Journal of
Environmental & Analytical Toxicology

Ensibi, et al., J Environ Anal Toxicol 2015, 5:3
DOI: 10.4172/2161-0525.1000272

Research Article Open Access

Effects of Semi-Static Exposure to Carbofuran in Liver Phase I and Phase II Enzymes of Common Carp (*Cyprinus carpio*)

Ensibi C[1]*, Pérez-López M[2], Soler Rodríguez F[2], Míguez-Santiyán MP[2], Daly Yahya MN[1] and Hernández-Moreno D[2]

[1]*Biodiversity and functioning of aquatics systems, Faculty of Sciences of Bizerte, University of Carthage, Zarzouna, Tunisia*
[2]*Toxicology Unit, Faculty of Veterinary Medicine (UEX), Caceres, Spain*

Abstract

The effect of semi-static exposition of common carp (Cyprinus carpio L) to different concentrations (0, 10, 50 and 100 µg/L) of the pesticide carbofuran has been evaluated. The following biological responses were measured: (1) ethoxyresorufin-O-deethylase (EROD) and benzyloxy-4-[trifluoromethyl]-coumarin-O-debenzyloxylase (BFCOD) activities, as a phase I biotransformation parameters; and (2) uridine diphospho-glucuronosyltransferase (UDPGT) activity as phase II conjugation enzyme. A clear inhibition of both EROD and BFCOD activities was observed in fish exposed to carbofuran with respect to controls, whereas in the case of UDPGT activity, values of exposed animals showed a clear increase during the experiment with respect to controls.

Keywords: Cyprinus carpio L; Carbofuran; Liver; Phase I Metabolism

Introduction

The aquatic environment is continuously being contaminated with chemicals from agriculture activities. Hundreds of pesticides of different chemical structures are extensively used to control a wide variety of agricultural pests and can contaminate aquatic habitats due to leaching and runoff water from treated areas. The pesticides may produce an immense disruption of the ecological balance causing damage to non-target organisms including fish of commercial importance [1]. Pesticides are found to damage vital organs of fish [2], skeletal system and they cause biochemical alteration in the exposed fishes [3-5].

Carbofuran (2,3-dihydro-2,2-dimethylbenzofuran- 7-yl methyl-carbamate) is a broad-spectrum systemic insecticide, nematicide, and acaricide used throughout the world. As a result of its widespread use, carbofuran has been detected in ground, surface and rain waters, soils, air, foods and wildlife [6,7]. Carbofuran is classified as very toxic to bees, fish, and other aquatic animals, and dangerous to wild birds [8].

Several biochemical and physiological responses occur when a toxicant enters into an organism, being able to involve an acclimation of the organism or lead to toxicity [9].

The majority of detoxification occurs in the liver via a multitude of enzyme systems. Phase I, usually reactions of oxidation, reduction, or hydrolysis, and phase II conjugation reactions biochemically transform toxicants into more water-soluble, and therefore excretable, substances [10]. Phase I enzymes include primarily cytochrome P450 (CYP450), which is a superfamily of heme-containing proteins involved in the biotransformation of many endogenous compounds and exogenous substrates. The proteins belonging to the first three families of CYP450: CYP1, CYP2, and CYP3 are closely associated with drug activation and metabolism [11]. Currently, CYP1A is the most studied xenobiotic-metabolizing isoform in fish and is often used as a biomarker for early effects of pollutants in aquatic organisms [12]. The CYP3A enzymes represent the family responsible for metabolism of many drugs [13]. In addition to enzymes that participate in the phase I of metabolism of xenobiotics, most fish possess a second group of biotransformation enzymes referred to as conjugation or phase II enzymes, which are also induced by exposure to different type of pollutants [12]. UDPGT (uridine diphospho-glucuronosyl transferase) is involved in the conjugation of the xenobiotic parent compound or its metabolites with an endogenous substrate [14] thus facilitating the excretion of chemicals by the addition of more polar groups to the molecule [5]. Phase II enzyme's answer to environmental xenobiotics is less pronounced than that of phase I [15]. However, even minor alterations in phase II activity, such as changes in phase II co-substrate levels in response to exposure to xenobiotics, can be harmful to aquatic organisms [16]. As previously indicated for phase I-biotransformation enzymes, different studies have demonstrated that the expression of phase II enzymes can be modulated in vivo after exposure to a broad variety of xenobiotic compounds [17,18].

Fish have proven to be useful experimental models for the evaluation of the health of aquatic ecosystems exposed to environmental pollution, because it responds with great sensitivity to changes in the aquatic environment [19]. The common carp, Cyprinus carpio, is probably one of the few aquaculture species assumed to be domesticated. Common carp is widespread and presently cultured all over Asia, in most parts of Europe, and in a small scale in some countries of Africa and Latin America. It has also been introduced in North America and Australia. Though considered to be a sport fish as well, its main importance is as a food fish [20].

This study was undertaken to evaluate the effect of carbofuran subchronic exposure in fish on two CYP450-mediated reactions: ethoxyresorufin O-deethylation (EROD, CYP1A), 7-benzyloxy-4-trifluoromethylcoumarin O-debenzylation (BFCOD, CYP3A), and UDPGT.

***Corresponding author:** Ensibi C, Biodiversity and functioning of aquatics systems, Faculty of Sciences of Bizerte, University Carthage, Zarzouna, Tunisia, Tel: 72 590 175; E-mail: ensibi.cherif@fsb.rnu.tn

Received January 05, 2014; **Accepted** January 29, 2015; **Published** February 02, 2015

Citation: Ensibi C, Pérez-López M, Soler Rodríguez F, Míguez-Santiyán MP, Daly Yahya MN, et al. (2015) Effects of Semi-Static Exposure to Carbofuran in Liver Phase I and Phase II Enzymes of Common Carp (*Cyprinus carpio*). J Environ Anal Toxicol 5: 272. doi:10.4172/2161-0525.1000272

Materials and Methods

Chemicals

All the chemicals and reagents used were of analytical grade from Sigma–Aldrich (Germany). Technical carbofuran (95% product) was provided by Faesal-Spain. Stock solution was prepared by dissolving pesticide in absolute ethanol in a standard volumetric flask.

Experimental design

Fish: Fresh water juvenile common carp (11–13 cm in length and with an average weight of 63.5 ± 6.8 g) were obtained from the local fish hatchery "Piscifactoría Vegas del Guadiana" in Badajoz (Spain), belonging to the Regional Government (Junta de Extremadura). They were acclimated to laboratory conditions for 12 days prior to the experiment. The fish were kept in batches of 28 individuals and were fed once daily ad libitum with a commercial dry diet for fish (OVN Dibaq-Diproteg) until the end of the experiment. Fish were starved for 24 h before sacrifice.

Water Exposure Experiments: Fish were divided into 4 groups with the first group serving as control and the others as experimental groups. The experimental groups were exposed to different sub-lethal concentration of pesticide : 10, 50 and 100 µg/L corresponding to 2, 10 and 20% of the 96 h LC50 value for commercial carbofuran in a test with C. carpio [21] for 0, 4, 15, and 30 days. The amount of solvent in the aquaria was negligible.

Water in each aquarium was weekly gently siphoned out and replaced by freshly water with the same concentrations of pesticide. Previously to water removing, water samples (250 mL) were taken from each tank for carbofuran analysis in order to assess the effective concentrations of the pesticide by using an HPLC technique with acetonitrile and water as mobile phase and a Nucleosyl 120 C18 column [22]. Water quality during the experiment was evaluated according to the American Public Health Association [23]. Temperature, pH, alkalinity, conductivity and hardness of aquatic media averaged 19–23°C, 7.5 ± 0.4 (Crison pHmeter), 3.2 ± 0.8 mmol/L, 0.45–0.93 mOhm and 178 ± 35 mg/L (as $CaCO_3$), respectively, and dissolved oxygen content was never situated below 5 mg/L.

Exposed and control animals (n=7 from each aquarium) were removed at the beginning of the experiment and at 4, 15 and 30 days of exposure. Fish were anesthetized with a solution of MS222 (Sigma) at a concentration of 0.1 g/L [24]. Animals were killed by decapitation, measured, weighed and sexed. Their liver were immediately dissected, weighed and individually frozen at -80°C until biochemical assays. All procedures were performed in accordance with the OECD guidelines for testing chemicals (OECD/Test guideline no 203, 1992).

Analytical methods

Prior to biochemical determinations, samples were prepared as follows: livers were homogenizedin a 0.1 M buffer phosphate pH 7.4 (KH2PO4/K2HPO4, Panreac).The homogenate was centrifuged at 825xg for 5 min at 4ºC, and the supernatant was centrifuged at 13200xg for 20 min at 4ºC in order to obtain the supernatant corresponding to the post-mitochondrial fraction. Levels of protein, EROD, BFCOD, and UDPGT enzymatic activities were measured in this post-mitochondrial fraction.

Hepatic CYP1A-mediated EROD activity was determined at 30˚C as described by Burke and Mayer [25] using 7-ethoxyresorufin as substrate. The reaction was stopped by adding 400 µL of acetonitrile and after centrifugation at low speed [12]. The 7-hydroxyresorufin fluorescence was determined using a Gemini XPS SpectraMax Plus microplate reader (Molecular Devices Corporation) at 537/583 nm excitation/emission wavelengths [26]. EROD blanks included microsomal samples plus acetonitrile to simulate the baseline noise.

Hepatic CYP3A-mediated BFCOD activity was analyzed according to the procedure described by [12] were 7-benzyloxy-4-trifluoromethyl-coumarin (BFC) is used as substrate. The fluorescence was read in a 96-multiwell plate at the excitation/emission wavelength pairs of 409 and 530 nm. Quantification was made using a calibration curve of the 7-hydroxy-4-trifluofluoromethyl-coumarin authentic standard, and several blanks containing the tested substance were done to remove possible fluorescence emitted by the pesticide.

UDPGT activity was measured according to the method of Clarke et al. [27] with p-nitrophenol (pNP) as substrate. The pNP was measured spectrophotometrically at 405 nm.

Protein content was measured in the post-mitochondrial fraction by the method of Bradford using bovine serum albumin (Sigma-Aldrich) as standard [28]. In all cases, reproducibility was based on the relative standard deviation of triplicate samples.

Statistical analysis

Data were analyzed with one-way analysis of variance (ANOVA) using SPSS for Windows (version 13, SPSS Inc., Chicago, IL). In cases of significant differences, the ANOVA was followed by the LSD (Least Significant Difference) post-hoc test. Significance limit was set at $p< 0.05$.Data was expressed as mean ± S.D.

Results

Figures 1-3 show the EROD, BFCOD and UDPGT activities, respectively in carp's liver during the whole experiment. For graphical representation, those enzymatic activities were converted in percentage of inhibition/induction with respect to enzymatic levels determined in control fish, which were assigned at 100%.

Liver EROD activity was significantly decreased after 4 and 15 days of exposure to all the carbofuran concentrations (10, 50 and 100µg/L)

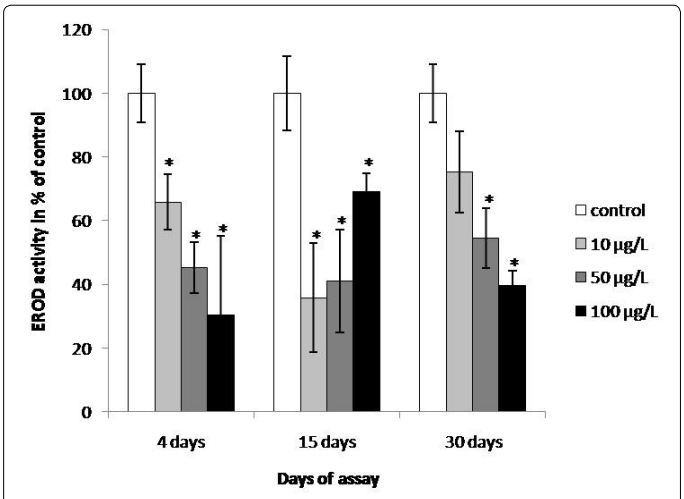

Figure 1: Evolution of liver EROD activity in juvenile common carp exposed to different concentrations of carbofuran for 4, 15, and 30 days. Data represent the mean ± SE (n=7). Note: *indicates significant difference between control and pesticide group (p<0,05).

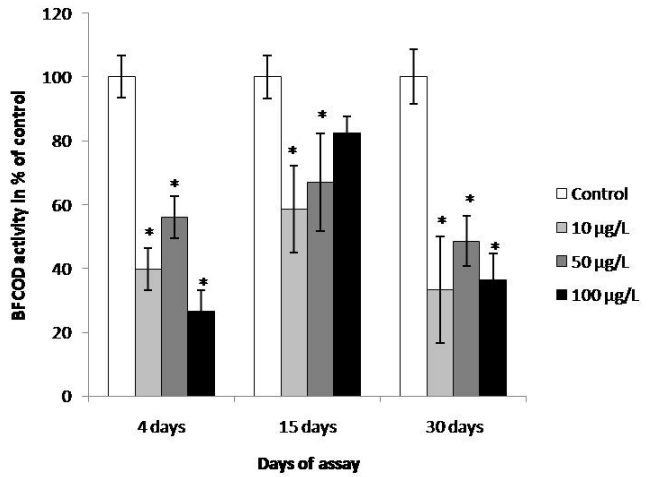

Figure 2: Evolution of liver BFCOD activity in juvenile common carp exposed to different concentrations of carbofuran for 4, 15, and 30 days. Data represent the mean ± SE (n=7). Note: *indicates significant difference between control and pesticide group (p<0,05).

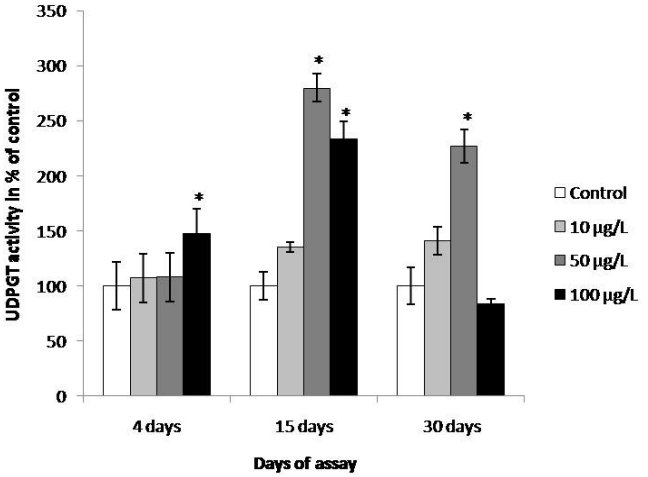

Figure 3: Evolution of liver UDPGT activity in juvenile common carp exposed to different concentrations of carbofuran for 4, 15, and 30 days. Data represent the mean ± SE (n=7). Note: *indicates significant difference between control a

compared to controls. However, this significant decrease was only recorded after 30 days of exposure to 50 and 100 µg/L (Figure 1).

The evolution of BFCOD activity during exposition to carbofuran showed the existence of an inhibitory effect. Almost all exposed groups presented a significant inhibitory effect on BFCOD activity (p-value <0.05), except for those animals exposed to 100 µg/L during 15 days (Figure 2).

Effects of carbofuran exposure on the UDPGT activity in liver tissues of common carp are presented in Figure 3. Low induction of UDPGT activity was recorded after 4 days of treatment and this induction was only significant in the exposure to a concentration of 100 µg/L. This induction of the activity is clearly observed after 15 days of treatment, being statistically significant for the concentrations of 50 and 100 µg/L. After 30 days of treatment, the induction of UDPGT activity remained in the fish exposed to 10 and 50 µg/L, being significant only for the second concentration.

Discussion

The liver is primarily responsible for the metabolism of toxic substances, including carbofuran. In particular the liver is the major site of CYP expression in teleost fish. Therefore, the knowledge of the effect of carbofuran on liver-detoxifying enzymes is very important. In the present study, we investigated the changes in EROD, BFCOD and UDPGT activities in the liver of common carp exposed to different concentrations of carbofuran.

In the present study, exposure to carbofuran caused decrease in EROD activity when compared to controls. The decrease of CYP1A catalytic activity might be due to the inhibition of its mRNA expression by carbofuran [29]. Many xenobiotic compounds, such as heavy metals at high concentrations, have shown effects of inhibition and/or decrease of liver microsomal cytochromes P4501A (CYP1A) activity in fish [30]. Inhibition of CYP1A activity by carbofuran is quite well investigated. The inhibition was explained by competitive inhibition at the substrate-binding site [31]. Liver EROD activity decrease may be due to increased reactive metabolites of carbofuran in the cytosol. It must also be considered that CYP1A catalytic activity varies between different xenobiotics, and other variables such as species, sex, reproductive stage, compound, route of exposure, time of exposure, mixed exposure and/or dose may influence the observed effects [32]. Exposure of salmonid species to pesticides, for example the organophosphate chlorpyrifos, produced a CYP1A protein level suppression of 30% with respect to control fish [33]. Nevertheless, in general all studies related to CYP1A enzymatic activity in fish exposed to xenobiotics have shown induction-processes, for example towards polybrominated diphenyl ethers (PBDEs) in livers of Carassius auratus [34], the fungicides propiconazole and ketoconazole in brown trout and Atlantic cod [35], β-naphthoflavone, dioxins and benzo[a]pyrene in rainbow trout and carps [36,37] or paraquat in Nile tilapia [38].

BFCOD activity is used as a marker of CYP3A in humans [39] and pigs [40]. In fish, BFCOD is believed to be the specific substrate for CYP3A [41]. Our study showed that BFCOD activity was inhibited by carbofuran (Figure 2). This is in agreement with the results from in vitro inhibition studies using liver microsomes from Atlantic cod, where inhibition of BFCOD activity by ketoconazole was observed [35]. Also, Burkina et al. [42] reported inhibition in BFCOD activity in hepatic microsomes of rainbow trout after exposure to clotrimazole in a concentration range of 1-100 µM. Similarly, exposure to ketoconazole resulted in an inhibition of BFCOD activity in the Atlantic salmon [43]. In contrast, dioxins and polycyclic aromatic hydrocarbons strongly induced BFCOD activity (up to 30-fold) in a time- and concentration-dependent manner, both in vitro in all cell lines and in vivo in zebrafish embryos [44]. In a previous study fish exposure to carbofuran demonstrated no response of BFCOD [12]. The conflicting results from exposure between fish species and xenobiotics [12,36] suggest the need for further studies.

UDPGT is one of the key enzymes indicative of exogenous substances present in the liver microsomes. The main function of UDPGT is to catalyze exogenous substances and metabolites using glucuronic acid or glutathione binding reactions, accelerating their excretion for detoxification [45]. Our results showed that UDPGT activity in liver of fish have been shown to be induced by carbofuran exposure which is in accordance with other authors [46]. UDPGT has been found to be induced by 3-methylcholanthrene in immune organs of carp (Cyprinus carpio) [47]. However, a carbofuran dose of 100 µl/L resulted in a marked inhibition of UDPGT activity in carp [12]. Changes in the expression of UDPGT activity due to environmental or

physiological factors have been partially studied in fish. Parameters such as water temperature, salinity level, feed availability and composition, have been analyzed to determine their possible implications on UDPGT activity in fish [15].

Conclusion

In the present study, the activities of three enzymes, EROD, BFCOD and UDPGT, were investigated in liver of Cyprinus carpio under varying concentrations of carbofuran and time of exposure. This study shows that the considered biomarkers might be good biomonitoring tools for assessment of the hazardous effects of pesticides on fish. EROD and BFCOD inhibition in livers appears as an early process in response to sublethal concentrations of carbofuran exposure. With regards to UDPGT activity, significant effects were observed later in animals exposed to carbofuran. The experimental data obtained with carps, under controlled laboratory conditions can be considered as a useful reference for comparisons with biomarkers response of organisms living in polluted environments.

Acknowledgments

The authors express special thanks to Extremadura Regional Government (Consejeria de Industria, Energia y Medio Ambiente, Junta de Extremadura, Spain) by supplying the fish for the experiment. Special thanks to the Tunisian Ministry of Scientific Research and Technology who allowed this study to be carried out. This research was supported by the Spanish Government within the framework of the project "PCI-Programa de Cooperación Interuniversitaria Tunez-España" (references A/015933/08 and A/024757/09).

References

1. Bretaud S, Toutant JP, Saglio P (2000) Effects of carbofuran, diuron, and nicosulfuron on acetylcholinesterase activity in goldfish (Carassius auratus). Ecotoxicol Environ Saf 47: 117-124.
2. Velmurugan B, Selvanayagam M, Cengiz EI, Unlu E (2007) The effects of monocrotophos to different tissues of freshwater fish Cirrhinus mrigala. Bull Environ Contam Toxicol 78: 450-454.
3. Havelková M, Randák T, Blahová J, Slatinská I, Svobodová Z (2008) Biochemical markers for the assessment of aquatic environment contamination. Interdiscip Toxicol 1: 169-181.
4. Schreiber EA, Otter RR, Van den Hurk P (2006) A biomarker approach to measure biological effects of contaminant exposure in largemouth bass from Lake Conestee, South Carolina, U.S.A. Environ Toxicol Chem 25: 1926-1932.
5. van der Oost R, Beyer J, Vermeulen NP (2003) Fish bioaccumulation and biomarkers in environmental risk assessment: a review. Environ Toxicol Pharmacol 13: 57-149.
6. Arráez-Román D, Segura-Carretero A, Cruces-Blanco C, Fernández-Gutiérrez A (2004) Determination of aldicarb, carbofuran and some of their main metabolites in groundwater by application of micellar electrokinetic capillary chromatography with diode-array detection and solid-phase extraction. Pest Manag Sci 60: 675-679.
7. Caldas SS, Demoliner A, Costa FP, D'Oca MGM, Primel EG (2010) Pesticide Residue Determination in Groundwater using Solid-Phase Extraction and High-Performance Liquid Chromatography with Diode Array Detector and Liquid Chromatography-Tandem Mass Spectrometry. J Braz Chem Soc 21: 642-650.
8. Dobsikova R (2003) Acute toxicity of carbofuran to selected species of aquatic and terrestrial organisms. Plant Protect Sci 39: 103-108.
9. Begum G (2004) Carbofuran insecticide induced biochemical alterations in liver and muscle tissues of the fish Clarias batrachus (linn) and recovery response. Aquat Toxicol I66: 83-92.
10. Liska D, Lyon M, Jones DS (2006) Detoxification and biotransformational imbalances. Explore (NY) 2: 122-140.
11. Uno T, Ishizuka M, Itakura T (2012) Cytochrome P450 (CYP) in fish. Environ Toxicol Pharmacol 34: 1-13.
12. Hernández-Moreno D, Soler-Rodríguez F, Míguez-Santiyán MP, Pérez-López M (2008) Hepatic monooxygenase (CYP1A and CYP3A) and UDPGT enzymatic activities as biomarkers for long-term carbofuran exposure in tench (Tinca tinca L). J Environ Sci Health B 43: 395-404.
13. Yan J, Cai Z (2010) Molecular evolution and functional divergence of the cytochrome P450 3 (CYP3) Family in Actinopterygii (ray-finned fish). PLoS One 5: e14276.
14. Berdikova Bohne VJ, Hamre K, Arukwe A (2007) Hepatic metabolism, phase I and II biotransformation enzymes in Atlantic salmon (Salmo Salar, L) during a 12 week feeding period with graded levels of the synthetic antioxidant, ethoxyquin. Food Chem Toxicol 45: 733-746.
15. George SG (1994) Enzymology and molecular biology of phase II xenobiotic-conjugating enzymes in fish. In Aquatic Toxicology; Molecular, Biochemical and Cellular Perspectives. Malins DC, Ostrander GK, (Eds) Lewis Publishers: Boca Raton, FL 37-85.
16. Van der Oost R, Goksøyr A, Celander M, Heida H, Vermeulen NPE (1996) Biomonitoring of aquatic pollution with feral eel (Anguilla anguilla) II. Biomarkers: pollution-induced biochemical responses. Aquat Toxicol 36: 189-222.
17. Vaccaro E, Meucci V, Intorre L, Soldani G, Di Bello D, et al. (2005) Effects of 17-estradiol, 4-nonylphenol and PCB 126 on the estrogenic activity and phase 1 and 2 biotransformation enzymes in male sea bass (Dicentrarchus labrax). Aquat Toxicol 75: 293-305.
18. Buet A, Banas D, Vollaire Y, Coulet E, Roche H (2006) Biomarker responses in European eel (Anguilla anguilla) exposed to persistent organic pollutants. A field study in the Vaccarès lagoon (Camargue, France). Chemosphere 65: 1846-1858.
19. Xing H, Li S, Wang Z, Gao X, Xu S, et al. (2012) Oxidative stress response and histopathological changes due to atrazine and chlorpyrifos exposure in common carp. Pest Biochem Physiol 103: 74-80.
20. Chistiakov D, Voronova N (2009) Genetic evolution and diversity of common carp Cyprinus carpio L. Cent Eur J Biol 4: 304-312.
21. Tomlin CDS (1997) The pesticide manual (11th Ed) BCPC (British crop Protection Council) UK.
22. Hernández-Moreno D, Soler Rodrguez F, M guez Santiyan MP, Perez-Lopez M, (2010) Brain acetylcholinesterase, malondialdehyde and reduced glutathione as biomarkers of continuous exposure of tench, Tinca tinca, to carbofuran ordeltamethrin. Sci Total Environ 408: 4976-4983.
23. APHA (1985) Standard Methods for the Examination of Water and Wastewater. American Public Health Association (18th Edition) Washington, DC.
24. Oropesa AL, García-Cambero JP, Soler F (2009) Glutathione and malondialdehyde levels in common carp after exposure to simazine. Environ Toxicol Pharmacol 27: 30-38.
25. Burke MD, Mayer RT (1974) Ethoxyresorufin: Direct fluorimetric assay of a microsomal-O-deethylation which is preferentially inducible by 3-methylcholantrene. Drug Metab Dispos 2: 583-588.
26. Solé M, Porte C, Barceló D (2000) Vitellogenin induction and other biochemical responses in carp, Cyprinus carpio, after experimental injection with 17 alpha-ethynylestradiol. Arch Environ Contam Toxicol 38: 494-500.
27. Clarke DJ, George SG, Burchell B (1992) Multiplicity of UDP-glucuronosyltransferases in fish. Purification and characterization of a phenol UDP-glucuronosyltransferase from the liver of a marine teleost, Pleuronectes platessa. Biochem J 284 : 417-423.
28. Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72: 248-254.
29. Ghosh MC, Ray AK (2012) Regulation of cytochrome P4501A by protein kinase C: the role of heat shock protein70. J Cell Commun Signal 6: 37-44.
30. Bozcaarmutlu A, Arinç E (2004) Inhibitory effects of divalent metal ions on liver microsomal 7-ethoxyresorufin O-deethylase (EROD) activity of leaping mullet. Mar Environ Res 58: 521-524.
31. Kharasch ED, Herrmann S, Labroo R (1992) Ketamine as a probe for medetomidine stereoisomer inhibition of human liver microsomal drug metabolism. Anesthesiology 77: 1208-1214.
32. Hasselberg L, Meier S, Svardal A, Hegelund T, Celander MC (2004) Effects of alkylphenols on CYP1A and CYP3A expression in first spawning Atlantic cod (Gadus morhua). Aquat Toxicol 67: 303-313.
33. Wheelock CE, Eder KJ, Werner I, Huang H, Jones PD, et al. (2005) Individual variability in esterase activity and CYP1A levels in Chinook salmon (Oncorhynchus tshawytscha) exposed to esfenvalerate and chlorpyrifos. Aquat Toxicol 74: 172-192.

34. Lu GH, Qi PD, Chen W (2013) Integrated biomarker responses of Carassius auratus exposed to BDE-47, BDE-99 and their mixtures. Int J Environ Res 7: 807-816.

35. Hasselberg L, Grøsvik BE, Goksøyr A, Celander MC (2005) Interactions between xenoestrogens and ketoconazole on hepatic CYP1A and CYP3A, in juvenile Atlantic cod (Gadus morhua). Comp Hepatol 4: 2.

36. Celander M, Förlin L (1991) Catalytic activity and immunochemical quantitation of hepatic cytochrome P-450 in β-naphthoflavone and isosafrole treated rainbow trout (Oncorhynchus mykiss). Fish Physiol Biochem 9: 189-197.

37. Van der Weiden MEJ, Bleumink R, Seinen W, van den Berg M (1994) Concurrence of P4501A induction and toxic effects in the mirror carp (Cyprinus carpio) after administration of a low dose of 2,3,7,8- tetrachorodibenzo-dioxin (TCDD). Aquat Toxicol 29: 147-162.

38. Figueiredo-Fernandes A, Fontaínhas-Fernandes A, Rocha E, Reis-Henriques MA (2006) The effect of paraquat on hepatic EROD activity, liver, and gonadal histology in males and females of Nile tilapia, Oreochromis niloticus, exposed at different temperatures. Arch Environ Contam Toxicol 51: 626-632.

39. Appiah-Opong R, Commandeur JN, van Vugt-Lussenburg B, Vermeulen NP (2007) Inhibition of human recombinant cytochrome P450s by curcumin and curcumin decomposition products. Toxicology 235: 83-91.

40. Nannelli A, Chirulli V, Longo V, Gervasi PG (2008) Expression and induction by rifampicin of CAR- and PXR-regulated CYP2B and CYP3A in liver, kidney and airways of pig. Toxicology 252: 105-112.

41. Christen V, Oggier DM, Fent K (2009) A microtiter-plate-based cytochrome P450 3A activity assay in fish cell lines. Environ Toxicol Chem 28: 2632-2638.

42. Burkina V, Zlabek V, Zamaratskaia G (2013) Clotrimazole, but not dexamethasone, is a potent in vitro inhibitor of cytochrome P450 isoforms CYP1A and CYP3A in rainbow trout. Chemosphere 92: 1099-1104.

43. Zlabek V, Zamaratskaia G (2012) Comparison of three fluorescent CYP3A substrates in two vertebrate models: pig and Atlantic salmon. Animal 6: 633-640.

44. Creusot N, Brion F, Piccini B, Budzinski H, Porcher JM, et al. (2014) BFCOD activity in fish cell lines and zebrafish embryos and its modulation by chemical ligands of human aryl hydrocarbon and nuclear receptors. Environ Sci Pollut Res Int .

45. Yang S, Xu F, Zheng B, Wu F, Wang S (2013) Multibiomarker responses upon exposure to tetrabromobisphenol A in the freshwater fish Carassius auratus. Aquat Toxicol 142-143: 248-56.

46. Solé M, Fortuny A, Mañanós E (2014) Effects of selected xenobiotics on hepatic and plasmatic biomarkers in juveniles of Solea senegalensis. Environ Res 135: 227-235.

47. Taysse L, Chambras C, Marionnet D, Bosgiraud C, Deschaux P (1998) Basal level and induction of cytochrome P450, EROD, UDPGT, and GST activities in carp (Cyprinus carpio) immune organs (spleen and head kidney). Bull Environ Contam Toxicol 60: 300-305.