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Fish Cholinesterases as Biomarkers of Organophosphorus and Carbamate Pesticides

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1. Introduction

Due to reasons that last for decades, environmental monitoring of pesticides is an urgent need. Contamination by pesticides is an important public health problem, mainly in developing countries. It is estimated that only 0.1% of the applied pesticides in fact reach the target pests, while the rest spreads throughout the environment (Hart and Pimentel, 2002). In addition, among the 500,000 deaths a year related to pesticides in the developing world, approximately 200,000 occur due to the use of organophosphorus (OP) and carbamates (CB) pesticides (Eddleston et al., 2008). These are among the most important classes of insecticides/acaricides in usage and billing (Nauen and Bretschneider, 2002). The primary and most known target for the action of organophosphorus and carbamate compounds is a family of enzymes (Cholinesterases; ChEs) formed by: acetylcholinesterase (AChE, EC 3.1.1.7) and butyrylcholinesterase (BChE, EC 3.1.1.8). The first is synthesized in hematopoiesis, occurs in the brain, endplate of skeletal muscle, erythrocyte membrane, and its main function is to regulate neuronal communication by hydrolyzing the ubiquitous neurotransmitter acetylcholine in synaptic cleft (Quinn, 1987; Silman and Sussman, 2005). The second is synthesized in liver and is present in plasma, smooth muscle, pancreas, adipocytes, skin, brain and heart (Çokugras, 2003). Although its physiological function is not well defined, BChE is pointed as one of the main detoxifying enzymes able to hydrolyze or scavenge a broad range of xenobiotic compounds like cocaine, heroine, anaesthetics, and pesticides (Soreq and Zakut, 1990; Taylor, 1991; Çokugras, 2003; Nicolet et al., 2003). Some studies hypothesize that one of the functions of BChE is to protect AChE against anticholinesterasic agents (Whitaker, 1980; Whitaker, 1986). Pezzementi and Chatonnet (2010) reported that ChEs emerged from a family of proteins with adhesion properties. Both play other roles in the neuronal tissue, particularly in neuronal differentiation and development, cell growth, adhesion and signalling. In addition, AChE participates even in hematopoietic differentiation (Chatonnet and Lockridge, 1989; Taylor, 1991; Johnson and Moore, 2000; Silman and Sussman, 2005).

Moreover, AChE and BChE are different concerning several other aspects: while AChE has an *in vivo* half-life of 120 days, BChE lasts 7-12 days. AChE is inhibited by substrate excess and BChE is activated by substrate excess (Lopez-Carillo and Lopez-Cervantes, 1993; Çokugras, 2003). AChE is selectively inhibited by propidium, DDM, caffeine, Nu1250, 62c47

and BW284c51 while BChE is selectively inhibited by percaine, isopestox, ethopropazine, Iso-OMPA, bambuterol and haloxon (Adams and Thompson, 1948; Austin and Berry, 1953; Aldridge, 1953; Bayliss and Todrick, 1956; Chatonnet and Lockridge, 1989; Harel et al., 1992; Kovarik et al., 2003). BChE has a larger space in its active site, which can hydrolize or be inhibited by a range of compounds. AChE has a more specific active site (Çokugras, 2003). Some of these features are governed by crucial differences in the structure of the enzymes such as: 1) the difference in size of active site can be explained by six aromatic residues lining the active site of AChE that are missing in BChE; 2) two of these (Phe-288 and Phe-290) are replaced by leucine and valine, respectively, in BChE. This feature prevents the entrance of butyrylcholine in the AChE active site; 3) peripheral site specific-ligands such as propidium does not inhibit BChE because the residue Trp-279, which is part of the peripheral anionic site located at the entrance of the active site gorge in AChE, is absent in BChE (Harel et al., 1992). According to Rosenberry (1975), AChE is more sensitive to the size of the acyl group than to the alcohol moiety (whether charged or neutral) of the substrate, while for BChE the opposite is observed. Both are inhibited by 50 μM of physostigmine (eserine), which is a condition that affords to discriminate cholinesterases (ChEs) from other esterases (Augustinsson, 1963).

The class of AChEs is more homogeneous in terms of their primary structure than the class of BChEs (Rosenberry, 1975). Despite of these differences, the amino acid sequence identity between AChE and BChE from vertebrates ranges from 53 to 60%, even in evolutionarily distant species (Chatonnet and Lockridge, 1989; Taylor, 1991). In addition, a study promoted the replacement of only two amino acids by site-directed mutagenesis in AChE for it to develop BChE activity (Harel et al., 1992). Both enzymes present the active site within a deep and narrow gorge, approximately in the middle of its globular structure, which apparently could disturb the substrate traffic. However, in fact this structure follows a rational organization which entraps substrate and transports it to the active site through the arrangement of amino acids lining the gorge. And all this occurs very efficiently (Quinn, 1987; Tõugo, 2001).

To characterize ChE, some studies used the kinetic parameters Km and Vmax, more specifically the Km and Vmax ratios for acetyl and butyrylcholine hydrolysis and their analogues by the enzymes. According to the expected values for these ratios, AChE has a low Vmax **ratio** and a Km **ratio** ≥ 1, because it presents excess substrate inhibition. BChE does not show this feature, its Vmax ratio is ≥ 1, and Km ratio < 1. (Pezzementi et al., 1991; Rodríguez-Fuentes and Gold-Bouchot, 2004).

Table 1 summarizes Km and Vmax of fish AChEs from brain, muscle and electric organ reported in the literature. The Km values varied from 0.085 (Rainbow trout brain) up to 3.339 mM (Brazilian flathead brain), whereas Vmax ranged from 0.116 (arapaima brain) up to 0.524 U/mg protein (female hornyhead turbot muscle).

Table 2 presents the values for optimum pH and maximum temperature of fish enzymes. pH values ranged from 7.5 to 8.5 for all reported species, while temperatures varied from 26ºC (bluegill brain) to 45ºC (tambaqui and pirarucu brains).

The Km values of fish BChEs presented in table 3 ranged from 0.033 (Nile tilapia liver) to 1.61 mM (tambaqui brain) and Vmax were from 0.04 (tambaqui brain) up to 0.231 U/mg protein (piaussu serum). Several studies have described that AChE accounts for most of the brain cholinesterasic activity (Rodríguez-Fuentes, 2004; Varò et al., 2004; Varò et al., 2007; Jung et al., 2007). However, our studies on brain ChEs from some fish reveal that certain

Scientific and common name	Km (mM)	Vmax (U/mg protein)	Source	Reference
Ictalurus punctatus – Channel catfish	0.375 ± 0.002	0.212 ± 0.002	Brain	Carr and Chambers, 1996
Oreochromis niloticus – Nile tilapia	0.101 ± 0.03	0.229 ± 0.014	Brain	Rodríguez-Fuentes and Gold-Bouchot, 2004
Pseudorasbora parva – topmouth gudgeon, Stone moroko	0.113 ± 0.11	0.490 ± 0.024	Brain	Shaonan et al., 2004
Carassius auratus – goldfish	0.112 ± 0.09	0.504 ± 0.027	Brain	Shaonan et al., 2004
Oncorhynchus mykiss – rainbow trout	0.085 ± 0.06	0.266 ± 0.023	Brain	Shaonan et al., 2004
Genidens genidens – guri sea catfish	0.236	nd	Brain	Oliveira et al., 2007
Paralonchurus brasiliensis – banded croaker	0.228	nd	Brain	Oliveira et al., 2007
Haemulon steindachneri – chere-chere grunt	1.035	nd	Brain	Oliveira et al., 2007
Pagrus pagrus – red porgy, common seabream	1.087	nd	Brain	Oliveira et al., 2007
Menticirrhus americanus – Southern kingcroaker	1.579	nd	Brain	Oliveira et al., 2007
Cynoscion striatus – striped weakfish	1.595	nd	Brain	Oliveira et al., 2007
Dules auriga (*Serranus auriga*)	1.624	nd	Brain	Oliveira et al., 2007
Merluccius hubbsi – Argentinean hake	3.259	nd	Brain	Oliveira et al., 2007
Percophis brasiliensis - Brazilian flathead	3.339	nd	Brain	Oliveira et al., 2007
Limanda yokohomae – Marbled sole	0.365 ± 0.16	nd	Brain	Jung et al., 2007
Limanda yokohamae – Marbled sole	0.18 ± 0.11	nd	Muscle	Jung et al., 2007
Pleuronectes vetulus - English sole	1.689 ± 0.26	0.482 ± 0.034	Muscle	Rodríguez-Fuentes et al., 2008
Pleuronichthys verticalis – hornyhead turbot	0.303 ± 0.07 (female); 0.226 ± 0.06 (male)	0.524 ± 0.032 (female); 0.120 ± 0.008 (male).	Muscle	Rodríguez-Fuentes et al., 2008
Colossoma macropomum – tambaqui	0.43 ± 0.02	0.129 ± 0.005	Brain	Assis et al., 2010
Arapaima gigas - pirarucu	0.42 ± 0.09	0.116 ± 0.002	Brain	not published results
Rachycentron canadum - cobia	0.43 ± 0.14	0.243 ± 0.02	Brain	not published results
Oreochromis niloticus – Nile tilapia	0.39 ± 0.2	0.218 ± 0.007	Brain	not published results

U = µmol of substrate hydrolyzed per minute; and nd = not determined

Table 1. Kinetic parameters of AChE from several freshwater and marine species

Scientific and common name	Optimum Temp	Optimum pH	Source	Reference
Solea solea – common sole	nd	7.5	Brain	Bocquené et al.,1990
Pleuronectes platessa – plaice	32 – 34ºC	8.5	Brain	Bocquené et al.,1990
Scomber scomber – mackerel	nd	7.5 – 8.5	Brain	Bocquené et al.,1990
Lepomis macrochirus – bluegill	26 – 27ºC	nd	Brain	Beauvais et al., 2002
Clarias gariepinus – African sharptooth catfish	nd	8.0	plasma	Mdegela et al., 2010
Colossoma macropomum – tambaqui	40 - 45ºC	7.0 – 8.0	Brain	Assis et al., 2010
Oreochromis niloticus – Nile tilapia	35ºC	8.0	Brain	not published results
Arapaima gigas - pirarucu	45ºC	8.0	Brain	not published results
Rachycentron canadum - cobia	35ºC	8.0	Brain	not published results

nd = not determined

Table 2. Values of optimal pH and temperature for AChE from several species of fish

Scientific and common name	Km (mM)	Vmax (U/mg protein)	Source	Reference
Oreochromis niloticus – Nile tilapia	0.033± 0.004	0.063 ± 0.001	Liver	Rodríguez-Fuentes and Gold-Bouchot, 2004
Oreochromis niloticus – Nile tilapia	0.123± 0.051	0.224 ± 0.016	Muscle	Rodríguez-Fuentes and Gold-Bouchot, 2004
Leporinus macrocephalus – piaussu	0.047	0.231 ± 0.008	Serum	Salles et al., 2006
Limanda yokohamae – Marbled sole	0.068 ± 0.35	nd	Muscle	Jung et al., 2007
Colossoma macropomum – tambaqui	1.61 ± 0.01	0.04 ± 0.001	Brain	not published results

U = µmol of substrate hydrolyzed per minute; nd + not determined.

Table 3. Kinetic parameters of BChE from several freshwater and marine species

species can present brain BChE or AChE with wider active sites. This is in accordance with Pezzementi and Chatonnet (2010), who reported atypical ChE activity in some fish species. Data about optimal pH and temperature of fish BChE are not presented here due to scarcity.

2. Organophosphorus and carbamates action on fish cholinesterases

OPs and CBs act by phosphorylating or carbamoylating the serine residue at the active site of the ChEs. Their structures present either similarities to the substrates or their hydrolytic intermediates and interact very slowly with the enzyme by forming stable conjugates (Quinn, 1987; Tõugu, 2001). This mechanism hinders the normal functioning of the enzyme, which cannot prevent the accumulation of the neurotransmitter in the synaptic cleft. The overstimulation caused by acetylcholine continuously firing its receptors generates a range of signs and symptoms. Because of their low environmental persistence and high toxicity, particularly to aquatic organisms, water must be continuously monitored (Beauvais et al., 2002).

Environmental monitoring may be chemical and/or biological. Chemical monitoring is the set of chemical analysis that quantify waste contaminants in a compartment or environmental matrix in a temporal or spatial scale. On the other hand, when the focus is to determine the magnitude of the effects of this contamination on organisms at individual or population level, biological monitoring is adopted (Henriquez Pérez and Sánchez-Hernández, 2003). The combined use of chemical and biological approaches in environmental monitoring is an important task for the assessment of contamination and its effects on an ecosystem. This is the basis of the concept of bioindicators.

In this scenario, when determining chemical characteristics of pollutants and their concentrations, organisms and their biomolecules represent a useful choice as bioindicators, since they afford to employ both the chemical and the biological approaches in environmental biomonitoring. Moreover, they also allow estimating the impact of these pollutants to such species that provide the target molecules (Wijesuriya and Rechnitz, 1993; Watson and Mutti, 2003). Among these compounds, enzymes play an important role due to their degree of specificity and fast response to relevant changes in the surrounding medium. The use of enzymes as bioindicators is based on the inhibition or negative interference in catalytic activity triggered by analytes (Marco and Barceló, 1996). Cholinesterase inhibition has been used as biomarker of organophosphorus and carbamate exposure. AChE is one of the oldest environmental biomarkers (Payne et al., 1996).

In general, the higher the concentration of pesticides and longer exposure time, the greater are the negative impacts, since these are the conditions when higher levels of biological organization, such as communities and ecosystems, are affected by pesticides. The effects of contaminants on low levels of biological organization (e. g., molecular and biochemical responses) occur more quickly, and the specificity of responses is generally higher. The effects on such levels can be directly related to exposure to pollutants. The presence of chemical residues and metabolites is a direct indicator of the availability of contaminants to organisms (Arias et al., 2003). In the monitoring of pesticides and other contaminants in water resources, several techniques that use organisms as bioindicators have been developed, either by estimation of population density and behavioral changes or by assessment of physiological characteristics of these organisms that make them sensitive to certain pollutants. These organisms are chosen based on features like habitat, ecology, food habits, species abundance and ease of capture (Henríquez Pérez and Sánchez-Hernández, 2003). There are two main approaches: 1) The *in vivo* approach, which exposes live specimens to the analyzed substance and collect tissues for analysis after the exposure period and 2) the *in vitro* approach, which exposes tissues or purified biomolecules directly to the analytes.

Each technique has its own advantages. In the first approach, the slow interaction between enzyme and pesticides is behind the ability ChEs has to signal inhibition several days or weeks after exposure, even when the concentrations in the water are negligible. On the other hand, the *in vitro* approach makes it possible to gain more precision in the correlation between pesticide concentrations and the resulting inhibition. In addition, the *in vitro* conditions avoid the contact between pesticides and the detoxificant complex of other tissues, allowing the use of target cholinesterases enzymes as biocomponents in electrochemical and optical devices and increasing the accuracy of data acquisition in biosensors.

In the aquatic environment pesticides and other xenobiotics can attach to suspended matter, sediments in bed of water body or be absorbed by the aquatic organisms where they undergo detoxification or bioaccumulation (Nimmo, 1985). Thus, AChE from aquatic organisms has been used due to its ability to assess the environmental impact when these compounds are not present in the water (Morgan et al., 1990; Sturm et al., 1999; Ferrari et al., 2004; Wijeyaratne and Pathiratne, 2006). Among these organisms are fish (Rodríguez-Fuentes and Gold-Bouchot, 2000; Fulton and Key, 2001; Oliveira et al, 2007; Rodríguez-Fuentes, Armstrong and Schlenk, 2008). Fish are part of ecosystems that are constantly affected by pollution from various sources, including crop fields and their pesticides and fertilizers. They occupy intermediate or higher positions in their food chains, thus undergoing accumulation of xenobiotics in their tissues and becoming a feasible alternative for environmental biomonitoring. Though it is unlikely that significant amounts of organophosphorus compounds could persist after the digestion and therefore be stored successively by higher members of the food chain, the position in the chain can influence strongly the pesticide bioaccumulation (Flint and Van der Bosch, 1981). And though the persistence of OPs in the environment is relatively short, residual life of some OP pesticides such as leptophos and fenamiphos is longer. Moreover, in general OPs may have their half-lives extended multiple times in acidic pH (WHO/IPCS/INCHEM, 1986a).

There is a lack of specificity in cholinesterase inhibition by pesticides. Several compounds are capable of inhibit them in a manner almost indistinguishable at first sight. However, such substances show different patterns of enzyme inhibition represented by time for covalent binding and type or duration of recovery. Some anticholinesterasic pesticides can interact with both active and allosteric sites of the enzyme expressing mixed inhibition mechanisms.

ChE inhibition by OP compounds follows different behaviors depending on pesticide chemical structure. OP compounds include esters, amides or thiol derivatives of phosphoric, phosphonic, phosphorotioic or phosphonotioic acids (WHO/IPCS/INCHEM, 1986a). As for the phosphoester moiety, two main groups of organophosphorus pesticides are present, the phosphate group (oxon form; P=O) and the phosphorothioate group (thion form; P=S). The first exerts direct inhibition, due to the greater electronegativity of oxygen in relation to sulphur when interacting in the active domain of the enzyme. The second group is less toxic and requires biotransformation to their oxo-analogues to become biologically active. This biotransformation occurs by oxidative desulfuration mediated by cytochrome P450 (CYP450) isoforms and flavin-containing mono-oxigenase enzymes, by N-oxidation and S-oxidation (WHO/IPCS/INCHEM, 1986a; Vale, 1998). The second group is synthesized in this form in order to resist the environmental factors and to increase the residual power of the compound, since OPs, in general, present a short half-life in the environment after biotransformation.

OPs effects can also be divided in terms of the kind of phosphorylation that takes place in the active site. Most of these pesticides contain two methyl or two ethyl (less often isopropyl) ester groups bonded to the phosphorus atom (Table 4). Depending on their structure, they can dimethyl- or diethyl-phosphorylate the serine hydroxyl group in the active center. After the release of the leaving group, dimethyl-phospho-ChE can be spontaneously reactivated slowly (starting from 0.7 hours) while diethyl-phosphoenzymes can recover their activity spontaneously in 31 hours. However, in diethyl OP compounds this recovery occurs in a minor fraction of the enzyme and this fraction can be reinhibited so that it is necessary to use oximes or other reactivation agents. On the other hand, diisopropyl-phospho-ChE has no measurable recovery (WHO/IPCS/INCHEM, 1986a; Vale, 1998; Eddlestone, 2002; Paudyal, 2008). It means that diethyl and diisopropyl-organophosphorus are able to inhibit the enzyme in long term.

Dimethyl OP	Diethyl OP	Diisopropyl OP
Dichlorvos	Diazinon	Diisopropyl fluorophosphates (DFP)
Temephos	Chlorpyrifos	Diisopropyl methylphosphonate (DIMP)
Methyl parathion	Tetraethyl pyrophosphate (TEPP)	
Malathion	Parathion	
Fenthion	Coumaphos	
Dimethoate	Sulfotepp	
Methamidophos	Ethion	

Table 4. Examples of organophosphorus pesticides according to ester groups bonded to phosphorus atom

Another feature of the interaction of OP compounds with the tissues is that most of them are lipophilic. According to Vale (1998), they are rapidly absorbed and accumulated in fat, liver, kidneys and salivary glands. Phosphorothioate compounds are more lipophilic than phosphates (Table 5).

More lipophilic	Less lipophilic
Chlorpyrifos, Diazinon, Temephos, Malathion, Parathion, Methyl-Parathion, Fenthion, Coumaphos, Dimethoate, Ethion, Sulfotepp	Tetraethyl pyrophosphate (TEPP), Trichlorfon, Dichlorvos, Methamidophos, Fenamiphos, Phosphamidon, Monocrotophos

Table 5. Examples of organophosphorus pesticides according to the lipophilicity

The loss of an alkyl group from the phosphoester bond in the enzyme-OP complex leads to the so-called aging process, which is time dependent. This process is mainly influenced by type of OP compound, pH and temperature. Since dimethyl OPs present less time for recovery, its aging half life is also short (3.7 hours). On the other hand, for diethyl OPs long time for recovery implies a longer aging half life, which may be up to 33 hours (Worek et al., 1997; Worek et al., 1999).

Oximes are nucleophilic agents which present more affinity for the OP molecules than the active center of cholinesterases. They catalyze the reactivation of enzyme and decrease the availability of enzymes subjected to the process of aging (Eddlestone, 2002). After aging, the

enzyme is not responsive to oximes treatment. Wilson (1951) reported reactivation of tetraethyl pyrophosphate-inhibited AChE by choline and hydroxylamine.

Some organophosphorus coumarinic compounds such as haloxon and coroxon present a type of inhibition which acts by phosphorylating the active site of AChE, concomitantly interacting with the peripheral site responsible for the inhibition by substrate excess. Despite being a more efficient inhibitor for BChE, haloxon and its analogues display unusual inhibition kinetics for AChE (Aldridge and Reiner, 1969).

CB pesticides are *N*-substituted esters of carbamic acid capable of readily inhibiting cholinesterases without metabolic activation, so they can induce acute toxicity effects faster than most of OP compounds. Although most CBs are not very stable in aquatic environments, some are soluble in water and can bioaccumulate in trophic levels, being particularly toxic to fish because they are metabolized slowly in such animals (Vassilieff and Ecobichon, 1982). Compared to OP compounds, CBs require larger doses to produce mortality or poisoning symptoms, because they do not bind to cholinesterases as stable as OP and do not promote aging. The half life of carbamoylated cholinesterases ranges from 0.03 to 4 h, depending on the compound (WHO/IPCS/INCHEM, 1986b).

There are two main reasons to use fish cholinesterase as biomarker. The first concerns the availability of this source: in 2009, the world fisheries and aquaculture production was 145.1 million tones, and most of the fish waste reused comes from tissues other than those that provide ChEs (FAO, 2010). Moreover, studies found very high AChE concentrations in the electric organs of the ray *Torpedo marmorata* and the eel *Electrophorus electricus* (Nachmansohn and Lederer, 1939; Leuzinger and Baker, 1967). Up to now the electric organs of *Torpedo* rays and *Electrophorus* eels (actually, they are Gymnotiformes, closer to knifefish than true eels) are still considered the most abundant source of this enzyme. These tissues are composed of structural units called electrocytes, electroplaques or electroplax, which consist in thin, flat plates of modified muscle that assemble as two large, wafer-like, roughly circular or rectangular surfaces. Each single *E. electricus* electroplaque generates a small charge because they present a potential difference of 100 mV. However, when they are piled in rows as a Voltaic pile (the arrangement in its body) they can generate a potential of approximately 600 V since there are from 5,000 to 6,000 electroplaques in its electric organ, which constitutes around 4/5 of its length. The sensitivity of fish ChEs under OP and CB exposure can be seen in tables 6, 7 and 8, which shows some differences between species *in vitro* and *in vivo*.

When measuring cholinesterases activity and inhibition, numerous differences between methodologies and laboratories become apparent, and many concerns rouse about what could be a normal level of activity for each species (Fairbrother and Bennet, 1988). In order to address these differences, some studies expressed results in terms of percentage of residual activity (Cunha Bastos et al., 1999; Villatte et al., 2002; Assis et al., 2007; Assis et al., 2010) or percentage of inhibition. According to the Food and Agriculture Organization (2007), 20% inhibition of brain AChE activity is considered the endpoint to identify the no-observed-adverse-effect-level (NOAEL) in organisms, while signs and symptoms appear when AChE is inhibited by 50% or more. Death occurs above 90% inhibition.

The most used assay for ChE activity is the Ellman method (1961). It consists in a dye-binding reaction occurring when the chromogenic reagent DTNB joins the choline or thiocholine moieties released after cholinesterases substrates breakdown. Over the years, the assay has been improved by the contribution of several works and some will be listed here.

Species	IC_{50} (μmol/L)	Ki (μmol/L)	Source	Reference
ORGANOPHOSPHATE				
Azinphos ethyl				
Cyprinus carpio	34.6	-	Muscle	Sato et al., 2007
Azinphos methyl				
Cyprinus carpio	53.7	-	Muscle	Sato et al., 2007
Chlorpyrifos				
Cyprinus carpio	810	-	Brain	Dembélé et al., 2000
Colossoma macropomum	7.6	2.61×10^{-2}	Brain	Assis et al., 2010
Arapaima gigas	7.87	2.69×10^{-2}	Brain	not published results
Rachycentron canadum	30.24	5.94×10^{-2}	Brain	not published results
Oreochromis niloticus	26.78	0.161	Brain	not published results
*Electrophorus electricus***	0.03	2.18×10^{-4}	Electric organ	not published results
Chlorpyrifos-oxon				
Gambusia affinis	0.05	-	Brain	Boone and Chambers, 1997
Gambusia affinis	0.006	-	Muscle	Boone and Chambers, 1997
Chlorpyrifos ethyl				
Cyprinus carpio	9.12	-	Muscle	Sato et al., 2007
Chlorpyrifos methyl				
Cyprinus carpio	35.48	-	Muscle	Sato et al., 2007
Chlorfenvinfos				
Cyprinus carpio	19	-	Brain	Dembélé et al., 2000
Clarias gariepinus	0.03	-	Brain	Mdegela et al., 2010
DEP				
Cyprinus carpio	12.02	-	Muscle	Sato et al., 2007
Diazinon				
Pimephales promelas	5000	-	Muscle	Olson and Christensen, 1980
Oncorhynchus mykiss	2.5	-	Brain	Keizer et al., 1995
Danio rerio	20.0	-	Brain	Keizer et al., 1995
Poecilia reticulate	7.5	-	Brain	Keizer et al., 1995
Cyprinus carpio	0.2	-	Brain	Keizer et al., 1995
Cyprinus carpio	19	-	Brain	Dembélé et al., 2000
Cyprinus carpio	2.95	-	Muscle	Sato et al., 2007
Clarias gariepinus	0.15	-	Brain	Mdegela et al., 2010
Colossoma macropomum	-	-	Brain	Assis et al., 2010
Arapaima gigas	1500	5.13	Brain	not published results
Rachycentron canadum	-	-	Brain	not published results
Oreochromis niloticus	-	-	Brain	not published results
*Electrophorus electricus***	0.3	2.18×10^{-3}	Electric organ	not published results
Diazoxon				
Cyprinus carpio	0.019	-	Muscle	Sato et al., 2007

Species	IC_{50} (μmol/L)	Ki (μmol/L)	Source	Reference
Dichlorvos				
Alburnus alburnus	0.63	-	Brain	Chuiko, 2000
Leuciscus idus	0.31	-	Brain	Chuiko, 2000
Esox lucius	0.31	-	Brain	Chuiko, 2000
Dicentrarchus labrax	33.4	-	Brain	Varò et al., 2003
Dicentrarchus labrax	44.8	-	Muscle	Varò et al., 2003
Cyprinus carpio	1.78	-	Muscle	Sato et al., 2007
Colossoma macropomum	0.04	1.37×10^{-4}	Brain	Assis et al., 2010
Arapaima gigas	2.32	7.92×10^{-3}	Brain	not published results
Rachycentron canadum	6.9	1.36×10^{-2}	Brain	not published results
Oreochromis niloticus	5.4	3.26×10^{-2}	Brain	not published results
*Electrophorus electricus***	0.16	1.16×10^{-3}	Electric organ	not published results
Dimethoate				
Clarias gariepinus	190	-	Brain	Mdegela et al., 2010
EPN oxon				
Cyprinus carpio	0.055	-	Muscle	Sato et al., 2007
Ethoprofos				
Cyprinus carpio	37.15	-	Muscle	Sato et al., 2007
Fenitrothion				
Clarias gariepinus	0.2	-	Brain	Mdegela et al., 2010
Iprobenfos				
Limanda yokohamae	1.11	-	Muscle	Jung et al., 2007
Isoxathion oxon				
Cyprinus carpio	0.00068	-	Muscle	Sato et al., 2007
Leptophos				
Cyprinus carpio	26.02	-	Muscle	Sato et al., 2007
Malaoxon				
Pimephales promelas	18	-	Muscle	Olson and Christensen, 1980
Oreochromis niloticus	0.02	-	Brain	Pathiratne and George, 1998
Pseudorasbora parva	0.81	-	Brain	Shaonan et al., 2004
Carassius auratus	0.76	-	Brain	Shaonan et al., 2004
Oncorhynchus mykiss	0.34	-	Brain	Shaonan et al., 2004
Cyprinus carpio	0.049	-	Muscle	Sato et al., 2007
Malathion				
Pimephales promelas	5700	-	Muscle	Olson and Christensen, 1980
Oreochromis niloticus	1000	-	Brain	Pathiratne and George, 1998
Cyprinus carpio	169.8	-	Muscle	Sato et al., 2007
MEP oxon				
Cyprinus carpio	2.14	-	Muscle	Sato et al., 2007

Species	IC_{50} (μmol/L)	Ki (μmol/L)	Source	Reference
Monocrotophos				
Sciaenops ocellatus	0.72	-	Brain	Ru et al., 2003
Paraoxon				
Gambusia affinis	0.27	-	Brain	Boone and Chambers, 1997
Gambusia affinis	0.06	-	Muscle	Boone and Chambers, 1997
Paraoxon ethyl				
Cyprinus carpio	0.14	-	Muscle	Sato et al., 2007
Paraoxon methyl				
Gambusia affinis	8.4	-	Brain	Boone and Chambers, 1997
Gambusia affinis	0.54	-	Muscle	Boone and Chambers, 1997
Cyprinus carpio	0.60	-	Muscle	Sato et al., 2007
Genidens genidens	0.45	-	Brain	Oliveira et al., 2007
Paralomchurus brasiliensis	0.47	-	Brain	Oliveira et al., 2007
Haemulon steindachneri	0.27	-	Brain	Oliveira et al., 2007
Pagrus pagrus	0.12	-	Brain	Oliveira et al., 2007
Menticirrhus americanus	0.29	-	Brain	Oliveira et al., 2007
Cynoscion striatu	0.21	-	Brain	Oliveira et al., 2007
Dules auriga	0.16	-	Brain	Oliveira et al., 2007
Merluccius hubbsi	0.11	-	Brain	Oliveira et al., 2007
Percophis brasiliensis	0.10	-	Brain	Oliveira et al., 2007
Parathion ethyl				
Cyprinus carpio	380	-	Muscle	Sato et al., 2007
Parathion methyl				
Cyprinus carpio	602.5	-	Muscle	Sato et al., 2007
Phoxim				
Cyprinus carpio	3.80	-	Muscle	Sato et al., 2007
Pirimiphos methyl				
Clarias gariepinus	0.003	-	Brain	Mdegela et al., 2010
Profenofos				
Clarias gariepinus	0.002	-	Brain	Mdegela et al., 2010
Temephos				
Colossoma macropomum	ne	-	Brain	Assis et al., 2010
Arapaima gigas	ne	-	Brain	not published results
Rachycentron canadum	ne	-	Brain	not published results
Oreochromis niloticus	ne	-	Brain	not published results
*Electrophorus electricus***	7.6	5.51×10^{-2}	Electric organ	not published results
TEPP				
Colossoma macropomum	3.7	1.27×10^{-2}	Brain	Assis et al., 2010
Arapaima gigas	0.009	3.07×10^{-5}	Brain	not published results

Species	IC_{50} (µmol/L)	Ki (µmol/L)	Source	Reference
Rachycentron canadum	8.1	1.59×10^{-2}	Brain	not published results
Oreochromis niloticus	20.75	0.125	Brain	not published results
*Electrophorus electricus***	0.06	4.35×10^{-4}	Electric organ	not published results
Triazophos oxon				
Pseudorasbora parva	0.13	-	Brain	Shaonan et al., 2004
Carassius auratus	0.16	-	Brain	Shaonan et al., 2004
Oncorhynchus mykiss	0.042	-	Brain	Shaonan et al., 2004
CARBAMATES				
BPMC				
Cyprinus carpio	0.76	-	Muscle	Sato et al., 2007
Carbaryl				
Pimephales promelas	10.0	-	Muscle	Olson and Christensen, 1980
Colossoma macropomum	33.8	0.116	Brain	Assis et al., 2010
Arapaima gigas	12.25	4.18×10^{-2}	Brain	not published results
Rachycentron canadum	8.31	1.63×10^{-2}	Brain	not published results
Oreochromis niloticus	9.2	5.55×10^{-2}	Brain	not published results
Electrophorus electricus	0.6	-	Electric organ	Tham et al., 2009
Clarias batrachus	0.59	-	Muscle	Tham et al., 2009
Clarias gariepinus	0.003	-	Brain	Mdegela et al., 2010
Carbofuran				
Cyprinus carpio	0.45	-	Brain	Dembélé et al., 2000
Colossoma macropomum	0.92	3.15×10^{-3}	Brain	Assis et al., 2010
Arapaima gigas	0.75	2.56×10^{-3}	Brain	not published results
Rachycentron canadum	0.082	1.61×10^{-4}	Brain	not published results
Oreochromis niloticus	0.19	1.15×10^{-3}	Brain	not published results
*Electrophorus electricus***	0.005	3.63×10^{-5}	Electric organ	not published results
Electrophorus electricus	0.02	-	Electric organ	Tham et al., 2009
Clarias batrachus	0.03	-	Muscle	Tham et al., 2009
MPMC				
Cyprinus carpio	0.98	-	Muscle	Sato et al., 2007
MTMC				
Cyprinus carpio	3.89	-	Muscle	Sato et al., 2007
NAC				
Cyprinus carpio	0.93	-	Muscle	Sato et al., 2007
PHC				
Cyprinus carpio	0.95	-	Muscle	Sato et al., 2007
XMC				
Cyprinus carpio	2.24	-	Muscle	Sato et al., 2007

ne – negligible effect.

Table 6. Pesticide IC_{50} and Ki* values for *in vitro* AChE from freshwater and marine fish.

Species	IC_{50} (μmol/L)	Source	Reference
Dichlorvos			
Alburnus alburnus	0.0063	Serum	Chuiko, 2000
Leuciscus idus	0.0016	Serum	Chuiko, 2000
Abramis ballerus	0.0008	Serum	Chuiko, 2000
Abramis brama	0.001	Serum	Chuiko, 2000
Rutilus rutilus	0.0016	Serum	Chuiko, 2000
Blicca bjoerkna	0.0008	Serum	Chuiko, 2000
Iprobenfos			
Limanda yokohamae	0.306	Muscle	Jung et al., 2007
Malathion			
Ictalurus furcatus	31	Liver	Aker et al., 2008
Ictalurus furcatus	50.2	Muscle	Aker et al., 2008
Parathion			
Gasterosteus aculeatus	0.00343a	Liver	Wogram et al., 2001
Gasterosteus aculeatus	0.00343b	Muscle	Wogram et al., 2001
Gasterosteus aculeatus	0.00343c	Gills	Wogram et al., 2001

a – 60% inhibition; b – 30% inhibition; c – 30% inhibition.

Table 7. Pesticide IC_{50} and Ki* values for *in vitro* BChE from freshwater and marine fish.

Species	Inhibition report	Source	Reference
ORGANOPHOSPHATES			
Azinphos methyl			
Sparus aurata	IC_{50} 72h - 0.0096 μM	Larvae	Arufe et al., 2007
Chlorpyrifos			
Oreochromis mossambicus	LC_{50} 96h – 0.07 μM Caused 88% inhibition in brain and gill	Brain and gill	Rao et al., 2003
Gambusia yucatana	0.43 μM 96h inhibited 80 and 50% (muscle and head, respectively)	Muscle and head	Rendón-von Osten et al., 2005
Oreochromis niloticus	IC_{50} 48 h - 0.011 μM	Brain	Chandrasekara and Pathiratne, 2007
Chlorpyrifos methyl			
Poecilia reticulate	LC50 96 h – 4.89 μM	-	Selvi et al., 2005
Diazinon			
Micropterus salmoides	295 μM 24h - 48.2%	Brain	Pan and Dutta, 1998
Cyprinus carpio	LC_{50} 96h for larvae –	Embryos and	Aydin and

Species	Inhibition report	Source	Reference
	5.03 μM and for embryos – 3.25 μM	larvae	Köprücü, 2005
Oreochromis niloticus	67% inhibition at 0.33 μM on the first day	Muscle	Durmaz et al., 2006
Oreochromis niloticus	3.3 μM - 62,5% inhibition after 24h	Brain	Üner etal., 2006
Cyprinus carpio	55.51% inhibition at 0.00012 μM after 5 days	Muscle, gill and kidney	Oruç and Usta, 2007
Dichlorvos			
Dicentrarchus labrax	LC_{50} 96h – 15.83 μM	Fingerling	Varò et al., 2003
Sparus aurata	0.23 μM 24h - 40.95% inhibition	Fingerling brain + dorsal muscle	Varò et al., 2007
Malathion			
Oreochromis niloticus	LC_{50} 96h – 6.66 μM	Brain	Pathiratne and George, 1998
Monocrotophos			
Oreochromis mossambicus	LC_{50} 96h – 51.5 μM This concentration caused 79 (brain), 89 (gill) and 43.8% (muscle) inhibition, in 24h exposure	Brain, gill and muscle	Rao, 2004
Oreochromis mossambicus	1/10 LC_{50} 96h caused 21 (liver), 40 (brain) and 28.6% (gill) inhibition in 24h exposure	Brain, liver and gill	Rao., 2006a
Parathion			
Danio rerio	0.0007 μM after 142 days inhibited 27.4%	Whole fish	Roex et al., 2003
RPR-II			
Oreochromis mossambicus	LC_{50} 96h – 0.75 μM This concentration caused 58 (brain), 90.2 (gill) and 68.5% (muscle) inhibition, in 24h exposure	Brain, gill and muscle	Rao., 2004
Oreochromis mossambicus	1/10 LC_{50} 96h caused approx. 33 (brain), 57 (gill) and 43% (muscle) inhibition, in 72h exposure	Brain, gill and muscle	Rao., 2006c
RPR-V			
Oreochromis mossambicus	LC_{50} 96h – 0.78 μM	Brain, gill and	Rao., 2004

Species	Inhibition report	Source	Reference
	This concentration caused 70.6 (brain), 86.3 (gill) and 54.8% (muscle) inhibition, in 24h exposure	muscle	
Oreochromis mossambicus	1/10 LC_{50} 96h caused approx. 30 (brain), 50 (gill) and 36% (muscle) inhibition, in 72h exposure	Brain, gill and muscle	Rao., 2006c
Temephos			
Oreochromis niloticus	ne	Head	Antwi, 1987
Sarotherodon galilaea	ne	Head	Antwi, 1987
Alestes nurse (*Brycinus nurse*)	ne	Head	Antwi, 1987
Schilbe mystus	ne	Head	Antwi, 1987
Trichlofon			
Cyprinus carpio	0.97 µM 24h - 52% inhibition	Brain	Chandrasekara and Pathiratne, 2005
Oreochromis niloticus	0.97 µM 8h - 73,6% inhibition	Axial muscle	Guimarães et al., 2007
CARBAMATES			
Aldicarb			
Danio rerio	LC_{50} 96h – 52.9 µM	-	Gallo et al., 1995
Poecilia reticulata	LC_{50} 96h – 3.5 µM	-	Gallo et al., 1995
Carbaryl			
Oncorhynchus mykiss	1.24 µM 96h inhibited 60.8%	Brain	Zinckl et al., 1987
Danio rerio	LC_{50} 96h – 46 µM	-	Gallo et al., 1995
Poecilia reticulata	LC_{50} 96h – 12.5 µM	-	Gallo et al., 1995
Oncorhynchus mykiss	3.72 µM 96h inhibited 50%	Larvae	Beauvais et al., 2001
Oncorhynchus mykiss	EC_{50} 96h – 0.095 µM	Brain and muscle	Ferrari et al., 2007
Carbofuran			
Oreochromis niloticus	LC_{50} 24h – 1.13 µM 96h – 2.17 µM	-	Stephenson et al., 1984
Carassius auratus	0.22 µM 48h inhibited 28% (brain) and 2.26 µM 48h inhibited 92% (muscle)	Brain and muscle	Bretaud et al., 1999
Gambusia yucatana	1.13 µM 24h	Muscle and	Rendón-von Osten

Species	Inhibition report	Source	Reference
	inhibited 50 and 30% (muscle and head, respectively)	head	et al., 2005
Tinca tinca	60% inhibition after 20 days of exposure of *Tinca tinca* to carbofuran at 0.1 µg/mL	Brain	Hernández-Moreno et al., 2010

ne – negligible effect.

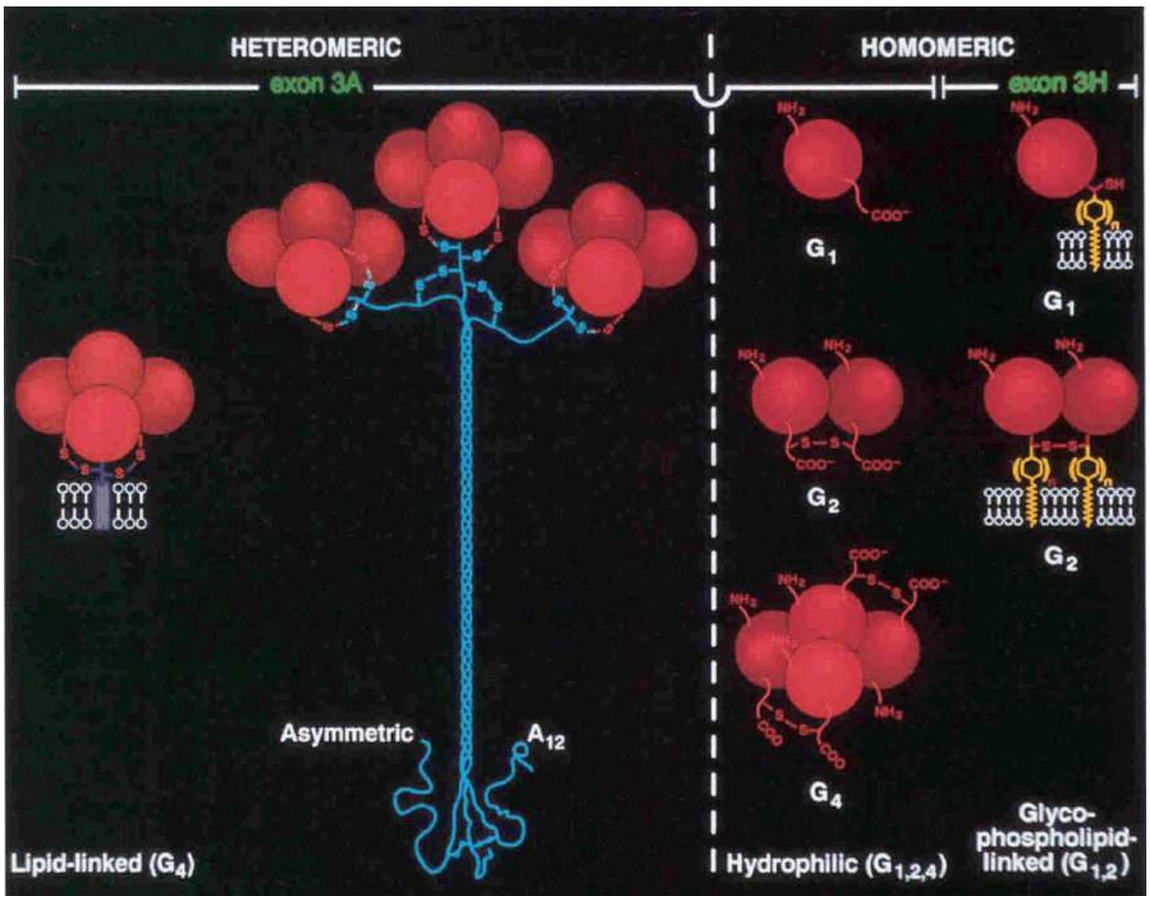
Table 8. Pesticide inhibition for *in vivo* AChE from freshwater and marine fish.

In 1960, Blaber and Creasey used ethopropazine in crude extract to prevent BChE activity when measuring AChE recovery *in vivo* (control with ethopropazine inhibited AChE by 13.7%, while BChE was inhibited by 91.5%).

Ions can alter cholinesterase activity inhibiting or activating so that some authors even propose the enzymes as biomarkers of heavy metals and other pollutants (Abou-Donia and Menzel, 1967; Mukherjee and Bhattacharya, 1974; Olson and Christensen, 1980; Tomlinson et al., 1981; Hughes and Bennett, 1985; Gill et al., 1990; 1991; Payne et al., 1996; Devi et al., 1996; Najimi, 1997; Reddy et al., 2003). This fact is not always taken into account during the use of cholinesterases as biomarkers of pesticides and can lead to false positives or negatives and misinterpretation of results. Tomlinson et al. (1981) described that activation by ions is only observed in conditions of low ionic strength, while inhibition can be noted in both low and high ionic strength.

Thus, heavy metals and ions can be present in samples of environmental matrices, as well as in food samples. Also, they are important interfering components in pesticide analysis using cholinesterases, since some of them are inhibitors or positive effectors. Nevertheless, the use of non-inhibitor chelating agents and ions with protecting enzyme activity effect could overcome these interferences.

Bocquené, Galgani and Truquet (1990) found that Tris buffer was the best extractor for fish AChE. Najimi and coworkers (1997) reported that using Tris the increasing doses of heavy metals resulted in different AChE activities though such result was not observed with phosphate buffer. It could be concluded that phosphate is the best buffer for pesticide assays and that Tris is the best alternative for heavy metals assays. However, Tomlinson et al. (1981) reported that EDTA has a protective action against divalent metallic cations which can cause some interference. Chatonnet and Lockridge (1989) reviewed cholinesterases and reported the different extracting strategies caused by ChEs molecular polymorphism: the globular forms G1, G2 and G4 are extractable in low ionic strength buffers (G2 glycophospholipid-linked is the form found in erythrocytes and in *Torpedo* electric organ, while G4 lipid-linked is present in vertebrates brain). The globular forms tightly bound to membranes require detergent for solubilization. Asymmetric forms (found mainly in vertebrate muscle and in some electric organs) are solubilized with buffers with high salt concentration. These forms contain tetrameric subunits (A4, A8 and A12) attached by disulphide bonds to a collagen-like tail (Figure 1).



Source: Taylor (1991)

Fig. 1. Molecular polymorphism of cholinesterases

Working with brain AChE, Ho and Ellman (1969) were able to solubilize the enzyme using triton X-100 and treatment with proteases. Nevertheless, in cholinesterase assays with pesticides, triton X-100 interacts with OP (oxon-form) and CB compounds or influences its rate of AChE inhibition (Marcel et al., 2000; Rosenfeld, Kousba and Sultatos, 2001).

For pesticides with larger acyl chains or higher lipophilic characteristic (for which only a small fraction reaches the target tissues), BChE can be more sensitive than AChE. The use of BChE offers some advantages, such as the facilitated plasma (its main source) separation from the other blood components and the possibility to collect samples without killing specimens. Furthermore, several studies have tried, with some success, to establish sharp correlations between inhibition in blood cholinesterases and in peripheral and central nerve tissues cholinesterases (Pope et al., 1991; Pope and Chakraborti, 1992; Chauldhuri et al., 1993; Padilla et al., 1994). Padilla (1995) working with paraoxon and chlorpyrifos, described that the strongest correlations occurred when measuring cholinesterase activity in steady-state inhibition, which is the peak inhibition time. This time depends on the inhibitor under analysis (4 hours post-dosing for paraoxon and 1-3 weeks post-dosing for chlorpyrifos).

Another concern about using fish cholinesterase as biomarker of organophosphorus and carbamate pesticides is that cyanobacterial blooms are very common in rivers, lakes and reservoirs when eutrophication raises nutrient contents in water. Some species of

cyanobacteria (*Anabaena flos-aquae* and *Anabaena lemmermannii*) produce anticholinesterasic metabolites such as anatoxin-a(s), which can be considered natural OP compounds and whose toxicity can be approximately 1000-fold higher than that of the insecticide paraoxon (Mahmood and Carmichael, 1986; Villatte et al., 2002). Moreover, cholinesterases inhibited by anatoxin-a(s) cannot be reactivated by oximes, because they are true irreversible inhibitors of these enzymes. The structure of anatoxin-a(s) resembles the shape of the organophosphorus dealkylated within the active site of the enzyme forming almost instantly an aged complex. A study obtained aged cholinesterase after 20-min incubation with this toxin (Villatte et al., 2002). However, by washing the brains before (with a solvent that does not transport it into the cells and does not affect enzymatic activity), such toxins do not interfere on *in vivo* assays using cholinesterase from this tissue, since it was observed that anatoxin-a(s) does not cross the blood-brain barrier (Cook et al., 1988; Rodríguez et al., 2006).

When comparing the use of crude extract to the use of purified enzyme, advantages and disadvantages can be observed in both, depending on the purpose. First of all, purified enzymes allow determining activity and inhibition more acutely without endogenous interfering agents. Moreover, they can be immobilized on a range of materials in particles or electrodes in order to produce electrochemical devices. Nevertheless purified enzymes require a medium to mimetize *in vivo* conditions and stabilize its activity. Besides, they are more susceptible to exogenous ions and non target compounds. The crude extract has the disadvantage of exposing the enzyme not only to the analyte. However, as mentioned before, much of OP pesticides are produced in the thion form (P=S), requiring bioactivation to reach their full toxic potential. Before biotransformation, the thion group exhibits little power of inhibition (WHO/IPCS/INCHEM, 1986a) which could hinder the correlation between pesticide concentration and ChE inhibition. Considering this, many studies use brain homogenates, since they also provide enzymatic complexes such as CYP P450 capable to transform the pesticide to its oxo-form (Mesnil, Testa and Jenner, 1984; Iscan et al., 1990; Ghersi-Egea et al., 1993).

According to Zahavi et al. (1971) and Carr and Chambers (1996), the reasons behind the species' differences in inhibitory potency has been reported to be the result of steric exclusion of the inhibitor from the active site of the enzyme. However, the difference in sensitivity between species occurs not only due to the structural diversity of inhibitors and between species cholinesterases, but also due to the balance between the activities of the detoxication complex and enzymes that promote the biotransformation of OPs. This balance can be part of enantiostatic responses to external agents which act as a device protecting against intoxication (Cunha Bastos et al., 1999; Monserrat et al., 2007).

Several attempts have been reported worldwide, in search for the best enzyme and fish source to establish methods to detect diverse organophosphorus and carbamate pesticides. In this sense, it is possible to improve monitoring protocols, obtaining data about the activation/detoxification complex of each species in use.

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4. References

Abou-Donia, M.S.; Menzel, D.B. 1967. Fish brain cholinesterase: its inhibition by carbamates and automatic assay. Comp. Biochem. Physiol. 21: 99-108.

Adams, D.H.; Thompson, R.H.S. 1948. The Selective Inhibition of Cholinesterases. Biochem. J. 42, 170.

Aker, W.G.; Hu, X.; Wang, P.; Hwang, H.-M. 2008. Comparing the Relative Toxicity of Malathion and Malaoxon in Blue Catfish *Ictalurus furcatus*. Environ Toxicol 23: 548–554.

Aldridge, W.N. 1953. The Differentiation of True and Pseudo Cholinesterase by Organophosphorus Compounds. Biochem. J. 53: 62-67.

Aldridge, W.N.; Reiner, E. 1969. Acetylcholinesterase – Two types of inhibition by an organophosphorus compound: one the formation of phosphorylated enzyme and the other analogous to inhibition by substrate. Biochem. J. 115: 147-162.

Antwi, L. A. K. 1987. Fish head acetylcholinesterase activity after aerial application of temephos in two rivers in Burkina Faso, west Africa. Bulletin of Environmental Contamination and Toxicology. 38:461-466.

Arias, A.R.L.; Buss, D.F.; Albuquerque, C.; Inácio, A.F.; Freire, M.M.; Egler, M.; Mugnai, R.; Baptista, D.F. Utilização de bioindicadores na avaliação de impacto e no monitoramento da contaminação de rios e córregos por agrotóxicos. Ciência e Saúde Coletiva, 12, n. 1, p. 61-72, 2007.

Arufe, M.I.; Arellano, J.M.; García, L.; Albendín, G.; Sarasquete, C. 2007. Cholinesterase activity in gilthead seabream (*Sparus aurata*) larvae: Characterization and sensitivity to the organophosphate azinphosmethyl. Aquat Toxicol 84: 328–336.

Assis, C.R.D.; Castro, P.F.; Amaral, I.P.G.; Maciel Carvalho, E.V.M.; Carvalho Jr, L.B., Bezerra, R.S. 2010. Characterization of acetylcholinesterase from the brain of the Amazonian tambaqui (*Colossoma macropomum*) and in vitro effect of organophosphorus and carbamate pesticides. Environ Toxicol Chem 29: 2243–2248.

Augustinsson, K.B. Cholinesterase and anticholinesterase agents. In: Handbook of experimental pharmacology. Koelle, G.B. (org). Springer-Verlag, Berlin and New York, p. 89-128, 1963.

Austin, L.; Berry, W.K. Two selective inhibitors of cholinesterase. Biochem J. 1953 July; 54(4): 695–700.

Aydın, R.; Köprücü, K. 2005. Acute toxicity of diazinon on the common carp (*Cyprinus carpio* L.) embryos and larvae. Pesticide Biochemistry and Physiology 82: 220–225.

Bayliss, B. J. & Todrick, A. (1956). The Use of a Selective Acetylcholinesterase Inhibitor in the Estimation of Pseudocholinesterase Activity in Rat Brain. Biochem. J. 62, 62-67.

Beauvais, S. L.; Cole, K. J.; Atchison, G. J.; Coffey, M. Factors affecting brain cholinesterase activity in Bluegill (*Lepomis macrochirus*). Water, Air, and Soil Pollution, v. 135, p. 249–264, 2002.

Beauvais, S. L.; Jones, S. B.; Parris, J. T.; Brewer, S. K.; Little, E. E. 2001. Cholinergic and Behavioral Neurotoxicity of Carbaryl and Cadmium to Larval Rainbow Trout (*Oncorhynchus mykiss*) Ecotoxicology and Environmental Safety 49: 84-90.

Blaber, L.C.; Creasey, N.H. 1960. The Mode of Recovery of Cholinesterase Activity in vivo after Organophosphorus Poisoning - 2. Brain cholinesterase. Biochem. J. 77: 597-604.

Bocquené G, Galgani F, Truquet P. 1990. Characterization and assay conditions for use of AChE activity from several marine species in pollution monitoring. Mar Environ Res 30: 75-89.

Boone, J.S.; Chambers, J.E. 1997. Biochemical factors contributing to toxicity differences among chlorpyrifos, parathion, and methyl parathion in mosquitofish (*Gambusia afinis*). Aquatic Toxicology, 39: 333-343.

Bretaud, S.; Toutant, J.-P.; Saglio, P. 1999. Effects of carbofuran, diuron, and nicosulfuron on acetylcholinesterase activity in goldfish (Carassius auratus). Ecotoxicology and Environmental Safety 47: 117-124

Cairns, J. Jr., Pratt, J.R. A history of biological monitoring using benthic macroinvertebrates. In: Rosenberg, D.M, Resh, V.H. Freshwater biomonitoring and benthic macroinvertebrates. New York: Chapman & Hall; 1993. p. 10-27.

Carr, R.L.; Chambers, J.E. 1996. Kinetic Analysis of the in Vitro Inhibition, Aging, and Reactivation of Brain Acetylcholinesterase from Rat and Channel Catfish by Paraoxon and Chlorpyrifos-oxon. Toxicol appl Pharmacol, 139: 365–373.

Chandrasekara, H. U.; Pathiratne, A. 2005. Influence of low concentrations of Trichlorfon on haematological parameters and brain acetylcholinesterase activity in common carp, *Cyprinus carpio* L. Aquaculture Research, 36, 144-149.

Chandrasekara, L.W.H.U.; Pathiratne, A. 2007. Body size-related differences in the inhibition of brain acetylcholinesterase activity in juvenile Nile tilapia (*Oreochromis niloticus*) by chlorpyrifos and carbosulfan. Ecotoxicology and Environmental Safety 67: 109–119.

Chatonnet, A.; Lockridge, O. 1989. Comparison of butyrylcholinesterase and acetylcholinesterase. Biochem. J. 260: 625-634.

Chauldhuri, J., Harp, P., Chakraborti, T. and Pope, C. (1993) Comparative dose-related effects of parathion and chlorpyrifos on cholinergic markers and behavior. Presented at the 1993 HERL Symposium on Biological Mechanisms and Quantitative Risk Assessment.

Cheng, Y.; Prusoff, W.H. 1973. Relationship between the inhibition constant (Ki) and the concentration of inhibitor which causes 50 per cent inhibition (I50) of an enzymatic reaction. *Biochem. Pharmacol*. 22: 3099–3108.

Chuiko, G.M. 2000. Comparative study of acetylcholinesterase and butyrylcholinesterase in brain and serum of several freshwater fish: specific activities and in vitro inhibition by DDVP, an organophosphorus pesticide. Comparative Biochemistry and Physiology Part C, 127: 233–242.

Çokugras, A. N. Butyrylcholinesterase: Structure and Physiological Importance. Turkish Journal of Biochemistry. 2003; 28 (2); 54-61.

Cook, W.O.; Beasley, V.R.; Dahlem, A.M.; Dellinger, J.A.; Harlin, K.S.; Carmichael, W.W. 1988. Comparison of effects of anatoxin-a(s) and paraoxon, physostigmine and pyridostigmine on mouse brain cholinesterase activity. Toxicon. 26 (8): 750-753.

Cunha Bastos, V. L. F.; Silva Filho, M. V.; Rossini, A.; Cunha Bastos, J. The Activation of Parathion by Brain and Liver of a Brazilian Suckermouth Benthic Fish Shows Comparable in Vitro Kinetics. Pesticide Biochemistry and Physiology 64, 149–156 (1999).

Dembélé, K.; Haubruge, E.; Gaspar, C. 2000. Concentration Effects of Selected Insecticides on Brain Acetylcholinesterase in the Common Carp (*Cyprinus carpio* L.). Ecotoxicology and Environmental Safety 45: 49-54.

Devi, M., Thomas, D.A., Barber, J.T., Fingerman, M., 1996. Accumulation and Physiological and Biochemical Effects of Cadmium in a Simple Aquatic Food Chain. Ecotox. Environ. Safe. 33, 38–43.

Durmaz, H.; Sevgiler, Y.; Üner, N. 2006. Tissue-specific antioxidative and neurotoxic responses to diazinon in *Oreochromis niloticus*. Pesticide Biochemistry and Physiology, 84: 215–226

Eddleston, M.; Buckley, N. A.; Eyer, P.; Dawson, A. H. Management of acute organophosphorus pesticide poisoning. Lancet. 2008 February 16; 371(9612): 597–607.

Eddleston, M; Szinicz, L.; Eyer, P.; Buckley, N. Oximes in acute organophosphorus pesticide poisoning: a systematic review of clinical trials. Q J Med 2002;95:275-83.

Ellman, G.L.; Courtney, K.D.; Andres, V.; Featherstone, R.M. 1961. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochemical Pharmacology, 7: 88-95.

Fairbrother, A.; Bennett, J.K. 1988. The usefulness of cholinesterase measurement, J. Wildl. Dis. 24: 587-590.

FAO. 2007. Pesticides in food report 2007. FAO plant production and protection paper 191. Food and Agriculture Organization, Rome, IT.

FAO. 2010. The State of World Fisheries and Aquaculture 2010. Rome, IT. 197p.

Ferrari, A., Venturino, A., Pechen de D'Angelo, A.M., 2004. Time course of brain cholinesterase inhibition and recovery following acute and subacute azinphosmethyl, parathion and carbaryl exposure in the goldfish (*Carassius auratus*). Ecotoxicol. Environ. Saf. 57, 420–425.

Ferrari, A.; Venturino, A.; D'Angelo, A. M. P. 2007. Muscular and brain cholinesterase sensitivities to azinphos methyl and carbaryl in the juvenile rainbow trout Oncorhynchus mykiss. Comparative Biochemistry and Physiology, Part C 146: 308–313.

Flint, M.L. and R. van Der Bosch, 1981. Introduction to Integrated Pest Management. Plenum Press, New York, pp: 237.

Fulton, M. H.; Key, P. B. 2001. Acetylcholinesterase inhibition in estuarine fish and invertebrates as an indicator of organophosphorus insecticide exposure and effects. Environ Toxicol Chem 20: 37-45.

Gallo, D.; Merendino, A.; Keizer, J.; Vittozzi, L. 1995. Acute toxicity of two carbamates to the Guppy (*Poecilia reticulata*) and the Zebrafish (*Brachydanio rerio*). The Science of the Total Environment, 171: 131-136.

Ghersi-Egea, J. F.; Perrin, R.; Leininger-Muller, B.; Grassiot, M. C.; Jeandel, C.; Floquet, J.; Cuny, G.; Siest, G.; Minn, A. Subcellular localization of cytochrome P450, and activities of several enzymes responsible for drug metabolism in the human brain, Biochem. Pharmacol. 45, 647 (1993).

Gill, T.S., Tewari, H., Pande, J., 1990. Use of the fish enzyme system in monitoring water quality: effects of mercury on tissue enzymes. Comp. Biochem. Physiol. 97C, 287-292.

Gill, T.S., Tewari, H., Pande, J., 1991. In vivo and in vitro effects of cadmium on selected enzymes in different organs of the fish *Barbus conchonius* Ham. (Rosy barb). Comp. Biochem. Physiol. 100C, 501-505.

Guimarães, A. T. B.; Silva de Assis, H. C.; Boeger, W. 2007. The effect of trichlorfon on acetylcholinesterase activity and histopathology of cultivated fish *Oreochromis niloticus*. Ecotoxicology and Environmental Safety 68: 57–62.

Harel, M.; Sussman, J.L.; Krejci, E.; Bon, S.; Chanal, P.; Massoulié, J.; Silman, I. 1992. Conversion of acetylcholinesterase to butyrylcholinesterase: Modeling and mutagenesis. Proc. Natl. Acad. Sci. USA. 89: 10827-10831.

Hart KA, Pimentel D. Environmental and economic costs of pesticide use. In: Encyclopedia of Pest Management. Pimentel D. ed.; Marcel Dekker: New York, NY, 2002: 237-239.

Henríquez Pérez, A.; Sánchez-Hernández, J. C. 2003. La amenaza de los plaguicidas sobre la fauna silvestre de las islas canarias. El indiferente, 14: 42-47.

Hernández-Moreno,D.; Soler, F.; Míguez, M.P.; Pérez-López, M. 2010. Brain acetylcholinesterase, malondialdehyde and reduced glutathione as biomarkers of continuous exposure of tench, *Tinca tinca*, to carbofuran or deltamethrin. Science of the Total Environment 408: 4976–4983

Ho, I.K.; Ellman, G.L. Triton solubilized acetylcholinesterase of brain. 1969. Journal of Neurochemistry. 16: 1505-1513.

Hughes, R.J., Bennett, J., 1985. Effect of metal ions on the activity of acetylcholinesterase. Biochem. Soc. Trans. 13, 219-220.

Iscan, M.; Reuhl, K.; Weiss, B.; Maines, M. D. Regional and subcellular distribution of cytochrome P450-dependent drug metabolism in monkey brain: The olfactory bulb and mitochondrial fraction have high levels of activity, Biochem. Biophys. Res. Commun. 169, 858 (1990).

Johnson, G.; Moore, S.W. Cholinesterases modulate cell adhesion in human neuroblastoma cells in vitro. Int. J. Dev. Neurosci., v. 18, p. 781–790, 2000.

Jung, J-H; Addison, RF; Shim, WJ. 2007. Characterization of cholinesterases in marbled sole, *Limanda yokohamae*, and their inhibition in vitro by the fungicide iprobenfos. Marine Environmental Research 63: 471–478.

Keizer, J.; D'Agostino, G.; Nagel, R.; Volpe, T.; Gnemid, P.; Vittozzi, L. 1995. Enzymological differences of AChE and diazinon hepatic metabolism: correlation of in vitro data with the selective toxicity of diazinon to fish species. The Science of the Total Environment 171: 213-220.

Kovarik, Z.; Bosak, A.; Sinko, G.; Latas, T. 2003. Exploring the Active Sites of Cholinesterases by Inhibition with Bambuterol and Haloxon. Croatica Chemica Acta. 76: 63-67.

Leuzinger, W.; Baker, A. L. 1967. Acetylcholinesterase, I. Large-scale purification, homogeneity and amino acid analysis. Proc. Nac. Acad. Sci. 57: 446-451.

Lopez-Carillo, L.; Lopez-Cervantes, M. 1993. Effect of exposure to organophosphate pesticides on serum cholinesterase levels. Archives of environmental health. 48: 359-363.

Mahmood, N.A.; Carmichael, W.W. 1986. The pharmacology of anatoxin-a(s), a neurotoxin produced by the freshwater cyanobacterium Anabaena flos-aquae NRC 525-17. Toxicon, 24: 425-434.

Marcel, V.; Estrada-Mondaca, S.; Magné, F.; Stojan, J.; Klaébé, A.; Fournier, D. 2000. Exploration of the Drosophila Acetylcholinesterase Substrate Activation Site Using

a Reversible Inhibitor (Triton X-100) and Mutated Enzymes. J Biol Chem. 275: 11603–11609.

Marco, M.-P.; Barceló, D. Environmental applications of analytical biosensors. Measuring Science Technology, v. 7, p. 1547–1562, 1996.

Mdegela, R.H.; Mosha, R.D.; Sandvik, M.; Skaare, J.U. 2010. Assessment of acetylcholinesterase activity in *Clarias gariepinus* as a biomarker of organophosphate and carbamate exposure. Ecotoxicology 19: 855–863

Mesnil, M.; Testa, B.; Jenner, P. Xenobiotic metabolism by brain monooxigenases and other cerebral enzymes, Adv. Drug Res. 13, 95 (1984).

Monserrat, J. M.; Martínez, P. E.; Geracitano, L. A.; Amado, L L.; Martins, C. M. G.; Pinho, G. L. L.; Chaves, I. S.; Ferreira-Cravo, M.; Ventura-Lima, J.; Bianchini, A. Pollution biomarkers in estuarine animals: Critical review and new perspectives. Comparative Biochemistry and Physiology, Part C 146 (2007) 221–234.

Morgan, M.J., Fancey, L.L., Kiceniuk, J.W., 1990. Response and recovery of brain AChE activity in Atlantic salmon exposed to fenitrothion. Can. J. Fish Aquat. Sci. 47, 1652–1654.

Mukherjee, S., Bhattacharya, S., 1974. Effect of some industrial pollutants on fish brain cholinesterase activity. Environ. Physiol. Biochem. 4, 226-231.

Nachmansohn, D.; Lederer, E. 1939. Sur la biochimie de la cholinesterase. I. Preparation de l'enzyme. Groupements-SH. Bull. Soc. Chim. Biol. 21: 797-808

Najimi, S., Bouhaimi, A., Daubèze, M., Zekhnini, A., Pellerin, J., Narbonne, J.F., Moukrim, A., 1997. Use of acetylcholinesterase in *Perna perna* and *Mytilus galloprovincialis* as a biomarker of pollution in Agadir marine bay (south of Morocco). Bull. Environ. Contam. Toxicol. 58, 901-908.

Nauen R, Bretschneider T. 2002. New modes of action of insecticides. *Pesticide Outlook* 13: 241-245.

Nicolet, Y.; Lockridge, O.; Masson, P.; Fontecilla-Camps, J. C.; Nachon, F. 2003. Crystal Structure of Human Butyrylcholinesterase and of Its Complexes with Substrate and Products. Journal of Biological Chemistry. 278: 41141–41147.

Nimmo, D.R. Pesticides. In: Rand, G.M.; Petrocelli, S.R., eds. Fundamentals of aquatic toxicology: methods and applications, New York: Hemisphere, p. 335-373, 1985.

Oliveira, M. M.; Silva Filho, M. V.; Cunha Bastos, V. L. F.; Fernandes, F. C.; Cunha Bastos, J. Brain acetylcholinesterase as a marine pesticide biomarker using Brazilian fishes. Marine Environmental Research 63 (2007) 303–312.

Olson, D.L., Christensen, G.M., 1980. Effects of water pollutants and other chemicals on fish acetylcholinesterase (in vitro). Environ. Res. 21, 327-335.

Oruç, E. O.; Usta, D. 2007. Evaluation of oxidative stress responses and neurotoxicity potential of diazinon in different tissues of *Cyprinus carpio*. Environmental Toxicology and Pharmacology 23: 48–55.

Padilla, S. Regulatory and research issues related to cholinesterase inhibition. Toxicology 102 (1995) 215 -220.

Padilla, S.; Moser, V.C.; Pope, C.N.; Brimijoin. W.S. (1992) Paraoxon toxicity is not potentiated by prior reduction in blood acetylcholinesterase. Toxicol. Appl. Pharmacol. 117, 110-115.

Pan, G.; Dutta, H. M. 1998. The Inhibition of Brain Acetylcholinesterase Activity of Juvenile Largemouth Bass *Micropterus salmoides* by Sublethal Concentrations of Diazinon. Environmental Research, Section A. 79: 133-137.

Pathiratne, A.; George, S.G. 1998. Toxicity of malathion to nile tilapia, *Oreochromis niloticus* and modulation by other environmental contaminants. Aquatic Toxicology 43: 261–271.

Paudyal, B.P. 2008. Organophosphorus poisoning. J Nepal Med Assoc. 47: 251-258.

Payne J F, Mathieu A, Melvin W, Fancey L L. Acetylcholinesterase, an old biomarker with a new future? field trials in association with two urban rivers and a paper mill in Newfoundland. Mar Pollut Bull, Vol. 32, No. 2, pp. 225-231, 1996.

Pezzementi, L., Chatonnet, A. 2010. Evolution of cholinesterases in the animal kingdom. Chemico-Biological Interactions 187: 27–33.

Pezzementi, L., Sanders, M., Jenkins, T., & Holliman, D. (1991). In J. Massouli e, F. Bacou, E. Barnard, A. Chatonnet, B. Doctor, & D. Quinn (Eds.), Cholinesterases: structure, function, mecanism, genetics and cell biology (pp. 24–31). USA: American Chemical Society.

Pope, C.N., Chakraborti, T.K., Chapman, M.L.. Farrar J.D. and Arthun, D. 1991. Comparison of in vivo cholinesterase inhibition in neonatal and adult rats by three organophosphorothioate insecticides. Toxicology 68: 51-61.

Pope, C.N.; Chakraborti, T.K. 1992. Dose-related inhibition of brain and plasma cholinesterase in neonatal and adult rats following sublethal organophosphate exposures. Toxicology 73: 35-43.

Quinn, D.M. Acetylcholinesterase: enzyme structure, reaction dynamics, and virtual transition states. Chemical Reviews, 87, p. 955-979, 1987.

Rao, J.V. 2004. Effects of monocrotophos and its analogs in acetylcholinesterase activity's inhibition and its pattern of recovery on euryhaline fish, *Oreochromis mossambicus*. Ecotoxicology and Environmental Safety 59: 217–222.

Rao, J.V. 2006a. Biochemical alterations in euryhaline fish, *Oreochromis mossambicus* exposed to sub-lethal concentrations of an organophosphorus insecticide, monocrotophos. Chemosphere 65: 1814–1820.

Rao, J.V. 2006b. Sublethal effects of an organophosphorus insecticide (RPR-II) on biochemical parameters of tilapia, *Oreochromis mossambicus*. Comparative Biochemistry and Physiology, Part C 143: 492–498.

Rao, J.V. 2006c. Toxic effects of novel organophosphorus insecticide (RPR-V) on certain biochemical parameters of euryhaline fish, *Oreochromis mossambicus*. Pesticide Biochemistry and Physiology 86: 78–84.

Rao, J.V.; Rani, C. H. S.; Kavitha, P.; Rao, R. N.; Madhavendra, S. S. 2003. Toxic effects of Chlorpyrifos to the fish *Oreochromis mossambicus*. Bulletin of Environmental Contamination and Toxicology, 70:985–992.

Rao, JV; Kavitha, P. 2010. In vitro effects of chlorpyrifos on the acetylcholinesterase activity of euryhaline fish, *Oreochromis mossambicus*. Z Naturforsch C. 65: 303-306

Reddy, G.R., Basha, M.R., Devi, C.B., Suresh, A., Baker, J.L., Shafeek, A., Heinz, J., Chetty, C.S., 2003. Lead induced effects on acetylcholinesterase activity in cerebellum and hippocampus of developing rat. Int. J. Devl. Neuroscience. 21, 347–352.

Rendón-von Osten, J.; Ortíz-Arana, A.; Guilhermino, L.; Soares, A. M. V. M. 2005. In vivo evaluation of three biomarkers in the mosquitofish (*Gambusia yucatana*) exposed to pesticides. Chemosphere, 58: 627–636.

Rodríguez, V.; Moura, S.; Pinto, E.; Pereira, C.M.P.; Braga, R.C. 2006. Aspectos toxicológicos e químicos da anatoxina-a e seus análogos. Quim. Nova, Vol. 29, No. 6, 1365-1371.

Rodríguez-Fuentes G, Armstrong J, Schlenk D. 2008. Characterization of muscle cholinesterases from two demersal flatfish collected near a municipal wastewater outfall in Southern California. Ecotoxicol Environ Saf 69: 466–471.

Rodríguez-Fuentes G, Gold-Bouchot G. 2004. Characterization of cholinesterase activity from different tissues of Nile tilapia (Oreochromis niloticus). Mar Environ Res 58: 505-509.

Rodríguez-Fuentes, G.; Gold-Bouchot, G. 2000. Environmental monitoring using acetylcholinesterase inhibition in vitro. A case study in two Mexican lagoons. Mar Environ Res 50: 357-360.

Roex, E.W.M.; Keijzers, R.; van Gestel, C.A.M. 2003. Acetylcholinesterase inhibition and increased food consumption rate in the zebrafish, *Danio rerio*, after chronic exposure to parathion. Aquatic Toxicology 64: 451-/460

Rosenberry, T.L. 1975. Acetylcholinesterase. Adv Enzymol Relat Areas Mol Biol. 43: 103-218.

Rosenfeld, C.; Kousba, A.; Sultatos, L.G. 2001. Interactions of rat brain acetylcholinesterase with the detergent Triton X-100 and the organophosphate paraoxon. Toxicol Sci 63: 208–213.

Ru, S.; Wei, X.; Jiang, M.; Li, Y. 2003. In vivo and in vitro inhibitions of red drum (Sciaenops ocellatus) brain acetylcholinesterase and liver carboxylesterase by monocrotophos at sublethal concentrations. Water, Air, and Soil Pollution 149: 17–25.

Salles, J.B.; Cunha Bastos, V.L.F.; Silva Filho, M.V.; Machado, O.L.T.; Salles, C.M.C.; Giovanni de Simone, S.; Cunha Bastos, J. 2006. A novel butyrylcholinesterase from serum of Leporinus macrocephalus, a Neotropical fish. Biochimie. 88: 59–68.

Sato, R.; Mitani, K.; Matsumoto, T.; Takahashi, S.; Yamada, R-h; Kera, Y. 2007. Effects of Insecticides In Vitro on Acetylcholinesterase Purified from Body Muscle of Koi Carp (*Cyprinus carpio*). Jpn. J. Environ. Toxicol. 10: 31-38.

Selvi, M.; Sarıkaya, R.; Erkoç, F.; Koçak, O. 2005. Investigation of acute toxicity of chlorpyrifos-methyl on guppy *Poecilia reticulata*. Chemosphere 60: 93–96.

Shaonan, L.; Xianchuan, X.; Guonian, Z.; Yajun, T. 2004. Kinetic characters and resistance to inhibition of crude and purified brain acetylcholinesterase of three freshwater fishes by organophosphates. Aquatic Toxicology 68: 293–299.

Silman, I.; Sussman, J. L. Acetylcholinesterase: 'classical' and 'non-classical' functions and pharmacology. Current Opinion in Pharmacology, 5, p. 293–302, 2005.

Soreq H, Zakut H. 1990. Cholinesterase genes: multileveled regulation. In Monographs in Human Genetics, ed. RS Sparkes, 13:1-102. Basel: Karger.

Sturm, A.; Wogram, J.; Hansen, P.D.; Liess, M. 1999. Potential use of cholinesterase in monitoring low levels of organophosphates in small streams: natural variability in threespined stickleback (Gasterosteus aculeatus) and relation to pollution. Environ. Toxicol. Chem. 18: 194–200.

Taylor, P. 1991. The cholinesterases. J. Biol. Chem. 266: 4025-4028.

Tham, L.G.; Perumal, N.; Syed, M.A.; Shamaan, N.A.; Shukor, M.Y. 2009. Assessment of Clarias batrachus as a source of acetylcholinesterase (AChE) for the detection of insecticides. J Environ Biol. 30: 135-138.

Tomlinson, G., Mutus, B., McLennan, I., 1981. Activation and inactivation of acetylcholinesterase by metal ions. Can. J. Biochem. 59, 728-735.

Tõugu V. 2001. Acetylcholinesterase: mechanism of catalysis and inhibition. Curr Med Chem - Cent Nerv Syst Agents 1: 155-170.

Üner, N.; Oruc, E. O.; Sevgiler, Y.; Şahin, N.; Durmaz, H.; Usta, D. 2006. Effects of diazinon on acetylcholinesterase activity and lipid peroxidation in the brain of *Oreochromis niloticus*). Environmental Toxicology and Pharmacology 21: 241–245.

Vale, J. A. 1998. Toxicokinetic and toxicodynamic aspects of organophosphorus (OP) insecticide poisoning. Toxicol. Let. 102-103: 649-652.

Varò, I.; Navarro, J.C.; Amat, F.; Guilhermino, L. 2003. Effect of dichlorvos on cholinesterase activity of the European sea bass (*Dicentrarchus labrax*). Pestic Biochem Phys 75: 61–72.

Varò, I.; Navarro, J.C.; Nunes, B.; Guilhermino, L. 2007. Effects of dichlorvos aquaculture treatments on selected biomarkers of gilthead sea bream (*Sparus aurata* L.) fingerlings. *Aquaculture* 266: 87–96.

Vassilieff, I.; Ecobichon, D.J. 1982. Stability of Matacil® in aqueous media as measured by changes in anticholinesterase potency. Bull. environ. Contam. Toxicol., 29: 366-370.

Villatte, F.; Schulze, H.; Schmid, R.D.; Bachmann, T.T. 2002. A disposable acetylcholinesterase-based electrode biosensor to detect anatoxin-a(s) in water. Anal Bioanal Chem., 372 :322–326.

Watson, W. P.; Mutti, A. Role of biomarkers in monitoring exposures to chemicals: present position, future prospects. Biomarkers, v. 9, n. 3, p. 211-242, 2004.

Whittaker, M. Cholinesterase. Karger, Basel, New York., p. 1-126, 1986.

Whittaker, M. Plasma cholinesterase variants and the anaesthetist. Anaesthesia, v. 35, p. 174-97, 1980.

WHO/IPCS/INCHEM. 1986a. Organophosphorus insecticides: a general introduction. Environmental Health Criteria 63. Geneva, CH.

WHO/IPCS/INCHEM. 1986b. Carbamate pesticides: a general introduction. Environmental Health Criteria 64. Geneva, CH.

Wijesuriya, D.C.; Rechnitz, G.A. Biosensors based on plant and animal tissues. Biosensors and Bioelectronics. V. 8, n. 3-4, p. 155-160, 1993.

Wijeyaratne, W.M.D.N., Pathiratne, A., 2006. Acetylcholinesterase inhibition and gill lesions in Rasbora caverii, an indigenous fish inhabiting rice field associated waterbodies in Sri Lanka. Ecotoxicology 15, 609–619.

Wilson, I.B. Acetylcholinesterase. XI. Reversibility of tetraethyl pyrophosphate inhibition. J Biol Chem. 1951, 190(1):111–117.

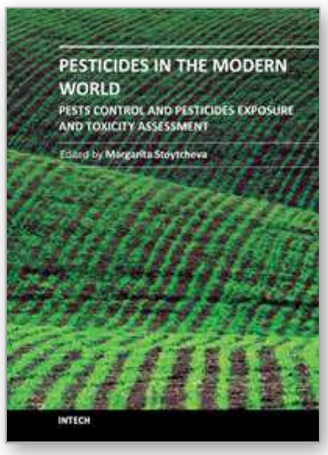
Wogram, J.; Sturm, A.; Segner, H.; Liess, M. 2001. Effects of parathion on acetylcholinesterase, butyrylcholinesterase, and carboxylesterase in three-spined stickleback (*Gasterosteus aculeatus*) following short-term exposure. Environ Toxicol Chem. 20: 1528-1531.

Worek, F.; Backer, M.; Thierman, H. 1997. Reappraisal of indications and limitations of oxime therapy in organophosphate poisoning. Hum Exp Toxicol. 16: 466-472.

Worek, F.; Diepold, C.; Eyer, P. 1999. Dimethylphosphoryl-inhibited human cholinesterases: inhibition, reactivation and ageing kinetics. Arch Toxicol. 73: 7-14.

Zahavi, M., Tahori, A. S., and Klimer, F. (1971). Insensitivity of acetylcho linesterases to organophosphorus compounds as related to size of esteratic site. Mol. Pharmacol, 7: 611–619.

Zinkl, J.G.; Shea, P.J.; Nakamoto, R.J.; Callman, J. 1987. Brain cholinesterase activity of rainbow trout poisoned by carbaryl. Bulletim of Environmental Contamination and Toxicology. 38: 29-35.



Pesticides in the Modern World - Pests Control and Pesticides Exposure and Toxicity Assessment

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The present book is a collection of selected original research articles and reviews providing adequate and up-to-date information related to pesticides control, assessment, and toxicity. The first section covers a large spectrum of issues associated with the ecological, molecular, and biotechnological approaches to the understanding of the biological control, the mechanism of the biocontrol agents action, and the related effects. Second section provides recent information on biomarkers currently used to evaluate pesticide exposure, effects, and genetic susceptibility of a number of organisms. Some antioxidant enzymes and vitamins as biochemical markers for pesticide toxicity are examined. The inhibition of the cholinesterases as a specific biomarker for organophosphate and carbamate pesticides is commented, too. The third book section addresses to a variety of pesticides toxic effects and related issues including: the molecular mechanisms involved in pesticides-induced toxicity, fish histopathological, physiological, and DNA changes provoked by pesticides exposure, anticoagulant rodenticides mode of action, the potential of the cholinesterase inhibiting organophosphorus and carbamate pesticides, the effects of pesticides on bumblebee, spiders and scorpions, the metabolic fate of the pesticide-derived aromatic amines, etc.

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