

1 **Evaluation of methods to determine adsorption of polycyclic**  
2 **aromatic hydrocarbons to dispersed carbon nanotubes**

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4 Berit Glomstad<sup>1</sup>, Lisbet Sørensen<sup>2,†</sup>, Jingfu Liu<sup>3</sup>, Mohai Shen<sup>3,§</sup>, Florian Zindler<sup>1,||</sup>, Bjørn M.  
5 Jønsen<sup>1</sup> and Andy M. Booth<sup>2,\*</sup>

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7 <sup>1</sup> Department of Biology, Norwegian University of Science and Technology, Trondheim NO-  
8 7491, Norway

9 <sup>2</sup> SINTEF Ocean, Trondheim NO-7465, Norway

10 <sup>3</sup> State Key Laboratory of Environmental Chemistry and Ecotoxicology, Research Center for  
11 Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing, 100085, China

12

13 <sup>†</sup> Current address: Institute of Marine Research, Bergen NO-5817, Norway.

14 <sup>§</sup> Current address: School of Environment, Key Laboratory for Yellow River and Huai River  
15 Water Environment and Pollution Control, Ministry of Education, Henan Key Laboratory for  
16 Environmental Pollution Control, Henan Normal University, Xinxiang 453007, China.

17 <sup>||</sup> Current address: Aquatic Ecology and Toxicology Section, Centre for Organismal Studies  
18 (COS), University of Heidelberg, Im Neuenheimer Feld 504, D-69120 Heidelberg,  
19 Germany.

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21 \*Corresponding author. E-Mail: andy.booth@sintef.no; Tel.: +47 93089510.

22 **Abstract:** A number of methods have been reported for determining hydrophobic organic  
23 compound adsorption to dispersed carbon nanotubes (CNTs), but their accuracy and reliability  
24 remain uncertain. We have evaluated three methods to investigate the adsorption of  
25 phenanthrene (a model polycyclic aromatic hydrocarbon; PAH) to CNTs with different  
26 physicochemical properties; dialysis tube (DT) protected negligible depletion solid phase  
27 microextraction (DT-nd-SPME), ultracentrifugation and filtration using various types of filters.  
28 Dispersed CNTs adhered to the unprotected PDMS-coated fibers used in nd-SPME. Protection of  
29 the fibers from CNT adherence was investigated with hydrophilic DT, but high PAH sorption to  
30 the DT was observed. The efficiency of ultracentrifugation and filtration to separate CNTs from  
31 the water phase depended on CNT physicochemical properties. While non-functionalized CNTs  
32 were efficiently separated from the water phase using ultracentrifugation, incomplete separation  
33 of carboxyl functionalized CNTs was observed. Filtration efficiency varied with different filter  
34 types (composition and pore size), and non-functionalized CNTs were more easily separated  
35 from the water phase than functionalized CNTs. Sorption of phenanthrene was high (<70%) for  
36 three of the filters tested, making them unsuitable for the assessment of phenanthrene adsorption  
37 to CNTs. Filtration using a hydrophilic polytetrafluoroethylene (PTFE) filter membrane (0.1  $\mu\text{m}$ )  
38 was found to be a simple and precise technique for the determination of phenanthrene adsorption  
39 to a range of CNTs, efficiently separating all types of CNTs and exhibiting a good and highly  
40 reproducible recovery of phenanthrene (82%) over the concentration range tested (70-735  $\mu\text{g/L}$ ).

41

42 **Keywords:** Carbon nanotubes, adsorption, negligible depletion solid phase microextraction,  
43 filtration, polycyclic aromatic hydrocarbons, nanomaterials.

## 44 INTRODUCTION

45 Carbon nanotubes (CNTs) have many possible applications, including composite materials in  
46 electronics (De Volder et al. 2013; Petersen et al. 2011) and in medicine (Kesharwani et al.  
47 2015). CNTs also interact strongly with hydrophobic organic compounds (HOCs) (Glomstad et  
48 al. 2016; Kah et al. 2011; Kah et al. 2014; Yang and Xing 2010; Yang et al. 2006b; Zindler et al.  
49 2016), making them interesting sorbents e.g. in water treatment (Mauter and Elimelech 2008; Yu  
50 et al. 2014). Concurrent with an increase in CNT production and use, is a growing concern  
51 regarding their environmental effects (Alloy and Roberts 2011; Edgington et al. 2010; Schwab et  
52 al. 2011). Furthermore, the high adsorption capacity of CNTs and other carbon nanomaterials  
53 (CNMs) for HOCs has been shown to influence HOC fate, bioavailability and toxicity in the  
54 environment (Baun et al. 2008; Glomstad et al. 2016; Schwab et al. 2013; Shen et al. 2014; Shen  
55 et al. 2012; Zindler et al. 2016).

56

57 While hydrophobic, pristine CNTs are prone to agglomeration and sedimentation in  
58 freshwater (Pan and Xing 2008), stable CNT dispersions can be formed under certain conditions.  
59 CNT physicochemical properties (e.g. surface oxygen content), and environmental factors (e.g.  
60 natural organic matter; NOM), have been reported to enhance CNT dispersibility (i.e. the  
61 dispersed CNT concentration) and dispersion stability (Hyung et al. 2007; Schwyzer et al. 2012).  
62 The increased available specific surface area (SSA) of dispersed CNTs enhances adsorption of  
63 HOCs compared to agglomerated CNTs (Zhang et al. 2012). CNTs dispersed in aquatic matrices  
64 may therefore have increased potential for influencing the fate of co-adsorbed HOCs. Assessing  
65 HOC adsorption to agglomerated rather than dispersed CNTs could lead to an underestimation of  
66 their environmental effects.

67

68 In recent years, several studies have investigated the adsorption of polycyclic aromatic  
69 hydrocarbons (PAHs) to CNTs (Cho et al. 2008; Glomstad et al. 2016; Kah et al. 2011; Kah et al.  
70 2014; Wang et al. 2009; Yang et al. 2006a; Yang et al. 2006b; Zhang et al. 2012; Zindler et al.  
71 2016), with phenanthrene being one of the most frequently used compounds (Apul et al. 2012).  
72 The adsorption of other HOCs to a range of CNTs with different physical and chemical  
73 properties has also been studied [e.g. (Carabineiro et al. 2011; Carabineiro et al. 2012; Chen et  
74 al. 2007; Cho et al. 2011; Dai et al. 2009; Huffer et al. 2015; Schwab et al. 2014)]. Most studies  
75 have focused on agglomerated rather than dispersed CNTs, although a few studies using CNTs  
76 dispersed by sonication have been reported (Kah et al. 2014; Zhang et al. 2012). Studying  
77 dispersed CNTs is challenging because of the difficulty in separating the dispersed CNTs from  
78 the water phase prior to determination of the freely dissolved PAH concentration in the water  
79 phase ( $C_{\text{free}}$ ), and the PAH concentration adsorbed by CNTs ( $C_{\text{CNT}}$ ) (Petersen et al. 2016; Yang et  
80 al. 2011). Centrifugation has commonly been employed in adsorption studies to sediment  
81 agglomerated CNTs before the supernatant is withdrawn and  $C_{\text{free}}$  determined (Cho et al. 2008;  
82 Kah et al. 2011; Wang et al. 2009; Yang et al. 2006a; Yang et al. 2006b). A few studies have  
83 also used centrifugation to assess PAH adsorption to dispersed CNTs (Linard et al. 2015; Su et  
84 al. 2013). However, centrifugation may not be sufficient to fully sediment well-dispersed CNTs,  
85 possibly causing errors in the determination of  $C_{\text{free}}$  and  $C_{\text{CNT}}$ .

86

87 Solid phase extraction (SPE) using polyoxymethylene sheets has been reported to successfully  
88 determine PAH adsorption to partly dispersed CNTs, but requires a 28 d equilibrium period (Kah  
89 et al. 2011; Kah et al. 2014; Zhang et al. 2012). Negligible depletion solid phase microextraction

90 (nd-SPME) has also been used to study HOC adsorption to aqueous suspensions of the fullerene  
91 C<sub>60</sub> (Hu et al. 2008; Hu et al. 2010), but has, to our knowledge, not been validated for CNTs.  
92 Here, a negligible fraction of the target analyte proportional to C<sub>free</sub> is extracted onto the fiber  
93 without affecting the equilibrium between C<sub>free</sub> and the adsorbent (Heringa and Hermens 2003).  
94 Whilst separation of dispersed CNTs from the water phase is avoided, nd-SPME could lead to  
95 the adherence of PAH-loaded CNTs to the fiber, causing an overestimation of C<sub>free</sub>.

96  
97 In a different approach, filtration has been used to study the adsorption of e.g. the herbicide  
98 diuron to CNTs (Schwab et al. 2014), and to investigate the adsorption of HOCs, including the  
99 PAH phenanthrene, to suspended agglomerates of C<sub>60</sub> (Baun et al. 2008). No studies have  
100 evaluated the use of filtration to study PAH (or HOC) C<sub>free</sub> in the presence of different types of  
101 CNTs. The importance of understanding the HOC adsorption to dispersed CNTs and the need to  
102 develop appropriate separation techniques has previously been highlighted (Pan and Xing 2008).  
103 However, no systematic evaluation of methods to determine C<sub>free</sub> in the presence of CNTs has  
104 been performed. Furthermore, the separation efficiency of centrifugation and filtration is likely to  
105 depend on CNT physicochemical properties (e.g. diameter and surface oxidation) which  
106 influence their dispersion state (Schwyzer et al. 2012). The role of CNT properties on the  
107 suitability of different separation techniques has not been considered to date.

108  
109 We have evaluated different methods for studying the adsorption of the model compound  
110 phenanthrene to CNTs, with a focus on identifying the most reliable way of determining C<sub>free</sub>  
111 values of phenanthrene remaining in the water phase. Three methods for determining C<sub>free</sub> were  
112 evaluated (i) dialysis tube (DT) protected nd-SPME (DT-nd-SPME), (ii) ultracentrifugation, and

113 (iii) filtration. The objective for the ultracentrifugation and filtration methods was a complete  
114 separation of dispersed CNTs from the water phase. A suite of CNTs, including one single-  
115 walled CNT (SWCNT), two non-functionalized multi-walled CNTs of different diameters and  
116 lengths (MWCNT-15 and MWCNT-30) and two functionalized MWCNTs (MWCNT-OH and  
117 MWCNT-COOH) were included in the study to ensure that the method would be suitable for a  
118 range of CNTs exhibiting varying physicochemical properties. CNT dispersions were prepared in  
119 the presence of environmentally relevant concentrations of NOM to increase the environmental  
120 relevance of the study and enhance the dispersion of CNTs.

121

122

## 123 **MATERIALS AND METHODS**

124 A schematic overview of the nd-SPME, DT-nd-SPME, ultracentrifugation and filtration  
125 methods evaluated for their suitability in the determination of  $C_{\text{free}}$  and subsequent calculation of  
126  $C_{\text{CNT}}$  is presented in **Figure 1**.

127

### 128 *Chemicals and materials*

129 A suite of five different CNTs (SWCNT, MWCNT-15, MWCNT-30, MWCNT-OH and  
130 MWCNT-COOH (purity >95%) was purchased from Timesnano (Chengdu Organic Chemicals  
131 Co., Ltd, Chinese Academy of Sciences). Phenanthrene ( $\geq 99.5\%$ ) and pyrene (>99%) (included  
132 in evaluation of nd-SPME) were purchased from Sigma Aldrich and stock solutions prepared in  
133 methanol. Fluorene-*d*10 was supplied by Chiron AS. Suwannee River natural organic matter  
134 (SR-NOM; Reference Aquatic NOM; Catalog number 2R101N) was purchased from the  
135 International Humic Substance Society (IHSS). The organic solvents; methanol (Fluka

136 Analytical), dichloromethane (Rathburn Chemicals Ltd), *n*-hexane (Fluka Analytical),  
137 acetonitrile (Fluka Analytical) and isopropanol (Lab Scan), were of analytical grade and tested  
138 in-house for any impurities before use. Polydimethylsiloxane-coated (PDMS) fused silica fibers  
139 (diameter 110  $\mu\text{m}$ ; coating thickness 30  $\mu\text{m}$ ) was purchased from Polymicro Technologies  
140 (Switzerland). Hydrophilic cellulose ester membrane dialysis tubing (DT) was purchased from  
141 Spectrum Laboratories, Inc.

142

#### 143 *CNT characterization*

144 CNT properties as specified by the manufacturer are available in Table S1 in the  
145 Supplementary Data. Several CNT parameters, including outer diameter and purity (transmission  
146 electron microscopy; TEM), surface chemistry (X-ray photoelectron spectroscopy; XPS) and  
147 specific surface area (SSA; calculated by Brunauer-Emmett-Teller method), were further verified  
148 by in-house characterization.

149

#### 150 *Media and media-NOM preparation*

151 Two freshwater media, commonly used in aquatic ecotoxicity tests; EPA moderately hard  
152 reconstituted water (MHRW) (U.S. Environmental Protection Agency 2002) and OECD algal  
153 growth media TG201 (OECD 2011), were used throughout the study. These media were selected  
154 in order to ensure that the methods being evaluated would subsequently be appropriate for use in  
155 standardized aquatic ecotoxicological tests (freshwater algae and *Daphnia magna*). It has been  
156 shown that dispersed CNT concentrations are positively related to NOM concentration [e.g.  
157 (Kennedy et al. 2008; Smith et al. 2012)]. In order to achieve improved dispersion of the CNTs,  
158 the NOM concentration utilized in the current study is at the higher end of the range found in

159 natural waters (Thurman 1985). The preparation of media containing SR-NOM (nominally 20  
160 mg/L), further referred to as media-NOM, or MHRW-NOM and TG201-NOM, has been  
161 previously reported (Glomstad et al. 2016) and is summarized in the Supplementary Data.  
162 Briefly, SR-NOM was stirred into the media for 24 h to maximize dissolution, followed by  
163 filtration to remove any residual particulate NOM that could represent an alternative adsorption  
164 site for the organic pollutants.

165

#### 166 *Preparation and quantification of CNT dispersions*

167 Details on the CNT dispersion procedure have been previously published (Glomstad et al.  
168 2016; Zindler et al. 2016). Briefly, bath sonication (Bandelin Sonorex Super RK 510H, 640W,  
169 35 kHz) was used to prepare 0.2 L stock dispersions of CNTs (100 mg/L) in media-NOM. The  
170 CNT stock dispersions were then added to 1.8 L of media-NOM, giving a final CNT  
171 concentration of 10 mg/L. A settling period of 24 h was applied to allow natural settling of any  
172 undispersed CNTs. This also allowed the settling of large CNT agglomerates which could  
173 influence subsequent UV-vis absorbance measurements as previously described (Cerrillo et al.  
174 2015; Li et al. 2006; Petersen et al. 2016). The preparation of and use of media solutions took  
175 place at room temperature ( $20 \pm 2$  °C). In cases where the CNT dispersions were not used  
176 immediately, they were stored in the dark at 4°C.

177

178 A spectral analysis was conducted to verify the absorbance maxima of both the studied CNTs  
179 and background media solutions in order to identify a wavelength where the influence of other  
180 matrix components (e.g. NOM and PAHs) on the absorbance was minimized. The dispersed  
181 CNT concentration (after 24 h settling) was determined by measuring absorbance at 800 nm with



182 UV-vis spectrophotometry (Hitachi U-2000) using quartz cuvettes (3.5 mL; 10 mm light path,  
183 Hellma Analytic). A series of external calibration standards with known CNT concentrations was  
184 used to generate individual UV-vis calibration curves for each of the CNTs. At the selected  
185 wavelength no significant absorbance by the two media solutions, the media-NOM solutions, or  
186 PAH solutions was observed. As the CNTs were dispersed in media-NOM, blank samples  
187 consisted of media-NOM without CNTs. The limit of detection (LOD) and limit of quantification  
188 (LOQ) was calculated as the blank mean (MHRW-NOM or TG201-NOM) plus three (LOD) or  
189 ten (LOQ) times the standard deviation (SD). Dispersed CNT concentrations were determined  
190 immediately after preparation of the dispersions or immediately before use if the dispersions had  
191 been stored.

192

### 193 *Negligible depletion solid phase microextraction (nd-SPME) approaches*

194 Another model PAH, pyrene, was used in addition to phenanthrene in evaluation of the nd-  
195 SPME method. Although pyrene was not used in the evaluation of other methods presented  
196 herein, the pyrene nd-SPME data provide important supplementary information to that generated  
197 for phenanthrene. Preliminary studies showed that CNTs adhered to the PDMS-coated fibers  
198 used as the extraction medium in the nd-SPME studies and affect the determination of  $C_{free}$ . To  
199 protect the PDMS-coated fibers, hydrophilic cellulose ester membrane dialysis tubing (DT) with  
200 a molecular weight cut-off of 0.5-1.0 kD and a width of 10 mm was used. The DT was cut into 3  
201 cm pieces and rinsed in deionized water before use. The PDMS-coated fibers were carefully cut  
202 into pieces of 1 cm (using a surgical scalpel) and rinsed in acetone before use. The fibers were  
203 placed inside DT filled with a few drops of deionized water before the DT ends were folded and

204 closed with metal clam clips (Figure S1, Supplementary Data). The extraction units were  
205 prepared daily and kept in deionized water until use.

206

207 To determine the PAH equilibration time for DT-nd-SPME, extraction of phenanthrene and  
208 pyrene (100  $\mu\text{g/L}$ ) at various time points; 1, 2, 4, 6 and 10 days, was performed. Linearity of the  
209 method was investigated in the range 5-100  $\mu\text{g/L}$ . Extraction was performed in individual  
210 solutions and mixtures of pyrene and phenanthrene to evaluate the possibility of using DT-nd-  
211 SPME for analysis of PAH mixtures. The effect of NOM on the extraction efficiency was  
212 investigated by performing the extraction in the presence and absence of NOM. This was  
213 performed both with and without DT protection (using DT of 15 cm). Comparison of PAH  
214 extraction onto the PDMS-coated fibers using DT-nd-SPME and nd-SPME was used to  
215 investigate the effect of DT on the extraction. Furthermore, liquid-liquid extraction (LLE) of the  
216 MHRW solutions with dichloromethane (3 x 20 mL) followed by gas chromatography mass  
217 spectroscopy (GC-MS) analysis was performed to estimate the loss of pyrene after extraction  
218 with nd-SPME and DT-nd-SPME. Uptake equilibrium studies showed that the adsorption  
219 equilibrium of PAHs onto the DT protected PDMS-coated fibers was established after 2 and 4  
220 days for phenanthrene and pyrene, respectively (Figure S2, Supplementary Data). To ensure  
221 equilibrium, and to standardize the method, an extraction time of 5 days was therefore  
222 implemented in all studies.

223

224 The detailed DT-nd-SPME method is presented in the Supplementary Data. Preliminary  
225 studies showed no effect of NOM on extraction of PAHs onto PDMS-coated fibers, with or  
226 without DT (Figure S3, Supplementary Data). Therefore, all tests for validation of the DT-nd-

227 SPME method were performed in MHRW. Validation of the method was performed in the  
228 absence of CNTs. Briefly, the extraction units were inserted into the sample solutions (MHRW  
229 spiked with PAHs) and extracted for 5 days. The fiber was then removed and placed in *n*-hexane  
230 for desorption (24 h) prior to analysis by GC-MS to determine PAH concentration.

231

### 232 *Ultracentrifugation*

233 Visual inspection of CNT dispersions after performing regular centrifugation (Eppendorf  
234 Centrifuge 5804 R, 2200 g, 30 min) showed that this was not sufficient to sediment the suite of  
235 CNTs tested. Therefore, ultracentrifugation was employed for further testing. Dispersions of  
236 each CNT type were placed in 36 mL centrifugation tubes and centrifuged at 40,000g for 30 min  
237 and 50,000g for 30 min; (T-865 Fixed Angle Rotor, Sorvall™ WX Ultracentrifuge, Thermo  
238 Scientific). After centrifugation, the supernatant was carefully removed using a glass pipette,  
239 taking care not to re-suspend settled CNTs. UV-vis absorbance measurements of the supernatant  
240 (800 nm) were the performed to assess the separation efficiency of CNTs from water phase.

241

### 242 *Filtration*

243 A glass vacuum filter flask setup was employed for filtration of CNT dispersions. Several  
244 filter membrane materials were investigated, including polycarbonate (PC; pore size 0.1 μm),  
245 mixed cellulose ester (MCE; 0.2 μm), polyvinylidene fluoride (PVDF; 0.2 μm) and hydrophilic  
246 polytetrafluoroethylene (PTFE; 0.1 μm). In addition, two glass fiber filters (GF/F; 0.7 μm, GF-  
247 75; 0.3 μm) were evaluated. The selected filters were assessed for (i) their ability to retain CNTs,  
248 and (ii) the degree of sorption of phenanthrene to the filter. The CNT dispersions were placed in  
249 the glass tower, gently drawn through the filter and the filtrate collected in 20 mL glass vials

250 placed inside the Erlenmeyer filter flask. Removal of CNTs was evaluated based on UV-vis  
251 absorbance measurements (800 nm) of the filtrate. Single replicates of each CNT type were used  
252 for all filters except for PTFE where triplicate samples were filtered owing to the promising data  
253 generated by the single replicate. Blanks (TG201-NOM) were filtered to verify that the filtration  
254 process itself did not contribute to the measured absorbance.

255  
256 Sorption of phenanthrene to the filters was evaluated by determining the phenanthrene  
257 concentration in the filtrate after filtration of solutions with known phenanthrene concentrations.  
258 Analysis was performed either by solid phase extraction (SPE; Bond Elut® PPL columns, 500  
259 mg, Agilent Technologies Inc.) of the filtrate followed by GC-MS, or by direct injection of the  
260 filtrate using high-performance liquid chromatography with photodiode-array UV detection  
261 (HPLC-UV) (Glomstad et al. 2016; Zindler et al. 2016). Details on the chemical analysis are  
262 provided in Supplementary Data. Quantification of target analytes by GC-MS offers the lowest  
263 limits of detection and is therefore necessary for samples with low  $C_{\text{free}}$  concentrations. Where  
264  $C_{\text{free}}$  of the target analyte is sufficiently high, HPLC-UV represents the quickest and least  
265 expensive method for quantification.

266

267

## 268 **RESULTS AND DISCUSSION**

### 269 *CNT properties and dispersibility*

270 A summary of the CNT physicochemical properties determined by in-house characterization  
271 are presented in **Table 1**. Significant differences in mean diameter, SSA and surface oxygen  
272 content were observed between the suite of CNTs tested. SWCNT exhibited a much smaller

273 outer diameter and a higher SSA (483.7 m<sup>2</sup>/g) than the MWCNTs. MWCNT-30 had a somewhat  
274 higher SSA (177.4 m<sup>2</sup>/g) than the other MWCNTs (~140 m<sup>2</sup>/g), however this was not  
275 statistically significant. MWCNT-COOH had the highest surface oxygen content (5.7%)  
276 followed by MWCNT-OH (3.9%). The non-functionalized CNTs had lower surface oxygen  
277 content as expected (<2.0%). Differences in CNT dispersibility were also observed, with final  
278 dispersed concentrations increasing in the order SWCNT (0.7 mg/L) < MWCNT-15 = MWCNT-  
279 30 (1.6 mg/L) < MWCNT-OH (2.9 mg/L) < MWCNT-COOH (6.0 mg/L). The results are in  
280 good agreement with other studies which have shown that outer diameter and oxygen-containing  
281 surface functional groups increase CNT dispersibility (Schwyzer et al. 2012). Initial  
282 characterization of the CNT dispersion using dynamic light scattering (DLS) to determine  
283 particle size distributions and polydispersity index resulted in large uncertainties in the  
284 measurements. This is consistent with the findings of other studies with high aspect ratio CNTs  
285 (Petersen and Henry 2012), which is due to DLS employing modelling assumptions based on  
286 monodisperse spheres. The different dispersion concentrations generated for each CNT type also  
287 represent an additional uncertainty when attempting to compare DLS data. No further assessment  
288 of CNT particle size distribution was conducted in the current study.

289

#### 290 *Dialysis tube protected negligible depletion solid phase microextraction (DT-nd-SPME)*

291 SEM images of the PDMS-coated fibers collected after exposure to CNT dispersions revealed  
292 significant quantities of CNTs adhered to the fiber surface (Figure S4, Supplementary Data).  
293 Preliminary studies using pyrene and phenanthrene with unprotected PDMS-coated fibers in the  
294 presence of CNTs also indicated little or no adsorption of phenanthrene to CNTs had occurred  
295 (Figure S5, Supplementary Data). In some cases, the determined C<sub>free</sub> was higher in the presence

296 of CNTs than when no CNTs were present. Phenanthrene adsorption by CNTs is well  
297 documented (Kah et al. 2011; Yang et al. 2006b). While Yang et al., (2006b) studied adsorption  
298 by CNTs using classical two-phase batch experiments, Kah et al., (2011) investigated both  
299 centrifugation and a three-phase system using a polyoxymethylene passive sampler. In all  
300 studies, high adsorption of the target PAH to the selected CNTs was observed. This is in contrast  
301 to the negligible level of phenanthrene adsorption calculated in the three-phase system  
302 investigated in the current study. Seen in relation with the SEM images, this supports the  
303 suggestion that the adherence of phenanthrene-loaded CNTs to the PDMS-coated fibers  
304 generates erroneously low  $C_{CNT}$  values when phenanthrene desorbs from the CNTs in the *n*-  
305 hexane. Given that the principle of the nd-SPME approach is based upon adsorption of negligible  
306 amounts of the target PAH, and considering the high adsorption affinity of CNTs towards PAHs,  
307 any adherence of PAH-loaded CNTs onto the PDMS-coated fibers will significantly influence  
308 the data generated.

309  
310 Although, nd-SPME has been used to determine PAH adsorption to  $C_{60}$  by inserting the  
311 PDMS-coated fibers directly into  $C_{60}$  dispersions (Hu et al. 2008), the role of PAH-loaded  $C_{60}$   
312 adherence to the PDMS-coated fibers was not investigated in the study. The current study shows  
313 that adherence of PAH-loaded CNTs to the PDMS fiber can lead to an overestimation of the true  
314  $C_{free}$  value. Modification of the method is necessary for use in PAH-CNT adsorption studies, and  
315 should be evaluated for other CNMs such as  $C_{60}$ . The DT-nd-SPME method showed promising  
316 features, as a linear relationship between PAH uptake onto the PDMS-coated fiber ( $C_{fiber}$ ) and  
317  $C_{nominal}$  was observed for both phenanthrene and pyrene in the concentration range tested (5-100  
318  $\mu\text{g/L}$ ; Figure S6). This showed that  $C_{fiber}$ , when protected inside the DT, was directly

319 proportional to PAH  $C_{\text{nominal}}$  in the MHRW media. In the presence of CNTs,  $C_{\text{fiber}}$  can therefore  
320 be related to  $C_{\text{free}}$ . DT-nd-SPME also proved to be suitable for extraction of phenanthrene and  
321 pyrene mixtures, as no difference in the amount of either PAH extracted onto the PDMS-coated  
322 fiber was observed (Figure S7, Supplementary Data). This indicates that DT-nd-SPME has  
323 potential for studying the adsorption of a range of PAHs (and possibly other organic compounds)  
324 to CNTs, either individually or as mixtures.

325  
326 A comparison of PAH extraction onto the PDMS-coated fibers with and without DT  
327 protection showed that the use of DT influenced the amount of PAH extracted onto the fibers. A  
328 significant reduction in  $C_{\text{fiber}}$  was observed when using DT-nd-SPME as compared to nd-SPME  
329 (Figure S3, Supplementary Data). However, these data were generated in initial studies using 15  
330 cm DTs rather than the 3 cm DTs used in the rest of the study. Therefore, the influence of DT  
331 protection on pyrene extraction was further investigated using 3 cm DTs. Again, a large decrease  
332 in  $C_{\text{fiber}}$  was observed with DT-nd-SPME compared to nd-SPME (**Figure 2A**). Furthermore, the  
333  $C_{\text{free}}$  determined from DT-nd-SPME by LLE after 5 days extraction showed a significant  
334 reduction in pyrene  $C_{\text{free}}$  (>70%) compared to nd-SPME (**Figure 2B**). The decrease in pyrene  
335  $C_{\text{free}}$  occurred at both the  $C_{\text{nominal}}$  values tested (1 and 100  $\mu\text{g/L}$ ), and is attributed to sorption  
336 onto the DT. Pyrene sorption to the DT appeared to be concentration dependent and did not  
337 result in a complete depletion of the solution, indicating an equilibrium was established between  
338  $C_{\text{free}}$  pyrene and DT-sorbed pyrene. Sorption of PAHs to the DT could theoretically be reduced  
339 by significantly decreasing the amount of DT used, and/or by increasing the water sample  
340 volume. However, reduction in DT length is not feasible unless other sealing techniques are  
341 identified, and increasing water volume presents challenges regarding sample throughput and

342 waste volumes. The degree of adsorption to the DT is also likely to be highly dependent on the  
343 specific PAH being studied. The high sorption of PAHs to the DT will affect the overall  
344 partitioning equilibrium in the system, possibly leading to a desorption of CNT-bound PAHs as  
345  $C_{\text{free}}$  is reduced and a new equilibrium becomes established.

346

#### 347 *Separation of CNTs from the water phase*

348 A complete removal of dispersed CNTs from the water phase is necessary to achieve an  
349 accurate determination of phenanthrene  $C_{\text{free}}$  in the water phase and a subsequent calculation of  
350  $C_{\text{CNT}}$  through mass balance. Therefore, the separation efficiency of ultracentrifugation and  
351 filtration was investigated in the current study using the suite of CNTs.

352

353 *Ultracentrifugation.* Absorbance measurements of the CNT dispersion supernatant after  
354 ultracentrifugation are presented in Figure S8 in the Supplementary Data. Ultracentrifugation  
355 showed good removal of four of the five CNTs from the water phase. No absorbance was  
356 detected for the non-functionalized CNTs, while an absorbance of 0.004, slightly above LOD,  
357 indicated that low concentrations of MWCNT-OH remained after the procedure. In contrast, the  
358 supernatant remaining after ultracentrifugation of MWCNT-COOH exhibited a relatively high  
359 absorbance (0.017), which was well above the LOQ. Approximately 10% of the initial MWCNT-  
360 COOH concentration remained in the supernatant after ultracentrifugation, showing that  
361 MWCNT-COOH could not be efficiently removed by ultracentrifugation. In previous studies,  
362 standard centrifugation (1500-3000g) has been employed to sediment dispersed CNTs (Linard et  
363 al. 2015; Su et al. 2013). The CNTs used in these studies were, however, non-functionalized. In  
364 the current study, even ultracentrifugation could not successfully separate dispersions of -COOH



365 functionalized CNTs, indicating the efficiency of (ultra)centrifugation is highly dependent on  
366 CNT physicochemical properties. Furthermore, the dispersion technique employed (i.e.  
367 sonication) and the presence of dispersants (e.g. NOM) are likely to influence CNT dispersion  
368 state and their sedimentation efficiency (Cerrillo et al. 2015).

369  
370 Ideally, any centrifugation method employed should offer a consistent level of separation  
371 efficiency, irrespective of CNT properties. At the very least, studies employing  
372 (ultra)centrifugation for the removal of dispersed CNTs from an aqueous phase should verify the  
373 method for its applicability for a broad range of CNT types and for the specific dispersion and  
374 media conditions employed in the study. However, centrifugation techniques do offer some  
375 advantages over nd-SPME and filtration by being independent of the target HOC analyte. While  
376 the suitability of both nd-SPME and filtration needs to be evaluated for individual analytes,  
377 centrifugation is suitable for all HOCs. Ultracentrifugation was not considered a suitable  
378 separation technique for the CNT suite tested in the current study owing to the incomplete  
379 separation observed for the functionalized MWCNTs. An improved removal of CNTs may be  
380 achieved by increasing the ionic strength of the solution immediately prior to ultracentrifugation.  
381 Increased ionic strength promotes agglomeration of CNTs (Petersen et al. 2016; Yang et al.  
382 2011), which could aid in their sedimentation during ultracentrifugation. However, change in  
383 ionic strength and agglomeration state may result in PAH desorption, and should therefore be  
384 evaluated further.

385  
386 *Filtration.* The absorbance measured in the filtrate, after filtration of CNT dispersions through  
387 a range of different filter types, is presented in Figure 3. The sorption of phenanthrene to the  
388 filters is presented in Figure 4. Filtration has been used to study diuron adsorption to dispersed

389 CNTs (Schwab et al. 2014), and phenanthrene adsorption to C<sub>60</sub> agglomerates (Baun et al. 2008).  
390 To our knowledge, this is the first study that systematically evaluates the efficiency of a range of  
391 filters for removing dispersed CNTs, and tests their adsorption towards a model compound. No  
392 absorbance was measured in blank samples (no CNTs present) after filtration with any of the  
393 filters, showing that the filtration step itself did not contribute to any measured absorbance. The  
394 efficiency of filters to retain CNTs clearly depended on both CNT type and filter type (Figure 3).  
395 All filter types could effectively remove the SWCNTs, whilst the two non-functionalized  
396 MWCNTs could be removed effectively except by the glass fiber filters. The two functionalized  
397 MWCNTs, in particular MWCNT-COOH, were more difficult to remove by filtration, with high  
398 absorbance measured for four (MCE, PVDF, GF/F and GF-75) out of the six filters tested.  
399 Assessment of all CNT and filter combinations indicated that only two filters, PC and PTFE,  
400 could adequately retain all five CNT types. No absorbance could be detected for any CNT after  
401 filtration by PC, and MWCNT-COOH exhibited only a slight absorbance (0.005) when the PTFE  
402 filters were used. In a previous adsorption study, PTFE filters with a pore size of 0.45 µm were  
403 used for the separation of dispersed CNTs (Schwab et al. 2014) and the current study suggests  
404 such filters can be appropriate for specific CNT types. SWCNTs were efficiently removed by  
405 GF filters with pore sizes up to 0.7 µm, but all of the MWCNTs had low retention capacities.  
406 This indicates that GF filters are suitable for studies addressing PAH adsorption to SWCNTs  
407 only. Similarly, PC, MCE, PDVF and PTFE filter membranes, with pore sizes up to 0.2 µm, all  
408 proved to remove SWCNTs and non-functionalized MWCNTs efficiently from the water phase  
409 but not the functionalized MWCNTs. However, the results show only filters with a pore size of  
410 0.1 µm were capable of effectively retaining all CNT types and therefore these filters have the  
411 broadest application.

412

413 To investigate if the detected amount of MWCNT-COOH after filtration with the PTFE filter  
414 could significantly affect the determination of  $C_{\text{free}}$ , the concentration of MWCNT-COOH at  
415 LOQ was calculated and the contribution of CNT bound phenanthrene to  $C_{\text{free}}$  estimated using  
416 adsorption data for MWCNT-COOH calculated in our previous study (Glomstad et al. 2016).  
417 The MWCNT-COOH concentration at the LOQ corresponded to <2% of the initial MWCNT-  
418 COOH concentration. The estimated contribution to  $C_{\text{free}}$  was <4% over the phenanthrene  
419 concentration range of 6-800  $\mu\text{g/L}$ . The contribution of phenanthrene adsorbed by MWCNT-  
420 COOH detected in the filtrate to the measured  $C_{\text{free}}$  is considered negligible, and both PC and  
421 PTFE are suitable for determination of  $C_{\text{free}}$ .

422

423 A number of factors could be contributing to the differences in filtration efficiency observed  
424 between CNT types. Although all CNT dispersions were prepared with identical nominal  
425 concentrations (10 mg/L), the dispersed concentrations determined after the 24 h settling period  
426 (i.e. the dispersibility) varied significantly due to the physicochemical properties of the different  
427 CNTs. The non-functionalized CNTs exhibit the greatest degree of agglomeration and settling  
428 (Petersen et al. 2016; Petersen et al. 2011), leading to lower final dispersed concentrations. The  
429 propensity for a particular CNT to form agglomerates will aid in its retention on a filter, possibly  
430 explaining the observed high degree of retention for the non-functionalized CNTs. It is also  
431 possible that there are differences in the proportion of individually dispersed CNTs, with those  
432 forming small or loose agglomerates being trapped more efficiently during filtration. Again,  
433 increasing the ionic strength of the solution prior to filtration should promote CNT  
434 agglomeration and may result in improved retention (Yang et al. 2011).

435

436 The difference in dispersed concentration was also considered as a possible factor influencing  
437 filtration efficiency. SWCNT was present in the lowest concentration (0.7 mg/L), yet was most  
438 easily removed by filtration. In contrast, MWCNT-COOH was present in the highest  
439 concentration (6.0 mg/L) and proved the most difficult to remove from the aqueous phase  
440 (**Figure 3**). To investigate if the concentration of dispersed CNTs affected the efficacy of the  
441 filtration, the influence of both dispersion dilution and the use of a secondary filtration of the  
442 filtrate was performed with MWCNT-COOH using selected filters that showed insufficient  
443 separation of this particle; MCE, PVDF and GF-75 (Figure S9, Supplementary Data). Neither  
444 dilution nor secondary filtration resulted in a satisfactory increase in CNT retention. This  
445 indicates initial CNT concentration does not significantly influence filtration efficiency and is  
446 consistent with the findings of a previous study (Yang et al. 2011).

447

448 The CNT dispersion method can have a significant impact on the final dispersion properties  
449 and aggressive dispersion techniques can damage the CNTs (Cerrillo et al. 2015; Hennrich et al.  
450 2007; Stegen 2014). Although SEM images of the pristine CNTs showed they all exhibited  
451 lengths significantly above 1  $\mu\text{m}$  (Figure S10), suggesting the potential for high retention on  
452 filters with pore sizes in the range 0.1-0.2  $\mu\text{m}$ , this is not consistent with results in the current  
453 study. However, a detailed assessment of the mean CNT lengths was not conducted in the  
454 current study. The use of a low-energy bath sonicator (as opposed to high-energy probe  
455 sonication) should ensure the formation of CNT fragments is minimized, consistent with  
456 previous studies into CNT damage and breakage (Cerrillo et al. 2015; Hennrich et al. 2007;  
457 Stegen 2014). Whilst it is also possible that other carbonaceous fragments could be present in

458 some of the samples, the common CNT synthesis method suggests significant differences  
459 between the different CNT types are unlikely. It is suggested that differences in CNT retention  
460 are predominantly driven by their surface chemistry and its role in dispersibility.

461  
462 In addition to effectively retaining the CNTs, filters must also have a low sorption potential  
463 for the target analytes. The current study investigated the sorption of phenanthrene, as a model  
464 PAH, to the filters. A low phenanthrene recovery (<30%) was observed for three of the filters  
465 (PC, MCE, PVDF; **Figure 4**), indicating they have high adsorption affinities for phenanthrene.  
466 Nearly 100 % adsorption of phenanthrene was observed for the MCE filter. This is somewhat  
467 surprising as cellulose ester membrane filters have previously been used to assess phenanthrene  
468 adsorption to suspended agglomerates of C<sub>60</sub> (Baun et al. 2008). The reason for the contrasting  
469 results observed is unknown, but shows that testing of selected filters is important to avoid  
470 erroneous measurements. A high recovery was seen for the glass fiber filters (>93%) and the  
471 PTFE filter membrane (~80%).

472  
473 Although the PC filter retained all CNT types well, the high sorption of phenanthrene made  
474 this filter unsuitable for subsequent determination of phenanthrene C<sub>free</sub>. The two glass fiber  
475 filters exhibited the lowest degree of adsorption but could not efficiently retain CNTs owing to  
476 their large pore sizes (0.3 and 0.7 μm, respectively). Considering the two criteria for selecting an  
477 appropriate filter, CNT retention and low phenanthrene adsorption, the PTFE filter membrane  
478 exhibited the best overall performance. To investigate the suitability of the PTFE filter further,  
479 phenanthrene recovery was tested at concentrations ranging from 70-735 μg/L (**Figure 5**). The  
480 filtration method showed very high precision regarding recovery values, with a coefficient of

481 variation (CV) of <2.3% for the six replicates tested at each concentration. Furthermore, the  
482 recovery ( $82.5 \pm 1.5\%$ ) was very similar over the concentration range tested. A recovery of  
483 >80% is considered to be acceptable, especially considering the high precision of the method.  
484 However, adsorption to the filter may vary for different PAHs, with increased adsorption likely  
485 for larger, more hydrophobic PAHs (e.g. pyrene) despite the hydrophilic nature of the filter. A  
486 lower recovery could still be acceptable provided that the PAH loss can be adequately corrected  
487 for, either through a predictable loss in the filtration step as observed in the current study, or by  
488 the use of internal standards. Importantly, the method should be verified when applied to new  
489 target analytes, with the most appropriate filter being selected optimized on a case-by-case basis.

490

491 Filtration using 0.1  $\mu\text{m}$  hydrophilic PTFE membrane proved to be a simple, time and cost  
492 efficient method for separating a range of CNTs from the water phase prior to determination of  
493 phenanthrene  $C_{\text{free}}$ . Subsequent to validation, the method has been successfully applied to  
494 investigate phenanthrene adsorption to dispersed CNTs (Glomstad et al. 2016; Zindler et al.  
495 2016). Adsorption isotherms were established and fitted to the Dubinin-Ashtakhov model that  
496 has previously been used in several other studies to describe PAH adsorption to CNTs (Kah et al.  
497 2014; Yang et al. 2006a; Yang and Xing 2010). The determined adsorption capacity was  
498 somewhat higher than that observed in other studies using agglomerated or partly agglomerated  
499 CNTs (Kah et al. 2011; Yang et al. 2006a), but were consistent with previous studies reporting  
500 higher adsorption capacities of PAHs for dispersed CNTs (Zhang et al. 2012). As the method  
501 was applicable to a range of CNTs, the study could evaluate the influence of CNT  
502 physicochemical properties on phenanthrene adsorption, and subsequently the effect on

503 bioavailability and toxicity to the freshwater algae *Pseudokirchneriella subcapitata* (Glomstad et  
504 al. 2016) and *Daphnia magna* (Zindler et al. 2016).

505

506

## 507 **CONCLUSIONS**

508 The current study shows that PAH adsorption to dispersed CNTs, using phenanthrene as a  
509 model compound, can be accurately determined using simple filtration methods. A 0.1  $\mu\text{m}$   
510 hydrophilic PTFE filter membrane efficiently separated a suite of dispersed CNTs with a range  
511 of physicochemical properties from the water phase without significantly retaining the dissolved  
512 phenanthrene. The resulting aqueous samples contained negligible quantities of CNTs allowing  
513 for accurate determination of phenanthrene  $C_{\text{free}}$  values. Ultracentrifugation was unable to fully  
514 sediment all of the studied CNT types from the water phase, limiting its use as a broadly  
515 applicable approach for CNT-PAH adsorption studies. The nd-SPME and DT-nd-SPME  
516 approaches evaluated highlighted significant problems with the adherence of CNTs to the  
517 PDMS-coated fibers and sorption of PAHs to the dialysis tubing, which negatively influence the  
518 determination of accurate  $C_{\text{CNT}}$  values. In addition, filtration offers a time and cost benefit to the  
519 other methods evaluated, especially the nd-SPME techniques. Filtration also has potential for  
520 investigating the adsorption of other HOCs, as a wide range of filter materials is available.  
521 However, careful selection of filters based on their composition and pore size is necessary to  
522 achieve efficient separation of CNTs from the water phase while minimizing PAH/HOC sorption  
523 to the filter. As sorption to the filter can vary for different target analytes, it is necessary to  
524 validate all filters for their suitability prior to use in adsorption studies.

525

526

527 *Supplementary data* – The Supplementary Data are available at DOI:

528

529

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540

541 *Conflict of Interest* – The authors declare no conflict of interest.

542

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693

694 **Tables**

695 **Table 1.** Physicochemical properties of the CNTs. Variations are presented as standard  
 696 deviations. Differing letters in the columns indicate statistically significant differences among the  
 697 CNTs (ANOVA:  $p > 0.05$ ).

CNT	Average diameter (nm)	Length ( $\mu\text{m}$ )*	Specific surface area ( $\text{m}^2/\text{g}$ )	Surface oxygen content (%)	Surface carbon content (%)
SWCNT	$2.4 \pm 1.1^a$	5-30	$483.7 \pm 23.5^a$	1.6	98.4
MWCNT-15	$14.8 \pm 5.6^b$	~50	$140.5 \pm 0.4^b$	1.5	98.5
MWCNT-30	$16.6 \pm 8.6^c$	10-30	$177.4 \pm 3.0^b$	2.0	98.0
MWCNT-OH	$14.3 \pm 5.8^b$	~50	$140.2 \pm 2.8^b$	3.9	96.1
MWCNT-COOH	$20.3 \pm 7.5^d$	~50	$139.7 \pm 3.5^b$	5.7	94.3

698 \*Supplied by the manufacturer.

699

700

701 **Figure legends**

702 **Figure 1.** Overview of the methods evaluated for the determination of CNT adsorption of  
 703 phenanthrene ( $C_{\text{CNT}}$ ). The method encircled with dotted lines is the final method developed for  
 704 determination of phenanthrene  $C_{\text{free}}$  in the presence of different types of CNTs.

705

706 **Figure 2.** (A) Concentration of pyrene on PDMS-coated fiber with and without DT protection at  
 707  $C_{\text{nominal}} 100 \mu\text{g/L}$ . Error bars represent standard deviation of replicate samples ( $n=3$ ) (B) Pyrene  
 708 concentration in the water determined by LLE after extraction with nd-SPME or DT-nd-SPME.  
 709 Error bars show standard deviation for replicate samples with DT protection ( $n=2$ ). A significant

710 reduction in the  $C_{\text{fiber}}$  (A) and  $C_{\text{free}}$  (B) was seen when using DT-nd-SPME compared to nd-  
711 SPME.

712

713 **Figure 3.** Absorbance measured at 800 nm in the filtrate of the CNT dispersions after filtration  
714 of five types of CNTs through various filters. Error bars represent the standard deviation of  
715 replicate samples using PTFE filters (n=3). For all other filters, only single samples were  
716 prepared and analyzed (n=1). Dotted lines show limit of detection (LOD) and limit of  
717 quantification (LOQ).

718

719 **Figure 4.** Sorption of phenanthrene by various filters. A high sorption by the filters would  
720 impede further analysis of the filtrate to determine  $C_{\text{free}}$ . Error bars represent the standard  
721 deviation of triplicate samples for the PTFE filters (n=3) and of duplicate samples for all other  
722 filter types (n=2).

723

724 **Figure 5.** Percentage of phenanthrene sorbed to PTFE filter membrane at different phenanthrene  
725  $C_{\text{nominal}}$ . Error bars represent the standard deviation of replicate samples (n=6). The percentage of  
726 phenanthrene sorbed was highly reproducible over the range of  $C_{\text{nominal}}$  tested.