



HAL
open science

Use of low density polyethylene membranes for assessment of genotoxicity of PAHs in the Seine River

Françoise Vincent-Hubert, Emmanuelle Uher, Carole Di Giorgio, Cécile Michel, Michel de Méo, Catherine Gourlay-Francé

► **To cite this version:**

Françoise Vincent-Hubert, Emmanuelle Uher, Carole Di Giorgio, Cécile Michel, Michel de Méo, et al..
Use of low density polyethylene membranes for assessment of genotoxicity of PAHs in the Seine River.
Ecotoxicology, 2017, 26 (2), pp.165-172. 10.1007/s10646-016-1751-6 . hal-02314910

HAL Id: hal-02314910

<https://amu.hal.science/hal-02314910v1>

Submitted on 12 Jan 2024

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Copyright

Use of low density polyethylene membranes for assessment of genotoxicity of PAHs in the Seine River

Vincent-Hubert Françoise^{1,2,*}, Uher Emmanuelle¹, Di Giorgio Carole³, Michel Cécile¹,
De Meo Michel³, Gourlay-France Catherine¹

¹ IRSTEA, UR Hydrosystèmes et Bioprocédés Antony cedex, France

² IFREMER, Laboratoire de Microbiologie - LNR, rue de l'île d'Yeu Nantes, France

³ Biogénotoxicologie et Mutagenèse Environnementales (EA 1784) Université de la Méditerranée Marseille, France

* Corresponding author : Françoise Vincent-Hubert, email address : francoise.vincent@ifremer.fr

Abstract :

The genotoxicity of river water dissolved contaminants is usually estimated after grab sampling of river water. Water contamination can now be obtained with passive samplers that allow a time-integrated sampling of contaminants. Since it was verified that low density polyethylene membranes (LDPE) accumulate labile hydrophobic compounds, their use was proposed as a passive sampler. This study was designed to test the applicability of passive sampling for combined chemical and genotoxicity measurements. The LDPE extracts were tested with the umu test (TA1535/pSK1002 ± S9) and the Ames assay (TA98, TA100 and YG1041 ± S9). We describe here this new protocol and its application in two field studies on four sites of the Seine River. Field LDPE extracts were negative with the YG1041 and TA100 and weakly positive with the TA98 + S9 and Umu test. Concentrations of labile mutagenic PAHs were higher upstream of Paris than downstream of Paris. Improvement of the method is needed to determine the genotoxicity of low concentrations of labile dissolved organic contaminants.

Keywords : Genotoxicity, River water, Passive samplers, PAHs, Ames assay, Umu test

44

45 1. Introduction

46

47 Water pollution by chemicals as a result of industrialization and increasing urbanization is a factor that
48 threatens the preservation of aquatic ecosystems and also human health. Polycyclic Aromatic
49 Hydrocarbons (PAHs) constitute a ubiquitous class of environmental chemical pollutants identified in
50 surface waters and sediments. Many of them are mutagenic and can impact aquatic organisms (Ohe
51 et al., 2004). Most studies report the mutagenic activity of dissolved and particulate PAHs after grab
52 sampling of large volumes of water. As a consequence, the genotoxicity of river waters or effluents
53 could be either underestimated or overestimated, depending upon the recent discharge of pollutants
54 into the river water.

55 More representative information of water contamination can now be obtained with passive samplers
56 that allow a time-integrated sampling of contaminants (Lebo et al., 1992; Vrana et al., 2005). Once
57 immersed, passive sampler devices accumulate contaminants from the water. For dissolved
58 hydrophobic contaminants, a semi-permeable membrane device (SPMD) was developed in the 1990's.
59 The low density polyethylene (LDPE) membrane has been also proposed as a passive sampler as it has
60 been confirmed that it can accumulate dissolved hydrophobic compounds that are bioavailable to
61 some aquatic organisms (Booij et al., 2003; Carls et al., 2004; Gourlay et al., 2005). As a single-phase
62 sampler, LDPE has been gaining interest because it avoids the toxicity of triolein, a molecule contained
63 in the lipidic phase of SPMD (Sabaliunas et al., 1999).

64 Even if passive samplers' coupling to bioassays might be a promising tool, few studies have
65 been performed to determine the limits of this coupling. Regarding SPMDs, it has been shown that
66 oleic acid derived from triolein induces a positive response in bioassays (Sabaliunas et al., 2000).
67 Presently, no data are available for LDPE membranes and their release of compounds when immersed
68 in organic solvent. They may induce toxicity during bioassays, or reduce the bioavailability of the tested

69 contaminants by adsorbing them during the bioassay, and thus lower the response of the bioassay.
70 The impact of purification procedures on the test response needs to be evaluated.

71 In France, evidence of mutagenic activities have been demonstrated in the water column and
72 in the **sediment of the Seine estuary**. Suspended particulate matter collected downstream of various
73 petrochemical industries **was** positive with the Ames assay (TA98+S9) and the comet assay (HepG2+S9)
74 and sediment from the estuary was also genotoxic and mutagenic (Cachot et al., 2006; Vincent-Hubert
75 et al., 2012). The principal sources of pollution in the Seine River basin come from high industrial and
76 urban activities and intensive agriculture. This area of France (75,000 km²) is inhabited by about 16
77 million people, concentrated within the Paris area. This population density leads to elevated levels of
78 pollutants in water, including PAHs (Blanchard et al., 2007; Meybeck et al., 2007; Tusseau-Vuillemin et
79 al., 2007). **However, the mutagenicity of surface water contaminants from the Paris area has not been**
80 **reported.**

81 A large number of different assays have been recommended to assess the presence of
82 genotoxic contaminants in surface water, wastewater and industrial effluents (Ohe et al., 2004). Most
83 of the published studies employed the *Salmonella typhimurium* mutagenicity test with strains TA98
84 and/or TA100, with and without metabolic activation (S9 mix). Data obtained with the *Salmonella*
85 strains can provide information on the classes of mutagens present in the water. For example, the
86 YG1041 strain is more sensitive to nitroaromatic compounds than the parent strain TA98 (Watanabe
87 et al., 1989). Nitro-PAHs have been detected in the extracts of environmental materials containing
88 diesel emissions and in urban river waters (Murahashi et al., 2001). The umu test was developed as an
89 alternative to the Ames test by Oda (Oda et al., 1985). Activation of the SOS repair system by genotoxic
90 compounds is measured by photometric determination of the β -galactosidase enzyme activity. The
91 umu test is widely used for routine monitoring of water samples as the results are available in a single
92 day with minimal advance preparation. The umu test proved to be very sensitive for the detection of
93 **mutagens in surface waters in Germany and Japan** (Dizer et al., 2002; Ohe, 1996).

94

95 This study was designed to develop a method for coupling the use of LDPE passive samplers
96 with genotoxicity assays. The umu test was chosen as a pre-screening test for its capacity of induction
97 of the SOS-system. For the *Salmonella* mutagenicity assay, three strains were chosen, the TA 98, TA
98 100 and YG1041 strains. Two field applications of the developed protocol are presented here. In the
99 urban area of Paris, LDPEs were deployed for three weeks in two different years at four river sites,
100 then genotoxicity testing and chemical analyses of LDPE extracts were performed.

101

102

103 2. Materials & Methods

104

105 2.1 Chemicals

106 Dimethylsulfoxide (DMSO) for molecular biology, 4-nitroquinoline-1-oxide (4NQO), 1-nitropyrene (1-
107 NP), chlorophenol red- β -D-galactopyranoside (CPRG), sodium azide (SA), 2 aminoanthracene, and
108 benzo[a]pyrene (BaP) were purchased from Sigma (France). Rat S9 was from Trinova (Germany).
109 Heptane Picograde[®] for residue analysis was purchased from LGC Promochem (France) and ethyl
110 acetate Suprasolv[®] for Gas Chromatography from VWR (France).

111

112 2.2 Preparation and deployment of LDPE membranes

113 Pieces (10x30 cm) of LDPE membranes (membrane thickness of 80 μ m) were cut from a roll (Manutan,
114 France). A cleaning procedure was performed to avoid the potential effects of impurities contained in
115 LDPE membranes, as preliminary tests revealed a slight toxicity of non-cleaned LDPE. This procedure
116 consisted of immersing LDPE sheets into a heptane/ethyl acetate mix (50/50, v/v) twice for 24h each.
117 The solvents were refreshed after the first 24-h period. LDPE sheets were then rinsed by immersion in
118 ultrapure water for 12 days to remove solvent traces. Water was changed every four days. A
119 preliminary three weeks field deployment study, on contaminated river sites, revealed that LDPE
120 extracts were **genotoxic** in the umu test in these conditions (data not shown).

121

122 2.3 Field studies

123 Four river sites were selected for the deployment of **four LDPE membranes per site at 1.50 m depth**
124 (Fig.1). One river site was not impacted by the urban activity of Paris (Marnay-sur-Seine) and the three
125 others were highly impacted by the urban area of Paris: Saint-Maurice, Triel-sur-Seine and Bougival.
126 Bougival is also impacted by the Seine-Aval wastewater treatment plant (Blanchard et al., 2007;
127 Meybeck et al., 2007; Tusseau-Vuillemin et al., 2007). Two field studies were performed, the first one,

128 in spring 2009, was limited to Marnay-sur-Seine and Bougival, and the second one was conducted on
129 the four sites during the spring and autumn 2010.

130

131 **2.4 Extraction and purification of the PAHs**

132

133 After field deployment, membranes were thoroughly cleaned of biofilm. They were then immersed in
134 250 ml heptane/ethylacetate mix (50/50) in closed amber glass bottles. Bottles were stirred on an
135 orbital shaker at 100 rpm for 48h. A preliminary study showed that 94 to 99% of the compounds were
136 removed from the membranes with this procedure. Membranes were then removed from the solvent
137 extract. The solvent mix was reduced to 1 mL with a rotary evaporator. The extract was purified with
138 a florisil column (Phenomenex, France) pre-conditioned with 5 mL of 94/6 heptane/ethylacetate mix.
139 Elution was performed by transferring 4x5 mL of a 94/6 heptane/ethylacetate mix. Solvent was
140 evaporated under a gentle steam of N₂. The evaporation was stopped when a 1 mL volume was
141 reached: 200 µL were reserved and spiked with the internal standards mix (Naphtalene-d8;
142 Acenaphtalene-d10; Phenanthrene-d10; Chrysene-d12; Perylene-d12) and stored at -20°C for chemical
143 analysis. The last 800 µL were evaporated until near dryness (5 µl) and transferred into 200 µL of DMSO
144 for genotoxicity testing.

145

146 **2.5 Chemical analysis of LDPE**

147 PAH analyses were performed on a GC/MS (Thermo electron, Les Ulis, France) operating in Selected
148 Ion Monitoring mode. The ionisation mode was electronic impact and the analyser was a simple
149 quadrupole. It was equipped with a Zebron ZB-5MS capillary GC column (60 m length x 0,25 mm ID x
150 0.2 µm film thickness). PAHs were quantified with internal calibration. The target PAHs were those on
151 the US EPA list (fluoranthene, fluorene, anthracene, benzo(b)fluoranthene, benz(a)anthracene,
152 indeno(1,2,3-cd)pyrene, benzo(a)pyrene, dibenzo(a,h)anthracene, benzo(g,h,i)perylene,
153 phenanthrene, benzo(k)fluorothene, pyrene and chrysene) excluding the three with lowest molecular

154 weights (naphthalene, acenaphtylene, acenaphtene). Detection limits were 10 ng.L⁻¹ in the final
155 extract for each PAH.

156

157 **2.6 Matrix effect**

158 Non-deployed LDPE membranes were extracted as blank extracts. These blank extracts were spiked
159 with the reference genotoxic components 1-NP and BaP. The umu test and Ames test were performed
160 with these spiked extracts.

161

162 **2.7 umu test**

163 The genotoxicity of PAHs accumulated on LDPE membranes was assessed with the umu-microplate
164 test using the bacterial strain TA1535/pSK1002 according to Oda (2004) and slightly modified by using
165 Chlorophenol red-D-galactopyranoside (CPRG (4 mg/ml) instead of O-nitrophenyl-β-D
166 galactopyranoside (Oda et al., 2004). The cytotoxic effect of the samples was determined by measuring
167 OD_{600 nm} changes and the genotoxic effects were evaluated by OD_{570 nm} changes. Samples with a growth
168 ratio (G) < 0.8 were considered cytotoxic and samples with an induction ratio (IR) > 1.5 were considered
169 to be genotoxic (Dizer et al., 2002). Determination of growth factor G, relative enzyme activity (REA)
170 and induction rate (IR) were calculated as described in Oda et al (.2004). The results are presented as
171 the mean ± SD of two or three independent experiments. Because most PAHs required metabolic
172 activation to be genotoxic, an activation system was used containing S9 of rats (4% S9, 0.4 M MgCl₂,
173 1.65 M KCL, 1.0 M Glucose 6-Phosphate, 0.1 M NADPH, 0.1 M NADH, and 0.2 M Na-Phosphate buffer
174 pH 7.4). Final concentrations of positive controls were: 4 NQO (0.132 μM), 1 NP (0.8μM) and BaP (5
175 μM) with S9 mix.

176

177

178

179 **2.8 Ames test on TA98, TA100 and YG1041 ± S9 mix**

180 The mutagenicity of PAHs extracted from LDPE membranes was assessed by the microsuspension
181 version of the *Salmonella* mutagenicity test (De Méo et al., 1995) using three strains of *Salmonella*
182 *typhimurium*, strains TA98, TA100 and YG1041. TA98 has been shown to detect PAHs in the presence
183 of S9 mix (De Méo et al., 1995) (Nikoyan et al., 2007). N-heterocyclic or aromatic amines have shown
184 elevated mutagenic activities in the presence of S9 mix on the metabolically enhanced YG1041 that
185 expresses *O*-acetyl transferase (Hagiwara et al., 1993). Nitroarenes have displayed a high mutagenic
186 activity without metabolic activation in the nitroreductase-enhanced YG1041 strain (Nikoyan et al.,
187 2007).

188 Each experiment included triplicate plates of four tested doses (4, 6, 8 and 10 µL). A solvent (DMSO)
189 control was added to detected the spontaneous frequency of revertants (quadruplicate plates). Two
190 positive controls were also included to ensure the performance of the tester strains and the S9 Mix:
191 20 ng/plate 2,4,7-trinitrofluorenone (TNFone) for TA98 and YG1041 and 5 µg/plate sodium azide for
192 TA100, 0.5 and 1.0 µg/plate of BaP for TA98 and YG1041 with S9 Mix.

193 A two-step analysis was performed to interpret data. The Dunnett test (Wahrendorf et al., 1985) was
194 performed to determine the significance of differences between the mean number of induced
195 revertants and the mean number of spontaneous revertants. If the Dunnett test was positive for at
196 least one tested concentration, non-linear regression analysis was carried out using two arbitrary
197 models as described previously (Kim and Margolin, 1999) with Table Curve 2D® software (version 5.0,
198 Jandel Scientific Software, San Rafael, CA, USA):

199 **MAR-1: rev / plate = (a + b * D) * (2 - e^(c*D²))** and **MAR-2: rev / plate = (a + b * D) * e^(-c*D²)**

200 With: rev/plate: number of revertants by plate

201 D: dose

202 a, b and c : calculated coefficients

203

204

205

206 **3. Results**

207
208 Preliminary tests performed to develop the coupling of mutagenicity tests and passive sampling. No
209 matrix effect of LDPE membranes was detected in our experimental conditions (data not shown)
210 indicating that molecules from LDPE membranes did not modify the genotoxicity of BaP.

211 Two field studies were conducted, the first one in 2009 and the second in 2010. Samples obtained in
212 2009 did not present any genotoxicity in the Umu test, with or without metabolic activation (Table 1).
213 As we did not have enough LDPE extracts, we tested them on TA98+S9 and YG1041+S9 only, which are
214 more sensitive to mutagenic activity of PAHs, a class of contaminants predominant in urban areas. The
215 sample from Marnay-sur-Seine site was statistically mutagenic in the Ames test (TA 98+S9) (Table 1),
216 indicating the presence of frameshift mutagens. No mutagenic activity was detected with YG1041,
217 indicating that no nitroaromatic compounds were present in these extracts.

218
219 For samples obtained in 2010, only one sample, Bougival, was positive in the umu test, indicating the
220 presence of direct acting genotoxic compounds in the river water there (Table 2). All samples displayed
221 statistically significant mutagenic activity in the Ames test (TA98 with S9 mix) (Table 2) indicating the
222 presence of frameshift mutagens in the river water at the four sites and for both seasons; two out of
223 eight samples were statistically mutagenic (TA98 without S9 mix) indicating the presence of direct
224 frameshift mutagens at Marnay-sur-Seine and Saint-Maurice. No mutagenic activity was detected with
225 the TA100 with or without S9 mix indicating the absence of mutagens inducing base substitutions.

226
227 The concentrations of total labile PAHs in LDPE measured in the 2010 samples shown that the Marnay-
228 sur-Seine site was the least contaminated and the Bougival and Triel-sur-Seine sites were the most
229 contaminated (Table 3). The highest concentrations of labile mutagenic and carcinogenic PAHs were
230 observed at Bougival at both seasons, followed by Triel-sur-Seine, while Marnay-sur-Seine and St-
231 Maurice presented the lowest concentrations. The highest concentrations of PAHs were for Pyrene at
232 St-Maurice (autumn), Bougival and Triel-sur-Seine and for Fluoranthene at Marnay-sur-Seine and St-

233 Maurice (spring). Benzo(a)pyrene and dibenz(a,h)anthracene, which are considered as the more
234 mutagenic PAHs, had the highest concentrations at Bougival. No correlation was noted between PAHs
235 concentrations and Ames assay data (Spearman correlation).

236

237 4. Discussion

238

239 This study evaluated the coupling of mutagenicity bioassays and with LDPE membranes in evaluating
240 the genotoxicity of Seine River water. Investigation of the genotoxicity of water contaminants is
241 generally limited to dissolved contaminants collected after spot sampling (Houk, 1992; Umbuzeiro et
242 al., 2001) or to particulate contaminants which represent the greatest fraction of the hydrophobic
243 contaminants. Despite low genotoxicity, our data confirmed the predominance of direct and S9-
244 activated frameshift-type mutagens in surface water of the Seine River as frequently reported after
245 spot sampling (Ohe et al., 2004; Vincent-Hubert et al., 2012).

246 Mutagenic PAHs sampled with passive samplers may inform on the level of bioavailable compounds
247 to some aquatic organisms. However, the mutagenicity of bioavailable compounds is still difficult to
248 estimate probably for two main reasons, the very low concentration, compared with concentrations
249 usually used in standard genotoxicity assays, and the mixture of contaminants sampled with passive
250 samplers. Very few studies have reported on the genotoxicity and the mutagenicity of river water
251 contaminants collected with passive samplers even though their toxicity has been reported (Allan et
252 al., 2012; Liscio et al., 2014). Gilli *et al.* reported that SPMD extracts from the Po River were not
253 mutagenic (Gilli et al., 2005). Sabaliunas *et al.* tested the genotoxicity of river water in Lithuania with
254 SPMD coupled to the Mutatox assay and found a positive response only without S9 metabolic
255 activation, which indicated the presence of direct acting mutagens (Sabaliunas et al., 2000).

256 The low level of mutagenicity of LDPE extract in our study can be explained by the low concentration
257 of labile mutagenic PAHs, ranging from 13 to 126 ng/LDPE extract. For example for BaP, which is among
258 the more mutagenic PAHs, the highest concentration of BaP was 62-fold below the lower BaP

259 concentration reported positive in the Ames test (1 µg/plate). Improvement of the detection could be
260 obtained in future studies by increasing the number of deployed membranes and the volume of sample
261 to be tested.

262 The concentrations of labile PAHs measured here are consistent with previous published data showing
263 that the Seine River sites under the influence of Paris area are more polluted than sites upstream of
264 Paris (Bourgeault and Gourlay-France, 2013; Michel et al., 2013).

265 The *umu*-assay is recognized as the most sensitive standard method for estimating the genotoxicity of
266 polluted waters such as industrial wastewater, suspended matter and surface water of rivers (Dizer et
267 al., 2002; Rao et al., 1995; Vahl et al., 1997). For the 2010 samples, the SOS induction gave a different
268 aspect of the genotoxicity than the Ames assay, as only one sample was positive in the Umu test
269 (Bougival) while all samples were mutagenic in the Ames assay (TA98+S9 mix). The *umuC* gene is
270 induced by DNA lesions but this does not lead necessarily to mutations which may explain the absence
271 of correlation between the two tests. The low reaction of the *umu*-test may be ascribed to the
272 presence of compounds that inhibit components of the SOS-system or the indicative enzyme, *β*-
273 galactosidase. The complexity of the mixtures makes the presence of SOS-inhibiting compounds
274 possible as reported in the literature (Vahl et al., 1997), even though the low concentration of PAHs
275 might be the major explanation of the low genotoxicity of LDPE extracts.

276
277 In conclusion, we developed a method for coupling the use of LDPE passive samplers with genotoxicity
278 assays. Field application of this new method revealed that labile dissolved PAHs extracted from passive
279 samplers were weakly positive with the TA98+S9 and Umu test. Concentrations of labile mutagenic
280 PAHs were higher upstream of Paris than downstream of Paris. Improvement of the method is needed
281 to determine the genotoxicity of low concentrations of labile dissolved organic contaminants.

282

283 **Acknowledgment**

284 We thank Dr. Y. Oda (Japan) who gave the bacterial strain TA1535/pSK1002, Dr. B.N. Ames (Berkeley,
285 CA, USA) who gave the bacterial strains TA98 and TA100 and Dr T. Nohmi (Tokyo, Japan) who gave the
286 strain YG1041.

287 We thank R. LOUAILECHE and C. VARRET for technical assistance.

288

289

290

291

292 **Compliance with Ethical Standards**

293 Cécile MICHEL had a Ph.D. Grant from the Ile-de-France Regional Council (R2DS program). This work
294 was supported by the PIREN- Seine research program.

295 Authors do not have Conflict of interest

296 Ethical approval: This article does not contain any studies with human participants or animals

297 performed by any of the authors.

298

299

300 **Figure legend:**

301

302 Figure 1: Localisation of the study sites in the Seine River Basin

303

304 Sampling sites on the Seine River are Marnay-sur-Seine, Bougival and Triel-sur-Seine. Saint-Maurice is
305 on the Marne River.

306 Bougival and Triel sur Seine are subject to diffuse urban contamination of Paris city (Paris: 48° 51' 12"
307 N, 2° 20' 56" E)

308 ▲Seine-Aval wastewater treatment plant.

309

310

311

312 **References**

- 313 Allan, S. E., Smith, B. W., Tanguay, R. L., and Anderson, K. A. (2012). Bridging environmental
314 mixtures and toxic effects. *Environmental Toxicology and Chemistry* 31, 2877-2887.
- 315 Blanchard, M., Teil, M. J., Guigon, E., Larcher-Tiphagne, K., Ollivon, D., Garban, B., and
316 Chevreuil, M. (2007). Persistent toxic substance inputs to the river Seine basin (France) via
317 atmospheric deposition and urban sludge application. *Sci Total Environ* 375, 232-243.
- 318 Booij, K., Hofmans, H. E., Fischer, C. V., and Van Weerlee, E. M. (2003). Temperature-
319 dependent uptake rates of nonpolar organic compounds by semipermeable membrane devices
320 and low-density polyethylene membranes. *Environmental Science & Technology* 37, 361-366.
- 321 Bourgeault, A., and Gourlay-France, C. (2013). Monitoring PAH contamination in water:
322 Comparison of biological and physico-chemical tools. *Sci Total Environ* 454, 328-336.
- 323 Cachot, J., Geffard, O., Augagneur, S., Lacroix, S., Le Menach, K., Peluhet, L., Couteau, J.,
324 Denier, X., Devier, M. H., Pottier, D., and Budzinski, H. (2006). Evidence of genotoxicity
325 related to high PAH content of sediments in the upper part of the Seine estuary (Normandy,
326 France). *Aquat Toxicol* 79, 257-267.
- 327 Carls, M. G., Holland, L. G., Short, J. W., Heintz, R. A., and Rice, S. D. (2004). Monitoring
328 polynuclear aromatic hydrocarbons in aqueous environments with passive low-density
329 polyethylene membrane devices. *Environmental Toxicology and Chemistry* 23, 1416-1424.
- 330 De Méo, M., Laget, M., Di Giorgio, C., Guiraud, H., Botta, A., Castegnaro, M., and Duménil,
331 G. (1995). Optimization of the Salmonella/mammalian microsome assay for urine mutagenesis
332 by experimental designs. *Mutation Research - Reviews in Genetic Toxicology* 340, 51-65.
- 333 Dizer, H., Wittekindt, E., Fischer, B., and Hansen, P. D. (2002). The cytotoxic and genotoxic
334 potential of surface water and wastewater effluents as determined by bioluminescence, umu-
335 assays and selected biomarkers. *Chemosphere* 46, 225-233.
- 336 Gilli, G., Schiliro, T., Pignata, C., Traversi, D., Carraro, E., Baiocchi, C., Aigotti, R., Giacosa,
337 D., and Fea, E. (2005). Application of semipermeable membrane device for assessing toxicity
338 in drinking water. *Chemosphere* 61, 1691-1699.
- 339 Gourlay, C., Miege, C., Noir, A., Ravelet, C., Garric, J., and Mouchel, J. M. (2005). How
340 accurately do semi-permeable membrane devices measure the bioavailability of polycyclic
341 aromatic hydrocarbons to *Daphnia magna*? *Chemosphere* 61, 1734-1739.
- 342 Hagiwara, Y., Watanabe, M., Oda, Y., Sofuni, T., and Nohumi, T. (1993). Specificity and
343 sensitivity of salmonella typhimurium YG1041 and YG1042 strains possessing elevated levels
344 of both nitroreductase and acetyltransferase activity *Mutat Res* 291, 171-180.
- 345 Houk, V. S. (1992). The genotoxicity of industrial wastes and effluents: A review. *Mutation*
346 *Research - Reviews in Mutation Research* 277, 91-138.
- 347 Kim, B. S., and Margolin, B. H. (1999). Statistical methods for the Ames Salmonella assay: a
348 review. *Mutation Research-Reviews in Mutation Research* 436, 113-122.
- 349 Lebo, J. A., Zajicek, J. L., Huckins, J. N., Petty, J. D., and Peterman, P. H. (1992). Use of semi-
350 permeable membrane devices for in situ monitoring of polycyclic aromatic hydrocarbons in
351 aquatic environments *Chemosphere* 25, 697-718.
- 352 Liscio, C., Abdul-Sada, A., Al-Salhi, R., Ramsey, M. H., and Hill, E. M. (2014). Methodology
353 for profiling anti-androgen mixtures in river water using multiple passive samplers and
354 bioassay-directed analyses. *Water Research* 57, 258-269.
- 355 Meybeck, M., Lestel, L., Bonte, P., Moilleron, R., Colin, J. L., Rousselot, O., Herve, D., de
356 Ponteves, C., Grosbois, C., and Thevenot, D. R. (2007). Historical perspective of heavy metals
357 contamination (Cd, Cr, Cu, Hg, Pb, Zn) in the Seine River basin (France) following a DPSIR
358 approach (1950-2005). *Sci Total Environ* 375, 204-231.

359 Michel, C., Bourgeault, A., Gourlay-France, C., Palais, F., Geffard, A., and Vincent-Hubert, F.
360 (2013). Seasonal and PAH impact on DNA strand-break levels in gills of transplanted zebra
361 mussels. *Ecotoxicol Environ Saf* 92, 18-26.

362 Murahashi, T., Ito, M., Kizu, R., and Hayakawa, K. (2001). Determination of nitroarenes in
363 precipitation collected in Kanazawa, Japan. *Water Research* 35, 3367-3372.

364 Nikoyan, A., De Meo, M., Sari-Minodier, I., Chaspoul, F., Gallice, P., and Botta, A. (2007).
365 Evaluation of a battery of Salmonella typhimurium tester strains for biomonitoring of
366 mutagenic polycyclic aromatic hydrocarbons, nitroarenes and aromatic amines. *Mutation*
367 *Research - Genetic Toxicology and Environmental Mutagenesis* 626, 88-101.

368 Oda, Y., Funasaka, K., Kitano, M., Nakama, A., and Yoshikura, T. (2004). Use of a High-
369 Throughput umu-Microplate Test System for Rapid Detection of Genotoxicity Produced by
370 Mutagenic Carcinogens and Airborne Particulate Matter. *Environ Mol Mutagen* 43, 10-19.

371 Oda, Y., Nakamura, S. I., and Oki, I. (1985). Evaluation of the new system (umu-test) for the
372 detection of environmental mutagens and carcinogens. *Mutat Res* 147, 219-229.

373 Ohe, T. (1996). Evaluation of SOS-inducing activity with an O-acetyltransferase-
374 overexpressing strain Salmonella typhimurium NM2009 for municipal river water and the
375 identification of 1-nitropyrene. In *Water Science and Technology*, pp. 313-320.

376 Ohe, T., Watanabe, T., and Wakabayashi, K. (2004). Mutagens in surface waters: A review.
377 *Mutation Research - Reviews in Mutation Research* 567, 109-149.

378 Rao, S. S., Burnison, B. K., Efler, S., Wittekindt, E., Hansen, P. D., and Rokosh, D. A. (1995).
379 Assessment of genotoxic potential of pulp mill effluent and an effluent fraction using Ames
380 mutagenicity and Umu-C genotoxicity assays. *Environmental Toxicology and Water Quality*
381 10, 301-305.

382 Sabaliunas, D., Ellington, J., and Sabaliuniene, I. (1999). Screening bioavailable hydrophobic
383 toxicants in surface waters with semipermeable membrane devices: Role of inherent oleic acid
384 in toxicity evaluations. *Ecotoxicol Environ Saf* 44, 160-167.

385 Sabaliunas, D., Lazutka, J. R., and Sabaliuniene, I. (2000). Acute toxicity and genotoxicity of
386 aquatic hydrophobic pollutants sampled with semipermeable membrane devices.
387 *Environmental Pollution* 109, 251-265.

388 Tusseau-Vuillemin, M. H., Gourlay, C., Lorgeoux, C., Mouchel, J. M., Buzier, R., Gilbin, R.,
389 Seidel, J. L., and Elbaz-Poulichet, F. (2007). Dissolved and bioavailable contaminants in the
390 Seine river basin. *Sci Total Environ* 375, 244-256.

391 Umbuzeiro, G. D. A., Roubicek, D. A., Sanchez, P. S., and Sato, M. I. Z. (2001). The
392 Salmonella mutagenicity assay in a surface water quality monitoring program based on a 20-
393 year survey. *Mutation Research - Genetic Toxicology and Environmental Mutagenesis* 491,
394 119-126.

395 Vahl, H. H., Karbe, L., and Westendorf, J. (1997). Genotoxicity assessment of suspended
396 particulate matter in the Elbe river: comparison of Salmonella microsome test, arabinose
397 resistance test, and umu-test. *Mutation Research-Genetic Toxicology and Environmental*
398 *Mutagenesis* 394, 81-93.

399 Vincent-Hubert, F., Heas-Moisan, K., Munsch, C., and Tronczynski, J. (2012). Mutagenicity
400 and genotoxicity of suspended particulate matter in the Seine river estuary. *Mutation Research*
401 *- Genetic Toxicology and Environmental Mutagenesis* 741, 7-12.

402 Vrana, B., Mills, G. A., Allan, I. J., Dominiak, E., Svensson, K., Knutsson, J., Morrison, G.,
403 and Greenwood, R. (2005). Passive sampling techniques for monitoring pollutants in water.
404 *Trac-Trends in Analytical Chemistry* 24, 845-868.

405 Wahrendorf, J., Mahon, G. A. T., and Schumacher, M. (1985). A non parametric approach to
406 the statistical analysis of mutagenicity data *Mutat Res* 147, 5-13.

407 Watanabe, M., Nohmi, T., and Ishidate, M. (1989). Establishment of new strains of *S-*
408 *typhimurium* highly sensitive to nitroarenes and amine aromatic- TA98 and TA100 derivatives
409 with high levels of nitroreductase or acetyltransferase activities *Mutat Res* 216, 383-383.
410
411
412

Table 1: Genotoxicity and Mutagenicity of LDPE **sampler deployed in the Seine River** water in 2009

Sample	Dilution	Umu test (TA1535/pSK1002)		Dose (μ l)	Ames-test (TA 98 +S9)	Ames test (YG 1041+S9)
		-S9 mix	+ S9 mix			
Marnay	1/8	1.2 \pm 0.2	1.4 \pm 0.1	4	42 \pm 10	120 \pm 24
	1/4	1.3 \pm 0.2	1.35 \pm 0.1	6	51 \pm 6	125 \pm 6
	1/2	0.9 \pm 0.4	-	8	61 \pm 7	nd
				10	49 \pm 4	nd
Bougival	1/8	1.21 \pm 0.2	1.33 \pm 0.2	4	29 \pm 4	107 \pm 9
	1/4	1.42 \pm 0.41	1.33 \pm 0.2	6	36 \pm 5	100 \pm 4
	1/2	0.94 \pm 0.33	-	8	34 \pm 2	NC
			-	10	33 \pm 6	97 \pm 11
BaP			1.97 \pm 0.4		654 \pm 8	161 \pm 6
DMSO 1%		1.22 \pm 0.1	1.1	10	31 \pm 6	106 \pm 11
LDPE control		0.94 \pm 0.5	1.07 \pm 0.07		35 \pm 7	130 \pm 24
4NQO		3.08\pm0.7				
1-NP		2.11\pm0.4				
Control		1	1			

Umu test: induction rate of β galactosidase activity (mean \pm SD). Genotoxic sample = value >1.5
 Ames test: number of revertants/plate (mean \pm SD). Bold faced values are significantly different from control value (DMSO1%). Spontaneous revertants: TA98= 25 \pm 5 ; YG1048=111 \pm 8
 Mean of three replicates.

Table 2: Genotoxicity and Mutagenicity of LDPE sampler deployed in the Seine River in 2010

Sites	Season	Umu test (TA1535/pSK1002)	Ames test (TA 98) (nb/rev/plate)		Ames test (TA100) (nb rev/plate)	
			- S9 mix	+ S9 mix	- S9 mix	+ S9 mix
Marnay-sur-Seine	Spring	1.17 ± 0.29	12 ± 5	39 ± 2	110 ± 7	101 ± 18
	Autumn	1.18 ± 0.3	96 ± 8	24 ± 6	77 ± 18	38 ± 20
St-Maurice	Spring	1.36 ± 0.27	27 ± 2	33 ± 0.5	121 ± 13	108 ± 12
	Autumn	1.38 ± 0.36	164 ± 89	35 ± 14	81 ± 8	35 ± 20
Bougival	Spring	2.07 ± 0.5	17 ± 7	30 ± 2	142 ± 15	131 ± 33
	Autumn	2.18 ± 0.7	14 ± 2	39 ± 4	82 ± 2	96 ± 13
Triel-sur-Seine	Spring	1.13 ± 0.18	16 ± 4	29 ± 15	125 ± 2	85 ± 20
	Autumn	1.53 ± 0.37	12 ± 3	42 ± 3	118 ± 22	153 ± 21
DMSO 1%		1.1	26 ± 6	10 ± 1	167 ± 16	127 ± 9
Positive control		7.85 ± 2	197 ± 21	371 ± 181	371 ± 295	1671 ± 108
Spontaneous revertants			13 ± 7	11 ± 0.5	55 ± 14	95 ± 30

Umu test: induction rate of β galactosidase activity (mean \pm SD). Genotoxic sample = value >1.5

Ames test: positive samples are two fold the DMSO value (bold letters). Control=DMSO1%

Positive controls for Ames test were: 20 ng/plate 2,4,7-trinitrofluorenone for TA98-S9 mix and 5 μ g/plate sodium azide for TA100-S9 mix. 0.5 and 1 μ g/plate of BaP for TA98 with S9 Mix. 10 μ L is the volume of LDPE extract tested with the Ames assay. Mean of three replicates.

Table 3: Concentrations of Labile PAHs in LDPE

PAHs (ng/LDPE extract)	Marnay sur Seine		St-Maurice		Bougival		Triel sur Seine
	Spring	Autumn	Spring	Autumn	Spring	Autumn	Spring
Acenaphtene	0.42	0.89	0.53	0.57	2.41	2.09	1.42
Fluorene	0.65	1.37	1.07	0.85	2.63	2.02	2.06
Phenanthrene	2.20	4.03	3.00	2.23	8.66	6.43	6.84
Anthracene	0.59	0.96	1.06	1.86	5.08	6.24	2.77
Fluoranthene	5.04	8.36	6.56	4.39	21.20	13.64	18.77
Pyrene	1.99	4.62	6.00	7.18	31.05	28.14	27.52
Benzo(a)anthracene	0.10	0.34	1.01	0.43	5.52	4.93	5.32
Chrysene	0.46	0.78	1.72	1.75	6.93	4.71	6.83
Benzo(b)fluoranthene	1.03	2.33	1.84	4.35	13.05	21.04	4.42
Benzo(k)fluoranthene	0.27	0.50	1.05	1.42	4.97	8.73	1.98
Benzo(a)pyrene	0.24	0.56	0.80	1.37	9.09	16.04	2.58
Indeno(1.2.3-cd)pyrene	0.43	0.72	0.81	1.06	3.05	5.77	0.84
Dibenz(a.h)anthracene	0.08	0.24	0.22	0.49	1.19	1.68	0.51
Benzo(g.h.i)perylene	0.34	0.65	0.57	1.19	3.13	4.87	1.39
Σ PAHs	13.88	26.41	26.29	29.22	118.02	126.40	83.31
labile mutagenic PAHs	24.86	22.45	22.81	24.86	104.83	110.26	76.20
labile carcinogenic PAHs	2.63	5.50	7.47	10.92	43.83	62.93	22.49

Sum of mutagenic PAHs: phenanthrene. pyrene. fluoranthene. benzo(a)anthracene. chrysene. benzo(b)fluoranthene. benzo(k)fluoranthene. benzo(a)pyrene. dibenz(a.h)anthracene. benzo(g.h.i)perylene.

Sum of the PAHs classified as probable human carcinogens according to the U.S. EPA classification. The PAHs included in this calculation belong to groups 1, 2B and 2A: benzo[a]anthracene (BaA), benzo[a]pyrene (BaP), benzo[b]fluoranthene (BbF), benzo[k]fluoranthene (BkF), chrysene (CHRY), dibenz[a,h]anthracene (DBahA), and indeno[1.2.3-c,d]pyrene (I123cdP).

Figure 1: Localisation of the study sites in the Seine River Basin

