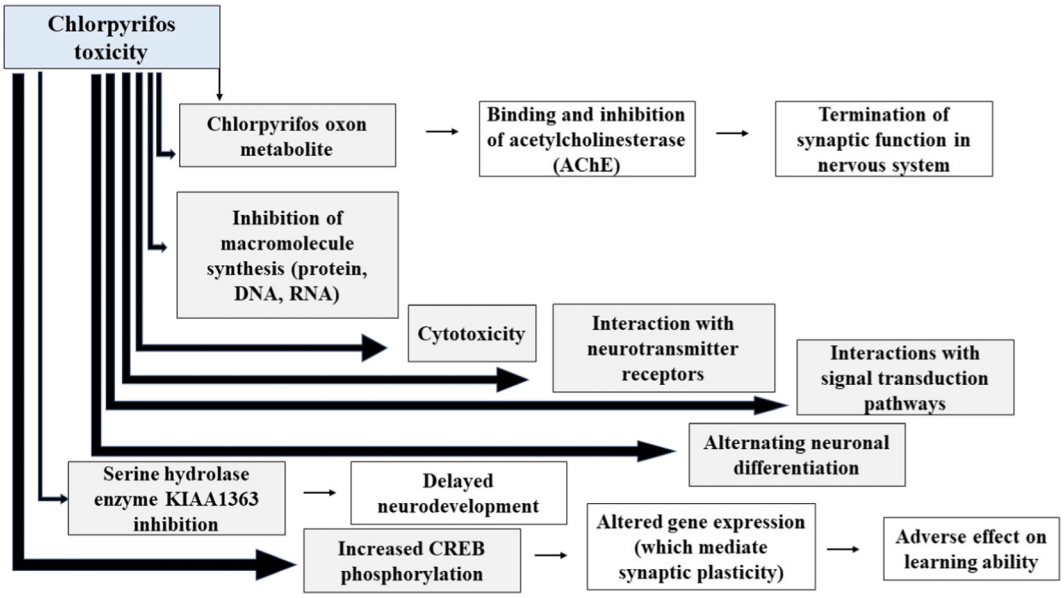


Fig. 3. Purported mechanisms of CPF toxicity.

In addition to the aquatic animals, land animals have also been exposed to the CPF toxicity. For example, a CPF dose of 10–40 mg/kg was reported as 'lethal' for cats (Blodgett, 2006). Additionally, CPF exposure also resulted in defects of the nervous system, respiratory muscles, diarrhea, and muscle weakness in dogs (Hopper et al., 2003). CPF has also been reported to be toxic for common grackles (*Quiscalus quiscula*) (LD_{50} = 5.62 mg/kg) and ring-necked pheasants (*Phasianus colchicus*) (LD_{50} = 8.41 mg/kg). Moreover, CPF exposure caused severe toxicity in common pigeons (*Columba livia*) and house sparrows (*Passer domesticus*), as well as the American robin (*Turdus migratorius*) (LD_{50} = 10 mg/kg). Furthermore, oral exposure of CPF (LD_{50} = 32–102 mg/kg) caused toxicity in chickens and mallard ducks (*Anas platyrhynchos*) (LD_{50} = 490 mg/kg) (EPA, 1999; Tomlin, 2006). The details for CPF toxicity in various animal species have been provided in Table 3.

CPF has been reported in the human seminal fluid, breastmilk, cervical fluid, and meconium of newborns (Watts, 2012). Watts (2012) further reported that as per an EPA report, around 127 cases of acute CPF toxicity were recorded between 2002 and 2009, with close to 17 of those being in children. Using the comet assay in human lymphocytes, an *in vitro* study revealed that 10 μM of CPF manifested in extensive DNA damage (Sandal and Yilmaz, 2011). The presence and exposure ranges for CPF in humans are usually determined through analyses of urine samples for TCP concentration, and this analysis serves as the fundamental test procedure for the presence, and the extent of exposure to the toxicant (Eaton et al., 2008). A double-blind, placebo-controlled

Table 2
Toxicity levels of CPF metabolites in some well-studied animal species.
Source = EFSA (European Food Safety Authority) (2019).

Organism	TCP	TMP	3,6-DCP	Desethyl chlorpyrifos	CPO
Rat	LD_{50} (females): 3129 mg/kg b.w. (oral)	LD_{50} (females): >2000 mg/kg b.w. (oral)	LD_{50} (females): >2000 <5000 mg/kg b.w. (oral)	LD_{50} (females) cut-off value: 500 mg/kg b.w. (oral)	LD_{50} (males and females): 100/300 mg/kg b.w. (oral)
	90-day NOAEL: 30 mg/kg b.w. per day	–	–	LD_{50} (females): 920 mg/kg b.w. (oral)	–
	NOAEL (maternal): 50 mg/kg b.w. per day	–	–	–	–
	NOAEL (developmental): 150 mg/kg b.w. per day	–	–	–	–
Dog	1-year NOAEL: 12 mg/kg b.w. per day	–	–	–	–
	ADI: 0.06 mg/kg b.w. per day (derived from the 1-year NOAEL study of dogs with 12 mg/kg b.w. per day and applying an uncertainty factor of 200)	–	–	–	–
Rabbit	NOAEL (maternal): 100 mg/kg b.w. per day	–	–	–	–
	NOAEL (developmental): 25 mg/kg b.w. per day	–	–	–	–
	ARfD: 0.25 mg/kg b.w. (derived from the NOAEL study of rabbits with 25 mg/kg b.w. per day and applying an uncertainty factor of 100)	–	–	–	–
Fish	31-day NOAEC (long-term toxicity): 0.808 mg/L				
Earthworm	14-day LC_{50} (acute toxicity): 9.8 mg/kg 56-day NOAEC (reproductive toxicity): 4.60 mg/kg (dry soil)				

LD = lethal dose, LC = lethal concentration, NOAEL = no-observed-adverse-effect-level, NOAEC = no observed adverse effect concentration, ARfD = acute reference dose, ADI = acceptable daily intake.

Table 3
The LD_{50}, LC_{50}, NOAEL and NOAEC values of CPF in various animal species.
Sources = Christensen et al. (2009); EFSA (2019); Lewis et al. (2016); National Center for Biotechnology Information (2020).

Organism	LD_{50}	LC_{50}	NOAEL	NOAEC
Rat (Sprague-Dawley)	66–223 mg/kg b.w. (oral) 1250–2000 mg/kg b.w. (dermal)	>1.0 mg/L air per 4 h (inhalation, whole body)	90-day: 0.1 mg/kg b.w. per day (oral) 21-day: >5 mg/kg b.w. per day (dermal) 14-day: $>0.296 \times 10^{-3}$ mg/L air (inhalation: nose-only) 0.1 mg/kg b.w. per day (maternal) 2.5 mg/kg b.w. per day (developmental) 2-years: 0.1 mg/kg b.w. per day (long-term) 2-years: 10 mg/kg b.w. per day (highest dose tested, carcinogenicity)	
Mouse	60 mg/kg (oral) 120 mg/kg (dermal)		90-day: 1 mg/kg b.w. per day (oral) 18-month: 0.9 mg/kg b.w. per day (long-term) 18-month: 47.1 mg/kg b.w. per day (highest dose tested, carcinogenicity)	
Dog (beagle)			90-day & 2-year: 0.1 mg/kg b.w. per day (oral)	
Rabbit (New Zealand White)	1000 mg/kg (oral) 2000 mg/kg (dermal)		81 mg/kg b.w. per day (maternal) 81 mg/kg b.w. per day (developmental)	
Mallard duck (*Anas platyrhynchos*)	490 mg/kg (acute, oral)	203 ppm (short-term dietary)		25 ppm
Northern bobwhite (*Colinus virginianus*)	39.2 mg/kg (acute)	423 ppm		125 ppm
Rainbow trout (*Oncorhynchus mykiss*)		96-hour: 0.025 mg/kg (acute)	1.6 μg/L	21-day: 0.00014 mg/kg (chronic)
Aquatic invertebrate (*Daphnia magna*)		EC_{50} 48-hour: 0.00010 mg/kg (acute)		0.056 μg/L
Aquatic invertebrate (*Americamysis bahia*)		96-hour: 0.00004 mg/kg (acute)		
Harlequin fly (*Chironomus riparius*)				28-day: 0.0001 mg/kg (chronic, static, water)
Lesser duckweed (*Lemna minor*)		EC_{50} 7-day: 0.53 mg/kg (acute, biomass)		
Green algae		EC_{50} 72-hour: 0.48 mg/kg (acute, growth)		96-hour: 0.043 (chronic, growth)
Honeybee (*Apis mellifera*)	24, 48, 72-hour: 0.059 μg (contact acute) 24, 48, 72-hour: 0.25 μg (oral acute)			
Bumblebee (*Bombus terrestris*)	24, 48, 72-hour: >1.58 μg (contact acute) Range: 0.09–2.39 μg 24, 48, 72-hour: 0.23 μg (oral acute)			
Mason bee (*Osmia bicornis*)	24, 48, 72-hour: 4.19 μg (contact acute)			
Earthworm (*Eisenia foetida*)		14-day: 129 mg/kg (acute)		12.7 mg/kg (chronic, reproduction)
Springtail (*Folsomia candida*)		35-day: 0.2 mg/kg (acute)		
Bluegill sunfish (*Lepomis macrochirus*)		96-hour: 0.002–0.010 mg/L		
Fathead minnow (*Pimephales promelas*)		96-hour: 0.12–0.54 mg/L		0.57 μg/L
Korean shrimp (*Palaemon macrodactylus*)		0.05 μg/L		
Chicken	32–102 mg/kg (oral)			
Common grackle (*Quiscalus quiscula*)	5.62 mg/kg			
Ring-necked pheasants (*Phasianus colchicus*)	8.41 mg/kg			
Common pigeon (*Columba livia*)	10 mg/kg			
House sparrow (*Passer domesticus*)	10 mg/kg			
Guinea pig (*Cavia porcellus*)	504 mg/kg (oral) 100 mg/kg (subcutaneous)			
Humans	300 mg/kg (oral)		28-day: 0.2 mg/kg b.w. per day 21-day: 0.3 mg/kg b.w. per day	
Goat (*Capra aegagrus hircus*)	500 mg/kg			
Common quail (*Coturnix coturnix*)	13.3 mg/kg b.w.			
Sheep	800 mg/kg b.w.			
Rhesus monkey (*Macaca mulatta*)			5 mg/kg bw per day	

LD = lethal dose, LC = lethal concentration, EC_{50} = half maximal effective concentration; NOAEL = no-observed-adverse-effect-level, NOAEC = no observed adverse effect concentration.

clinical study involving 12 human volunteers (6 males and 6 females) measuring the withdrawal capacity of CPF through the application of single oral doses of CPF at 0.5, 1, or 2 mg/kg of their body weight, concluded that the recovery of CPF in urine samples in the form of TCP ranged only between 20 and 35% of the dose administered (Timchalk et al., 2002). This suggested two possibilities; either the maximum fraction was not getting absorbed or, was being retained by the adipose tissues of the body. Development of symptoms such as disorientation, headaches (cephalgia), nausea, dizziness, and vomiting (emesis), was observed in the case of accidental exposures involving elevated levels of CPF, in under 1 h, indicating rapid absorption and diffusion of the toxicant to the brain tissues (Cochran, 2002), and this distribution pattern may owe in part, to the lipophilic nature of CPF. Additionally, animal models also provide evidence regarding the toxic effects, leading to mortality, of CPF exposure. For instance, Costa et al. (2018) reported the effect of CPF exposure on *Ceratophyrys ornata* tadpoles and pointed out that CPF exposure caused alterations in the sounds, swimming patterns, severe abnormalities, and ultimately death due to its cholinesterase-inhibiting action.

Significant evidence for carcinogenicity due to CPF exposure is lacking, considering the clinical and investigative studies conducted to date. However, CPF has been increasingly linked with carcinogenicity in humans (Watts, 2012), with a study by Ventura et al. (2012) describing the pesticide as a potential risk factor for breast cancer. The team reported that a 0.05 μM dose of CPF produced estrogenic effects through the proliferation of human estrogen-dependent breast cancer cell lines. However, higher dosing at 50 μM resulted in reduced cell proliferation, brought forth by cell cycle arrest. It is pertinent to mention that the CPF concentration measured in various soils and water bodies is also close to 0.05 μM. Andersen et al. (2002) reported similar estrogenic effects with human breast cancer cell proliferation induced by CPF doses of <50 μM. Kojima et al. (2004) observed CPF-induced estrogenic effects at 10^{-5} M, resulting in estrogen-dependent cell proliferation in Chinese hamster ovary (CHO) cells. According to Grunfeld and Bonefeld-Jorgensen (2004), a minute increase in mRNA of estrogen receptors for human breast cancer cells was recorded in their study. Horton et al. (2012) in their experiment, found CPF in blood sampled from the umbilical cord and reported working memory deficits in those children upon reaching 7 years of age, with male children more adversely affected compared to female children.

Karunanayake et al. (2012) in a case-control study conducted in males across six regions of Canada, showed a significant association between CPF exposure and Hodgkin's lymphoma, although the recorded cases were few. Another study on male pesticide applicators likewise reported a comparatively higher incidence of intensity-dependent lymphohematopoietic cancer, as well as brain, lung, and kidney cancers in the workers who were exposed to CPF (Watts, 2012).

Engel et al. (2005) similarly reported in an experimental study that the spouses of pesticide application workers were at a higher risk of developing breast cancer, owing to nonoccupational exposure to CPF. Alavanja et al. (2003) indicated that the male pesticide applicators were at an increased risk of developing prostate cancer. Gotoh et al. (2001) conducted a study on medical examination reports of pest and termite control workers exposed to CPF and observed acute impairment of BuChE, as well as erythrocyte cholinesterase (RBC-ChE) activities, and abnormalities in levels of blood urine nitrogen (BUN), and white blood cell (WBC) count.

A long-term investigation into the peripheral immune system (PIS) of 12 patients following exposure to CPF revealed several immune system-associated abnormalities, such as atopy, antibiotic susceptibilities, increased number of CD26 cells, and elevated autoimmunity levels, resulting in increased concentration of autoantibodies targeted towards smooth muscles, thyroid gland, parietal cells, brush border, anti-nuclear antibodies (ANA), and myelin (Thrasher et al., 1993). Another study by Thrasher et al. (2002) in 29 people with chronic CPF contact, further substantiated the previous findings, with increased expression of CD26, decreased mitogenesis, and raised levels of autoantibodies. In another investigation, Joly et al. (2013) reported the effect of low-dose chronic exposure of CPF on human intestinal microbiota through SHIME (simulator of the human intestinal microbial ecosystem) and rat modeling. The colonic segments were exposed to 1 mg CPF for 30 days, whereas rats were exposed to the same concentration for 60 days. The findings exposed that dysbiosis occurred in the microbial community along with a decrease in the number of several microbial populations. A cohort study of human infants with *in utero* exposure to CPF, partially attributed to residential use of the pesticide, established a positive association between maternal and umbilical cord blood CPF levels, as well as potentially, neurodevelopmental and cognitive defects (Eaton et al., 2008). Hence, the following subsections provide a comprehensive review based on classical *in vitro* and *in vivo*/clinical (animal modeling and human trials) studies encompassing the various types of CPF-induced toxicities.

6.1. Genotoxicity

The genotoxicity induced by OP pesticides has the potential to be mutagenic or carcinogenic to humans and has been associated with heritable genetic disorders, reproductive dysfunction, carcinogenesis, as well as various birth defects. In this regard, the cleavage of DNA into its constituent inter-nucleosomal fragments is a highly significant cellular event during the process of development and is pivotal to the production of genotoxicity by OP pesticides such as CPF (Li et al., 2015). Wellman and Kramer (2004) conducted an *in vitro* genotoxicity investigation regarding the binding capacity of CPF and CPO to calf thymus DNA (CT DNA), and this involved the incubation of the CT DNA with CPF. The examination of thermal denaturation patterns of DNA revealed no fluctuations in denaturation curves, indicating that either there is no direct binding between these chemicals and the DNA, or the binding occurs with a weak affinity. Cui et al. (2006), for their *in vitro* study involving the incubation of CPF with CT DNA, or with the isolated mouse hepatocytes, reported similar findings of no DNA denaturation taking place, or the formation of any DNA-adducts.

However, in a study by Zhang et al. (2010), the researchers investigated the interaction between CPF and CT DNA (in a physiological buffer with pH 7.4) by using fluorescence spectroscopy coupled with the melting point effect of DNA, and ethidium bromide (EB) as a fluorescence probe. The results of the study indicated that CPF is capable of quenching the fluorescence of the EB-CT DNA complex by way of aggressively competing with EB to bind with CT DNA, in turn removing EB from the complex, and ultimately the production of DNA adducts as a direct consequence of CPF binding with the base pairs of CT DNA, implying that CPF is capable of inducing chemical damage to the DNA of various organisms.

Li et al. (2015) examined the effects of CPF on nuclear DNA in the human HeLa and HEK293 cells utilizing quantitative detection assays, against the damage in the S2 cells from the common fruit fly, *Drosophila melanogaster* as a positive control. The findings of the study indicated that exposure to CPF induced DNA migration (in the alkaline comet assay). Also, when exposed to 200 μm CPF for a time interval of 10 mins, a marked increase in the concentration of gamma-H2AX-positive HeLa cells was observed. Increasing the time of exposure further increased the concentration of the gamma-H2AX-positive HeLa cells, indicating that CPF induced DNA double-strand breaks (DSBs) in a time-dependent manner. Furthermore, there was increased DNA fragmentation (relative to control) in a concentration- and time-dependent manner post-exposure with CPF. This study conclusively indicated that CPF is a powerful genotoxic agent.

Earlier, Ojha and Gupta (2015), also reported similar findings when they investigated the genotoxic effects of CPF in peripheral blood lymphocytes of rats. The study reported that CPF exposure induced both single-strand breaks (SSBs) and double-strand breaks in DNA, along with the formation of DNA protein cross-links (DPC), and a significant

increase in the mean DNA damage index (relative to control). The study further postulated that CPF may also inactivate some repair enzymes, inducing DNA strand breaks. Sharma et al. (2019) also reported that CPF intoxication induced both genotoxicity and oxidative stress in the blood, kidney, and liver of rats, with the DNA damage most pronounced in the liver. This damaged DNA has been linked with the generation of the reactive oxygen species (ROS), and these oxygen radicals, when produced in excess has been shown to cause further damage in the form of lipid peroxidation, enzyme inactivation, damage to DNA, and imbalances of the antioxidant levels, and the scavenger systems in the body of the organism.

Blakley et al. (1999) tested male Fischer 344 rats for certain immune system alterations with oral gavage dose of CPF at 5 mg/kg, administered twice a week for 28 days. No changes in body weight and the organ/body weight ratios, as well as B-lymphocyte blastogenesis, were detected. However, the CPF-treated rats exhibited impaired T-lymphocyte blastogenesis, with an increase in total splenic lymphocyte count, and reduced humoral immunity, as well as an enhanced expression of specific cell marker antigens, normal antibodies, and phagocytic responses, evidencing immunotoxicity.

Comet assay or single-cell gel electrophoresis (SCGE) assay has found wide-ranging application as a standard biomarker to quantify and analyze DNA damage (single- or double-stranded breaks)/repair in cells, as well as evaluate the extent of such damage. A rapid, sensitive, versatile, and comparatively simple technique (Olive and Banath, 2006), the test requires fewer number of sample cells and has applications for various types of tissues (Speit et al., 2009). Sandal and Yilmaz (2011) exposed cultured primary human lymphocytes to three different concentrations (1, 5, and 10 μM) of CPF, cypermethrin, endosulfan, and 2,4-D, and reported the DNA damage (assessed by the Alkaline Comet assay technique) in the peripheral human lymphocytes, indicating CPF as a potential genotoxicant.

Sandhu et al. (2013), similarly, reported lymphocyte damage with minute doses of CPF and elaborated that the damage was more pronounced in males rather than females due to the presence of androgens. However, the researchers did not comment that whether female androgen levels were higher due to adrenal problems, or can the damage be equally higher as in male individuals. According to Farag et al. (2010), the administration of CPF doses at 10 mg/kg/day, and even higher at 15 mg/kg/day did not induce carcinogenicity in male and female rats. However, a dose of 25 mg/kg/day adversely affected reproductive performance in male rats, caused maternal toxicity, and proved to be embryo lethal, producing embryo/fetotoxic, and teratogenic effects (Table 4).

Yin et al. (2009) used Chinese toads (*Bufo gargarizans*) in their study for assessing the sublethal dose-dependent DNA damage in erythrocytes and liver cells of tadpoles employing Comet assay, and genotoxicity in erythrocytes using the Micronucleus test (MN). The consequent increase in comet tail length indicated mutagenicity at the cytological level in a concentration-dependent manner. Ali et al. (2008) tested the genotoxicity by acute CPF exposure in vivo in the spotted snakehead (*Channa punctata*), a freshwater fish common throughout South Asia, using a biomarker combination of the Alkaline Comet assay (erythrocytes) and the Micronucleus assay (lymphocytes and gill cells) techniques. The results demonstrated that the highest DNA damage exhibited at LC50 of CPF through comet tail length measurement was observed on day 5 of dose administration, also proving that CPF has significant potential for the induction of genotoxicity in various aquatic organisms. Furthermore, Watts (2012) reported that the induction of DNA damage from exposure to both acute and chronic CPF doses in rats occurred after 24 h of administration. However, the damage appeared to repair at 48 and 72 h post-administration.

Many studies over the years have investigated and reported mutagenic effects due to CPF exposure in humans, mice, rats (brown rats, *Rattus norvegicus*), fish (both freshwater and marine), fruit flies (*Drosophila melanogaster*), plants, and bacteria. For instance, a dose-dependent increase in the micronucleus (MN) frequency was reported in 3-day old mouse embryos (early preimplantation phase), after maternal exposure to a single dose of CPF on day 0 of the pregnancy (Tian and Yamauchi, 2003). DNA damage and the formation of micronuclei were also seen as a result of CPF exposure in the spotted snakehead, a freshwater fish (Ali et al., 2008).

Rahman et al. (2002), in their study for evaluation of CPF as a genotoxicant, treated mice with oral doses of CPF at 0.28 to 8.96 mg/kg and observed comet tail length increase in the blood leukocytes 24 h postexposure. These variations, however, started to subside after 48 and 72 h, disappearing entirely after 96 h of exposure. In a case-control study, Ramirez and Cuenca (2002) investigated DNA damage in the lymphocytes isolated from the blood samples of 30 female workers involved in the banana packaging operations with occupational exposure to CPF, as well as probably other pesticides. SCGE assays of blood lymphocytes of test subjects with exposure periods of up to 5, 5–10, 10–15, and over 15 years were performed. However, no significant overall differences were evident between the case and control cohorts, although, the subgroups with exposure of between 5 and 15 years did exhibit a significant increase in DNA strand breaks, generally attributed to DNA damage, and thus, genotoxicity.

Micronuclei (MN) formation after CPF exposure is indicative of the potential mutagenicity of the pesticide. Tian and Yamauchi (2003) studied this phenomenon, whereby CPF was injected intraperitoneally at doses of 40 and 80 mg/kg on day 0 of pregnancy in mice, and the production of the micronuclei in the 3-day old blastocysts was investigated. The treatment manifested in a 15% increase in the number of blastocysts with at least 1 micronucleus formed. A follow-up study by Tian et al. (2007) involved the use of the Giemsa staining technique. The results recorded were similar to those obtained from the DAPI staining technique used for the 2003 study. Owing to the similarity of the outcomes of the two studies, any viable conclusion cannot be drawn as the doses administered were sufficient to induce toxicity in maternal cells, and that the fetal cells are more susceptible to the formation of micronuclei due to their elevated metabolic and proliferation rates.

The action of ROS, in general, has been implicated by various earlier studies (Rahman et al., 2002; Giray et al., 2001) as a possible mechanism of action in terms of mediating the DNA damage, and in turn, inducing the genotoxicity manifested by exposure to CPF. It is fairly plausible, as evidenced by various *in vivo* studies, and further substantiated by *in vitro* investigations, that the generation of ROS contributed significantly towards inducing chromosomal aberrations (CA), micronuclei (MN), and sister chromatic exchanges (SCEs), consequent DNA damage, as well as cell cycle perturbations such as the arrest of cells at G_0 and G_1 phases, and apoptosis (Chauhan et al., 2016). However, conclusive evidence linking the formation of CPF-induced DNA adducts or oxidative stress to genotoxicity remains elusive and demands further research.

6.2. Developmental and reproductive toxicity

CPF exposure during pregnancy has been linked to the induction of various undesirable effects in the developing organism, as CPF can cross the placenta, a trait similar to other hydrophobic compounds (Burke et al., 2017). It is generally implied that the inhibition of AChE is the purported mechanism underlying the developmental toxicity induced by OP pesticides such as CPF. However, this does not seem to be the case as many studies report other non-cholinesterase-associated mechanisms as well (Abreu-Villaça and Levin, 2017). For instance, a significant upregulation of the serotonin (5HT) receptor subtypes 5HT1, and 5HT2 was reported in multiple brain regions of adult rats that were exposed neonatally to CPF (1 mg/kg/day) on postnatal days (PND) 1–4, the effect being mere pronounced in males than females (Burke et al., 2017). Moreover, various other studies have reported that CPF is capable of interacting, and consequently altering the activity of many serine hydrolases (e.g., carboxylesterases), m2 muscarinic, and

Table 4
Effects of various doses and exposure durations of CPF on the endocrine and other physiological functionalities of biological systems.

Exp. subject	CPF dose level	Exposure route	Exposure duration	Effects observed	Citation
Effects on thyroid functionality					
Rats	10^{-5} M	Simulated cell line culture	–	Thyroid hormone-dependent rat pituitary GH3 cells proliferation	Ghisari and Bonefeld-Jorgensen (2005)
Pregnant mice	6 mg/kg	Oral	Prenatal+ postnatal exposure	Reduced serum T4, impaired thyroid, and adrenal glands in dams (more pronounced in males) reduced T3 levels in female dams	De Angelis et al. (2009)
Female mice	1–5 mg/kg	Subcutaneous injection	17–20 days	Increased thyroid hormone levels in pregnant rats, thyroid impairment in newborn	Haviland et al. (2010)
Neuroendocrine disruption and developmental abnormalities					
Frog tadpoles (*Rana dalmatina*)	0.025, 0.50, 0.1 mg/L	Simulated ecological	30 days	Developmental abnormalities at larval stages	Bernabo et al. (2011)
Male and female mice	Pre- and postnatal exposure	In utero	5 months	Neuroendocrine abnormalities in the brain. Increased oxytocin and reduced vasopressin (more pronounced in males), prolactin unaffected	Venerosi et al. (2012)
Rainbow Trout (*Oncorhynchus mykiss*)	10–40 μg/L	Simulated ecological	7 days	Neurotoxicity	Greer et al. (2019)
Catfish *Heteropneustes fossilis* (Bloch 1794)	1.92 mg/L	Simulated ecological	4 days	Neuro-degenerative effect	Mishra et al. (2020)
Whiteleg shrimp *Litopenaeus vannamei*	0.7 and 1.3 μg/L	Simulated ecological	4 days	Oxidative stress, and decreased brain activity	Duarte-Restrepo et al. (2020)
Reproductive toxicity					
Male and female rats	CPF (7 and 14 mg/kg), endosulfan (4.5 and 9)	–	15 days	Reduced weight of testes and ovaries, reduced concentrations of testosterone and estradiol	Ahmad et al. (1993)
Male mice	10–25 mg/kg	Oral	4 weeks	Reduced sperm number and motility	Farag et al. (2010)
Fish – Nile tilapia (*Oreochromis niloticus*)	5, 10, 15 ppb	Simulated ecological	15–30 days	Reduced estradiol, cortisol, and testosterone levels	Oruc (2010)
Male rats	–	Simulated cell line culture	–	Reduced testosterone level, decreased LH receptor-stimulated cAMP production	Viswanath et al. (2010)
Male rats	9 mg/kg	Oral	70 days	Reduced testosterone level, sperm abnormalities, increased lipid profile	El-Mazoudy et al. (2011)
Male rats	10.6 mg/kg	Oral	15 weeks	Altered enzymatic activities in testes and pituitary glands	Shittu et al. (2012)
Rats	8.5 mg/kg	Oral	28 days	Increased malonaldehyde and reduced enzyme activities in testes and pituitary gland	Umosen et al. (2012)
Rats	3 mg/kg	Oral	20 weeks	Reproductive toxicity	Li et al. (2019)
Mammary gland carcinogenicity					
Female Wistar rats	0.1–2.5 mg/kg/day	Oral	8 weeks	Oxidative stress, increase in ductal thickness, ovarian surface epithelium height, myometrium thickness, and endometrial gland diameter, negative effect on reproductive organs indicating cancer beginning	Nishi and Hundal (2013)
Female Sprague-Dawley rats	0.01–1.0 mg/kg/day	Oral	100 days	Higher number of ducts and alveolar structures, the onset of breast diseases (adenosis, hyperplasia), endocrine imbalance, decreased level of luteinizing hormone and progesterone, breast cancer development	Ventura et al. (2016)
Modulation of gut microbiota and intestinal health					
Zebrafish (*Danio rerio*)	30 μg/L, 100 μg/L, 300 μg/L	Simulated ecological	21 days	Increased malondialdehyde levels and decreased glutathione contents in the gut, changed the gut microbial community at the genus level	Wang et al. (2019)
Preweaning mice	–	Oral	–	Modulation of gut microbiota and alterations in the genotypes of intestinal microflora	Guardia-Escote et al. (2020)
Rats	1 mg/kg/mL/day	Oral	–	Gut microbiome dysbiosis	Perez-Fernandez et al. (2020)
Miscellaneous (endocrine, immune and physiological disruptions)					
Sheep	12.5 mg/kg	Oral	36 days	Reduced T4 and increased cortisol	Rawlings et al. (1998)
Pregnant rats	1 mg/kg	–	GD 16	Increased GnRH mRNA levels	Gore (2001)
Rats	250 mg/kg chlorpyrifos-methyl	Subcutaneous injection	10 days	Reduced T4 levels and body weight, increased liver weight	Kang et al. (2004)
Rats	100 mg/kg	Oral	7 weeks	Adrenal vacuolation, reduced weight of thyroid, testes, and ventral prostate gland increased liver weight	Jeong et al. (2006)
Rats	50 mg/kg	Subcutaneous injection	Single-dose	Hyperglycemia and hyperlipidemia	Acker and Nogueira (2012)
Humans[a]	Accidental environmental exposure	–	–	A statistically significant correlation between urine TCP and T4 levels in the blood	Fortenberry et al. (2012)
Male and female Wistar rats	3 and 12 mg/kg/day	Oral	7 to 14 days	Increase in bi-nucleus cell formation, more comet formation, and olive movement in male rats, DNA damage in both genders	Sandhu et al. (2013)

[a] Clinical trial.

CB1 cannabinoid receptors, and structural proteins such as tubulin and kinesin (Terry, 2012; Burke et al., 2017).

Some other studies have suggested the disruption of the axonal transport and outgrowth, with tubulin and similar structural proteins as mediators, to be the underlying cause of the disruptions associated with the structural and functional integrity of the developing brain, as a result of exposure to CPF, and/or CPF-oxon. These disruptions have been shown to produce anomalous patterns of neuronal connectivity, thereby contributing to the neurobehavioral changes reportedly attributed to developmental exposure to CPF (Burke et al., 2017). Some *in vitro* studies have also reported that CPF can induce developmental neurotoxicity by way of altering the downstream signaling pathways involving neurotrophins, leading to the suppression of neurite outgrowth, cell differentiation, and neuronal repair, processes that are regulated by these neurotrophins to a significant extent (Bernd, 2008; Burke et al., 2017). Other mechanisms that have been proposed in this regard include aggravated oxidative stress levels, imbalances of the intracellular Ca^{2+} homeostasis, escalated levels of signaling mediated by inflammatory mediators such as interleukins (ILs), and cytokines, and the increased expression of protein kinases, including protein kinase C (PKC), and mitogen-activated protein kinases (MAPK) (Tian et al., 2015; Zhang et al., 2015; Burke et al., 2017).

Many research studies have been conducted in rats and mice for evaluating the toxicity of CPF during developmental stages. Farag et al. (2003) treated 30 female Fischer 344 rats with CPF doses (0, 5, 15, and 25 mg/kg-day) between gestation days 6 to 15, followed by evaluation of fetuses on day 21, and reported clinical toxicity symptoms in dams that received doses of 15 and 25 mg/kg-day, exhibiting loss in body weight and impairment of AChE activity, as well as fetal weight reduction and death, revealing a progression of CPF toxigenicity, from developmental stages into postnatal life, as well as fetotoxicity and teratogenicity. Sanghi et al. (2003) reported the presence of CPF (mean 0.230 mg/L, range 0.085–0.355 mg/L) in the breastmilk samples collected from 12 women in Bhopal, India. Given that CPF is inherently lipophilic, its presence alongside other pesticides (endosulfan, malathion, parathion) in these samples can be attributed to the fat component of the milk.

According to Saulsbury et al. (2009), an investigation in Mexican American children revealed reduced gestational birth weight and birth length due to chronic CPF exposure. The decrease in birth weight could be linked to the impairment of the thyroid hormone function and could lead to diminished fetal brain development (Watts, 2012). However, in this regard, the study on rats (Breslin et al., 1996b) showed no definitive treatment-related effects on reproduction. Moreover, there was no effect on the histopathology of reproduction tissue and their physiology, however, in the F1 generation, CPF did induce fetotoxicity and deaths. These embryo-lethal effects were not observed in the subsequent generations. Hence, this toxicity could be due to the presence of CPF in the milk.

The exposure to various environmental toxicants, including pesticides such as CPF, has been linked with the induction of various reproductive system defects including the deterioration of the male reproductive system function, and infertility (Sharma et al., 2014). Various studies have reported that CPF acts as an androgen receptor (AR) antagonist, thereby suppressing the gene expression pathways associated with hypothalamic gonadotropin synthesis (involving luteinizing hormone, and follicle-stimulating hormone), or steroidogenesis. This suppression has in turn been attributed to the lowered levels of testosterone (due to probable oxidative damage to the Leydig cells, that produce testosterone), defects of the gonads, and the reduced activity of the luteinizing hormone (LH), and follicle-stimulating hormone (FSH) (Alaa-Eldin et al., 2017). A major reproductive defect that has been shown to manifest in males as a result of the CPF exposure is the reduction in the testicular weight, which in turn has been attributed to the degenerative and necrotic alterations in the seminiferous tubules, widening of the interstitial spaces, and the reduction in the number of germ cells, or spermatozoa. Moreover, a decrease in the motility and viability of the sperms has been demonstrated as well, along with a higher incidence of sperm head abnormalities (Farag et al., 2010; Mosbah et al., 2016; Alaa-Eldin et al., 2017).

The sperm DNA is vital to the transmission of genetic information during the process of reproduction, and if its structural and functional integrity is compromised by way of abnormalities associated with chromatin or damage to DNA itself, the likelihood of paternal fertility problems increases manifold. OPs such as CPF have proven to be highly potent phosphorylating agents, capable of inducing genotoxicity by binding themselves to protamines and DNA, making the DNA more vulnerable to *in situ* denaturation, and resulting in alterations to the structure of chromatin (Alaa-Eldin et al., 2017). Given that ROS is a product of normal cell metabolism, sperm cells are also capable of generating free oxygen radicals. At lower concentrations, these ROS contribute to sperm cell capacitation, acrosomal reactions, and the binding of the sperms to zona pellucida. However, at higher generation frequencies, ROS have been implicated as contributors to various sperm cell anomalies, degradation of spermatogenic cells, and ultimately infertility (Babazadeh and Najafi, 2017).

In the case of females, the reproductive toxicity effects induced by OP insecticides mainly include changes in the reproductive system and the germ cells, and range from the reduced ovarian weight, and follicle production, disruptions of the ovarian cycle, and estrous cycle arrest, to alterations associated with steroidogenic enzymes, and prolonging of the pregnancy duration, stillbirths, as well as infertility (Kara and Öztaş, 2020). According to Nishi and Hundal (2013), CPF has been shown to induce alterations in the weight and morphology, by contributing to the thickening of the surface epithelium, and myometrium. Moreover, as Ventura et al. (2016) reported, the ovotoxic and embryotoxic mechanism of action of CPF involves altering the processes such as embryonic hatching, cell proliferation, and apoptosis by way of mimicking estrogen. Moreover, CPF has also been linked to reducing serum sex hormones including LH, progesterone, and estrogen (Nandi et al., 2011; Kara and Öztaş, 2020).

The toxicity-associated reproductive effects of CPF in the female rats include the fetuses with reduced birth weights as well as fetal mortality, though, the manifestations were dose-dependent. CPF has also been shown to induce apoptotic effects in placental cells by eliciting nuclear condensation and fragmentation, loss of mitochondrial potential, and an overall reduction in cell growth and proliferation (Saulsbury et al., 2008). CPF has the potential to induce toxic effects in glial cells as well, in particular, the oligodendrocyte progenitor cells (OPCs) (also known as oligodendrocyte precursor cells, NG^{2+} glia, or polydendrocytes), that play a significant role in brain development (Saulsbury et al., 2009). The studies on rats have further revealed problems such as reproductive toxicity, fetotoxicity, and teratogenicity, long-term cognitive, and behavioral anomalies, resorptions, decrease in fetal weight, and incomplete development of the spine in the newborns at the dose rate of 0.3 mg/kg-day on gestation days 0–7 (Muto et al., 1992).

Ovarian cancer is emerging as a significant female reproductive malignancy leading to many deaths worldwide. Several studies indicate that various environmental and genetic factors are involved in the development of ovarian cancer. In this regard, excessive use of pesticides is considered as one of the leading causes of this lethal ailment. It is hypothesized that different endocrine-disrupting chemicals present in the pesticides have the potential to induce several epigenetic variations that play a catalytic part in the progression of ovarian cancer (Samtani et al., 2018). In an investigation, male rats were exposed to CPF (3.0 mg/kg) for 20 weeks (Table 4) and it was observed that CPF significantly decreased the sperm count, testosterone levels, gonadotropin concentration, thereby increasing the oxidative damage in testis (Li et al., 2019). Studies based on animal modeling also provide evidence that elevated exposure of CPF can enhance the oxidative degradation in female albino rats creating an adverse impact on the reproductive organs that can be a precursor of reproductive carcinogenicity as discussed in Table 4 (Nishi and Hundal, 2013).

6.3. Endocrine toxicity

The principal mechanism for CPF-induced nervous system damage involves the inhibition of the AChE enzyme, primarily found in chemical synapses and postsynaptic neuromuscular bundles, particularly in muscles and nerves, as well as the red blood cells (RBCs). A cholinergic serine hydrolase enzyme, AChE hydrolyzes the neurotransmitter acetylcholine, into choline, and acetic acid, thereby preventing ACh dispersal and consequent activation of nearby receptors (Colovic et al., 2013; Li et al., 2015). Before suppressing brain cholinesterase, CPF inhibits plasma cholinesterases, and this inhibition pathway tends to be the most plausible indicator of CPF-induced toxicity (De Coster and Larebeke, 2012). However, apart from the inhibition of the cholinesterases, there are various other routes of action for CPF toxicity, such as interference with components for cell signaling, altering oxidative stress parameters, thereby triggering variations in the expression and functioning of antioxidant genes, genital defects, as well as weakened estrogenic activity *in vivo*, as studied in the case of Mediterranean bivalve mussel, *Mytilus galloprovincialis* (Canesi et al., 2011; Matthiessen et al., 2018).

Usmani et al. (2003, 2006) in their two studies reported CPF as a potent inhibitor of the liver metabolism of both testosterone and estradiol hormones in humans. Furthermore, CPF intoxication has been shown to affect the key enzymes of the liver's carbohydrate metabolism, and Goel et al. (2006) investigated these effects by treating male SD rats with an oral regimen of CPF at 13.5 mg/kg body weight. The team recorded a marked increase in the activities of the enzymes, glucose-6-phosphatase (G6pase), and glycogen phosphorylase (GP), while a significant decrease in the case of hexokinase, lactate dehydrogenase (LDH), succinate dehydrogenase (SDH), and the reserves of glycogen. In terms of oxidative stress induced by CPF exposure *in vivo*, Saulsbury et al. (2009) reported that exposure to the pesticide resulted in the generation of the reactive oxygen species (ROS), in turn manifesting in the form of lipid peroxidation (LPO); a free radical-mediated chain of reactions that could lead not only to the disintegration of the cellular membranes but may also yield lipid hydroperoxides that have the potential to induce modifications in other cellular macromolecules.

Pregnant mice, in a study by Haviland et al. (2010), were exposed to CPF doses of 0, 1, or 5 mg/kg on gestational days 17 to 20, followed by the testing of the *in utero* exposed newborns for the extent of thyroid hormone levels present. Although males remained unaffected, the female mice exhibited diminished foraging ability in a unique foraging behavior assaying maze. The reduction in thyroid hormone levels in the female mice owing to the dose-dependent CPF exposure indicated that *in utero* exposure of the pesticide can cause sex-selective impairment of the thyroid gland, as well as behavioral abnormalities (Table 4).

CPF exhibits enhanced toxicity when coupled, either concurrently, or sequentially, with other insecticides/pesticides. This characteristic interactive relationship between CPF and other pesticides, concurrent in this case, was demonstrated by Kacham et al. (2006) in their study. Neonatal rats at 7 days old, were dosed orally with CPF (8 mg/kg), and parathion (0.5 mg/kg) both concurrently and sequentially, for the examination of the interactive toxicological response. Concurrent dosing exhibited a comparatively higher lethality ratio of 15/24, compared to that of 7/23 for sequential dosing in the case of the C/P group (CPF administration followed by parathion after an interval of 4 h), and for that of the P/C group (parathion treatment followed by CPF after an interval of 4 h), the ratio was 1/24. Furthermore, the inhibition of the brain, diaphragm, and plasma cholinesterase activity was notably higher in the C/P group (59%), as compared to the P/C group (28%) (Kacham et al., 2006). Cui et al. (2011) similarly studied the interactive effect of CPF and cypermethrin in ICR (Institute of Cancer Research strain) mouse hepatocytes, using the comet assay technique, and reported the induction of DNA strand breakage and DNA hypomethylation.

Recently, Mishra et al. (2020) assessed the histopathological changes in the brain cells and associated behavioral modifications using air-breathing catfish after CPF exposure (1.92 mg/L) for four days (Table 4). The findings revealed that fish with CPF exposed brains showed loss of equilibrium, less locomotive activities reduced erratic movements as compared to the non-CFP exposed fish. The concluded that CPF exposure to the brain cells can induce significant downregulation in the brain cell performance resulting in altered brain activities, poor swimming performance, and reduced locomotion.

6.3.1. CPF mechanisms of endocrine disruption

The endocrine-disruption effects of CPF could be induced through many pathways: 1. CPF has been noted as the primary inhibitor of the enzyme, AChE, inhibiting its activity by binding the enzyme through non-reversible phosphorylation, and ultimately blocking the active binding site. Acetylcholine (ACh) is a neurotransmitter present at the neuromuscular junctions, all autonomic ganglia, the synapses of the visceral motor system, and various other locations within the CNS. AChE, a serine hydrolase enzyme, is found in the synaptic cleft between nerve and muscle cells, and actions the rapid hydrolysis of ACh to choline and acetic acid, its two constituent parts, effectively terminating the synaptic/impulse transmission. The inhibition of AChE by OP pesticides such as CPF results in the concentration of ACh in the synaptic cleft, leading to the failure of translation of hormonal regulation impulses, and consequently the impairment of endocrine homeostasis in the body (EPA, 1992). Moreover, CPF has also been reported to induce deficits in the functioning of adenylyl cyclase, as well as impacting the activity of the GTP binding proteins or the G-proteins, that link the neurotransmitter to the hormone receptors for coordinating the signaling cascade activity in the impulse-receiving cells. This manifests in the accumulation of ACh in postsynaptic cells, thereby impeding the signal transduction, and ultimately the impairment of many endocrine system-associated functions. 2. The CPF-induced endocrine toxicity has been extensively investigated in parents as well as their progenies. However, these epigenetic/transgenerational studies report that the mechanism for transmission may involve the germline in some cases, or it may be entirely nongenomic in others. This effectively means that the transmission may not be due to the mutations in DNA sequences, but by way of modifications in the factors responsible for the regulation of gene expression, for instance, DNA methylation, and acetylation of histones (Diamanti-Kandarakis et al., 2009). 3. A study conducted on marine bivalve mussels, *M. galloprovincialis*, revealed that CPF exposure of 4.5 mg/L/animal for 72 h adversely affected the lysosomal parameters of the digestive gland with a noticeable reduction in lysosomal membrane stability (LMS), as well as a significant increase in the neutral lipid content. Moreover, CPF also induced estrogenic impairment, through the interference with the expression of genes (the transcription for these genes being modulated by chemicals with estrogenic activity (EA)). These genes include that for biotransformation, as well as for antioxidant defenses (glutathione-S-transferase (GTS) and catalase), and the receptors for the hormones, estrogen, and serotonin (5-HT). This indicates that CPF, apart from inducing the cholinesterase inhibition, can also trigger the impairment of estrogenic activity, by down-regulating 5-HT receptors, and therefore, elicits an endocrine-disrupting effect on estrogen (Canesi et al., 2011). 4. CPF-induced endocrine disruption can also be associated with sex-dependent dysfunctions, whereby the male sexual dysfunctions are more androgen-dependent when compared with females (Diamanti-Kandarakis et al., 2009). The two probable pathways are, a. Direct influence on androgenic and estrogenic activities, triggered by alterations in the estrogen receptors (ERs), primarily the estrogen receptor alpha (ERα) of the hypothalamus, uterus, and epididymis (Diamanti-Kandarakis et al., 2009). b. Indirect influence involving effects on endogenous steroid production, action, or metabolism, resulting in over or underproduction of steroid hormones vital for sexual differentiation mechanisms (Diamanti-Kandarakis et al., 2009).

6.4. Hypothalamic-pituitary-gonadal (HPG) axis

A global decrease in fertility levels and malignant forms of cancer is a major concern for developing countries, with fewer opportunities for a

full investigation. The reasons may include a decline in male semen quality and problems with the female reproductive system. However, less emphasis has been given to the transgenic food, man-made chemicals, and pesticides producing environmental contamination, and ultimately inducing endocrine disruption in both humans and other organisms. The animals exposed to CPF exhibited a noticeable decline in plasma testosterone levels and an increase in follicle-stimulating hormone (FSH), as well as luteinizing hormone (LH). As the secretion of testosterone is controlled by LH, this influences the Leydig cells for the production of testosterone (Umosen et al., 2012). Issam et al. (2009) however, observed a reduction in plasma testosterone levels, as well as decreased LH, and FSH production in male rats after 60 days of treatment with deltamethrin (DM), an α-cyano pyrethroid insecticide. CPF was also reported to affect the HPG axis in male rats, and reduced gonadotropin-releasing hormone (GnRH) gene expression with lowered LH and FSH production as mentioned in Table 4 (Gore, 2001). The decrease in testosterone production could be due to the fact, that the CPF target is steroidogenic acute regulatory proteins (StAR), liable for transporting cholesterol from the external to the internal mitochondrial membrane (Manna et al., 2001). The low production of testosterone could also be attributed to the testicular atrophy, and high levels of plasma LH or because no/less testosterone is present to induce negative feedback for LH production. Moreover, after the treatment with CPF, there was a decline in the formation of 5α-dihydrotestosterone (5α-DHT), which contributed to the lower levels of androstenedione. These findings suggest that this decrease in testosterone production could be due to the CPF or its metabolites and has a direct or indirect effect on the HPG axis in the male rats.

In a two-generation CPF-induced toxicity study conducted by Breslin et al. (1996a) on female mice, no reproductive toxicity, ovum damage, changes in estrous cycle and ovulation, and no endocrine complications were observed. When CPF at dose rates of 0, 0.5, 1, and 2 mg/kg-day was administered in the pubertal assay of male and female mice, there was no dose-related effect on preputial separation and vaginal opening when compared to the control rats. Moreover, there was no effect of treatment on the age of the first estrous and estrous cycle length, and the testosterone levels also remained unchanged in males. However, at higher doses, brain cholinesterase activity was inhibited with no deleterious effects on the reproductive hormones of the body (Juberg et al., 2013).

CPF as a potential endocrine disruptor has been linked with alterations in the serum androgen and estrogen levels in a sex-selective manner. Furthermore, the levels of estradiol were greatly affected by TCP (a CPF metabolite), CPM, and carbaryl, as well as the naphthols, 1-naphthol (1N), and its isomer 2-naphthol. Estradiol, although the major female sex hormone, has considerable significance for regulating male reproductive health as well (Watts, 2012). Sex-associated differences, due to pesticide toxicity, have been reported in many research studies, and females have been found to be more sensitive to CPF toxicity. LD_{50} levels for females have been reported to be almost half than those mentioned for males (Mansour and Mossa, 2009).

Jeong et al. (2006) treated female rats with CPM at 100 mg/kg and reported reduced fertility index, mating ratio, number of fetal implantations, and pups born alive. Reduced ovarian weight and increased spleen weight was observed in the F1 female rats exposed to 100 mg/kg of CPM. However, Sandhu et al. (2013) reported a significant increase in comet formation, and increased tail length, and more DNA percentage in comet tail among male rats rather than the females (Table 4). The adult male rats showed a reduced sperm count, a decrease in weight of seminal vesicles, increased weight of liver, and kidneys at 100 mg/kg dose levels (Jeong et al., 2006). More recently, Hazarika et al. (2019) studied the mechanistic approach involved in the molecular interaction of CPF and its various degradation products on the sex hormone-binding globulin (SHBG) through the molecular docking simulation approach and proposed that CPF degradation products have a strong ability to bind with SHBG and disturb the androgen signaling.

6.5. Hypothalamic-pituitary-thyroid (HPT) axis

CPF has been reported to potentially induce long-term changes in endocrine function and is known to disrupt the thyroid gland and its secretions the most, causing various clinical and subclinical malfunctions. The study of thyroid functioning has witnessed significant interest, as the thyroid gland is involved in many physiological activities and acts on almost all body tissues. Thyroid hormones, triiodothyronine (T_3) and thyroxine (T_4) are involved in thermoregulation, normal reproductive functions, heartbeat regulation, metabolism of proteins, carbohydrates, and lipids, gastrointestinal (GI) functions, and emotional stability. Adult male rats were tested for alterations induced in thyroid hormone levels by CPF dosed daily at 5 mg/kg body weight orally for 14 days. Both the live body weight, as well as the thyroid weight were significantly reduced (Leemans et al., 2019).

The loss of body weight may indicate systemic intoxication due to inhibition of the AChE and may be due to the intoxication of the thyroid gland. Furthermore, the reduced size of thyroid follicles, follicular denaturation, vacuolation in the cytoplasm, mitochondrial degeneration, and condensed nuclei were reported in the thyroid gland (Shady and Noor El-Deen, 2010). Similar observations of histopathology and thyroid hormones imbalance were stated by De Angelis et al. (2009) when CD-1 mice were treated with 6 mg/kg CPF, indicating that CPF induces serious and long-lasting histopathological alterations in the gland (Table 4). Thyroid follicles of CPF-treated rats also showed micronuclei formation with abnormal chromatin distribution (Shady and Noor El-Deen, 2010). Moreover, no changes in mean serum levels of T4, or thyroid-stimulating hormone (TSH) levels at any dose in either sex were observed, as well as no pathological findings in the ovaries, uterus, testis, epididymis, and thyroid after CPF treatment (Juberg et al., 2013). Sparling and Fellers (2009) reported that CPF exposure reduced body weight and tadpole length and induced a delayed metamorphosis. The authors correlated the delay in metamorphosis with decreased levels of thyroid hormone. However, the delay may be due to the stress conditions, and that stress is regardless of thyroid hormone signaling/effects.

Reduced thyroglobulin protein (Tg) (precursor protein for thyroid hormones T_3 and T_4), due to a decrease in the amount of colloid created a drop in the plasma T_3 and T_4 levels and increased production of TSH from the adenohypophysis in the male rats treated with CPF. The rats exposed to CPF also showed strong expression of a caspase-3 protein (encoded by CASP3 gene) which helps in the execution of apoptosis (Shady and Noor El-Deen, 2010) and may also be the cause of decreased thyroid function. Effects of CPF on the thyroid and adrenal functions of CD-1 deficient mice in both gestational and postnatal periods were reported by De Angelis et al. (2009). No signs of maternal toxicity were observed. However, F1 generation mice showed reduced T_4 levels and thyroid follicular necrosis at a dose of 6 mg/kg which was more pronounced in males. The histology of both adrenal and thyroid glands showed vacuolation in a dose-dependent manner.

6.6. Hypothalamic-pituitary-adrenal (HPA) axis

A major and important endocrine system, the HPA axis takes part in the stress response mechanism and maintains different body functions. Jeong et al. (2006) tested the effects of CPM on maternal and fetal thyroid and adrenal hormones at dose levels 1, 10, and 100 mg/kg. After exposure to CPM during pre-breeding, gestation and lactation, the F1 generation showed low levels of T_3, T_4, estradiol, and testosterone, while cortisol and TSH levels were higher in both male and female pups. Histopathological necrosis and vacuolation were found in the thyroid and adrenal glands. Vacuolation in the zona glomerulosa, zona fasciculata, and zona reticularis were observed in a dose-dependent manner. It is thought that OPs act on dexamethasone target tissues/

cells, however, CPF does not draw out its outcome through stress or events on the HPA axis (Table 4).

Seidler and Slotkin (2011) reported that early exposure to CPF/common toxicant chemicals helps in damage to the hormone path those maintain and have critical in the body metabolic homeostasis. The proposed mechanisms of CPF-induced increased plasma glucose levels are due to the activation of the HPA axis. Studies also provide considerable evidence regarding the potential of organophosphate pesticides in causing several neurodevelopment disorders leading to learning disabilities and delayed neurodevelopment due to inhibition of AChE activity after prenatal and postnatal exposure (Sapbamrer and Hongsibsong, 2019). Primarily organophosphate pesticides were thought to be neurotoxic only, but they can also induce diabetes-like alterations. Pesticides especially organophosphate are known to induce stress and these stress responses intimidate body homeostasis resulting in activation of the HPA axis which leads to hyperglycemia (Mechanick, 2006). Under stress conditions, the adrenal gland gets activated and secretes glucocorticoids in response to ACTH release from the anterior pituitary gland. These adrenal cortical glucocorticoids act on the hepatocytes for activation of the gluconeogenesis pathway and in turn increase blood glucose levels. Insulin secreted by β-cells of pancreases is responsible to maintain plasma glucose levels and internalization of glucose in the myocytes and other vital organs of the body, however, increased levels of glucocorticoids interact with this uptake of insulin-mediated glucose uptake by these myocytes. Stress is also recognized for the release of cytokines that take part in the increased production of hepatic glucose and induce glucose resistance in peripheral tissue (Hotamisligil, 2003). Other hormones produced in response to stress are glucagon from α-cells of pancreases, catecholamines from the adrenal medulla, and growth hormone from the somatotrophs of the adenohypophysis also take part in the increased glycogenolysis and gluconeogenesis from hepatocytes (Gustavson et al., 2003). These are the normal physiological mechanisms to maintain circulating glucose for use of the brain and to support the energy requirements of insulin-independent cell types (Mizock, 2001). Catecholamines produced in the stress induce lipolysis and liberate free fatty acids in the circulation and enhance its blood levels and take part in insulin signaling inhibition and glycogen production as well (Itani et al., 2002).

6.7. CPF and pancreas toxicity

The mammalian pancreas is composed of exocrine and endocrine systems for controlling enzymatic intestinal digestion and glucose metabolism. Along with the liver, the pancreas is the most important organ that performs a significant role in glucose homeostasis (Schinner et al., 2005). The OP pesticides can induce pancreatic inflammation through the role of immunocytes (monocytes, granulocytes, and lymphocytes) and neutrophils infiltration, as well as increased levels of inflammatory mediators such as tumor necrosis factor alpha (TNF-α), interleukin-1 (IL-1β) interleukin-6 (IL-6), interleukin-8 (IL-8), platelet-activating factor (PAF), intercellular adhesion molecule 1 (ICAM-1), monocyte chemotactic protein-1 (MCP-1), substance P (SP), hydrogen sulfide (H2S), complement component c5a, and neutral endopeptidase (NEP) (Shrivastava and Bhatia, 2010). Some studies have also demonstrated endocrine and exocrine acute pancreatitis (AP) in rats, resulting from acinar cell-associated damage induced by exposure to OP pesticides (Gulalp et al., 2007).

The primary mechanism involving CPF-induced acute pancreatitis revolves around excessive stimulation of the pancreatic acinar cells. This stimulation is a direct consequence of the release in excess concentrations of ACh from pancreatic nerves. Studies have reported the deterioration and necrosis of acini, and the β-cells of the Islets of Langerhans, the proliferation of interlobular ducts, the widening of the interlobular spaces, as well as the infiltration of these spaces by various inflammatory cells (Kammon et al., 2011; Khayal and Ahmed, 2019). Various investigations have linked these factors to the causation of hyperglycemic episodes, whereby CPF induced a decrease in the serum insulin levels, with the consequent increase in serum glucose levels (Orabi et al., 2013; Hamza et al., 2014; Khayal and Ahmed, 2019). Other studies have also cited the role of muscarinic receptors located on the pancreatic β-cells associated with the production of insulin and its release. By way of inhibiting the activity of AChE, CPF increases the accumulation of ACh leading to greater stimulation of these muscarinic receptors and their eventual downregulation, resulting in reduced insulin synthesis. Furthermore, the stimulation by AChE for extended periods may also manifest in the reduction of β-cell sensitivity to glucose (Khayal and Ahmed, 2019).

Diazinon, other than CPF, also tends to inhibit glucose-mediated insulin secretion from β-cells *in vitro* (Pourkhalili et al., 2009). Malathion at the dose rate of 200–400 ppm for 4 weeks decreased the insulin production to 69.6% (Abdollahi et al., 2004). However, there are very little data available on the effects of CPF on endocrine pancreatic toxicity. Since the β-cells of the pancreas contain preproinsulin which is clipped into insulin by specific proteases prohormone convertases 1 and 2. This conversion occurs in the Golgi apparatus and insulin secretory granules and the endoplasmic reticulum. CPF effects the inhibition of AChE via phosphorylation of the serine. Similarly, PC-1 and 2 are also known as serine proteinases of the subtilisin/kexin-type family (Casida and Quistad, 2004) and have similar catalytic triad at the active site. Due to that, we can hypothesize that CPF not only has an inhibitory effect on AChE but also on the proinsulin processing enzymes. If it is true, this may be one of the major reasons for environmental toxicant-induced diabetes mellitus. Also, that may account for one of the reasons for CPF-induced hyperglycemia.

6.8. CPF and mammary carcinogenesis

The mammary gland is a complex organ that is responsible for milk production or lactation. The development of mammary glands is a complex process that undergoes a sequence of various coordinated developmental phases from birth to senescence and is influenced by hormonal stimulation (Maffini et al., 2006). The primary hormones responsible for the development of mammary glands are progesterone and estradiol (Atwood et al., 2000; Clarke, 2006). This hormonal regulation is mediated by estrogen and progesterone receptors which are associated with ductal elongation and development of lobuloalveolar units in the mammary glands (Mallepell et al., 2006).

Breast cancer has emerged as a major health risk for women worldwide and is second only to skin cancer in terms of cases reported. It is estimated that approximately one in every eight women is diagnosed with invasive breast cancer during their lifetime. As the statistics indicate that only 5–10% of the cases related to breast cancer are hereditary, the likelihood of involvement of lifestyle and environment-associated risk factors increases significantly. One of these risk factors is the agrochemicals such as CPF, although, the relationship between CPF and the causation of mammary carcinogenesis is not well elucidated. Some of the proposed mechanisms for the explanation of the link between CPF and increasing mammary carcinogenic potential include AChE inhibition, endocrine disruption, altering the expression of cell cycle proteins, as well as cell adhesion molecules vital for breast development, elevated oxidative stress levels, and decreased apoptotic signaling (Yang et al., 2020). CPF has been shown to induce increased cell proliferation, elevated histone deacetylase (HDAC) mRNA levels, the greater number of ducts (and hyperplastic ducts), as well as a higher incidence of tumors, and a decrease in the latency period related to tumor formation (Ventura et al., 2019; Yang et al., 2020).

Studies have revealed that CPF triggers the cellular proliferation in MCF-7 breast cancer cells through estrogen receptor alpha (ER) (Ventura et al., 2012). The results stated that CPF (0.05 μM) has the ability to induce cellular proliferation in hormone-dependent breast cancer cells. The findings of the investigation have also revealed that CPF (50 μM) modifies the cellular proteins by changing the redox balance of MCF-7 and causes intra-S arrest. Additionally, CPF (50 μM) binds

tubulin sites and alters the polymerization of microtubules by inducing an arrest in the G2/M phase. Conclusively, the study provides evidence on the potential of CPF as an environmental risk factor of breast cancer by modulating the proliferation of breast cells. In another exploration, the same group (Ventura et al., 2016) investigated the effect of CPF (@ 0.01 mg and 1 mg/kg/day) on mammary gland health, level of serum steroid hormones, and expression of hormone receptors by exposing female rats (Sprague-Dawley) to experimental CPF doses for 100 days. The study demonstrated that a proliferating ductal network with an increased level of adenosis and hyperplasia was observed along with an increased expression of the progesterone receptor in the experimental rats after a 100-day exposure of CPF. On the contrary, reduced levels of progesterone, luteinizing hormone, and estradiol were observed in rats exposed to CPF (Table 4). These findings confirm the harmful effect of CPF on the mammary glands and suggest that this pesticide may act as a potential risk factor for inducing mammary carcinogenicity.

6.9. Effect of CPF exposure on gut microbiota and intestinal health

The human intestine has a vast range of microbial communities performing various physiological functions in the body. The types and populations of these gut microflora can be changed by various genetic and environmental factors. Organophosphate pesticides have been reported to modulate the microbial community in the mammalian intestinal tract. Studies have claimed that the toxic effects of these pesticides can significantly alter the microbial populations and their activities (Roman et al., 2019). For example, Wang et al. (2019) studied the effect of CPF exposure (30 μg/L, 100 μg/L, 300 μg/L) for 21 days on the gut microbial dysbiosis and oxidative stress in zebrafish. The results stated that CPF exposure increased oxidative stress in the gut of zebrafish by increasing the malondialdehyde levels and decreasing the glutathione contents. Additionally, a considerable change in the relative abundance of Proteobacteria was also seen in the CPF exposed zebrafish, Furthermore, CPF exposure also caused changes in about 25 types of gut bacteria at the genus level.

Recently, Guardia-Escote et al. (2020) investigated the effect of postnatal CPF exposure on the modulation of gut microbiota and found significant alterations in the genotypes of intestinal microflora. Also, Perez-Fernandez et al. (2020) explored the effects of low-dose (1 mg/kg/mL/day) CPF exposure on gut microbiota in rats and concluded that CPF exposure resulted in momentous gut microbiome dysbiosis. These studies support the evidence regarding the capability of CPF to induce a considerable impact on the number and genotypic/phenotypic characteristics of mammalian gut microorganisms which ultimately negatively affect the intestinal health.

7. Pharmacological treatment and possible non-pharmacological remedies

Keeping in view the health hazards of CPF, various studies have also been conducted to discover such agents or mineral elements that can modulate the damages caused by this environmental contaminant. Some of those remedies are mentioned below. Additionally, some important strategies to reduce CPF concentrations have been summarized in Table 5.

7.1. Clinical signs and symptoms of CPF exposure and pharmacological treatment

Some studies have reported that there is a likelihood that humans are much more sensitive to the acute- and short-term oral exposure as well as acute dermal exposure of CPF as compared to animals, with significant evidence indicating the inhibition of plasma cholinesterase (ChE) activity, and possible clinical manifestation (Rathod and Garg, 2017). The mode of action, in terms of production of systemic toxic effects, for all OPs, involves the inhibition of acetylcholinesterase, with the affected individuals consequently exhibiting symptoms of initial cholinergic hyperstimulation (followed by depression) as a consequence of the accumulation of acetylcholine, the neurohumoral mediator of the cholinergic junctions (Rauh, 2018). Moreover, OPs such as CPF, as a result of acute exposure, have been shown to induce a toxidrome which comprises of muscarinic receptor (postganglionic parasympathetic junctions) manifestations, including salivation, urination, lacrimation (runny eyes), miosis, diarrhea, gastric cramps, bronchorrhea, emesis, bronchospasm, and bradycardia, as well as nicotinic receptors (neuromuscular junctions) symptoms such as muscle fasciculations, muscle weakness, cramping, mydriasis, hypertension, and tachycardia. Furthermore, the CNS effects of OP poisoning may manifest in the form of anxiety, restlessness, emotional instability, confusion, ataxia, seizures, tremors, and coma. Consequent acute respiratory failure due to severe poisoning may ultimately contribute to mortality in humans (Nurulain, 2012; Jafarzadeh et al., 2013; Peter et al., 2014). Organophosphate-induced delayed neuropathy (OPIDN) has been reported to be associated with the occurrence of neuropathy target esterase (NTE) inhibition several weeks after the exposure, as well as the expression of the extrapyramidal signs such as dystonia, cogwheel rigidity, and marked Parkinsonian features possibly associated with dopamine blockade (Eddleston and Chowdhury, 2016; Reji et al., 2016).

Poisoning with rapidly active OP pesticides can manifest in a respiratory arrest within 30 min of exposure, and it has been widely reported that many individuals do not survive to reach the healthcare facilities where they can receive primary treatment. Affectees who do reach a hospital, need resuscitation (and airway control), oxygenation, fluids, and

Table 5
Strategies to reduce the residual concentration of CPF.

Strategy	Subject	Method applied	Effect	Reference
Water washing	Rice and maize	Soaking in water before cooking	59–100% reduction in CPF concentration	Tejada et al. (1990)
	Red pepper fruits	Sonication in water for 5 min	30–40% reduction in CPF concentration	Lee (2001)
	Spinach, cauliflower, potato, eggplant, tomato, okra	Tap water washing for 30 s	15–33% reduction in CPF concentration	Randhawa et al. (2007)
Ozone treatment	Wheat and corn	Soaking	Reduced concentration up to 15–20 ppm with 12.3% reduction/day	Zhanggui et al. (2003)
Cooking	Spinach, cauliflower, potato, eggplant, tomato, okra	Cooking of sliced vegetables in boiling water for 10–12 min.	12–48% reduction in CPF concentration	Randhawa et al. (2007)
Pasteurization	Milk	At 62.8 °C for 0.5 h.	44.68% reduction in CPF concentration	El-Hoshy (1997)
Processing	Apples	Frozen apple slices, apple juice, apple sauce, and apple concentrate	78–98% reduction in CPF concentration	Zabik et al. (2000)
Microbial biodegradation	*In vitro*	*Bacillus cereus* inoculation in liquid medium containing 150 mg/L CPF	80% catabolic biodegradation after 5 days of incubation	Liu et al. (2012)
	In vitro	2% *Pseudomonas nitroreducens* AR-3 inoculum in mineral salt medium (MSM) containing 100 mg/L CPF	42% reduction after 2 h. of incubation	Aswathi et al. (2019)

atropine, and ventilatory support, within the first hour of exposure, usually termed as the 'Golden Hour' from the perspective of clinical intervention. Any more delays, and the characteristic 'ageing' of the toxicants in blood sets in. Benzodiazepines can be employed for the control of seizures. In the situations involving ocular exposures, irrigation of the patients' eyes with isotonic sodium chloride (NaCl) solutions, and lactated Ringer's solutions could prove beneficial. Also, Morgan lenses can be used for irrigation purposes as well (Goud et al., 2012; Detweiler, 2014; Eddleston and Chowdhury, 2016).

Traditionally, the mainstay of the clinical treatment for CPF poisoning (as well as other OPs) has involved the use of two antidotes, atropine (blocker of muscarinic receptors), and oximes, in particular, pralidoxime (2-PAM), as well as obidoxime, and TMB-4, that first gained clinical prominence during the late 1950s. Atropine is a potent nonspecific antagonist with a good CNS penetration capability and acts to block the OP-induced hyperstimulation of both the central and muscarinic cholinergic nerve terminals, while the action of pralidoxime involves restoration of the strength of the respiratory and skeletal muscles. In addition, supplemental antidotes, such as magnesium, sodium, and clonidine bicarbonates have also been studied in various trials, although have not produced the desired outcomes. Moreover, novel antidote solutions have been sought in the form of nicotinic receptor antagonists, beta-adrenergic agonists (e.g. Salbutamol/Albuterol), or beta-blockers, as well as lipid emulsions, and have been undergoing testing in large animal models, and pilot clinical trials (Detweiler, 2014; Eddleston and Chowdhury, 2016; Vale and Lotti, 2015). Intravenous (IV) administration of magnesium sulfate ($MgSO_4$), and calcium channel blocking (CCB) drugs such as Nimodipine, a 1,4-dihydropyridine CCB, have been investigated, and various studies have reported the reversion of neuromuscular junction (NMJ) effects induced by exposure to CPF and other OPs (Detweiler, 2014; Brvar et al., 2018).

Initial IV-administered atropine bolus loading dose can be set at 0.6 to 3 mg, and then repeated q3-5min PRN (pro re nata or as the circumstance demands), until the patient is atropinized, i.e. adequate cardiorespiratory function has been re-established (the reversal of bradycardia, and restoration of systolic blood pressure to over 80 mm Hg, as well as oxygenation), a process often called, 'atropinization'. Excessive atropine, however, has been linked with antimuscarinic toxicity, resulting in hyperthermia episodes, tachycardia, delirium, and ileus (Eddleston and Chowdhury, 2016). Employed as a nucleophilic agent, pralidoxime takes part in reversing the muscle paralysis resulting from CPF poisoning and is administered (an IV loading dose of 20–30 mg/kg over 15–30 min in 100 mL isotonic NaCl and then repeated q6-8h) in accompaniment with atropine for effectively resolving the effects of the OP poisoning (Eddleston and Chowdhury, 2016; Blumenberg et al., 2018).

Penehyclidine is a more recently developed anticholinergic agent, with M1 and M3 cholinergic receptors as its main targets. Clinical studies have indicated that atropine and penehyclidine, when administered together, is a very effective therapy for the treatment of patients with OP poisoning, both in terms of cure rate, and the reduction in the incidence of adverse drug reactions (ADR) associated with atropine (Yu et al., 2020). Further treatment of the chronic toxicity due to OPs involves detoxification supports such as the nutrition-associated therapy with docosahexaenoic acid (DHA), an omega-3 fatty acid isolated from various types of fish, and that aids in enhancing the antioxidant activity in the brain, thereby preventing further OP-induced damage. Other antioxidants, for instance, vitamin C, vitamin E, and alpha-lipoic acid (ALA), as well as glycine, taurine, and *N*-acetylcysteine have also been proposed for the reduction of OP-induced oxidative stress in patients (Detweiler, 2014).

7.2. Non-pharmacological remedies

7.2.1. Effect of various processing/storage approaches on CPF residues in foods

Food safety is a very important area of concern as it directly relates to human health. The presence of pesticide residues in various foods is common and most of the pesticides are present in the quantities that can cause serious harm to human health. Given that CPF has been extensively used on crops to exterminate insect pests, the residual limits of CPF have been reported as toxic. Hence, it is vital to tackle this issue of food safety through strategic interventions. Several post-harvest processing treatments are generally claimed to reduce the residual concentrations of CPF and other pesticides in food matrices. The most commonly employed approaches include washing, peeling, cooking, fermentation, and other thermal processing techniques (Kaushik et al., 2009).

Simple household food processing techniques can be improved to reduce the concentration of CPF and its residues on fruits and vegetables. Randhawa et al. (2007) recorded the effect of washing, peeling, and cooking of fruits and vegetables on the concentration of CPF and its metabolite TCP. The results revealed a 15–33 percent reduction in CPF residues by washing, 65–85% by peeling, and further 12–48% by cooking. However, cooking increased contamination levels of TCP due to the thermal break down of CPF into its residual forms. Similar results were reported by Zafar et al. (2012) when they found a significant reduction in pyrethroid pesticide levels on eggplant and okra fruit after washing with tap water. Treating the food crops with acidic or alkaline water also reduces the pesticide residues from the crops (Ahmed et al., 2011).

Studies have also concluded that the development of different products from raw agricultural commodities also lowers down the concentrations of pesticides in a significant way. In this regard, Zabik et al. (2000) processed apples sprayed with CPF and other pesticides into different products such as frozen apple slices, apple juice, apple sauce, and apple concentrate. Results explored that processing of apples into these products reduced the concentrations of CPF and other pesticides from 78 to 98% which affirmed that processing can considerably reduce the residual concentrations of pesticides from fruits and vegetables. in another exploration, Whangchai et al. (2011) investigated the effect of ozone treatment on CPF concentrations in lychee fruit. Purposely, the fruits were dipped in CPF solution (10 mg/L) for 10 min. Followed by ozone treatment (gaseous form and ozonated water) using various concentrations (80, 160, 200, 240 mg/L) for different time intervals (10, 20, 30, 60 min). and subsequent storage for 6 days at room temperature. The findings explained that ozone treatment of fruits was effective in reducing CPF concentrations. Likewise, Partha et al. (2006) employed different processing approaches viz. simple water washing, salt water washing, cooking, washing, and cooking, and treatment with a detergent solution to reduce the CPF concentrations from cauliflower curds and reported that various treatments momentously reduced (27.9 to 73.3%) CPF concentrations in cauliflower curds.

7.2.2. Dietary administrations to reduce the toxicity of ingested CPF concentrations

Several nutritive and non-nutritive components have been by researchers to overcome the toxic effects of consumed CPF through food. In this regard, vitamins, minerals, and other phytochemicals with antioxidant properties have shown their potential to minimize the hazardous effects of exposed CPF. In an investigation, the combined dose containing CPF and zinc was administered to rats to check the effect of zinc against CPF toxicity. Rats treated with both CPF and zinc showed no significant difference in enzymatic activity form the control group, which was exposed only to vehicle, revealing that zinc treatment shows ameliorating effects by neutralizing oxygen free radicals which are produced due to CPF intoxication (Goel et al., 2006).

In addition to zinc, a major secretory product of the pineal gland called melatonin is also a potential agent in reducing the toxicity induced by CPF. Melatonin and its metabolites act as effective antioxidants to scavenge the reactive oxygen species and reduce the oxidative stress that is produced by CPF. Melatonin protects DNA, proteins, and lipids from destruction, which is usually caused by

reactive oxygen species, as it can easily pass through the blood-brain barrier and cell membranes. These findings were figured out by Umosen et al. (2012) experimented on rats.

The ameliorating effect of vitamin C on the oxidative stress caused by CPF was studied by Shittu et al. (2012) on Wistar rats. CPF effect was observed on rat pituitary gland which resulted in increased levels of malonaldehyde and reduced superoxide dismutase and catalase levels. These effects were ameliorated by vitamin C treatment.

Another effective antioxidant that has been discovered to reduce the oxidative stress of CPF is propolis. Propolis is a natural product extracted from cracks in tree bars and leaf buds which contain enriched salivary enzymes of honeybees. It contains about >300 biochemical components including some minerals like Mg, Ca, I, K, Na, Cu, Zn, Mn, Fe, vitamins B, C, E, and some enzymes and a large number of fatty acids. It is extensively used in food products. El-Mazoudy et al. (2011) tested the effect of CPF on male rats' fertility which was greatly reduced, and sperm physiology was also affected to a large extent. In a subsequent test, pretreatment with propolis restored most of the fertility parameters to normal. Furthermore, CPF induced testicular histopathology was alleviated by propolis pre-administration.

7.2.3. Microbial degradation of CPF

Recently, it has also been suggested that some bacterial (*Enterobacter* sp., *Pseudomonas* sp., *Stenotrophomonas* sp., *Bacillus* sp., *Klebsiella* sp., *Staphylococcus* sp., etc.) and fungal (*Verticillium* sp., *Aspergillus* sp., *Penicillium* sp., *Cladosporium* sp., etc.) species are quite beneficial in the biotransformation of environmental CPF. Microbial biodegradation is found a cost-effective and safe method for the removal of CPF-based environmental toxins (Supreeth and Raju, 2017). A generalized pathway describing the mechanism involved in the microbial biotransformation of CPF has been illustrated in Fig. 4.

In an exploration, Aswathi et al. (2019) reported that Pseudomonas nitroreducens AR-3 can be used for about 97% removal of CPF from the soil. Purposely, they applied 2% inoculum in mineral salt medium (MSM) containing yeast extract (0.5 b/L), glucose (1 g/L), and CPF (100 mg/L) and found a very lower concentration of TCP (degradation product of CPF) after 8 h of incubation. They also revealed that organophosphate hydrolase (OPH) enzyme was mainly responsible for the rapid degradation of CPF (42% removal in 2 h) which showed the potential of *P. nitroreducens* for rapid bioremediation of CPF. Likewise, Liu et al. (2012) used *Bacillus cereus* isolated from contaminated soil for biodegradation of CPF and reported about 80% catabolic biodegradation after 5 days in a liquid medium. Moreover, Chishti et al. (2013) also reviewed the work of several researchers on microbial degradation of CPF. Based on these findings, it is recommended that the microbial species having the capability to mineralize CPF and other pesticides without developing toxic substances must be explored and adopted to minimize the hazardous environmental and health effects of CPF and its by-products.

8. Conclusion

Chlorpyrifos, an organophosphate pesticide, has been used on food crops, households, and other workplaces for the extermination of insect pests. It is one of the most commonly used pesticides worldwide. However, keeping in view its toxic effects such as genotoxicity, immunotoxicity, cytotoxicity, oxidative stress, neurotoxicity, and mutagenicity, etc., CPF has been banned in various countries. The present review provides a brief explanation of CPF-induced toxic effects and possible mechanisms of action on animal and human health e.g., acetylcholinesterase inhibition, modification of gene-regulating factors, or the effects thereof on the estrogenic receptors. Studies have concluded that humans are more sensitive to the oral and dermal exposure of CPF with several clinical manifestations resulting in the symptoms of cholinergic hyperstimulation, dystonia, eye irritation, cogwheel rigidity, respiratory distress and Parkinsonian disease. Keeping in view its endocrine and other toxicological effects, several pharmacological and non-pharmacological approaches have been employed such as the use antidotes, administration of different drugs and nutritional therapies which include dietary administration of vitamin C, melatonin, and zinc which are capable of ameliorating the toxic effects of CPF. Additionally, the microbial degradation/biotransformation can be a useful strategy to mitigate CPF-induced toxicity in the environment. Moreover, food processing methods are quite effective in reducing CPF concentrations in foods. Conclusively, gap exists regarding proper baseline studies highlighting the safety/toxicity comparison of CPF with other members of the group to support the advantageous usage of CPF over other OP pesticides. Therefore, the question about the potential toxicity or safety of CPF can be resolved by the use of proper quantitative tests through animal experiments followed by prospective human studies with known CPF and other OP pesticide exposures. These improvements in the analysis should be brought about and correlated with the absorbed doses of CPF and other OP pesticides in the light of proper mechanistic understanding. In this aspect, intellectual safety assessments can be made to bridge this gap in order to mitigate its harmful environmental and health effects.

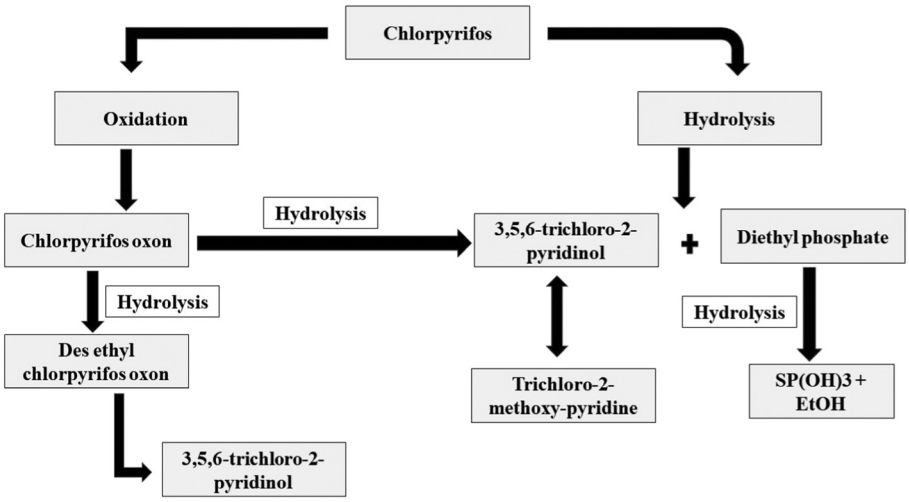


Fig. 4. Generalized pathway for microbial degradation of CPF.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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