Supporting Information

Correlative radioimaging and mass spectrometry imaging: a powerful combination to study ^{14}C-graphene oxide in vivo biodistribution

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1. Characterization

1.1. ^{12}C and ^{14}C-Graphene Oxide Nanoribbons

^{12}C-Graphene Oxide Nanoribbons

A. Transmission Electron Microscopy analysis of ^{12}C-Graphene Oxide Nanoribbons
B. Raman Analysis of ^{12}C-Graphene Oxide Nanoribbons
C. XPS analysis of ^{12}C-Graphene Oxide Nanoribbons

^{14}C-Graphene Oxide Nanoribbons

A. Transmission Electron Microscopy analysis of ^{14}C-Graphene Oxide Nanoribbons

1.2. ^{12}C and ^{14}C-Graphene Oxide

^{12}C-Graphene Oxide

A. Transmission Electron Microscopy analysis of ^{12}C-Graphene Oxide
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^{14}C -Graphene Oxide

A. Transmission Electron Microscopy analysis of ^{14}C-Graphene Oxide.
B. AFM analysis of ^{14}C-Graphene Oxide

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1. Characterization

1.1. ^{12}C and ^{14}C-Graphene Oxide Nanoribbons

^{12}C-Graphene Oxide Nanoribbons

A. Transmission Electron Microscopy analysis of ^{12}C-Graphene Oxide Nanoribbons. Graphene Oxide Nanoribbons were analysed by transmission electron microscopy (Philips CM 30; CEA Saclay, DEN-LM2E, France and LVEM5 Low-voltage Transmission Electron Microscope, CEA-Saclay, SCBM, France) and found to be mainly composed of large micrometer scale nanoribbons of graphene oxide.

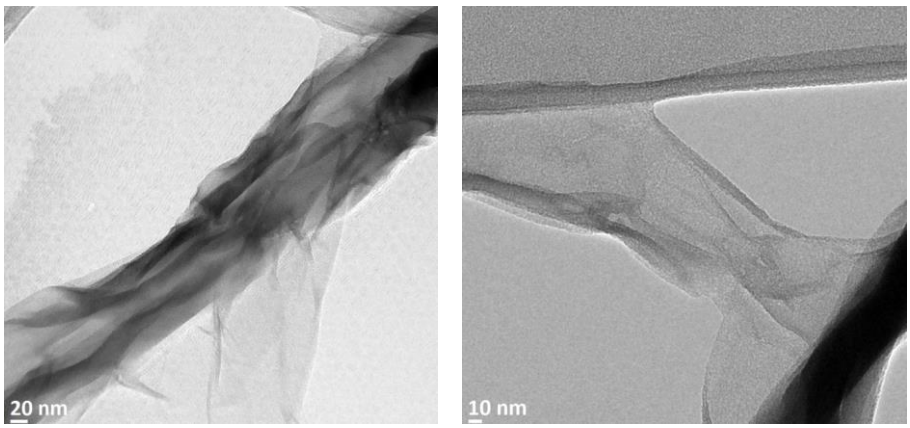


Figure S1. Transmission electron microscopy (TEM) analysis of ^{12}C-Graphene oxide Nanoribbons

B. Raman Analysis of ^{12}C-Graphene Oxide Nanoribbons. Raman spectra were recorded on a Horiba Jobin-Yvon LabRam ARAMIS equipped with a laser excitation at 532 nm.

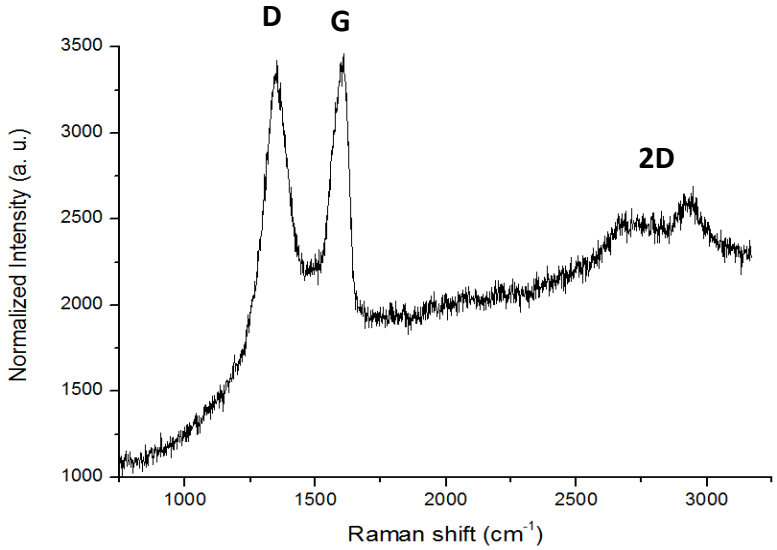


Figure S2. Raman Analysis of ^{12}C-Graphene Oxide Nanoribbons

C. XPS analysis of ^{12}C-Graphene Oxide Nanoribbons. The chemical composition of the non-radiolabelled Graphene Oxide Nanoribbons was evaluated by XPS (X-ray induced photoelectron spectroscopy) analysis using a Kratos Analytical Axis Ultra DLD spectrometer with monochromatic Al KR X-ray radiation (hν = 1486.6 eV). Quantitative analysis of Graphene oxide nanoribbons indicated that the C content was ca. 67.5 %, the O content was ca. 31.5 %, and the Nitrogen was ca. 1.0 % with a major contribution of Csp^3 and epoxy groups, minor contributions of Csp^2 and carboxylic acid groups.

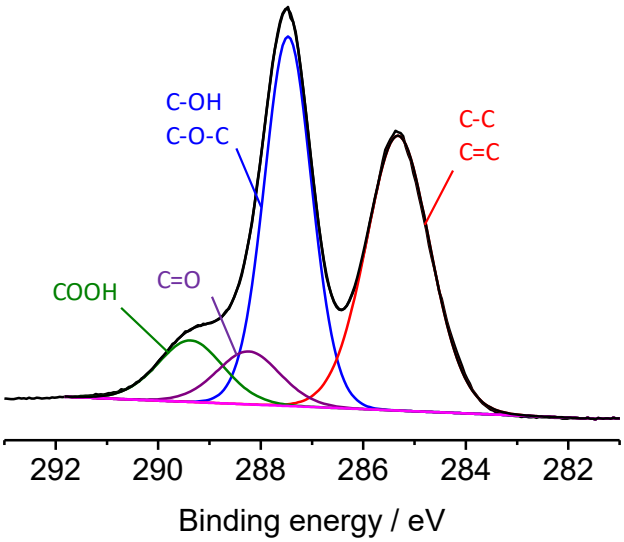


Figure S3. XPS analysis of Carbon composition in ^{12}C-Graphene Oxide Nanoribbons indicating a content of 67.5 %

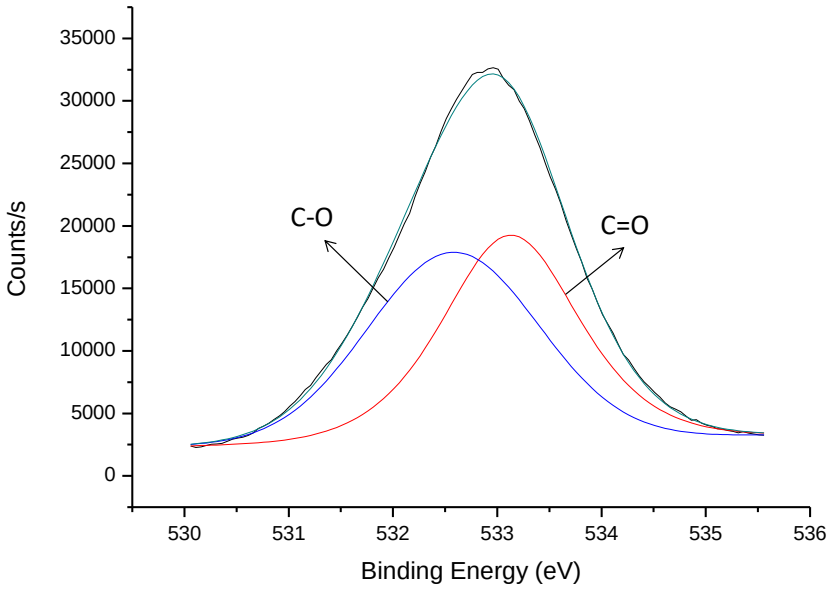


Figure S4. XPS analysis of Oxygen composition in ^{12}C-Graphene Oxide Nanoribbons indicating a content of 31.5 %

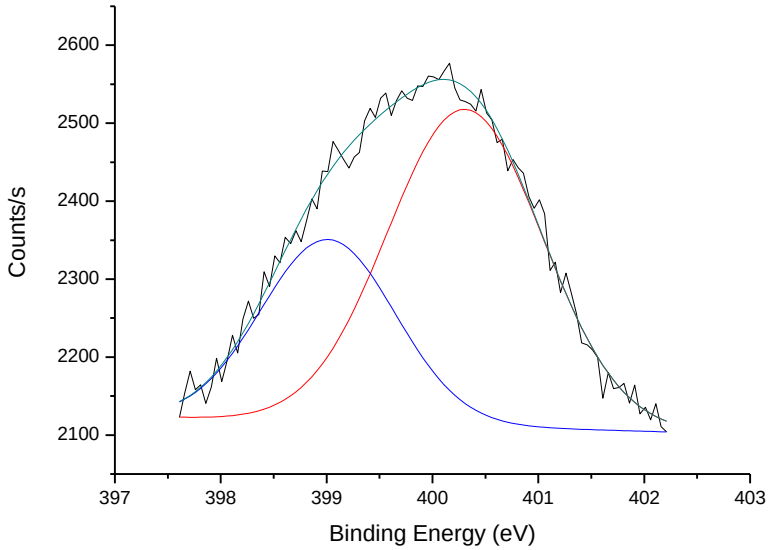


Figure S5. XPS analysis of Nitrogen composition in ^{12}C-Graphene Oxide Nanoribbons indicating a content of 1.0 %

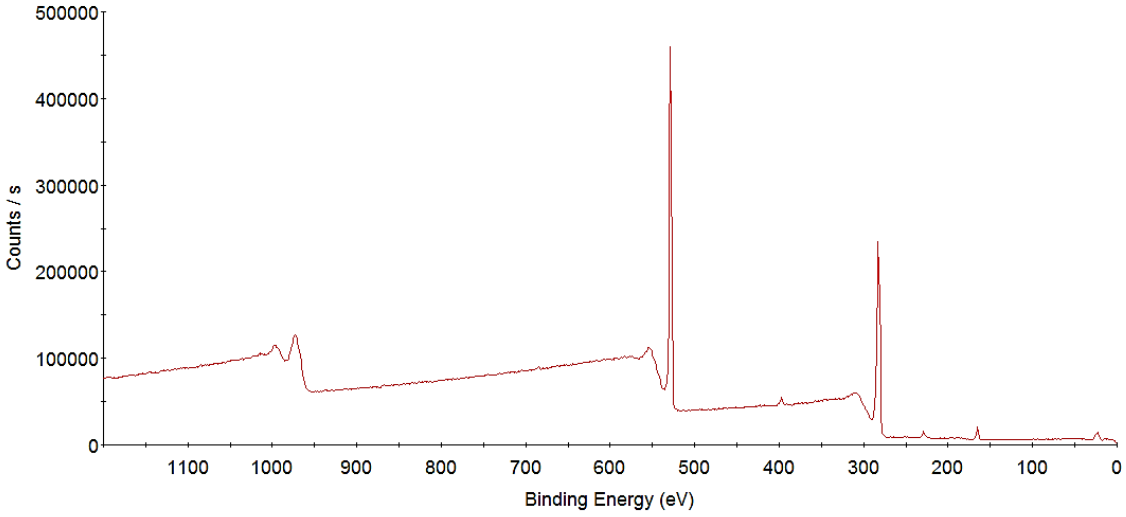


Figure S6. XPS survey spectra of ^{12}C-GO nanoribbons

^{14}C-Graphene Oxide Nanoribbons

A. Transmission Electron Microscopy analysis of ^{14}C-Graphene Oxide Nanoribbons. ^{14}C-Graphene Oxide Nanoribbons were analysed by transmission electron microscopy (LVEM5 Low-voltage Transmission Electron Microscope, CEA-Saclay, SCBM, France) and found to be mainly composed respectively of large micrometer scale nanoribbons of graphene oxide.

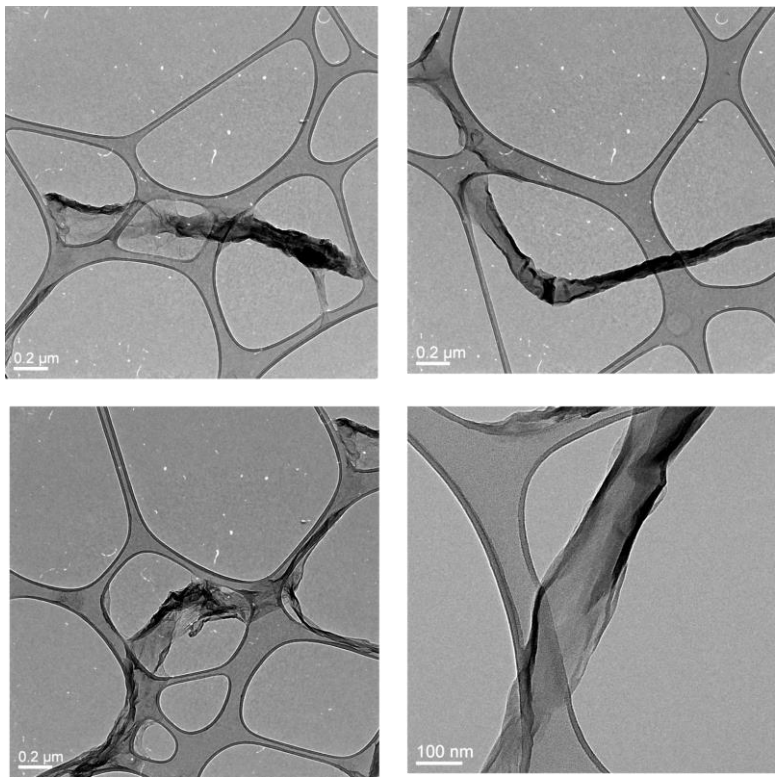


Figure S7. Transmission electron microscopy (TEM) analysis of ^{14}C-Graphene oxide Nanoribbons

1.2. ^{12}C and ^{14}C-Graphene Oxide

^{12}C-Graphene Oxide

A. Transmission Electron Microscopy analysis of ^{12}C-Graphene Oxide. Graphene Oxide were analysed by transmission electron microscopy (Philips CM 30; CEA Saclay, DEN-LM2E, France and LVEM5 Low-voltage Transmission Electron Microscope, CEA-Saclay, SCBM, France) and found to be mainly composed of nanometer (< 100 nm) scale graphene oxide particles.

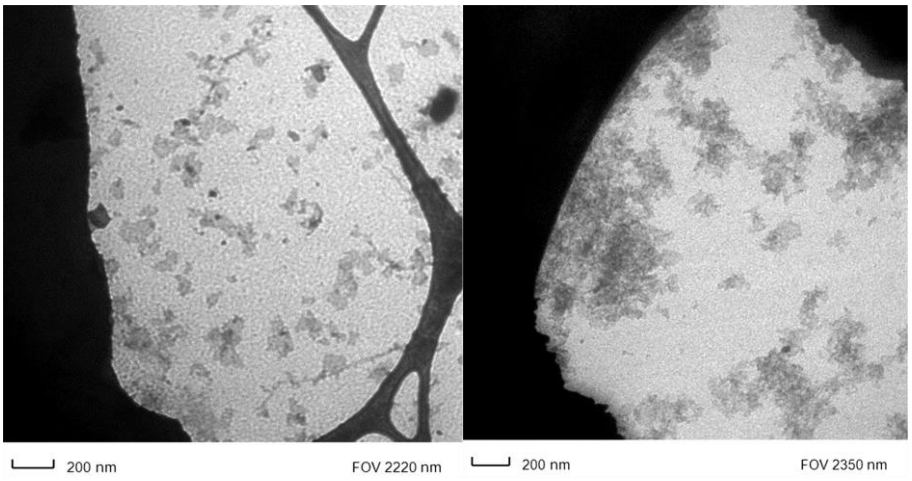
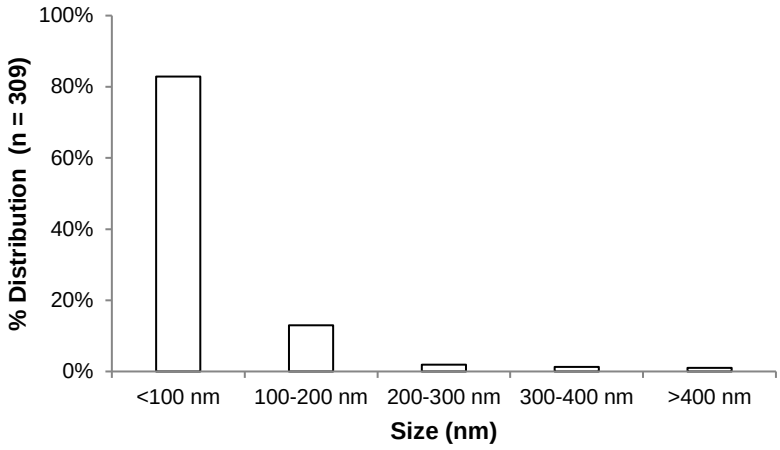


Figure S8. Transmission electron microscopy (TEM) analysis of ^{12}C-Graphene oxide

B. Size distribution of ^{12}C-Graphene Oxide



Size	Distribution
<100 nm	83 %
100-200 nm	13 %
200-300 nm	2 %
300-400 nm	1 %
>400 nm	1 %

Figure S9. Particle size distribution of ^{12}C-Graphene Oxide determined with 309 measurements showing majority of the particles (>80%) are smaller than 100 nm.

C. Raman Analysis of ^{12}C-Graphene Oxide. Raman spectra were recorded on a Horiba Jobin-Yvon LabRam ARAMIS equipped with a laser excitation at 532 nm

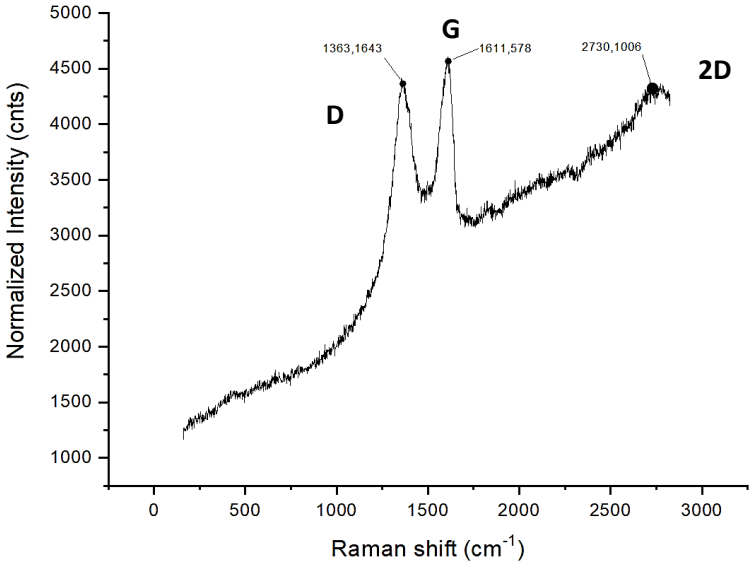


Figure S10. Raman Analysis of ^{12}C-Graphene Oxide

D. XPS analysis of ^{12}C-Graphene Oxide. The chemical composition of the non-radiolabelled Graphene Oxide was evaluated by XPS (X-ray induced photoelectron spectroscopy) analysis using a Kratos Analytical Axis Ultra DLD spectrometer with monochromatic Al KR X-ray radiation (hv = 1486.6 eV). Quantitative analysis of Graphene oxide nanoribbons indicated that the C content was ca. 69 %, the O content was ca. 30.3 %, and the Nitrogen was ca. 0.7 % with a major contribution of Csp^3 and epoxy groups, minor contributions of Csp^2 and carboxylic acid groups.

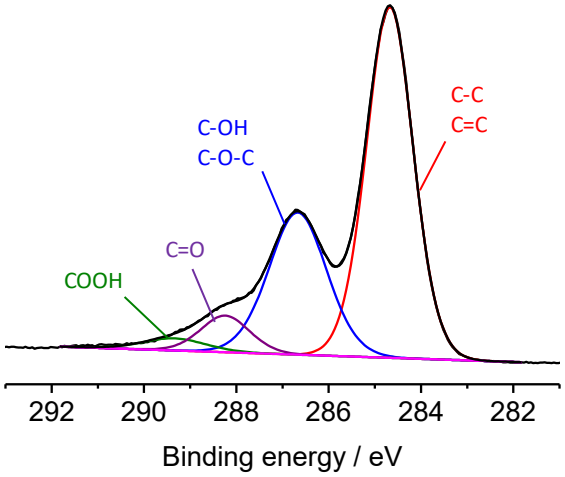


Figure S11. XPS analysis of Carbon composition in ^{12}C-Graphene Oxide indicating a content of 69.0 %

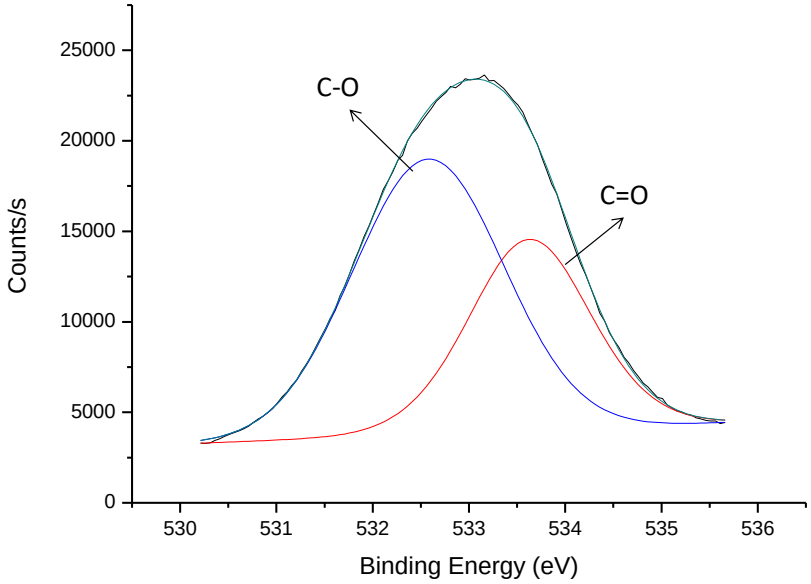


Figure S12. XPS analysis of Oxygen composition in ^{12}C-Graphene Oxide indicating a content of 30.3 %

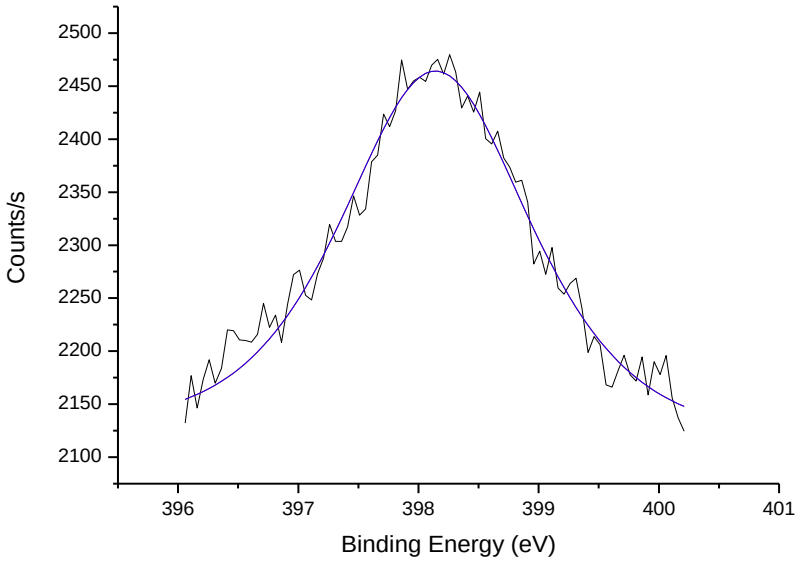


Figure S13. XPS analysis of Nitrogen composition in ^{12}C-Graphene Oxide indicating a content of 0.7 %

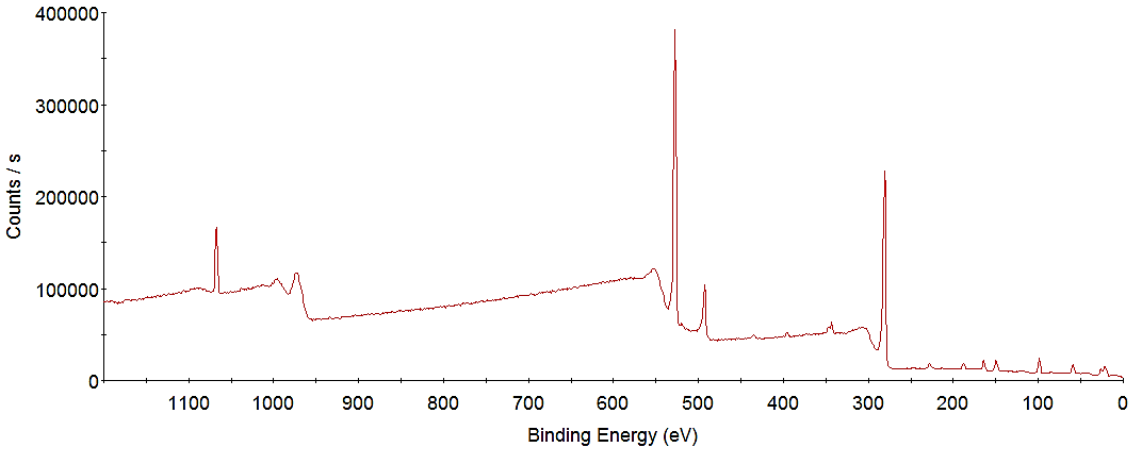


Figure S14. XPS survey spectra of ^{12}C-GO

^{14}C-Graphene Oxide

A. Transmission Electron Microscopy analysis of ^{14}C-Graphene Oxide. Graphene Oxide were analysed by transmission electron microscopy (LVEM5 Low-voltage Transmission Electron Microscope, CEA-Saclay, SCBM, France) and found to be mainly composed nanometer (< 100 nm) scale graphene oxide particles.

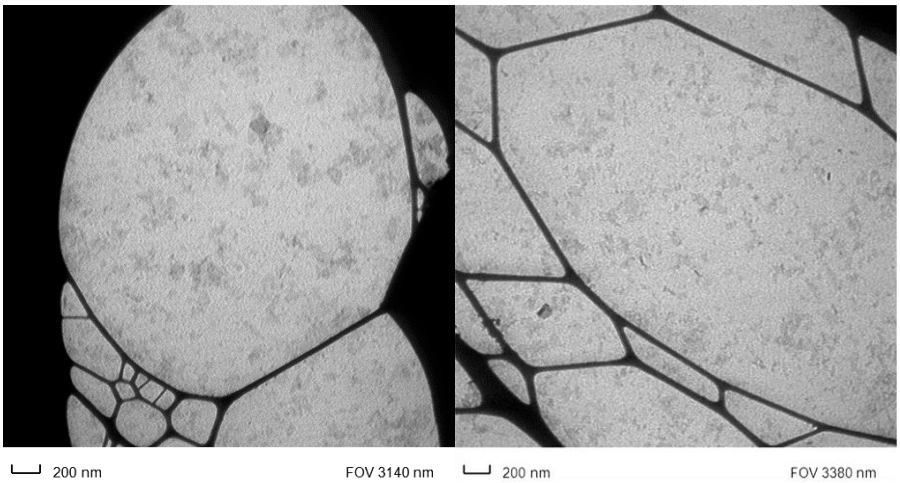


Figure S15. Transmission electron microscopy (TEM) analysis of ^{14}C-Graphene oxide

B. AFM analysis of ^{14}C-Graphene Oxide

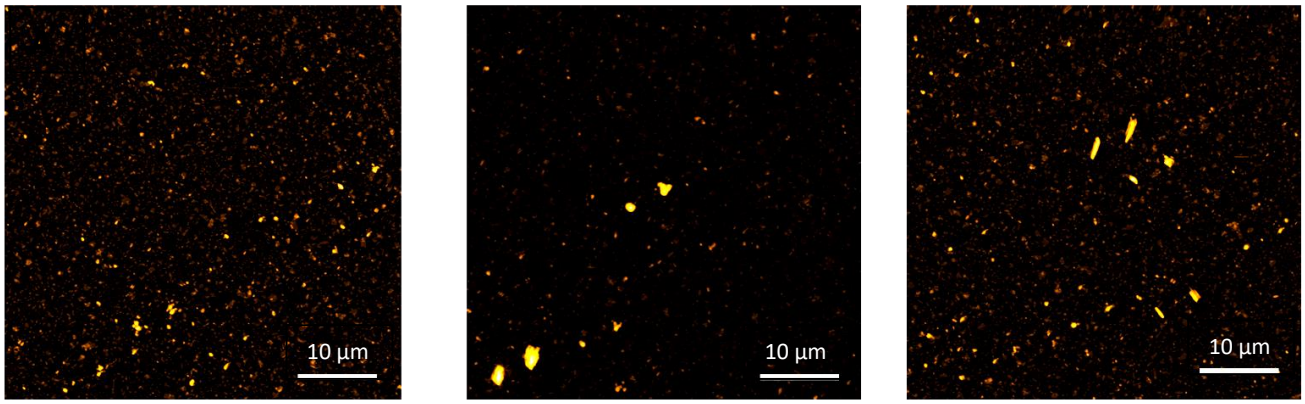


Figure S16. Atomic Force Microscope analysis of ^{14}C-Graphene oxide.

2. Method

2.1. Tissue staining

Table S1. Tissue staining protocol

Solution	Time (s)	Role
Formaldehyde (10% in EtOH)	120	Fixation
EtOH	60	Hydration
EtOH 90% (in water)	60	
EtOH 70% (in water)	60	
Water	60	
Haematoxylin	30	Nucleus coloration
Water (x2)	60	Rinsing
Eosin 0.5% (in water)	30	Cytoplasm coloration
EtOH 90% (in water)	60	Dehydration
EtOH 70% (in water)	60	
EtOH	60	
Xylene	120	Clarification

2.2. Radioimaging

Table S2 Acquisition parameters for β-imaging experiments

Dose	Organs	Accumulation	Controls
50 µg	Lung, Liver, Spleen, Kidney	0.5 h 3 h	-3.7 Bq, -17.87 Bq
75 µg	Lung, Liver, Spleen, Kidney	0.5 h 3 h	

2.3. MS-Imaging

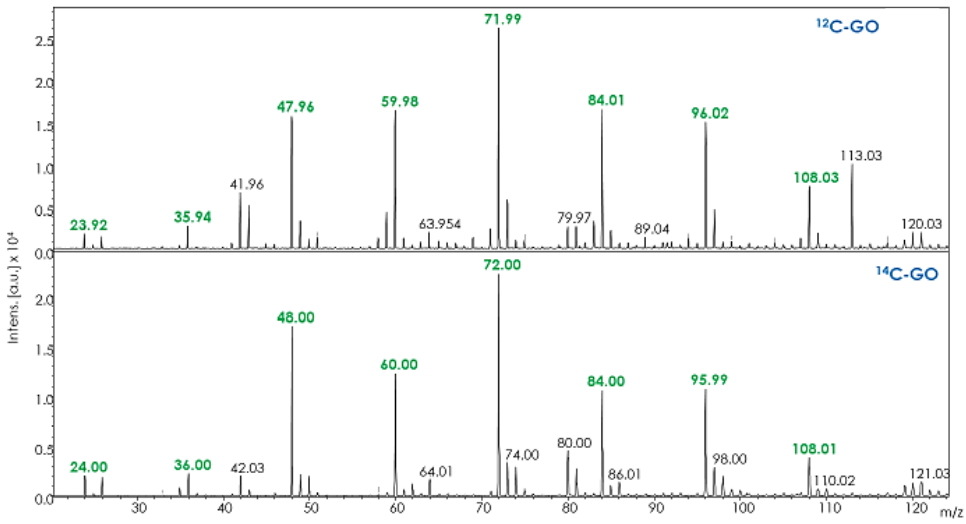


Figure S17. LDI-MS spectra of ^{14}C-labelled and unlabelled Graphene Oxide. Results were collected after 4000 shots per spectra at 2 kHz, with a delayed extraction time of 10 ns and a laser fluence of 90%.

3. Biodistribution

3.1. Radioimaging quantification

Table S3: Amount of radiolabelled Graphene Oxide detected in organs.

	Doses (µg)	Mean values (ng/slice, n = 3)	Standard deviation	ng/mg	Slice volume (mm^3)	Organ weight (g)	µg per organs	% per organs
Lungs	75	142.6	8.0	151.8	1.1	0.25	29.3	39.1
	50	52.9	14.7	26.8	0.5	0.24	22.4	44.9
Liver	75	25.4	4.9	29.2	1.2	2.13	42.6	56.8
	50	21.1	2.4	28.6	1.4	1.81	25.4	50.9
Spleen	75	2.2	0.4	1.3	0.61	0.13	0.5	0.60
	50	1.0	0.08	0.5	0.56	0.12	0.2	0.36
Kydneys	75	0.5	0.09	0.5	1.03	1.11	0.5	0.64
	50	0.4	0.03	0.4	1.07	0.94	0.3	0.60

The cpm/slice were first converted to nCi/slice from the references and then to ng/slice from the stock solution. The averages were determined on three sections of each organs and for each dose injected. The ng/mg were calculated from the density of each tissue and the size of the sections. From this value, the µg/organ and the % of injected dose were determined.

3.2. β-Imaging

The different accumulation time (Table S3) between the different organs explains why the signal in spleen and kidney looks as intense as for lung and liver, even though the amount of $^{14/12}$C-GO is much smaller in spleen and kidney.

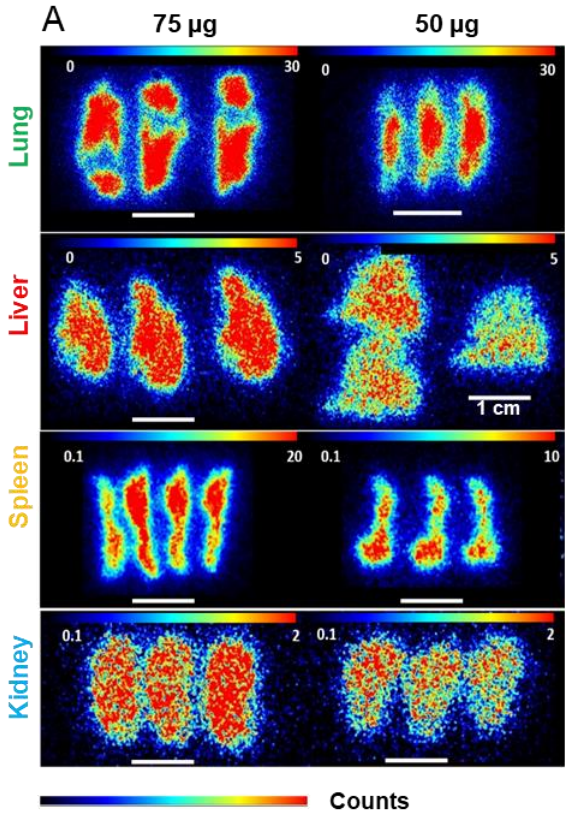


Figure S18. Radioimaging of exposed organs using β-imager **A.** Biodistribution of $^{14/12}$C-GO in liver, Lungs, Spleen and kidneys. Lungs and livers were exposed to 75 and 50 µg of $^{14/12}$C-GO with an accumulation time of 30 minutes versus 3 hours for spleen and kidney.

3.3. MSI - Spatial Resolution

a. 100 µm resolution: Lungs

MSI analysis of two lung sections from mice exposed to 50 µg and 75 µg of $^{14/12}$C-GO with a spatial resolution of 100 µm.

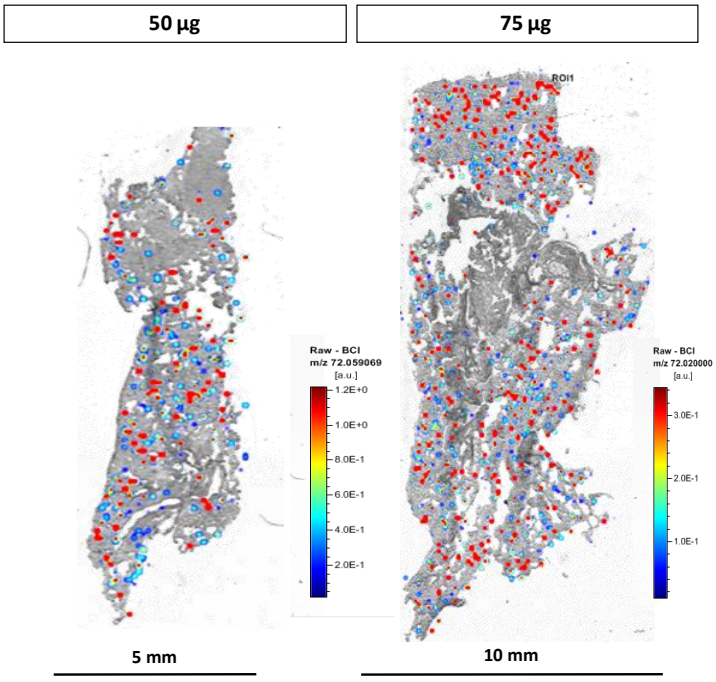


Figure S19. MSI analysis of two lung sections from mice exposed to 50 µg and 75 µg of $^{14/12}$C-GO with a spatial resolution of 100 µm. The lung section are the same as those used for quantification analysis with a pixel acquisition shift of 50 µm (x,y raster). The MS image, merged with the corresponding optical image, was acquired with an accumulation of 1000 shots per spectrum and per pixel at 2 kHz with an extraction delay of 10 ns, small laser focus and 90% laser fluence. Molecular image of GO was represented using m/z 72 ion.

b. 100 µm resolution: Liver

MSI analysis of two liver sections from mice exposed to 50 µg and 75 µg of $^{14/12}$C-GO with a spatial resolution of 100 µm.

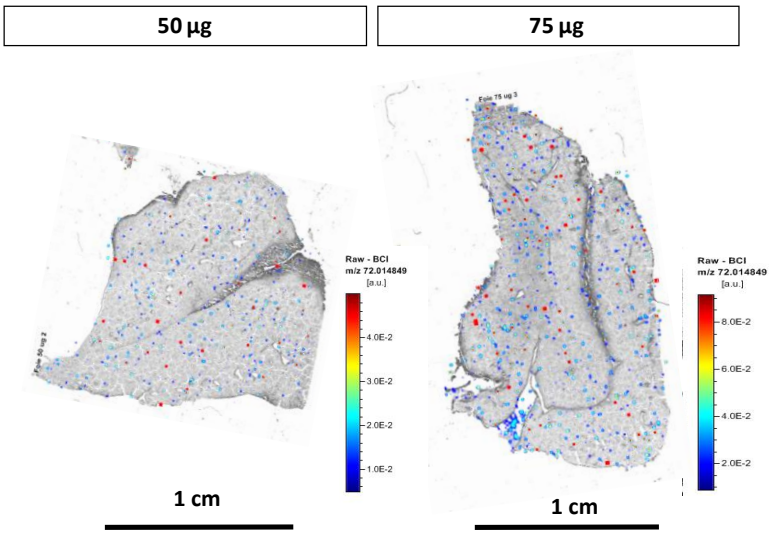


Figure S20. MSI analysis of two liver sections from mice exposed to 50 µg and 75 µg of $^{14/12}$C-GO with a spatial resolution of 100 µm. The lung section are the same as those used for quantification analysis with a pixel acquisition shift of 50 µm (x,y raster). The MS image, merged with the corresponding optical image, was acquired with an accumulation of 1000

shots per spectrum and per pixel at 2 kHz with an extraction delay of 10 ns, small laser focus and 90% laser fluence. Molecular image of GO was represented using m/z 72 ion.

c. 25 µm resolution: Lungs

MSI analysis of two liver sections from mice exposed to 50 µg and 75 µg of $^{14/12}$C-GO with a spatial resolution of 25 µm.

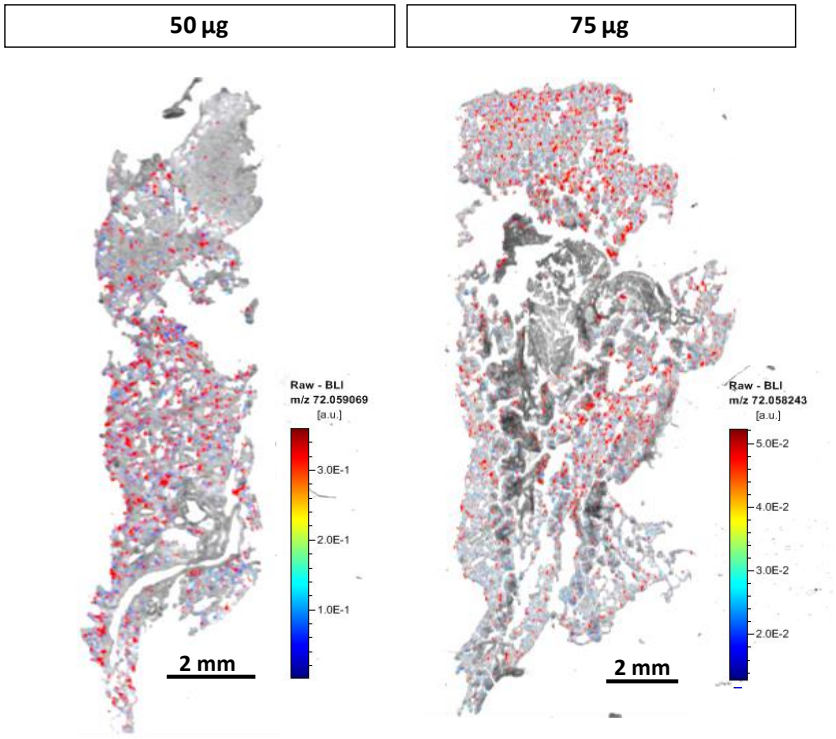


Figure S21. MSI analysis of two lung sections from mice exposed to 50 µg and 75 µg of $^{14/12}$C-GO with a spatial resolution of 25 µm. The lung section are the same as those used for quantification analysis with a pixel acquisition shift of 50 µm (x,y raster). The MS image, merged with the corresponding optical image, was acquired with an accumulation of 1000 shots per spectrum and per pixel at 2 kHz with an extraction delay of 10 ns, small laser focus and 90% laser fluence. Molecular image of GO was represented using m/z 72 ion.