Original article

Different enzymatic activities in carp (*Cyprinus carpio* L.) as potential biomarkers of exposure to the pesticide methomyl

David Hernández-Moreno[1,2], Irene de la Casa-Resino[1], José María Flores[1], Manuel José González-Gómez[1], Carlos María Neila[1], Francisco Soler[1], and Marcos Pérez-López[1]

Toxicology Unit, Veterinary School of Caceres, Caceres, Spain[1], Universidad Autónoma de Chile, Chile[2]

This study investigated the influence of the pesticide methomyl on different enzymatic activities in carp. The fish were exposed to a sub-lethal concentration (0.34 mg L^{-1}) of methomyl for 15 days. On days 4 and 15, catalase (CAT) and glutathione-S-transferase (GST) activities were measured in the liver and gills. Acetylcholinesterase (AChE) activity in brain and muscle was also determined. Liver catalase activity slightly increased in exposed fish when compared to controls, but it was statistically significant only at the beginning of the experiment. No changes in CAT activity in the gills of exposed and control animals were observed (mean values were in the range 10.7-11.7 nmol min^{-1} per mg of protein). Liver GST activity was slightly inhibited in the exposed animals at the beginning of the study; however, it was significantly inhibited in the gills. Brain AChE activity was diminished throughout the experiment and significantly decreased after 96 h of exposure compared to controls (0.041 *vs.* 0.075 nmol min^{-1} per mg of protein; $p<0.001$). Our findings suggest that CAT, GST, and AChE are reliable biomarkers of effect after exposure to methomyl.

KEY WORDS: *acetylcholinesterase; carbamate; catalase; fish; glutathione S-transferase*

Common carp (*Cyprinus carpio* L.) is one of the most important fish cultured in the world, either as food or for recreational fishing. Carp is often recommended for the baseline evaluation of emerging pollutants in aquatic ecosystems (1) and commonly used in experimental models due to its availability and good adaptation to laboratory conditions (2).

Methomyl [S-methyl N-((methylcarbamoyl)oxy) thioacetimidate], a carbamate insecticide, has been classified as a highly toxic pesticide for aquatic organisms (3). Its acute toxicity to various freshwater fish, with 96-h LC_{50} values ranging from 0.9 mg L^{-1} (bluegill sunfish) to 3.4 mg L^{-1} (rainbow trout), was reported in several studies (4, 5). It is widely used to control insect pests in many countries around the world, and by entering water bodies directly or indirectly it affects both surface and ground waters.

In spite of advancements in chromatographic techniques, the detection of low pesticide levels in water can be difficult because they may fall below detection limits (6). In such instances, the effects of chronic (or sub-lethal) concentrations of pesticides on non-target organisms can be studied by detecting changes in organisms at the physiological, biochemical, or molecular levels, providing "early warning" tools in environment quality monitoring (7, 8). The inhibition and induction of these biomarkers are good approaches to measuring the potential impact of pollutants on environmental organisms. For example, acetylcholinesterase (AChE) activity in fish has proven useful for monitoring the contamination of marine and freshwater ecosystems and this specific inhibitory effect has been directly associated to carbamate and organophosphorus pesticides (9).

Moreover, many of these potentially toxic compounds or their metabolites have shown toxic effects related to oxidative stress in fish (4, 9-11). Cells have a variety of defence mechanisms to neutralize the harmful effects of free radicals (10). One of the main antioxidative enzymes that serve to detoxify reactive oxygen species are catalases (CAT). CAT is a ubiquitous heme protein that degrades hydrogen peroxide (H_2O_2) to oxygen and water and is involved with the Haber–Weiss reaction (12). Similarly, glutathione S-transferases (GST) are a group of widely distributed enzymes that catalyse the conjugation of glutathione (GSH) with various electrophilic substances. GST-mediated conjugation is involved in the detoxification of many xenobiotics, playing an important role in protecting tissues from oxidative stress (13).

Taking into account the great relevance of effect-biomarkers when no levels of pesticides can be detected in the environment, the aim of this study was to evaluate the alterations in different biochemical parameters in several tissues of carp (cholinesterase activity in muscle and brain, as well as glutathione S-transferase and catalase enzymatic activities in liver and gills) exposed to methomyl.

MATERIALS AND METHODS

Chemicals and reagents

All chemicals and reagents were of analytical grade and purchased from Sigma-Aldrich (St. Louis, MO, USA). Tested substance methomyl (CAS No. 16752-77-5) was of 99 % purity. Its stock solution was prepared by dissolving the pesticide in 1 mL of absolute ethanol in a standard volumetric flask.

Fish

Carp specimens with a weight of 30.0±5.2 g and length of 8-11 cm were acquired from a local fish hatchery in Badajoz (Spain) and acclimated to laboratory conditions for 15 days prior to the beginning of the experiment. Fish were kept in two batches (control and exposed group) of 15 individuals into independent tanks (1 m×0.4 m×0.5 m, 160 effective litres). During the experiment, fish were fed daily with a commercial dry diet (OVN Dibaq-Diproteg, Segovia, Spain) *ad libitum* until the end of the experiment. Fish were starved for 24 h before sacrifice.

Experimental design

The experimental group was exposed to a sub-lethal concentration of the pesticide (0.34 mg L^{-1}) during 15 days. This concentration represented 10 % of LC_{50} previously established for rainbow trout (14), being similar to the environmental level found in water samples taken near Cáceres, Spain (unpublished data).

Although the stock solution of pesticide was prepared in ethanol, the final amount of solvent in the aquaria, considering the total volume of the tanks, was negligible, as recommended by OCDE test guidelines (15).

Water quality characteristics (temperature, alkalinity, conductivity, total hardness, dissolved oxygen, ammonia, nitrate and nitrite) were routinely monitored during the experiment and all were within acceptable limits. Furthermore, once a week, water samples (V=250 mL) were taken from each tank and subjected to high-performance liquid chromatography (HPLC) analysis in order to assess the effective concentrations of the pesticide. Standard HPLC technique with acetonitrile and water as mobile phase and a Nucleosyl 120 C18 column was applied, according to the method previously described for other carbamate pesticides (11).

Animal maintenance and experimental procedures were conducted in accordance with the Guide for Use and Care of Laboratory Animals (16), and efforts were made to minimize animal suffering and reduce number of specimens. The experimental design was approved by the Ethics Committee of the University of Extremadura (Spain) and carried out in accordance with current legislation (17).

Exposed and control animals (n=5) were removed on three occasions from each aquarium: at the beginning of the experiment (day 0), and after 4 and 15 days of the experiment.

Fish were anesthetized with a solution of tricaine methanesulfonate (MS222; Sigma-Aldrich, St. Louis, MO, USA) at a concentration of 0.1 g L^{-1}. (11). Animals were sacrificed; and liver, gills, brain and a portion of muscle were removed, weighed, and individually frozen at -80 °C until biochemical analyses.

Liver and gills of each animal were homogenised in 1:10 (w/v) ice cold potassium phosphate buffer (0.1 mol L^{-1}, pH 7.4). The homogenate was centrifuged for 20 min at 10000 *g* (4 °C) to obtain the post-mitochondrial supernatant. Brain and muscle tissues were homogenized in cold potassium phosphate buffer

(0.1 mol L^{-1}, pH 7.2). The obtained homogenate was centrifuged at 6000 *g* for 15 min at 4 °C. The supernatant was used to determine AChE activity (nmol min^{-1} per mg of protein). AChE activity was spectrophotometrically determined according to Ellman (18), adapted to microplate, by measuring the increase in absorbance of the sample at 405 nm in the presence of 1 mmol L^{-1} acetylthiocholine as substrate, and 0.1 mmol L^{-1} 5,5,-dithiobis-2-dinitrobenzoic acid (DTNB) as chromogen.

CAT activity assay was carried out by spectrophotometric measurement at 240 nm following the method of Clairborne (19). The enzymatic GST activity was measured at 340 nm according to Habig et al. (20). Specific enzyme activity of was defined as nmol min^{-1} per mg of protein. Protein content was estimated by the Bradford method (21), using bovine serum albumin as standard.

Statistical analyses were performed using GraphPad Prism v.5 software. All values are represented as mean±standard deviation (SD). Data were treated by means of the non-parametric Kruskal Wallis test followed by the post-hoc Dunn's test in order to delimitate the groups in which significant differences occurred.

RESULTS AND DISCUSSION

Recent environmental monitoring in the Cáceres area indicated the presence of methomyl residues. We therefore decided to perform an experiment to investigate whether an environmentally relevant methomyl concentration, when tested in laboratory conditions, poses risk to carp, a typical fish species that inhabits local freshwater bodies.

In the course of our experiment, despite exposure to the pesticide, no mortality or visual changes on fish indicating potential impairments in the nervous system appeared in any of the considered experimental groups. Methomyl presence in the tested water samples was monitored during the experiment and confirmed by HPLC to be within 20 % of the nominal concentration, with the same retention time as the commercial standard.

The effects of methomyl exposure on CAT, GST, and AChE activity in the tissues of common carp are shown in Figure 1.

Whereas hepatic CAT activity in the exposed fish increased compared to controls both on day 4 ($p<0.05$) and day 15 of exposure, branchial CAT activity did not show any significant difference between control and exposed animals during the whole experiment (mean values 10.7-11.7 nmol min^{-1} per mg of protein).

In the course of the experiment, liver CAT activity increased both in the control and exposed fish, which was statistically significant only after 4 days of exposure (Figure 1). The higher results appeared after 15 days, which had no relevance with respect to the previous sampling day due to the increased value of CAT in the controls at the same sampling. Ensibi et al. (22) also reported that CAT values in carp exposed to carbamates did not change and tended to be similar to controls after 15 days of treatment, exhibiting changes after only 4 days of exposure. Increased values in controls from the last sampling with respect to controls from day 4 can be explained by external conditions influencing animal behaviour, thus showing the importance of having a control group for each day of experiment.

With respect to hepatic GST activity, significant inhibition in the exposed animals ($p<0.05$) was observed at the beginning of the experiment compared to control animals (mean GST activity of 72.4 and 87.0 nmol min^{-1} per mg of protein, respectively), with highly significant inhibition in the gills ($p<0.01$) of the exposed fish. However, the hepatic inhibitory effect disappeared at the end of the experiment (control and exposed animal GST activities were within 68-72 nmol min^{-1} per mg of protein), whereas the same initial effect quantified in gills remained, although at a lower level of significance ($p<0.05$).

The differences between the levels of GST activity in the liver of the control fish measured on days 4 and 15 were statistically significant. On the contrary, in the exposed fish no significant differences were observed in the activities of this enzyme on day 4 and day 15 (Figure 1). These changes might have been generated by environmental changes other than the ones covered by this study, which is for control animals more because the antioxidant system of exposed fish is already affected by the pesticide. Indeed, it was reported that the unchanged GST activity after insecticide exposure may indicate enzyme inactivation by a pesticide or GSH depletion, inducing GST to lose its activity (22).

Some authors reported a lack of effect in CAT levels in gills of fish exposed to carbamates, appearing only after exposure to high concentrations (23). However, decreased CAT levels were found in *C. punctatus* exposed to pyrethrins (24). Even when gills are the first contact-point with xenobiotics, they do
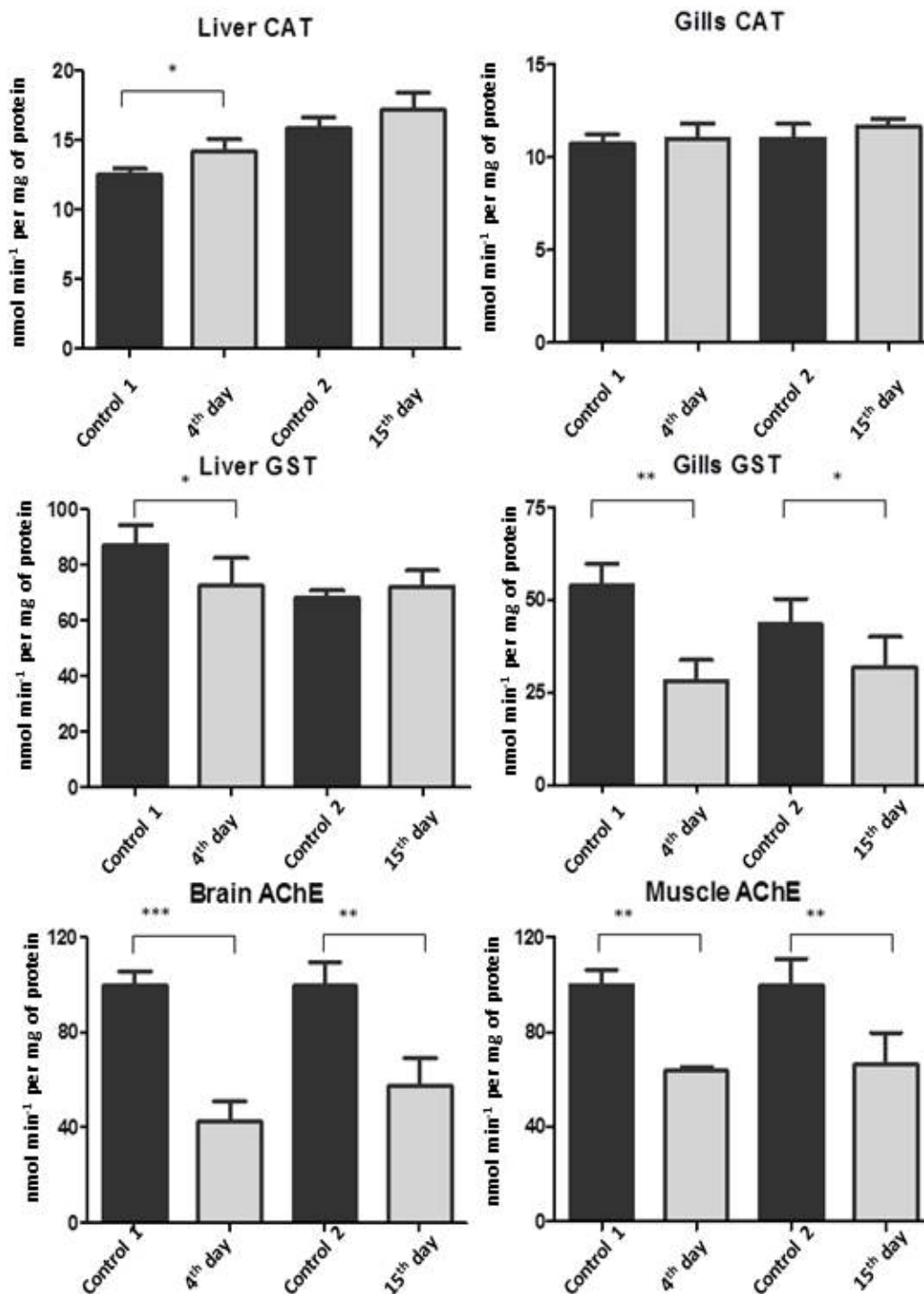
Figure 1 *Levels of catalase (CAT), glutathione-S-transferase (GST), and acetylcholinesterase (AChE) measured in tissues of common carp exposed to 0.34 mg L^{-1} of methomyl in laboratory conditions.*
*Control 1 corresponds to results from day 4, as it was established at the beginning of the experiment and Control 2 corresponds to results from day 15, as it was established at the end of the experiment. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$*

not always have an effect over this tissue, passing directly through this barrier and acting on other tissues like liver. In fact, CAT levels were reported higher in liver as a result of breaking down toxins present in the blood and processing metabolic products for degradation (25, 26).

Ferrari et al. (27) showed that different pesticides induced hepatic CAT activity during the first 48 h of exposure, and similarly, Capkin and Altinok (28) observed that rainbow trout exposed to 25 µg L^{-1} of carbamate carbosulfan for 60-days significantly increased liver CAT activity when compared to controls.

However, oxidative stress levels in fish from waters contaminated by different concentrations of pesticides were entirely different (29), and in some cases a clearly inhibitory effect was observed. Crestani et al. (30) for instance, observed a reduction in CAT activity in the liver of silver catfish exposed to the herbicide clomazone. Similar effects were found by Pandey et al. (31) in the liver of the freshwater fish *Channa punctatus* evaluated after 24 h of treatment with endosulfan. Blahová *et al.* (32) observed a significant decline in CAT activity in zebrafish exposed to atrazine. Curiously, this same xenobiotic was assayed by Nwani et al. (33), who noticed an elevation of liver CAT activity after the exposure of *Channa punctatus*.

The first line of defence against oxidative stress consists of antioxidant enzymes (e.g., CAT) and a decrease in their activity changes the redox status of the cells. Thus, it is possible that an increase in the activity of these enzymes contributes to the elimination of ROS induced by cells exposed to pesticides (34, 35).

GST has also been widely used as an environmental biomarker. In some studies, hepatic GST activity decreased after 5 days of exposure to a mixture of pesticides (36). Similarly, liver of fish fed with a standard diet and exposed to the herbicide quinclorac showed a marked inhibition of GST activity (2). Nevertheless, some studies have also observed an opposite effect, as for example low level exposure of *Galaxias maculatus, Galaxias truttaceus*, and *Salmo gairdneri* to the fungicide tetrachloroisophthalonitrile resulted with the induction of the liver GST activity (37).

When considering studies focused on carbamate pesticides and fish, a clear inhibition of GST activity was spotted after 96 h of exposition to methomyl (4). These results are in agreement not only with ours, but also with those of Rendón-von Osten et al. (9), who found a significant inhibition of GST activity in mosquito fish (*G. yucatana*) gills after 24 h exposure to 0.06 mg L^{-1} of carbofuran. GST inhibition has also been reported in gills of mosquito fish exposed to carbofuran and liver of *Ancistrus multispinis* exposed to deltamethrin (38). The observed decrease of GST activity may have occurred because liver and gills are two of the first organs in the contact with pesticides. However, this decrease may lead to a different response where the antioxidant defence is disrupted by pesticides or its derived products, indicating a toxic situation caused by insecticide exposure (39).

As shown in Figure 1, methomyl exposure caused a clear and significant decrease in AChE activity compared to controls both in brain (*p*<0.001 on day 4; *p*<0.01 on day 15) and muscle (*p*<0.01 on day 4 and 15). Thus, our results indicate that AChE activity varied according to tissue type at least during the first days of exposure, with brain tissue being more susceptible. This phenomenon could be related to the higher number of neurons in the brain than in the muscles, i.e. more receptors were available directly. After first contact with the pesticide, and thus after saturation of the synaptic sites in both tissues, the inhibition rate reached equilibrium.

There were no significant differences in the AChE activities measured in brain and muscle of control fish on days 4 and 15. Comparison of the values measured in the exposed fish indicate that brain AChE activity was significantly lower on day 4 as compared to day 15 (Figure 1). Several studies reported a similarity between the AChE activity of exposed fish and controls after an initial period of inhibition (11, 40). Reactivations of the carbamate-mediated anticholinesterase effect, or even an enzyme superproduction as a response to inhibition, have been reported as cause for that phenomenon (41).

The highest inhibition in brain samples took place at day 4 (0.041 nmol min^{-1} per mg of protein in exposed animals, and 0.075 nmol min^{-1} per mg of protein in control), clearly showing the inhibitory effect of carbamate pesticides and rendering AChE activity as the most adequate biomarker for exposition to carbamates in fish. The same was reported in several studies on freshwater and marine fish (42-44). For instance, Hernández-Moreno et al. (43) observed that brain and muscle cholinesterase activities in seabass (*Dicentrarchus labrax*) exposed to 250 mg L^{-1} of carbofuran were significantly inhibited, showing a 47 % of inhibition in brain and 41 % in muscle, compared to the control. Moreover, Bretaud et al. (40)

reported a significant inhibition of both brain and muscle AChE activity in the goldfish (*Carassius auratus*) after 48 h of exposure to 500 mg L^{-1} of carbofuran. The same pesticide also caused AChE activity inhibition in tench (*Tinca tinca*) exposed to 100 μg L^{-1} of carbofuran, whereas an exposure to deltamethrin did not affect AChE activity (11). Li et al. (4) evaluated the acute toxicity of the pesticide methomyl on the topmouth gudgeon (*Pseudorasbora parva*). In our study, the pesticide caused a sharp decrease in specific brain AChE activity (around 48 %) with concentrations between 0.043 and 0.213 mg L^{-1}. These results were similar to those obtained by other authors, with *C. auratus* exposed *in vitro* to methomyl for 8 min at 30 °C in which AChE activity was sharply inhibited (44).

To conclude, catalase, GST and AChE showed to be good biomarkers of effect after exposure to methomyl, whereas carps proved suitable for biomonitoring the environments they inhabit. Although limited by only one concentration tested, our study suggests that exposing the common carp to methomyl at sub-lethal levels could provoke changes in enzyme activity, which is in accordance with previously published literature on the subject.

Acknowledgements

REFERENCES

1. United States Environmental Protection Agency (US EPA) Fish Sampling and Analysis: Guidance for Assessing Chemical Contamination Data for Use in Fish Advisories (EPA 823-B-00-008). Washington: US EPA; 2000.
2. Cavalheiro de Menezes C, Leitemperger J, Santi A, Lópes T, Veiverberg CA, Peixoto S, Bohrer Adaime M, Zanella R, Vargas Barbosa NB, Loro VL. The effects of diphenyl diselenide on oxidative stress biomarkers in *Cyprinus carpio* exposed to herbicide quinclorac (Facet®). Ecotoxicol Environ Saf 2012;81:91-7. DOI:10.1016/j.ecoenv.2012.04.022
3. International Program on Chemical Safety (IPCS). Health and safety guide No. 97 (IPCS INCHEM Home) (HSG 97). 1995.
4. Li H, Jiang H, Gao X, Wang X, Qu W, Lin R, Chen J. Acute toxicity of the pesticide methomyl on the topmouth gudgeon (*Pseudorasbora parva*): mortality and effects on four biomarkers. Fish Physiol Biochem 2008;34:209-16. doi: 10.1007/s10695-007-9178-x
5. Tomlin CDS. The Pesticide Manual, 13th ed. Alton Hampshire: British Crop Protection Council; 2003.
6. Chandrasekara LW, Pathiratne A. Body size-related differences in the inhibition of brain acetylcholinesterase activity in juvenile Nile tilapia (*Oreochromis niloticus*) bychlorpyrifos and carbosulfan. Ecotoxicol Environ Saf 2007;67:109-19. doi: 10.1016/j.ecoenv.2006.04.002
7. Crane M, Maltby L. The lethal and sub-lethal responses of *Grammarus pulex* to stress: sensitivity and sources of variation in a situ bioassay. Environ Toxicol Chem 1991;10:1331-9. doi: 10.1002/etc.5620101012
8. Miren P, Cajaraville MJ, Bebianno JB, Cinta P, Carmen S, Aldo V. The use of biomarkers to assess the impact of pollution in coastal environments of the Iberian Peninsula: a practical approach. Sci Total Environ 2000;247:295-311. doi: 10.1016/S0048-9697(99)00499-4
9. Rendón-von Osten J, Ortiz-Arana A, Guilhermino L, Soares AMVM. *In vivo* evaluation of three biomarkers in the mosquitofish (*Gambusia yucatana*) exposed to pesticides. Chemosphere 2005;58:627-36. doi: 10.1016/j.chemosphere.2004.08.065
10. Monteiro DA, Almeida JA, Rantin FT, Kalinin AL. Oxidative stress biomarkers in the fresh water characid fish, *Brycon cephalus*, exposed to organophosphorus insecticide Folisuper 600 (methyl parathion). Comp Biochem Physiol C 2006;143:141-9. PMID: 16546452
11. Hernández-Moreno D, Soler F, Míguez, MP, Pérez-López M Brain acetylcholinesterase, malondialdehyde and reduced glutathione as biomarkers of continuous exposure of tench, *Tinca tinca*, to carbofuran or deltamethrin. Sci Total Environ 2010;408:4976-83. doi: 10.1016/j.scitotenv.2010.07.044
12. Storey KB. Oxidative stress: animal adaptations in nature. Braz J Med Biol Res 1996;29:1715-33. PMID: 9222437
13. Fournier D, Bride JM, Poirie M, Berge JB, Plapp FW. Insect glutathione S-transferases. Biochemical characteristics of the major forms of houseflies susceptible and resistant to insecticides. J Biol Chem 1992;267:1840-5. PMID: 1730722
14. Kamrin MA. Pesticide Profiles: Toxicity, Environmental Impact, and Fate. Boca Raton (FL): CRC/Lewis Publishers; 1997.
15. Organisation for Economic Co-operation and Development (OECD). Test 203: Fish acute toxicity test. In: OECD Guidelines for testing of chemicals. Paris: OECD; 1992.
16. ETS123 European convention for the protection of vertebrate animals used for experimental and other scientific purposes. [displayed 20 May 2014] Available at http://conventions.coe.int/Treaty/Commun/QueVoulezVous.asp?NT=123&CM=8&DF=9/5/2006&CL=ENG
17. DIRECTIVE 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes [displayed 28 August 2014]. Available at http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2010:276:0033:0079:en:PDF
18. Ellman GL, Courtney KD, Andres VJ, Featherstone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol 1961;7:88-95. doi: 10.1016/0006-2952(61)90145-9
19. Clairborne A. Catalase activity. In: Greenwald RA, editor. CRC handbook of methods in oxygen radical research. Boca Raton (FL): CRC Press; 1985. p. 283-4.

20. Habig WH, Pabst MJ, Jakoby WB. Glutathione S-transferase. The first enzymatic step in mercapturic acid formation. J Biol Chem 1974;249:7130-9. PMID: 4436300

21. Bradford M. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 1976;72:248-54. PMID: 942051

22. Ensibi C, Hernández Moreno MD, Soler F, Daly Yahya MN, Míguez-Santiyán MP, Pérez-López M. Effects of subchronic exposure to carbofuran on antioxidant defence system and malondialdehyde levels in common carp (*Cyprinus carpio* L.). Toxicol Environ Chem 2011;94:748-59. doi: 10.1080/02772248.2012.672821

23. Kubrak O, Atamaniuk TM, Husak VV, Drohomyretska IZ, Storey JM, Storey KB, Lushchak VI. Oxidative stress responses in blood and gills of *Carassius auratus* exposed to the mancozeb-containing carbamate fungicide Tattoo. Ecotoxicol Environ Saf 2012;85:37-43. doi: 10.1016/j.ecoenv.2012.08.021

24. Sayeed I, Parvez S, Pandey S, Bin-Hafeez B, Haque R, Raisuddin S. Oxidative stress biomarkers of exposure to deltamethrin in freshwater fish, *Channa punctatus* Bloch. Ecotoxicol Environ Saf 2003;56:295-302. doi: 10.1016/S0147-6513(03)00009-5

25. Aebi HE. Catalase. In: Bergmeyer HU, editor. Methods of enzymatic analysis, vol. 3 K. Weinheim (Germany): Verlag Chemie; 1984. p. 273-86.

26. Chelikani P, Fita I, Loewen PC. Diversity of structures and properties among catalases. Cell Mol Life Sci 2004;61:192-208. doi: 10.1007/s00018-003-3206-5

27. Ferrari A, Venturino A, Pechén de D'Angelo AM. Effects of carbaryl and azinphos methyl on juvenile rainbow trout (*Oncorhynchus mykiss*) detoxifying enzymes. Pestic Biochem Physiol 2007;88:134-42. doi: 10.1016/j.pestbp.2006.10.005

28. Capkin E, Altinok I. Effects of chronic carbosulfan exposure on liver antioxidant enzyme activities in rainbow trout. Environ Toxicol Pharmacol 2013;36:80-7. doi: 10.1016/j.etap.2013.03.022

29. Hai DQ, Vargas SI, Matkovic B. Organophosphate effects on antioxidant system of carp (*Cyprinus carpio*) and catfish (*Ictalurus nebulosus*). Comp Biochem Physiol 1997;117:83-8. doi: 10.1016/S0742-8413(96)00234-4

30. Crestani M, Menezes C, Glusczak L, dos Santos Miron D, Spanevello R, Silveira A, Gonçalves FF, Zanella R, Loro VL. Effect of clomazone herbicide on biochemical and histological aspects of silver catfish (*Rhamdia quelen*) and recovery pattern. Chemosphere 2007;67:2305-11. doi: 10.1016/j.chemosphere.2006.09.070

31. Pandey S, Ahmad I, Parvez S, Bin-Hafeez B, Haque R, Raisuddin S. Effect of endosulfan on antioxidants of freshwater fish *Channa punctatus* Bloch: 1. Protection against lipid peroxidation in liver by copper preexposure. Arch Environ Contam Toxicol 2001;41:345-52. PMID: 11503072

32. Blahová J, Plhalová L, Hostovský M, Divišová L, Dobšíková R, Mikulíková I, Stěpánová S, Svobodová Z. Oxidative stress responses in zebrafish *Danio rerio* after subchronic exposure to atrazine. Food Chem Toxicol 2013;61:82-5. doi: 10.1016/j.fct.2013.02.041

33. Nwani CHD, Lakra WS, Nagpure NS, Kumar R, Kushwaha, B, Srivastava SK. Toxicity of the herbicide atrazine: effects on lipid peroxidation and activities of antioxidant enzymes in the freshwater fish *Channa punctatus* (Bloch). Int J Environ Res Publ Health 2010;7:3298-312. doi: 10.3390/ijerph7083298

34. Stara A, Machova J, Velisek J. Effect of chronic exposure to simazine on oxidative stress and antioxidant response in common carp (*Cyprinus carpio* L.). Environ Toxicol Pharmacol 2012;33:334-43. doi: 10.1016/j.etap.2011.12.019

35. Ural MŞ. Chlorpyrifos-induced changes in oxidant/antioxidant status and haematological parameters of *Cyprinus carpio carpio*: ameliorative effect of lycopene. Chemosphere 2013;90:2059-64. doi: 10.1016/j.chemosphere.2012.12.006

36. Botté ES, Jerry DR, King SC, Keune CS, Negri AP. Effects of chlorpyrifos on cholinesterase activity and stress markers in the tropical reef fish *Acanthochromis polyacanthus*. Mar Pollut 2012;65:384-93. doi: 10.1016/j.marpolbul.2011.08.020

37. Davies PE. The toxicology and metabolism of chlorothalonil in fish. Metabolism, enzymatic and detoxication in Salmo spp. and Galaxias spp. Aquat Toxicol 1985; 7:277-99. doi: 10.1016/0166-445X(85)90045-1

38. Pimpão CT, Zampronio AR, deAssis S. Effects of deltamethrin on hematological parameters and enzymatic activity in *Ancistrus multispinis* (Pisces, Teleostei). Pestic Biochem Phys 2007;88:122-7. doi: 10.1016/j.pestbp.2006.10.002

39. Clasen B, Leitemperger J, Murussi C, Pretto A, Menezes Ch, Dalabona F, Marchezan E, Adaime MB, Zanella R, Loro VL. Carbofuran promotes biochemical changes in carp exposed to rice field and laboratory conditions. Ecotoxicol Environ Saf 2014;101:77-82. doi: 10.1016/j.ecoenv.2013.12.012

40. Bretaud S, Toutant JP, Saglio P. Effects of carbofuran, diuron, and nicosulfuron on acetylcholinesterase activity in goldfish (*Carassius auratus*). Ecotoxicol Environ Saf 2000;47:117-24. doi: 10.1006/eesa.2000.1954

41. Blaber LC, Creasey NH. The mode of recovery of cholinesterase activity *in vivo* after organophosphorus poisoning. Biochem J 1960;77:597-604. PMC1205081.

42. Silva Filho MV, Oliveira MM, Salles JB, Cunha Bastos VLF, Cassano VPF, Cunha Bastos J. Methyl-paraoxon comparative inhibition kinetics for acetylcholinesterases from brain of neotropical fishes. Toxicol Lett 2004;153:247-54. doi: 10.1016/j.toxlet.2004.04.026

43. Hernández-Moreno D, Pérez-López M, Soler F, Gravato C, Guilhermino L. Effects of carbofuran on the sea bass (*Dicentrarchus labrax* L.): study of biomarkers and behaviour alterations. Ecotoxicol Environ Saf 2011;74:1905-12. doi: 10.1016/j.ecoenv.2011.07.016

44. Yi MQ, Liu HX, Shi XY, Liang P, Gao XW. Inhibitory effects of four carbamate insecticides on acetylcholinesterase of male and female *Carassius auratus in vitro*. Comp Biochem Physiol C Toxicol Pharmacol 2006;143:113-6. doi: 10.1016/j.cbpc.2005.12.008

Sažetak

Razlike u enzimskim aktivnostima u tkivima šarana (*Cyprinus carpio* L.) kao mogući biološki pokazatelji izloženosti pesticidu metomilu

Istražen je utjecaj pesticida metomila na razine enzimskih aktivnosti u tkivima šarana izloženih subletalnoj koncentraciji od 0,34 mg L^{-1} tijekom 15 dana. Nakon 4 i 15 dana u jetrima i škrgama pokusnih životinja izmjerene su aktivnosti katalaze i glutation S-transferaze (GST), a aktivnost acetilkolinesteraze (AChE) u mozgu i mišićima. Aktivnost katalaze u jetrima blago se smanjila u izloženih riba u usporedbi s kontrolnom skupinom, no to je smanjenje bilo statistički značajno tek na početku pokusa. Istovremeno nisu zamijećene značajne promjene u aktivnosti katalaze u škrgama izloženih i kontrolnih riba (srednje vrijednosti bile su u rasponu 10,7-11,7 nmol min^{-1} po miligramu proteina. Dok je aktivnost enzima GST u jetrima izloženih riba bila tek blago inhibirana na početku pokusa, u škrgama je utvrđena značajna inhibicija. Aktivnost acetilkolinesteraze u mozgu s vremenom se smanjivala i pokazala značajan pad nakon 96 sati izloženosti (0,041 *vs.* 0,075 nmol min^{-1} po miligramu proteina; $p<0.001$). Rezultati upućuju na zaključak da su katalaza, GST i AChE pouzdani biološki pokazatelji učinka izloženosti metomilu.

KLJUČNE RIJEČI: *acetilkolinesteraza; karbamat; katalaza*; *ribe; glutation S-transferaza*

CORRESPONDING AUTHOR:

Marcos Pérez-López
Toxicology Unit, Veterinary School of Caceres
Avda de la Universidad s/n.
10003 Caceres, Spain
E-mail: *marcospl@unex.es*