

SAFETY ASSESSMENT OF COMMERCIAL ANTIMICROBIAL FOOD PACKAGING: TRICLOSAN AND MICROPLASTICS, A CLOSER LOOK.

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1. INTRODUCTION.

Active packaging is a technology that improves the safety and quality of packaged foods (Wyrwa & Barska, 2017). In particular, the incorporation of antimicrobial agents in food packaging prevents the growth of pathogenic and/or spoilage microorganisms and can therefore extend the shelf-life of food products (Firouz, Mohi-Alden, & Omid, 2021). Therefore, antimicrobials are one of the classes of active agents present in the largest number of commercial food storage products (Vilela et al., 2018).

Triclosan (2,4,4'-trichloro-2'-hydroxydiphenyl ether, TCS) (Table S1, Supplementary Material) has been widely used as an antimicrobial additive in food storage containers and kitchen utensils, commonly marketed under the trademark Microban® (Ahmed et al., 2017; Schumann & Schmid, 2018). In those household articles, TCS is generally incorporated directly into the polymer matrix before curing, in concentrations up to 1% (w/w) (Beiras, Verdejo, Campoy-López, & Vidal-Liñán, 2021; Petersen, 2016).

However, growing concern about suspected adverse effects of TCS both on human health (e.g. endocrine disruption Dodson, et al., 2020; Wong & Durrani, 2017, liver tumor promotion Yueh et al., 2014 and antibiotic resistance, among others) and the environment (TCS is classified as very toxic to aquatic life, $EC_{50} < 1 \text{ mg L}^{-1}$, where EC_{50} means the concentration reducing by 50% the endpoint compared to control in bioassay-based toxicity tests Beiras, Verdejo, Campoy-López, & Vidal-Liñán, 2021), led in 2010 to the removal of this substance from the list of authorized additives in materials in contact with food in the European Union (European Commission, 2010). This action only applies to companies and products manufactured within the EU. However, regulations concerning TCS in other

26 countries differ from those in Europe. For example, antimicrobials in food
27 packaging are regulated as food additives by the Food and Drug Administration
28 (FDA) in the USA (Restuccia et al., 2016). Thus, although TCS has been banned in
29 certain health care products, such as hand-soaps and body-washes (Food and Drug
30 Administration, 2016), it is still used in household articles (including food
31 containers) (Halden, 2014, Restuccia et al., 2016).

32 The adoption of e-commerce around the world has allowed consumers to access
33 through online purchase to all kinds of plastic food packaging manufactured outside
34 the EU; however, due to confidentiality issues, producers often do not disclose the
35 chemical composition of plastic materials (Hoekstra, et al., 2015). This is a serious
36 limitation, since the identification of plastic objects including prohibited or
37 restricted additives, is not feasible. These items must then be tested to ensure their
38 compliance with standards set by the European legislation on food contact materials.
39 The Regulation EU 10/2011 (Commission Regulation, 2011), and its respective
40 amendments accept migration tests using representative food simulants, as a valid
41 and reliable method for the verification of regulatory compliance of plastics in
42 contact with food. On the other hand, although not included in the EU Regulation it
43 is also advisable to evaluate the migration behavior of these materials under realistic
44 conditions of use (e.g. microwave heating), due to the daily use of microwave safe
45 food containers (Bhunia, Sablani, Tang, & Velasco, 2013).

46 Furthermore, the release of microplastics from food packaging, which in turn might
47 end up in packed food, depends on processing and storage conditions (Du, Cai,
48 Chen, & Shi, 2020; Kedzierski, Lechat, Sire, Le Maguer, Le Tilly & Bruzard, 2020;
49 Fadare, Wan, Guo, & Zhao, 2020). Although the presence of microplastics as food
50 contaminants is not currently regulated by EU legislation, in 2016 the European

51 Food Safety Authority (EFSA) requested more data on the impact of food
52 processing on the formation of microplastics; however only a few studies
53 investigating this topic have been published ever since (Rainieri & Barranco, 2019;
54 Kieran, Covernton, Davies, Dower, Juanes & Dudas, 2019).

55 The concern is not only about the risks posed to human health and the environment
56 by microplastics themselves, but also on the release of additives incorporated into
57 plastics. In fact, some recent studies have shown that plastic chemical additives,
58 such as TCS, are responsible for the joint aquatic toxicity of microplastics (Beiras,
59 Verdejo, Campoy-López, & Vidal-Liñán, 2021; Webb, Gaw, Marsden, & McRae,
60 2020; Zhu, Wang, Zhao, Wang, Liu, & Liu, 2019).

61 The aim of the present study was to test different commercial antimicrobial food
62 packages sold through online sales platforms to verify compliance with TCS ban on
63 food contact materials. In this context, migration studies were carried out in
64 accordance with EU Regulation 10/2011, and the influence of test conditions (e.g.
65 food simulants, temperature, duration of contact, repeated use and microwave
66 heating) has been studied.

67 In addition, the potential release of microplastic particles to food simulants was
68 investigated by testing the plastic food containers under microwave or conventional
69 heating conditions. This is especially relevant, as microplastics containing TCS
70 might enter food in contact with antibacterial containers during typical daily storage
71 and use conditions, posing potential health risks to consumers and acting as a new
72 entry route of emerging pollutants into the environment.

73

74

75 **2. MATERIALS AND METHODS**

76 **2.1. Reagents and samples**

77 Triclosan (TCS) was obtained from Sigma-Aldrich (St. Louis, MO, USA). Individual
78 stock solutions (200 mg L⁻¹) were prepared in methanol, and stored at 4°C in amber
79 vials. Working solutions of TCS (0.5-10 mg L⁻¹) were prepared in mobile phase
80 MeOH/H₂O (78:22, v/v).

81 Acetic acid from Panreac (Barcelona, Spain) and methanol (HPLC grade) from SDS
82 (Peypin, France) were employed. HPLC grade (acetonitrile, absolute ethanol and
83 chloroform) and LC-MS grade (acetonitrile, water and ammonium acetate) reagents
84 were purchased from Scharlab (Barcelona, Spain). Ultrapure water used in all
85 experiments was obtained from a Milli-Q water purification system (Merck Millipore,
86 Bedford, MA, USA).

87 Reusable Microban[®] polypropylene food containers from different brands (namely,
88 Lock and Store, Neoflam, Prepology and Micronware) and geographical areas (e.g.
89 USA and Southeast Asia) were acquired through online sales platforms (Figure S1,
90 Supplementary Material). All products lack information on the antimicrobial additive
91 used, but were marketed as suitable for microwave heating and food storage at
92 refrigerated and frozen conditions.

93 **2.2. Chromatographic analysis**

94 **2.2.1. LC-UV**

95 Chromatographic analysis of TCS was performed with an HPLC system PU-2089
96 (Jasco Analítica, Spain), equipped with an Agilent Zorbax SB-C₁₈ column (4.6 × 150
97 mm, 3.5 μm), and a UV-Vis detector set at 280 nm. The mobile phase used for the
98 elution was ultrapure water (22%) and methanol (78%) that was delivered at 1 mL min⁻¹
99 ¹, resulting a total analysis time of 7 minutes. A sample injection volume of 20 μL was

100 used. The calibration curves were built with standard solutions of TCS (0.5-10 mg L⁻¹)
101 in methanol/H₂O (78:22, v/v).

102 **2.2.2. Capillary-LC-MS**

103 Capillary-LC mass spectrometry (cLC-MS) analysis was performed for confirmation of
104 TCS presence in sample extracts. An Agilent 1100 series instrument (Agilent
105 Technologies, California, USA) equipped with a binary capillary pump and a degasser
106 was used; coupled to an Agilent 6120 Quadrupole Mass spectrometer. A micro-ESI
107 source was used and N₂ was used both as drying gas (temperature: 325 °C, flow rate: 8.0
108 L min⁻¹) and nebulizer gas (pressure: 17 psi). Capillary voltage was set at 3.5 kV in
109 negative polarity mode.

110 A Luna C18 (2) capillary column (150 mm × 0.3 mm, 3 μm) (Phenomenex, California,
111 USA) was employed for cLC separation and kept at 30 °C in a Beckman oven
112 (Fullerton, CA, USA). Gradient elution was applied at a flow rate of 10 μL min⁻¹, using
113 as mobile phase a combination of A: 2.5 mM ammonium acetate (NH₄Ac) and B:
114 acetonitrile (ACN). Initial composition at 30% B was maintained for 3 min, followed by
115 linear increase to 95% B in 10 min, held for 5 min, return to initial conditions in 2 min
116 and held for 5 min. A 10 μL stainless steel loop was employed for sample injection,
117 using 10% ACN in 2.5 mM NH₄Ac as on-column focusing solution.

118 Agilent Chemstation software was employed for acquisition and data processing.
119 Acquisition of MS full scan combined with selected ion monitoring (SIM) detection of
120 m/z 287 for specific monitoring of TCS was applied.

121 **2.3. Detection and estimation of TCS content in food packaging.**

122 A dissolution-precipitation/extraction procedure (Alin & Hakkarainen, 2011) was used
123 to quantify the concentration of TCS in the food packages. Briefly, 1.0 x 1.0 cm plastic
124 pieces were weighed and placed in 40 mL glass vials containing 15 mL of chloroform.

125 The vials were closed and heated to 60°C in an ultrasonic bath. Then, 5 mL of ethanol
126 was added and the vials were stored for at least 1 night in a refrigerator. Aliquots of 5
127 mL were taken and evaporated to dryness, followed by further reconstitution in
128 methanol/H₂O (78:22, v/v) and filtration through nylon 0.22 µm syringe filters prior to
129 chromatographic analysis. The determinations were carried out in triplicate for each
130 polymer sample.

131 **2.4. Migration experiments**

132 The migration tests were carried out in accordance with EU Regulation 10/2011 and the
133 guidelines for testing kitchenware articles drawn up by the Joint Research Center (JRC)
134 of the EU Commission (Jakubowska, Beldi, Robouch & Hoekstra, 2020).

135 **2.4.1. Conventional oven heating**

136 Polypropylene food containers were cut into small pieces (1.5 x 1.5 cm, surface area of
137 ~2.2 cm²; 2 mm thickness) and dipped into 40 mL glass vials each containing 3 mL of
138 food simulant, namely ultrapure water, 3% (v/v) acetic acid, 10% (v/v) ethanol and 95%
139 (v/v) ethanol, representing aqueous, acidic and fatty foods, respectively. A homemade
140 stainless steel accessory was placed over the plastic pieces to keep them submerged in
141 the solution, and the vials were tightly closed.

142 Standardized migration tests included 10 days at 20°C; 10 days at 40°C and 2 hours at
143 70°C, depending on the intended use.

144 The experiments were performed in triplicate and blank samples were also included.

145 After heating, the polypropylene pieces were removed and the extracts evaporated to
146 dryness, followed by further reconstitution in the mobile phase before chromatographic
147 analysis. Migration results were expressed in mg of migrated TCS per dm² of surface
148 area.

149

150 **2.4.2. Microwave heating**

151 Migration tests were carried out on a Samsung apparatus at 700 W for 2 min to simulate
152 standard microwave heating conditions. Small pieces of the plastic containers (1.5 x 1.5
153 cm, surface area of $\sim 2.2 \text{ cm}^2$) were soaked in 10 mL of absolute ethanol in 200 mL
154 polytetrafluoroethylene (PTFE) vessels that were tightly closed. The experiments were
155 performed in triplicate and blank samples were also included. After the heating time, the
156 plastic pieces were removed and the extracts were transferred to 40 mL glass vials,
157 evaporated to dryness and finally reconstituted in the methanol/H₂O (78:22, v/v) before
158 chromatographic analysis.

159 **2.4.3. Repeated migration experiments**

160 The influence of the repeated use of reusable food containers on the migration of TCS
161 has been explored with successive extraction cycles both in a conventional oven (2 h,
162 70°C) and in a microwave oven (2 min, 700 W), as described in Sections 2.4.1 and
163 2.4.2.

164 TCS migration during four consecutive assays was evaluated using the same piece of
165 plastic and adding portions of fresh food simulant (95% v/v ethanol) in each assay.
166 Finally, the extracts were analyzed by HPLC as described above.

167 **2.5. Fourier transform infrared spectrophotometry (FTIR) analyses**

168 Microban[®] polypropylene food container samples were analyzed using an attenuated
169 total reflection Fourier transform infrared spectrophotometer (ATR-FTIR Nicolet iS50,
170 Thermo Fisher Scientific, USA). All spectra were recorded in the absorbance mode in
171 the 000–500 cm^{-1} region with a resolution of 4 cm^{-1} and 64 scans. Spectra were
172 acquired and compared to isotactic polypropylene reference spectra.

173 **2.6. Scanning electron microscopy (SEM) analyses**

174 SEM images of the surface of Microban® food packaging samples and their migration
175 solutions were collected on a JEOL JSM-6335F emission scanning electron microscope,
176 equipped with an energy dispersive X-ray analyzer (EDX). Before observation under
177 the microscope, the surface of the polymer samples was sputter-coated with a thin
178 conductive gold layer. Regarding the migration solutions, sample preparation was done
179 by dropwise-pouring the solutions onto a coverslip, air drying and coating with a layer
180 of gold. The SEM operating parameters were: acceleration voltage 20 kV and working
181 distance 15 mm. The SEM images were taken at different magnifications ranging from
182 2000 to 8500 X.

183 **3. RESULTS AND DISCUSSION**

184 **3.1. Detection and quantification of TCS in food packaging**

185 TCS was found to be present in all Microban® antimicrobial food containers tested.
186 Firstly, typical HPLC-UV chromatograms of a standard solution of TCS and ethanolic
187 extracts from food containers show a clear peak at elution times of ~ 6 min
188 corresponding to TCS (Figure S2, Supplementary Material).

189 The identity of TCS in the extracts was further confirmed by cLC-MS. Figure 1 shows
190 representative examples of single ion monitoring (SIM) chromatograms at m/z values of
191 287 obtained from both, a pure TCS standard and a sample extract. Retention times
192 (14.7 min) that match both, in standards and in sample extracts, in addition to the
193 experimental isotopic pattern that adequately matches with the theoretical one, allowed
194 us to confirm the presence of TCS in the antimicrobial food containers.

195 Once confirmed the presence of TCS, its concentration in the food containers was
196 determined by HPLC-UV analysis of the extracts after dissolution/extraction of the
197 plastic pieces. The mean values obtained were 11 ± 2 and 9 ± 2 mg/dm² for the brands

198 Lock and Store and Prepology, respectively; and 3.02 ± 0.02 and 2.5 ± 0.5 mg/dm² for

199 Neoflam and Micronware brands, respectively.

200

201 **3.2. Migration experiments**

202 The key factors involved in migration from food packaging are related to the
203 physicochemical properties of the migrant, the type of food or food simulants, the
204 contact time-temperature conditions and the nature of the food packaging material
205 (Poças, 2018).

206 Comparison of the specific migration of TCS between different food simulants under
207 accelerated conditions (70°C for 2 h) suggests that the amount of TCS released from the
208 plastic food containers to aqueous/acidic simulants is very small compared to fatty food
209 simulants (e.g. 95% v/v ethanol) (Figure 2). TCS is a very hydrophobic compound
210 (expressed as the octanol-water partition coefficient, $\log K_{ow} = 4.8$), and is therefore
211 easily soluble in 95% (v/v) ethanol (Table S1, Supplementary Material). Therefore, the
212 synergistic effects of the hydrophobicity of the analyte, the swelling of the polymer that
213 facilitates TCS diffusion and the high temperature might contribute to the migration of
214 TCS from the polymer matrix.

215 Furthermore, the amount of migrated TCS also depends on its concentration in the
216 polymeric material. The brands Lock and Store and Prepolgy provided the highest
217 migration values (Figure 2) because these food containers present TCS concentrations
218 around three times higher than Neoflam or Micronware brands. Since 95% (v/v) ethanol
219 causes the highest degree of TCS migration, further migration tests were carried out in
220 this solvent.

221 In addition to accelerated tests at 70°C/2 h, EU Regulation 10/2011 also includes
222 migration tests at 20°C/10 days and 40°C/10 days, which represent food in frozen and
223 refrigerated conditions and long-term storage at room temperature or below, or with a
224 short warm-up, respectively. Then, the amount of migrated TCS was compared as a
225 function of contact time and temperature (Figure 3).

226 The migration of TCS increases both with temperature and contact time. Higher
227 temperature increases the solubility of TCS and improves the swelling of the polymeric
228 material, which facilitates both diffusion and mass transfer of TCS from the polymer to
229 the food simulant (Vilaplana, Ribes-Greus, & Karlsson, 2009; Sanches Silva, Cruz
230 Freire, & Paseiro Losada, 2010). Thus, the test conditions leading to the highest amount
231 of TCS release correspond to 40°C/10 days.

232 Furthermore, the kinetic curves performed at 40°C/10 days under static conditions,
233 followed a similar trend as can be seen in Figure 4. The amount of migrated TCS
234 gradually increased during the first few days until it reached the plateau after
235 approximately 7-8 days. These plots suggest that TCS located on the outer layer of the
236 polymeric surface is easily accessible by the food simulant allowing a rapid release of
237 TCS from the polymer, while the migration of TCS present in the inner layers is is
238 controlled by the diffusion of this additive within the plastic and therefore, migrates
239 slowly (Alin & Hakkarainen, 2011; Li, 2020).

240 However, migration rates differ greatly between the Microban® food packages,
241 depending in part on the initial concentration of TCS in each polymer matrix (a high
242 concentration of TCS in the Lock and Store and Prepology containers enhances the
243 amount of TCS released from these polymer matrices). Furthermore, the diffusion
244 coefficients of migrants depend on the nature of the polymer, among others.

245 Of the three different grades of polypropylene that are commonly used in plastic
246 packaging, namely polypropylene homopolymer (PP), propylene-ethylene random
247 copolymer (PP-R) and propylene-ethylene block copolymer (PP-C), PP has been the
248 most resistant to migration of additives, especially in contact with fatty food simulants
249 (Alin & Hakkarainen, 2010).

250 Thus, the “upper bound” diffusion coefficients (D_p^*) of TCS in the different classes of
251 polypropylene have been calculated according to the Arrhenius type equation (1),
252 following the recommendations given by the European Commission (Hoekstra, et al.,
253 2015).

$$254 \quad D_p^* = 10^4 \exp(A_p^* - 0.1351M_r^{2/3} + 0.003M_r - 10454R/RT) \quad (\text{cm}^2/\text{s}) \quad (1)$$

255 and

$$256 \quad A_p^* = A_p'^* - \tau/T \quad (2)$$

257 where: A_p^* describes the basic diffusion behavior of the polymer matrix in relation to
258 the migrants; $A_p'^*$, is a specific parameter for a given class of polymer (13.1 and 13.5
259 for PP+PP-R and PP-C, respectively Hoekstra, et al., 2015); M_r , is the migrant relative
260 molecular mass (Da); τ , a polymer specific “activation temperature” increment (K)
261 (1577 and 0 for PP+PP-R and PP-C, respectively Hoekstra, et al., 2015); T, is the
262 temperature (K); R, the gas constant (8.3145 J mol⁻¹ K⁻¹) and 10454R a reference
263 activation energy (J mol⁻¹). The asterisk* indicates an upper bound value and the
264 apostrophe' indicates the parameter is temperature independent.

265 Accordingly, D_p^* values of 6.7 x 10⁻¹⁰ cm²/s and 2.1 x 10⁻⁸ cm²/s (at 40°C) have been
266 obtained for TCS in PP/PP-R and PP-C, respectively. These numbers illustrate how the
267 type of polypropylene material significantly influences the migration rate of TCS.

268 Fourier transform infrared spectroscopy (FTIR) was used to characterize the type of
269 polypropylene in Microban[®] food packages. As shown in Figure S3 (Supplementary
270 Material) the FTIR spectra of the samples from all food packages match the reference
271 spectra of isotactic polypropylene homopolymer. Therefore, the differences observed in
272 migration rates of TCS from the Microban[®] food containers (Figure 4) cannot be
273 attributed to the type of polymer, but most likely to differences in matrix crystallinity

274 due to the manufacturing process, which is another determining factor which controls
275 the release of migrants from a polymer matrix.

276 As previously reported, an enhancement in the crystallinity of the polymer matrix
277 makes

278 TCS be locked into a compact polymer network, leading to a decrease in its release rate
279 (Kamalipour, Masoomi, Khonakdar & Razavi, 2016). This could explain the lower TCS
280 migration rates observed for Micronware samples compared to other brands with a very
281 similar TCS concentration (e.g. Neoflam).

282 **3.3. Repeated migration experiments**

283 Since Microban[®] food containers are reusable articles, migration of TCS was also
284 investigated in a repeated migration regime. According to the EU Legislation
285 (Commission Regulation 10/2011), the influence of repeated use on TCS migration has
286 been explored with four consecutive tests (at 70°C, 2 h), using a single plastic sample
287 and adding new portions of 95% (v/v) ethanol each time.

288 As shown in Figure 5A, the migration values are much higher in the first cycle
289 compared to subsequent cycles, which means that TCS present on the outer layer of the
290 polymeric surface is released in the first cycle, while in the subsequent cycles the
291 migration of TCS (which is highly soluble in 95% v/v ethanol) is controlled by the
292 diffusion of this additive within the polymer matrix.

293 On the other hand, since food is often heated in the microwave directly inside the
294 polymeric packaging, the influence of microwave heating on the level of TCS migration
295 has also been studied in a repeated migration regime. A similar trend to that observed
296 for repeated migration at 70°C 2h, has been observed in the decrease of TCS amount
297 during successive microwave extractions (Figure 5B). However, large differences in
298 migration levels in the first cycle have been obtained between microwave- and oven-

299 heated samples. Microwave-heated samples resulted in TCS migration values two to
300 three times lower than those obtained during conventional heating.

301 As indicated above, contact times and temperature are the most relevant factors for TCS
302 migration from food packaging. In an ordinary microwave oven, under real conditions
303 of use, the exact temperature reached is unknown, but the duration of heating (usually 1
304 or 2 minutes) is very short compared to conventional heating tests (2 hours) which leads
305 to lower amounts of migrated TCS. However, microwaves could also increase the
306 diffusivity of small additives in the polymer and/or create hot spot areas on the plastic
307 surface (Alin & Hakkarainen, 2011) that can facilitate TCS migration and compensate
308 for short warm-up times. This is particularly true from the second cycle onwards where
309 the migration levels are lower but relatively constant (Figure 5B).

310 **3.4. Investigation of the release of microplastics from food packaging**

311 Human exposure to microplastics through plastic food packages has been reported in
312 previous studies (Du, Cai, Chen, & Shi, 2020; Fadare, Wan, Guo, & Zhao, 2020;
313 Rainieri & Barranco, 2019). The release of microplastics from food contact materials
314 mainly depends on both the type of plastic material and the processing conditions (Du,
315 Cai, Chen, & Shi, 2020; Fadare, Wan, Guo, & Zhao, 2020).

316 Therefore, Microban[®] food containers were examined by SEM to investigate whether
317 they are a potential source of microplastics. The surface of food containers were studied
318 before and after migration tests (e.g. conventional heating or microwave heating with
319 repeated use) to detect any morphological changes in their surface (such as cracks,
320 roughness or flakes).

321 SEM images of unexposed food packaging samples (Figure S4, Supplementary
322 Material) show the presence of various protrusions and cracks in the plastic surface.
323 After conventional heating (70°C, 2 h, 4 cycles), the plastic samples show some degree

324 of surface deterioration (e.g. consistent roughness and a flaky surface, Figure S4,
325 Supplementary Material). In contrast, no obvious changes have been observed in the
326 surface of samples exposed to microwave heating (data not shown).

327 However, the presence of micron-sized particles (between 8-45 μm) has been observed
328 in the extracts after either microwave (Figure 6) or conventional heating (data not
329 shown). The elemental analysis of these microparticles confirms that they are
330 microplastics since the predominant elements are oxygen and carbon, while Cl peaks
331 can be attributed to TCS which has three chlorine atoms in its molecule. Cu and Zn
332 peaks belong to the metal coverslip used to deposit migration solutions. Therefore, it
333 can be concluded that microplastics containing TCS can leach out of Microban[®] food
334 containers when exposed to either conventional or microwave heating.

335 So far, it is still difficult to evaluate and confirm the actual risks of microplastics on
336 human health, although there are many studies that show the damage caused in animals
337 (Deng, Zhang, Lemos, & Ren, 2017; Huang et al., 2021).

338 The concern is not just limited to microplastics themselves, but also to the associated
339 chemicals (e.g. additives) that leach out and could be absorbed and then transferred into
340 different tissues after ingestion of microplastics in food. In this regard, as TCS has a
341 relatively high octanol-water partition coefficient ($\log K_{ow} = 4.8$), it has a high
342 bioaccumulation potential.

343 In addition, a recent study has shown that brief exposure to TCS, in relatively low
344 doses, could have adverse effects on colonic inflammation and associated colonic
345 tumorigenesis in mice (Yang et al., 2018).

346

347 **4. CONCLUSIONS**

348 Although current EU legislation prohibits the presence of TCS in food contact
349 materials, due to its toxic potential to humans, TCS has been found to be present in
350 several antimicrobial food containers purchased through e-commerce.

351 Migration tests with food simulants according to EU Regulation 10/2011 showed a
352 significant TCS release under all the tested conditions, with the highest TCS migration
353 rates corresponding to 95% ethanol (v/v) at 40°C for 10 days (simulating fatty food,
354 long-term storage at room temperature or below, or with a short warm-up). This could
355 be due to the synergistic effects of the hydrophobicity of the analyte, the swelling of the
356 polymer that facilitates TCS diffusion, as well as the combination of temperature and
357 long contact times, contributing to the migration of TCS.

358 Microwave heating also showed significant migration rates of TCS to fatty food
359 simulants, although lower than those for conventional migration tests, which can be
360 attributed to the shorter duration of microwave heating (usually 1-2 minutes) compared
361 to oven heating time (2 h).

362 Repeated-use migration tests have shown a continuous release of TCS during 4 cycles,
363 with a significant drop in the amount of TCS released between the first cycle and the
364 rest. This implies that TCS present in the outer layer of the polymeric surface is quickly
365 released in the first cycle and, while in subsequent cycles, migration is mainly
366 controlled by diffusion through the polymer.

367 Changes in the surface morphology of food containers (increased surface roughness and
368 flakes), after exposure to conventional heating (70°C, 2 h, 4 cycles) have been observed
369 by SEM. In addition, microplastics (between 8 and 45 µm) containing TCS were found
370 to leach from the inner surface of food containers when exposed to either oven or
371 microwave heating, which can contaminate packaged foodstuff and reach consumers.

372 Therefore, a harmonization of global policies is needed regarding the prohibition of
373 TCS in food contact materials and the control of antimicrobial food packaging from e-
374 commerce.

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498

499 **FIGURE CAPTIONS**

500 **Figure 1.** SIM chromatograms at m/z 287 for: (A) TCS 20 $\mu\text{g L}^{-1}$ standard solution and
501 (B) Microban[®] food packaging extract; (C) mass spectra for the peak at $t_r = 14.7$ min;
502 (D) Theoretical isotopic pattern for the $[\text{M-H}]^-$ ion for TCS.

503 **Figure 2.** Migration of TCS from Microban[®] antimicrobial containers to different food
504 simulants at 70°C for 2 h (n=3).

505 **Figure 3.** Migration of TCS from Microban[®] antimicrobial containers to 95% (v/v)
506 ethanol at 20°C/10 days; 40°C/10 days and 70°C/2 h (n=3).

507 **Figure 4.** Kinetic migration curves of TCS from Microban[®] antimicrobial containers to
508 95% (v/v) ethanol at 40°C/10 days (n=3).

509 **Figure 5.** Migration of TCS from Microban[®] antimicrobial containers to 95% (v/v)
510 ethanol under repeated use conditions. (A) Conventional heating (2 h, 70°C, n=3) and
511 (B) microwave heating (2 min, 700W, n=3).

512 **Figure 6.** (A) SEM images of microplastics released from food packaging samples in
513 95% (v/v) ethanol after microwave heating at 700 W for 2 min and 4 migration cycles
514 (magnification between 2000 and 8500X). (B) EDX analysis of the microplastics. SEM
515 operating parameters: acceleration voltage 20 kV; working distance 15 mm;
516 magnification 2000-8500X.

517 **Figure S1.** Types of PP food containers with Microban[®] antibacterial protection used in
518 this study. (A) Lock and Store; (B) Neoflam; (C) Prepology and (D) Micronware.

519 **Figure S2.** Typical HPLC-UV chromatograms of Microban[®] food packaging extracts.
520 (A) TCS standard solution of 10 mg L^{-1} , (B) Lock and Store and (C) Prepology extracts.

521 **Figure S3.** Comparison of FTIR spectra of Microban[®] food packaging samples with
522 isotactic polypropylene reference spectra.

523 **Figure S4.** SEM images of the surface of food packaging samples (Lock and Store and
524 Micronware) before (**A, C**) and after (**B, D**) conventional heating with 95% (v/v)
525 ethanol at 70°C/2h and 4 migration cycles. SEM operating parameters: acceleration
526 voltage 5 kV; working distance 14.1-14.9 mm; magnification (5000-15000X).
527

Figure 1

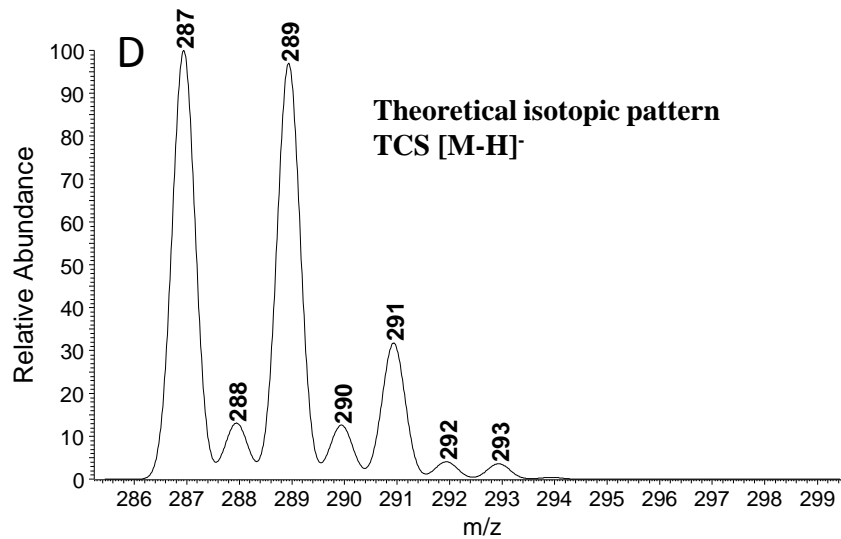
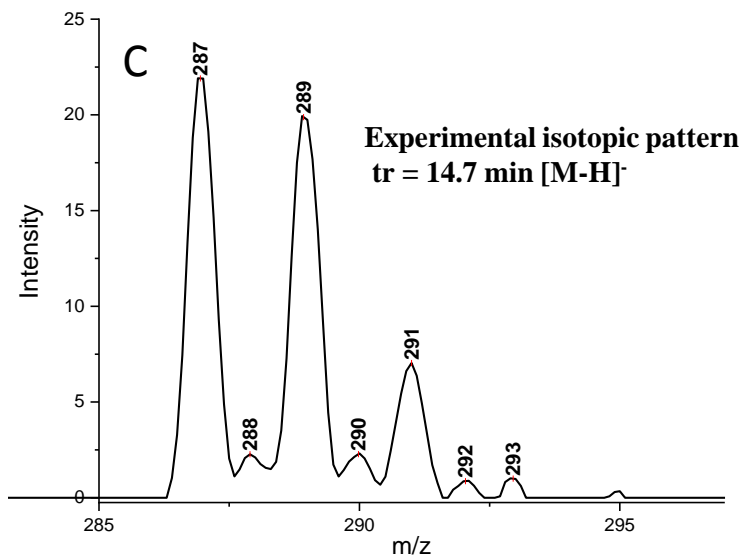
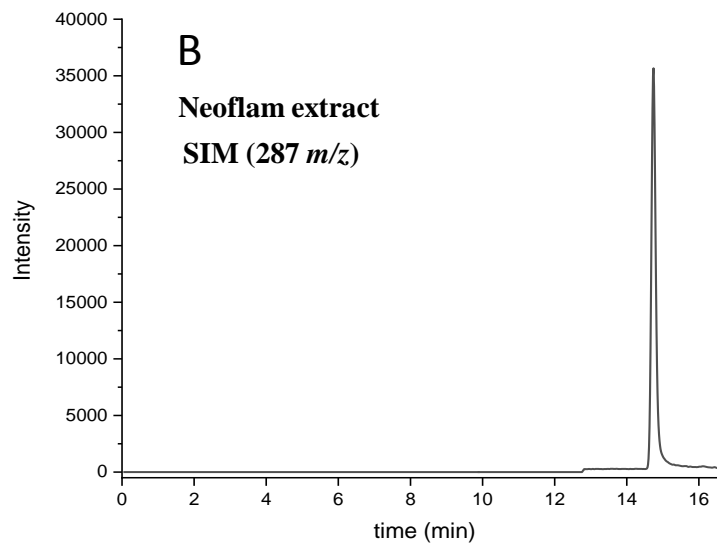
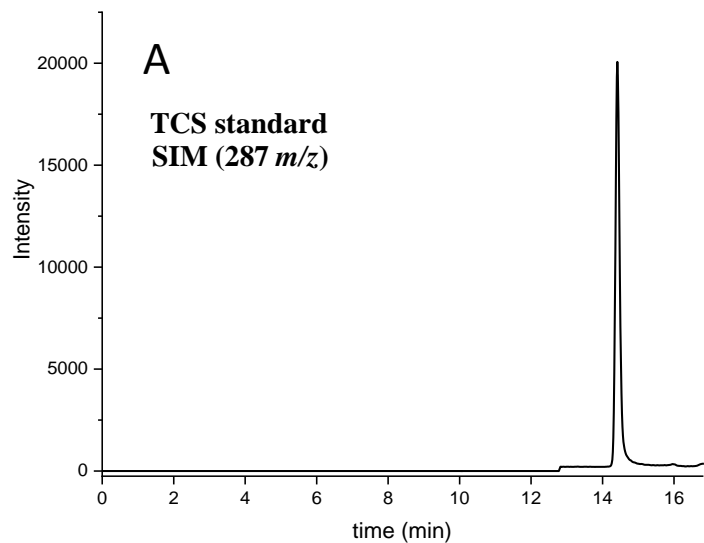


Figure 2

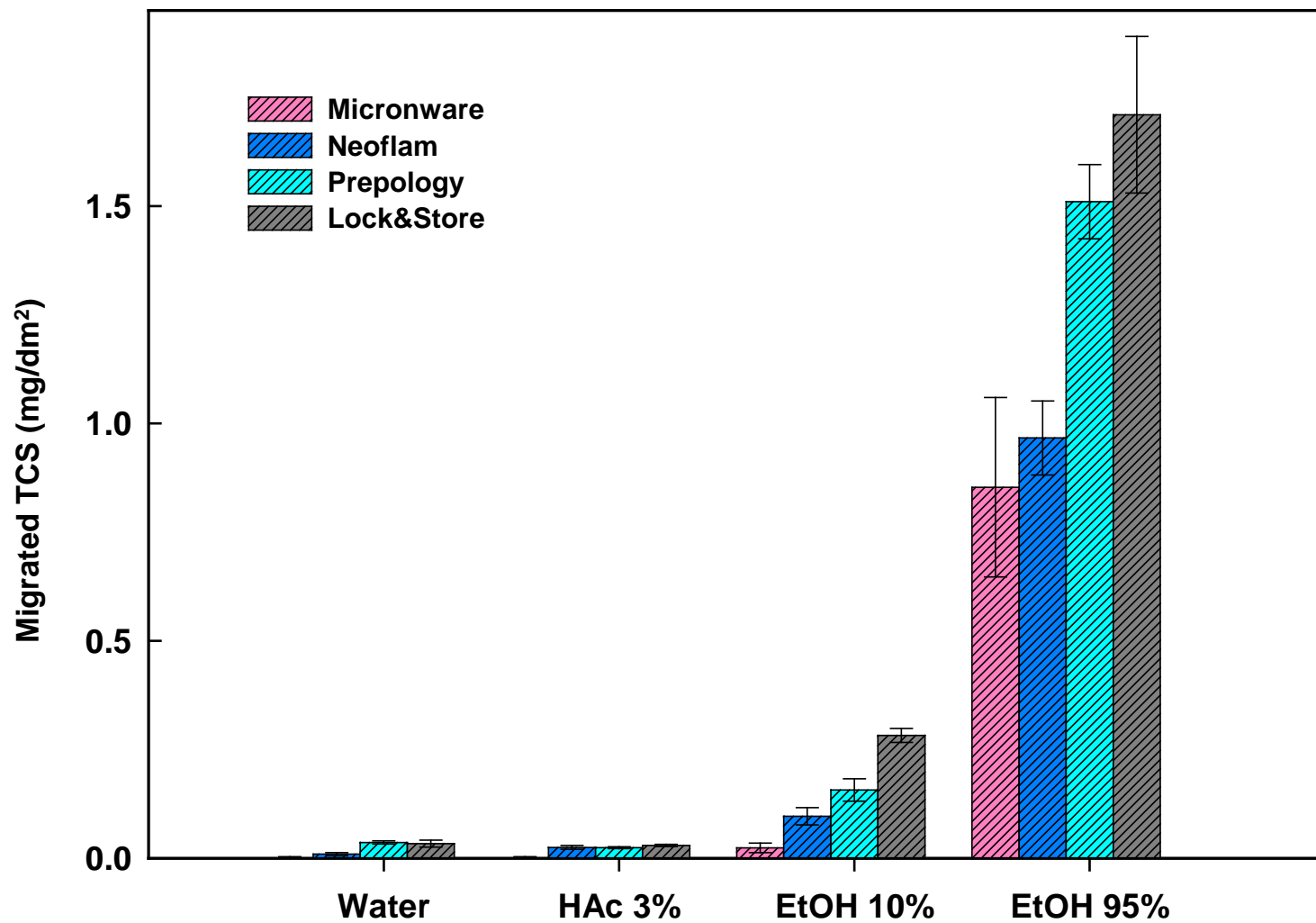


Figure 3

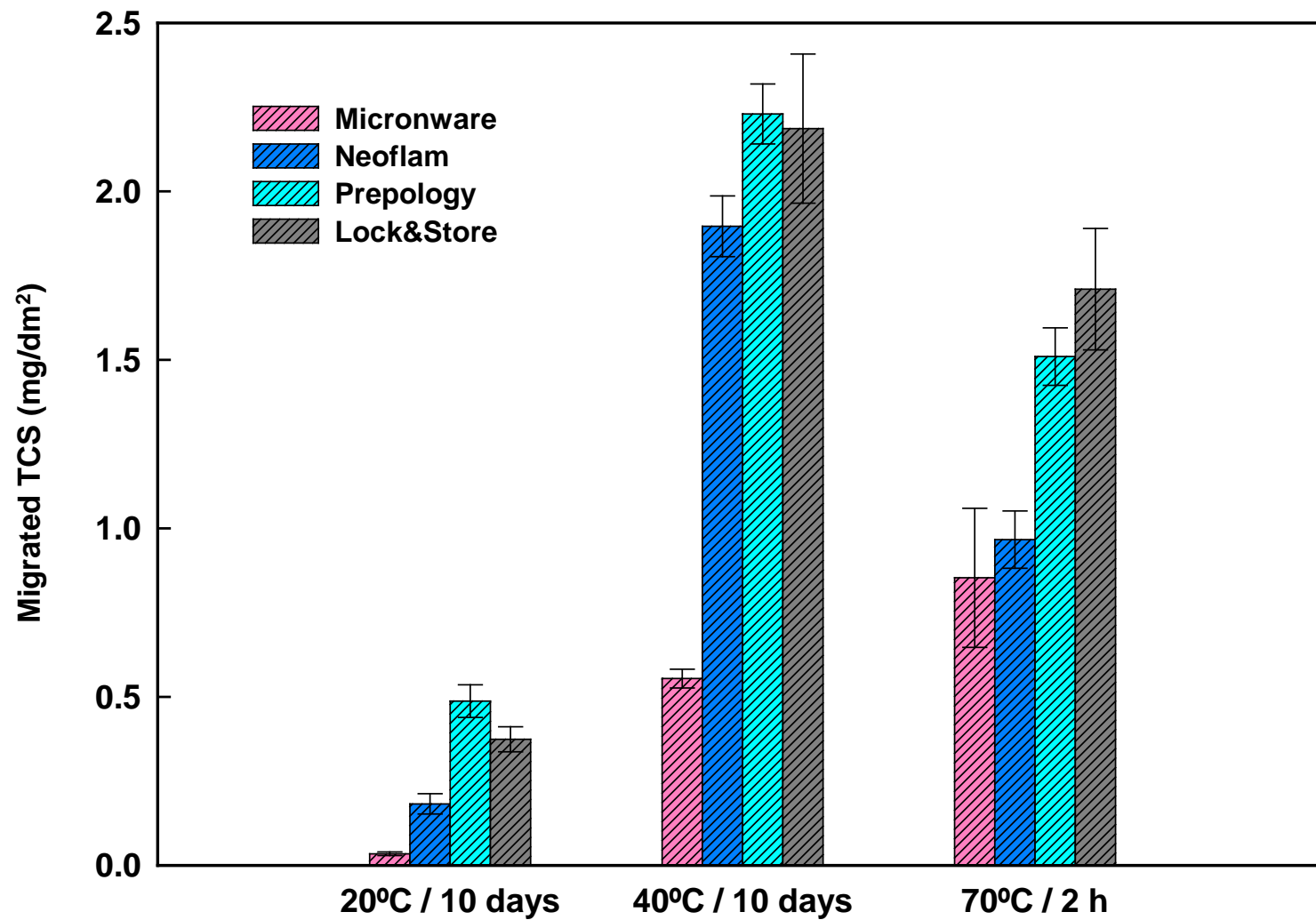


Figure 4

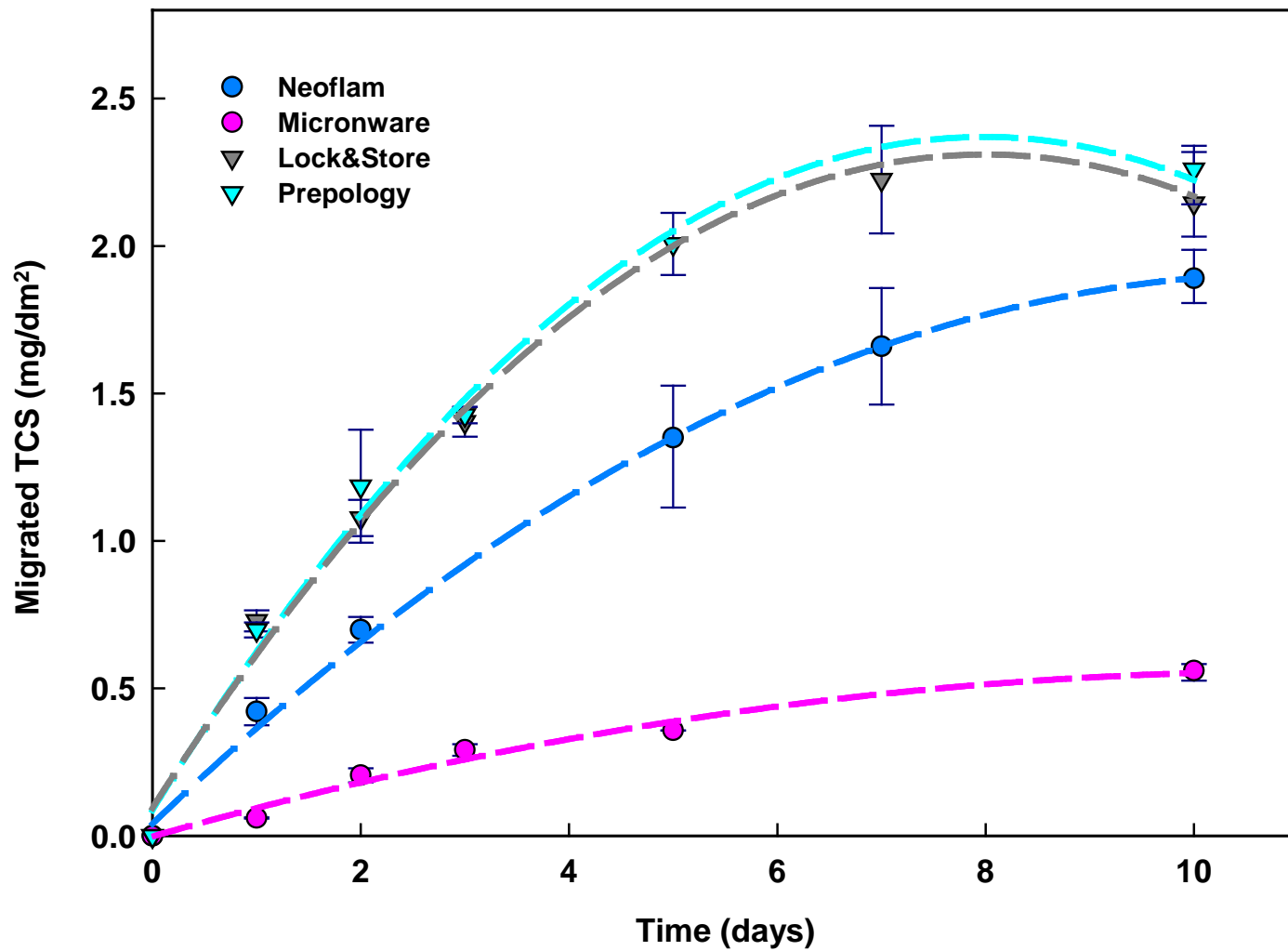


Figure 5

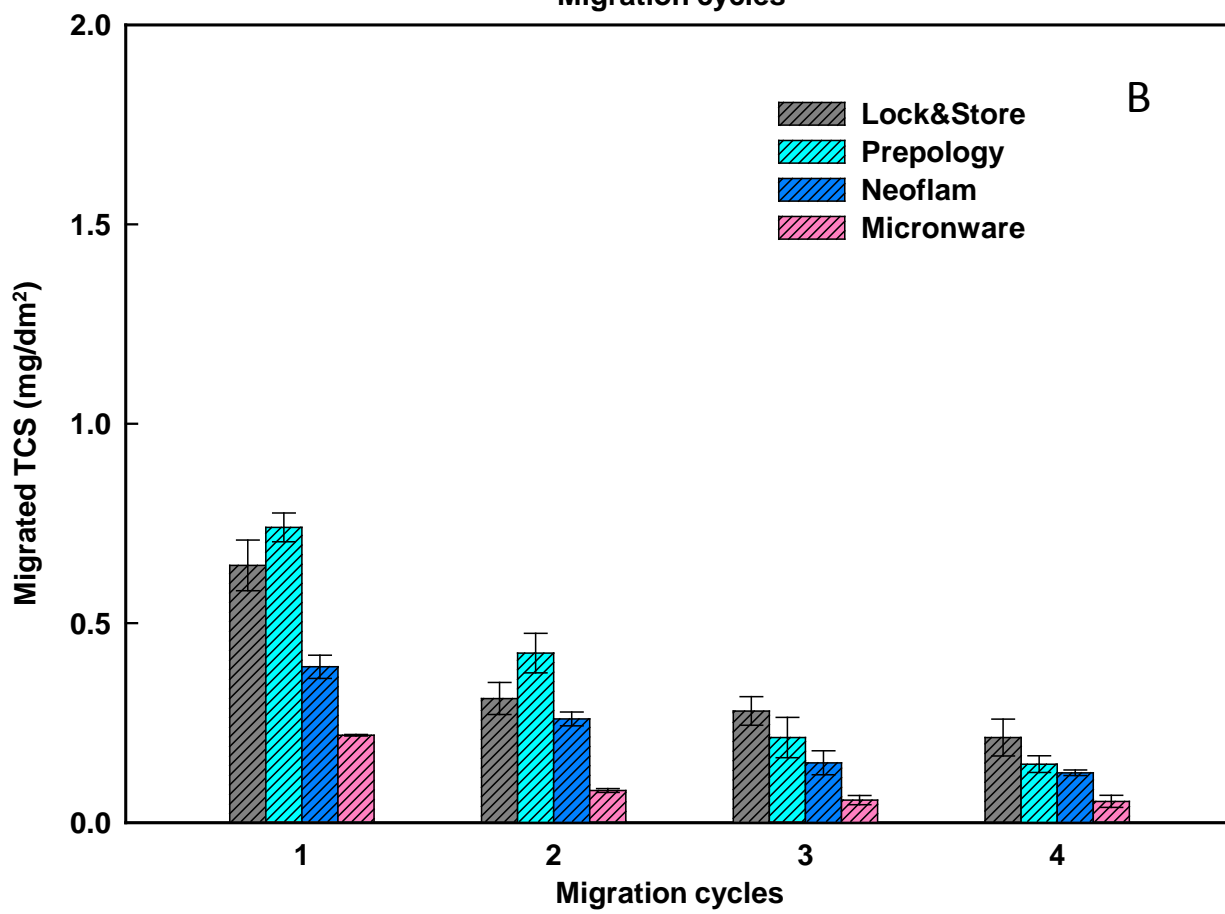
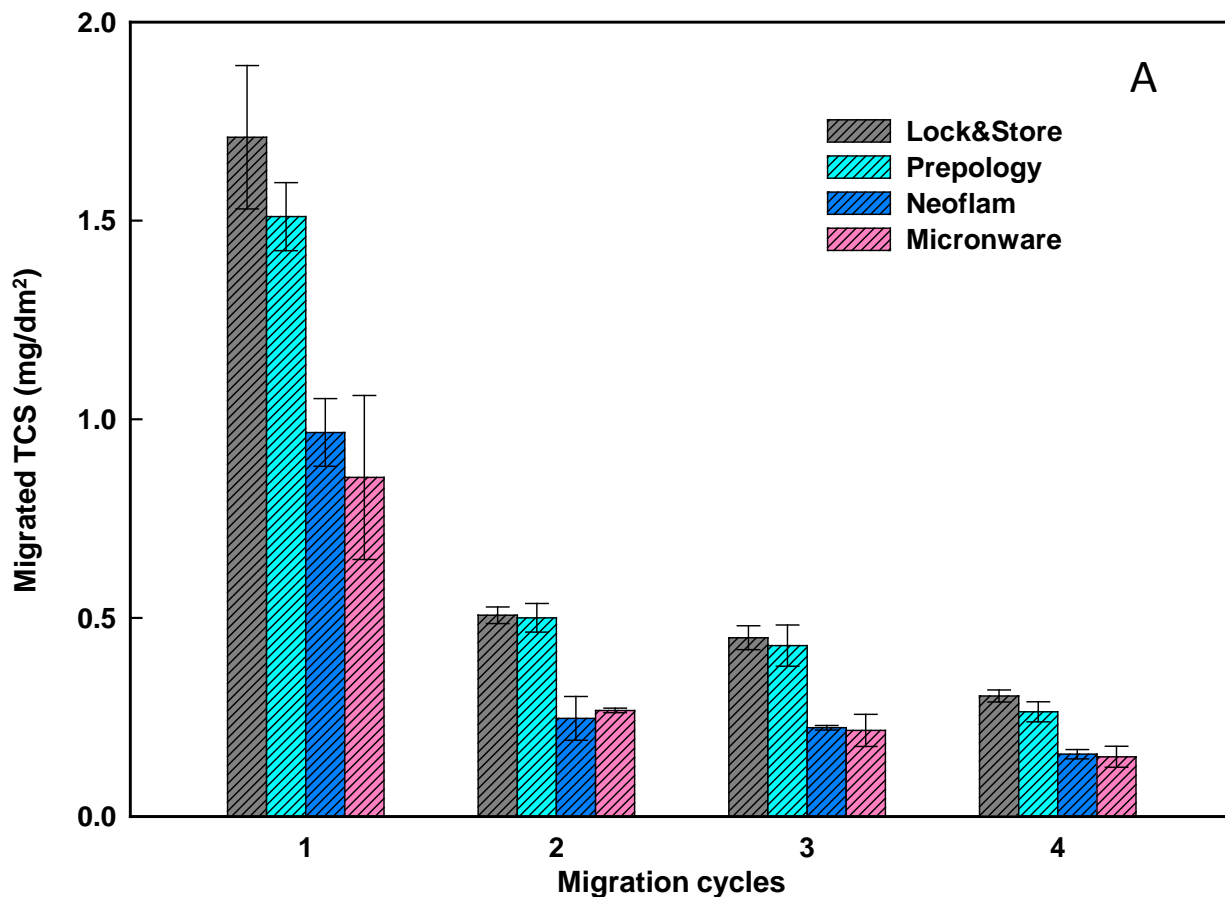


Figure 6

