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1 **Microplastics enhance *Daphnia magna* sensitivity to the pyrethroid insecticide**
2 **deltamethrin: effects on life history traits.**

3

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1 **Abstract**

2 The aim of the study was to investigate the influence of microplastics (MPs) on the ecotoxicity
3 of common contaminants of aquatic ecosystems. As a model contaminant, the hydrophobic
4 pesticide deltamethrin (DM) was chosen, and its effects on life history traits of *Daphnia magna*
5 were studied in the presence or absence of polyethylene MPs. Commercialized DM and MPs
6 obtained as dry powder were used in the experiment. According the manufacturer (Cospheric,
7 Santa Barbara, CA, USA) MPs were spherical (1-4 μm in diameter), had a density of 0.96 g/cm^3
8 and were without any solvent. Three concentrations of polyethylene MPs were tested (0, 1, 10
9 mg/L) with two realistic concentrations of DM (0 and 40 ng/L) and a solvent control (acetone).
10 During the 21 d experiment, *D. magna* neonates were individually exposed to the treatments,
11 and the effects of MPs and DM alone and together were evaluated by assessing survival, number
12 of cumulative molts, days to first brood, number of broods, number of neonates per surviving
13 adult, and body length. Significant detrimental effects on survival were only observed for the
14 two mixture treatments. DM alone (40 ng/L) delayed the days to first brood and reduced the
15 number of neonates per surviving adult, whereas MPs alone (10 mg/L) induced significant
16 reduction in the number of juveniles by surviving adults. The combined exposure to DM and
17 MPs clearly had a synergistic effect on survival, brood number, and number of neonates per
18 surviving female. For example, compared to exposure to 40 ng/L of DM alone, the addition of
19 1 mg/L of MPs resulted in a 51.1% reduction in number of neonates per surviving female and
20 a 46% reduction in brood number. These results suggest the potential drastic effects of this kind
21 of mixed exposure on daphnid populations, which are key components of freshwater food webs.

22

23 **Keywords:**

24 Polyethylene microplastics, deltamethrin, *Daphnia magna*, survival, growth, reproduction,
25 chronic ecotoxicity, interaction, mixture

26

27

28 **1. Introduction**

29 Plastic pollution is a major environmental concern worldwide. According to Geyer et al. (2017),
30 8300 million metric tons (Mt) of virgin plastics have been produced as of 2017, around 76% of
31 which now are plastic wastes. Annual waste production is projected to rise to 3.4 billion Mt in
32 the next 30 years (Silpa et al., 2018). As plastic is designed to be durable, it persists in the
33 environment for long periods of time. Inappropriate dumping of disused or abandoned plastic
34 wastes leads to contamination of the environment, affecting terrestrial, freshwater, and marine
35 ecosystems (Rezania et al., 2017; Chae & An, 2018; Li et al., 2018).

36 Among plastics, macroplastics are defined as large item plastics that are > 5 mm in size,
37 microplastics (MPs) are plastic particles ranging in size from 0.1 μm to 5 mm, and nanoplastics
38 are plastic particles ranging from 1 to 100 nm in size (Bråte et al., 2017). MPs are ubiquitous,
39 and large quantities of MPs have been detected in freshwater, marine, and terrestrial ecosystems
40 (in soil, sediment, water, ice, snow: Rezania et al., 2017; Chae & An, 2018; Wagner & Lambert,
41 2018; Bergmann et al., 2019), even in remote areas (i.e., from the Arctic to Antarctica; Waller
42 et al., 2017; Bergmann et al., 2019). Their presence is related to inputs from other ecosystem
43 compartments (e.g., terrestrial, rivers, sediment relocation from shorelines, wet and dry
44 atmospheric deposition; Chubarenko et al., 2018; Bergmann et al., 2019; Klein & Fisher, 2019;
45 Wetherbee et al. 2019). Concentrations of MPs vary from 10^{-5} to 10^5 MPs/m^{-3} in surface water
46 and from 40 to 400 MPs/L in sediments (reviewed in Li et al., 2018), and concentration in snow
47 samples can be considerable even in remote areas (Bergmann et al., 2019: European sites 154
48 $\times 10^3$ MPs/L , Artic snow : 14.4×10^3 MPs/L). Among MPs found in natural environments,
49 polyethylene (PE) MPs are generally predominant (Wagner & Lambert, 2018; Bergmann et al.,
50 2019). To date, most ecotoxicological studies of MPs have focused on marine organisms,
51 especially on marine fish, which highlights the need to acquire more information about
52 freshwater organisms (de Sà et al., 2018; Wagner & Lambert, 2018).

53 Because MPs consist of numerous types, compositions, sizes, and shapes of plastics, which
54 influence their bioavailability and then their ecotoxicity, results in the literature are highly

55 variable. The most consistent reported effect of MPs was the reduction in natural prey
56 consumption in the presence of MPs, but negative effects on growth, reproduction, and survival
57 were also reported for some taxa (Foley et al., 2018: meta-analysis). Accordingly, many studies
58 reported that MPs are transferred into the aquatic food web (Setälä et al., 2014; Nelms et al.,
59 2018). Ingestion of MPs (number of particles per individual, proportion of individuals ingesting
60 plastic) was taxon- and life stage-specific, and depended on MP size, shape, and aging (Vroom
61 et al., 2017). MPs ingested by organisms (feeding and/or ventilation processes) can have
62 mechanical effects due to their attachment to external surfaces (e.g., reducing mobility and
63 clogging the digestive tract) as well as chemical effects (e.g., inflammation, neurotoxicity,
64 genotoxicity, hepatic stress, oxidative stress, disturbance of energy metabolism) (Setälä et al.
65 2016; de Sà et al., 2018; Wagner & Lambert, 2018; Prokić et al., 2019).

66 MPs also provide large surface areas that can adsorb, concentrate, and transport a range of
67 pollutants in aquatic systems, especially persistent organic pollutants (POPs) and hydrophobic
68 organic chemical (HOCs), and this process affects the mobility and bioavailability of pollutants
69 for organisms (Wagner & Lambert, 2018; Wang et al., 2018). The interactive effect of MPs and
70 other pollutants is problematic for the assessment of risks linked to MPs (Auta et al., 2017;
71 Galloway et al., 2017; Wang et al., 2018). The sorbent capacity of MPs is influenced by the
72 type of polymer and its size and state, stressing the need to conduct more studies on interactive
73 effects, especially for freshwater organisms (de Sà et al., 2018, Wang et al., 2018; Prokić et al.,
74 2019). To date, studies of the interactive effects of MPs and other pollutants have been focused
75 mainly on pollutant accumulation or organism survival, whereas few studies have tested
76 interactive effects on organism growth and reproduction, which are key parameters for
77 population dynamics.

78 Pyrethroid insecticides are considered to be of low acute toxicity to mammals (Grosman &
79 Diel, 2005), but they are highly ecotoxic to insects and non-target organisms (Saha & Kaviraj,
80 2008). They are widely used in households, for field pest control (agriculture, forestry), and for
81 malaria control programs (Villarini et al., 1998; Ansari & Razdan, 2001). According to Mordor

82 Intelligence (2017), pyrethroid use has been increasing during the last two decades, and it
83 accounts for more than 35% of the world market for insecticides. Deltamethrin (DM) is a
84 hydrophobic and synthetic type II pyrethroid that can be found in diverse compartments,
85 including air, water, and sediment, as well as in plants and animals (Pawlisz et al., 1998). DM
86 concentrations in water were reported to range from 0.04 to 24 µg/L in Canadian agricultural
87 areas (Pawlisz et al., 1998) and from 2 to 58.8 ng/L in the Ebro Delta in Spain (Feo et al., 2010).
88 DM primarily acts on the nervous system, especially on the voltage-dependent ion channel in
89 excitable membranes, but it also impacts various secondary targets involved in signal
90 transduction (Narahashi, 1992) and in up and down regulation of the proteome (Toumi et al.,
91 2014). DM is known to be toxic to diverse aquatic organisms (Fojut et al., 2012; US EPA,
92 2013), especially to daphnids, which are the crustacean zooplankton taxon most affected by
93 exposure to DM (Crane et al., 2011; Toumi et al., 2013, 2015).

94 The cladoceran *Daphnia magna* is a standard organism in ecotoxicology studies and is
95 routinely used as a model species to determine ecotoxicity of chemicals for risk assessment
96 (Pereira et al., 2010) due to a suite of interesting features (Baird & Barata, 1998). In addition,
97 *D. magna* was found to be sensitive to a large number of chemicals (Hanazato, 1998) such as
98 pyrethroids (Ren et al., 2009; Toumi et al., 2013). Moreover, ingestion of MPs can have
99 negative effects on *D. magna* (Rehse et al., 2016).

100 The aim of this study was to test the chronic ecotoxicity of DM in the presence or absence
101 of MPs to test the potential interaction between both compounds and assess its outcome on *D.*
102 *magna* life history traits. We first evaluated MP behavior in the water column of the
103 experimental units, and then we analyzed the life history traits of *D. magna* (survival, number
104 of cumulative molts, days to the first brood, number of broods, number of neonates per
105 surviving adult, and body length). Lastly, we assessed the potential sensitivity modification
106 (using a stress on stress study) in juveniles born under exposure to MPs or DM.

107
108

109 **2. Materials and Methods**

110 **2.1. Materials**

111 *2.1.1. DM*

112 DM used in this study was the technical active substance of diverse formulations
113 commercialized by Bayer (DECIS EC25; Leverkusen, Germany). The chemical name of DM
114 ($C_{22}H_{19}Br_2NO_3$) is (s)-Cyano-(3-phenoxyphen) methyl (1R)-cis-3-(2,2-dibromovinyl)-2,2-
115 dimethylcyclopropanecarboxylate). DM is a hydrophobic compound (log Kow = 6.2; Hansch
116 et al., 1995).

117

118 *2.1.2. PE MPs*

119 We used the same MPs as those used by Khan et al. (2015), Batel et al. (2016), and Rehse et al.
120 (2016). They were PE MP particles, 1–4 μm in diameter (clear PE microspheres 0.96 g/cm^3),
121 obtained as dry powder from Cospheric (Santa Barbara, CA, USA). As described by Rehse et
122 al. (2016), i) these particles are well defined with a narrow size distribution and uniform
123 spherical shape, ii) they consist of pure PE material with no additives such as pigments, iii) no
124 solvents are used during manufacturing processes, iv) 1 mg of 1–4 μm particles contains
125 between 3.1×10^7 and 2.0×10^9 particles, and particles tend to v) attach to surfaces and vi) float
126 close to the water surface in the *Daphnia* culture medium they used.

127

128 **2.2. Organisms and breeding**

129 The experiment was conducted using *D. magna* (Straus 1820) identified as clone A by the
130 laboratory of Professor Calow (Sheffield University, UK). This biological material has been
131 maintained for more than 40 years at the LIEC laboratory (University of Lorraine in Metz,
132 France) in parthenogenetic cultures carried out in 1 L aquaria at 20 °C with LCV medium (a
133 20/80 mixture of Lefevre-Czarda (LC) medium (Ionescu et al., 2006) and French (Volvic)
134 mineral water) under a 16-8 h light-dark photoperiod and at a density of 40 animals/L (Manar
135 et al., 2009). The medium was supplemented with a mixture of vitamins (0.1 mL/L) containing

136 thiamine HCl (750 mg/L), vitamin B12 (10 mg/L), and biotin (7.5 mg/L), and was renewed
137 three times a week. During breeding, the organisms were fed with a mixture of three algal
138 species (5×10^6 *Raphidocelis subcapitata*, 2.5×10^6 *Desmodesmus subspicatus*, and 2.5×10^6
139 *Chlorella vulgaris* cells per *Daphnia* per day).

140

141 **2.3. Preparation of stock and test solutions**

142 The medium of each treatment was renewed three times a week (every 2 d). During this step,
143 for each treatment, new exposure media were prepared and placed in another set of tanks. Adult
144 *D. magna* then were gently transferred from the old exposure medium to the renewed exposure
145 medium specific to each replicate number of each exposure treatment.

146

147 *2.3.1. DM*

148 Before each experiment and medium renewal, stock solutions (80 ng DM/L) were prepared by
149 dissolving the pesticide directly in acetone and afterwards in the *Daphnia* medium (LVC, see
150 above). Briefly, 10 mg of DM were dissolved in 20 mL of pure acetone followed by dilution of
151 1/50, then 1/50, 1/5, 1/100, and 1/5 in LVC medium; thus, obtaining a solution of 80 ng DM/L.
152 The acetone control (MP0-DM0ac) was prepared using the same procedure, but without DM.
153 A last dilution of 1/2 was performed in LVC medium containing 20 mg MPs/L or 2 mg MPs/L
154 to obtain MP10-DM40 or MP1-DM40 treatments (final acetone percentage: 8×10^{-7} %; v:v),
155 whereas a 1/2 dilution was performed in LVC medium without MPs to obtain the MP0-DM40
156 treatment.

157

158 *2.3.2. MPs*

159 A bottle containing 200 mg of MPs was transferred to a 2 L-jauged flask. This bottle was rinsed
160 five times with LCV medium. Next, LCV medium was gradually added under homogenization
161 (500 mL by 500 mL). After each medium addition, the flask was ultrasonicated for 15 min to
162 minimize aggregation of particles (cf. Rehse et al., 2016). This stock solution (100 mg MPs/L)

163 was used for the six medium renewals of the experiment. It was kept under agitation during the
164 duration of the experiment (21 d) and ultrasonicated for 15 min every 2 d (before renewal of
165 the medium).

166 All labware was rinsed with LCV medium. To prepare the exposure solutions at each
167 renewal, a solution was prepared from the stock solution using the LCV medium in order to
168 obtain 2 or 20 mg MPs/L. This solution was constantly homogenized during the preparation of
169 MP-DM mixtures.

170 All MP-DM solutions (see Table 1) were prepared immediately before the beginning of the
171 experiment and the medium renewals. The final volume of our experimental units was 50 mL.
172 To prepare the treatment MP10-DM40 (10 mg MPs/L with 40 ng DM/L), we transferred 25 mL
173 of the 20 mg MPs/L solution and, using the same 25 mL-jauged flask, we transferred 25 mL of
174 culture medium containing 80 ng DM/L, algae, and daphnids. To prepare the treatment with 1
175 mg MPs/L, we transferred 2.5 mL of 20 mg MPs/L⁻¹ in a 25 mL-jauged flask and, using the
176 same micropipette, we transferred 22.5 mL of daphnid culture medium. Then, we transferred
177 the content in the experimental unit with 25 mL of daphnid culture medium containing 80 ng
178 DM/L (MP1-DM40) or not (MP1-DM0).

179

180 **2.4. DM and MP analysis**

181 *2.4.1. DM analysis*

182 DM was extracted from exposure solutions with dichloromethane (CH₂Cl₂). After extraction,
183 the solvent was concentrated to 1 mL in a rotary evaporator maintained at 45 °C and 800 mbar
184 to evaporate the CH₂Cl₂. DM was analyzed using a gas chromatograph equipped with an ion
185 trap mass spectrometer (FOCUS-ITQ 700, Thermo Scientific Inc.) in electronic impact mode.
186 Each DM extract was injected (2 µL) in the splitless mode (1 min) on a Varian VF 5 capillary
187 column (30 m × 0.25 mm × 0.25 µm film thickness), and detection was performed in MSMS
188 mode (parent ion 181 m/z; daughter ion: 153 m/z). Detection and quantification limits were
189 calculated for the extract as 0.2 and 0.5 µg/L respectively, with an uncertainty of 8%. After 24

190 and 48 h, nominal concentrations in treatments without MPs were decreased by 45% and 74%,
191 respectively. To limit this concentration variation, and in accordance with the OECD 211
192 (2012) guideline recommendations, media were renewed every 2 d.

193

194 *2.4.2. Measurement of MP concentration*

195 We filtered 770 mL of 20 mg MPs/L on glass microfibre filters (GF/F Whatmann filters; 47
196 mm diameter; porosity 0.7 μm). The filters were weighed before and after filtration (Sartorius
197 analytic A120S; accuracy: 0.1 mg; Goettingen, Germany). We detected a concentration of 19.58
198 mg MPs/L instead of a concentration of 20 mg MPs/L.

199

200 *2.4.3. Assessment of MP behavior*

201 Water was sampled using a micropipette at one centimeter from the surface of the liquid in units
202 without daphnids and algae. We used a spectrophotometer to follow the MP distribution in the
203 water column (Analytikjena Specord 205, Jena, Germany). The most discriminating optical
204 density for following MPs was 900 nm. We measured the absorbance just after solution
205 preparation and after 3 d in experimental units containing 0, 20, 40, 60, 80, and 100 mg MPs/L.
206 There was a linear and significant relationship between MP concentration (x) and absorbance
207 at 900 nm (y; $y = 0.0022x$; $R^2 = 0.9988$). However, after 3 d of rest, MP concentrations in the
208 water column (1 cm from the surface) were 4.92 and 4.78 mg/L in 20 and 40 mg/L solutions,
209 respectively, and 12.6, 10.06, and 10.62 mg/L in 60, 80, and 100 mg/L solutions, respectively.
210 The increase of turbidity along the concentration gradient was less pronounced at the end of the
211 test than at the beginning.

212

213 *2.4.4. MP distribution in the water column*

214 At the beginning and after 2 d in 60 mL test beakers (the same experimental units as used for
215 daphnid exposure) in the absence of algae and daphnids, we measured the absorbance of
216 solutions containing 10 and 20 mg MPs/L at the bottom of the units and at 1 cm from the surface

217 of the liquid (from 190 to 1100 nm; we focused our analyses on 900 nm). No differences were
218 observed between the two sampling zones (10 mg MPs/L: 0.0134 vs. 0.0136; 20 mg MPs/L:
219 0.0236 vs. 0.0241). However, after 2 d of rest, we observed a loss of 50% of MPs in the water
220 column. These MPs were floating on the surface of the water due to their low density. This
221 effect was *de facto* limited because the exposure solutions were renewed every 2 d.

222

223 2.4.5. Assessment of the effect of acetone on MPs

224 As acetone was used to solubilize DM (final acetone percentage: 8×10^{-7} %; v:v), we checked
225 under a microscope (100x and 400x) for the possible alteration of MPs after 2 d in the
226 experimental units (without food and daphnids) in the different treatments (i.e., MP10-DM0,
227 MP10-DM40, MP1-DM0 and MP1-DM40). A drop of water was placed on a microscope slide.
228 Particular attention was paid to homogenizing the water before analysis and to slightly pressing
229 the slide to avoid bias due to thickness of items. Ten fields were chosen randomly at 100×
230 magnification and then viewed at different magnifications from 100× to 400× to detect potential
231 modifications in MP concentration (dissolution), shape (loss in sphericity), and color. No loss,
232 deformation, or abnormality of MPs were observed in the MP10-DM40 and MP1-DM40
233 treatments compared to MP10-DM0 and MP1-DM0 treatments.

234

235 2.5. Chronic ecotoxicity

236 The experimental procedure was conducted in accordance with the OECD 211 (2012) guideline
237 recommendations, except that we did not check the quantity of total organic carbon added due
238 to the food (algae) supply, and we used LCV medium (see Section 2.2) instead of M4 or M7
239 medium.

240 Briefly, *D. magna* were exposed individually in 60 mL glass beakers containing 50 mL of
241 test solution composed of LCV medium with algae and mixtures of DM and MPs at desired
242 nominal concentrations. LCV medium was used because it is the usual culture medium for
243 daphnids (and thus it did not induce stress), and it met the validity criteria for the test (OECD

244 211, 2012). During the test, daphnids were fed with a mixture of three algal species (5×10^6 *R.*
245 *subcapitata*, 2.5×10^6 *D. subspicatus*, and 2.5×10^6 *C. vulgaris* cells per *Daphnia* per day).
246 Temperature was controlled at 20 °C, photoperiod was maintained at 16-8 h light-dark, and the
247 test solution (with food) was renewed every 2 days.

248 The assessment endpoints were survival, number of cumulative molts, days to first brood,
249 number of broods, number of neonates per surviving adult, and body length (measured from
250 the top of the head to the base of the tail spine using the image analysis software Motic Image
251 Plus 2.0 (Motic China Group Ltd, Xiamen, China).

252 To minimize DM adsorption, each exposure vessel was previously saturated for 24 h with
253 the corresponding DM concentration before each test. Seven combinations of experimental
254 conditions were studied (Table 1): a control (LCV medium only, MP0-DM0) and a solvent
255 control (MP0-DM0ac; medium + acetone at 8×10^{-7} %), three MP concentrations (MP: 0, 1,
256 and 10 mg/L) mixed with two DM concentrations (DM: 0 and 40 ng/L). At least ten replicates
257 were carried out for each treatment.

258 In the present study, the DM concentrations used for the experiment were based on the
259 $IC_{20_{21days}}$ value obtained in a previous study (Toumi et al., 2013) in which DM at 40 ng/L
260 exerted no effect on survival, but had slight but significant effects on different sublethal
261 parameters (number of cumulative molts, days to the first brood, and number of neonates per
262 surviving adult). The MP concentrations (1 and 10 mg/L) were selected based on Rehse et al.
263 (2016) in order to test a concentration high enough to cause death and a lower concentration
264 without expected death. This experimental design allowed us to assess the interactive effects of
265 the DM and MPs.

266

267 **2.6. Sensitivity of juveniles born during the experiment: stress on stress experiment**

268 Acute tests with potassium dichromate were performed according to the International
269 Organization for Standardization procedure 6341 (ISO, 1996). Four replicates of five neonates
270 (< 24 h) born during the chronic test under different exposure conditions were placed in glass

271 test tubes containing 10 mL of each of the following test and control solutions: MP0-DM0;
272 MP0-DM0ac; MP0-DM40; and MP1-DM0. The neonates were not fed during the test. The
273 assessment endpoint was immobilization of *D. magna*. The juveniles that were able to swim
274 were considered to be mobile, and those that still moved their antennae, but did not swim within
275 15 sec after gentle shaking were considered to be immobile. This experiment was not possible
276 for the MP1-DM40 and MP10-DM40 treatments because too few juveniles were produced.

277

278 **2.7. Statistical analyses**

279 As very slight and not significant differences were reported between the two controls (MP0-
280 DM0 and MP0-DM0ac: no acetone effect) regardless of the tested endpoint, these two groups
281 were pooled before further analysis (Mann-Whitney test: $p > 0.68$; Table 1). Data analyses were
282 conducted within the R statistical environment (R Core Team, 2013) using the *drc* package for
283 concentration-response modelling. $P < 0.05$ was considered to be statistically significant.

284

285 *2.7.1. Survival*

286 Time-to-event data analysis (Hosmer et al., 2008) was used to investigate the effects of MP and
287 DM concentrations on survival. This approach considers events occurring during the whole
288 study period (not only at the final time point) and takes into account right-censored values (i.e.,
289 organisms for which death was not observed within the study period). Kaplan-Meier curves
290 were used for time-to-event data representation. Log-rank tests were performed to compare
291 survival curves between pairwise treatments (Peto & Peto, 1972). A Bonferroni correction was
292 applied for multiple comparisons ($n = 15$ comparisons). Interaction significance on survival
293 was assessed using the two-way Scheirer-Ray-Hare test on *D. magna* longevity response (time
294 alive). Because this test requires a perfectly balanced design, data were randomly selected to
295 obtain the same number of replicates per treatment based on the lowest number of replicates
296 among treatments (minimum of 13 replicates for longevity data).

297

298 2.7.2. *Number of neonates per surviving female and body length*

299 For these endpoints, the normality of data and homoscedasticity were checked, and
300 subsequently parametric statistical methods were used. To compare the treatments, one-way
301 analysis of variance (ANOVA) was performed followed by *post-hoc* Tukey tests for pairwise
302 comparisons. Next, two-way ANOVA was performed to investigate the effect of the two factors
303 under study (MP and DM) as well as their interaction. Because all organisms in the MP10-
304 DM40 treatment died, data from the 10 mg MP/L treatments (MP10-DM0 and MP10-DM40)
305 were discarded for the two-way analyses.

306

307 2.7.3. *Cumulative number of molts, days to the first brood, and number of broods*

308 For these endpoints, the normality of data and homoscedasticity were not checked, so non-
309 parametric statistical methods were used. To compare the treatments, Kruskal-Wallis tests were
310 performed followed by Mann-Whitney tests with Bonferroni correction for pairwise
311 comparisons. Next, two-way Scheirer-Ray-Hare tests (used as a non-parametric equivalent of
312 two-way ANOVA) were performed to investigate the effect of the two factors under study (MP
313 and DM) as well as their interaction. Because these tests require a perfectly balanced design,
314 data were randomly selected to obtain the same number of replicates per treatment based on the
315 lowest number of replicates among treatments. Because all organisms in the MP10-DM40
316 treatment died, data from 10 mg MP/L treatments (MP10-DM0 and MP10-DM40) were
317 discarded for the two-way analyses.

318

319 2.7.4. *Stress on stress*

320 Acute potassium dichromate LC₅₀s (with 95% confidence interval) were calculated for neonates
321 born during the chronic experiment (see Section 2.6) and further used in the acute toxicity test.
322 For each time point (24 and 48 h) and for each pre-exposure treatment (MP0-DM0, MP1-DM0,
323 MP10-DM0, MP0-DM40), two-parameter log-logistic models were fitted to survival data
324 assuming a binomial error model.

326 **3. Results**327 **3.1. *D. magna* survival**

328 All *D. magna* survived in controls during the 21 d of the experiment (MP0-DM0; Figure 1).
329 This result satisfies one validity criterion of the test ($\leq 20\%$ mortality; OECD, 2012). Exposure
330 to 40 ng DM/L alone (MP0-DM40) or 1 mg MP/L alone (MP1-DM0) for 21 d resulted in
331 slightly lower survival (93.3%), but survival curves did not differ significantly from that of the
332 control. When the two pollutants were combined (MP1-DM40), much lower survival was
333 detected (20%), and the survival curve was significantly different from those of the three
334 previously mentioned ones. Additionally, earlier first event of death (day 5 vs. day 15) was
335 observed in the MP1-DM40 treatment. Survival of *D. magna* in the 10 mg MP/L alone (MP10-
336 DM0) treatment was intermediate (69.2%), but the survival curve was similar to these of the
337 control, MP1-DM0, and MP0-DM40 treatments. However, when 10 mg MP/L was combined
338 with 40 ng DM/L (MP10-DM40), all daphnids were dead by day 9, and this survival curve was
339 highly significantly different from those of the other five curves, again with earlier first event
340 of death (day 3). Survival of *D. magna* was reduced by 78.6% in the MP1-DM40 treatment
341 compared to unique exposure to DM (MP0-DM40) or MP (MP1-DM0). Finally, the combined
342 effect of MP and DM was much greater than the sums of individual exposures (mortality of
343 6.66% [MP1-DM0] and 6.66% [MP0-DM40] vs. 80% [MP1-DM40]; mortality of 31.8 %
344 [MP10-DM0] and 6.66% [MP0-DM40] vs. 100% [MP10-DM40]), which clearly suggests
345 synergy between MP and DM.

346 Significant differences in longevity were detected among the six tested treatments
347 (Kruskall-Wallis ANOVA; $H_{5,90} = 67.18$; $P = 2.45 \times 10^{-13}$). *D. magna* exposed to control
348 treatment lived for the entire 21 d of the experiment, which was similar to the longevity
349 observed for the MP1-DM0, MP10-DM0, and MP0-DM40 treatments, but higher than that of
350 the MP1-DM40 (11.60 ± 5.62 d) and MP10-DM40 (5.15 ± 2.23 d) treatments (Table 1; Figure
351 2a). The two-way Scheirer-Ray-Hare test performed on the six treatments revealed that

352 longevity was significantly affected by DM exposure ($H_{1,77} = 21.81$; $P = 3 \times 10^{-6}$), MP exposure
353 ($H_{2,77} = 30.69$; $P = 2.17 \times 10^{-7}$), and the interaction of MP and DM exposure ($H_{1,77} = 11.08$; P
354 $= 0.0039$).

355 In subsequent sections, data for the MP10-DM40 treatment are not given, as all of the *D.*
356 *magna* died early in the experiment.

357

358 **3.2. Cumulative number of molts**

359 *D. magna* exposed to control treatment during the 21 d experiment underwent eight molts,
360 which was similar to the values for MP1-DM0 and MP10-DM0 treatments, but higher than
361 those of the MP0-DM40 (7.86 ± 0.36) and MP1-DM40 (7.33 ± 0.58) treatments (Table 1;
362 Figure 2b). Significant difference in cumulative number of molts was detected among the five
363 test treatments (Kruskall-Wallis ANOVA; $H_{4,60} = 21.37$; $P = 0.0003$). The cumulative number
364 of molts in the MP1-DM40 treatment was reduced by 6.67% and 8.33% compared to unique
365 exposure to DM (MP0-DM40) or MP (MP1-DM0), respectively. The two-way Scheirer-Ray-
366 Hare test performed on MP0-DM0, MP1-DM0, MP0-DM40, and MP1-DM40 treatments
367 revealed that the cumulative number of molts was not affected by DM exposure ($H_{1,11} = 2.2$; P
368 $= 0.14$), MP exposure ($H_{1,11} = 2.2$; $P = 0.14$), or the interaction of MP and DM exposure ($H_{1,11}$
369 $= 2.2$; $P = 0.14$).

370

371 **3.3. Days to the first brood**

372 In the control treatment (MP0-DM0), the first brood of *D. magna* appeared close to day 11
373 (Table 1; Figure 2c). There was a significant difference in the days to the first brood among the
374 five tested treatments (Kruskall-Wallis ANOVA; $H_{4,67} = 46.42$; $P = 2 \times 10^{-9}$). Mann-Whitney
375 tests followed by Bonferroni correction revealed that compared to control and MP1-DM0
376 treatments, for which values were similar, the days to the first brood of *D. magna* were
377 significantly delayed in MP0-DM40 and in MP1-DM40 treatments, whereas values for the
378 MP10-DM0 treatment fell in the middle and were not significantly different from values in the

379 other treatment. Days to the first brood in the MP1-DM40 group were 14.13% and 26.51%
380 higher compared to single exposures (i.e., MP0-DM40 and MP1-DM0), respectively. These
381 results suggest synergy between MP and DM effects, as days to the first brood for the mixture
382 MP1-DM40 was delayed by 3 d (26.51% longer compared to MP1-DM0 and the control), and
383 the delay was much greater than that observed for MP0-DM40 (1.22 d). The absence of
384 significant difference between days to the first brood in MP0-DM40 and MP1-DM40
385 treatments seems to be partly related to the low survival in the latter treatment (20%). The two-
386 way Scheirer-Ray-Hare test performed on MP0-DM0, MP1-DM0, MP0-DM40, and MP1-
387 DM40 treatments revealed that the endpoint of days to the first brood was significantly affected
388 by DM exposure ($H_{1,15} = 11.36$; $P = 0.0007$), but not by MP exposure ($H_{1,15} = 0.45$; $P = 0.5$) or
389 by interaction of MP and DM exposure ($H_{1,15} = 1.82$; $P = 0.18$).

390

391 **3.4. Number of broods**

392 In the control treatment (MP0-DM0), *D. magna* produced four broods on average during the 21
393 d experiment (Table 1; Figure 2d). There was a significant difference in number of broods
394 among the five tested treatments (Kruskall-Wallis ANOVA; $H_{4,62} = 27.13$; $P = 2 \times 10^{-5}$). Mann-
395 Whitney tests followed by Bonferroni correction revealed that compared to control and MP1-
396 DM0 treatments, for which values were similar, the number of broods of *D. magna* was
397 significantly lower in the MP1-DM40 treatment. Although lower than the control group value,
398 the number of broods in the MP10-DM0 and M0-DM40 treatments did not differ significantly
399 from values in the other groups. The number of broods in the MP1-DM40 treatment was 46%
400 and 50% lower compared to the single exposure groups (M0-DM40 and MP1-DM0),
401 respectively. These results suggest synergy between MP and DM for the following reasons: i)
402 the number of broods of daphnids exposed only to MP (MP1-DM0 and MP10-DM0) were 0%
403 and 8.25% lower than that of the control, respectively; ii) the value for daphnids exposed to
404 DM did not differ significantly from that of the control (M0-DM40: 7.25% lower than control);
405 and iii) the number of broods of *D. magna* exposed to the mixture treatment MP1-DM40 was

406 50% lower than that of the control (2 vs 4 broods). The absence of significant difference
407 between MP0-DM40 and MP1-DM40 seems to be partly related to the low survival in the latter
408 treatment (20%). The two-way Scheirer-Ray-Hare test performed on MP0-DM0, MP1-DM0,
409 MP0-DM40, and MP1-DM40 treatments revealed that the number of broods was significantly
410 affected by DM exposure ($H_{1,59} = 22$; $P = 2.72 \times 10^{-6}$), MP exposure ($H_{1,59} = 12.6$; $P = 0.00039$),
411 and the interaction of MP and DM exposure ($H_{1,59} = 8.58$; $P = 0.0034$).

412

413 **3.5. Number of neonates per surviving adult**

414 In the control treatment (MP0-DM0), the number of neonates produced by surviving females
415 was 93.90 ± 3.84 (Table 1; Figure 2e). This result satisfies one validity criteria of the test (≥ 60
416 neonates produced by surviving female; OECD, 2012). There was a significant difference in
417 the number of neonates produced among the five tested treatments (ANOVA; $F_{4,60} = 37.16$; $P =$
418 2×10^{-16}). *Post-hoc* Tukey tests revealed that compared to the control and MP1-DM0
419 treatments, for which values were similar (difference of 0.64%), the number neonates per
420 surviving adult was significantly lower in the MP10-DM0 (-15.75 %), MP0-DM40 (-19.36%),
421 and MP1-DM40 treatments (-60.6 %), but no significant differences were detected among these
422 three treatments. The number of neonates per surviving adult in the MP1-DM40 treatment was
423 51.13% and 60.85% lower than that in the single exposure (MP0-DM40 and MP1-DM0)
424 treatments, respectively. Again, these results suggest synergy between MP and DM effects
425 because the number of neonates for daphnids exposed to 1 mg MP/L (MP1-DM0) was similar
426 to that of the control, whereas individuals exposed to the mixture MP1-DM40 was 60.85%
427 lower than that of *D. magna* exposed to DM alone (MP0-DM40). The two-way ANOVA
428 performed on MP0-DM0, MP1-DM0, MP0-DM40, and MP1-DM40 treatments revealed that
429 the number of neonates per surviving adult was significantly affected by DM exposure ($F_{1,47} =$
430 50.91 ; $P < 0.0001$), MP exposure ($H_{1,47} = 30.28$; $P < 0.0001$), and the interaction of MP and
431 DM exposure ($H_{1,47} = 11.71$; $P = 0.001294$).

432

433 **3.6. Body length**

434 In the control treatment (MP0-DM0), the length of daphnids after 21 d of exposure was $3.74 \pm$
435 $0.06 \mu\text{m}$ (Table 1; Figure 2f). There was a significant difference in daphnid length among the
436 five tested treatments (ANOVA; $F_{4,60} = 10.81$; $P = 0.0288$), but *post-hoc* Tukey's tests revealed
437 no pairwise differences. Body length in the MP1-DM40 treatment was reduced by 1.14% and
438 2.54% compared to unique exposure (MP0-DM40 or MP1-DM0) treatments, respectively.
439 Again, the lowest value was found in the MP1-DM40 treatment ($3.65 \pm 0.04 \mu\text{m}$; -2.54%),
440 whereas the mean length value was slightly higher in the MP1-DM0 treatment ($+0.2\%$)
441 compared to the control. The mean value was lower in the MP0-DM40 treatment (-1.2%)
442 compared to the control. The two-way ANOVA performed on MP0-DM0, MP1-DM0, MP0-
443 DM40, and MP1-DM40 treatments revealed that body length was significantly affected by DM
444 exposure ($F_{1,47} = 12.5$; $P = 0.000944$), but not by MP exposure ($H_{1,47} = 0.7$; $P = 0.41$) or by the
445 interaction of MP and DM exposure ($H_{1,47} = 1.7$; $P = 0.19$).

446

447 **3.7. Sensitivity of juveniles born during the experimentation: stress on stress experiment**

448 Due to the high mortality in mixture treatments and the low production of juveniles, $LC_{50,24h}$
449 and $LC_{50,48h}$ tests were only performed on the neonates (< 24 h) from parents that survived to
450 the former experiment with MP and DM (Table 2). No significant differences in potassium
451 dichromate LC_{50} were detected among treatments, as indicated by the overlapping 95%
452 confidence intervals. However, the potassium dichromate $LC_{50,48h}$ for the MP10-DM0 and
453 MP0-DM40 treatments were lower than that of the control (0.6 and 0.575 mg/L, respectively,
454 compared to 0.647 for the control MP0-DM0: -7.3% and -11.1% , respectively).

455

456 **4. Discussion**

457 The interaction effects of PE MPs and the pyrethroid insecticide DM on *D. magna* were
458 evaluated by i) analyzing the behavior of MPs, ii) analyzing the life history traits of *D. magna*

459 exposed to MPs, DM, and combinations of the two, and iii) assessing the acute sensitivity
460 modification in juveniles born from parents previously exposed to MPs or DM.

461

462 **4.1. Behavior of DM and MPs**

463 DM nominal concentrations in treatments without MPs decreased over time (24h: -45%; 48 h:
464 -74%). This loss in DM concentration was also reported in Toumi et al. (2013) under the same
465 conditions. This loss was non-exclusively linked to DM degradation and uptake by daphnids,
466 and it was partly counteracted by renewing the medium every 2 d.

467 No loss, deformation, or abnormality of MPs were reported due to use of acetone as the
468 solubilizing solvent for DM concentration preparation. MP concentration assessment was
469 conducted using filtration through glass microfiber filters and weighing, and there was a linear
470 and significant relationship between MP concentration and absorbance. No differences in MP
471 concentration were observed between measurement at the bottom of the units and at one
472 centimeter from the surface of the liquid. The prepared MP concentration was very close to the
473 expected value (i.e., 19.58 vs. 20 mg MP/L; difference of 2.1%). However, after 2 or 3 d of rest,
474 MP concentration in the water column had decreased, and the turbidity along the concentration
475 gradient was less pronounced than at the beginning of the test. This phenomenon is related to
476 floating of MPs on the surface of the water due to MPs density. This trend was also observed
477 by Rehse et al. (2016) using the same MPs. This effect was *de facto* limited because the
478 exposure solutions were renewed every 2 d.

479

480 **4.2. Effects of DM**

481 Acute effects of DM on *D. magna* were previously described by several researchers who
482 reported effective (lethal) concentration for 50% of organisms after 48h of exposure
483 [E(L)C₅₀_{48h}] values ranging from 0.031 to 5 µg/L (see Table 2 in Toumi et al., 2013), which is
484 far above concentrations potentially found in freshwater ecosystems (see introduction: from 2
485 ng/L to 24 µg/L). Toumi et al. (2013), who used the same exposure conditions and the same *D.*

486 *magna* strain, reported a E(L)C50_{48h} of 0.32 µg DM/L. This value is high compared with
487 concentrations known to exert chronic effects. Toumi et al. (2013) also reported a 20 %
488 inhibitory concentration after 21 d of exposure (IC20_{21d}) of 22 ng/L for the number of neonates
489 per surviving adult, 34 ng/L for the cumulative number of molts, 48 ng/L for the number of
490 broods, 152 ng/L for the population growth rate, and 160 ng/L for the longevity. They reported
491 the lowest 21 d No-Observed-Effect Concentration (NOEC) as 9 ng /L which was in the same
492 range as values reported by McNamara (1991; 4–9 ng/L) and the NOEC reported by Hanson et
493 al. (2007) for freshwater communities using mesocosms (9.6 ng/L). In the present study,
494 significant chronic effects of DM (MP0-DM40) included reduced longevity, number of broods,
495 number of neonates per surviving adult, and length as well as delayed days to first brood. These
496 results were in accordance with Toumi et al. (2013), who reported the same significant
497 responses for the same daphnid strain (strain 1) exposed to the same concentration. Finally, our
498 results revealed that the choice of 40 ng DM/L as the test concentration was relevant, as it had
499 no effect on survival (and a very slight effect on longevity), but slight and/or significant sublethal
500 effects on other parameters (see Section 2.5).

501

502 **4.3. Effects of MPs**

503 Many authors have reported that toxicity of MPs differs due to their composition, shape, age,
504 and size (Ma et al., 2016; de Sà et al., 2018), as well as the species or the clone considered
505 (Imhof et al., 2016; Foley et al., 2018). In *D. magna*, ingestion of MPs is concentration- and
506 exposure time-dependent (Scherer et al., 2017; Canniff & Hoang, 2018). According to Scherer
507 et al. (2017), the presence of natural particles (including food, such as algae) reduced MP
508 uptake. They also found that *D. magna* consumed 1 and 10 µm MPs depending of their life
509 stage (larger daphnids ingested more and larger MPs), but not 90 µm MPs, and that *D. magna*
510 ingested more MPs (up to 6180 particles/h) than other organisms due to their filtering feeding
511 mode of food acquisition.

512 In the present study, the significant effects on *D. magna* induced by MPs alone were reduced
513 longevity, number of broods, and number of neonates by surviving adults. The only difference
514 found by pairwise comparisons of MP0, MP1, and MP10 treatments was a significant reduction
515 in number of neonates by surviving adults, and this was only for the highest tested MP
516 concentration (10 mg/L compared to the control; gap of -15.75%). No significant differences
517 were observed between the 1 mg MP/L treatment compared to the control, whereas exposure
518 to 10 mg MP/L led to a slight, but not significant decrease in survival (69.2% vs. 100%; gap of
519 -30.8%), longevity (21 ± 0 vs. 20.6 ± 1.55 d; gap of -1.9 %), number of broods (3.67 ± 0.50
520 vs. 4 ± 0 ; gap of -8.25%), and length (3.706 ± 0.052 vs. 3.736 ± 0.061 μm ; gap of -0.8%) and
521 to an increase in days to the first brood (11.69 ± 0.85 vs. 11.05 ± 0.22 ; gap of + 5.79%). Reshe
522 et al. (2016), who used the same MPs in a more acute test (4 vs. 21 d in our study), reported a
523 $E(L)C_{50-96h}$ of 57.43 mg/L for *D. magna*.

524 Some reports described detrimental effects related to organic pollutants added during plastic
525 production (Bakir et al., 2014b; Wagner & Lampert, 2018). However, confounding effects due
526 to additives were not an issue in our study because the manufacturer did not use them in the
527 production of the MPs used in our experiments. Even at higher concentrations than the ones we
528 tested, many researchers reported weak or absent effects of MPs on *D. magna* reproduction,
529 growth, and survival; however, they often reported other confounding factors, such as aging,
530 food presence, relative abundance, and organism age. For example, Ma et al. (2016) observed
531 no effect (immobilization) on *D. magna* exposed for 48 h to polystyrene MPs at sizes of 5, 10,
532 or 15 μm , even at concentration up to 100 mg/L, but they found a strong effect of
533 polystyrene nanoplastics on immobilization of *D. magna*. This result highlights the
534 importance of MP size on toxicity. Rist et al. (2017) reported that exposure of *D. magna* to 1
535 mg/L of 1 μm polystyrene MPs had no effect on feeding rate, reproduction (time to first
536 offspring, number of broods, number of neonates per brood), growth (number of molts, size of
537 the animals at the end of the test), and mortality. Although the gut of *D. magna* was filled with
538 green fluorescent PE microbeads (size range: 63–75 μm ; particle density: 0.99–1.01 g/cm^3), no

539 effects of MPs on *D. magna* survival and reproduction were reported for concentration between
540 25 and 100 mg/L (Canniff & Hoang, 2018). Imhof et al. (2017) found that a mixture of four
541 MP polymers had no effects on mortality, reproduction, and morphology (body length, body
542 width, and tail spine length) of adult *D. magna*, but they had limited and significant effects on
543 juvenile morphology (body length, body width, and tail spine length). After 48 h of exposure,
544 adult *Daphnia* exhibited alterations in expression of genes related to stress responses (HSP60,
545 HSP70, and GST) and body function and composition (sarco(endo)plasmic reticulum calcium
546 ATPase) (Imhof et al., 2017).

547 MPs can induce a reduction in natural prey consumption (Foley et al., 2018), but many
548 studies reported the effect of food quantity or food/MP ratios on MP toxicity. For example,
549 Aljaibachi and Callaghan (2018) tested polystyrene MPs and reported that daphnid mortality
550 and reproduction were linked to availability of food rather than to MP concentration. Using the
551 same MPs as those used in this study, Ogonowski et al. (2016) showed that at high concentration
552 relative to the feeding suspension (10^5 particles/mL contributing to 74% of suspended material),
553 exposure of *D. magna* to secondary MPs led to high mortality, longer inter-brood period, and
554 lower reproduction, whereas no effects were reported for the control treatment and primary
555 MPs. Jemec et al. (2016) showed that ground PE terephthalate textile microfibers increased
556 immobilization/mortality of *D. magna* after 48 h only when daphnids were not pre-fed with
557 algae prior to the experiment (20–40% at exposure doses from 12.5 to 100 mg/L, with the
558 maximum value for an exposure of 25 mg/L). If daphnids were fed before the experiments, no
559 effect was reported.

560 In contrast, Canniff and Hoang (2018) observed that the algae *Raphidocelis subcapitata*
561 grew more in the presence of MPs, suggesting that MPs could serve as substrates for *R.*
562 *subcapitata* growth; subsequent intake of MPs by *R. subcapitata* could indirectly provide
563 energy for survival and reproduction. Organisms serving as prey to larger predators (such as
564 zooplankton) may be particularly susceptible to negative impacts of MPs, and they could have
565 potential impacts throughout the food web (Foley et al., 2018) because MPs move up the food

566 chain from lower to higher trophic organisms (Hollman et al., 2013; Setälä et al., 2014; Nelms
567 et al., 2018).

568

569 **4.4. Interactive effects of MPs and DM**

570 In the present study, a significant interaction (synergy) between MPs and DM was found for *D.*
571 *magna* longevity. Significant differences in longevity between single exposures to MPs or DM
572 and mixtures were observed: a reduction of 78.6% in the MP1-DM40 treatment was observed
573 compared to MP0-DM40 or MP1-DM0 treatments, and 100% mortality within 9 d occurred in
574 the MP10-DM40 treatment. In contrast, mortality was only 30.8% and 6.7% in the MP10-DM0
575 and MP0-DM40 treatments, respectively.

576 A synergistic effect was also reported for number of broods and number of neonates per
577 surviving adult. No differences were observed among the control and the unique exposure to
578 DM or MP treatments, except for number of neonates per surviving adult, for which a
579 statistically significant 19.36% reduction was detected between control and MP0-DM40
580 treatments. Compared to the MP0-DM40 and MP1-DM0 treatments, exposure to the mixture
581 MP1-DM40 resulted in a reduction up to 50% in number of neonates per surviving adult and a
582 reduction of close to 50% in number of broods. Other tested endpoints showed the same trends,
583 but effects were not detected through Sheirer-Ray-Hare tests and two-way ANOVA because
584 the number of replicates was very low (3 to 13). Compared to unique exposure to DM or MP,
585 exposure to the mixture MP1-DM40 resulted in the following: i) 14.13% and 26.51% increase
586 in days to the first brood, respectively; ii) 6.67% and 8.33% reduction in cumulative number of
587 molts; and iii) 1.14% and 2.54% reduction in body length. As is often the case, the number of
588 neonates per surviving adult was the most sensitive tested endpoint.

589 Batel et al. (2016) used the same MPs as those used in our study and showed that food-
590 borne MP-associated POPs (benzo[a]pyrene) can desorb in the fish intestine and then be
591 transferred to the intestinal epithelium and liver. Bakir et al. (2014a) showed that
592 polyvinylchloride and PE MPs can sorb and desorb C-14-DDT, C-14-phenanthrene (Phe), C-

593 14-perfluorooctanoic acid, and C-14-di-2-ethylhexyl phthalate. According to the authors,
594 desorption rates were faster under simulated gut conditions compared to seawater (could be up
595 to 30 times greater). Rochman et al. (2013a) showed that chemicals sorbed on MPs (marine
596 fragments containing polycyclic aromatic hydrocarbons, polychlorinated biphenyls, and
597 polybrominated diphenyl ethers) were ingested by the marine fish *Oryzias latipes* through
598 vectorization by MP particles; they then bioaccumulated and induced pathological and
599 oxidative stress as well as inflammation of the liver. When the fish exposed to PE MPs with
600 low adsorbed chemicals (control) also showed signs of stress, they were less severe than in fish
601 fed marine PE MPs.

602 Synergistic interaction effects of chemicals and MPs on aquatic organisms was reported in
603 many studies. For example, Pacheco et al. (2018) showed that a mixture of high concentrations
604 of 5 nm gold nanoparticles and 5 μm red microsphere MPs induced mortality that was higher
605 than the sum of the lethal effects induced by gold nanoparticle and MPs alone. However, they
606 also reported an antagonistic relationship on mortality when daphnids were exposed to a low
607 concentration of the mixture as well as more pronounced effects than each type of particles
608 when tested individually. Mixture treatments also damaged some reproduction endpoints (e.g.,
609 resulting in a higher number of aborted eggs), suggesting toxicological interactions. Toumi et
610 al. (2018) also reported differences in type of interaction (antagonism, synergy) related to low
611 or high exposure concentration. Kim et al. (2017) described the interactive effect of a mixture
612 of polystyrene MPs and nickel on *D. magna* immobilization, but the type of interaction
613 depended on the MPs used in the mixture: a slight synergistic effect was observed when using
614 MPs coated with a carboxyl group, but a slight antagonistic effect was detected when using
615 MPs without the carboxyl group coating. However, Horton et al. (2018) found no interactive
616 effect when *D. magna* was acutely and conjointly exposed to polystyrene MPs and pesticides
617 (dimethoate: 0.15–5 mg/L or DM: 0.016–10 $\mu\text{g/L}$). They concluded that polystyrene MPs are
618 unlikely to act as a significant sink or as a vector for increased uptake of pesticides by aquatic
619 organisms. There are several potential explanations for this difference from our results. For

620 example, Horton et al. (2018) used polystyrene MPs, which have lower affinity for hydrophobic
621 chemical sorption than PE (Rochman et al., 2013b, Wang & Wang, 2018; Wagner & Lambert,
622 2018). Additionally, they conducted an acute test rather than a chronic one. Finally, they tested
623 the interaction between polyamide MPs and bisphenol on immobilization of daphnids, Reshe
624 et al. (2017) showed that chemical uptake through MPs is negligible compared to pollutant
625 uptake through water for acute tests at high tested concentrations.

626 A growing body of studies illustrates the potential for plastic debris to sorb, concentrate,
627 and transport POPs and metals (Bakir et al., 2014b; Wagner & Lambert, 2018; Prozić et al.,
628 2019). Thus, MPs are considered to be a vector for hazardous HOCs (such as DM) in the aquatic
629 environment, and therefore, as an important potential exposure route as well (Browne et al.,
630 2013; Teuten et al., 2007). However, because MPs currently are less represented in the
631 environment than other media that transfer HOC (e.g., colloids, phytoplankton, zooplankton),
632 Koelmans et al. (2019) reported that the fraction sorbed by plastic remains low compared to the
633 fraction held by other media. Nevertheless, MPs represent a new route of exposure for aquatic
634 organisms that merits further study.

635 In the present study, PE microsphere MPs were used as a standardizable model to assess
636 the interactive effect of MPs and DM on life history traits of *D. magna*. PE MPs are
637 predominantly found in aquatic ecosystems, but more as fragments linked to secondary
638 production than as microspheres (Wagner & Lambert, 2018). We tested higher MP
639 concentrations than those found in the natural environment, but MP concentrations in the field
640 are expected to increase in the near future due to atmospheric deposition, especially in lentic
641 environments. Clear interactive effects of MPs and DM were found without any confounding
642 effects linked to the food/MP ratio, as the ratios were comparable among treatment groups with
643 or without exposure to DM. Although a recent WHO report (WHO, 2019) noted the absence of
644 risk linked to the presence of MPs in drinking water, it is important to highlight that
645 micropollutants in drinking water and water in natural ecosystems were not analyzed at the
646 ng/L level and that a lot of the water is consumed by organisms throughout their life (i.e.,

647 chronic exposure). Therefore, caution is needed because the effects of micropollutant can be
648 strongly enhanced in presence of MPs.

649 To better assess the risk linked to pollutant vectorization by MPs, further studies are needed.
650 They should include more environmentally realistic MP concentrations, shapes/ages (fragments
651 secondarily produced), and exposure conditions (e.g., mesocosms, in situ). Additionally, more
652 pollutants, chemical and biological species, and effect of/on trophic routes should be considered.

653

654 **5. Conclusions**

655 MPs encompass a family composed of numerous types, compositions, sizes, and shapes of
656 plastics, and all of these factors influence their bioavailability and ecotoxicity. Recent studies
657 highlighted the capacity of MPs to vectorize toxicants due to their sorbing ability. The present
658 study reports (for mortality, longevity, number of broods, and number of neonates per surviving
659 adult) or suggests (for cumulative number of moult) a synergistic effect of DM and PE MPs on
660 *D. magna*. Life history traits of *D. magna* were dramatically modified by exposure to a mixture
661 of DM and MPs compared to exposure to MPs or DM alone. The detrimental effects of this
662 synergy on daphnid survival, growth, and reproduction suggest potential drastic effects of these
663 kinds of mixtures on daphnid populations, which are at the base of aquatic food chain. Literature
664 suggests that MPs can reach all ecosystem on Earth through wet and dry atmospheric
665 deposition. Thus, MP concentration in ecosystems with high residence time (such as lentic
666 ecosystems) will increase over time, especially as plastic wastes will also increase over time.
667 Hydrophobic micropollutants (defined as xenobiotic chemicals that have strong effects at low
668 concentrations, such as pesticides and POPs) have a new route of exposure through ingestion
669 or inhalation of MPs, which could pose a serious threat to organisms and ecosystems. In our
670 study, sublethal effects of DM on daphnids were observed at ng/L concentrations. The same
671 exposure in combination with MPs caused greater lethal effects. Therefore, caution is needed
672 because the effects of micropollutants can be strongly enhanced by the presence of MPs.

673

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679

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858 distinct population segment, and delta smelt (*Hypomesus transpacificus*), and the federally
859 endangered California clapper rail (*Rallus longirostris obsoletus*), California freshwater
860 shrimp (*Syn caris pacificus*), California tiger salamander (*Ambystoma californiense*),
861 Sonoma county distinct population segment and Santa Barbara county distinct population
862 segment, San Francisco garter snake (*Thamnophis sirtalis tetrataenia*), and tidewater goby
863 (*Eucyclogobius newberryi*) available at <[https://www.epa.gov/espp/litstatus/effects/redleg-](https://www.epa.gov/espp/litstatus/effects/redleg-frog/2013/deltamethrin/analysis.pdf)
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Figure 1: Kaplan-Meier curves for the time-to-death of organisms exposed to 0, 1, or 10 mg MPs/L with 0 or 40 ng DM/L. The plus sign (+) at the end of curves represents censored data. Overall, out of 91 organisms, 60 were right-censored. The numbers after DM and MP in the combined treatment correspond to deltamethrin (DM) and microplastic (MP) concentrations, respectively (in ng/L and in mg/L, respectively). Different lowercase letters indicate significantly different survival curves after the Log-rank test with Bonferroni correction ($n = 15$; $p < 0.00333$).

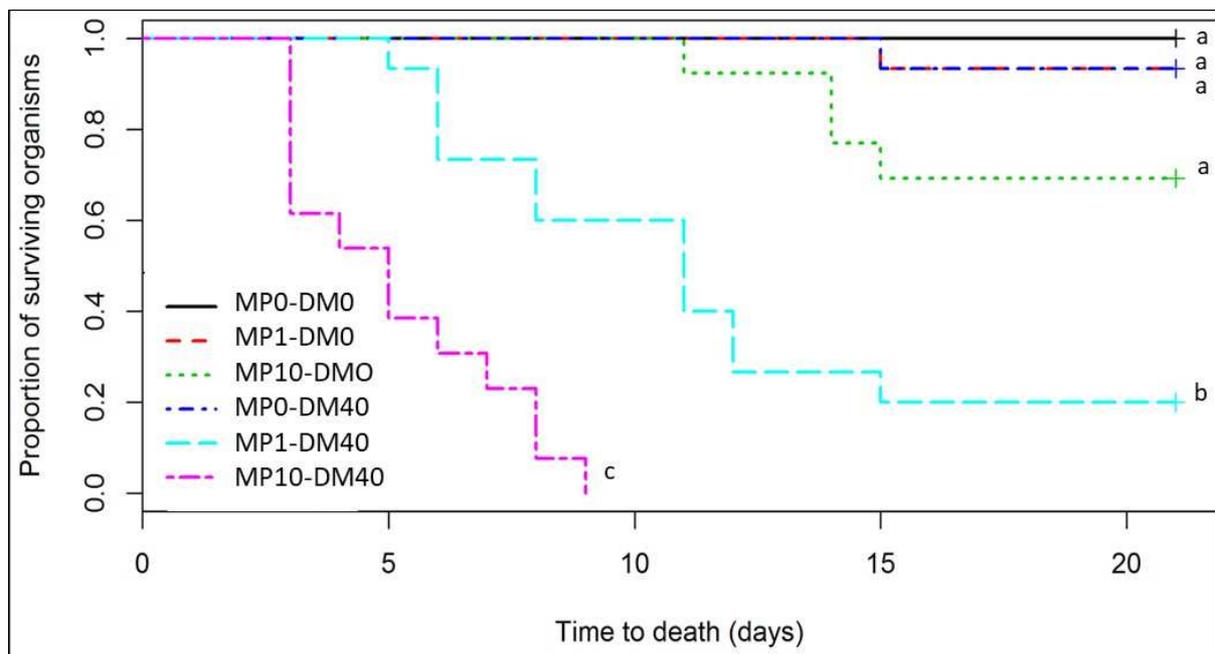
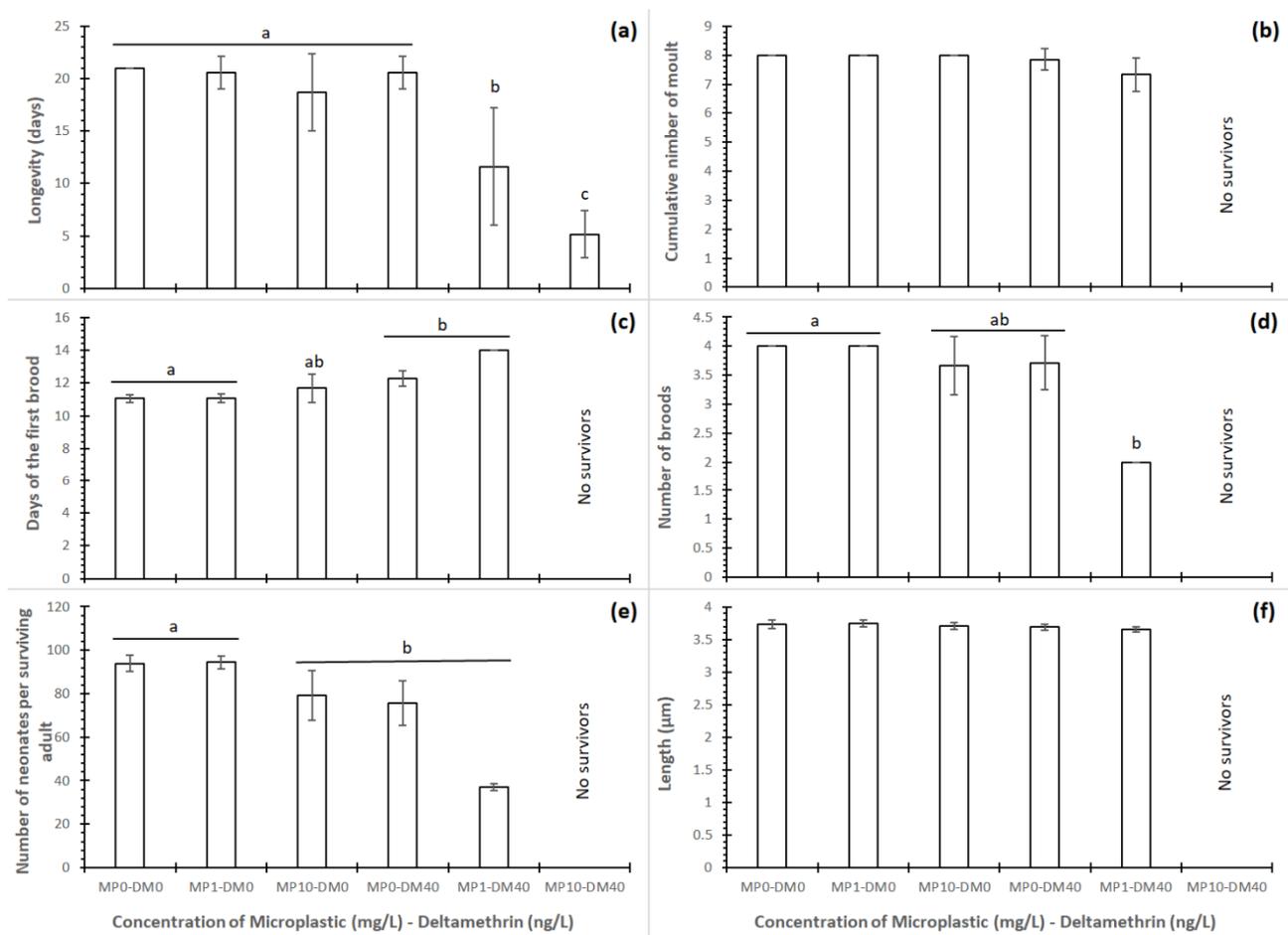


Figure 2: Mean (\pm standard deviation) of longevity (a), cumulative number of molts (b), days to the first brood (c), number of broods (d), number of neonates per surviving adult (e), and length (f) of *D. magna* exposed to MPs and DM for 21 days. The numbers after MP and DM in the combined treatment correspond to MP and DM concentrations, respectively (in mg/L and in ng/L, respectively). No significant differences were observed between the control (MP0-DM0) and solvent control (with only acetone MP0-DM0ac), so the data were pooled to constitute the control (MP0-DM0). Different lowercase letters indicate significantly different groups after *post-hoc* Tukey's tests ($p < 0.05$: for length, number of neonates) or the Mann-Whitney test with Bonferroni correction ($n = 15$; $p < 0.00333$; longevity, cumulative number of molts, days to the first brood, number of broods). No *D. magna* survived in the MP10-



DM40 treatment.

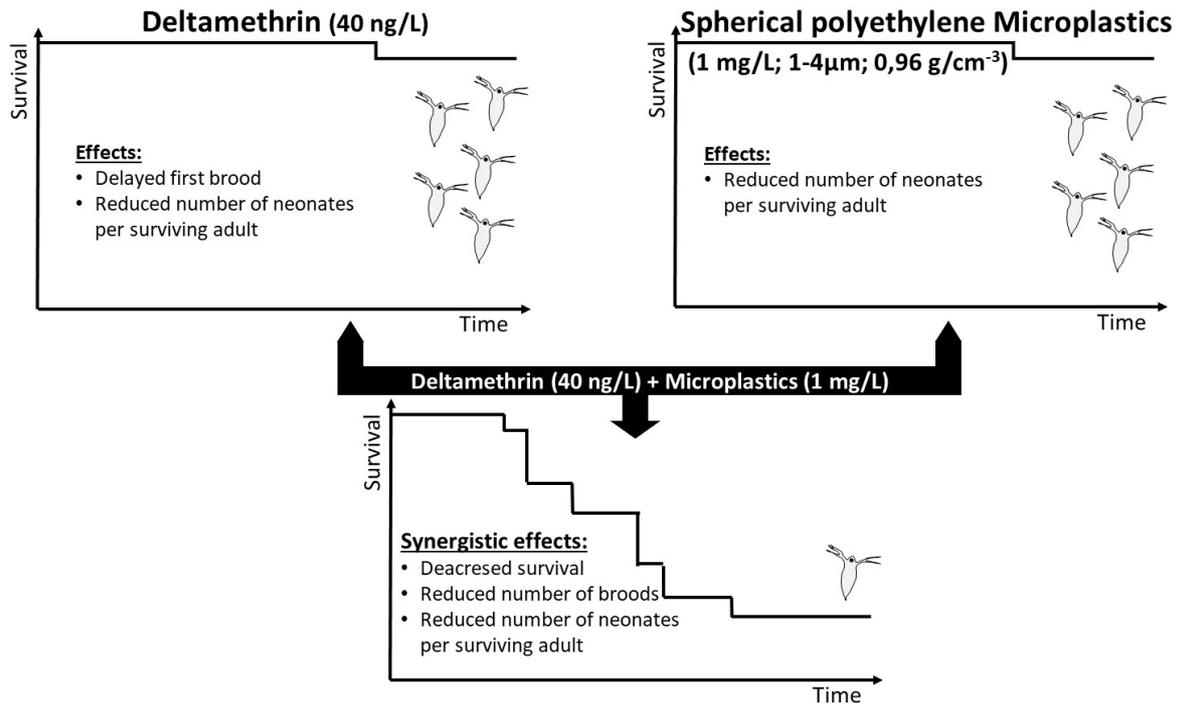
Felten *et al.*, Figure 2

Table 1: Mean (\pm standard deviation) of longevity, cumulative number of molts, days to the first brood, number of broods, number of neonates per surviving adult, and length of *D. magna* exposed for 21 d to MPs and/or DM. The numbers after MP and DM in the combined treatment groups correspond to MP and DM concentrations, respectively (in mg/L and in ng/L, respectively). DM0ac is the solvent control treatment containing only acetone. Ns: no significant differences between the two controls (MP0-DM0 and MP0-DM0ac), which were pooled to constitute the control (MP0-DM0). For the considered endpoints, different lowercase letters indicate significantly different groups after *post-hoc* Tukey's tests ($p < 0.05$: for length, number of neonates per surviving adult) or the Mann-Whitney test with Bonferroni correction ($n = 15$; $p < 0.00333$; longevity, cumulative number of molts, days to the first brood, number of broods).

Labels	Microplastics (mg/L)	Deltamethrin (mg/L)	Survival (%)	Living daphnids after 21 days	Longevity (days)	Number of cumulative molts	Days to the first brood	Number of broods	Number of neonate per surviving adult	Length (μm)
<i>Controls</i>										
MP0-DM0	0	0	100	10	21.0 \pm 0.00	8.00 \pm 0.00	11.00 \pm 0.00	4.00 \pm 0.00	94.10 \pm 4.25	3.733 \pm 0.055
MP0-DM0ac	0	0	100	10	21.0 \pm 0.00	8.00 \pm 0.00	11.10 \pm 0.32	4.00 \pm 0.00	93.70 \pm 3.59	3.738 \pm 0.069
<i>Mean (MP0-DM0)</i>	0	0	100	20	21.0 \pm 0.00	8.00 \pm 0.00	11.05 \pm 0.22	4.00 \pm 0.00	93.90 \pm 3.84	3.736 \pm 0.061
<i>Treatments</i>										
MP1-DM0	1	0	93.3	14	20.6 \pm 1.55	8.00 \pm 0.00	11.07 \pm 0.26	4.00 \pm 0.00	94.50 \pm 3.01	3.745 \pm 0.051
MP10-DM0	10	0	69.2	9	18.7 \pm 3.71	8.00 \pm 0.00	11.69 \pm 0.85	3.67 \pm 0.5	79.11 \pm 11.42	3.706 \pm 0.052
MP0-DM40	0	40	93.3	14	20.6 \pm 1.55	7.86 \pm 0.36	12.27 \pm 0.46	3.71 \pm 0.47	75.71 \pm 10.18	3.692 \pm 0.049
MP1-DM40	1	40	20	3	11.6 \pm 5.62	7.33 \pm 0.58	14.00 \pm 0.00	2.00 \pm 0.00	37.00 \pm 1.73	3.650 \pm 0.040
MP10-DM40	10	40	0	0	5.15 \pm 2.23	no survivor				

Table 2: LC₅₀ values and their 95% confidence intervals for neonates (whose parents were exposed to MPs or DM) after 24 h (LC50_{24h}) and 48 h (LC50_{48h}) of exposure to potassium dichromate. No mixture (MP + DM) effects were tested because mortality was too high under these conditions. The numbers after MP and DM in the combined treatment correspond to MP and DM concentrations, respectively (in mg/L and in ng/L, respectively). MP0-DM0ac is the solvent control treatment containing only acetone.

Treatment (Microplastic-Deltamethrin)		MP0-DM0	MP0-DM0ac	MP1-DM0	MP10-DM0	MP0-DM40
LC50_{24h}		0.834	0.830	0.815	0.855	0.873
95 % confidence interval	97.5%	0.881	0.883	0.877	0.906	0.921
	2.5%	0.788	0.777	0.751	0.804	0.825
LC50_{48h}		0.647	0.684	0.641	0.600	0.575
95 % confidence interval	97.5%	0.692	0.740	0.698	0.655	0.631
	2.5%	0.602	0.627	0.584	0.544	0.520



Felten *et al.*, Graphical abstract