1	Degradation of ¹⁴ C-labeled Few Layer Graphene via Fenton
2	Reaction: Reaction Rates, Characterization of Reaction
3	Products, and Potential Ecological Effects
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21 ABSTRACT

22 Graphene has attracted considerable commercial interest due to its numerous potential 23 applications. It is inevitable that graphene will be released into the environment 24 during the production and usage of graphene-enabled consumer products, but the 25 potential transformations of graphene in the environment are not well understood. In this study, ¹⁴C-labeled few layer graphene (FLG) enabled quantitative measurements 26 27 of FLG degradation rates induced by the iron/hydrogen peroxide induced Fenton reaction. Quantification of ¹⁴CO₂ production from ¹⁴C-labeled FLG revealed 28 significant degradation of FLG after 3 days with high H_2O_2 (200 mmol L⁻¹) and iron 29 (100 μ mol L⁻¹) concentrations but substantially lower rates under environmentally 30 relevant conditions (0.2 to 20 mmol L^{-1} H₂O₂ and 4 µmol L^{-1} Fe³⁺). Importantly, the 31 32 carbon-14 labeling technique allowed for quantification of the FLG degradation rate 33 at concentrations nearly four orders of magnitude lower than those typically used in 34 other studies. These measurements revealed substantially faster degradation rates at 35 lower FLG concentrations and thus studies with higher FLG concentrations may 36 underestimate the degradation rates. Analysis of structural changes to FLG using 37 multiple orthogonal methods revealed significant FLG oxidation and multiple reaction 38 byproducts. Lastly, assessment of accumulation of the degraded FLG and 39 intermediates using aquatic organism Daphnia magna revealed substantially 40 decreased body burdens, which implied that the changes to FLG caused by the Fenton 41 reaction may dramatically impact its potential ecological effects.

43 Keywords: ¹⁴C-labeled few layer graphene; Fenton reaction; degradation kinetics;
 44 ¹⁴CO₂ generation; quantification; characterizations

46 Abbreviations

¹⁴ C	carbon-14
FLG	few layer graphene
CNMs	carbon nanomaterials
GO	graphene oxide
TEM	transmission electron microscopy
SEM	scanning electron microscopy
XPS	X-ray photoelectron spectroscopy
FTIR	Fourier transform infrared
AFM	atomic force microscopy
LSC	liquid scintillation counting
HPLC	high-performance liquid chromatography
LC-MS/MS	liquid chromatography coupled with tandem
	mass spectrometry
GC-MS	gas chromatography-mass spectrometry

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50 1. Introduction

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Graphene, a nanomaterial with a honeycomb lattice structure composed of planar sp^2 52 53 bound carbon atoms (Geim and Novoselov, 2007; Novoselov et al., 2004), has 54 become one of the most intensively studied carbon nanomaterials (CNMs) for a 55 number of potential applications (Geim, 2009; Stankovich et al., 2006; Sun et al., 56 2008). Since the application of graphene and its derivate has been greatly developed, 57 it is inevitable that graphene will be released into the environment during the 58 production and usage of graphene-enabled consumer products. It has been shown that 59 graphene can induce cytotoxic effects such as cell membrane damage and bacterial 60 toxicity and genotoxic effects on mammalian cells (Akhavan and Ghaderi, 2010; 61 Bianco, 2013; Yang et al., 2013; Yang et al., 2010) and can accumulate in Daphnia 62 magna (Guo et al., 2013).

One critical question regarding the fate of graphene in the environment is the extent and rate of degradation (Bai et al., 2014; Kotchey et al., 2012). If graphene can be degraded to CO_2 , graphene may not accumulate in the environment with time, which would decrease the likelihood of potential adverse effects. A recent study showed that treatment of pristine multilayered graphene by H_2O_2 at physiologically

68	and environmentally relevant concentrations (1 to $10000 \times 10^{-6} \text{ mol } \text{L}^{-1}$) was found to
69	cause holes and defects in the surface of graphene (Xing et al., 2014). The hydroxyl
70	radical (•OH) was considered to play an important role in the oxidative degradation of
71	graphene (Xing et al., 2014; Zhou et al., 2012). In addition, the distinctly different
72	reactivities have been observed for graphene-based materials with varying oxygen
73	contents, defects and numbers of layers (Figure S1). For example, researchers have
74	found that graphene oxide (GO), a precursor of graphene, can be degraded by
75	photo-Fenton reaction (Bai et al., 2014; Zhou et al., 2012), or by enzymatic reaction
76	with horseradish peroxidase and H_2O_2 (Kotchey et al., 2011; Kotchey et al., 2012).
77	However, reduced graphene oxide resisted degradation under the same experimental
78	condition (Kotchey et al., 2011). Moreover, photoreaction of GO under sunlight or
79	UV irradiation resulted in reduced graphene oxide nanosheets with many holes and
80	defects (Hou et al., 2015; Matsumoto et al., 2011). It was also found that the
81	intermediate photoproducts which have reduced sizes, decreased oxygen
82	functionalities and low molecular-weight are more slowly degraded by photoreaction
83	compared to parent GO (Hou et al., 2015). Overall, the conclusions regarding the
84	degradation of graphene-based nanomaterial were mostly drawn from qualitative
85	results and did not provide quantitative degradation rates (Bai et al., 2014; Hou et al.,
86	2015; Kotchey et al., 2011; Kotchey et al., 2012; Matsumoto et al., 2011; Xing et al.,
87	2014; Zhou et al., 2012).

88	Fenton chemistry ($Fe^{2+}/Fe^{3+}/H_2O_2$) is well known as a strong oxidizing agent
89	with the production of •OH. It has been developed and applied in wastewater
90	treatment, especially in treating aromatic organic pollutants (Ensing et al., 2003;
91	Neyens and Baeyens, 2003; Pignatello et al., 2006). Recently, the Fenton and
92	photo-Fenton reactions were found to have significant implication on the treatment of
93	carbon nanotubes (CNTs) (Allen et al., 2009; Fan et al., 2007) and GO (Bai et al.,
94	2014; Zhou et al., 2012). Researchers have demonstrated that this simple and
95	economical approach could effectively degrade CNTs and GO into CO ₂ and some
96	intermediate products which largely consist of adjacent aromatic rings with carboxylic
97	acid groups (Allen et al., 2009; Bai et al., 2014; Zhou et al., 2012). In addition, Fenton
98	chemistry can functionalize CNMs and was shown to cut GO into graphene quantum
99	dots via photo-Fenton reaction (Zhou et al., 2012). Furthermore, the reaction of
100	CNMs by Fenton chemistry may undertake similar oxidation processes as by
101	enzymatic biodegradation systems. Therefore, Fenton chemistry was applied to
102	understand the degradation mechanism of CNMs in biodegradation systems,
103	especially for the enzymatic-catalyzed systems which with ferric heme iron (Fe ^{$3+$}) in
104	the catalytic active center (Allen et al., 2009; Andon et al., 2013; Bai et al., 2014;
105	Kagan et al., 2010). Moreover, the naturally occurring Fenton chemistry plays a
106	significant role in the environmental fate of the pollutants (Fukushima and Tatsumi,
107	2001; Sawyer et al., 1996). Thus, the reaction of CNMs by Fenton chemistry could
108	reveal important information on the environmental fate of CNMs.

109	In previous studies, several techniques have been applied to characterize and
110	quantify the degradation of CNMs (see Table S1 for a summary of studies on CNMs
111	degradation). However, most of these techniques utilized (e.g., Raman spectroscopy,
112	transmission electron microscopy (TEM) and scanning electron microscopy (SEM)
113	and UV-vis-IR spectroscopy) may have a limited capacity for CNMs quantification in
114	environmentally relevant matrices as a result of interferences or an insufficiently low
115	detection limit (Fores-Cervantes et al., 2014; Petersen et al., 2011b). In contrast, the
116	¹⁴ C isotope, a long-life radioactive isotope, is a sensitive tracer and ¹⁴ C-isotopic
117	quantification methods can overcome the limitations described above for other
118	techniques. ¹⁴ C-labeling has been applied to quantitatively tracking the biodistribution
119	of CNMs (Georgin et al., 2009; Guo et al., 2013; Petersen et al., 2009; Petersen et al.,
120	2008; Petersen et al., 2011a; Petersen et al., 2011b). Recently, ¹⁴ C-labeling was also
121	applied to study the microbial degradation of multiwall carbon nanotubes under
122	complex reaction conditions (Zhang et al., 2013). Microbial degradation of multiwall
123	carbon nanotubes resulted in ${}^{14}\text{CO}_2$ as the end product under some conditions (Zhang
124	et al., 2013).

125 In this study, we treated ¹⁴C-labeled few layer graphene (FLG) by 126 iron/H₂O₂-driven Fenton chemistry. We demonstrate that FLG can be completely 127 degraded by the Fenton reaction by measuring the FLG degradation rates under a 128 range of conditions using ¹⁴C-labeling to quantify FLG residues and ¹⁴CO₂ release. By 129 measuring ¹⁴C, FLG degradation kinetics with various concentrations of iron, 130 hydrogen peroxide, and FLG were quantitatively evaluated. FLG degradation was 131 tested using iron and hydrogen peroxide concentrations relevant for wastewater 132 treatment of organic pollutants (Kusic et al., 2007; SafarzadehAmiri et al., 1997) and 133 lower and more environmentally relevant conditions (Xing et al., 2014). In addition, 134 degradation products were identified using multiple orthogonal techniques to fully 135 understand the reaction and determine if the techniques were in agreement. Moreover, 136 the accumulation of FLG before and after the Fenton reaction was tested using D. 137 magna, a standard test aquatic organism (USEPA, 2002; OECD, 2004), to probe the 138 potential ecological impacts of degraded FLG.

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- 141 **2.** Materials and methods
- 142 2.1. Materials

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144 Phenol (\geq99.5% purity) and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>, 30% wt) were purchased from
145 Sigma-Aldrich Co. (St. Louis, MO, USA). <sup>14</sup>C-ring-labeled phenol in an ethanol
146 solution (\geq 97% purity, determined by HPLC radiochromatogram) was purchased
147 from Moravek Biochemicals and Radiochemicals (CA, USA). Analytical-grade
148 tetrahydrate (FeCl<sub>2</sub>•4H<sub>2</sub>O), ammonium dihydrogenphosphate (NH<sub>4</sub>H<sub>2</sub>PO<sub>4</sub>), ferric
149 chloride (FeCl<sub>3</sub>•6H<sub>2</sub>O), hydrochloric acid (HCl) and anhydrous sodium sulfate
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150 (NaSO₄) were purchased from Nanjing Chemical Reagent Co., Ltd (Nanjing, China). 151 Ultrapure water was used in this experiment (>18 M Ω). Dichloromethane and 152 methanol of HPLC/SPECTRO grade were purchased from TEDIA (Fairfield, USA). 153 Certain commercial equipment, instruments and materials are identified in order to 154 specify experimental procedures as completely as possible. In no case does such 155 identification imply a recommendation or endorsement by the National Institute of 156 Standards and Technology nor does it imply that any of the materials, instruments or 157 equipment identified are necessarily the best available for the purpose.

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159 2.2. Synthesis, purification, and characterization of ¹⁴C-labeled FLG

¹⁴C-labeled FLG was synthesized as described in our previous study (Guo et al., 161 2013). A full description of synthesis process and characterization of ¹⁴C-labeled FLG 162 is provided in the Supplementary Information (SI) and summarized here. Briefly, 163 164 FLG was successfully synthesized by graphitization and exfoliation of sandwich-like 165 FePO₄/dodecylamine hybrid nanosheets and then purified using hydrochloric acid to 166 remove the iron catalysts to below the detection limit (0.005 mg/L) by inductively 167 coupled plasma optical emission spectroscopy (Optima 5300DV). TEM and SEM 168 showed crumpled nanosheets of FLG and agglomeration (Figure S2). Atomic force 169 microscopy (AFM) measurements showed the thickness of FLG is 1.05 to 4.05 nm

170	and mostly was in the range of 1.35 to 1.95 nm, which would correspond to graphene
171	that is four layers thick, and that the lateral size distribution ranged from 60 to 590 nm
172	with two major peaks at 90 and 365 nm (Lu et al., 2015). High resolution TEM image
173	indicates that the interlayer distance is about 0.344 nm, which is the interlayer
174	distance of graphite. The strong D (1343 cm ⁻¹), G (1579 cm ⁻¹), and 2D (2686cm ⁻¹)
175	bands in Raman spectra are common in graphene with multilayers structure.
176	Elemental analysis by X-ray photoelectron spectroscopy (XPS) indicates that the
177	atomic ratio of FLG is C: O: H: N is 89: 6: 1: 4. Also, XPS-peak-fitting analysis of
178	the average carbon element content shown that most of carbon participate in C=C and
179	C-C bonds. UV-vis spectra indicate no obvious UV-vis absorption region of this FLG.
180	The specific radioactivity of the purified FLG was 16.12 \pm 0.59 mCi g ⁻¹ (the
181	uncertainty indicates the standard deviation of triplicate samples). To measure
182	potential carbon-14 impurities in FLG, the dispersed FLG were extracted using
183	dichloromethane and <i>n</i> -hexane mixtures, and the extracts were analyzed using liquid
184	scintillation counting (LSC) and gas chromatography-mass spectrometry (GC-MS).
185	Results did not show a significant increase in the radioactivity or obvious chemical
186	peaks in GC-MS chromatograms of the extracted solutions, indicating that
187	carbon-14 byproducts were not formed from the synthesis, purification, or dispersion
188	processes of FLG (Guo et al., 2013).

189 2.3. Assessment of FLG degradation under varying reaction conditions.

191 The following experiments were conducted to determine the degradation behavior of FLG by Fenton chemistry at environmentally realistic concentrations of Fe³⁺ and 192 H₂O₂ (Xing et al., 2014). Briefly, 20 mL of ¹⁴C-FLG suspensions with initial 193 concentrations of 20, 50, 100 or 500 μ g L⁻¹ were prepared and 100 μ mol L⁻¹ Fe³⁺ and 194 195 2 mM H₂O₂ were added to initiate the reaction. These FLG concentrations are at least three orders of magnitude lower than that (100 mg FLG L^{-1}) used in previous studies 196 197 (Bai et al., 2014; Zhou et al., 2012). To study this reaction using a broader range of conditions, treatment of 500 μ g L⁻¹ of ¹⁴C- FLG with 4 μ mol L⁻¹ Fe³⁺ and 0.2 or 20 198 199 mM H₂O₂ was also evaluated. Experiments were also performed to assess FLG transformation through reactions mediated by Fenton reagents under different reaction 200 conditions with higher concentrations of iron and hydrogen peroxide (Fe^{3+} and H_2O_2) 201 concentrations were varied from 25 to 200 μ mol L⁻¹ or 0.2 to 200 mmol L⁻¹, 202 203 respectively) potentially relevant for treatment of wastewater with FLG (Kusic et al., 2007; SafarzadehAmiri et al., 1997). Similar experiments were also performed with 204 FLG and either H_2O_2 or Fe^{3+} . Lastly, an experiment was conducted testing 500 µg L⁻¹ 205 14 C-FLG with 200 mmol L⁻¹ H₂O₂ and 50 µmol L⁻¹ Fe³⁺ for 10 days to assess if the 206 207 FLG would be completely converted to CO₂. 208

The reactions were carried out in 40-mL glass vials equipped with polytetrafluoroethylene (PTFE)-lined screw caps and incubated on a rotary shaker operating at 150 rpm at 25 °C. Each reactor contained a 20 mL reaction medium that was adjusted to pH 4; the reasons for using this pH value are that ferric ions can form

212	iron hydroxides, which will not react with hydrogen peroxide, at neutral and alkaline
213	conditions (Lu et al., 2001) and that this pH was used in related previous studies (Bai
214	et al., 2014; Zhou et al., 2012). H ₂ O ₂ was added last to each reactor to initiate the
215	reaction. To quantify the production of ¹⁴ CO ₂ , gas in the reaction vial headspace
216	was sampled after 0, 4, 8, 12, 24, 36, 48, 60, 72 h, 96, 120 and 144 h using a syringe
217	with valve, and then added to 2-mL of a 5 M NaOH solution. The NaOH solution was
218	transferred to 3 mL of alkaline scintillation cocktail (Oxysolve C-400; Zinsser
219	Analytic, Frankfurt, Germany). At each of the times listed above, 100 μ L of the
220	reaction suspension from each vial was removed and mixed with 3 mL of alkaline
221	scintillation cocktail. The corresponding radioactivity in the samples was analyzed by
222	LSC (LS6500; Beckman Coulter, USA). The limit of detection of this experimental
223	system was approximately 54 ng 14 C L ⁻¹ . Three replicates were tested at each reaction
224	condition. All experiments were performed in the dark to prevent photolysis reactions.
225	

226 2.4. Characterizations of FLG changes and products identification.

Experiments were also conducted to characterize changes to FLG remaining in the aqueous phase after the Fenton reaction and to identify the possible formation of intermediate products. Samples at two initial FLG concentrations, 500 μ g L⁻¹ and 100 mg L⁻¹, were prepared to characterize changes to FLG via the Fenton reaction using instruments with different sensitivities. The 500 μ g L⁻¹ samples were prepared using

233	the procedure described above in 40-mL glass vials with unlabeled FLG and Fe^{3+} and
234	H_2O_2 concentrations of 50 µmol L ⁻¹ and 200 mmol L ⁻¹ , respectively. These samples
235	were analyzed using UV-vis spectroscopy, Raman spectroscopy, AFM, and TEM. For
236	techniques that required higher FLG concentrations, the reactions were conducted in
237	100-mL beakers and stirred with a magnetic stir bar at room temperature. Unlabeled
238	FLG, Fe ³⁺ , and H ₂ O ₂ concentrations of 100 mg L ⁻¹ , 50 μ mol L ⁻¹ , and 200 mmol L ⁻¹ ,
239	respectively, were used. At set reaction times, FLG was sampled and analyzed using
240	FTIR Spectroscopy, XPS, HPLC, and LC-MS/MS (detailed information on the
241	analysis method and samples preparation are presented in the SI). In experiments
242	designed to measure intermediate products, 200 mmol L^{-1} H ₂ O ₂ was additionally
243	added to the reaction suspensions at day 3 to increase the mass of degradation
244	byproducts. After incubation for 3 days or 5 days, 10 mL of the aqueous phase from
245	the reaction mixture was sampled and sequentially extracted three times using 10 mL
246	dichloromethane each time. The extracted solutions were combined, dried by rotary
247	evaporation, and then the residue was dissolved in methanol. The reconstituted
248	samples were completely transferred into 1.8-mL vials and then each was reduced to
249	0.5 mL using a gentle stream of nitrogen gas. It should be noted that this method
250	could not detect any volatile products that were created. This sample was sequentially
251	analyzed by HPLC to identify any peaks and LC-MS/MS to determine the molecular
252	weight of the possible products. HPLC was performed on an Agilent 1200 instrument
253	equipped with the variable wavelength detector (VWD) by a 4.6×250 mm Eclipse

254	XDB C18 reverse-phase column (Agilent, USA) at 30 °C. The detection wavelength
255	of VWD was 230 nm. The isocratic mobile phase was made up of methanol (70%)
256	and water (30%) with a flow rate of 1.0 mL min ⁻¹ and the injection volume was 20 μ L.
257	LC-MS/MS analysis was carried out on a Thermo liquid chromatograph connected to
258	a Thermo LCQ Advantages (Quest LCQ Duo, USA) mass spectrometer through an
259	ESI interface. 10 μL of concentrated sample was injected into a Eclipse XDB C18
260	reverse-phase column (Agilent, USA) via a split injector (split ratio 1:5). The
261	composition and operation of mobile phase were kept identical to those mentioned
262	previously for HPLC. The mass spectrometer was set in negative ionization mode
263	over the range $m/z = 50-1200$. Capillary voltage and cone voltage were 4.5 kV and 25
264	V, respectively. Desolvation and source temperatures were 300 and 120 °C,
265	respectively. Nitrogen was used as sheath gas at a flow rate of 35 arb units and as
266	auxiliary gas at a flow rate of 5 arb units. In addition, to quantitatively track the
267	possible intermediate resulting from FLG degradation at different incubation times,
268	reactions were performed with 14 C-FLG at 500 µg L ⁻¹ . At predetermined intervals (0,
269	6, 12, 36, 60, 72, and 84 hours after the reaction was initiated), 1 mL samples of the
270	reaction solution were removed, filtered using a 0.45-µm Millipore membrane
271	(cellulose acetate), and the radioactivity in the filtrate was quantified by LSC. In
272	addition, the filtrate obtained from the 3 days reaction sample was extracted as
273	described above and the radioactivity of extracts was also analyzed by LSC.

276 D. magna neonates (< 24 h old) were exposed in untreated or treated FLG 277 suspensions to explore the potential ecological effects of FLG after treatment with the 278 Fenton reaction. The exposure suspensions used in these experiments were prepared using the following method: 500 μ g L⁻¹ ¹⁴C-labeled FLG was first treated by the 279 Fenton reagent (50 μ mol L⁻¹ Fe³⁺ and 200 mmol L⁻¹ H₂O₂). After allowing the 280 281 reaction to occur for 3 days, the reaction was stopped by adding NaOH solution at a final concentration of 150 μ mol L⁻¹ to ensure Fe³⁺ was fully precipitated as Fe(OH)₃. 282 Residual H_2O_2 was eliminated by addition of catalase (5.0 µg L⁻¹, reaction time was 2 283 284 h) and by confirming the hydrogen peroxide removal by monitoring the concentration of residual H₂O₂ using UV-vis spectrophotometry at 220 nm. Preliminary data 285 suggested that the added catalase was sufficient to consume residual H₂O₂ and had no 286 adverse impact on the Daphnia. Then reaction solutions were passed through a 287 288 0.45-µm syringe filter (cellulose acetate, 25mm, Sterile, Fisher Scientific, Pittsburgh, PA) to remove larger FLG particles and precipitated iron. The residual Fe^{3+} content in 289 290 the filtrate was analyzed by acidifying the filtrate with HCl and then quantified using the potassium thiocyanate chromogenic reaction and inductively coupled 291 292 plasma-optical emission spectroscopy(details can be found in the SI). The results showed that Fe^{3+} could be decreased to below the limit of detection (0.005 mg/L) for 293 294 both measurements.

The residual radioactivity in the filtrate was measured as (6878.5 ± 460.4) dpm mL⁻¹ by LSC which corresponds to a FLG concentration of $(192.2 \pm 12.9) \ \mu g \ L^{-1}$. CaCl₂·2H₂O, MgSO₄·2H₂O, NaHCO₃ and KCl were added into the filtrate as the artificial freshwater (AF) at the final concentration of 58.8 mg L⁻¹, 24.7 mg L⁻¹, 13.0 mg L⁻¹ and 1.2 mg L⁻¹, respectively (hardness [Ca²⁺]+ [Mg²⁺] = 0.5 mmol L⁻¹), pH= 6.5±0.2. In addition, a dispersion of 192.2 $\mu g \ L^{-1}$ of unreacted FLG was prepared in AF for comparison.

302 Ten D. magna neonates (<24 h) were exposed to 30 mL of the reacted and 303 unreacted FLG samples. Triplicate containers were sampled after 0.5, 4, 10, and 24 h. 304 There was no feeding during the exposure experiments. Assessment of Daphnia 305 immobilization was conducted on the organism to evaluate the toxicity of degradation 306 intermediates during the exposure period. After the exposure duration, D. magna were 307 placed in a beaker containing 30 mL clean water and pipetted vigorously to remove FLG particles attached to their carapaces. This step was repeated three times. We 308 309 demonstrated in our previous study that this procedure can effectively minimize the 310 contributions from the attached FLG to the total mass of FLG associated with the D. 311 magna (Guo et al., 2013). Then, the D. magna from each container were added to 312 scintillation vials with 3 mL of Gold Star cocktail, ultrasonicated for 20 min (100 W, $P = 7.52 \text{ J s}^{-1}$), allowed to sit for at least 24 h in the dark at room temperature, and 313 314 then analyzed using LSC. The radioactivity from blank samples (i.e., D. magna not 315 exposed to FLG) was subtracted from the neonate uptake results. After D. magna 316 removal, aqueous-phase radioactivity was also measured as described above to317 determine the concentration of FLG remaining suspended.

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319 2.6. Data analysis.

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Data on ¹⁴CO₂ generation during the examined times was fitted to the zero-order kinetics C_t - C_0 =kt, using Origin 8.5 software, where C_0 is the initial percentage of ¹⁴CO₂ generated from Fenton reagent mediated FLG reaction, C_t is ¹⁴CO₂ percentage at time t, and k is ¹⁴CO₂ generation rate constant. When determining the rate constants for the degradation processes, all data points for each time point were used. Statistical analyses (t-tests) were performed using SPSS 18.0 (PASW Statistics, IBM Company); differences were considered statistically significant at p < 0.05.

- 329 **3. Results and Discussion**
- 330

331 3.1. Assessment of FLG removal at varying reaction conditions.

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We first explored the degradation of FLG by Fe^{3+}/H_2O_2 -driven Fenton reaction at environmentally relevant conditions by measuring ¹⁴CO₂ generation (Figure 1). FLG can be slowly transformed into CO₂ by 4 µmol L⁻¹ Fe³⁺ and 0.2 or 20 mmol L⁻¹ H₂O₂ after a 6 day reaction (Figure 1 (a)). The total production of ¹⁴CO₂ at day 6 was

337	around 0.1 % and 0.6 % of the initial $^{14}\text{C}\text{-labeled}$ FLG for 0.2 or 20 mmol L^{-1} H_2O_2
338	with 4 μ mol L ⁻¹ Fe ³⁺ , respectively. In contrast, significantly faster degradation can be
339	observed after increasing the Fe^{3+} concentration to 100 µmol L ⁻¹ , in which case the
340	total production of 14 CO ₂ after day 3 was 1.5 % and 12 % of the initial 14 C-labeled
341	FLG for 0.2 or 20 mmol L^{-1} H ₂ O ₂ , respectively. In experiments testing the impact of
342	the initial FLG concentration on the degradation rate (Figure 1 b), the total amount of
343	14 CO ₂ produced after 5 days was approximately 6.2, 3.8, 2.9 and 1.3 % of the initial
344	20, 50, 100 and 500 μ g L ^{-1 14} C-labeled FLG. This suggests that measurements made
345	using higher FLG concentrations, as done in all previous studies (see Table S1) may
346	significantly underestimate the rate of Fenton reactions at lower concentrations. This
347	finding highlights the importance of highly sensitive quantitative measurements using
348	carbon-14 labeled FLG.

Although we have demonstrated that Fe³⁺/H₂O₂-driven Fenton reaction could 349 cause FLG degradation as measuring the production of ¹⁴CO₂ at environmentally 350 351 relevant conditions, it is important to study the degradation kinetics of FLG via 352 Fenton reaction across a broader range of conditions to quantitatively evaluate the 353 degradation kinetics. These conditions could potentially be used, for example, to treat 354 wastewater with graphene. As shown in Figure 2 and Figure 3, when testing different initial Fe^{3+} and H_2O_2 concentrations, a significant fraction of FLG, ranging from 2.0% 355 to 50%, was transformed into ¹⁴CO₂, and ¹⁴CO₂ generation increased with longer 356 reaction times. Total ¹⁴CO₂ generation increased from 1.5 % to 32.8 % of the total 357

initial radioactivity in a dose-dependent manner as the H₂O₂ dosages from 0.2 to 200 358 mmol L^{-1} at a constant Fe³⁺ dosage (100 µmol L^{-1}) (Figure 2a and Figure 3a). We also 359 fitted the kinetics of relative generation of ${}^{14}\text{CO}_2$ using a zero-order model (C_t - C_0 =kt) 360 361 to obtain the reaction rate (k) for each reaction condition, as listed in Table 1. While 362 the reaction rate increased with increasing H_2O_2 concentrations, a different trend was observed when testing various concentrations of Fe^{3+} (25 to 200 µmol L⁻¹) with a 363 fixed H_2O_2 concentration (200 mmol L⁻¹). In this case, the maximum mineralization 364 rate was measured at a Fe^{3+} dosage of 50 µmol L⁻¹ (Figure 2c, Figure 3b and Table 1). 365 However, raising the Fe^{3+} dosage (from 75 to 200 µmol L⁻¹) did not result in further 366 367 increases in the degradation rate. The radioactivity remaining in the aqueous phase decreased at rates consistent with ¹⁴CO₂ generation (Figure 2b and 2d). When 368 369 incubating FLG with only H₂O₂, approximately 1.8% of the initial FLG was transformed into ${}^{14}CO_2$ with 200 mmol L⁻¹ H₂O₂ after 72 h incubation (Figure S3a, b). 370 371 This result was in agreement with a previous result that demonstrated FLG 372 degradation by H₂O₂ (Xing et al., 2014). Conversely, degradation was not observed with any concentration of Fe^{3+} tested in the absence of H₂O₂ (Figure S3c, d). 373

The reaction kinetics studies showed that Fenton chemistry driven by H_2O_2 and Fe³⁺ can cause FLG degradation into CO₂. As shown in Scheme S1, Fenton-like reactions can generate reactive oxygen species (ROS) such as •OH and •OOH. These reactive oxygen species are considered the critical factor in the transformation of the FLG (Pignatello et al., 2006). These generated reactive oxygen species could attack

379	and destroy FLG to form defect sites, and finally decompose FLG into CO ₂ (Fan et al.,
380	2007; Zhou et al., 2012). The H_2O_2 concentration is directly proportional to the
381	generation rate of ROS in Fenton-like reaction (Ensing et al., 2003; Neyens and
382	Baeyens, 2003). With increasing Fe^{3+} dosage, ROS can be formed at higher rates by
383	the increased reaction rates with H ₂ O ₂ . However, excess iron ions may consume the
384	active oxygen species (Equations. 3 and 5 in Scheme S1) and thus reduce the Fenton
385	reaction efficiency (Lin and Gurol, 1998).

387 3.2. Characterization of FLG after Fenton reaction: intermediate products.

388

When treating 500 μ g L^{-1 14}C-FLG with 200 mmol L⁻¹ H₂O₂ and 50 μ mol L⁻¹ Fe³⁺ for 389 390 10 days, we determined that the residual radioactivity in reaction solutions was below the detection limit (< 54 ng 14 C L⁻¹), thus showing that FLG was completely 391 392 transformed into CO₂. While the ultimate end product of FLG degradation is CO₂, 393 degradation products other than, or as precursors of CO₂, are also likely to be 394 produced prior to complete conversion to CO_2 . As such, the residual radioactivity in 395 the aqueous phase as shown in Figure 2 may contain oxidized FLG and degradation 396 intermediates. As shown in Figure S4, approximately 15% of the remaining 397 radioactivity was measured in the filtrate from the 3 day reaction sample while the 398 radioactivity in the filtrate from the unreacted FLG was not detectable. In addition, 399 the filtrate obtained from the 3 day reaction sample (containing 15% radioactivity) 400 was extracted, and the remaining radioactivity in the reconstituted solution was 401 determined to be 3.6% of the initial radioactivity using LSC revealing that. FLG 402 degradation generated low molecular weight products. The extraction solutions from the degradation of a high concentration of FLG (100 mg L^{-1}) were analyzed by 403 404 HPLC and LC-MS/MS to identify the possible products. HPLC and LC-MS/MS 405 chromatograms and LC-MS base peak chromatograms, which usually monitors only 406 the most intense peaks in each spectrum, for FLG degradation products after 3 or 5 407 days of reaction showed a series of new peaks compared to unreacted FLG (Figure 408 S5, S6, and S7). Larger magnitude signals were present for the reaction samples for 409 the LC-MS base peak chromatograms after a 3 day treatment compared to the 5 day 410 treatment. The majority of peaks are large molecular weight components in the 411 solution of 3 day of reaction, such as m/z 497.15, 610.36, 723.38, 836.43, 949.49 and 412 1062.45 (Figure S7). In the extract from the 5 day reaction sample, the low molecular 413 weight components, such as m/z 118.06. 215.07, 282.15, 222.17, 301.03 and 425.25 414 appeared as the most intense peaks (Figure S7). Based on the analysis of related 415 studies (Allen et al., 2009; Bai et al., 2014; Hou et al., 2015; Zhang et al., 2013; Zhou 416 et al., 2012), and according to the MS/MS spectra (Figure S7) of the detected 417 intermediate product, possible molecular formulas and structures of these low 418 molecular weight intermediates were deduced, as shown in Figure S6.

419



422

423	incubation by showing how the color of the vial decreases after incubation with Fe^{3+}
424	and H ₂ O ₂ . UV-Vis spectrometry analysis between 280 and 800 nm of FLG samples
425	incubated for 0, 12, 24, 36, 48, 60, and 72 h showed a similar decrease (Figure S8).
426	Progress of the Fenton reaction of FLG was also assessed by TEM and AFM
427	(Figure S9 and Figure 4d, e). At 0 days, the FLG was observed to be integrated on the
428	basal plane and many of them were agglomerated. After 1 day reaction, the
429	agglomerated FLG became more dispersed, a phenomenon that could be explained by
430	an increased concentration of hydrophilic functional groups on the surface of FLG.
431	After reaction for 3 days, holes in the FLG were observed. The results in Figure 4d
432	show that the height of the flat FLG sheet is \approx 1.4 nm, and after the Fenton reaction
433	for 3 days, FLG was oxidized to contain holes but the height of the FLG was still \approx
434	1.4 nm (Figure 4e). By day 5, the FLG plane was barely visible using TEM,
435	indicating that the majority of FLG has been completely oxidized. A similar
436	degradation process was also observed in the enzymatic oxidation of GO with
437	increased incubation time (Kotchey et al., 2011).
438	Analysis of the treated FLG samples with Raman spectroscopy also showed
439	substantial degradation (Figure 4b). The observed D and G bands are distinctive of
440	graphitic materials: the D band represents the disorder present in sp^2 -hybridized

Figure 4a provides visual evidence of FLG degradation after 3 and 5 days of

441 carbon systems, while the G band represents the stretching of C-C bonds (Malard et

442 al., 2009; Wang et al., 1990). The ratio of the D band to G band can be used to 443 evaluate the defects in graphitic materials (Pimenta et al., 2007). For day 0 and 2, the 444 D:G ratio increased from (0.91 ± 0.07) :1.0 to (1.37 ± 0.22) :1.0 (p =0.021, t test), and at 445 day 5, both the D and G bands disappeared. This result revealed that Fenton oxidation 446 of the graphitic lattice would result in an increased frequency of defect sites and that the carbon atoms were transformed from sp^2 to sp^3 hybridization (Dresselhaus et al., 447 2010). At day 5, the disappearance of the D and G was attributed to the complete 448 449 oxidation of the graphitic lattice.

FT-IR analysis revealed several peaks at 3400 cm⁻¹ (O-H), 1710 and 1678 450 cm⁻¹ (C=O), 1590 cm⁻¹ (C=C), 1370 cm⁻¹ (C=C or C-O-H), 1220 and 1060 cm⁻¹ (C-O) 451 452 on the surface of FLG after 3 d reaction (Figure S10) (Hwang and Li, 2010; Sun et al., 453 2012; Zielke et al., 1996). These results indicate that the oxygen-containing groups (hydroxyl, carboxyl, carbonyl, and epoxy groups) were introduced on the surface of 454 455 FLG via the Fenton reaction. The XPS spectra in Figure S11 show the increase of O 456 and decrease of C element on the surface of FLG with increasing reaction time up to 3 457 days; analyzing samples after 5 days was infeasible as a result of an inability to obtain 458 a sufficient mass of FLG particles by centrifugation. Figure 4c and Table S2 459 summarize the surface elemental composition and oxygen species distribution that 460 was determined by XPS (corresponding O1s spectra (Yu et al., 2011; Zielke et al., 1996) are provided in Figure S12). After 3 day reaction, the O:C ratio of FLG 461 increased markedly from 0.04 to 0.17, indicating that FLG was oxidized and 462

463 oxygen-containing groups were introduced to the surface of FLG. Moreover, the
464 oxygen species and carbon species distribution was significantly changed, the oxygen
465 in OH, C-O and O-C=O increased with the reaction time. No significant increase of
466 the O: C ratio was observed after reaction for 1 day.

467

- 468 3.4. Daphnia magna results.
- 469

470 Previous research on the degradation products from CNMs has suggested that the 471 structures of the byproducts are polycyclic aromatic hydrocarbons (PAHs) (Allen et 472 al., 2009; Bai et al., 2014; Hou et al., 2015; Zhang et al., 2013; Zhou et al., 2012), a 473 result similar to the findings of this study on FLG degradation. However, these 474 intermediate PAHs may have important toxicological implications (Bai et al., 2014). 475 For example, it has been demonstrated that the oxidation products (e.g., MW < 3000476 Da) of horseradish peroxidase-catalyzed degradation of single-wall carbon nanotubes 477 will induce DNA damage (Pan et al., 2013). It was suggested that the products from 478 photoreaction of GO are likely to exhibit different accumulation and toxic properties 479 compared to parent GO (Hou et al., 2015), but the potential toxicological impacts of 480 FLG degraded by the Fenton reaction and its byproducts are not yet well understood. 481 Our previous study revealed FLG accumulation in the gut tract of *D. magna* 482 (Guo et al., 2013), a result similar to studies with other CNMs (Pakarinen et al., 2013; 483 Petersen et al., 2009; Petersen et al., 2011a). However, the smaller size of degraded

484	FLG and low molecular weight compounds generated via the Fenton reaction showed
485	different potential ecological effects (Figure 5). Substantial accumulation (17 μ g mg ⁻¹
486	of dry tissue) of FLG was measured in the D. magna exposed to unreacted FLG,
487	while accumulation of the degradation intermediates after exposure for 24 h was
488	nearly two orders of magnitude smaller (<0.02 μ g mg-1) (Figure 5a). No D. magna
489	immobilization was observed in the exposure of the unreacted FLG or after exposure
490	to the reacted FLG during the 24 h exposure period. Settling measurements of
491	unreacted and reacted FLG suspensions after Daphnia removal showed significantly
492	different behaviors (Figure 5b). After subtracting the fraction of unreacted and reacted
493	FLG accumulated by the Daphnia, around 57% of the untreated FLG agglomerated
494	and settled out of suspension over the 24 h period, while the settling percentage was
495	less than 2% in the suspensions from the FLG treated by the Fenton reaction.

497 **4.** Conclusion

498

This study provides unambiguous experimental evidence that the Fe²⁺/ Fe³⁺/ H₂O₂ -driven Fenton reaction can effectively degrade FLG. FLG can be completely transformed into CO₂ by high concentrations of Fenton reagents (e.g., 200 mM H₂O₂ and 50 μ M Fe³⁺), which implies that Fenton reaction can be potentially used for the complete degradation of FLG. In addition, by measuring the ¹⁴CO₂ release, FLG 504 degradation kinetics under various reaction conditions were quantitatively evaluated. 505 This study highlights a highly sensitive approach to quantitatively measure CNMs 506 degradation for environmentally realistic conditions with low CNMs concentrations. 507 Moreover, identification of degradation intermediates and characterization of the FLG 508 changes using multiple orthogonal techniques provided critical information to fully 509 understand the degradation process of FLG; results from each of these techniques 510 were also in agreement. Importantly, we found that FLG degradation products, 511 became more stable in water compared to the unreacted FLG, and were less easily 512 accumulated in D. magna even though the organisms were exposed to higher average 513 concentrations over the exposure period. Therefore, an important implication of this 514 study is that FLG may be transformed via naturally occurring Fenton-like reactions, 515 and the resulting change of FLG on the morphology, properties and the degradation 516 intermediates should be taken into account when assessing its potential ecological 517 risks.

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519

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- 525 Appendix A. Supplementary Data
- 526
- 527 Supplementary data related to this article can be found at the Water Research website.

528

529 **References**

- 530
- 531 Akhavan, O. and Ghaderi, E., 2010. Toxicity of graphene and graphene oxide
 532 nanowalls against bacteria. Acs Nano 4 (10), 5731-5736.
- 533 Allen, B.L., Kotchey, G.P., Chen, Y.N., Yanamala, N.V.K., Klein-Seetharaman, J.,
- Kagan, V.E. and Star, A., 2009. Mechanistic investigations of horseradish
 peroxidase-catalyzed degradation of single-walled carbon nanotubes. J. Am.
 Chem. Soc. 131 (47), 17194-17205.
- 537 Andon, F.T., Kapralov, A.A., Yanamala, N., Feng, W.H., Baygan, A., Chambers,
- 538 B.J., Hultenby, K., Ye, F., Toprak, M.S., Brandner, B.D., Fornara, A.,
- 539 Klein-Seetharaman, J., Kotchey, G.P., Star, A., Shvedova, A.A., Fadeel, B. and
- 540 Kagan, V.E., 2013. Biodegradation of single-walled carbon nanotubes by
- eosinophil peroxidase. Small 9 (16), 2721-2729.
- 542 Bai, H., Jiang, W., Kotchey, G.P., Saidi, W.A., Bythell, B.J., Jarvis, J.M., Marshall,
- 543 A.G., Robinson, R.A. and Star, A., 2014. Insight into the mechanism of graphene

- 544 oxide degradation via the photo-Fenton reaction. J. Phys. Chem. C 118 (19),
 545 10519-10529.
- 546 Bianco, A., 2013. Graphene: Safe or toxic? The two faces of the medal. Angew.
 547 Chem. Int. Edit. 52 (19), 4986-4997.
- 548 Dresselhaus, M.S., Jorio, A., Hofmann, M., Dresselhaus, G. and Saito, R., 2010.
- 549 Perspectives on carbon nanotubes and graphene Raman spectroscopy. Nano Lett.
 550 10 (3), 751-758.
- 551 Ensing, B., Buda, F. and Baerends, E.J., 2003. Fenton-like chemistry in water:
- 552 Oxidation catalysis by Fe(III) and H_2O_2 . J. Phys. Chem. A 107 (30), 5722-5731.
- 553 Fan, C., Li, W., Li, X., Zhao, S., Zhang, L., Mo, Y. and Cheng, R., 2007. Efficient
- photo-assisted Fenton oxidation treatment of multi-walled carbon nanotubes.
 Chin. Sci. Bull. 52 (15), 2054-2062.
- 556 Fores-Cervantes, D.X., Maes, H.M., Schaffer, A., Hollender, J. and Kohler, H.P.E.,
- 557 2014. Slow biotransformation of carbon nanotubes by horseradish peroxidase.
- 558 Environ. Sci. Technol. 48 (9), 4826-4834.
- 559 Fukushima, M. and Tatsumi, K., 2001. Degradation pathways of pentachlorophenol
- by photo-Fenton systems in the presence of iron(III), humic acid, and hydrogen
 peroxide. Environ. Sci. Technol. 35 (9), 1771-1778.
- 562 Geim, A.K., 2009. Graphene: Status and prospects. Science 324(5934), 1530-1534.
- 563 Geim, A.K. and Novoselov, K.S., 2007. The rise of graphene. Nat. Mater. 6 (3),
- 564 183-191.

- 565 Georgin, D., Czarny, B., Botquin, M., Mayne-L'Hermite, M., Pinault, M.,
 566 Bouchet-Fabre, B., Carriere, M., Poncy, J.L., Chau, Q., Maximilien, R., Dive, V.
- and Taran, F., 2009. Preparation of C-14-labeled multiwalled carbon nanotubes
- for biodistribution investigations. J. Am. Chem. Soc. 131 (41), 14658-14659.
- 569 Guo, X.K., Dong, S.P., Petersen, E.J., Gao, S.X., Huang, Q.G. and Mao, L., 2013.
- 570 Biological uptake and depuration of radio-labeled graphene by *Daphnia magna*.
 571 Environ. Sci. Technol. 47 (21), 12524-12531.
- 572 Hou, W.C., Chowdhury, I., Goodwin, D.G., Henderson, M., Fairbrother, D.H.,
- 573 Bouchard, D. and Zepp, R.G., 2015. Photochemical transformation of graphene 574 oxide in sunlight. Environ. Sci. Technol. 49 (6), 3435–3443.
- Hwang, Y.S. and Li, Q.L., 2010. Characterizing photochemical transformation of
 aqueous nC(60) under environmentally relevant conditions. Environ. Sci.
 Technol. 44 (8), 3008-3013.
- 578 Kagan, V.E., Konduru, N.V., Feng, W.H., Allen, B.L., Conroy, J., Volkov, Y.,
- 579 Vlasova, I.I., Belikova, N.A., Yanamala, N., Kapralov, A., Tyurina, Y.Y., Shi,
- 580 J.W., Kisin, E.R., Murray, A.R., Franks, J., Stolz, D., Gou, P.P.,
- 581 Klein-Seetharaman, J., Fadeel, B., Star, A. and Shvedova, A.A., 2010. Carbon
- 582 nanotubes degraded by neutrophil myeloperoxidase induce less pulmonary
- 583 inflammation. Nat. Nanotechnol. 5 (5), 354-359.

- 584 Kotchey, G.P., Allen, B.L., Vedala, H., Yanamala, N., Kapralov, A.A., Tyurina, Y.Y.,
- 585 Klein-Seetharaman, J., Kagan, V.E. and Star, A., 2011. The enzymatic oxidation
 586 of graphene oxide. Acs Nano 5 (3), 2098-2108.
- 587 Kotchey, G.P., Hasan, S.A., Kapralov, A.A., Ha, S.H., Kim, K., Shvedova, A.A.,
- 588 Kagan, V.E. and Star, A., 2012. A natural vanishing act: The enzyme-catalyzed
- degradation of carbon nanomaterials. Accounts Chem. Res. 45 (10), 1770-1781.
- 590 Kusic, H., Bozic, A.L. and Koprivanac, N., 2007. Fenton type processes for
- 591 minimization of organic content in coloured wastewaters: Part I: Processes592 optimization. Dyes Pigm. 74 (2), 380-387.
- Lin, S.S. and Gurol, M.D., 1998. Catalytic decomposition of hydrogen peroxide on
 iron oxide: Kinetics, mechanism, and implications. Environ. Sci. Technol. 32
 (10), 1417-1423.
- Lu, K., Huang, Q., Wang, P. and Mao, L., 2015. Physicochemical changes of
 few-layer graphene in peroxidase-catalyzed reactions: Characterization and
 potential ecological effects. Environ. Sci. Technol.
 dx.doi.org/10.1021/acs.est.5b02261.
- 600 Lu, M.C., Lin, C.J., Liao, C.H., Ting, W.P. and Huang, R.Y., 2001. Influence of pH
- on the dewatering of activated sludge by Fenton's reagent. Water Sci. Technol.
 44 (10), 327-332.
- Malard, L.M., Pimenta, M.A., Dresselhaus, G. and Dresselhaus, M.S., 2009. Raman
 spectroscopy in graphene. Phys. Rep. 473 (5-6), 51-87.

- 605 Matsumoto, Y., Koinuma, M., Ida, S., Hayami, S., Taniguchi, T., Hatakeyama, K.,
- Tateishi, H., Watanabe, Y. and Amano, S., 2011. Photoreaction of graphene
- 607 oxide nanosheets in water. J. Phys. Chem. C 115 (39), 19280-19286.
- 608 Neyens, E. and Baeyens, J., 2003. A review of classic Fenton's peroxidation as an
- advanced oxidation technique. J. Hazard. Mater. 98 (1-3), 33-50.
- 610 Novoselov, K.S., Geim, A.K., Morozov, S.V., Jiang, D., Zhang, Y., Dubonos, S.V.,
- 611 Grigorieva, I.V. and Firsov, A.A., 2004. Electric field effect in atomically thin
- 612 carbon films. Science 306 (5696), 666-669.
- 613 Organization for Economic Co-operation and Development (OECD), 2004. Test No.

614 202: *Daphnia sp.* acute immobilisation test. OECD Publishing.

- 615 Pakarinen, K., Petersen, E.J., Alvila, L., Waissi-Leinonen, G.C., Akkanen, J.,
- 616 Leppanen, M.T. and Kukkonen, J.V.K., 2013. A screening study on the fate of
- 617 fullerenes (nC60) and their toxic implications in natural freshwaters. Environ.
- 618 Toxicol. Chem. 32 (6), 1224-1232.
- 619 Pan, S.M., Sardesai, N.P., Liu, H.Y., Li, D.D. and Rusling, J.F., 2013. Assessing
- DNA damage from enzyme-oxidized single-walled carbon nanotubes. Toxicol.
 Res. 2 (6), 375-378.
- 622 Petersen, E.J., Akkanen, J., Kukkonen, J.V.K. and Weber, W.J., 2009. Biological
- 623 uptake and depuration of carbon nanotubes by *Daphnia magna*. Environ. Sci.
- 624 Technol. 43 (8), 2969-2975.

625	Petersen, E.J., Huang, Q. and Weber, W.J., 2008. Ecological uptake and depuration of
626	carbon nanotubes by Lumbriculus variegatus. Environ. Health. Perspect. 116 (4),
627	496-500.

- 628 Petersen, E.J., Pinto, R.A., Mai, D.J., Landrum, P.F. and Weber, W.J., 2011a.
- 629 Influence of polyethyleneimine graftings of multi-walled carbon nanotubes on
- 630 their accumulation and elimination by and toxicity to *Daphnia magna*. Environ.
- 631 Sci. Technol. 45 (3), 1133-1138.
- 632 Petersen, E.J., Zhang, L.W., Mattison, N.T., O'Carroll, D.M., Whelton, A.J., Uddin,
- 633 N., Nguyen, T., Huang, Q.G., Henry, T.B., Holbrook, R.D. and Chen, K.L.,
- 634 2011b. Potential release pathways, environmental fate, and ecological risks of
 635 carbon nanotubes. Environ. Sci. Technol. 45 (23), 9837-9856.
- 636 Pignatello, J.J., Oliveros, E. and MacKay, A., 2006. Advanced oxidation processes for
- 637 organic contaminant destruction based on the Fenton reaction and related
 638 chemistry. Crit. Rev. Env. Sci. Tec. 36 (1), 1-84.
- 639 Pimenta, M.A., Dresselhaus, G., Dresselhaus, M.S., Cancado, L.G., Jorio, A. and
- Saito, R., 2007. Studying disorder in graphite-based systems by Raman
 spectroscopy. Phys. Chem. Chem. Phys. 9 (11), 1276-1291.
- 642 SafarzadehAmiri, A., Bolton, J.R. and Cater, S.R., 1997. Ferrioxalate-mediated
- 643 photodegradation of organic pollutants in contaminated water. Water Res. 31 (4),
- 644 787-798.

- 645 Sawyer, D.T., Sobkowiak, A. and Matsushita, T., 1996. Metal [ML(x); M=Fe, Cu,
- 646 Co, Mn]/hydroperoxide-induced activation of dioxygen for the oxygenation of
 647 hydrocarbons: Oxygenated Fenton chemistry. Accounts Chem. Res. 29 (9),
 648 409-416.
- 649 Stankovich, S., Dikin, D.A., Dommett, G.H.B., Kohlhaas, K.M., Zimney, E.J., Stach,
- E.A., Piner, R.D., Nguyen, S.T. and Ruoff, R.S., 2006. Graphene-based
 composite materials. Nature 442 (7100), 282-286.
- 652 Sun, X.M., Liu, Z., Welsher, K., Robinson, J.T., Goodwin, A., Zaric, S. and Dai, H.J.,
- 653 2008. Nano-graphene oxide for cellular imaging and drug delivery. Nano Res. 1654 (3), 203-212.
- Sun, Y.B., Wang, Q., Chen, C.L., Tan, X.L. and Wang, X.K., 2012. Interaction
 between Eu(III) and graphene oxide nanosheets investigated by batch and
 extended X-ray absorption fine structure spectroscopy and by modeling
 techniques. Environ. Sci. Technol. 46 (11), 6020-6027.
- United States Environmental Protection Agency (USEPA), 2002. Methods for
 measuring the acute toxicity of effluents and receiving waters to freshwater and
 marine organisms, 5th ed. U.S. Environmental Protection Agency: Washington,
 DC.
- 663 Wang, Y., Alsmeyer, D.C. and Mccreery, R.L., 1990. Raman spectroscopy of carbon
- materials: Structural basis of observed spectra. Chem. Mater. 2 (5), 557-563.

- King, W., Lalwani, G., Rusakova, I. and Sitharaman, B., 2014. Degradation of
 graphene by hydrogen peroxide. Part. Part. Syst. Char. 31 (7), 745-750.
- 667 Yang, K., Li, Y.J., Tan, X.F., Peng, R. and Liu, Z., 2013. Behavior and toxicity of
- graphene and its functionalized derivatives in biological systems. Small 9 (9-10),
 1492-1503.
- Yang, K., Zhang, S.A., Zhang, G.X., Sun, X.M., Lee, S.T. and Liu, Z.A., 2010.
 Graphene in mice: Ultrahigh in vivo tumor uptake and efficient photothermal
 therapy. Nano Lett. 10 (9), 3318-3323.
- 673 Yu, X.-Y., Luo, T., Zhang, Y.-X., Jia, Y., Zhu, B.-J., Fu, X.-C., Liu, J.-H. and Huang,
- K.-J., 2011. Adsorption of lead (II) on O₂-plasma-oxidized multiwalled carbon
 nanotubes: thermodynamics, kinetics, and desorption. ACS Appl. Mater.
 Interfaces 3 (7), 2585-2593.
- 677 Zhang, L.W., Petersen, E.J., Habteselassie, M.Y., Mao, L. and Huang, Q.G., 2013.
- 678 Degradation of multiwall carbon nanotubes by bacteria. Environ. Pollut. 181,679 335-339.
- 680 Zhou, X.J., Zhang, Y., Wang, C., Wu, X.C., Yang, Y.Q., Zheng, B., Wu, H.X., Guo,
- S.W. and Zhang, J.Y., 2012. Photo-Fenton reaction of graphene oxide: A new
 strategy to prepare graphene quantum dots for DNA cleavage. Acs Nano 6 (8),
 6592-6599.
- 684 Zielke, U., Huttinger, K.J. and Hoffman, W.P., 1996. Surface-oxidized carbon fibers
- 685 .1. Surface structure and chemistry. Carbon 34 (8), 983-998.

687 Table 1. Kinetics results obtained by fitting ¹⁴CO₂ generation rate to zero-order

H ₂ O ₂				Fe ³⁺		FLG	
500 μg L ⁻¹ FLG, 100		500 μ g L ⁻¹ FLG, 4 μ M		$500 \ \mu g \ L^{-1} FLG$, 200 mM		2 mM H ₂ O ₂ , 100 μ M Fe ³⁺	
$\mu M \ Fe^{3+}$		Fe ³⁺		H_2O_2			
(mM)	k (% h ⁻¹)	(mM)	k (% h ⁻¹)	(µM)	k (% h ⁻¹)	$(\mu g L^{-1})$	k (% h ⁻¹)
0.2	0.02140±0.0016	0.2	0.0007±0.0001	25	0.3494±0.0489	20	0.05255±0.0073
2	0.02295±0.0023	20	0.0043±0.0005	50	0.633±0.0119	50	0.03365±0.0012
20	0.1732±0.0025			75	0.5516±0.0167	100	0.02415±0.0006
100	0.3173±0.0118			100	0.4563±0.0219	500	0.02295±0.0023
200	0.4549±0.0236			150	0.3836±0.0168		
				200	0.3788 ± 0.0085		

688 plots under different concentrations of H_2O_2 , Fe^{3+} and FLG.

The uncertainty value is the standard error for the calculated k.; the R^2 values were ≥ 0.92 for all condition.



Figure 1. Relative yield of ¹⁴CO₂ over time resulting from Fenton reagent mediated FLG reaction under various environmentally relevant conditions. (a), [FLG] = 500 μ g L⁻¹, [H₂O₂]= 0.2 or 20 mmol L⁻¹, [Fe³⁺] = 4 μ mol L⁻¹ for open symbols and 100 μ mol L⁻¹ for solid symbols, 25 °C, pH= 4.0; (b), [FLG] = 20, 50, 100 or 500 μ g L⁻¹, [H₂O₂]= 2 mmol L⁻¹, [Fe³⁺] = 100 μ mol L⁻¹, 25 °C, pH= 4.0. Error bars represent standard deviations (n= 3).



Figure 2. Relative yield of ¹⁴CO₂ and removal of FLG over time resulting from Fenton reagent mediated FLG reaction at different H₂O₂ (a) and Fe³⁺ (c) concentrations. The residual radioactivity over time in the reaction samples with varied concentrations of H₂O₂ (b) or Fe³⁺ (d). Experimental conditions: (a) and (b) [FLG] = 500 µg L⁻¹, [Fe³⁺] = 100 µmol L⁻¹, 25 °C, pH= 4.0; (c) and (d): [FLG] = 500 µg L⁻¹, [H₂O₂]= 200 mmol L⁻¹, 25 °C, pH=4.0. Error bars represent standard deviations (n= 3).



Figure 3. Mass recovery of ¹⁴C-FLG after 3 days Fenton reaction at different initial FeCl₃ and H₂O₂ dosages by combining the aqueous phase concentration and the mass of ¹⁴CO₂. Experimental conditions: (a) [FLG] = 10 μ g, [Fe³⁺] = 100 μ mol L⁻¹, 25 °C, pH= 4.0; (b): [FLG] = 10 μ g, [H₂O₂]= 200 mmol L⁻¹, 25 °C, pH=4.0. Error bars represent standard deviations (n= 3).



Figure 4. Characterization of FLG during the Fenton-like reaction. (a) Photograph of FLG in systems variously containing Fe³⁺ and/or H₂O₂, including a system containing Fe³⁺ at 50

 μ mol/L (G+Fe); a system containing 100 mmol/L H₂O₂ (G+H); and a system containing both 50 μmol/L Fe³⁺ and 100 mmol/L H₂O₂ for Day 3 or 5. (b) and (c) displays the Raman spectra and XPS analysis of the FLG reacted with Fenton reagent at different times. (d) and (e) show AFM images of FLG and FLG reacted with Fenton reagent at Day 3. The inserted dotted line in Figures (d) and (e) shows the height profile of FLG film. Experimental conditions: (a), (b), (d) and (e), [FLG]= 500 µg L⁻¹, [Fe³⁺] = 50 µmol L⁻¹, [H₂O₂] = 200 mmol L⁻¹; (c), [FLG]= 100 mg L⁻¹, [Fe³⁺]= 50 µmol L⁻¹, [H₂O₂]= 200 mmol L⁻¹; 25 °C, pH= 4.0.



Figure 5. The uptake of *D. magna* after exposure of unreacted FLG and its degradation intermediates up to 24 h (a). Measured residual concentrations and settling percentage of unreacted FLG and its degradation intermediates in uptake experiment suspensions after *D. magna* removal (b). Reaction conditions: [FLG] = 500 μ g L⁻¹, [Fe³⁺] = 50 μ mol L⁻¹, [H₂O₂] = 200 mmol L⁻¹, 25 °C, pH= 4.0, 3 days.