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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

1. Introduction

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

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

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

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

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

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

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

2. Materials & Methods

2.1 Chemicals

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

2.2 Preparation and deployment of LDPE membranes

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

2.3 Field studies

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

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

2.4 Extraction and purification of the PAHs

After field deployment, membranes were thoroughly cleaned of biofilm. They were then immersed in 250 ml heptane/ethylacetate mix (50/50) in closed amber glass bottles. Bottles were stirred on an orbital shaker at 100 rpm for 48h. A preliminary study showed that 94 to 99% of the compounds were removed from the membranes with this procedure. Membranes were then removed from the solvent extract. The solvent mix was reduced to 1 mL with a rotary evaporator. The extract was purified with a florisil column (Phenomenex, France) pre-conditioned with 5 mL of 94/6 heptane/ethylacetate mix. Elution was performed by transferring 4x5 mL of a 94/6 heptane/ethylacetate mix. Solvent was evaporated under a gentle steam of N_2. The evaporation was stopped when a 1 mL volume was reached: 200 µL were reserved and spiked with the internal standards mix (Naphtalene-d8; Acenaphtalene-d10; Phenanthrene-d10; Chrysene-d12; Perylene-d12) and stored at -20°C for chemical analysis. The last 800 µL were evaporated until near dryness (5 µl) and transferred into 200 µL of DMSO for genotoxicity testing.

2.5 Chemical analysis of LDPE

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

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

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

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

MAR-1: $\mathbf{rev / plate = (a + b * D) * (2 - e^{(c*D^2)})}$ and **MAR-2**: $\mathbf{rev / plate = (a + b * D) * e^{(-c*D^2)}}$

With: rev/plate: number of revertants by plate

D: dose

a, b and c : calculated coefficients

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 genotoxity 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-

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

4. Discussion

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

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

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

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

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

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

In conclusion, we developed a method for coupling the use of LDPE passive samplers with genotoxicity assays. Field application of this new method revealed that labile dissolved PAHs extracted from passive samplers were 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.

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Compliance with Ethical Standards

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Authors do not have Conflict of interest

Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.

Figure legend:

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

Sampling sites on the Seine River are Marnay-sur-Seine, Bougival and Triel-sur-Seine. Saint-Maurice is on the Marne River.
Bougival and Triel sur Seine are subject to diffuse urban contamination of Paris city (Paris: 48° 51’ 12” N, 2° 20’ 56” E)
Seine-Aval wastewater treatment plant.

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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 (µl)	Ames-test (TA 98 +S9)	Ames test (YG 1041+S9)
		-S9 mix	+ S9 mix			
Marnay	1/8	1.2 ± 0.2	1.4 ± 0.1	4	42 ± 10	120 ± 24
	1/4	1.3 ± 0.2	1.35 ± 0.1	6	**51** ± 6	125 ± 6
	1/2	0.9 ± 0.4	-	8	**61** ± 7	nd
				10	49 ± 4	nd
Bougival	1/8	1.21 ± 0.2	1.33 ± 0.2	4	29 ± 4	107 ± 9
	1/4	1.42 ±0.41	1.33 ± 0.2	6	36 ± 5	100 ± 4
	1/2	0.94 ±0.33	-	8	34 ± 2	NC
			-	10	33 ± 6	97 ± 11
BaP			**1.97 ±0.4**		**654** ± 8	**161** ± 6
DMSO 1%		1.22 ±0.1	1.1	10	31 ± 6	106 ± 11
LDPE control		0.94±0.5	1.07±0.07		35 ± 7	130 ± 24
4NQO		**3.08**±0.7				
1-NP		**2.11±0.4**				
Control		1	1			

Umu test: induction rate of β galactosidase activity (mean ± SD). Genotoxic sample = value >1.5
Ames test: number of revertants/plate (mean±SD). Bold faced values are significantly different from control value (DMSO1%). Spontaneous revertants: TA98= 25 ±5 ; YG1048=111±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 ± 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 µg/plate sodium azide for TA100-S9 mix. 0.5 and 1 µg/plate of B*a*P for TA98 with S9 Mix. 10 µL 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