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Accumulation of polystyrene nanoplastics and triclosan by a model tooth-carp fish, *Aphaniops hormuzensis* (Teleostei: Aphaniidae)

Saemi-Komsari, Maryam; Pashaei, Reza; Abbasi, Sajjad; Esmaeili, Hamid Reza; Dzingelevičienė, Reda; Shirkavand, Hadavand B; Pasalari, Kalako M; Szultka-Mlynska, Malgorzata; Gadzała-Kopciuch, Renata; Buszewski, Boguslaw; Turner, Andrew

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1 **Accumulation of polystyrene nanoparticles and triclosan by a model**
2 **tooth-carp fish, *Aphaniops hormuzensis* (Teleostei: Aphaniidae)**

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5 Maryam Saemi-Komsari ^{1,+}, Reza Pashaei ^{2,+}, Sajjad Abbasi ^{*,3,4}, Hamid Reza Esmaeili ¹, Reda
6 Dzingelevičienė ^{4,5}, Behzad Shirkavand Hadavand ⁶, Marzieh Pasalari Kalako ¹, Malgorzata Mlynska-
7 Szultka ⁷, Renata Gadzała-Kopciuch ⁷, Boguslaw Buszewski ⁷, Andrew Turner ⁸

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9
10 + These authors contributed equally.

11 ¹ *Ichthyology and Molecular Systematics Laboratory, Zoology Section, Biology Department, School of*
12 *Science, Shiraz University, Shiraz, Iran*

13 ² *Marine Research Institute of Klaipeda University, Klaipeda, Lithuania*

14 ³ *Department of Earth Sciences, School of Science, Shiraz University, Shiraz, 71454, Iran*

15 ⁴ *Centre for Environmental Studies and Emerging Pollutants (ZISTANO), Shiraz University, Shiraz, Iran*

16 ⁵ *Faculty of Health Sciences, Klaipeda University, H. Manto Street 84, 92294 Klaipeda, Lithuania*

17 ⁶ *Department of Resin and Additives, Institute for Color Science and Technology, P.O.Box: 16765-654,*
18 *Tehran, Iran*

19 ⁷ *Department of Environmental Chemistry and Bioanalytics, Faculty of Chemistry, Nicolaus Copernicus*
20 *University, Torun, Poland*

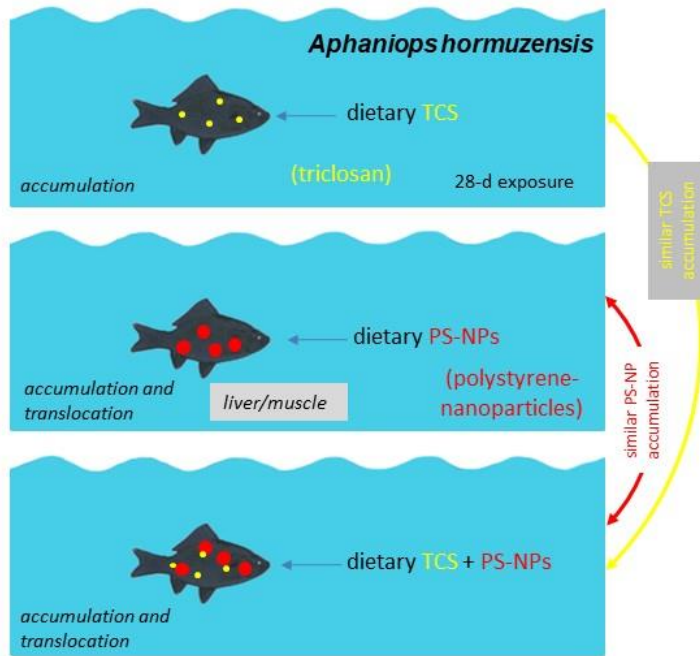
21 ⁸ *School of Geography, Earth and Environmental Sciences, University of Plymouth, PL4 8AA, UK*

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24 * Corresponding author. Department of Earth Sciences, College of Science, Shiraz University, 71454, Shiraz, Iran;
25 Centre for Environmental Studies and Emerging Pollutants (ZISTANO), Shiraz University, Shiraz, Iran. E-mail
26 address: sajjad.abbasi.h@gmail.com; sajjad.abbasi@shirazu.ac.ir

27 **Graphical Abstract**



28

29

30 **Highlights**

31 *Aphaniops hormuzensis* exposed to dietary polystyrene nanoparticles (PS-NP)

32 PS-NP accumulation observed in both digestive and non-digestive organs

33 Presence of triclosan had no measurable effect on PS-NP accumulation and vice versa

34 Results suggest PS-NPs and triclosan do not interact but former can be translocated on ingestion

35

36

37

38 **Abstract**

39 The presence and effects of nanoplastics (NPs; < 1 μm) in the aquatic environment are a growing
40 concern. In this study, a model tooth-carp fish, *Aphaniops hormuzensis*, has been exposed to
41 different concentrations of fluorescent polystyrene nanoplastics (PS-NP) in its diet (up to 5 mg kg⁻¹)
42 over periods of 28 d and the particle accumulation in different tissues determined. Accumulation
43 was observed in both digestive and non-digestive organs, with concentrations greater in the gut,
44 liver and gill (up to 400 $\mu\text{g kg}^{-1}$ dw) than in the skin and muscle (< 180 $\mu\text{g kg}^{-1}$ dw), but no
45 dependency on exposure time or dose was evident. The presence of the organic contaminant,
46 triclosan (TCS), in the diet and at concentrations up to 0.5 $\mu\text{g kg}^{-1}$ did not affect PS-NP uptake by
47 *A. hormuzensis*, while TCS accumulation in the whole body increased with time (up to 10 $\mu\text{g kg}^{-1}$)
48 and, likewise, appeared to be unaffected by the presence of PS-NPs. These observations
49 suggest that the two contaminants do not interact with each other or that any interactions have
50 no impact on accumulation. The results of this study add to the growing body of evidence that
51 NPs can be translocated by aquatic organisms after ingestion, and reveal that, for the species
52 and conditions employed, nanoparticles are accumulated more readily than a widely used organic
53 chemical.

54

55 **Environmental Implication**

56 This study has shown that Polystyrene-Nanoplastic (PS-NPs) of 100-300 nm in size are
57 accumulated by the tooth-carp fish, *A. hormuzensis*, over a period of dietary exposure of 28-days,
58 with particle translocation observed to both digestive and non-digestive organs. Specifically,
59 accumulation was greater in the gut, gill and liver than in the skin and muscle. The presence of
60 the organic co-contaminant, triclosan (TCS), did not significantly affect the uptake of PS-NP;
61 likewise, the accumulation of TCS appeared to be unaffected by the presence of PS-NPs,
62 suggesting that little interaction (e.g., adsorption and desorption) takes place between the two

63 types of contaminant. The results of this study add to the growing body of evidence that NPs can
64 be translocated by aquatic organisms after ingestion, and reveal that, for the species and
65 conditions employed, nanoparticles are accumulated more readily than a widely used organic
66 chemical.

67

68

69 **Keywords:** **bioconcentration**; exposure; nanoplastics; **organic pollutants**; tissue; translocation

70

71

72 **1. Introduction**

73 Long-term weathering (including abrasion and exposure to sunlight and microorganisms) causes
74 plastic waste to be broken down into progressively smaller particles in the environment (Belzagui
75 et al., 2020; Veerasingam et al., 2020). Microplastics (MPs), in the size range 1 μm nm to 5 mm,
76 have, therefore, received extensive attention in the scientific literature and with respect to
77 freshwater, seawater, sediments, groundwater, biota, soils, dusts and the atmosphere (Zhang
78 and Liu, 2018; Abbasi et al., 2019; Panno et al., 2019; Batel et al., 2020; Evangelidou et al., 2020;
79 Wang et al., 2020; Abbasi and Turner, 2021; Cincinelli et al., 2021; Tanentzap et al., 2021). Far
80 fewer studies, however, have been conducted on nanoplastics (NPs; < 1 μm in size), largely
81 because of analytical challenges in their identification and characterisation in aquatic and biotic
82 matrices at realistic concentrations (Ter Halle et al., 2017). Nevertheless, the smaller size and
83 surface area of NPs means that they have, potentially, more complex and harmful properties than
84 MPs that relate to transport, interactions with light, reactivity, bioavailability and migration of
85 additives (Gigault et al., 2021). Accordingly, NP research has focused on short-term interactions
86 of relatively high concentrations of well-defined, commercial or customised NPs with organic or
87 metallic pollutants or biota (or both) under controlled laboratory conditions (Liu et al., 2016; Liu et
88 al., 2021; Matthews et al., 2021).

89
90 Results of such studies suggest that, in aquatic organisms, NPs are able to accumulate,
91 translocate from digestive to non-digestive organs, including the brain, heart and gonads, exert
92 many and varied adverse impacts on health, biomagnify, and interact with organic and inorganic
93 co-contaminants (through adsorption) and affect the bioaccumulation of these chemicals
94 (Trevisan et al., 2022). Specific information in this respect is, however, distinctly lacking for
95 organisms from higher trophic levels, and in particular for freshwater fish (Barría et al., 2020;
96 Brandts et al., 2022).

97

98 **The aim of the present study is to investigate** the potential accumulation and toxicity of NPs
99 constructed of polystyrene (PS-NPs) in a model tooth-carp fish, *Aphaniops hormuzensis* (order
100 Cyprinodontiformes; family Aphaniidae), an endemic killifish found in the Persian Gulf Basin of
101 southern Iran (Teimori et al., 2018). *Aphaniops hormuzensis* is known for its ability to adapt to
102 widely different ecological conditions and, with a high reproductive rate and a physiological
103 similarity with zebrafish (*Danio rerio*), serves as an attractive model in environmental studies
104 (Motamedi et al., 2019). We determine PS-NP distributions in the digestive and non-digestive
105 organs arising from different periods and concentrations of exposure, and study the impacts that
106 particle exposure have on the accumulation of the organic pollutant, triclosan (TCS). TCS is an
107 aromatic ether (5-chloro-2-(2,4-dichlorophenoxy)phenol; CAS 3380-34-5) with a solubility of
108 about 10 mg L⁻¹ at 20 °C and a log *K*_{ow} of 4.76 (Yalkowsky et al., 2010). It is used as a preservative
109 and antimicrobial agent that has broad applications in clinical settings and in various personal
110 care and consumer products, including soaps, shampoos, toothpastes, medical devices, plastics,
111 textiles and shoes. Consequently, TCS is one of the more frequently detected and highly
112 concentrated contaminants in aquatic and terrestrial environments (Dhillon et al., 2015).

113

114

115 **2. Materials and methods**

116 **2.1. Fish sampling and acclimation**

117 *Aphaniops.hormuzensis*, with an average wet weight of 0.544 ± 0.061 g and an average length
118 of 3.40 ± 0.25 cm (and aged ~ 2 to 3 years based on scale ring counts of selected individuals),
119 were obtained from the Mehran River in the Hormozgan province of Iran in June 2021. In the
120 ichthyology laboratory at Shiraz University, Iran, fish were kept in dechlorinated tap water at 26 ±
121 1 °C and under a photoperiod of 12 h light:12 h dark in aquaria for two weeks, with feeding once
122 a day with 3 to 6% of their body weight of a BioMar commercial food (protein = 56%, crude lipids

123 = 18%, carbohydrates = 8.9%; digestible energy = 19.7 MJ kg⁻¹). Water temperature, pH and
124 dissolved oxygen concentration were maintained at 25 ± 1 °C, pH 7.5-7.9 and 6.9 mg L⁻¹,
125 respectively.

126

127 **2.2. Reagents and nanoparticles**

128 All reagents used in the study were purchased from Sigma-Aldrich, Merck or Seastar, and pure,
129 distilled-deionized water used for cleaning and dilution had a resistivity greater than 18 MΩ.cm.
130 Working solutions and dilutions of 97% TCS were prepared in a 1:1 mixture of distilled water and
131 ethanol.

132

133 Styrene was purified and neutralized by washing with a 5% w/v solution of NaOH followed by
134 several washings with pure water and subsequent distillation under vacuum. Polymerization was
135 performed in a three-port reactor, equipped with an additive funnel (to add styrene monomer
136 droplets), cooler and thermometer. Briefly, 400 ml of benzoyl peroxide (1 to 3 % w/v) and polyvinyl
137 alcohol (1 % w/v) in deionized water were mixed at 750-550 rpm for 20 min as nitrogen gas was
138 passed through to remove dissolved oxygen. The temperature was then raised to 90 °C and
139 styrene droplets were gradually added to the solution while being stirred for a period of 30 min.
140 The polymerization reaction continued for 8 h before synthesized polystyrene nanoparticles (PS-
141 NP) were fluorescently stained with rhodamine B (Vakili Tahami et al., 2016; Shohani et al., 2017).
142 The identity of the PS-NPs was confirmed by attenuated total reflection Fourier Transform Infra-
143 Red spectroscopy (FTIR) using a Bruker TENSOR II, and under an LEO-1455VT electron
144 microscope PS-NPs appeared to be spherically shaped, with a minimum, maximum and average
145 particle diameter of about 100 nm, 300 nm and 185 nm, respectively.

146

147 In order to load the fish diet with different concentrations of TCS and PS-NP, different quantities
148 of the compound in a water-ethanol mixture (500 mg L⁻¹) and/or a colloidal slurry in pure water

149 (1000 mg L⁻¹) were sprayed on to the Biomar food before the contents were sealed with a layer
150 of gelatin and dried at room temperature (Ramos et al., 2016).

151

152 **2.3. Exposures**

153 After a period of four days without feeding, 105 fish were randomly selected and seven individuals
154 were placed into a series of fifteen 10-L glass aquaria. The median lethal concentration (LC50) of
155 TCS was determined by exposing each aquarium to a different concentration of the compound
156 (0, 0.01, 0.1, 1, 1.5, 2, 2.5 mg L⁻¹), with controls based on corresponding volumes of ethanol as
157 the carrier solvent, for a period of 96 h and with water-contaminant changes performed daily.

158

159 The protocol above was repeated over a period of 96 h for fish in eight aquaria that were exposed
160 to different concentrations of PS-NP in water (0, 1, 5, 10, 25, 50, 100, 200 mg L⁻¹) and where
161 water (with PS-NPs) was changed daily, and in seven aquaria each that were fed different
162 concentrations of PS-NP or TCS in their daily diets (up to 200 mg kg⁻¹ and 500 mg kg⁻¹
163 respectively) and where half of the water was changed daily.

164

165 In a second experiment, 221 specimens in 33 aquaria were fed daily diets, in triplicate, of 0
166 (control), TCS (0.5, 0.1, 0.01 mg kg⁻¹), PS-NP (5, 1, 0.1, 0.01 mg kg⁻¹), and PS-NP + TCS added
167 concurrently (0.5 + 0.5, 0.5 + 0.1, 0.5 + 0.01 mg kg⁻¹). Every three days the bottom third of water
168 in each aquarium was replaced, and replicates were terminated after 3 d, 14 d and 28 d. On
169 termination, three individuals were retrieved from each aquarium before being rinsed to remove
170 particles from the skin, anaesthetized and sacrificed by cervical transection.

171

172 **2.4. Accumulation of PS-NPs**

173 To measure the accumulation of PS-NPs, tissue samples (skin, muscle, gut, liver, and gill) from
174 three individuals in each exposure were isolated and lyophilized in a freeze-dryer for 72 h before

175 being weighed. Different tissues from individuals or, where insufficient material was recovered,
176 the pooled contents of a given tissue from the three individuals, were digested in 1M HNO₃ for 2
177 h at 70 °C, **resulting in clear solutions with no particulate residue** (Lu et al., 2016). Digests were
178 diluted to 5 mL with pure water and PS-NP concentration was measured by fluorescent
179 spectrophotometry (excitation: 450 nm; emission: 530 nm) using a Lambda 365 Perkin Elmer
180 spectrometer **that had been calibrated with serial dilutions of PS-NPs in HNO₃. Analysis of the**
181 **digests of unexposed fish revealed no peak in the target wavelength of rhodamine b.**

182

183 **2.5. Accumulation of TCS**

184 From each exposure, three fish were retrieved, anaesthetized and freeze-dried. Whole fish were
185 powdered in a porcelain pestle and mortar before 0.2 g were weighed into a 5 mL centrifuge tube
186 with a screw cap. Ten mL of acetonitrile and 0.1 mL of 0.1 M ethylenediaminetetraacetic acid
187 were added to the frozen fish and the contents were homogenized using an IKA Ultra Turrax T125
188 digital homogenizer before being shaken for 15 min and centrifuged at 4000 rpm for 30 min. The
189 supernatant was transferred to a clean centrifuge tube and shaken for 10 min with 0.3 mL of *n*-
190 hexane before the solvent layer was discarded.

191

192 Two-mL extracts were cleaned and the analyte concentrated on methanol-conditioned Waters
193 Corp. Oasis HLB cartridges, with elution employing 1 mL of methanol/1% formic acid. Extracts
194 were reduced to 0.5 mL under nitrogen at room temperature before being filtered through a 0.22
195 µm PTFE syringe filter and analysed by high performance liquid chromatography using **an Agilent**
196 **Technologies 1100 series HPLC system coupled with 6410 triple quadrupole liquid**
197 **chromatography–mass spectrometry (LC/MS) (Waldbronn, Germany) (Pashael et al., 2022).**
198 **Percentage TCS recoveries for *A. hormuzensis* homogenates were 98.4 ± 1.5, 95.6 ± 2.3, 91.2 ±**
199 **5.8, 93.4 ± 4.3 and 82.6 ± 7.5 for spiking levels of 2 µg kg⁻¹, 5 µg kg⁻¹, 10 µg kg⁻¹, 100 µg kg⁻¹ and**

200 500 $\mu\text{g kg}^{-1}$, respectively. The detection limit for TCS was about 0.001 mg mL^{-1} , or about 2 $\mu\text{g kg}^{-1}$
201 on a dry weight tissue basis.

202

203 2.3. Statistical analysis

204 One-way ANOVA with Tukey's post-hoc test was employed in Minitab v19 to investigate
205 significant differences ($\alpha = 0.05$) between groups of data, while Pearson's moment correlations
206 were performed in Excel 365. Estimates of LC50 by the probit model were undertaken in SPSS
207 v19.0 software.

208

209 3. Results

210 3.1. Toxicity of PS-NPs and TCS

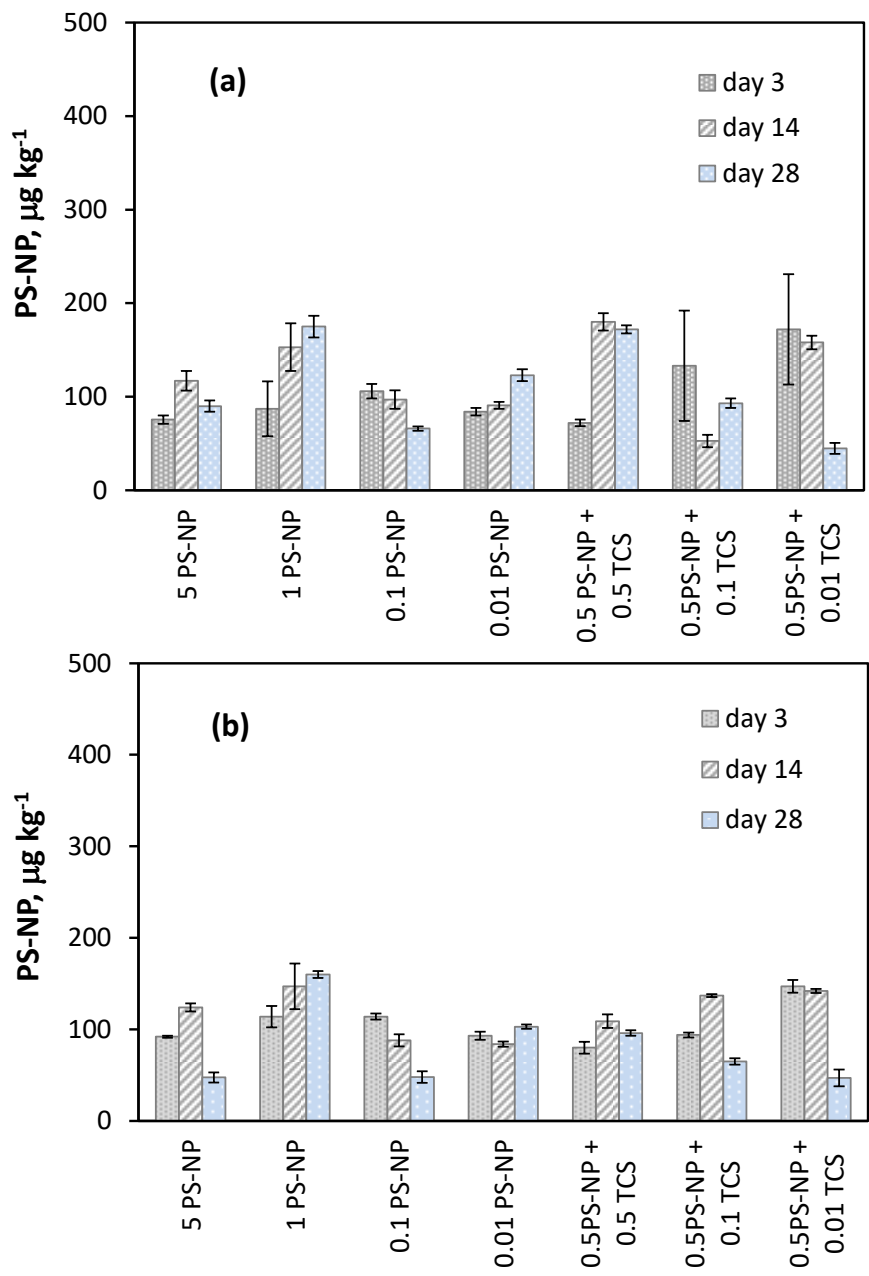
211 The estimated LC50 for *A. hormuzensis* exposed to aqueous TCS for 96 h was 0.924 mg L^{-1} , and
212 controls revealed that mortality was not affected by the presence or concentration of ethanol
213 present as a carrier solvent. The estimated LC50 for PS-NPs suspended in water for 96 h was
214 19.3 mg L^{-1} . In contrast, no mortality was observed for fish exposed to PS-NPs or TCS in their
215 diet and up to concentrations of 200 mg kg^{-1} and 500 mg kg^{-1} , respectively.

216

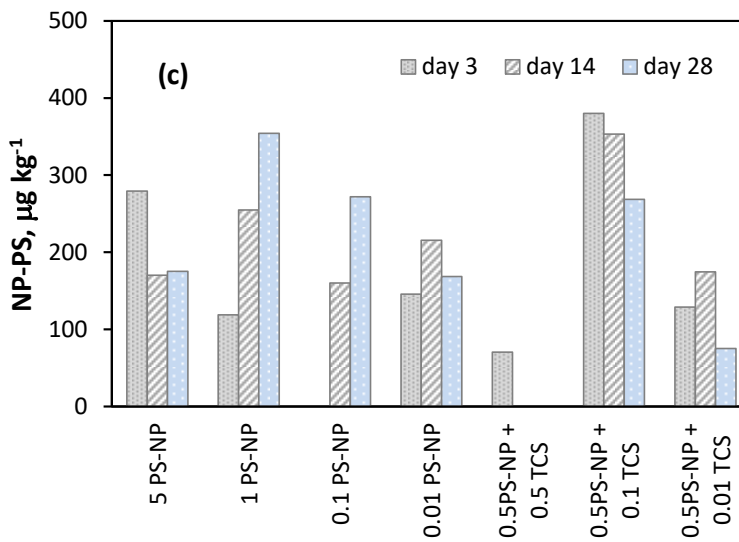
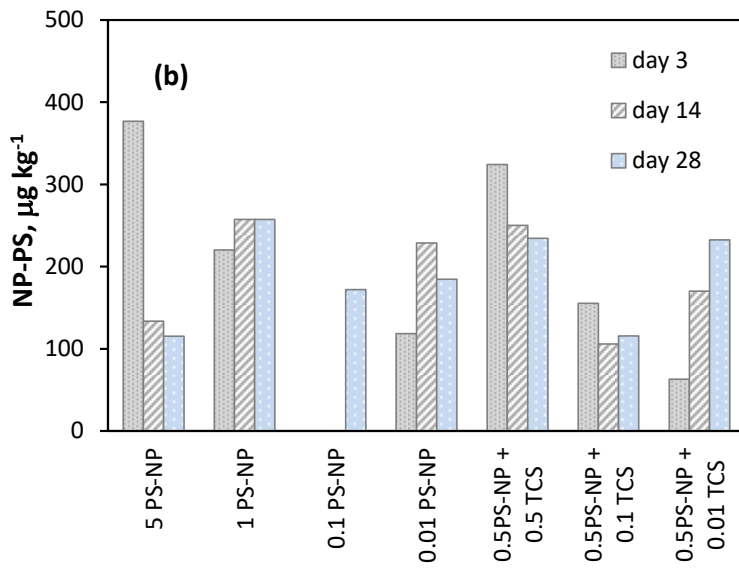
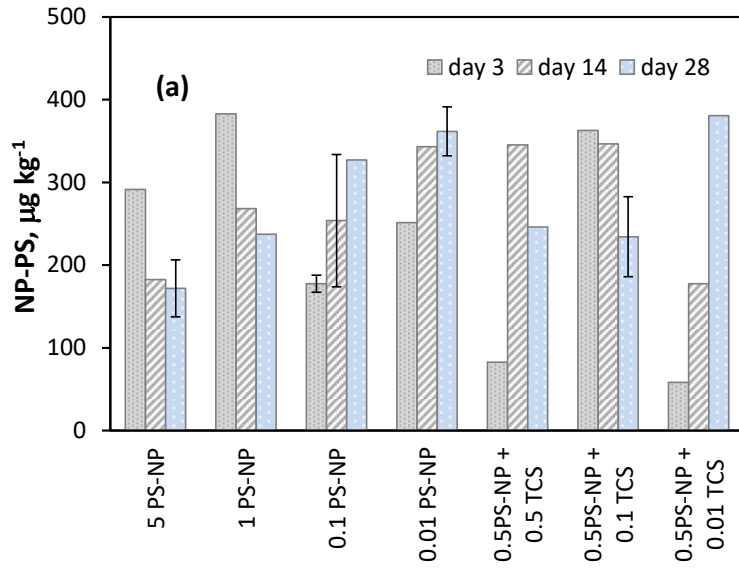
217 3.2. Accumulation of PS-NPs

218 When *A. hormuzensis* were exposed to relatively low concentrations of PS-NPs in their diet, and
219 both with and without TCS, particle accumulation was observed in all tissues considered. Figure
220 1 shows the accumulation of PS-NPs in the skin and muscle, or the tissues where sufficient
221 material was obtained for replication ($n = 3$) in each aquarium. For individuals in each aquarium,
222 variations (as relative standard deviation) were usually below 15%. However, mean
223 concentrations (ranging from about 50 to 180 $\mu\text{g kg}^{-1}$) exhibited no clear dependence on time of
224 exposure (3 to 28 d), concentration of PS-NPs, or presence or concentration of TCS.

225 Nevertheless, a significant correlation was observed between mean concentrations in muscle and
 226 skin ($y = 0.423x + 471$; $r = 0.619$, $p < 0.05$, $n = 21$) that was improved when exposures including
 227 TCS were excluded ($y = 0.590x$, $r = 0.787$, $p < 0.05$, $n = 12$).



228
 229 **Figure 1: Dry weight concentrations of PS-NP in the (a) skin and (b) muscle tissue of *A.***
 230 ***hormuzensis* under the different exposure conditions. Errors represent one standard deviation**
 231 **about the mean of three measurements.**



233 Figure 2: Dry weight concentrations of PS-NP in the (a) gut, (b) gill and (c) liver of *A. hormuzensis*
234 under the different exposure conditions. Errors represent one standard deviation about the mean
235 of three measurements of a pooled sample. Where no errors are shown, data represent a single
236 measurement or the mean of two measurements; where no data are present, insufficient material
237 was recovered for analysis.

238
239 Figure 2 shows the accumulation of PS-NPs in the gut, gill and liver of *A. hormuzensis* under
240 different exposure conditions. Note here that data are more limiting, with triplicates only present
241 from the pooled gut contents in five cases and measurements absent in two (gill) or three (liver)
242 cases. As above, accumulation exhibited no clear dependence on exposure period, concentration
243 of PS-NPs, or presence or concentration of TCS. However, compared with the skin and muscle,
244 accumulation was more variable, and no statistically significant correlations were observed
245 between the tissue types. According to one-way ANOVA, mean concentrations of PS-NPs (for all
246 exposure conditions and exposure times) in the skin and muscle, in the liver and gill, and in the
247 gut and liver were not significantly different, but concentrations in the gut, gill and liver were
248 significantly greater than concentrations in the skin and muscle, and concentrations in the gut
249 were significantly greater than in the gill.

250
251 The concentrations of PS-NPs in different tissue of *A. hormuzensis*, [PS-NP-tissue], were
252 normalised to concentrations in amended food, [PS-NP-diet], as dimensionless assimilation
253 efficiencies, AEs (Chong and Wang, 2000):

254
255
$$AE = \frac{[PS-NP-tissue]}{[PS-NP-diet]} \quad (1)$$

256
257 Table 1 provides values of AE for each tissue, averaged over the three exposure times, for the
258 four concentrations of dietary PS-NPs employed (in the absence of TCS). There is a clear

259 increase in AE with decreasing dietary concentration in all tissue types but that is greatest for the
260 gut.

261

262

263 Table 1: Mean assimilation efficiencies for PS-NPs in different tissues of *A. hormuzensis* exposed
264 to different dietary concentrations and calculated according to Equation 1.

265

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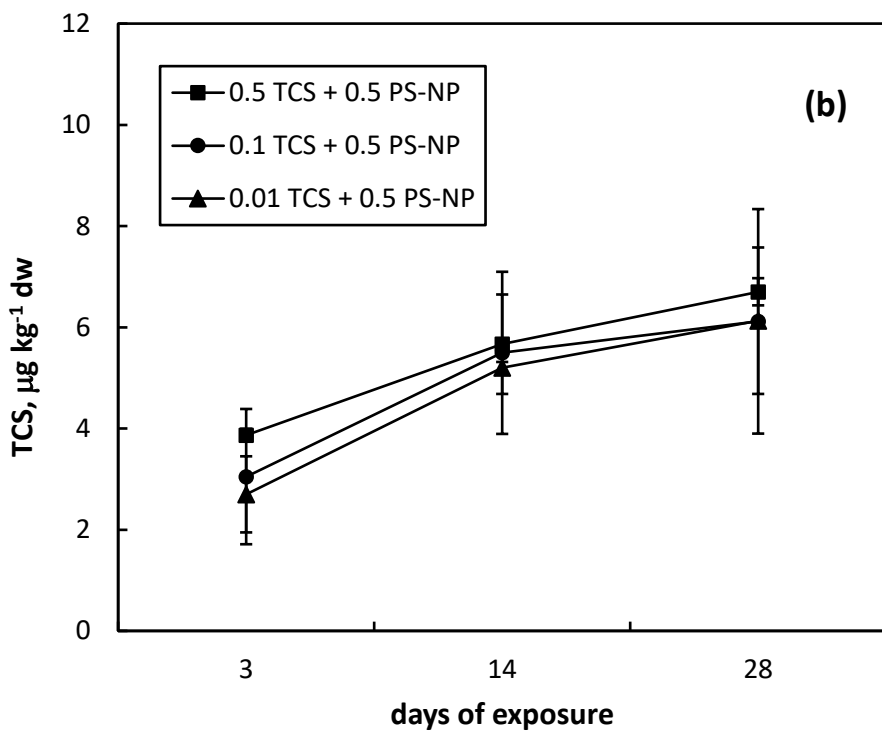
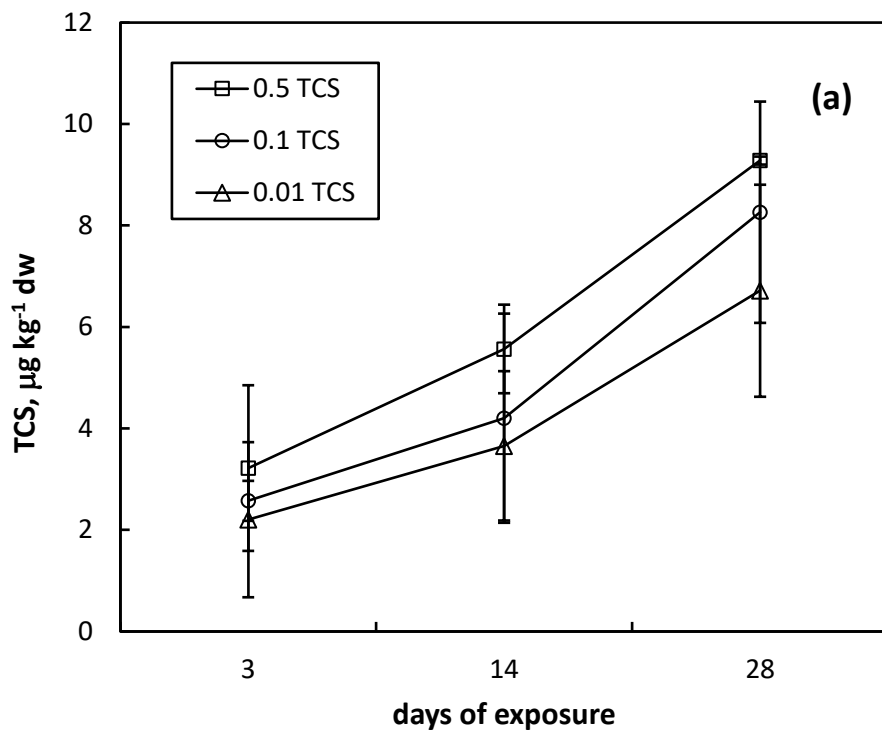
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270

tissue	PS-NP-diet, mg kg ⁻¹			
	5	1	0.1	0.01
skin	0.019	0.138	0.895	9.92
muscle	0.018	0.141	0.835	9.31
gut	0.043	0.296	2.53	31.9
gill	0.042	0.245	0.573	17.7
liver	0.042	0.243	1.44	17.7



271
 272 **Figure 3: Dry weight concentrations of TCS in individuals of *A. hormuzensis* exposed to different**
 273 **concentrations of TCS in the (a) absence and (b) presence of PS-NPs. Errors represent one**
 274 **standard deviation about the mean of three measurements.**

275

276 **3.3. Accumulation of TCS**

277 The accumulation of TCS by *A. hormuzensis* in exposures with and without PS-NPs is shown in
278 Figure 3. Note that no significant impacts on growth were observed in the presence of either
279 contaminant. TCS was not detected in the control exposures ($< 0.2 \mu\text{g kg}^{-1}$) and in the absence
280 of PS-NPs the mean concentration of accumulated TCS exhibited a progressive increase with
281 increasing time of exposure and a (non-significant) increase with increasing exposure
282 concentration. Likewise, in the presence of a constant concentration of PS-NPs, mean TCS
283 accumulation increased with increasing time of exposure and exhibited a (non-significant)
284 increase with increasing exposure concentration. After 28 d, mean concentrations of accumulated
285 TCS were lower in the presence of PS-NPs than in their absence, but at each TCS exposure
286 concentration differences were non-significant. At this time point, assimilation efficiencies for TCS
287 in whole *A. hormuzensis*:

288

$$289 \text{AE} = [\text{TCS-fish}]/[\text{TCS-diet}] \quad (2)$$

290

291 ranged from about 0.01 to 1 and are comparable with the lower values of AE for PS-NPs
292 reported in Table 1.

293

294 **4. Discussion**

295 **4.1. Accumulation and translocation of PS-NPs**

296 We did not observe any dependence of accumulation of PS-NPs in *A. hormuzensis* on time of
297 exposure, possibly because variations amongst conditions in the aquaria and concentrations in
298 amended food outweigh those arising from differences in accumulation. Nevertheless, the results
299 of this study suggest that PS-NPs of average diameter 100 nm are able to enter both digestive

300 and non-digestive organs and both contact and internal tissues of *A. hormuzensis* when the
301 animal is exposed to particles as part of its diet. Accumulation in internal organs requires ingested
302 PS-NPs to cross the intestinal barrier and enter the circulatory system via haemolymph.
303 Specifically, PS-NPs in the gastrointestinal tract would be delivered to the liver via the portal vein,
304 with particles not retained by the liver distributed elsewhere (Brandts et al., 2022). This would
305 explain the high levels of PS-NPs in the liver relative to the muscle, for example. Additionally, it is
306 also possible that PS-NPs in contact tissues like the gill and skin are captured more directly from
307 water. This would require a fraction of ingested PS-NPs to be egested or ventilated (through the
308 gill) and mobilized into the aqueous medium, with captured particles evading detachment during
309 washing. The role of epidermis infiltration in translocation is unclear but evidence from field
310 studies of larger plastic particles in fish muscle tissue than in the blood (Ma et al., 2022) and
311 infiltration of NPs (about 50 nm) through the epidermis into fish eyes (Sendra et al., 2021)
312 suggests that it is possible. The correlation between PS-NP accumulation in the skin and muscle
313 of *A. hormuzensis* that we have observe supports this possibility.

314
315 Translocation of nanoplastic and microplastics of (mainly) polystyrene or polyethylene
316 construction has been reported widely in the literature for aquatic invertebrates and vertebrates
317 and from both laboratory exposures and field studies (e.g., Browne et al., 2008; Brennecke et al.,
318 2015; Zhao et al., 2017; Bhargava et al., 2018). Regarding fish, microplastics have been reported
319 in different organs (including those not involved in digestion) from various freshwater and marine
320 species caught from the wild (Collard et al., 2017; Abbasi et al., 2018; McIlwraith et al., 2021).
321 Experimental studies have demonstrated the passage of PS-NPs of a similar size range to those
322 used herein across the intestinal epithelium (Clark et al., 2022; Vagner et al., 2022) and have
323 found evidence for their translocation in the Crucian carp, *Carassius carssius* (to the brain;
324 Mattsson et al., 2017), and the fathead minnow, *Pimephales promelas* (to the liver and kidney;
325 Elizalde-Velázquez et al., 2020). Lu et al. (2016) exposed zebra fish (*Danio rerio*) to 20 mg L⁻¹ of

326 fluorescent, waterborne PS-NPs and found that 5 μm particles accumulated in the gut, gills and
327 liver but 20 μm particles were not detected in the liver, while Zhang et al. (2019) treated red tilapia
328 (*Oreochromis niloticus*) with 0.1 μm PS-NPs and reported accumulation in the gut, gill, liver and
329 brain. This suggests that suspended PS-NPs can also be taken up through the gills and
330 translocated but that the process is limited by particle size. These studies also demonstrated
331 increasing, non-linear PS-NP accumulation over time in each organ. Brandts et al. (2022)
332 exposed goldfish (*Carassius auratus*) to waterborne PS-NPs of 44 nm in diameter and, via size
333 exclusion chromatography-mass spectrometry, found accumulation in the liver and muscle, but
334 not in the digestive tract.

335
336 Recent research has raised some concerns about the extent of translocation and the particle
337 sizes involved. For instance, Catarino et al. (2019) and Triebkorn et al. (2019) suggest that
338 markers of nanoplastic and microplastic particles, including fluorescent dyes, may leach into the
339 aqueous medium during exposures or during histological procedures when solvents are applied.
340 Triebkorn et al. (2019) also surmise that particles may be unintentionally relocated through the
341 dissection process in experimental studies, particularly when using high concentrations, or
342 through more general contamination in field studies. In carefully conducted exposures using *D.*
343 *rerio*, Batel et al. (2020) found that polyethylene particles above 4 μm in diameter were restricted
344 to the gut lumen. The authors argued that the translocation of larger particles was physiologically
345 unlikely but that smaller, nano-sized particles might have the propensity to cross the intestinal
346 epithelium.

347
348 In the present study, we observed no fluorescence in control fish, took care to avoid cross
349 contamination during dissection, and used particles smaller than the upper limit capable of being
350 taken up actively by tissues (about 1 μm ; Triebkorn et al., 2019). Moreover, the lack of a dose-

351 dependence on PS-NP accumulation (across two orders of magnitude) that we observed is not
352 consistent with leaching of a mass-dependent concentration of fluorescent dye. Rather, lack of
353 dose-dependency may reflect a limit to the quantity of plastics able to pass the intestinal
354 epithelium or the availability of active transporter sites (Clark et al., 2022), or a concentration-
355 dependent aggregation of PS-NPs. In the environment, however, where considerably lower
356 concentrations of nanoparticles are likely, such limiting factors are not predicted to be important.

357

358 *4.2. PS-NP-TCS interactions*

359 The role of micro- and nanoplastics in the accumulation of co-contaminants, **including TCS**, by
360 aquatic biota has received increasing attention over the past decade but results of laboratory
361 studies and theoretical modelling are often inconclusive or contradictory (Triebkorn et al., 2019).
362 Moreover, and in particular for fish, the focus has generally been on the desorption of adsorbed
363 or additive organic chemicals and metals from plastics and other engineered particles in the
364 digestive environment and their subsequent potential for uptake rather than the co-administration
365 of contaminants and plastics as part of the diet (Rochman et al., 2013; Chen et al., 2017; Yan et
366 al., 2017; Zhang et al., 2019).

367

368 Concentrations of TCS accumulated by *A. hormuzensis* exposed to sublethal concentrations in
369 the diet showed a dependence on time of exposure, but mean concentrations were not
370 significantly different among the different exposure concentrations. This suggests that some
371 maximum (saturated) concentration is attained in the fish overall, although any shifts in
372 concentration among the different organs are unknown, and that percentage TCS bioavailability
373 decreases with increasing amount in the diet. No significant differences in mean TCS
374 accumulation between equivalent exposures in the presence and absence of PS-NPs are partly
375 consistent with the limited, relevant information available in the literature regarding freshwater
376 and marine fish. Thus, in a study on *D. rerio* exposed to waterborne polyethylene beads (~ 10 to

377 100 μm) and Ag, Khan et al. (2015) showed that co-exposure had no impact on Ag accumulation
378 but when Ag had adsorbed to the polyethylene surface, reduced uptake occurred. In the European
379 seabass (*Dicentrarchus labrax*), Granby et al. (2018) showed that the uptake of various
380 polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers in the diet was similar in
381 the presence and absence of polyethylene particles (~ 100 to $250 \mu\text{m}$). However, enhanced
382 accumulation (and bioavailability) was observed when the organic contaminants had been pre-
383 sorbed to the microplastics for reasons that are unclear. By contrast, Grigorakis and Drouillard
384 (2018) found a reduction in accumulation of dietary PCBs by the goldfish, *C. auratus*, when
385 polyethylene beads (100 to $500 \mu\text{m}$) were added to food.

386

387 Unlike the studies above, our investigation employed much smaller plastics with higher surface
388 areas and that are able to cross the intestinal epithelium and translocate. Despite using more
389 reactive and bioavailable particles, however, there is no evidence that PS-NPs facilitate or inhibit
390 the uptake of TCS, at least by *A. hormuzensis*.

391

392

393 **5. Conclusion**

394 This study has shown that PS-NPs of 100-300 nm in size are accumulated by the tooth-carp
395 fish, *A. hormuzensis*, over a period of dietary exposure of 28-days, with particle translocation
396 observed to both digestive and non-digestive organs. Specifically, accumulation was greater in
397 the gut, gill and liver than in the skin and muscle, but accumulation did not display a dose-
398 dependence. The presence of the organic co-contaminant, TCS, did not significantly affect the
399 uptake of PS-NP; likewise, the accumulation of TCS appeared to be unaffected by the presence
400 of PS-NPs, suggesting that little interaction (e.g., adsorption and desorption) takes place
401 between the two types of contaminant. While the understanding of the interactions between NPs

402 and aquatic organisms (with or without co-contaminants) is improving, future studies are
403 recommended that involve more realistic plastic concentrations, coupled with a greater range in
404 their shape, condition (e.g., aging, fouling) and polymeric construction.

405

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417

418

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