



**HAL**  
open science

## Photodegradation of fluorene in aqueous solution : Identification and biological activity testing of degradation products

Said Kinani, Yasmine Souissi, Aziz Kinani, Svetlana Vujovic, Selim Ait-Aissa,  
Stéphane Bouchonnet

### ► To cite this version:

Said Kinani, Yasmine Souissi, Aziz Kinani, Svetlana Vujovic, Selim Ait-Aissa, et al.. Photodegradation of fluorene in aqueous solution : Identification and biological activity testing of degradation products. *Journal of Chromatography A*, 2016, 1442, pp.118-128. 10.1016/j.chroma.2016.03.012 . ineris-01862937

**HAL Id: ineris-01862937**

**<https://ineris.hal.science/ineris-01862937>**

Submitted on 28 Aug 2018

**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.

# 1 **Photodegradation of fluorene in aqueous solution: identification and** 2 **biological activity testing of degradation products**

3 Said Kinani<sup>a</sup>, Yasmine Souissi<sup>a</sup>, Aziz Kinani<sup>a</sup>, Zvetlana Vujović<sup>a</sup>, Sélim Aït-Aïssa<sup>b</sup> and  
4 Stéphane Bouchonnet<sup>a,\*</sup>

5 <sup>a</sup>Laboratoire de Chimie Moléculaire - École Polytechnique - 91128 Palaiseau, France

6 <sup>b</sup>Unité Écotoxicologie in vitro et in vivo, Institut National de l'Environnement Industriel et des  
7 Risques (INERIS), BP 2, F-60550 Verneuil en Halatte, France

8 \* Corresponding author, e-mail: [stephane.bouchonnet@polytechnique.edu](mailto:stephane.bouchonnet@polytechnique.edu)

## 9 **HIGHLIGHTS**

10 Degradation of fluorene under UV-Vis irradiation in water was investigated.

11 Twenty six photoproducts were characterized using GC-MS coupling.

12 Competing photodegradation pathways of fluorene under UV-Vis irradiation have been  
13 suggested.

14 Some photoproducts of fluorene are biologically much more active than the parent compound.  
15  
16

## 17 **ABSTRACT**

18 Degradation of fluorene under UV-Vis irradiation in water was investigated and structural  
19 elucidation of the main photoproducts was achieved using gas chromatography coupled with  
20 mass spectrometry. Twenty-six photoproducts were structurally identified, mainly on the basis  
21 of electron ionization mass spectra interpretation. The main generated transformation products  
22 are hydroxy derivatives. Some secondary photoproducts including fluorenone, hydroxy  
23 fluorenone, 2-biphenyl carboxylic acid, biphenylene, methanol fluorene congeners and hydroxy  
24 fluorene dimers were also observed. A photodegradation pathway was suggested on the basis  
25 of the chemical structures of photoproducts. Fluorene as well as its main photoproducts for

26 which chemical standards were commercially available were tested for their ability to elicit  
27 cytotoxic, estrogenic and dioxin-like activity by using *in vitro* cell-based bioassays. None of  
28 the tested compounds was cytotoxic at concentrations up to 100  $\mu\text{M}$ . However, 2-  
29 hydroxyfluorene and 3-hydroxyfluorene exerted significant estrogenic and dioxin-like activity  
30 on a concentration range of 3-30  $\mu\text{M}$ , while fluorene and 9-hydroxyfluorene were weakly or  
31 not active, respectively, in our assays. This supports the view that photodegradation processes  
32 can generate by-products of higher toxicological concern than the parent compound and  
33 strengthens the need to further identify transformation products in the aquatic environment.

34 **Keywords:** photolysis, photodegradation products, polycyclic hydrocarbons, fluorene, *in vitro*  
35 tests

36

37 **1. Introduction**

38 The industrial and urban development which occurred in the second half of the 20<sup>th</sup> century  
39 allowed the emergence of thousands of organic chemicals into aquatic environment without  
40 prior study of their toxicity. Several decades later, a large number of these compounds have  
41 shown negative impacts on human health and ecosystems even at very low concentrations.  
42 Polycyclic Aromatic Hydrocarbons (PAHs) are among the most widespread pollutants in the  
43 environment. They are of special concern due to their negative characteristics, including  
44 persistence, mobility in the environment and toxicity. The most environmental PAHs result  
45 from incomplete combustion of organic matter: forest fires, automobile exhaust, coal, oil  
46 refining processes, etc. [1,2,3] Many PAHs are toxic; their mainly adverse health effects are  
47 cancers in various tissues such as prostate, breast, pancreatic and cervical [4,5,6,7],  
48 cardiovascular diseases [8] and immunosuppression [9]. PAHs are also known to have endocrine  
49 disrupting capabilities with consequent alteration of fertility in terrestrial and aquatic organisms  
50 [10,11,12]. A number of PAHs are also found to bind the estrogen, androgen and aryl hydrocarbon  
51 receptors and either induce or inhibit the estrogen, antiandrogen and dioxin-like responses  
52 [13,14,15]. For these reasons, PAHs have been listed as priority pollutants by both the US  
53 Environmental Protection Agency (US EPA) and the European Union (EU). Despite a reduction  
54 in PAHs emissions from fuel combustion, resulting from the substitution of coal by fossil fuels  
55 since the 1960s and from the development of clean fuels and catalytic converters for diesel and  
56 gasoline engines in the recent years, the amounts of PAHs in aquatic environment remains  
57 high.<sup>[16]</sup> PAHs can be introduced into the aquatic environment through different routes  
58 including industrial and municipal wastewater, rainwater runoff, atmospheric deposition and  
59 sediment- and air-water exchange [17, 18,16]. The concentration of PAHs in aquatic  
60 environments varies widely depending on the nature of water (i.e. ground water *versus* surface  
61 water) and the sampling location. In European surface water, the concentrations of PAHs were  
62 reported to range from 0.6 to 171.3 ng.L<sup>-1</sup> in rainwater [19,20], from 2 to 587 ng.L<sup>-1</sup> in river water  
63 [21, 22,20], from 5 to 1930 ng.L<sup>-1</sup> in seawater [23,24], from 1.4 to 5 ng.L<sup>-1</sup> in lake and reservoir  
64 water [22,19] and from 4 to 1473 ng.L<sup>-1</sup> in wastewater [22,20]. In natural water environment,  
65 the PAHs are eliminated mainly through processes of sorption to sediments, biodegradation and  
66 photodegradation. Photodegradation remains the main route of PAHs degradation, especially  
67 for the most recalcitrant ones. It has been reported that photolysis processes induced under

68 natural sunlight can be significantly accelerated by dissolved organic matters. In advanced  
69 oxidation processes involving UV light for water and wastewater purification, the ultimate  
70 objective is to achieve complete mineralization of organic pollutants to carbon dioxide, water,  
71 or at least to produce small organic intermediates more soluble, readily biodegradable and thus  
72 less toxic than their parent compounds [25,26,27]. Numerous studies have been conducted on the  
73 photodegradation of PAHs. In most of these studies, much interest has been focused on the  
74 optimization of degradation conditions. The evaluation of the degradation efficiency only relies  
75 on the disappearance of the initial pollutants. In this way, the kinetics of disappearance are  
76 evaluated, as well as the monitoring of the mineralization rate achieved along the process.  
77 Unfortunately, in the course of the degradation of these pollutants, some by-products potentially  
78 more toxic and sometimes even more stable than the parent compounds may be formed [28,29].  
79 For example, studies carried out by Gala *et al.*, Bertilsson *et al.*, Shemer *et al.* and Woo *et al.*  
80 have shown that exposure to UV irradiation may increase the toxicity of many PAHs to a variety  
81 of aquatic organisms [30,25,31,27]. However, very little is known about the nature of the  
82 substances responsible for this increase in toxicity. In this regard, we were interested in the  
83 identification of the phototransformation by-products of PAHs. In the present study, fluorene  
84 (Flu) has been chosen as a model compound for several reasons. First, according to our  
85 knowledge, identification of photoproducts of this PAH in water has never been reported.  
86 Second, fluorene is one of the US EPA and EU priority PAHs because it has water solubility  
87 significantly higher than those of PAHs with larger molecular weights. Third, a previous study  
88 has shown that 2-hydroxyfluorene, which could be a possible photodegradation product of  
89 fluorene in water, exhibits estrogenic activity [32]. The primary objectives of the present  
90 research were the elucidation of the photoproducts chemical structures and that of the reaction  
91 pathways leading to their formation during UV-Vis irradiation of fluorene in aqueous solution.  
92 The kinetics of fluorene disappearance and those of appearance of some photoproducts were  
93 examined. *In vitro* bioassays based on human MELN and fish PLHC-1 cell lines were carried  
94 out to evaluate the estrogenic and dioxin-like potency of photoproducts for which chemical  
95 standards were commercially available.

96

## 97 2. Materials and methods

### 98 2.1 Chemicals, reagents and sample preparation

99 Standards of fluorene (Flu,  $\geq 99\%$  purity), 2-hydroxyfluorene (2OH-Flu,  $\geq 98\%$  purity), 3-  
100 hydroxyfluorene (3OH-Flu,  $\geq 98\%$  purity), 9-hydroxyfluorene (9OH-Flu,  $\geq 96\%$  purity), were  
101 purchased from Sigma-Aldrich (Saint-Quentin Fallavier, France). HPLC-grade methylene  
102 chloride (DCM), acetonitrile (ACN), methanol (MeOH), pyridine, dimethylsulfoxide (DMSO)  
103 and the silylation reagent: N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) with 1% of  
104 trimethylchlorosilane (TMCS) were also purchased from Sigma-Aldrich (France). Ultrapure  
105 water used for photolysis experiments and SPE extraction was obtained by purification of  
106 drinking water using a Millipore Milli-Q device (resistivity  $< 18 \text{ M}\Omega\cdot\text{cm}$  ; DOC  $< 0.1 \text{ mg}\cdot\text{L}^{-1}$ ;  
107 Millipore, CA, USA). A stock solution of individual compounds: Flu, 2OH-Flu, 3OH-Flu and  
108 9OH-Flu, each one at  $10 \mu\text{g}\cdot\text{mL}^{-1}$ , was prepared in amber vials by dissolving the appropriate  
109 amount of each chemical in anhydrous methylene chloride. It was used for the development of  
110 the GC-MS analytical method. All the standard chemicals were prepared in DMSO for bioassay  
111 testing.

### 112 2.2 Irradiation device

113 Photolysis experiments were conducted in a cylindrical pyrex glass reactor (200 mm long x 25  
114 mm internal diameter, effective reaction volume: 50 mL). This reactor is surmounted by two  
115 tubes closed by valves allowing constant pressure (1 atm), constant supply of oxygen and  
116 removal of the sample to be analysed. A UV lamp immersed in a cylindrical quartz water jacket  
117 tube was placed in the middle of the reactor. The lamp used in our experiments was a high-  
118 pressure mercury vapour lamp (HPL-N 125W/542 E27 SG) manufactured from Philips-France  
119 (Ivry sur Seine, France). It emits a polychromatic radiation with wavelengths ranging from 350  
120 nm to 750 nm. Approximately 15% of the emitted light power is in the range 350-400 nm with  
121 a further 85% between 400 and 750 nm. The luminous flux emitted from this lamp was reported  
122 by the manufacturer to be 6200 lm. Prior to photolysis experiments, the lamp was allowed  
123 warming up for 10 min to achieve equilibrium intensity. Homogeneity of the reaction medium  
124 was carried out using a magnetic stirrer. During irradiation period, the reaction temperature was  
125 maintained between 23 and 25 °C by adjusting the flow rate of cooling water. The reactor was  
126 wrapped in an aluminium foil to optimize UV-visible irradiation of the solution and to avoid  
127 emission outside the reactor. The control samples were continuously stirred and kept in the dark

128 under similar experimental conditions as the irradiated solution. For photolysis experiments, a  
129 fluorene solution at  $2 \text{ mg}\cdot\text{L}^{-1}$  was prepared by dissolving 1 mg of fluorene standard in 500 mL  
130 of Milli-Q water. It was prepared just prior to use to reduce any possible biological, physical  
131 and/or chemical transformation due to prolonged storage of fluorene.

### 132 *2.3 Extraction of residual fluorene and its photoproducts*

133 Solid phase extraction (SPE) has been used to extract and enrich fluorene and its generated  
134 photoproducts from water solutions. The protocol employed derives from methods described  
135 in our previous studies.<sup>[33]</sup> Briefly, Oasis<sup>®</sup> HLB cartridges (200 mg x 6 mL, Waters, France)  
136 were placed into a SPE vacuum manifold (Supelco, Bellefonte, PA, USA), conditioned with 3  
137 mL of ACN, then 3 mL of distilled water, and equilibrated with 3 mL of a mixture of  
138 methanol/water (5:95, v/v). Sample volumes of 50 mL were loaded on the cartridges, and the  
139 aqueous solution was eluted as waste at a rate of approximately  $2.5 \text{ mL}\cdot\text{min}^{-1}$ . The retained  
140 compounds (fluorene and its photodegradation products) were eluted by loading four times 2.5  
141 mL of methanol. The organic extracts were evaporated to complete dryness under a gentle  
142 nitrogen stream, and dry residues were resuspended in 0.5 mL of DCM, sonicated and then  
143 divided into two equal parts. The first one was completely dried under a gently stream of  
144 nitrogen and reconstituted into 50  $\mu\text{L}$  of DMSO for bioassay analysis. The second aliquot was  
145 subdivided again into two equal parts; the first one was directly analysed by GC/MS, while the  
146 second was derivatized with BSTFA according to the method already described by Kinani et  
147 al. prior to GC/MS analysis [33].

### 148 *2.4 GC-MS analysis*

149 Residual fluorene and generated photoproducts (derivatized and non-derivatized extracts) were  
150 analysed using a “450-GC” gas chromatograph equipped with a “CP-8400” autosampler and  
151 coupled to a “240-MS” ion trap mass spectrometer (Varian, Les Ulis - France). The  
152 chromatographic separation was performed on a 60 m “Factor four VF-10-MS” (10% phenyl,  
153 90% methylpolysiloxane) capillary column (internal diameter: 0.25 mm, film thickness: 0.25  
154  $\mu\text{m}$ ) from Varian. All experiments were performed by automatically injecting 1.0  $\mu\text{L}$  of sample  
155 in the splitless mode at a rate of  $50 \mu\text{L}\cdot\text{s}^{-1}$ . High purity (99.999%) helium was used as the carrier  
156 gas at a constant flow of  $1.4 \text{ mL}\cdot\text{min}^{-1}$  hold by electronic pressure control. The injector  
157 temperature was set at 280 °C. The split valve opened after 2.0 min, with a split ratio of 40/100.  
158 The capillary column was ramped from an initial temperature of 50 °C, held for 0.5 min, at 10  
159  $^{\circ}\text{C}\cdot\text{min}^{-1}$  up to 320 °C where it was held for 7.5 min. The total duration of GC analysis was 35.0

160 min. The manifold, ion trap electrodes and transfer line temperatures were set at 120 °C, 220  
161 °C and 300 °C, respectively. The mass spectrometer was operated in internal ionization source  
162 and the acquisition was performed in full scan mode. Both chemical ionization (CI) and electron  
163 ionization (EI) modes were used for a reliable structural elucidation of photodegradation  
164 products of fluorene. The instrument was automatically tuned using the ions resulting from  
165 electron ionization of perfluorotributylamine. In EI, the ionization energy was 70 eV and the  
166 filament current was 10  $\mu$ A. Spectra were recorded using the automatic gain control (AGC)  
167 function with a target value of 20.000. CI was performed using methanol as reagent gas.  
168 Automatic reaction control (ARC) parameters were 100  $\mu$ s for the maximum ionization time,  
169 and 2000 for the target value. In all experiments, the multiplier voltage was set to 1850 V ( $10^5$   
170 gain) by automatic tuning. Ions were scanned over a 50-600 m/z range, with a scan rate of 1  
171 scan. $s^{-1}$ . For the experiments carried out with fluorene at 0.1 ppm in raw lake water (see section  
172 3.3), GC-MS analyses were performed with the same chromatographic conditions, using  
173 selected ion storage in the EI mode. The two major ions (see Table 1) were selected for each  
174 compound. The sensitivity was also enhanced by increasing the filament current and multiplier  
175 voltage values up to 50  $\mu$ A and 2000 V, respectively.

176

## 177 *2.5 In vitro bioassays*

### 178 2.5.1 Estrogenic activity

179 The MELN reporter cell line has been used in order to assess the estrogenic activity of  
180 commercially available photoproducts of fluorene. This cell line was obtained by stable  
181 transfection of MCF-7 human breast cancer cells by an ERE-bGlob-Luc-SVNeo plasmid [<sup>34</sup>].  
182 The cells were routinely cultured in phenol red containing Dulbecco's Modified Eagle's  
183 Medium (DMEM), supplemented with 5% foetal calf serum (FCS), 1% nonessential amino  
184 acids and penicillin/streptomycin (50 U/mL each) in a 5% CO<sub>2</sub> humidified atmosphere at 37  
185 °C. For experiments, cells were left to incubate for 2 days in phenol red free DMEM  
186 supplemented with 3% dextran charcoal coated-FCS (DCC medium) before seeded in white  
187 opaque 96-wells culture plates at a density of 50,000 cells per well. Serial dilutions of reference  
188 or test chemicals were added in triplicates 24 h later and then left to incubate for 16 h. In all  
189 assays, DMSO in the culture medium was always at 0.1% v/v, including in cell controls. At this  
190 concentration, no effect on cell viability or luciferase activity was observed. After cell exposure,



191 the medium was removed and replaced by 50  $\mu$ L of DCC medium containing 0.3 mM of D-  
192 luciferin and the luminescence signal was measured in living cells for 2 s per well with a  
193 microtiter plate luminometer ( $\mu$ Beta, Wallac). Relative luminescence units (RLU) were  
194 converted to relative response units expressed as percent of maximal luciferase activity induced  
195 by the positive control (17 $\beta$ -Estradiol (E2) at 10 nM).

#### 196 2.5.2 Dioxin-like activity

197 The dioxin-like activity was monitored by using the fish hepatic PLHC-1 cell line (ATCC,  
198 #CRL-2406) as described by Louiz *et al.* [15]. The cells were routinely grown at 30 °C in E-  
199 MEM culture media supplemented with 10% FCS and 1% antibiotics in a 5% CO<sub>2</sub> humidified  
200 atmosphere. For experiments, cells were seeded in 96-well plates at a density of approximately  
201 50,000 cells per well. After 24 h of incubation, cells were exposed to test chemicals or sample  
202 extracts for 4 h incubation period. Then, plates were processed for 7-ethoxyresorufin-O-  
203 deethylase (EROD) activity in intact cells, as previously described.<sup>[15]</sup> Results were expressed  
204 as percent of EROD activity induced by the positive control (tetrachlorodibenzodioxin (TCDD)  
205 at 0.3 nM).

#### 206 2.5.3 Cytotoxicity

207 The ability of test chemicals to alter cell viability after a 24 h-exposure was assessed in the  
208 PLHC-1 cell line by using the MTT assay [<sup>35</sup>], as previously described [<sup>36</sup>]. The results were  
209 expressed as relative to DMSO treated control cells.

### 210 **3. Results and discussion**

#### 211 *3.1 Identification of fluorene photoproducts by GC-MS*

212 The comparison between chromatograms of irradiated and non-irradiated solutions of fluorene  
213 revealed the presence of twenty six photoproducts. The identified compounds are summarized  
214 in Table 1. Among the twenty six compounds, those which were commercially available, such  
215 as 9-fluorenone, 2-hydroxyfluorene (2OH-Flu), 3-hydroxyfluorene (3OH-Flu) and 9-  
216 hydroxyfluorene (9OH-Flu), were quickly and unambiguously identified by comparing  
217 retention times and mass spectra with those of reference compounds. The other compounds  
218 were identified on the basis on their EI and CI mass spectra interpretation. BSTFA  
219 derivatization was helpful since it permitted to establish the number of exchangeable hydrogen  
220 atoms for each photolysis product.

221 *Table 1*

222 Six products with  $M = 182$  were detected with close retention times (ranging from 25.7 to 29.3  
223 min.) and identical mass spectra. Compared to fluorene mass weight, those compounds are  
224 shifted by + 16 amu, suggesting addition of an oxygen atom. In derivatized extracts, the  
225 corresponding molecular and pseudo molecular ions are shifted by + 72 amu (one hydrogen  
226 atom replaced by  $\text{Si}(\text{CH}_3)_3$ ) in EI and CI, leading to the conclusion that these compounds are  
227 hydroxylated derivatives of fluorene (OH-Flu). Owing to the analysis of standard solutions, the  
228 chromatographic peaks at 26.4, 26.5 and 29.1 were unambiguously attributed to, 9OH-Flu,  
229 2OH-Flu and 3OH-Flu, respectively, and both other peaks were logically assumed to  
230 correspond to 1-hydroxyfluorene (1OH-Flu) and 4-hydroxyfluorene (4OH-Flu).

231 A product is detected at 26.5 min. Its main ions in EI are  $m/z$  180 and  $m/z$  152, corresponding  
232 to the molecular ion  $M^+$  and to  $[\text{M}-\text{CO}]^+$ , respectively. The EI and CI mass spectra and the  
233 retention time of this product were consistent with those observed for the authentic standard of  
234 9-fluorenone. This result suggests that a  $\text{CH}_2$  group of fluorene has been oxidized into CO, as  
235 depicted in Figure 1. A similar oxidation was reported by Rivas et al. who proposed a detailed  
236 mechanism of oxidation of fluorene by hydroxyl radicals. 9-fluorenone, 9-fluorenol and  
237 dibenzofuran were formed during the first step of the reaction; further oxidation of these  
238 products proceeded via hydroxylation and cleavage of the fluorene ring [37].

239 *Figure 1*

240 Nine products with  $M = 196$  were detected. They were divided into two groups on the basis of  
241 their EI mass spectra. The four first eluted compounds (27.8, 29.8, 31.8 and 31.9 minutes)  
242 correspond to isomers of hydroxyfluorenone (OH-Flu-one): EI mass spectra of some isomers  
243 of OH-Flu-one are displayed in the NIST mass spectra database [38]. These spectra are identical  
244 so that the different isomers could not be determined owing to the database. It is to be noted  
245 that 2-benzyl-1,4-benzoquinone provides the same EI mass spectrum [38]. Its formation has  
246 been considered since 1,4-naphthoquinone was detected as a photolysis product of naphthalene  
247 in several previous studies [39,40]. In the present work, BSTFA derivatization of the compounds  
248 of interest led to the addition of one trimethylsilyl (TMS) group to each compound ( $M = 268$ ),  
249 showing the presence of one exchangeable hydrogen atom, in agreement with OH-Flu-one  
250 isomers. The five other products with  $M = 196$  (RT = 30.1, 30.2, 30.3, 30.4 and 30.5 minutes)  
251 displayed EI mass spectra with ions at  $m/z$  178,  $m/z$  166 and  $m/z$  165, resulting from

252 eliminations of  $\text{H}_2\text{O}$ ,  $\text{H}_2\text{C}=\text{O}$  and  $\text{CH}_3\text{O}$ ; respectively. After many attempts to find likely  
253 chemical structures corresponding to  $\text{C}_{13}\text{H}_8\text{O}_2$ , we finally concluded that the only possibilities  
254 corresponding to these EI mass spectra were structures of fluorene methanol (MeOH-Flu), in  
255 agreement with the solution suggested by the NIST database. Since photolysis had been  
256 performed in pure water, the only explanation for the addition of a carbon atom to Flu was  
257 through the formation of a dimer species, for which a mechanism of formation is proposed in  
258 Figure 2. The first eluted isomer (RT = 30.1 min.) was determined to be 9MeOH-Flu. As a  
259 matter of fact, its EI mass spectrum displays a  $m/z$  178 ion (loss of  $\text{H}_2\text{O}$  from  $\text{M}^+$ ) in greater  
260 abundance than for other isomers (82% vs 24%). The supporting information file SI-1 shows  
261 the dissociation pathways proposed to interpret the EI mass spectrum of 9MeOH-Flu. The easier  
262 loss of water from 9MeOH-Flu than for other MeOH-Flu isomers is easily rationalized on the  
263 basis of the  $\alpha,\beta$  concerted elimination of  $\text{H}_2\text{O}$  involving a hydrogen atom of the  $\text{sp}^3$  carbon,  
264 which cannot occur for other isomers.

265 *Figure 2*

266 Four compounds with  $M = 348$  were detected. The first one elutes at 37.1 min. while the three  
267 other ones are coeluted between 38.4 and 38.6 minutes. All are assumed to be isomers of  
268 hydroxylated dimers of fluorene. Their formation is discussed below. Five products with  $M =$   
269 198 were detected, one eluted at 28.5 min. and three between 31.2 and 31.6 min. For the first  
270 one, the EI mass spectra obtained from non-derivatized extracts showed major fragment ions at  
271  $m/z$  181 ( $[\text{M}-\text{OH}]^+$ ) and  $m/z$  153 ( $[\text{M}-\text{COOH}]^+$ ), issued from characteristic losses for  
272 compounds containing a carboxylic group. In chemical ionization, this molecule exhibits a base  
273 peak at  $m/z$  181 consistent with water elimination. The EI mass spectrum obtained from  
274 derivatized extracts exhibits a molecular ion at  $m/z$  270 and a  $\text{MH}^+$  pseudo molecular ion at  $m/z$   
275 271 issued from auto protonation, a phenomenon classically observed in ion trap mass  
276 spectrometers. The major fragment ions are observed at  $m/z$  181  $[\text{M}-\text{OSi}(\text{CH}_3)_3]^+$ ,  $m/z$  153  
277  $[\text{MH}-\text{HOSi}(\text{CH}_3)_3-\text{CO}]^+$  and  $m/z$  73  $[\text{Si}(\text{CH}_3)_3]^+$ . The main ions of the CI mass spectrum are  
278  $m/z$  271 ( $\text{MH}^+$ ) and  $m/z$  181 corresponding to  $[\text{MH}-\text{HOSi}(\text{CH}_3)_3]^+$ . Based on these mass  
279 fragmentation patterns and on the results of MS databases researches, this compound was  
280 identified as 2-biphenylcarboxylic acid (BPCA). Two mechanisms can be advanced to explain  
281 the formation of this compound. The first one involves direct oxidation of fluorene while the  
282 second one involves oxidation of 9-fluorenone, which has been previously identified as a  
283 photoproduct of fluorene. The four other compounds with  $M = 198$  may correspond either to

284 dihydroxyfluorene congeners, either to structures such as those displayed in the supporting  
285 information file SI-2, which are dialdehydes analogous to those reported by several authors in  
286 their studies devoted to photocatalysis of naphthalene [39,<sup>41</sup>,<sup>42</sup>]. The molecular weight of these  
287 three compounds is shifted to  $M = 342$  after BSTFA derivatization, thus indicating that two  
288 exchangeable hydrogen atoms were replaced by a TMS group and allowing to conclude in favor  
289 of dihydroxyfluorene congeners.

### 290 *3-2 Mechanistic approach of the photodegradation of fluorene*

291 As demonstrated in what follows, the  $sp^3$  carbon atom of fluorene leads to a particular behavior  
292 under UV-Vis irradiation in comparison with other PAHs. As a matter of fact, one of the main  
293 mechanisms generally proposed to rationalize oxidation of PAHs under UV photolysis begins  
294 by the formation of a radical molecular cation from the irradiated molecule through electron  
295 removing [<sup>43</sup>]. The solvated electron released by the molecule is assumed to be transferred to  
296 dissolved oxygen yielding a superoxide anion which may, in turn, react with water to provide  
297  $H_2O_2$  (see the upper left side of Figure 1). UV photolysis of  $H_2O_2$  yields hydroxy radicals [<sup>44</sup>].  
298 The mechanism of electron removing in the aqueous phase may be compared with that of  
299 electron ionization in the gas phase, which is widely used in mass spectrometry.  $\pi$  electrons are  
300 known to be much more easier to abstract than  $\sigma$  electrons. That is why ionization is expected  
301 to occur on one of the aromatic rings, as observed in mass spectrometry. In this study, ionization  
302 on one of both aromatic rings can easily account for the formation of 1-, 2-, 3- and 4-  
303 hydroxyfluorene (Figure 1, left pathway) but cannot lead to the formation of 9-hydroxyfluorene  
304 (9OH-Flu) and fluorenone (Flu-one). The detection of large amounts of 9OH-Flu and Flu-one  
305 suggests a mechanism, competing with electron removing, consisting in the direct cleavage of  
306 one of the C-H bonds in which the  $sp^3$  carbon atom is involved, as suggested in the center  
307 pathway of Figure 1. In the present case, the elimination of  $H^\cdot$  from this carbon atom leads to a  
308 fully conjugated radical which is particularly stable. A direct reaction between this radical and  
309 a  $HO^\cdot$  radical leads to 9OH-Flu but also to other hydroxyfluorene isomers because of mesomeric  
310 effects. We suggest that fluorenone results from dehydrogenation of fluorenenol as the "kinetic"  
311 approach (see below) shows a good correlation between the increase in Flu-one concentration  
312 and the decrease in 9OH-Flu concentration. Furthermore, we observed when analyzing standard  
313 samples that 9OH-Flu is spontaneously converted into Flu-one in a few hours, at ambient  
314 temperature, even sheltered from light. It is to be noted that in a previous study, Dabrowska et  
315 al. established that the formation of fluorenone precedes that of fluorenenol when irradiating

316 fluorene not in water but in methylene chloride and hexane [45]. Sabaté et al. reached the same  
317 conclusion performing irradiation in an ethanol/water mixture [46]. Both studies focused on the  
318 stability of fluorene under photolysis and only Flu, OH-Flu and Flu-one were detected owing  
319 to a UV-vis spectrophotometer; other by-products were not detected or not considered. The  
320 formation of BPCA is assumed to result from oxidation of Flu-one through addition of a  
321 hydroxy radical. Since dihydroxyfluorene (diOH-Flu) isomers could not be identified, their  
322 formation has been assumed to result from a hydroxy radical addition onto 9OH-Flu and/or  
323 onto another OH-Flu isomer. Finally, the formation of hydroxy-9-fluorenone may result either  
324 from dehydrogenation of hydroxy-9-fluorenol, via a mechanism analogous to that involved in  
325 the reduction of 9OH-Flu into Flu-one, either from attack of a hydroxy radical onto the later.

326 We proposed the mechanism displayed in Figure 2 to rationalize the formation of 9MeOH-Flu  
327 through a dimer intermediate. The formation of other MeOH-Flu isomers with the CH<sub>2</sub>OH  
328 group bound to an aromatic ring in 1, 2, 3 or 4 position can be explained with analogous  
329 mechanisms considering a dimer with a C-C bond between the sp<sup>3</sup> carbon atom and a carbon  
330 atom of an aromatic ring (Reaction pathways are suggested in the supplementary information  
331 file SI-3). The detection of biphenylene (M = 152) at 18.0 min. is in agreement with the  
332 suggested mechanism.

333 With the aim to establish how photolysis products of Flu are related to each other and if the  
334 evolutions of relative amounts are in agreement with the proposed reaction pathways, we  
335 followed relative concentrations as a function of the irradiating time up to 120 minutes. A "real"  
336 kinetic study was not the purpose of its work; it would have required possessing standard  
337 compounds for all the photolysis products and would have been very complicated to achieve  
338 since several photolysis pathways are undoubtedly in competition. Given the unexpected  
339 evolution of the fluorene concentration as a function of the irradiation time, kinetics  
340 experiments were carried out three times and provided results with a good repeatability (RSD  
341 < 15%)". Figure 3 displays the evolution of the relative amounts of fluorene and its  
342 photoproducts. Since concentrations of isomeric compounds undergo the same evolution, sums  
343 of amounts were plotted for much clarity. It is to be noted that the amounts reported in figure 3  
344 correspond to the areas of chromatographic peak integrated on the total ionic current (TIC) in  
345 the EI mode and normalized to the initial TIC value which corresponds to the fluorene peak  
346 area at t<sub>0</sub>. Considering the likely differences in response factors between compounds, the  
347 relative amounts plotted allow the monitoring of a given photoproduct but they cannot be used  
348 to compare concentrations of different photoproducts.

349 The concentration of Flu decreases by a factor four during the first fifteen minutes of irradiation,  
350 then increases until one hour of irradiation and decreases again during the following hour.  
351 Figure 3 shows evidence that the fast decrease in Flu concentration corresponds to dimers  
352 formation. Dimers partially dissociate in turn to give biphenylene and MeOH-Flu isomers,  
353 which are assumed to eliminate  $\cdot\text{CH}_2\text{OH}$  radicals under irradiation to provide fluorene again  
354 (Figure 2), thus explaining the increasing relative amount of Flu after the first fifteen minutes  
355 of irradiation. In competition with this reaction pathway, the mechanisms suggested in Figure  
356 1 appear to be slower. The kinetics features are in good agreement with the proposed  
357 mechanisms since the concentrations in Flu-one and BCPA increase as that in 9OH-Flu  
358 decreases. Amounts of diOH-Flu isomers increase then slowly decrease as those of OH-Flu  
359 isomers increase.

360 *Figure 3*

### 361 *3.3 Irradiation in more realistic conditions*

362 The above experiments were performed with a relatively high concentration of fluorene to allow  
363 fullscan detection in GC-MS and thus characterization of photoproducts. To get closer of  
364 natural conditions, additional experiments were carried out with organic matter containing  
365 water taken from Lake Palaiseau (France) spiked with fluorene at  $100 \mu\text{g}\cdot\text{L}^{-1}$ . Water evaporation  
366 under mild conditions led to a dry matter residue of  $230 \text{ mg}\cdot\text{L}^{-1}$ . Experiments were conducted  
367 with irradiation times of 60 and 120 min. The sample preparation process was almost the same  
368 as the one described in section 2.3; a filtration step on a PTFE membrane (diam. 13 mm, pore  
369 size:  $0.45 \mu\text{m}$ , Sigma-Aldrich, France) has only been added to remove suspended matter prior  
370 to solid phase extraction. GC-MS analyses were performed with the chromatographic  
371 conditions previously described but using selected ion storage in the EI mode. The two major  
372 ions (see Table 1) were selected for each compound; the method sensitivity was enhanced by  
373 increasing both filament current and electron multiplier voltage (see section 3.2). For both  
374 irradiation times, the GC-MS chromatograms showed the photoproducts characterized earlier  
375 but those at  $M = 348$ , which were assumed to result from dimerization and hydroxylation of  
376 fluorene and those at  $M = 196$  corresponding to methanol-fluorene congeners. The  
377 disappearance of dimerized species using a solution at 0.1 ppm may be easily explained as the  
378 probability of reaction between two molecules decreases when the concentration decreases.  
379 Non-detection of methanol-fluorene congeners is logical as well, given that their formation  
380 requires a first dimerization step (see the mechanisms depicted in figure 1 and supporting

381 information file 3). Additional experiments were performed in the fullscan mode, with the aim  
382 to possibly detect photoproducts issued from photo-induced reactions between fluorene and  
383 some components of dissolved organic matter. None was detected, either because such  
384 reactions do not occur or, more likely, because detection in the fullscan mode provides too high  
385 detection thresholds in comparison with the expected concentration ranges for such products.

386

### 387 *3.4 In vitro activity of hydroxyfluorenes*

388 Amongst all the identified degradation products, 2OH-Flu, 3OH-Flu and 9OH-Flu were  
389 commercially available as chemical standards and could thus be tested for their biological  
390 activity using *in vitro* cell-based assays. While none of the test compounds was found to alter  
391 cell viability at concentrations up to 100  $\mu\text{M}$  (data not shown), two of them, i.e. 2OH-Flu and  
392 3OH-Flu, were able to activate the estrogen (ER) and aryl hydrocarbon (AhR) receptors. As  
393 shown in Figure 4, 2OH-Flu and 3OH-Flu exerted a significant estrogenic activity in the MELN  
394 cell line, yielding up 50% and 100% of luciferase induction, respectively. For both chemicals,  
395 the EC50 was found to be 10  $\mu\text{M}$ . Conversely, the parental compound fluorene was only weakly  
396 estrogenic (weak effect at  $> 30 \mu\text{M}$ ) while 9OH-Flu was not active at the test concentrations.  
397 The *in vitro* estrogenic activity of 2OH-Flu has been previously reported in yeast-based assays  
398 [47, 48], we confirm here its activity in a mammalian cell-based assay and report for the first time  
399 the estrogenic potency of 3OH-Flu. In addition, the position of the hydroxyl group on the  
400 molecule has a strong influence on the biological activity. Both 3OH-Flu and 2OH-Flu have a  
401 phenol ring in their structure which confers structural similarity with the endogenous hormone  
402 17 $\beta$ -estradiol and thus contributes to their ability to bind to and activate the ER [48].  
403 Conversely, 9OH-Flu, which has no phenolic group in its structure, was unable to activate the  
404 ER in our assay.

405

#### *Figure 4*

406 Interestingly, 2OH-Flu and 3OH-Flu were also potent inducers of EROD activity in the PLHC-  
407 1 cell line (Figure 5), with EC50s around 10 and 3  $\mu\text{M}$ , respectively. This induction was marked  
408 after 4 hours of cell exposure but it was strongly reduced after 24 h, only a weak induction (i.e.  
409 10% that of the positive control) remaining at that time point. Similar patterns of transient  
410 EROD induction were previously reported with weakly persistent AhR agonists such as various

411 PAHs, which suggested that the compounds were metabolized by cellular systems [49,15].  
412 Fluorene and 9OH-Flu were found weakly or non-active in this bioassay.

413 *Figure 5*

414 Overall, our results indicate that Flu photolysis could generate hydroxylated degradation  
415 products that are biologically much more active than is the parent compound. Further  
416 experiments assessing the biological activity of photolyzed aqueous solutions of fluorene will  
417 be necessary to characterize the whole toxicity of the mixture of photoproducts and thus to  
418 provide a more comprehensive identification of active fluorene by-products. For instance, the  
419 use of effect-directed analysis approach (EDA) [50], e.g. based on HPLC fractionation of the  
420 chemical mixture and chemical identification of bioactive fractions could nicely provide new  
421 information on active photoproducts.[51] Such approach has already proven useful to identify  
422 phototransformation products of key emerging aquatic pollutants such as diclofenac [52] or  
423 estrone [53]. Identifying biologically active oxygenated PAHs using EDA would doubtless  
424 provide crucial basic knowledge that is still needed, for instance, to better interpret estrogenic  
425 and dioxin-like activities of PAHs-associated fractions in environmental complex samples.

426

#### 427 **4. Conclusion**

428 Studies aiming to identify transformation products of pollutants are necessary to understand  
429 their behavior and impact on the environment, as well as their effects on human health. In the  
430 present work, the photodegradation of fluorene (Flu) under UV-Vis irradiation in water was  
431 investigated. The complete mineralization of Flu was not observed under our conditions. Using  
432 gas chromatography coupled with mass spectrometry, twenty-six photoproducts were  
433 characterized, mainly on the basis of electron and chemical ionization mass spectra  
434 interpretation. The main generated transformation products are monohydroxy derivatives.  
435 Some secondary photoproducts were also observed: fluorenone, hydroxy fluorenone, 2-  
436 biphenyl carboxylic acid, biphenylene, methanol fluorene congeners and hydroxy fluorene  
437 dimers. Structural elucidation of photoproducts led to suggest photodegradation pathways.  
438 Fluorene and the main photoproducts for which chemical standards were commercially  
439 available were tested for their ability to elicit cytotoxic, estrogenic and dioxin-like activity by  
440 using *in vitro* cell-based bioassays. None of the tested compounds was cytotoxic at



441 concentrations up to 100  $\mu\text{M}$ . However, 2-hydroxyfluorene and 3-hydroxyfluorene exerted  
442 significant estrogenic and dioxin-like activity on a concentration range of 3-30  $\mu\text{M}$ . This study  
443 demonstrates that UV-Vis irradiation of fluorene generates hydroxylated photoproducts that are  
444 biologically much more active than is the parent compound and strengthens the need to consider  
445 transformation products and not only identified pollutants in the environment.  
446

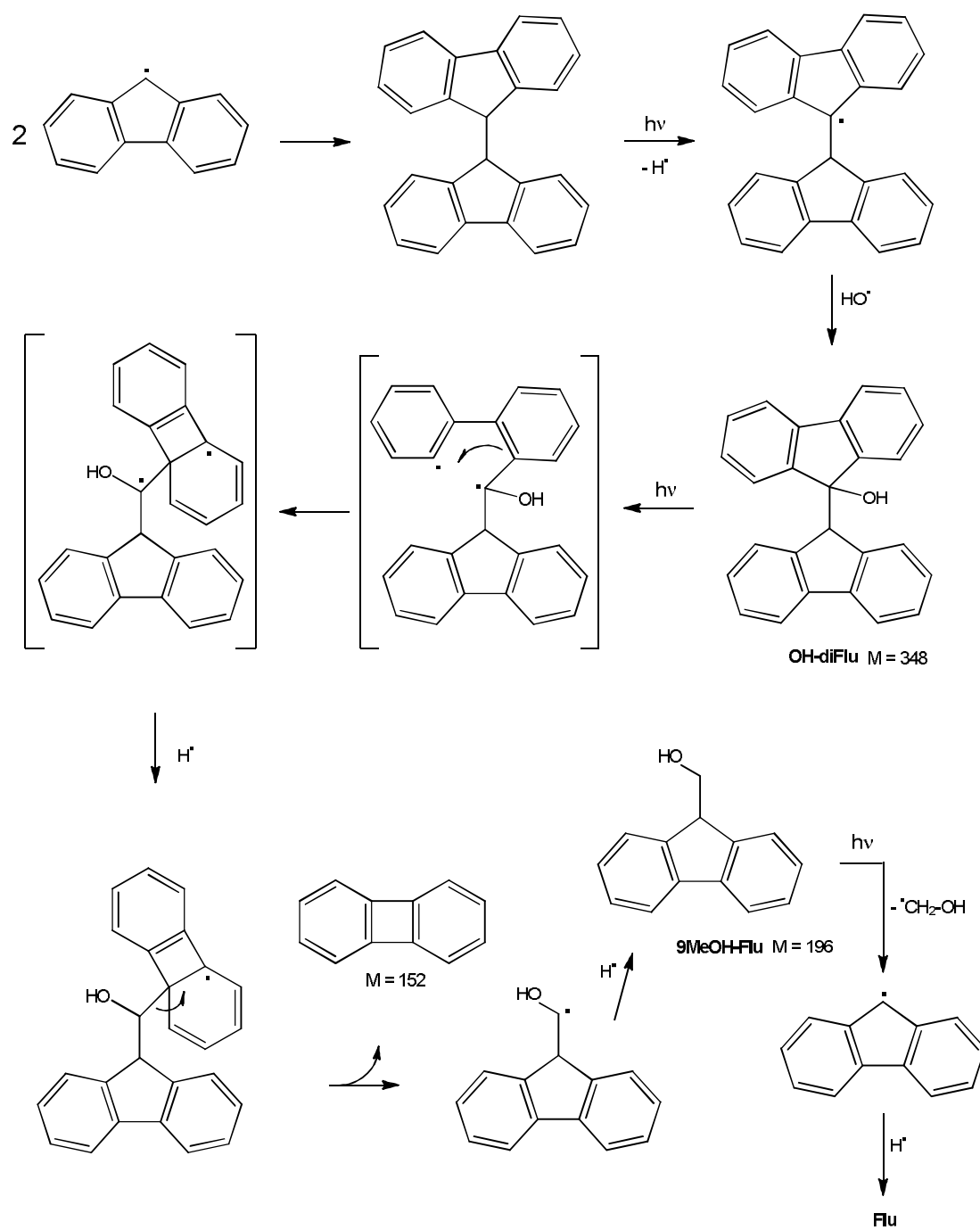


452 Figure 2. Reaction pathways proposed for the formation of OH-diFlu dimers and 9MeOH-Flu  
453 under UV-Vis irradiation in water

454 Figure 2. *The relative amounts reported correspond to the areas of chromatographic peak*  
455 *integrated on the total ionic current (TIC) in the EI mode and normalized to the initial TIC*  
456 *value which corresponds to the fluorene peak area at  $t_0$ .*

457

458

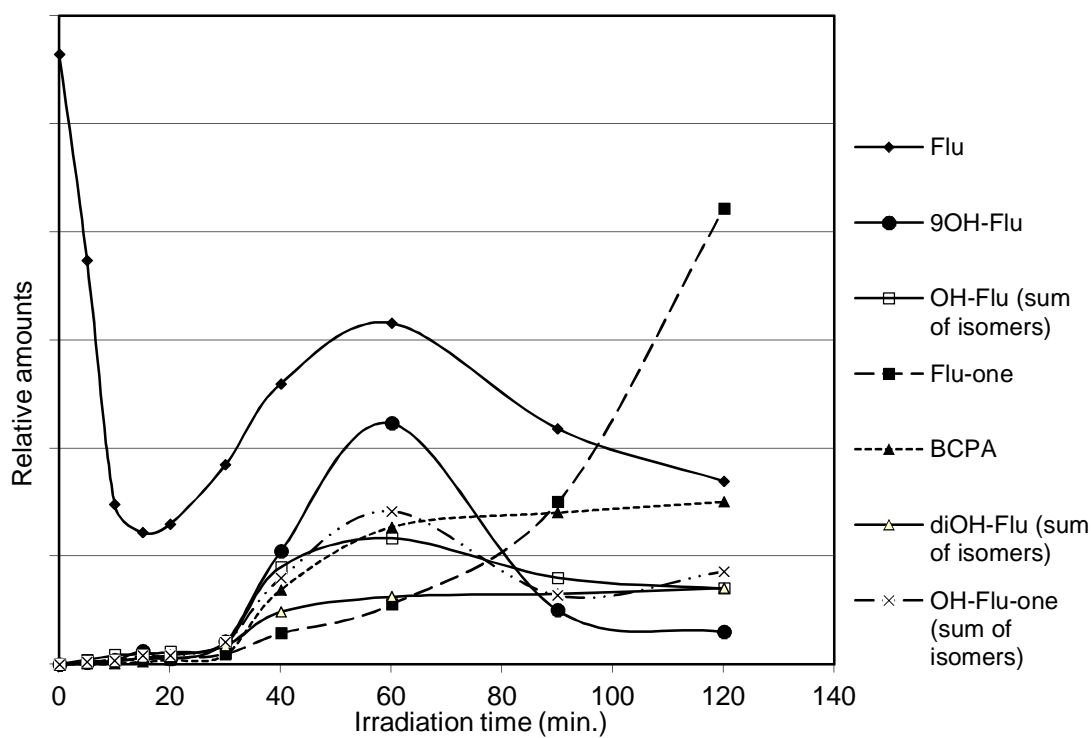


459

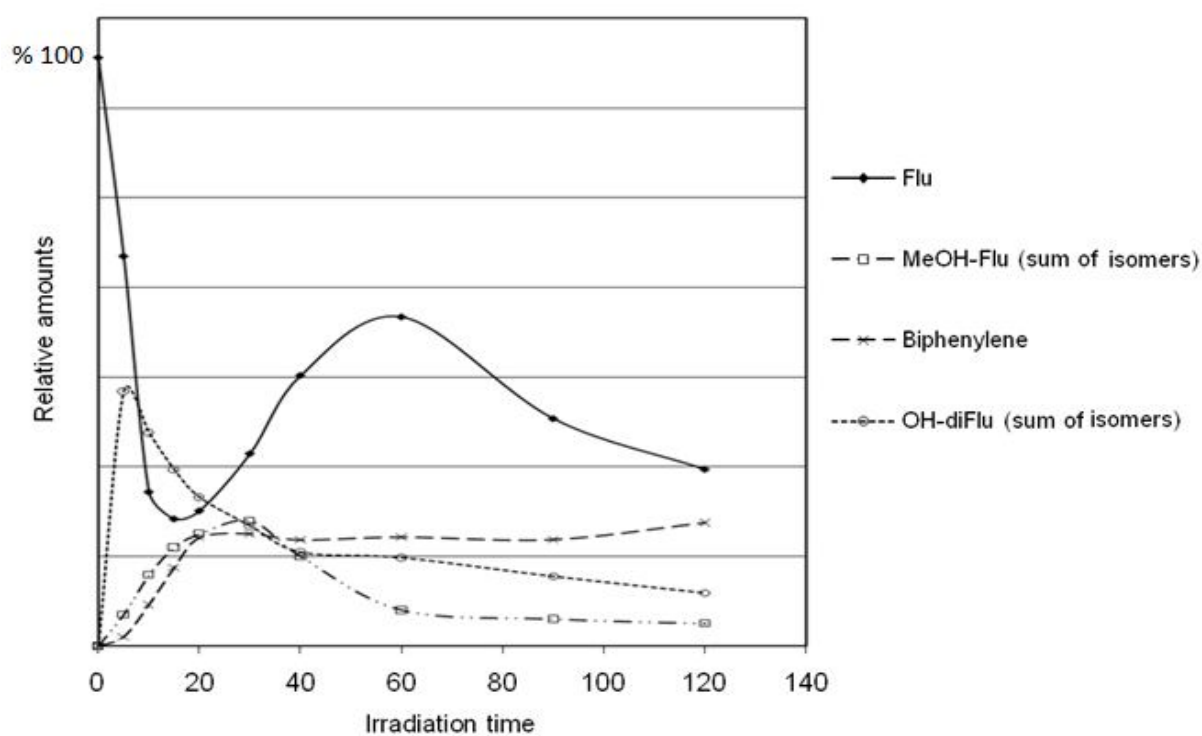
460

461

462 Figure 3. Relative amounts of fluorene photoproducts as a function of the irradiation time.  
 463 Upper part involves photoproducts displayed in Figure 1 while lower part involves those in



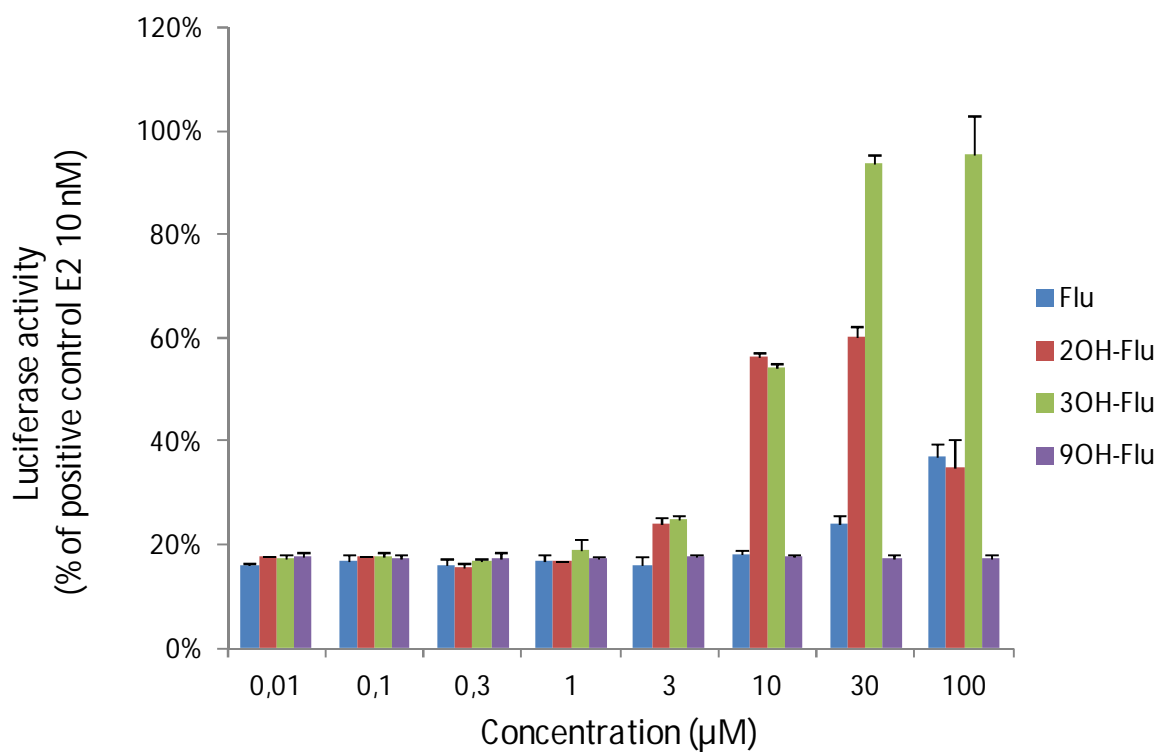
464



465

466

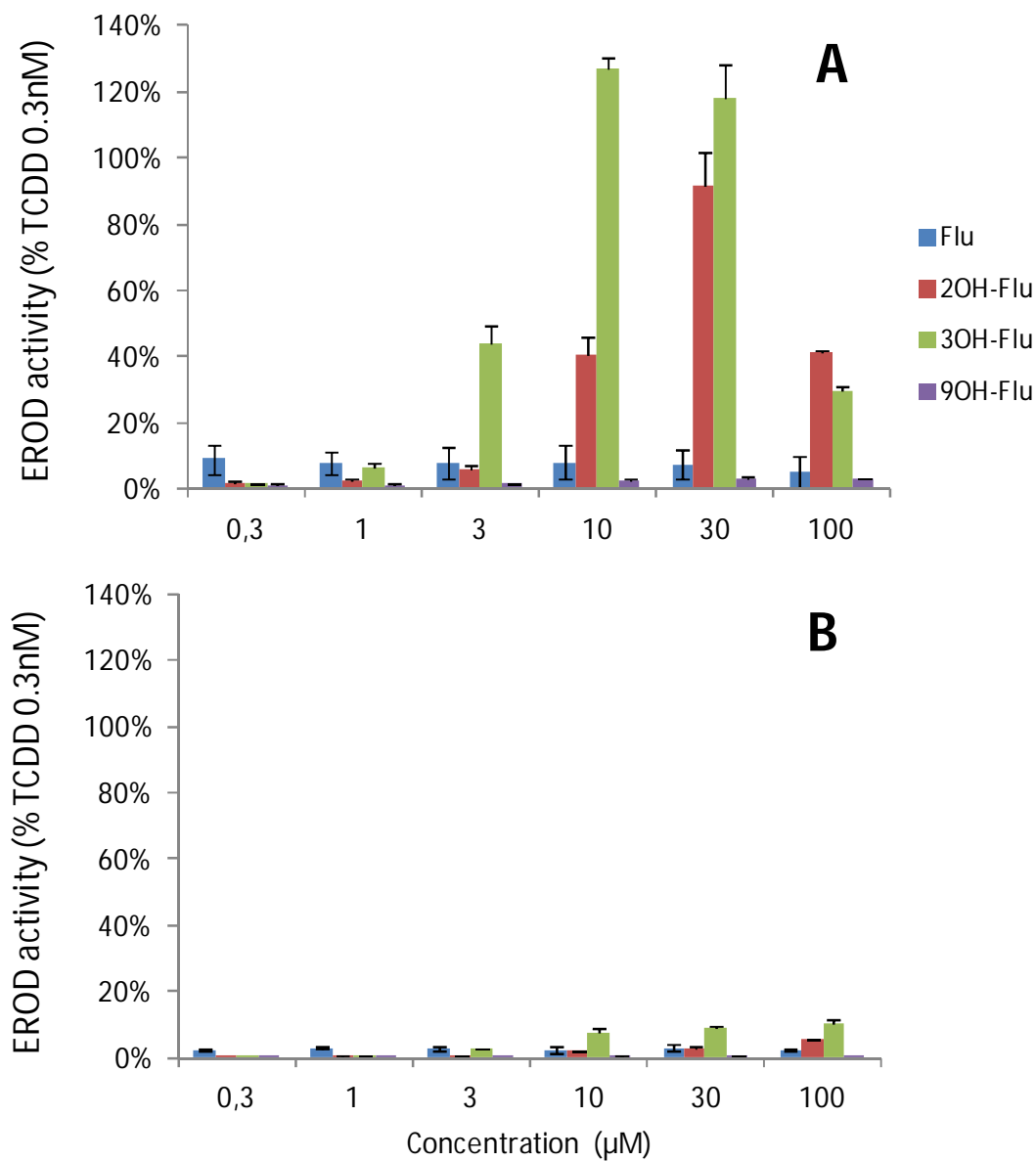
467 Figure 4. *In vitro* estrogenic activity of Flu, 2OH-Flu, 3OH-Flu and 9OH-Flu in the MELN  
468 reporter cell line. Results are expressed as % of luciferase induction relative to the positive  
469 control estradiol (E2, 10 nM)



470

471 Figure 5. *In vitro* dioxin-like activity of Flu, 2OH-Flu, 3OH-Flu and 9OH-Flu in the PLHC-1  
472 cell line after cell exposure for a) 4 h and b) 24 h. Results are expressed as % of EROD activity  
473 induction relative to the positive control dioxin (TCDD, 0.3 nM)

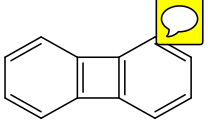
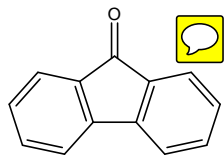
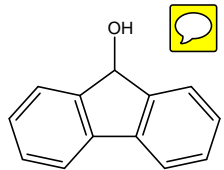
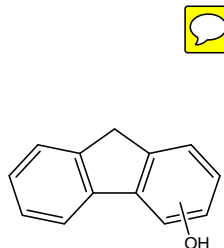
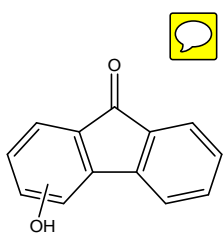
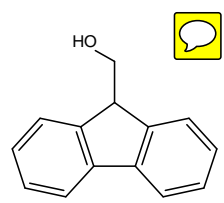
474



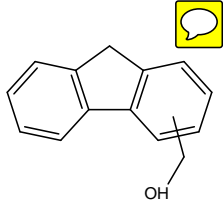
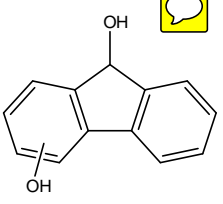
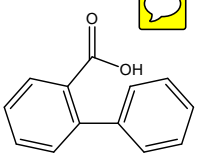
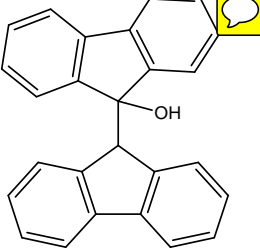
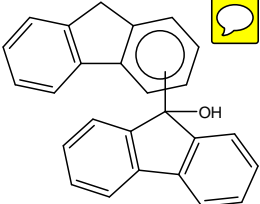
475  
476  
477  
478

479 Table 1. Identified photoproducts resulting from UV-Vis irradiation of fluorene in water

MW	Rt (min.)	Main ions in mass spectra <sup>a</sup>			Identified compounds	
		EI	CI	CI (DC) <sup>b</sup>	Structure	Name

152	18.0	152 (100), 150 (90), 122 (45), 133 (27), 91 (27)	nd <sup>c</sup>	nd		Biphenylene
180	26.5	180 (100), 152 (59), 151 (19)	181 (100)	nd		9-Fluorenone
182	26.4	182 (100), 181 (79), 165 (23), 153 (34), 152 (43)	183 (100)	255 (100)		9-Fluorenol
182	25.7 28.7 28.9 29.1 29.3	182 (100), 181 (49), 165 (9), 164 (18), 153 (21), 152 (36), 151 (11)	183 (100)	255 (100)		Hydroxy-fluorene congeners
196	27.8 29.8 31.8 31.9	196 (100), 168 (98), 140 (30), 139 (71), 117 (17), 91 (17)	197 (100)	269 (100)		Hydroxy-9- fluorenone congeners
196	30.1	196 (66), 178 (82), 166 (58), 165 (100), 152 (16)	197 (100)	269 (100)		9-Methanol- fluorene



196	30.2 30.3 30.4 30.5	196 (55), 178 (24), 166 (55), 165 (100), 152 (15)	197 (100)	269 (100)		Methanol-fluorene congeners
198	31.2 31.3 31.4 31.6	198 (100), 197 (78), 181 (69), 162 (15), 153 (42), 152 (45), 141 (25), 139 (22) 115 (36)	199 (100)	343 (100)		Hydroxy-9-fluorenone
198	28.5	198 (55), 197 (41), 181 (100), 169 (13), 153 (19), 152 (18), 141 (26), 139 (12), 115 (26)	199 (100)	271 (100)		2-Biphenyl carboxylic acid
348	37.1	348 (33), 174 (100)	349 (100)	421 (100)		Hydroxy fluorene dimer
348	38.4 38.5 38.6	348 (62), 174 (100)	349 (100)	421 (100)		Hydroxy fluorene dimer isomers

480 <sup>a</sup> relative abundances (%) are given in parentheses - <sup>b</sup> DC: Derivatized compound - <sup>c</sup> nd: non  
 481 detected  
 482

483

484 **References**

485

---

[<sup>1</sup>] A. Orecchio, PAHs associated with the leaves of *Quercus ilex* L.: Extraction, GC-MS analysis, distribution and sources: Assessment of air quality in the Palermo (Italy) area, *Atmos. Environ.* 41 (2007) 8669-8680.

[<sup>2</sup>] R. Boonyatumanond, M. Murakami, G. Wattayakorn, A. Togo, H. Takada, Sources of polycyclic aromatic hydrocarbons (PAHs) in street dust in a tropical Asian mega-city, Bangkok, Thailand, *Sci. Total Environ.* 384 (2007) 420-432.

[<sup>3</sup>] S. Orecchio, Assessment of polycyclic aromatic hydrocarbons (PAHs) in soil of a Natural Reserve (Isola delle Femmine) (Italy) located in front of a plant for the production of cement, *J. Hazard. Mater.* 173 (2010) 358-368.

[<sup>4</sup>] C.E. Bostrom, P. Gerde, A. Hanberg, B. Jernstrom, C. Johansson, T. Kyrklund, A. Rannug, M. Tornqvist, K. Victorin, R. Westerholm, Cancer risk assessment, indicators, and guidelines for polycyclic aromatic hydrocarbons in the ambient air, *Environ. Health Persp.* 110 (2002) 451-489.

[<sup>5</sup>] R. Kizu, K. Okamura, A. Toriba, H. Kakishima, A. Mizokami, K. Hayakawa, A role of aryl hydrocarbon receptor in the antiandrogenic effects of polycyclic aromatic hydrocarbons in LNCaP human prostate carcinoma cells, *Arch. Toxicol.* 77 (2003) 335-43.

[<sup>6</sup>] M.T. Wu, L.H. Lee, C.K. Ho, S.C. Wu, L.Y. Lin, B.H. Cheng, C.L. Lu, C.Y. Yang, H.T. Tsai, T.N. Wu, Environmental exposure to cooking oil fumes and cervical intraepithelial neoplasm, *Environ. Res.* 94 (2004) 25-32.

---

[<sup>7</sup>] M.D. Gammon, S.K. Sagiv, S.M., Eng, S. Shantakumar, M.M. Gaudet, S.L. Teitelbaum, J.A. Britton, M.B. Terry, L.W. Wang, Q. Wang, S.D. Stellman, J. Beyea, M. Hatch, G.C. Kabat, M.S. Wolff, B. Levin, A.I. Neugut, R.M. Santella, Polycyclic aromatic hydrocarbon-DNA adducts and breast cancer: a pooled analysis, *Arch. Environ. Health* 59 (2004) 640-649.

[<sup>8</sup>] J. Lewtas, Air pollution combustion emissions: Characterization of causative agents and mechanisms associated with cancer, reproductive, and cardiovascular effects, *Muta. Res.* 636 (2007) 95-133.

[<sup>9</sup>] ATSDR, 1995. Toxicological Profile for Polycyclic Aromatic Hydrocarbons, Atlanta: Agency for Toxic Substances and Disease Registry. Available at: [www.atsdr.cdc.gov/toxprofiles](http://www.atsdr.cdc.gov/toxprofiles).

[<sup>10</sup>] W.G. Jeong, S.M. Cho, The effects of polycyclic aromatic hydrocarbon exposure on the fertilization and larval development of the Pacific oyster, *Crassostrea gigas*, *J. Shellfish Res.* 24 (2005) 209-213.

[<sup>11</sup>] A. Ramesh, F. Inyang, D.D. Lunstrac, M.S. Niaz, P. Kopsombut, K.M. Jones, D.B. Hoode, E.R. Hills, A.E. Archibong, Alteration of fertility endpoints in adult male F-344 rats by subchronic exposure to inhaled benzo(a)pyrene, *Exp. Toxicol. Pathol.* 60 (2008) 269-280.

[<sup>12</sup>] Y. Han, Y., Xia, P. Zhu, S. Qiao, R. Zhao, N. Jin, S. Wang, L. Song, G. Fu, X. Wang, Reproductive hormones in relation to polycyclic aromatic hydrocarbon (PAH) metabolites among non-occupational exposure of males, *Sci. Total Environ.* 408 (2010) 768-773.

[<sup>13</sup>] H.S. Lee, E.M. Cho, J.H. Jung, A. Ohta, Evaluation on antagonist activities of polycyclic aromatic hydrocarbons using the yeast two-hybrid detection system for endocrine disruptors, *Environ. Monit. Assess.* 129 (2007) 87-95.

---

[<sup>14</sup>] A.M. Vinggaard, J. Niemela, E.B. Wedebye, G.E. Jensen, Screening of 397 chemicals and development of a quantitative structure-activity relationship model for androgen receptor antagonism, *Chem. Res. Toxicol.* 21 (2008) 813-823.

[<sup>15</sup>] I. Louiz, S. Kinani, M-E. Gouze, M. Ben-Atti, D. Menif, S. Bouchonnet, J.M. Porcher, O.K. Ben-Hassine, S. Aït-Aïssa, Monitoring of dioxin-like, estrogenic and anti-androgenic activities in sediments of the Bizerta lagoon (Tunisia) by means of in vitro cell-based bioassays: Contribution of low concentrations of polynuclear aromatic hydrocarbons (PAHs), *Sci. Total Environ.* 402 (2008) 318-329.

[<sup>16</sup>] K. Ravindar, R. Sokhi, R., Van Grieken, Atmospheric polycyclic aromatic hydrocarbons: Source attribution, emission factors and regulation, *Atmos. Environ.* 42 (2008) 2895-2921.

[<sup>17</sup>] E. Manoli, C. Samara, Polycyclic aromatic hydrocarbons in natural waters: sources, occurrence and analysis, *Trends Anal. Chem.* 18 (1999) 417- 428.

[<sup>18</sup>] C.L. Gigliotti, P.A. Brunciak, J. Dachs, T.R. Glenn IV, E.D. Nelson, L.A. Totten, S.J. Eisenreich, Air-water exchange of polycyclic aromatic hydrocarbons in the New York-New Jersey Harbor estuary, USA, *Environ. Toxicol. Chem.* 21 (2002) 235-244.

[<sup>19</sup>] M. Àngels Olivella, Polycyclic aromatic hydrocarbons in rainwater and surface waters of Lake Maggiore, a subalpine lake in Northern Italy, *Chemosphere* 63 (2006) 116-131.

[<sup>20</sup>] M.T. Pena, M.C. Casais, M.C. Mejuto, R. Cela, Development of an ionic liquid based dispersive liquid-liquid microextraction method for the analysis of polycyclic aromatic hydrocarbons in water samples, *J. Chrom. A* 1216 (2009) 6356-6364.

[<sup>21</sup>] R. Götz, O.H. Auer, P. Friesel, K. Roch, Organic trace compounds in the water of the River Elbe near Hamburg, *Chemosphere* 36 (1998) 2103-2118.

---

[<sup>22</sup>] A. Ballesteros-Gómez, S. Rubio, D. Pérez-Bento, Determination of priority carcinogenic polycyclic aromatic hydrocarbons in wastewater and surface water by coacervative extraction and liquid chromatography-fluorimetry, *J. Chrom. A* 1203 (2008) 168-176.

[<sup>23</sup>] E. Pérez-Cerrera, V.M. León A.G. Parra, E. González-Mazo, Simultaneous determination of pesticides, polycyclic aromatic hydrocarbons and polychlorinated biphenyls in seawater and interstitial marine water samples, using stir bar sorptive extraction-thermal desorption-gas chromatography-mass spectrometry, *J. Chrom. A* 1170 (2007) 82-90.

[<sup>24</sup>] A. Valavanidis, T. Vlachogianni, S. Triantafillaki, M. Dassenakis, F. Androustos, M. Scoullou, Polycyclic aromatic hydrocarbons in surface seawater and in indigenous mussels (*Mytilus galloprovincialis*) from coastal areas of the Saronikos Gulf (Greece), *Estuar. Coast. Shelf S.* 79 (2008) 733-739.

[<sup>25</sup>] S. Bertilsson, A. Widenfalk, Photochemical degradation of PAHs in freshwaters and their impact on bacterial growth - influence of water chemistry, *Hydrobiologia* 469 (2002) 23-32.

[<sup>26</sup>] C.D. Clark, W.J. De Bruyn, J. Ting, W. Scholle, Solution medium effects on the photochemical degradation of pyrene in water, *J. Photoch. Photobio. A* 186 (2007) 342-348.

[<sup>27</sup>] O.T. Woo, W.K. Chung, K.H. Wong, A.T. Chow, P.K. Wong, Photocatalytic oxidation of polycyclic aromatic hydrocarbons: intermediates identification and toxicity testing, *J. Hazard. Mater.* 168 (2009) 1192-1199.

[<sup>28</sup>] R. Dabestani, L.N. Ivanov, Invited Review: A compilation of physical, spectroscopic and photophysical properties of polycyclic aromatic hydrocarbons, *Photochem. Photobiol.* 70 (1999) 103-134.

---

[<sup>29</sup>] L. Rizzo, Bioassays as a tool for evaluating advanced oxidation processes in water and wastewater treatment, *Water Res.* 45 (2011) 4311-4340.

[<sup>30</sup>] W.R. Gala, J.P. Giesy, Flow cytometric determination of the photo induced toxicity of anthracene to the green alga *Selenastrum capricornutum*, *Environ. Toxicol. Chem.* 13 (1994) 831-840.

[<sup>31</sup>] H. Shemer, K.G. Linden, Aqueous photodegradation and toxicity of the polycyclic aromatic hydrocarbons fluorene, dibenzofuran and dibenzothiophene, *Water Res.* 41 (2007) 853-861.

[<sup>32</sup>] S. Kinani, S. Bouchonnet, N. Creusot, S. Bourcier, P. Balaguer, J.M. Porcher, S. Aït-Aïssa, Bioanalytical characterisation of multiple endocrine- and dioxin-like activities in sediments from reference and impacted small rivers, *Environ. Pollut.* 158 (2010) 74-83.

[<sup>33</sup>] S. Kinani, S. Bouchonnet, S. Bourcier, N. Creusot, J.M. Porcher, S. Aït-Aïssa, Extraction and purification procedures for simultaneous quantification of phenolic xenoestrogens and steroid estrogens in river sediment by gas chromatography/ion trap mass spectrometry, *Rapid Comm. Mass Spectrom.* 22 (2008) 3651-3661.

[<sup>34</sup>] P. Balaguer, A.M. Boussioux, E. Demirpence, J.C. Nicolas, Reporter cell lines are useful tools for monitoring biological activity of nuclear receptor ligands, *Luminescence* 16 (2001) 153-158.

[<sup>35</sup>] T. Mossman, Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays, *J. Immunol.* 65 (1983) 55-63.

[<sup>36</sup>] N. Laville, S. Aït-Aïssa, E. Gomez, C. Casellas, J.M. Porcher, Effects of human pharmaceuticals on cytotoxicity, EROD activity and ROS production in fish hepatocytes, *Toxicology* 2004, 196, 41-55.

---

[<sup>37</sup>] F.J. Rivas, F.J. Beltran, O. Gimeno, M. Carbajo, Fluorene oxidation by coupling of ozone, radiation, and semiconductors: A mathematical approach to the kinetics, *Ind. Eng. Chem. Res.* 45 (2006) 166-174.

[<sup>38</sup>] NIST Standard Reference Data. 2012. <http://www.nist.gov/srd/nist1a.cfm>.

[<sup>39</sup>] L. Hykrdova, J. Jirkovsky, G. Mailhot, M. Bolte, Fe(III) photoinduced and Q-TiO<sub>2</sub> photocatalysed degradation of naphthalene: comparison of kinetics and proposal of mechanism, *J. Photoch. Photobio. A* 151 (2002) 181-193.

[<sup>40</sup>] M.J. García-Martínez, L. Canoira, G. Blázquez, I. Da Riva, R. Alcántara, J.F. Llamas, Continuous photodegradation of naphthalene in water catalyzed by TiO<sub>2</sub> supported on glass Raschig rings, *Chem. Eng. J.* 110 (2005) 123-128.

[<sup>41</sup>] T. Ohno, T. Mitsui, M. Matsumura, Photocatalytic activity of S-doped TiO<sub>2</sub> photocatalyst under visible light, *Chem. Lett.* 32 (2003) 364-365.

[<sup>42</sup>] M. Muneer, M. Qamar, M. Saquib, D. Bahnemann, Heterogeneous photocatalysed reaction of three selected pesticide derivatives, propham, propachlor and tebuthiuron in aqueous suspension of titanium dioxide, *Chemosphere* 61 (2005) 457-468.

[<sup>43</sup>] A. Lair, C. Ferronato, J.M. Chovelon, J.M. Herrmann, Naphthalene degradation in water by heterogeneous photocatalysis: An investigation of the influence of inorganic anions, *J. Photoch. Photobio. A* 193 (2008) 193-203.

[<sup>44</sup>] J.S. Miller, D. Olejnik, Photolysis of polycyclic aromatic hydrocarbons in water, *Water Res.* 35 (2001) 233-243.

---

[<sup>45</sup>] D. Dabrowska, A. Kot-Wasik, J. Namiesnik, Stability studies of selected polycyclic aromatic hydrocarbons in different organic solvents and identification of their transformation products, *Pol. J. Environ. Stud.* 17 (2008) 17-24.

[<sup>46</sup>] J. Sabaté, J.M. Bayona, A.M. Solanas, Photolysis of PAHs in aqueous phase by UV irradiation, *Chemosphere* 44 (2001) 119-124.

[<sup>47</sup>] M. Kamiya, A. Toriba, Y. Onoda, R. Kizu, K. Hayakawa, Evaluation of estrogenic activities of hydroxylated polycyclic aromatic hydrocarbons in cigarette smoke condensate, *Food Chem. Toxicol.* 43 (2005) 1017-1027.

[<sup>48</sup>] T.W. Schultz, G.D. Sinks, Xenoestrogenic gene expression: Structural features of active polycyclic aromatic hydrocarbons, *Environ. Toxicol. Chem.* 21 (2002) 783-786.

[<sup>49</sup>] N.C. Bols, K. Schirmer, E.M. Joyce, D.G. Dixon, B.M. Greenberg, J.J. Whyte, Ability of polycyclic aromatic hydrocarbons to induce 7-ethoxyresorufin-O-deethylase activity in a trout liver cell line, *Ecotoxicol. Environ. Safety* 44 (1999) 118-128.

[<sup>50</sup>] W. Brack, Effect-directed analysis: a promising tool for the identification of organic toxicants in complex mixtures? *Anal. Bioanal. Chem.* 377 (2003) 397-407.

[<sup>51</sup>] N. Creusot, H. Budzinski, P. Balaguer, S. Kinani, J.M. Porcher, S. Aït-Aïssa, Effect directed analysis of endocrine disrupting compounds in multi-contaminated sediment: identification of novel ligands of estrogen and pregnane X receptors, *Anal. Bioanal. Chem.* 405 (2013) 2553-2566.

[<sup>52</sup>] T. Schulze, S. Weiss, E. Schymanski, P.C. von der Ohe, M. Schmitt-Jansen, R. Altenburger, G. Streck, W. Brack, Identification of a phytotoxic photo-transformation product of diclofenac using effect-directed analysis, *Environ. Pollut.* 158(5) (2010) 1461-1466.



---

[<sup>53</sup>] Y. Souissi, S. Kinani, S. Bouchonnet, S. Bourcier, C. Malosse, M. Sablier, N. Creusot, E. Mombelli, S. Aït-Aïssa, Photolysis of estrone generates estrogenic photoproducts with higher activity than the parent compound, *Environ. Sci. Pollut. R.* 21 (2014) 7818-7827.