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Impact of trichlorfon Organophosphate use in Pisciculture: a review

Impacto do uso de Organofosforados de Triclorfon na piscicultura: uma revisão

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ABSTRACT
Classified as a class II organophosphate and considered highly toxic, trichlorfon is a drug widely used in worldwide pisciculture for the treatment of parasites in farming ponds. It is used for the treatment of several fish species, both in natural and artificial ponds. The main action of trichlorfon occurs in the nervous system of fish by blocking the enzyme acetylcholinesterase and causing acetylcholine accumulation, which leads to the constant passage of neural transmission. However, several studies show that trichlorfon can be more harmful to the fish than to the parasite that the drug is trying to eliminate. Our article brings a review of the main effects of trichlorfon in several fish species around the world, in order to further evaluate these side effects and help researchers to understand this drug.

Keywords: Organophosphate, trichlorfon, pisciculture, fish.

RESUMO
Classificado como organofosforado classe II e considerado altamente tóxico, o triclorfon é um medicamento amplamente utilizado na piscicultura mundial para o tratamento de parasitas em tanques de cultivo. É usado para o tratamento de várias espécies de peixes, tanto em tanques naturais como artificiais. A principal ação do triclorfon ocorre no sistema nervoso dos peixes ao bloquear a enzima acetilcolinesterase e causar o acúmulo de acetilcolina, o que leva à passagem constante da transmissão neural. Entretanto, vários estudos mostram que o triclorfon pode ser mais prejudicial aos peixes do que ao parasita que a droga está tentando eliminar. Nosso artigo traz uma revisão dos principais efeitos do triclorfon em várias espécies de peixes ao redor do mundo, a fim de avaliar melhor estes efeitos colaterais e ajudar os pesquisadores a entender esta droga.

Palavras-chave: Organofosforado, triclorfon, piscicultura, peixes.

1 INTRODUCTION

Trichlorfon (dimethyl [2,2,2-trichloro-1-hydroxyethyl] phosphonate) is an antiparasitic frequently used in agriculture and pisciculture. It was introduced in the 1950s when it began to be used against plagues of insects and to control parasites and insects in domestic animals (International Program on Chemical Safety 1992). In pisciculture, the trichlorfon can be used as an acaricide, insecticide, and anthelmintic to treat fish through immersion baths where the compound is mixed with the water (Brasil 2018; Rauco 2002). In Brazil the compound can be found in several formulations, having in common the amount of trichlorfon in its composition, ranging from 95% to 98% plus carrier substances.

Acetylcholinesterase (AChE) is the target enzyme of the trichlorfon, which can be found in several tissues. Trichlorfon acts by blocking the action of AChE, inducing the accumulation of acetylcholine in the synaptic cleft, which promotes

uninterrupted signal transmission between the nerve cell and the muscle, triggering prolonged muscle contraction (International Program on Chemical Safety 1992). Thus, trichlorfon acts on the parasite and also on the host organism. This occurs mostly in fish, where several studies have been made about the action of trichlorfon and its effects on these organisms.

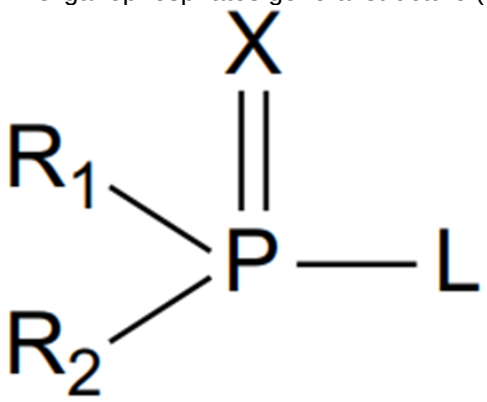
Considering trichlorfon negative effects on aquatic organisms, especially fish, and that these effects can disturb fish production, this paper aims to present a literature review about the main results of trichlorfon based drugs used in pisciculture, presenting organophosphates definition and action mechanisms, the uses of trichlorfon, and trichlorfon effects on parasites and fish, especially in the Amazonian region.

2 ORGANOPHOSPHATE COMPOUNDS: DEFINITION AND ACTION

Organophosphate pesticides (OPs) are widely used in agriculture to control plagues. However, most pesticides in this class are considered toxic because they can be bioaccumulated in the environment (Dzudzevic Cancar et al. 2016). In the mid-1950s, Malathion® was one of the first organophosphates to be considered safe, then at least 200 more compounds were produced and commercialized (Chambers et al. 2010*a*).

Organophosphate compounds are mainly recognized by the presence of phosphorus (P) in their structure with three other individually bonded atoms (H, O, and C) and a covalent bond supporting their structure (Chambers et al. 2010*c*). They are derived from two main groups: phosphoric acid (H_3PO_4) and phosphonic acid (H_3PO_3). This nomenclature is based on the atoms surrounding the central phosphorus. The leaving group (L) is the most reactive and variable group when OP phosphorylates the acetylcholinesterase within its structure. The least reactive groups within the structure are the R_1 and R_2, commonly composed of alkoxy groups (Chambers et al. 2010a) (Figure 1).

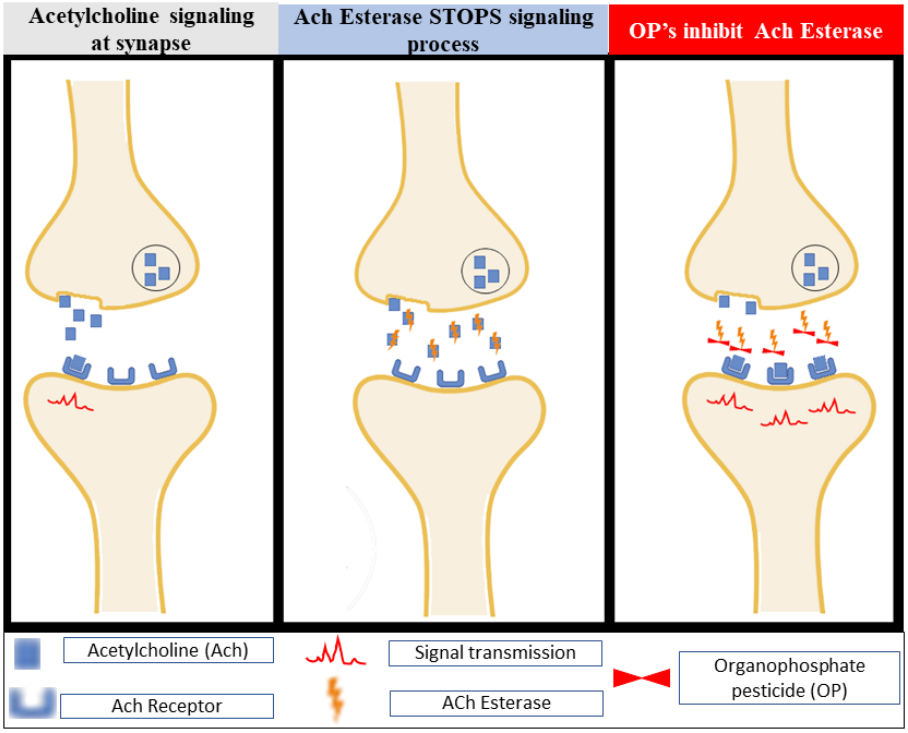
Figure 1: Organophosphates general structure (OPs).



For the organophosphates metabolization to occur they are subject to many metabolic pathways mediated by several groups of enzymes responsible for xenobiotic metabolism. This happens due to the variation caused by the atoms linked to phosphorus and carbon in the compound structure. The first group to be expelled is the leaving group, already mentioned above, which phosphorylates its esterase targets. Then, secondary metabolites are formed after the phosphorylation and also need to be metabolized. Therefore, the organophosphate compounds or their secondary metabolites need to go through two different metabolism phases: phase 1 and phase 2. The phase 1 reaction consists of the oxidation, reduction, and hydrolysis of the compound; phase 2 consists of conjugation reactions (Chambers et al. 2010b).

The main mechanism of action of organophosphate compounds is the acetylcholinesterase inhibition. Acetylcholinesterase (AChE) is an enzyme characterized as cholinesterase whose main function is to complete the transmission of the nerve impulse through the modification of the neutral transmitter acetylcholine (ACh) into acetic acid (Ac) and choline (Ch) by a hydrolysis process. When the organophosphate binds to AChE, it forms an irreversible complex, causing the enzyme to be blocked (Kubitza and Ono 2007). This leads to an accumulation of ACh in the Central Nervous System, which may cause illnesses such as Alzheimer's disease. In fish, it can result in physiological changes and intensify nerve impulse transmission (De Aguiar et al. 2004) (Figure 2).

Figure 2: Mechanism of action of OPs on the AChE enzyme. Acetylcholine signaling at the synapse: normal functioning of synaptic transmission through the bond of ChE binding itself to the receptor of the postsynaptic neuron. ACh Esterase STOPS signaling process: the action of AChE on ChE, stopping the synaptic transmission signal. OP inhibits ACh Esterase: the action of OPs on AChE, preventing the interruption of synaptic signal transmission.



Among the most used organophosphates, the main one is trichlorfon (dimethyl [2,2,2-trichloro-1-hydroxyethyl] phosphonate), also known as Dipterex 500®, Methyl Paration®, Neguvon® and Masoten® (Ordinance nr. 10 08.03.85 – DOU 14.03.85, Ordinance No. 318 06.23.87 – DOU 26.06.87) in its commercial versions. It is presented as an insecticide that is used both in agriculture to control pests and in pisciculture to control parasites, flatworms, leeches, aquatic insects, and nymph elimination through immersion baths that vary in concentration and time depending on the organism to be eliminated (Rauco 2002). Each of the commercial versions has a specific amount of trichlorfon in its composition and specific application modes.

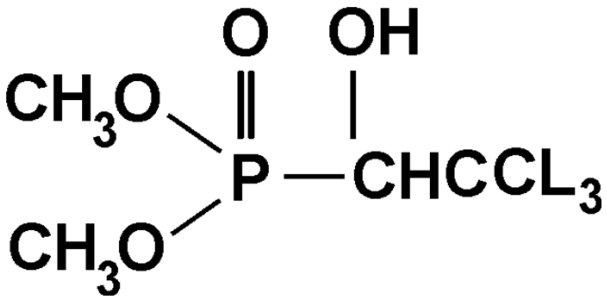
3 THE USE OF THE ORGANOPHOSPHATE TRICHLORFON

Trichlorfon (dimethyl [2,2,2-trichloro-1-hydroxyethyl] phosphonate) is classified as an organophosphate compound and is mostly used to control ectoparasites in pisciculture (Brazil 2008; Santana and Cavalcante 2016), with an established maximum concentration limit of 10µg/kg in farmed fish (Brazil 2019).

According to the National Health Surveillance Agency (ANVISA), trichlorfon is a compound classified in toxicological class II (highly toxic) with insecticidal, acaricide, and anthelmintic properties (Brazil 2018). The compound is presented as a colorless powder and is considered stable at room temperature and, when hydrolyzed in an acidic medium, its half-life is 526 days at pH 1-5 and 20ºC. In an alkaline medium, at pH 8 and 37.5ºC, trichlorfon is hydrolyzed into a compound called dichlorvos which is considered more toxic than trichlorfon itself. There are many studies evaluating the effects of dichlorvos on several organisms (International Program on Chemical Safety 1995).

Trichlorfon is an organophosphate derived from phosphoric acid and its homologs, being presented in different forms and different concentrations, depending on its purpose (Figure 3). As well as compounds in the organophosphate class, trichlorfon acts on the acetylcholinesterase enzyme (AChE). According to Duncan et al. (2020), AChE in fish brains decreases its activity by at least 90% when the animals are subjected to trichlorfon. As a result of the exposure, fish can also have a deformed body, loss of balance, and altered swimming ability (Silva et al. 2020). In this work, it was also observed that fish submitted to a 12hrs exposure with a 3.2 mg/L concentration of trichlorfon presented different organs colorations. Furthermore, as a result of AChE inhibition, intense muscle fibers contractions were detected (Kubitza and Ono 2007; Silva et al. 2020).

Figure 3: Trichlorfon chemical structure.



After the metabolization, trichlorfon generates secondary metabolites such as dimethyl trichlorfon, dimethyl dichlorvos, dimethyl hydrogen phosphate, methyl hydrogen phosphate, phosphoric acid, and trichloroethanol. With a long lifetime, the best form to degrade trichlorfon is by demethylation, through the break of the

P-C bond in the chemical structure and the steric hydrolysis of dichlorvos (International Program on Chemical Safety 1995).

Trichlorfon composed drugs are known by the trade names Neguvon® and Masoten®. Neguvon® is indicated for the treatment of internal and external parasitosis in cattle, horses, sheep, and swine. It is composed of 97% trichlorfon in 100g of the drug. This drug is indicated for parasitic diseases such as berne (*Dermatobia hominis*), gastric habronemosis (*Oestrus ovis*), stomach larvae (*Gasterophilus* spp.), gastric and cutaneous habronemosis (*Habronema* spp.), scabs, lice, flies, and worms. It can be applied orally to the animals as well as by spraying in the places where they are raised. Its dosage changes depending on the animal being treated.

As for Masoten®, it is best known for treating fish in pisciculture and each 100g of the drug contains 80% of the trichlorfon. This drug is indicated for the treatment of parasitosis in freshwater fish such as fish lice (*Argulus* sp.), gill crab (*Ergasilus* sp.), anchor worm (*Lernea* sp.), protozoa (*Trichodinas* sp.), gill worm (*Dactylogyrus* sp.), skin worm (*Gyrodactylus* sp.) and some trematode species. The dosage used in pisciculture depends on the organism to be eliminated, the size of the tanks in which the product will be applied, and if the treatment will take place before or after the occupation. Masoten® is first prepared and dissolved in distilled water for further immersion baths.

Frequently, aquatic animals treated with trichlorfon composed drugs are bathed in the substance, which is commonly known as immersion baths, where the drug is added to tank water (Rauco 2002). For immersion baths, the proportion between dosage and duration time is taken into account, because baths performed for a long period, combined with a high dose of the drug, can cause mortality in treated animals (Flores-Nava and Vizcarra-Quiroz 1988). Resolution nº 20 by the National Council for the Environment (CONAMA) established on July 18, 1986, that the dosage allowed for organophosphate compounds in freshwater is 1.0 mg/L.

4 THE EFFECTS OF THE ORGANOPHOSPHATE TRICHLORFON ON PARASITES

There is a wide range of susceptible species for which trichlorfon is

indicated. For that, some studies were carried out with the trichlorfon measuring values of $LC_{50\text{-}96h}$ in order to evaluate its effect on species of parasites and algae that can affect pisciculture and compromise production. Crustaceans of the genus *Daphnia* sp. are also known as water fleas, which affect several piscicultures and are one of the groups of organisms that have been studied in the laboratory when it comes to testing toxicological agents. Arauco et al. (2005) studied the toxicological response in three *Daphnia* species (*Daphnia magna*, *Daphnia similis*, and *Daphnia laevis*) about copper sulfate agents and trichlorfon, aiming to define the $LC_{50\text{-}96h}$ of the two compounds in these animals with and without the presence of sediment (free from other toxic compounds) in the tanks used.

According to the results found by Arauco et al. (2005), the three studied species of *Daphnia* did not show significant differences in the toxicity of trichlorfon in the absence or presence of the sediment. However, in its presence, a higher dose of trichlorfon (approximately 400 times more) was necessary to affect the species. The authors emphasize that this is due to the ability of trichlorfon to adsorb to the sediment, reducing its toxicological effect on the studied species. However, this turns out to be a concern because the trichlorfon binds to the sediment over the exposure time and can cause accumulation in the environment. The authors also highlighted that, according to the results found, trichlorfon proved to be more toxic than copper sulfate for the three studied species.

The acute toxicity of trichlorfon was measured by Qin and Dong (2004) through tanks containing the Australian freshwater crustacean *Cherax destructor*, also known as yabby. The work aimed to define the acute toxicity of trichlorfon on yabbys and also on some zooplankton species and other parasites that affect the breeding tanks of this crustacean, to inform producers about the safe trichlorfon concentrations that could be used in the production without compromising the yabbys. In addition to the crustacean, the $LC_{50\text{-}96h}$ of ostradocda *Newhamia* sp., cladocera *Daphnia carinata*, copepoda *Boeckella tricarticulata*, and two species of rotifers *Keratella quadrata* and *Filina longiseta* were also defined. The $LC_{50\text{-}96h}$ found were: *Cherax destructor* 0.055 mg/L^{-1}, *Newnhamia* sp. 0.0001 mg/L^{-1}, *D. carinata* 0.00001 mg/L^{-1}, *B. tricarticulata* 0.003 mg/L^{-1}, *K. quadrata* 0.016 mg/L^{-1} and *F. longiseta* 0.014 mg/L^{-1}. The $LC_{50\text{-}96h}$ results show that the parasites are more sensitive to trichlorfon than the yabbys, allowing their treatment without

compromising the organisms being raised.

Some studies were carried out with larger parasites, aiming to compare and explain the trichlorfon mechanism of action between these parasites and some higher organisms. For this purpose, Rajini et al. (2008) used the parasite *Caenorhabditis elegans* as the model organism, which is a species of nematode of the Rhabditidae family. This species was chosen as the model organism because there are studies that compare the toxic effects of xenobiotics in this species with some species of mammals, as well as the genetic and physiological similarity between the cholinergic system of these animals and higher organisms. For the tests, the authors used 10 types of organophosphate compounds: acephate, dimethoate, dichlorvos, dicrotophos, monocrotophos, methamidophos, phosphamidon, omethoate, phosdrin, and trichlorfon. As a result, the authors observed that all organophosphates studied ended up inhibiting about 50% of AChE activity in the studied organism and compromising its ability to move. These results may be predictive for further studies on the effects of organophosphates on the neurotoxicity of higher organisms, such as mammals.

Coelho et al. (2011) carried out a study where non-target organisms were exposed to trichlorfon, in order to evaluate its toxicological potential in these organisms. The potential of biomarkers such as cholinesterase (ChE), glutathione-S-transferase (GST), lactate dehydrogenase (LDH), and catalase (CAT) was also evaluated in species such as *Danio rerio* (embryos and adults), *Daphnia magna*, *Pseudokirchneriella subcapitada* (seaweed) and *Chlorella vulgares* (seaweed). Among these organisms the most sensitive to trichlorfon exposure was *D. magna*, presenting an $LC_{50\text{-}48h}$ of 0.29 µg/L. *P. subcapitata* had an $LC_{50\text{-}96h}$ of 274.5 mg/L, while *C. vulgares* had no observed effects. The phases of *Danio rerio* presented in the initial $CL_{50\text{-}96h}$ of 25.4 mg/L and in the adult $CL_{50\text{-}96h}$ of 28.8 mg/L. Among the biomarkers tested, the most sensitive was ChE, which was expected, however, all the biomarkers studied are presented as useful tools in terms of exposure and intoxication by trichlorfon.

5 THE EFFECTS OF THE ORGANOPHOSPHATE TRICHLORFON ON FISH

One of the first studies about trichlorfon effects on fish species was carried out by Veiga et al. (1997), in which the drug Dipterex 500® was used in juvenile

specimens of curimbatá (*Prochilodus scrofa*). Separated into the control and treated groups, the second one received a dosage of 0.2 µl/liter of the drug diluted in water with an exposure time of 24 hours. It was observed in the first group that the compound caused damage to the splenic tissue, causing tissue atrophy, as well as a significant decrease in the number of erythrocytes. Were observed damage to the pyknotic nuclei, necrotic foci, a drop in average hematocrit values, number of erythrocytes, average corpuscular hemoglobin rate, and average corpuscular hemoglobin concentration. It was also observed that neutrophils were more frequent in the exposed animals and lymphocytes in the control animals. Lymphocytes and monocytes decreased as time increased and neutrophils and monocytes increased. The meaning of these changes for fish physiology and their homeostasis remains uncertain.

Tavares-Dias et al. (1999) evaluated the effect of 0.4 mg of trichlorfon in 500 L of water in two days of treatment using the species pacu (*Piaractus mesopotamicus*) as an experimental animal parasitized with *Argulus* sp.. After 50 days of treatment, there were reductions in the number of red blood cells and hemoglobin in the blood. Yoshimura and Endoh (2005) evaluated the acute toxicity levels ($LC_{50\text{-}96h}$) of five antiparasitics in aquatic organisms: *Oryzias latipes*, *Daphnia magna*, and *Brachionus calyciflorus*. Neguvon® (Japan-Bayer) was the drug used with the trichlorfon compound. The authors observed that trichlorfon was rapidly decomposed and after 96 hours it showed 0.7% of its initial concentration in the form of dichlorvos. Thomaz et al. (2009) evaluated the cardiorespiratory function and oxidative stress markers in Nile tilapia (*Oreochromis niloticus*) that was exposed for 96 hours to a concentration of 0.5 mg/L^{-1} of trichlorfon and showed that the organ more sensitive to the drug was the heart, when in comparison with liver and gill. This was visualized from the analysis of glutathione S-transferase activity, which decreased its enzymatic activity, and hydroperoxide, which increased its activity during trichlorfon exposure. The liver and gills showed antioxidant mechanisms against trichlorfon exposure preventing lipid peroxidation.

Mataqueiro et al. (2009) defined the $LC_{50\text{-}96h}$ in pacu (*Piaractus mesopotamicus*) and observed histopathological changes in its gills, liver, and kidneys caused by exposure to the organophosphate trichlorfon. The calculated value for the $LC_{50\text{-}96h}$ was 0.1906 mg/L^{-1}. In the gills exposed to the concentrations

of 0.05 and 0.1 mg, the primary and secondary lamellae suffered hyperplasia and swelling, as well as subepithelial edema. These alterations found in the gills can cause damage to the fish's respiratory system and the ionic regulation mechanisms controlled by the tissue structure (Evans et al. 2005). After 7 days of exposure to the lowest concentration of 0.05 mg/$L^{-1,}$ it was possible to observe in the liver structure of these fish that the hepatocytes lost their conventional morphology when the cell nucleus was located peripherally and cell fusion was identified. These same damages were found in the other concentrations. However, at the concentration of 0.1 mg/L^{-1}, hepatocytes showed signs of necrosis with pyknotic nuclei, decreased cytoplasmic affinity for eosin, and hypertrophied cells. These alterations became irreversible, causing severe damage to the liver's metabolism. The kidneys of fish exposed to lower concentrations began to show changes after 2 days of trichlorfon exposure. Were observed thickening of the glomerular capsule and an increase in the intracapsular space with glomerular atrophy. During the experiment, the nephrological damage was severe, making the animals' survival unachievable.

The effects on gene expression levels of the genes heat shock protein (HSP70), growth hormone, acetylcholinesterase (AChE), trypsinogen, cytochrome P4501B (CYP1B), and cytochrome oxidase subunit 1 (COI) were evaluated by Sinha et al. (2010) in *Pangasiadon hypophthalmus* exposed to trichlorfon concentrations at 0.01 mg/L, 0.1 mg/L and 0.5 mg/L for 6h, 24h, 96h, 7 days, 14 days, 28 days and 56 days in liver and gills. The results found showed different levels of gene expression of the evaluated genes. After 56 days of exposure, for the AChE gene in the liver, there was an increase in expression, being higher at the concentration of 0.5 mg/L. In the gills, the expression levels drastically decreased after 96 hours of exposure for all concentrations. HSP70 levels rose in both tissues and at all the concentrations after 96 hours of exposure. As for growth hormone, there was a decrease in expression levels after 6 hours of exposure in both tissues and at all exposure concentrations. Trypsinogen expression levels decreased when evaluated in the liver after 24 hours of exposure, and in the gills, these levels were slightly higher when compared to the control group. COI showed higher expression levels in both tissues, at all concentrations after 24 hours of trichlorfon exposure. There was a variation in the gene expression levels of

CYP4501B in the liver when compared to the gills. The liver showed a lower expression level at all concentrations after 24 hours of exposure while the gills showed higher expression levels after 96 hours of exposure. Thus, with the results found it is possible to observe that the genes tested are strong candidates as biomarkers for monitoring trichlorfon use in pisciculture. It is also very important to note that trichlorfon exposure compromises the gene expression of growth hormone, which ends up causing damage to the growth of cultivated fish.

AChE enzymatic activity in the tambaqui (*Colossoma macropomum*) brain was evaluated by Assis et al. (2010), during exposure to five organophosphates: dichlorvos, diazinon, chlorpyrifos, and tetraethyl pyrophosphate. In this work, it was observed that the concentration of dichlorvos, chlorpyrifos, and tetraethyl pyrophosphate necessary to inhibit 50% of the enzyme activity was 0.04 µmol/L, 7.6 µmol/L, and 3.7 µmol/L, respectively. The results found indicated that AChE can be used as a possible sensory biomarker for evaluative studies of organophosphates. Coelho et al. 2011 established $LC_{50\text{-}96h}$ levels in organisms such as *Danio rerio* (zebrafish), *Daphnia magna*, and in seaweeds such as *Pseuudokirchneriella subcapitata* and *Chlorella vulgaris* in addition to studying the effects of these sub-lethal concentrations on proteins considered to be potential biomarkers such as cholinesterase (ChE), glutathione-S-transferase (GST), lactate dehydrogenase (LDH) and catalase (CAT). Fertilized eggs were exposed for 5 days to concentrations of 0, 2.5, 5.0, 10, 20, 40, 80 and 160 mg/L of trichlorfon; adult fish were exposed for 4 days to 0, 2.5, 5, 10, 20, 40, 60 and 80 mg/L of trichlorfon. Zebrafish in the early stages of life presented $LC_{50\text{-}96h}$ corresponding to 25.4 mg/L and the adults presented $LC_{50\text{-}96h}$ with a value of 28.8 mg/L. The authors also observed that trichlorfon was considered teratogenic to zebrafish embryos and after exposure, these embryos showed abnormalities in yolk sac absorption, spinal flexion, and pericardial edema. They also pointed out that among the biomarkers studied, ChE was the one that during the exposure showed the most intense loss of activity when compared to the other biomarkers.

Studies using the fish *Carassius auratus gibelio*, also known in Europe and Asia as Prussian carp, were carried out to study the effects of trichlorfon exposure on oxidative stress, hepatocyte apoptosis, and accumulation of hepatic lipids (Xu et al. 2012*a,b*). Xu et al. (2012*a*) observed, after exposing the fish to 0, 0.5, 1.0,

2.0, and 4.0 mg/L-1 of trichlorfon for 30 days, that the activity of the hepatic total nitric oxide synthesis enzyme (T-NOS), of xanthine oxidase enzyme (XOD), and the hepatocyte apoptosis rates increased according to the increase in trichlorfon concentration. Through the activity levels of the superoxide dismutase plasma enzymes (SOD), catalase (CAT), and vitamin E, it was evaluated whether there was an imbalance in the antioxidative balance of the plasma. It was observed that SOD and CAT showed an increase in enzyme activity when fish were exposed to 2 and 4 mg/L^{-1} of trichlorfon, and CAT activity was reduced when fish were exposed to $0.5mg/L^{-1}$. The vitamin E of the plasma increased at concentrations of 2 and 4 mg/L^{-1}. The results found in the work showed that the hepatocytes apoptosis occurred due to the imbalance in the antioxidative activities of the plasma, caused by the peroxidation of lipids.

Xu et al. (2012*b*) studied, also in Prussian carp (*Carassius auratus gibelio*), the trichlorfon effects on the accumulation of hepatic lipids. In this work, plasma and biochemical metabolism of hepatic lipids were analyzed. In treatments of 1.0, 2.0, and 4.0 mg/L, triglyceride levels increased in the liver and decreased in plasma. At exposure concentrations of 0.5, 1.0, and 4.0 mg/L of trichlorfon, plasma insulin levels increased. Regarding the lipids, it was observed that there was an accumulation in the liver. The lipase that is sensitive to the hepatic hormone did not differ when treated fish were compared to the control group. In fish exposed to 2.0 mg/L of trichlorfon, hepatic adenosine 3',5'-cyclic monophosphate, very low-density lipoprotein, and apolipoprotein B100 showed a decrease. When hepatocytes were visualized under an optical microscope, the rough endoplasmic reticulum showed mitochondrial dilatation and vacuolization. In conclusion to the data found during the work, the authors also mention that trichlorfon was able to damage the hepatic pathways of lipid metabolism and caused damage to the hepatocyte structure of the studied fish.

About the action of trichlorfon on *Piaractus mesopotamicus* (commonly known as pacu), Mataqueiro et al. (2014) evaluated the inhibition of the enzymatic activity of AChE making quantification of trichlorfon in both water and exposed fish, through gas chromatography. Regarding AChE activity, inhibition was observed in plasma and brain, as expected, but the enzyme retook activity after 7 days of fish recovery. According to the results found on gas chromatography, the trichlorfon

levels dissipated more markedly in the first 3 hours after the sampling and remained decreasing after every left hour. The results indicated that, in water with pH 7.7, trichlorfon has a short residual action, being completely dissipated after 35 to 40 hours.

Also using pacu (*Piaractus mesopotamicus*) as a study organism, Venturini et al. (2014) used Masoten® to evaluate its effects on the enzymatic activity of AChE, alkaline phosphatase (ALP), and acid phosphatase (ACP) enzymes in muscle, plasma, and liver. The fish were exposed to 10% of the $LC_{50\text{-}96h}$ of trichlorfon described for pacu and later the fish were submitted to recovery (without trichlorfon exposure). The results found showed that AChE activity was reduced during the exposure and continued to decrease even after the recovery period. The levels of ALP and ACP activity were stable during the exposure but after the recovery period, the ALP enzyme showed a high activity rate in the liver and muscles and a low activity rate in plasma, while ACP showed high liver activity and decreased muscle activity. The trichlorfon exposure also ended up affecting the energy metabolism of fish.

In one of the pioneering works about the trichlorfon ingestion by fish, Pucher et al. (2014) assessed trichlorfon and fenobucarb contamination capacity through the contaminated grasses ingestion by herbivorous carp from Vietnam. The fish were fed for 10 days with fish feed. As the fish were fed, the levels of trichlorfon in the water increased. Contamination of fish food, either by grass or feed, did not cause fish mortality and the trichlorfon did not cause fish feed rejection. However, there was a decrease in AChE activity and liver changes. When quantified, trichlorfon levels were low.

Some studies were carried out aiming at the reduction of the trichlorfon effects on fish because many of these effects are deleterious and can cause serious damage to their organisms and production. Yonar et al. (2015) used propolis (*Populus nigra* L.) to analyze whether it would be able to relieve the trichlorfon effects in common carp (*Cyprinus carpio*) regarding hematological damage and oxidant and antioxidant parameters caused by the exposure. The fish were exposed to a sublethal trichlorfon concentration corresponding to 11 and 22 mg/L^{-1}, and propolis was incorporated into their diet at a concentration of 10 mg/kg^{-1} according to their weight. Both products were simultaneously administered. The

experiment ran for 14 days and blood, liver, kidney, and gill samples were collected. The results found suggest that although trichlorfon caused damage to the fish in terms of hematological and antioxidant parameters, propolis had the ability to reduce these effects in the fish that received the enriched feed. Thus, propolis is evaluated as a good ally to reduce trichlorfon effects, because it is cheap and can be administered through the fish diet.

In order to understand how trichlorfon can be toxic not only for the treated fish species but also for non-target organisms of the antiparasitic, Ma & Li (2018) evaluated the effects of this antiparasitic on the transcriptome of *Rana chensinensis*, an anuran from the Asian continent. The brown frogs were exposed for 4 weeks at a concentration of 0.1 mg/L and after this period livers were collected from both the control group and the experimental group. Transcriptional analysis showed that trichlorfon exposure caused dysregulation in oxidative stress, lipid peroxidation, and liver damage in the frogs. Furthermore, enzymes related to the xenobiotics and organophosphates metabolism were found to have high transcriptional regulation. Among these, are CYP450 and GST.

The best-known trichlorfon treatment method to reduce parasite infestations is the immersion baths. However, these immersion baths result in serious damage to both fish and the environment, increasing the rates of pollution by organophosphates. In an attempt to reduce the pollution effects caused by this treatment method, Lu et al. (2018) tried to orally administer trichlorfon in fish and evaluated its effects after a single administration. After trichlorfon oral administration in *Carassius auratus gibelio* (Prussian carp) at concentrations of 0.5 g/kg, 1 g/kg, and 2 g/kg, it was observed that the absorption of trichlorfon in plasma and liver tissue occurred quickly. However, this trichlorfon had much lower levels in less than 24 hours of administration. Effects such as vacuolar degeneration, necrosis, and central vein congestion were seen in the liver after administration. Although the oral administration of trichlorfon is a safer way regarding environmental pollution, the work showed that there was an accumulation of trichlorfon in plasma and liver tissue, which can cause hematotoxicity and hepatotoxicity, causing serious physiological problems in fish even with a single oral application.

Woo et al. (2018) evaluated the effects of trichlorfon in common carp

(*Cyprinus carpio* L.) regarding hematological parameters, biochemical factors, and stress. They were exposed to concentrations of 0, 0.5, 1.0, 2.0, and 4.0 mg/L^{-1} during one and two weeks. The exposure was made at two different temperatures: 15ºC and 25ºC. After trichlorfon exposure at different temperatures, the parameters evaluated showed that trichlorfon can cause damage to erythropoietic tissue. The authors also evaluated the expression levels of genes such as HSP70 and cytochrome p450 1A, indicating that these genes can be used as possible biomarkers for these conditions.

Studies on trichlorfon show that, at a pH greater than 5.5, the compound is transformed into dichlorvos, which is considered to be 5 times more toxic than trichlorfon itself. Thus, several studies were guided to use the secondary metabolite dichlorvos. Altenhofen et al. (2019) used zebrafish as the study organism in order to analyze the effects of dichlorvos exposure in their early stages of life and during their development. Morphological parameters and also locomotor and social behavior were analyzed at 7, 14, 30, 70, and 120 days after fertilization of fish exposed to 1.5 and 10 mg/L of dichlorvos. The results found showed that in 7 days post-fertilization fish there was a reduction in body size and heart rate. It was also observed that they lost the abilities of escaping, traveling long distances, average speed, and mobility. Their social behavior was not affected, but the results evaluated showed that organophosphate exposure can cause behavioral damage and neural changes.

Baldissera et al. (2019) evaluated whether the phosphoryl transfer chain would be involved in the hepatic and branchial metabolic changes caused by trichlorfon exposure of the fish *Rhamdia quelen*. The results found showed that trichlorfon exposure compromised the phosphoryl transfer network, indicating that it is indeed involved in hepatic and branchial changes. In another work using the same experimental model *Rhamdia quelen*, Baldissera et al. (2019*b*) analyzed the neurotoxic effects of trichlorfon exposure in fish by analyzing the disruption of the blood-brain barrier and evaluating its effects on oxidative stress, cell viability, and brain neurotransmitters. The results found showed that the disruption of the blood-brain barrier is a very important pathway involved in neurotoxic damage caused by trichlorfon, causing cerebral oxidative damage and alterations in brain neurotransmitters.

In order to evaluate the trichlorfon exposure effects on tambaqui (*Colossoma macropomum*) and to determine the value of $LC_{50\text{-}96h}$ in juvenile fish, our research group used 5 initial concentrations to try to determine this value: C0 = 0 mg/L of trichlorfon, C1 = 0.4 mg/L, C2 = 0.8 mg/L, C3 = 1.6 mg/L and C4 = 3.2 mg/L (Silva et al. 2020). The value found for $LC_{50\text{-}96h}$ was 0.870 mg/L of trichlorfon for fish with an average of 13.52 g. The authors also described that fish swimming ability was disturbed when exposed to a higher concentration of trichlorfon (3.2 mg/L), as well as morphological differences in some organs when compared to animals that were not exposed to the compound. Duncan et al. (2020) also described the same $LC_{50\text{-}96h}$ value in tambaqui and analyzed the activity of acetylcholinesterase (AChE) and glutathione-S-transferase (GST) enzymes in the brain, muscle, intestine, and liver of fish exposed to $LC_{50\text{-}96h}$ 30% and 50% concentrations (0.26 mg/L and 0.43 mg/L, respectively). AChE showed inhibition of 90% in the brain, muscle, and intestine. However, GST did not show activity variation in any of the tissues studied. As in several other works previously described, the authors emphasize the possible use of AChE as a possible biomarker in conditions of trichlorfon exposure.

Several studies are guided in order to minimize the effects caused by the treatment with the antiparasitic trichlorfon in aquatic organisms. Thus, Li et al. (2020) used extracts from the plant *Angelica sinensis* in an attempt to minimize the treatment effects on Prucian carp (*Carassius auratus auratus*). As expected, trichlorfon treatment caused function loss and oxidative damage in fish muscles, through decreased energy metabolism and oxidation of lipids and proteins. When the *Angelica sinensis* extract was added to the fish diet there was a decrease in the generated oxidative damage, as well as enzymes related to antioxidant activity had higher levels. Therefore, the use of the plant extract in question was effective in minimizing the trichlorfon effects on the Prussian carp muscle.

In the same experiment carried out by Ma & Li, 2018, Ma et al. (2020) performed transcriptomic analysis of brown frogs' brains (*Rana chensinensis*) subjected to trichlorfon stress. The transcriptome sequencing of this species showed about 874 genes with differential expression after trichlorfon exposure, acting directly on some neural ion channels and as an agonist or antagonist at specific receptors, or interfering with signal transduction in the brain of brown frogs.

The effects triggered by this exposure, although believed to be reversible, can cause body damage as a whole, leading to neurological, metabolic, and immunological disorders. Therefore, this study, as well as that of Ma & Li (2018), shows that even non-target species can suffer from damage caused by trichlorfon use, even at doses considered low.

Still aiming for the relief of trichlorfon effects in fish, Baldissera et al. (2021) used the flavonoid rutin added to the diet of silver catfish (*Rhamdia quelen*) in order to verify whether this flavonoid would be able to relieve or prevent behavioral damage and oxidative stress in the brain. As a result, fish that received rutin-enriched feed had the prevention of the morphological effects caused by trichlorfon exposure, except for AChE brain activity, which remained lower compared to the non-control group. Thus, as well as the propolis evaluated by Yonar et al. (2015), the flavonoid rutin used in the fish diet is also a great ally in the search for the reduction of trichlorfon exposure effects.

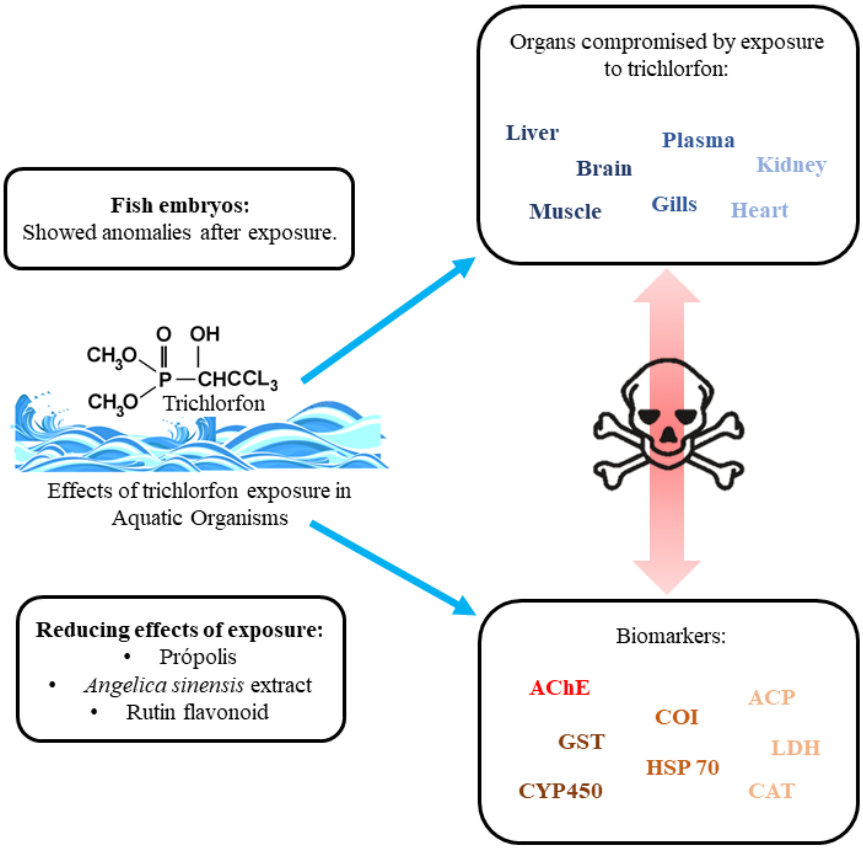
In a recent study, Cruz et al. (2021) performed an evaluation of trichlorfon effects on the parasite *Dawestrema cycloancistrum* (Monogenea), on the gills of the pirarucu (*Arapaima gigas*), and also evaluated the effects of this treatment on the fish. In *in vivo* and *in vitro* tests, trichlorfon proved to be highly efficient in eliminating this parasite. Contrary to several articles seen and discussed here, pirarucu did not show changes in plasma after immersion baths (60 minutes for two days) with trichlorfon at a concentration of 150 mg/L, but these immersion baths were highly efficient on the parasites located on the gills. Thus, pirarucu, until this review, was the only fish that did not suffer alterations due to treatment with this antiparasitic.

In a study evaluating the effects of trichlorfon exposure on *Pseudoplatystoma corruscans*, commonly known as pintado (also a species of great importance for pisciculture in Brazil) gills and liver of these fish were analyzed (Oliveira-Lima et al., 2021). Exposure to the antiparasitic showed histopathological, histomorphometric, and histochemical changes in the analyzed organs. Several works already cited showed the same responses to trichlorfon exposure in several fish species.

All the studies mentioned show the relevance of exploring and evaluating the trichlorfon effects on different species, both in fish and parasites, as well as

evaluating the bioaccumulation capacity of trichlorfon in the environment and the fish muscle, as this is the organ most consumed by humans. Therefore, the study of trichlorfon toxicological effects becomes a public health issue because it seeks to answer and analyze the extent to which humans are being contaminated or not with the compound through fish consumption. It is also relevant to monitor how this organophosphate is used in breeding tanks because, as it is quite volatile, it may affect those who handle it and repair it for use. The genes expression study related to metabolism and response to trichlorfon exposure is also extremely important for the definition of environmental biomarkers, as well as for measuring at the molecular level the damage caused by trichlorfon exposure. Figure 4 presents a summary about the consequences of trichlorfon use in aquatic organisms, the most affected organs by the exposure, and the biomarkers that can be used to evaluate these damages.

Figure 4: Summary of the studies described through this literature review. The fish embryos evaluated showed anomalies related to the bone part of the body after exposure. Propolis, *Angelica sinensis* extract and the flavonoid rutin showed a reduction in the exposure effects. The organs compromised by the exposure are presented with different color intensities: from the darkest, the most compromised organ, to the lightest, the least compromised organ, as well as biomarkers.



In addition, the NGS tools that had been developed so far are positively helping studies with this type of approach because analysis such as transcriptomics show a more complete response in terms of genes expression that can be compromised by trichlorfon exposure, both in target and non-target organisms.

Knowing the toxicological effects and considering the trichlorfon toxicity to non-target organisms, some strategies are also being studied to reduce the effects of the use of the compound in these organisms trying to reduce production loss when it comes to pisciculture. Thus, several studies are still needed to solve the doubts about trichlorfon toxicity, both in aquatic organisms and humans.

6 CONCLUSION

The organophosphate trichlorfon, despite being considered highly toxic, is hugely used for the treatment of parasites in aquatic organisms. Its main mechanism of action is by inhibiting the enzyme acetylcholinesterase and after blocking the synaptic passage in the nervous system it causes the death of the parasite. However, several studies had shown that this same mechanism of action also occurs in the treated organism, thus compromising its function. For the mass production of these organisms, this becomes a problem, because it can compromise the production health. According to the presented studies, several organs of these organisms are compromised by the treatment using trichlorfon: liver, gills, muscle, spleen, heart, brain and blood. From these organs, many enzymes and metabolites can be used as biomarkers to detect the trichlorfon use in aquatic organisms.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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