

Review

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Review

Neurotoxicity Biochemical Biomarkers in Fish Toxicology

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Simple Summary: Many aquatic pollutants may affect the functions of the fish nervous system, and in consequence – disturb various vital functions such as feeding, reproduction, or migration, leading to fish population decrease. Thus, evaluating the neurotoxic potential of chemical compounds is important for fish resource management. The present literature review indicates that the measurement of the activity of acetylcholinesterase (AChE, one of the key enzymes necessary for nervous system function) provides reliable information about the neurotoxicity of various aquatic pollutants in fish. Thus, evaluation of AChE activity may be recommended as a biomarker in fish toxicological studies.

Abstract: Acetylcholinesterase (AChE) activity is the most commonly used neurotoxicity biomarker in fish. It is measured mostly in the brain but can also be evaluated in muscle or (in the case of very small fish) in whole body homogenate. AChE activity is measured using standard methods, thus the results obtained by various authors are comparable. AChE seems to be a sensitive neurotoxicity biomarker since in most cases of fish exposure to various toxic agents a decrease in the enzyme activity was observed. The effects of toxic agents on AChE activity are concentration and time-related. An increase in AChE activity occurred very rarely. The results of the analysis showed that most aquatic pollutants may modulate AChE activity. Acetylcholinesterase activity can be also used to evaluate transgenerational neurotoxic effects.

Keywords: acetylcholinesterase; AChE; aquatic pollution; fish; neurotoxicity

1. Introduction

Development and use of neurotoxicity biomarkers is an important issue in toxicology since numerous emerging pollutants such as microplastics, pharmaceuticals, and other novel chemical compounds as well as “old” contaminants e.g. metals and pesticides, show a neurotoxic potential and may cause human neurodevelopmental disorders such as autism, attention-deficit hyperactivity disorder, dyslexia and others [1]. In polluted waters, neurotoxic compounds affect aquatic organisms first, thus measurement of neurotoxic effects in fish is necessary from the One Health perspective [2]. Neurotoxicity in fish may result in disturbed schooling, migration, spatial distribution, feeding, reproduction, and predator avoidance. Neurotoxicity biomarkers used in fish include histopathological [3,4] and morphometric [5] evaluation of brain, various behavioral endpoints [6–8], expression of marker genes associated with neuron development and growth [9], the levels of neurotransmitters in brain [10,11]. However, changes in activity of cholinesterases are the most often used biomarkers of aquatic pollution, because these enzymes are frequent targets for toxic effects of contaminants such as insecticides or metals [12]. The most commonly used biochemical biomarker of neurotoxicity in fish is acetylcholinesterase (AChE) activity measured in the brain, in the whole body (in the case of embryos and larvae), or in other organs (muscle, liver, and gills).

The present paper aims to review and summarize the recent literature data on the use of acetylcholinesterase activity as a neurotoxicity biomarker.

2. Acetylcholinesterase as a Neurotoxicity Biomarker

AChE is a serine hydrolase that terminates the impulse transmission in cholinergic synapses by hydrolysis and inactivation of the neurotransmitter acetylcholine (ACh). Although the primary function of AChE is to terminate neural transmission, it was also found that AChE plays a role in neural development [13]. AChE occurs in all tissues and is most abundant in the brain and muscles. AChE inhibition increases ACh concentration and its neurotransmitter action [14] causing cognitive and behavioral disturbances. According to de la Torre et al. [15], AChE activity is a sensitive biomarker of exposure of fish to toxic agents. However, Menendez-Helman et al. [16] pointed out that using AChE activity as a biomarker requires understanding its natural fluctuation. They measured the seasonal cycle of AChE activity in *Cnesterodon decemmaculatus* and reported the highest values in summer, and a considerable decrease in winter, probably related to different water temperatures. Also, an inverse relationship between enzymatic activity and animal size was established. Seasonal changes in AChE activity and sensitivity to pesticides (glyphosate and chlorpyrifos) were also reported by Bernal-Rey et al. [17] for the same fish species.

Scopus database was searched for the effects of toxic agents on fish AChE activity (Article title, Abstract, Keywords: “fish AND toxic* AND acetylcholinesterase”). The search resulted in 1308 documents, including 881 (67%) published in 2014-2023 (Oct. 24, 2023). These data were viewed and 100 of them (concerning only the brain or, in the case of embryos and larvae, whole body AChE) were selected and shown in Table 1.

Additionally, the search: “fish AND pesticide AND acetylcholinesterase” resulted in 49% of papers published in the last 10 years, “fish AND pharmaceutical or drug” AND acetylcholinesterase - 61%, “fish AND nanoparticle AND acetylcholinesterase” - 86% and “fish AND microplastic AND acetylcholinesterase” resulted in 98% of papers published in 2014-2023. These statistics show that AChE activity measurement is an increasingly important component of multibiomarker evaluation of the effects of toxic agents in fish, including emerging aquatic pollutants.

In Table 1 summarizing the effects of various toxic agents on AChE activity, 100 papers were included containing 119 data (in some studies, toxicity of more than one compound was evaluated). Most data (45%) concern pesticides (35% – insecticides, 8% – herbicides, 2% – fungicides). Other groups of data concern pharmaceuticals and disinfectants (12%), elements (10%), microplastics (7%) and nanoparticles (6%). The remaining 18% of data describe the effects of other compounds such as antifouling agents, cosmetic components, aromatic hydrocarbons, cyanotoxins, plastic components, and others. Most experiments (44%) were conducted on embryos, larvae, or adults of *Danio rerio*.

Analysis of the methods of AChE activity measurements (in 100 papers) revealed that most authors (66%) used the method developed and described by Ellman et al. [18], 23% used commercial biochemical kits, 4% - other methods and 7% did not specify the method used. Spectrophotometric cuvettes (16%) are often replaced by microplates (27%) and 57% of authors did not specify the reading method (but commercial kit users presumably used microplates, thus the microplates were probably used in most studies).

3. The effects of Toxic Agents on Acetylcholinesterase Activity

Analysis of the results shown in Table 1 revealed that in most cases (64%) AChE inhibition was reported, in 25% - no change, and only in 11% - an increase in AChE activity occurred. In case of no significant change observed, most data (44%) concerned lower concentrations of agents, while at higher concentrations changes occurred, in 35% no change was a single result for one concentration tested or observed at all levels of a studied agent, in 18% - after shorter times of exposure, while at longer times changes were observed, and only in 3% (1 case) nonlinear reaction occurred and no change was reported at the intermediate concentration of an agent. These data showed clear concentration- and time-related effects of chemicals on AChE activity, and a high responsiveness and sensitivity of this enzyme to various toxic agents. This confirms that AChE is a good neurotoxicity biomarker.

The results of many studies indicate that various aquatic pollutants may modulate AChE activity in fish, e.g., organophosphorus and carbamate insecticides are well-known AChE inhibitors that act

by specifically binding to the active site of AChE and blocking the access of the physiological substrate [14,19–21], as well as organophosphate esters used as plasticizers and flame retardants [22]. However, chemicals other than carbamates and organophosphates have also been documented to alter acetylcholinesterase activities (Table 1). Sato et al. [23] tested *in vitro* inhibition of common carp AChE by 35 various insecticides and their derivatives and found that various chemical forms of active compounds (e.g. oxon vs. thiono or diethyl vs. dimethyl) showed different inhibitory power. Also, combinations of different insecticides showed an additive inhibitory effect. An *in vitro* study of the effects of metal ions on *Diodon hystrix* brain AChE activity [24] revealed inhibition order: $\text{Cr}^{6+} < \text{Co}^{2+} < \text{Ag}^{2+} < \text{Cu}^{2+} < \text{Pb}^{2+} < \text{As}^{5+} < \text{Cd}^{2+} < \text{Zn}^{2+} < \text{Ni}^{2+} < \text{Hg}^{2+}$ and proved that AChE activity is a useful biomarker for evaluating metal toxicity to fish.

According to Colovic et al. [14], AChE inhibitors include irreversible and reversible groups. Reversible inhibitors, competitive or noncompetitive, mostly have therapeutic applications, while toxic effects are associated with irreversible AChE activity modulators.

The data of various studies showing the effects of aquatic pollutants on the fish brain or whole-body AChE activity (Table 1) reveal that most examined agents including microplastics, pharmaceuticals, pesticides, metals, etc., usually inhibited the enzyme but the effects were concentration- and time-related. AChE activation also sometimes occurred or non-linear alternate bidirectional changes were observed at various concentrations or different times of exposure to the same agent making interpretation of neurotoxic effect difficult. In most cases of microplastic exposures inhibition of AChE was observed, however at low concentrations enzyme activation sometimes occurred [25,26]. Also, most exposures of fish to pharmaceuticals resulted in AChE inhibition, except for some antidepressants (sertraline) that induced no changes or activated the enzyme. According to Muller et al. [27], antidepressants inhibited human AChE and opposite effects observed in fish are difficult to explain. Most pesticides: insecticides, herbicides, fungicides, and an antifouling agent caused AChE inhibition in fish, often concentration and/or time-related. According to Mladenovic et al. [28], many commonly used triazine, carbamate, organophosphate, neonicotinoid, methylurea, or phenylurea pesticides such as atrazine, simazine, propazine, carbofuran, monocrotophos, dimethoate, carbaryl, tebufenozide, imidacloprid, acetamiprid, diuron, monuron, and linuron are AChE inhibitors due to their binding to the AChE active site. For some pesticides that usually inhibited AChE, activation was reported in single cases [29–31] which might have been related to very low concentrations used. Inhibition of AChE activity by other chemicals was also reported: disinfectants, bisphenol A, cosmetic compounds, organic solvents, metals and other elements, nitrogenous metabolites, and other aquatic pollutants. It is noteworthy that in the case of exposure of fish to nanoparticle materials AChE activity was rarely inhibited, suggesting that environmentally relevant concentrations of these pollutants are little neurotoxic. According to the review by Olivares-Rubio and Espinosa-Aguirre [32], polycyclic aromatic hydrocarbons (e.g., benzo[a]pyrene, pyrene, and anthracene) inhibited AChE activity but PAHs with a low molecular weight did not induce changes or cause stimulation of AChE activity.

Acetylcholinesterase activity is sometimes measured in various tissues and the results are not always the same as for the brain and also differ among the organs examined. Fakhereddin and Doğan [33] observed different values and different time- and concentration-related patterns of splenic and cardiac AChE activities in rainbow trout treated with clothianidin (a neonicotinoid insecticide). Abegoda-Liyanage and Pathiratne [34] reported no alterations in brain AChE activities following TiO_2 and nano- TiO_2 exposures but an increase occurred in gill and liver. Different patterns of gill and muscle AChE activity changes over time of exposure to three pesticides, compared to the brain, were reported also by Amin et al. [29]. Bonansea et al. [35] observed no changes in brain AChE activity following low and high cypermethrin or chlorpyrifos exposures, while muscle AChE activities significantly decreased after exposures to high concentrations of both pesticides. According to Golombieski et al. [36], AChE activity in the brain and muscles of three cyprinid fish species exposed to diafuran decreased similarly. A considerable and significant decrease in AChE activity in muscle of trichlorfon-exposed tilapia was also reported by Guimaraes et al. [37]. Similar changes in brain and muscle AChE activities were observed by Modesto and Martinez [38] in *Prochilodus lineatus* exposed

to Roundup and by Benli and Celik [39] in zebrafish treated with sulfoxaflor. The decrease in AChE activities measured in the muscle, liver, and gill of tilapia exposed to dichloromethane followed the decrease in the brain. However, the control value of AChE activity and the difference between the values for the control and exposed fish were the highest in brain and muscle. Nayak and Patnaik [40] reported the highest activity of AChE in the brain and muscle of *Anabas testudineus* but the degree of enzyme inhibition following exposure to naphthalene occurred also in the gill and liver. According to Tilton et al. [41], chlorpyrifos reduced zebrafish muscle AChE activity in a concentration-dependent way but no such a relationship was observed for copper – a significant decrease in AChE activity occurred only at the lowest Cu concentration (6.3 µg/L). Ullah et al. [42] reported similar activities and inhibition of brain and muscle AChE of *Tor putitora* from various sampling sites of different pollution levels. The results obtained by Marinho et al. [43] showed an even higher sensitivity of muscle AChE, compared to the brain, to nano-Ag intoxication. These data show that AChE activity in muscle is probably an equally reliable biomarker as brain or whole-body AChE activity.

On the other hand, sometimes brain and muscle AChE may show opposite reactions to toxic agents. dos Santos Teixeira et al. [44] reported that AChE activity of pintado da Amazônia decreased in muscle but increased in the brain compared to the control group after Roundup exposure. According to Zhang et al. [45], AChE activity in various tissues of zebrafish was inhibited almost at the same time when the fish were exposed to high concentrations of toxic agents (Cd^{2+} or deltamethrin), while at lower concentrations inhibition showed a delay compared to the brain: brain > gill > muscle > liver. These data show that muscle AChE activity should be considered with caution. Caution is also necessary since for some toxic agents, AChE may be not a reliable biomarker of neurotoxicity. Agostini et al. [46] reported no changes in zebrafish brain AChE activity during treatment with 0.5% ethanol solution, while a significant decrease in acetylcholine level and choline acetyltransferase occurred.

Table 1. The effects of toxic agents on brain (or whole body – in embryos and larvae) acetylcholinesterase (AChE) activity in fish.

GCS	Fish species	Toxic agent	Concentration [mg/L]	Exp. dur. [d]	AChE activity	Author
M	<i>Oreochromis niloticus</i>	microplastic	100	21	↓	[47]
M	<i>Danio rerio</i> embryos	microplastic	0.1-10	4	↓	[48]
M	<i>Oreochromis mossambicus</i>	microplastic	100***	14	-	[49]
			500 ***		↓	
			1000***		↓	
M	<i>Danio rerio</i> embryos	microplastic	0.1-3	5	↓	[50]
M	<i>Danio rerio</i>	microplastic	10000@	5	↓	[51]
M	<i>Oryzias javanicus</i>	microplastic	0.5-5	21	↑	[25]
M	<i>Danio rerio</i>	microplastic	2	30	↑	[26]
E		Cu (as CuSO ₄ •5H ₂ O)	25*		↑	
M	<i>Dicentrarchus labrax</i>	microplastic	0.26 or 0.69	4	↓	[52]
E		Hg (as HgCl ₂)	0.010 or 0.016		↓	
E	<i>Cnesterodon decemmaculatus</i>	As (as NaAsO ₂)	0.5-5	4	-	[53]
E	<i>Anabas testudineus</i>	Cr VI (as CrO ₃)	2.75 or 5.5	72	↓	[54]
E	<i>Danio rerio</i>	Cr III (as	1	5	↓	[55]
E		CrCl ₃ •6H ₂ O)			↓	
E		Cr VI (as (K ₂ Cr ₂ O ₇)			↓	
E	<i>Hypophthalmichthys molitrix</i> larvae	Hg (as HgCl ₂)	1-10*	14	↓	[56]
E	<i>Danio rerio</i> embryos	Hg (as HgCl ₂)	10	1	-	[57]
			100	2-4	-	
				1	-	
E	<i>Danio rerio</i>	Al	5.5	15	↓	[58]
E	<i>Oreochromis niloticus</i>	Al (as Al ₂ (SO ₄) ₃)	1 or 3*	14	↑	[59]
E	<i>Danio rerio</i> embryos + larvae	Sb (as K ₂ Sb ₂ C ₈ H ₄ O ₁₂ •3 H ₂ O)	200-800	2	↓	[60]
N	<i>Oreochromis niloticus</i>	Ti (as TiO ₂ NPs)	0.05 or 0.1	7-14	-	[34]
E		Ti (as TiO ₂)			-	
N	<i>Oncorhynchus mykiss</i>	graphene nanoflakes	4	36	-	[61]
N		graphene oxide				
N		reduced graphene				
N		oxide silicon carbide nanofibers				
N	<i>Danio rerio</i>	Se NPs	0.5 or 10	4	↓	[62]
N	<i>Danio rerio</i>	Ag NPs	1	4	-	[43]
			3-5*		↓	
F	<i>Danio rerio</i>	paclobutrazol	10	4-14	↓	[63]
F	<i>Danio rerio</i>	thifuzamide	0.19	6	↓	[64]
			1.9 or 2.85		↓	
F	<i>Danio rerio</i> embryos	mancozeb	0.5*	4	↓	[65]
			5*		-	
			50*		↓	
H	<i>Prochilodus lineatus</i>	Roundup®	1 or 5	4	↓	[38]
H	<i>Danio rerio</i> larvae	Roundup®	4.8*	5	↑	[30]
H	<i>Danio rerio</i> embryos	Roundup®	0.25	2	↓	[66]

glyphosate						
H	<i>Danio rerio</i> larvae	haloxyfop-p-methyl	0.2-0.4	4	↑	[67]
H	<i>Oreochromis niloticus</i>	pendimethalin	0.52	28	↓	[68]
I	<i>Cyprinus carpio</i>	chlorpyrifos	25*	21	↓	[69]
H		glyphosate	3.5		↓	
I	<i>Tilapia nilotica</i>	Nemacur®	0.1-2		↓	
I		malathion	0.1-2	1	↓	[70]
H		diuron	1		↓	
DMA® 806 BR						
H	<i>Danio rerio</i>	(Fipronil)	63.5*	4	↑	[71]
I		Regent® 800 WG (2,4-D)	447*		↑	
I	<i>Danio rerio</i> embryos	chlorphoxim	2.5-7.5	4	↓	[72]
I	<i>Gambusia affinis</i>	chlorpyrifos	0.297	4	↓	[73]
I	<i>Oncorhynchus mykiss</i>	chlorpyrifos	2.25 or 4.5	1-4	-	[74]
			7.25*	1-2	-	
			7.25*	3-4	↓	
I	<i>Oreochromis niloticus</i>	chlorpyrifos	5-15*	30	↓	[75]
I	<i>Cyprinus carpio</i>	diafuran	1-3	4	↓	[36]
	<i>Ctenopharyngodon idella</i>					
	<i>Aristichthys nobilis</i>					
I	<i>Jenynsia multidentata</i>	cypermethrin	0.04 or 0.4*	4	-	[35]
I		chlorpyrifos	0.4 or 4*		-	
I	<i>Gambusia affinis</i>	cypermethrin	0.2 or 6.25**	7	↓	[76]
I	<i>Heteropneustes fossilis</i>	chlorpyrifos	0.09 or 0.192	7-30	↓	[77]
I	<i>Capoeta umbla</i>	chlorpyrifos	55	1	-	[4]
			110	4	↓	
				1	↓	
				4	↓	
I	<i>Cyprinus carpio</i>	chlorpyrifos	23 or 46*	14	↓	[78]
I	<i>Oreochromis niloticus</i>	malathion	1.425	1	↑	[29]
				2	↓	
		chlorpyrifos	0.125	1	↑	
				2	↑	
I		λ-cyhalothrin	0.0039	1	-	
				2	↓	
I	<i>Colossoma macropomum</i>	malathion	7.3	4	-	[79]
I	<i>Cyprinus carpio</i>	λ cyhalothrin	0.14 or 0.28*	15-45	↓	[80]
I	<i>Danio rerio</i>	chlorpyrifos	1.16*	5	↓	[81]
I		cyfluthrin	7.06 or 14.12*			
I	<i>Oncorhynchus mykiss</i> larvae	chlorpyrifos	0.3*	21	-	[82]
			3*		↓	
			2*		-	
I	<i>Oncorhynchus mykiss</i>	chlorpyrifos		7	-	[83]
				14-21	↓	
			4*	7-21	↓	
I	<i>Danio rerio</i>	dinotefuran	0.2	28	↓	[84]
			1		↓	
I	<i>Danio rerio</i>	imidacloprid	0.15*	4	-	[85]
		15 or 45*	↓			
I	<i>Danio rerio</i>	imidacloprid	0.05-20*	14-35	-	[86]

I		thiamethoxam			↓	
I	<i>Danio rerio</i>	sulfoxaflor	0.87-3.51	4	↑	[39]
I	<i>Gambusia affinis</i>	carbofuran	0.191 or 0.255	15-40	↓	[87]
I	<i>Oreochromis niloticus</i>	carbofuran	0.246	30	↓	[88]
I	<i>Danio rerio</i> larvae	fenpropathrin	0.016-0.064	4	↑	[89]
I	<i>Danio rerio</i> larvae	isoprocarb	1-2.5	6	↓	[90]
I	<i>Clarias batrachus</i>	thiamethoxam	6.93 or 13.86	45	↓	[91]
I	<i>Danio rerio</i>	methomyl	0.5-23.3	6	↓	[92]
I	<i>Oncorhynchus mykiss</i>	phosmet	5*	1-2	-	[93]
			5*	3-4	↓	
			25 or 50*	1-4	↓	
I	<i>Prochilodus lineatus</i>	fipronil	5.5 82^	15	- ↓	[94]
I	<i>Rhamdia quelen</i>	trichlorfon	11	21	↓	[95]
I	<i>Colossoma macropomum</i>	trichlorfon	0.26 or 0.43	1-4	↓	[96]
I	<i>Carassius auratus gibelio</i>	trichlorfon	0.5-2****	0.5-4	↓	[97]
I	<i>Oryzias latipes</i>	diazinon	10 or 20*	122	↓	[98]
I	<i>Channa punctatus</i>	triazophos	3.4 or 6.8*	4	↓	[99]
I		deltamethrin	0.36 or 0.72*		↓	
Pd	<i>Danio rerio</i> embryos + larvae	cloramine T	16	4	-	[100]
			32		-	
			64		↓	
			128		↓	
Pd	<i>Danio rerio</i> embryos + larvae	2, 5-dichloro-1, 4-benuinone	0.2 0.4 or 0.6	4	- ↓	[101]
Pd	<i>Danio rerio</i>	fluoxetine	5-16**	4	↓	[102]
Pd	<i>Danio rerio</i>	fluoxetine	0.1-10*	21	-	[103]
Pd	<i>Rhamdia quelen</i>	ciprofloxacin	1* 10 or 100*	28	- ↓	[3]
Pd	<i>Oreochromis mossambicus</i>	triclosan	0.131-1.046	4	↓	[104]
Pd	<i>Corydoras paleatus</i>	triclosan	189*	2	↓	[105]
Pd	<i>Gambusia affinis</i>	gestodene	4.4** 378.7**	60	↓ ↑	[106]
Pd	<i>Oreochromis niloticus</i>	synthetic progesterone	0.2-0.8	4	↓	[107]
Pd	<i>Danio rerio</i>	metformin	1, 20 or 40*	120	↓	[108]
Pd	<i>Danio rerio</i>	sertraline	1 10 or 100	28	- ↑	[109]
Pd	<i>Danio rerio</i> embryos + larvae	sertraline	1-100	10	-	[64]
Pd	<i>Danio rerio</i>	nortriptyline	0.88-500*	7	↓	[110]
Pd	<i>Danio rerio</i> embryos	moxidectin	1.5-5*	4	-	[111]
O	<i>Danio rerio</i>	tributyltin	10** 100-300**	42	- ↓	[112]
O	<i>Danio rerio</i> embryos	bisphenol A	11.4	1	↓	[113]
O	<i>Danio rerio</i>	bisphenol A	0.22-1.5*	4	↑	[31]
O	<i>Danio rerio</i>	bisphenol AF	0.05 or 0.5	4	↓	[114]
O	<i>Gambusia affinis</i>	bisphenol A	4.74 or 7.74	15-60	↓	[115]
O	<i>Oreochromis niloticus</i>	benzylparaben	0.005-5*	56	↓	[116]
O	<i>Danio rerio</i> embryos	methylparaben	0.1 or 1*	6	↓	[117]
O	<i>Danio rerio</i>	methylparaben	1 or 11*	30	↓	[118]

O	<i>Danio rerio</i> embryos	octocrylene	5* 50 or 500*	4	- ↓	[119]
O	<i>Gambusia affinis</i>	decabromodiphenyl ether	25 or 50*	2	↑	[120]
O	<i>Danio rerio</i> embryos	benzophenone-3	1 or 10*	3	↓	[121]
O	<i>Danio rerio</i> larvae	hexabromobenzene	30*	6	-	[122]
		pentabromobenzene	100-300*		↓	
O			30-100*		-	
			300*		↓	
O	<i>Clarias gariepinus</i>	benzene	0.762**	30	↓	[123]
O		toluene	26.614**		-	
O		xylene	89.403**		-	
O	<i>Anabas testudineus</i>	naphthalene	4.2-5.0	3	↓	[40]
O	<i>Cyprinus carpio</i>	ammonia NH ₃	30.7	4	↓	[124]
O		nitrite NO ₂ ⁻	153.7			
O	<i>Danio rerio</i> embryos + larvae	ammonia NH ₃	0.06-0.85	7	↓	[125]
O	<i>Oreochromis mossambicus</i>	ammonia NH ₃	1	28-56	↓	[126]
O	<i>Oreochromis niloticus</i>	guanitoxin	125 or 250#	4	↓	[127]
O	<i>Danio rerio</i>	ethanol	5	7-28	-	[46]
O	<i>Oreochromis mossambicus</i>	dichloromethane	730-790	4	↓	[128]
O	<i>Cirrhinus mrigala</i>	phenol	2.32 or 6.96	7-28	↓	[129]
O	<i>Clarias gariepinus</i>	burnt tyre ash	0.56-2.24	28	↓	[130]

*µg/L **ng/L ***mg/kg feed ****g/kg feed #algal extract @particles/L ^ µg/kg sediment . GCS – group of chemical substance; E – metals and other elements; F – fungicides; H – herbicides; I – insecticides; M – microplastics; N – nanoparticles; O – other substances; Pd – pharmaceuticals and disinfectants . Exp. dur. – exposure duration; d – days; m – minutes; n/a – not applicable; NPs – nanoparticle. ↓ – decrease, ↑ – increase, - - no change.

4. Transgenerational Effects of Toxic Agents on Acetylcholinesterase Activity

Interestingly, AChE activity can be used as a biomarker of transgenerational toxicity effects. Pompermaier et al. (2022) reported that zebrafish larvae obtained from adults exposed to an herbicide containing 2,4- dichlorophenoxyacetic acid (2,4-D) showed elevated AChE activity. According to Wan et al. (2022), parental exposure of medaka to benzo[a]pyrene (BaP) caused a significant reduction of AChE activity in offspring but not in the exposed adults. Molecular analysis revealed that the marker genes associated with neuron development were downregulated in the larvae. This indicates an epigenetic effect. A similar transgenerational epigenetic effect of chlorpyrifos-oxon on AChE activity was observed by Schmitt et al. [131]. According to Brander et al. [132], epigenetic mechanisms include acetylation or phosphorylation of histones, DNA methylation, and interactions of non-coding RNA during transcription that may affect gene and protein expression.

Inhibition of AChE activity is usually accompanied by oxidative stress. Issac et al. [133] found that oxidative stress experimentally induced by exposure of zebrafish embryos to H2O2 resulted in a considerable and significant decrease in AChE activity. However, the role of oxidative stress in the modulation of AChE activity is still unclear and according to various authors, it may be involved in both decrease [134,135] and increase [136] of AChE activity.

5. Other neurotoxicity Biochemical Biomarkers

In fish toxicology, other neurotoxicity biochemical biomarkers are also sometimes used such as activities of BChE (butyrylcholinesterase), CbE (carboxylesterase), and GABA (γ-aminobutyric acid) level [137]. Morsy [10] evaluated the concentrations of neurotransmitters: dopamine, norepinephrine, and acetylcholine in *Cyprinus carpio*, exposed to dithiopyridine herbicide, and reported a decrease in catecholamine levels, while ACh concentration increased (which was accompanied by AChE

inhibition). Bedrossiantz et al. (2021) reported a concentration- and time-dependent decrease in the brain levels of dopamine, norepinephrine, and serotonin in zebrafish exposed to methamphetamine. According to a meta-analysis by Santana et al. [138], fish ChE and BChE are common biomarkers of environmental contamination due to their sensitivity to many toxicants, including pesticides. The authors concluded that regardless of tissue, BChE response was more variable than AChE, and no difference between their average activities was detected. The effects of pesticides were size- and age-related, cholinesterases in adult fish being the least sensitive to intoxication. Insecticides were stronger inhibitors compared to herbicides and fungicides. Interestingly, the analytical-grade pure active compounds inhibited cholinesterase activities more than commercial formulations. Among the pesticide classes, organophosphorus insecticides had the strongest effect on the activity of fish AChE and BChE, followed by carbamates, organochlorines, and pyrethroids. Ortiz-Delgado et al. [139] reported that malathion inhibited AChE and BChE activities in Senegalese sole in a dose- and time-dependent manner, but the activity of BChE was about two orders of magnitude lower compared to AChE.

6. Conclusions

The activity of acetylcholinesterase is the most widely applied neurotoxicity biomarker in fish. The results obtained by many authors for various fish species and numerous chemicals showed that AChE activity is a sensitive, and useful but nonspecific biomarker of intoxication in fish. AChE activity can be affected by aquatic pollutants, that mostly cause enzyme inhibition. This results in disturbed acetylcholine hydrolysis, the consequences of which are disturbed sensory, cognitive, and motor functions.

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