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

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### 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 km<sup>2</sup>) 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  $\beta$ -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- $\beta$ -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<sup>®</sup> for residue analysis was purchased from LGC Promochem (France) and ethyl  
110 acetate Suprasolv<sup>®</sup> 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  $\mu$ 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 N<sub>2</sub>. The evaporation was stopped when a 1 mL volume was  
141 reached: 200 µL were reserved and spiked with the internal standards mix (Naphthalene-d8;  
142 Acenaphthalene-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<sup>-1</sup> 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<sub>600 nm</sub> changes and the genotoxic effects were evaluated by OD<sub>570 nm</sub> 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 MgCl<sub>2</sub>,  
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<sup>(c\*D<sup>2</sup>)</sup>)** and **MAR-2: rev / plate = (a + b \* D) \* e<sup>(-c\*D<sup>2</sup>)</sup>**

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

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288

289

290

291

292 **Compliance with Ethical Standards**

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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

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

Sample	Dilution	Umu test (TA1535/pSK1002)		Dose ( $\mu$ 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	<b>51</b> $\pm$ 6	125 $\pm$ 6
	1/2	0.9 $\pm$ 0.4	-	8	<b>61</b> $\pm$ 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			<b>1.97 <math>\pm</math> 0.4</b>		<b>654 <math>\pm</math> 8</b>	<b>161 <math>\pm</math> 6</b>
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		<b>3.08<math>\pm</math>0.7</b>				
1-NP		<b>2.11<math>\pm</math>0.4</b>				
Control		1	1			

Umu test: induction rate of  $\beta$  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	<b>39 ± 2</b>	110 ± 7	101 ± 18
	Autumn	1.18 ± 0.3	96 ± 8	<b>24 ± 6</b>	77 ± 18	38 ± 20
St-Maurice	Spring	1.36 ± 0.27	27 ± 2	<b>33 ± 0.5</b>	121 ± 13	108 ± 12
	Autumn	1.38 ± 0.36	<b>164 ± 89</b>	<b>35 ± 14</b>	81 ± 8	35 ± 20
Bougival	Spring	<b>2.07 ± 0.5</b>	17 ± 7	<b>30 ± 2</b>	142 ± 15	131 ± 33
	Autumn	<b>2.18 ± 0.7</b>	14 ± 2	<b>39 ± 4</b>	82 ± 2	96 ± 13
Triel-sur-Seine	Spring	1.13 ± 0.18	16 ± 4	<b>29 ± 15</b>	125 ± 2	85 ± 20
	Autumn	1.53 ± 0.37	12 ± 3	<b>42 ± 3</b>	118 ± 22	153 ± 21
DMSO 1%		1.1	26 ± 6	10 ± 1	167 ± 16	127 ± 9
Positive control		<b>7.85 ± 2</b>	<b>197 ± 21</b>	<b>371 ± 181</b>	<b>371 ± 295</b>	<b>1671 ± 108</b>
Spontaneous revertants			13 ± 7	11 ± 0.5	55 ± 14	95 ± 30

Umu test: induction rate of  $\beta$  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  $\mu$ g/plate sodium azide for TA100-S9 mix. 0.5 and 1  $\mu$ g/plate of BaP for TA98 with S9 Mix. 10  $\mu$ 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
$\Sigma$ 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

