MINISTRY OF RESEARCH, INNOVATION AND DIGITIZATION

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Effects of Chronic Exposure of Polyethylene on Common Carp (*Cyprinus Carpio*) and the Impact on Enzymatic Biomarkers

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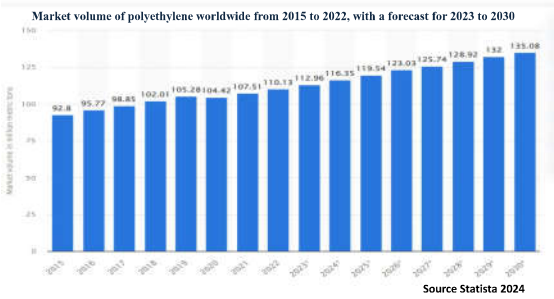
Introduction

Global plastic consumption has led to an increased and persistent microplastic pollution in aquatic environments. Due to their physical properties, plastic particles are omnipresent and are affecting aquatic biota. Polyethylene (PE) is a synthetic polymer and one of the most widely used plastics. Its presence in the environment endangers the health of aquatic organisms.



The aim of the study was to evaluate the effects of long-term exposure to polyethylene (PE) on the development, oxidative stress indicators, neurotoxicity, and reproduction in carp (*Cyprinus carpio*).

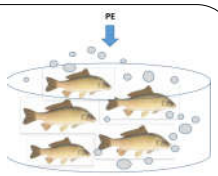
Materials and methods

***Chronic toxicity* - OECD 305 -** *Cyprinus carpio* - ≥ 2 year age, 10 individuals per each test or control

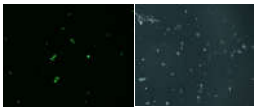
Objective of test: lethal effects / behavior, biometric indicators, physiological indices.

Test conditions:

- PE in two different particle sizes (40-48 µm and 125 µm, 0.6 mg/L for each type)
- density of the particles in the test:
  - 200 particles PE 40-48 µm/L,
  - 50 particles PE 125 µm/L
- Semi - static system
- Exposure time 75 days
- Daily feeding (1% of the lot's weight)
- Temperature 21.1-22.3°C
- Oxygen 6.53-7.55 mg/L
- pH 7.42 – 7.74
- Light / darkness 12-16 h/day
- Control with dilution water
- Test solution volume – 80 liters
- No mortality or behavioral changes were recorded at the end of the test.



Laboratory testing system



Microscopic identification of PE 40-48 µm and 125 µm in test solutions

Methods of enzymatic biomarkers

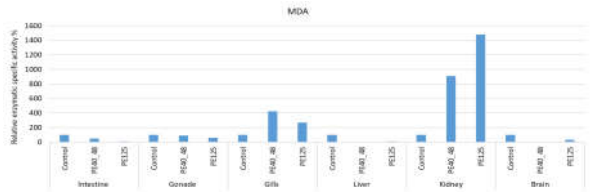
Biomarkers	Method	Principle	Test conditions
Protein concentration	The Lowry method	The formation of a copper complex when the protein reacts with an alkaline copper reagent (biuret reaction) and on the reduction of phosphomolybdates and phosphotungstates from the Folin-Ciocalteu reagent by the phenolic compounds in the protein.	Incubate for 10 min at 50 °C Sample volume 200 µl Absorbance reading 660 nm Standard curve of bovine serum albumin 10 mg %, range (0-60 µg/ml) $y = 0.0014192x + 0.0224$, $r^2 = 0.9997$
Catalase (CAT)	The Aebi Method (1971)	The dosing method is based on the property of catalase to catalyze the decomposition of H_2O_2 from the reaction medium in which we find: total protein extract, H_2O_2 0.059M and buffer K_2HPO_4/KH_2PO_4 0.1M, (pH = 7.1).	Working temperature 25 °C Sample volume 30 µl Absorbance reading 240 nm
Glutathione S-transferase (GST)	Sigma Aldrich kit (MAK153)	GST activity is measured in relation to the conjugation of 1-chloro-2,4-dinitrobenzene (CDNB) with reduced Glutathione.	Sample volume 20 µl Absorbance reading 340 nm (kinetic mode for 10 min)
Glutathione reductase (GRed)	Goldberg and Spooner method (1983)	The enzyme catalyzes the reduction reaction of oxidized glutathione 33 mM, with the participation of NADPH 2 Mm in buffer solution K_2HPO_4 / KH_2PO_4 0.1 M (pH = 7.8)	Working temperature 25 °C Sample volume 25 µl Absorbance reading 340 nm
Lipid peroxidation (MDA)	Sigma Aldrich MAK 085 kit	The test is based on the reaction of MDA with thiobarbituric acid (TBA) which forms a colorimetric product (532 nm), proportional to the MDA present	Incubation temperature: 95 °C Sample volume: 200 µl Absorbance reading 532 nm MDA standard curve (range 0-100 nmol/ml) $y = 0.0163x + 0.1571$, $r^2 = 0.9388$
Alanine aminotransferase (ALT / GPT)	RANDOX kit (IPCC Mansfi. AL 1205)	The method is based on the reaction between α- oxoglutarate and L-alanine, which through the catalytic action of the ALT enzyme produces L-glutamate and pyruvate.	Working temperature 30 °C Sample volume 200 µl Absorbance reading 340 nm
Aspartate aminotransferase (AST/GOT)	RANDOX kit (IPCC Mansfi. AL 1204)	The method is based on the reaction between 2-oxoglutarate and L-aspartate, which through the catalytic action of the AST enzyme produces L-glutamate and oxaloacetate.	Working temperature 30 °C Sample volume 200 µl Absorbance reading 340 nm
Acetylcholinesterase (AChE)	kit MAK119 Sigma-Aldrich	AChE reacts with 5,5-dithiobis (2-nitrobenzoic acid) to form a colorimetric product (412 nm), proportional to AChE activity present.	Working temperature 20-25 °C Sample volume 40 µl Absorbance reading 412 nm
EROD (7-ethoxy-resorufin-O-dectylase)	MyBioSource kit MBS1601687	ELISA tests use the specific binding between EROD or VTG antibodies for their quantification in samples. The wells of the test microplates are coated with capture antibodies that allow specific binding with EROD or VTG from the standard and the samples added to the wells. Later, another EROD or VTG specific detection antibody is added to create a sandwich between the enzyme and the antibody, which is detected with a secondary antibody labeled with the enzyme (horseradish peroxidase, HRP). Enzyme activity is determined by adding a substrate that forms a colored product, and the intensity of the color is directly proportional to the amount of EROD or VTG present in the sample.	Working temperature 37 °C Sample volume 40 µl Absorbance reading 450 nm EROD standard curve (range 1.5-24 ng/ml) $y = 0.0199x + 0.1459$, $r^2 = 0.9753$
Vitellogenine (VTG)	MyBioSource kit MBS1603087		Working temperature 37 °C Sample volume 40 µl Absorbance reading 450 nm VTG standard curve (range 15-240 µg/ml) $y = 0.0199x + 0.1459$, $r^2 = 0.9753$

Results and conclusions

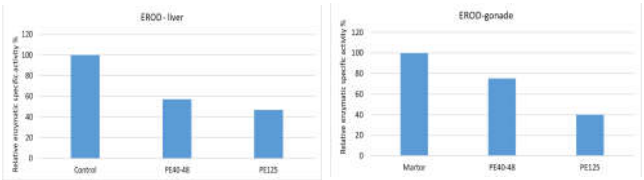
PE showed bioavailability mainly through ingestion with food, causing weight loss of fish, especially in the case of PE 125 µm. PE induced oxidative stress in fish organs.

Organ	CAT	Gred	GST	MDA	ALT	AST	AChE	EROD	VTG
PE 40-48 µm									
Gill	+(**)	+(**)	-(ns)	+(**)	NA	NA	NA	NA	NA
Liver	+ (**)	-(*)	+(ns)	-(**)	+(**)	+(*)	NA	-(ns)	+(ns)
Intestine	+ (ns)	-(**)	+(*)	-(*)	NA	NA	NA	NA	NA
Kidney	- (ns)	-(*)	-(ns)	+(**)	NA	NA	NA	NA	NA
Brain	-(**)	+(ns)	-(ns)	NA	NA	NA	-(ns)	NA	NA
Gonade	-(**)	NA	-(ns)	-(ns)	NA	NA	NA	-(ns)	-(ns)
Muscle	NA	NA	-(*)	NA	NA	NA	-(ns)	NA	NA
PE 125 µm									
Gill	+(**)	+(*)	-(ns)	+(**)	NA	NA	NA	NA	NA
Liver	+(**)	-(ns)	+(ns)	-(**)	-(ns)	-(ns)	NA	-(*)	+(*)
Intestine	+(*)	-(**)	+(*)	-(**)	NA	NA	NA	NA	NA
Kidney	-(*)	+(ns)	-(ns)	+(**)	NA	NA	NA	NA	NA
Brain	+(ns)	+(**)	-(ns)	-(*)	NA	NA	-(ns)	NA	NA
Gonade	-(*)	NA	-(ns)	-(ns)	NA	NA	NA	-(*)	-(*)
Muscle	NA	NA	-(*)	NA	NA	NA	-(ns)	NA	NA

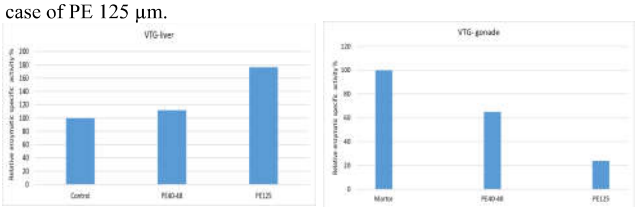
Note: + (increase in enzymatic activity); - (inhibition of enzymatic activity); ns – non-significant effect ($p>0.05$); * - significant effect ($p<0.05$); ** - highly significant effect ($p<0.01$); NA - analysis not conducted.



A significant increase in the activity of catalase (CAT) and glutathione reductase (GRED) enzymes correlated with lipid peroxidation in the gills and kidneys was observed. In the liver, increased CAT and glutathione S-transferase (GST) activity occurred without lipid peroxidation effects. Additionally, alanine transferase (ALT) and aspartate transferase (AST) activities showed significant changes, especially in the case of PE 40-48 µm.



Ethoxyresorufin - o- demethylase (EROD) was inhibited, especially in the case of PE 125 µm.



Vitellogenin (VTG) activity in the liver increased by 10% for PE 40-48 µm and by 80% in the case of PE 125 µm. Low levels of VTG were observed in the gonads.

The size of the particle influenced the level of effects but also the target organs. For example, PE 40-48 affected the gills, liver, and intestine through the increased expression of stress enzymes, while PE 125 could cause effects on the liver, intestine, gills, kidneys, gonads, and brain by inhibiting antioxidant enzymes.

Microplasfish - project logo credit @ Maria Alexandra Geanta

Acknowledgement

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