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# Quantitative detection of carbon nanotubes in biological samples by an original method based on microwave permittivity measurements

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## A B S T R A C T

Due to their nanoscale, morphology, and chemical composition, the tracking and the quantitative analysis of carbon nanotubes (CNTs) in biological samples still represent huge challenges. A new technique for the quantitative and accurate detection of CNTs in various biological samples at different scales (whole organisms to organs) was developed using amphibian larvae exposed to double-walled CNTs (DWCNTs). This technique is based on the dielectric relaxation of ultra-low volume suspensions under a microwave electromagnetic field. CNT concentrations were consequently extracted from complex permittivity measurements at 5 GHz, making possible to quantitatively assess the animal exposure to CNTs. Our results indicate a detection threshold of 0.02 µg of DWCNTs, which is the lowest achieved in the literature to date.

## 1. Introduction

The ingestion and the excretion of carbon nanotubes (CNTs) by aquatic organisms, such as amphibian larvae, daphnia, copepods, and fish exposed to CNTs have been widely reported. These phenomena could be observed simply by the naked eye or under a light microscope [1–7]. In previous works [1,8,9], the ingestion and excretion by the amphibian *Xenopus laevis* larvae of double-walled CNTs (DWCNTs) and multi-walled CNTs (MWCNTs) were observed during their exposure (semi-static conditions) to a large range of CNT

concentrations (0.1–50 mg/L). According to the darkening intensity of the intestine, the CNT amount in this organ seemed to rise with the CNT concentration in the exposure medium. Nevertheless, we could not assume, for example, that the CNT amount ingested by larvae exposed to 10 mg/L was less than after exposure to 50 mg/L based only on visual inspection. Both individual and agglomerated CNTs were evidenced in the gut lumen of exposed aquatic organisms, such as *Xenopus* larvae, by Transmission Electron Microscopy (TEM), which is commonly used to characterize CNT powders or CNT composites [1,10]. Other techniques such as Raman

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spectroscopy [8] and confocal microscopy [6,11] were also used. However, ecotoxicological studies using these techniques are limited to qualitative observations. Finally, isotopic labeling (radioactivity measurements for  $^{14}\text{C}$  [4,12,13], or isotopic ratio by GC-MS for  $^{13}\text{C}$  [14,15]) can be used and have the advantage of being quantitative, but are rather expensive because the amount of  $^{13}\text{C}$  or  $^{14}\text{C}$  to be included in the samples during their synthesis should be as high as possible to increase the chances of detection of the CNTs when they are finally diluted in a matrix (soil, water, organism). Moreover,  $^{14}\text{C}$  radiolabeling is restricted to accredited laboratories authorized to handle this radioactive isotope. Among other quantitative techniques that are currently in development, near infrared fluorescence and photoluminescence [16,17] are unfortunately only suitable for unbundled semiconducting SWCNTs (tracking and semi-quantitative analysis), and thermogravimetry [18], even if potentially interesting, is often extremely difficult in very complex matrices such as biological samples. It is thus necessary to develop a method more accessible than the latter in order to quantify the presence of CNTs in environmental samples such as for example aquatic organisms and potential accumulating organs.

To reach this target, we took advantage of the intrinsic high conductivity of CNTs at microwaves frequencies. They feature notably high shielding properties against electromagnetic (EM) interference (dissipation of the incident EM radiation as heat) [19], which make CNTs good candidates for the development of composite materials suitable for industrial EM applications (for example microwave absorption devices). They are generally incorporated in polymers, such as epoxy resins, [20–24], to design materials with improved electronic, thermal and mechanical properties compared with their components. The electrical properties of CNTs are often studied through their complex permittivity determined from transmission and/or reflection measurements in microwave range [20,24]. Although the values obtained vary widely according to the nature of the polymer and the analysis device [25], dielectric relaxation phenomena were observed when CNTs were submitted to an EM field in microwave frequencies [20,26].

In addition, research activities have consequently emerged from the convergence of high-frequency (HF) microsystems and microfluidics to develop new analytical and biological, medical and environmental diagnostic systems. HF biosensor which exploits the near-field interaction between EM waves and biological fluids, such as suspensions of cells in their culture medium has already been demonstrated [27]. A microfluidic channel designed to load biological fluids was integrated perpendicularly to a coplanar waveguide (coplanar line CPW) which propagates the EM field. When the EM waves that propagate through the channel interact with the fluid, a modulation of the EM signal (amplitude and phase) is recorded according to the dielectric characteristics of the fluid. Using this device, Grenier et al. [27] showed that the addition of (biological) cells in suspension in the culture medium created a decrease of the relative permittivity correlated with the increase of the cell density. Moreover, they observed a significant difference in the values of relative permittivity (both the real part and the imaginary part) between living and dead cells [28]. Finally, by working on cancer cell suspensions, Chen et al. [29] reported that for low cells concentration the sensor

response is proportional to the number of cells contained in the sensing area. The analysis technique has therefore many advantages. It allows non-invasive analysis of a very small volume of biological materials/liquids (microliters [28] or even nanoliters [29]), the detection and quantification of cells in suspensions, and their distinction according to their status (alive/dead or non-cancerous/cancerous).

We employed the developed technique and devices to examine the relationship between the CNT concentration in amphibian *X. laevis* larvae and the dielectric signature of the sample in the HF range in order to develop a quantification methodology suited to ecotoxicological studies made in laboratory conditions. The biological matrix consisted in samples of either entire larvae exposed to DWCNTs or only their intestines, where CNTs were mainly observed under classical binocular inspection of the larvae and appeared more concentrated. As the biological samples were analyzed in suspensions, the design of a dispersion and measurement protocol was required. The detection limit and the measurement accuracy were also estimated. The DWCNT concentrations in samples of larvae exposed to DWCNTs (whole larvae and intestines) measured with the HF device were compared to those obtained by the quantification of the catalytic by-products of the DWCNT synthesis (Co and Mo) by classical chemical analysis. Finally, the comparison between the content of DWCNTs in whole larvae and in the intestine only of larvae exposed to the same DWCNT concentration, but also of whole larvae exposed to different DWCNTs concentrations (10 or 50 mg/L) provides some interesting quantitative data in terms of accumulation in the body, and especially saturation in the intestine above some critical concentration.

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## 2. Experimental

### 2.1. Material

DWCNTs were produced by the catalytic chemical vapor deposition (CCVD) of methane on a  $\text{Mg}_{0.99}(\text{Co}_{3/4}\text{Mo}_{1/4})_{0.010}$  solid solution, as described earlier [30]. CNT batch was composed of 80% of DWCNTs, 15% of SWCNTs and 5% of MWCNTs, with external diameter and length ranging respectively from 1 to 3 nm and from 1 to 100  $\mu\text{m}$  or more (bundles) [31]. DWCNTs had a purity greater than 92% (carbon content of dry DWCNTs measured by flash combustion; heating up to 1000 °C during about 1 s, after preheating at 925 °C; measurement accuracy  $\approx \pm 2\%$ ). They contained only  $3.00 \pm 0.15\%$  m Co and  $0.90 \pm 0.04\%$  m Mo. For the elemental analysis of metals [40], a few milligrams of sample were weighed in a platinum crucible and placed in a quartz tube specially designed for our open system which is called a matra (proprietary design, French CNRS Central Service of Analysis). A 2 mL mixture of 1:1  $\text{HNO}_3/\text{H}_2\text{SO}_4$  was added. The matra was heated at 250 °C for 12 h. Sonication (bath, Branson 1510, VWR) during a few minutes was then necessary to unstuck black residual deposit. The matra was then introduced for a few seconds in an electric bunsen (VWR) pre-heated at 600 °C to enhance dissolution of residual particle and was finally heated again at 250 °C for 12 h. Metals quantification was performed by ICP AES, ICAP 6300 model

(ThermoFisher Scientific, Germany). The specific surface area of DWCNTs was 980 m<sup>2</sup>/g (Brunauer, Emmett and Teller (BET) method; Micrometrics Flow Sorb II 2300; 4 h-degassing at 120 °C in N<sub>2</sub> and adsorption of nitrogen gas at the temperature of liquid nitrogen; measurement accuracy  $\approx \pm 3\%$ ). Raman spectra of DWCNTs were recorded on a Horiba Jobin Yvon LabRAM HR800 Raman micro-spectrometer at 633 nm (red laser excitation, He/Ne), equipped with a thermoelectrically cooled CCD. Five spectra were averaged for each sample, after baseline correction, and the D-band was normalized with the G-band intensity of the corresponding spectrum mean.  $I_D/I_G$  peak intensity ratio of DWCNTs was only  $0.24 \pm 0.05$ .

Carboxymethylcellulose (CMC; [9004-32-4], carboxymethylcellulose sodium salt) was supplied by Fluka (Sigma Aldrich). This complex polysaccharide is characterized by an ultra low viscosity (15–50 mPa s), a nominal molecular weight ranging between ca. 15–50 kDa, a degree of polymerization of 60–90, a degree of substitution (DS) of 0.60–0.95 (i.e. 6–9.5 carboxymethyl groups per 10 anhydrous units), and a density of 1.59 g/cm<sup>3</sup>.

## 2.2. Biological material and exposure conditions of amphibian larvae to DWCNTs

*X. laevis* larvae (stage 50 [32]) were exposed to DWCNT suspensions prepared according to the protocol applied to raw MWCNTs and described by Bourdiol et al. [9].

On the one hand, larvae were exposed under static conditions (neither renewing of the media nor feeding of larvae) during 96 h to 10 or 50 mg/L of DWCNTs. The exposure includes a negative control (NC) condition corresponding to a medium only composed of standardized water [33] (distilled tap water to which nutritive salts were added [294 mg/L CaCl<sub>2</sub>, 2H<sub>2</sub>O; 123.25 mg/L MgSO<sub>4</sub>, 7H<sub>2</sub>O; 64.75 mg/L NaHCO<sub>3</sub>; 5.75 mg/l KCl]) and so free from DWCNTs. At the end of this exposure, larvae were frozen (–80 °C, organisms pooled by 10), then freeze dried (Alpha 2-4 10 Plus, Martin Christ; 0.12 mbar), and finally manually ground. These samples are subsequently identified respectively by “Lar10”, “Lar50” and “Lar0”. On the other hand, larvae were exposed during 12 days according to the standard procedures of the MicroNucleus test (MNT, [33]) under semi-static conditions (every 24 h the organisms were removed, then placed in fresh test suspensions, and finally fed) to 0 (NC) or 10 mg/L of DWCNT. At the end of the exposure, their intestines were dissected and pooled by 5 in order to be frozen, then freeze dried and finally manually ground. These samples are subsequently identified respectively by “Int0” and “Int10”.

## 2.3. Microfluidic and microwave-based device

The biosensor, presented in Fig. 1, is composed of a coplanar waveguide (CPW) transmission line and a polydimethylsiloxane (PDMS) microfluidic channel localized on top. This HF circuit was prepared on a quartz substrate. Information about the microfabrication of this device may be found in Grenier et al. [27]. The microfluidic channel is 2 mm in length, 300 μm in width, and 200 μm in thickness, so that the sensing area corresponds to a volume of 0.13 μL. Indeed, it serves as a microchamber in which the suspension to be analyzed is injected, and provides the interaction between the suspen-

sion and the electromagnetic (EM) field that propagates along the CPW line (Fig. 1B). Including the dead volume of the device, the total volume to inject for a single analysis is ranging between 0.5 and 1 μL.

To avoid external contamination, evaporation and change in temperature during the analysis (liquid heating linked to important dielectric losses in the microwave range), the microfluidic channel is closed and the EM/fluidic biosensor is supported by a chuck maintained at 20 °C. The filling, cleaning and refilling of the microfluidic channel are manually performed with a syringe and controlled using a microscope equipped with a CCD camera. Two coplanar microprobes are connected to the HF circuit on both sides of the microfluidic channel (Fig. 1) and to a vector network analyzer (thanks to coaxial cables) in charge of the microwave parameters measurements.

## 2.4. Preparation of biological suspensions

### 2.4.1. Suspensions to be analyzed (with unknown DWCNT concentration)

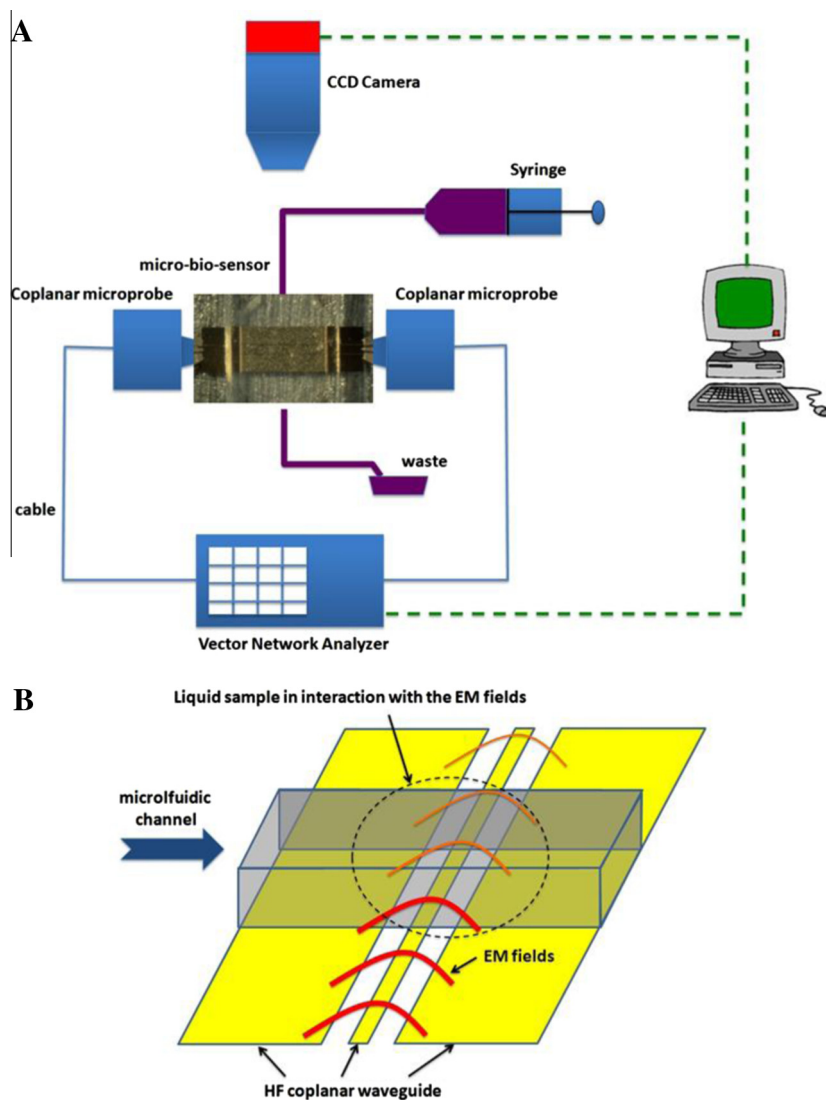
A tip sonicator (Vibra Cell 75042, 20 kHz, 500 W, 20% power with 5 s on/5 s off pulses) was used to prepare the biological suspensions to be analyzed. First of all, dielectric measurements were carried out on “Lar10” and “Int10” suspensions prepared only in deionised water (DiW, 5 mg/mL) by 1 min-ultrasonication. Even if this protocol was applied just before the suspension transfer into the microfluidic channel, it has proven ineffective to avoid the re-agglomeration of the biological matrix and the DWCNTs during the data acquisition. The agglomeration phenomena should disturb the suspensions flow through the microfluidic channel, but also its cleaning. Moreover, the heterogeneous distribution of the suspensions observed over the active front of sensor might be responsible for the important scattering of values obtained for the same suspension.

To improve the stability of suspensions, the next biological samples were dispersed in a CMC solution (“DiW + CMC”) and the ultrasonication time was increased. CMC powder was dissolved in deionised water (10 mg/mL, 60 °C, manual mixing) then “DiW + CMC” solution was dispersed with the ultrasonication tip (15 min), and finally each biological sample was dispersed in the appropriate volume of “DiW + CMC” to prepare 10 mg/mL suspensions.

A reference biological suspension free from DWCNTs, “Xen0” suspension, was prepared by dispersing both “Lar0” and “Int0” samples in the appropriate volume of “DiW + CMC” solution (10 mg/ml).

### 2.4.2. Calibration range of biological suspensions (with known DWCNT concentration)

A calibration range with a nominal DWCNT concentration ranging from 0.06 to 4.00 mg/mL was prepared by adding a known weight of dry DWCNTs to the “Xen0” suspension. This stock suspension was composed of 4.0 mg DWCNTs/mL (15 min of ultrasonication) and identified as “Xen0 + DWCNT4”. The calibration suspensions identified as “Xen0 + DWCNT2”, “Xen0 + DWCNT1”, etc until “Xen0 + DWCNT0.06” were prepared by successive dilutions (dilution factor of 2, keeping a fixed CMC concentration). An ultrasonication step (5 min) was applied prior to each dilution. A calibration model



**Fig. 1 – (A) Schematic view of the microfluidic and microwave-based device and (B) concept of the micro-bio-sensor. CCD: charge coupled device; EM: electromagnetic, HF: high-frequency. The actual volume under examination is 0.13  $\mu$ L. (A color version of this figure can be viewed online.)**

as well as the limit of detection of the presented technique were consequently proposed.

#### 2.4.3. Blind suspensions (with known DWCNT concentration)

Five suspensions composed of “Xen0” suspension (10 mg/mL) and a known concentration of DWCNTs were blind analyzed in order to validate the proposed modeling and the measurement accuracy of the reported DWCNT quantification technique by dielectric measurements in the microwaves frequency range. These suspensions, identified as “Blind1”, “Blind2”, “Blind3”, “Blind 4” and “Blind5”, were prepared by dilution from 4 different stock suspensions. Blind samples were prepared and measured by different people.

#### 2.5. Dielectric measurements

First of all, on-wafer short-open-load-through (SOLT) standards were used for vector-network-analyzer calibration,

which places the reference planes at the contact point of the microwaves probes. Then when microprobes connected to the HF circuit, a measurement was performed when the channel is empty. This step allows suppressing the contribution of both the CPW accesses and the polymer walls of the microfluidic channel, so that analytical calculations could be done to extract the intrinsic dielectric properties of the liquid suspension or the solution filled in the microfluidic channel.

The protocol established to perform the microwave measurements on the calibration suspensions, those with unknown DWCNT concentration and the blind ones is described below:

- (1) Dispersion of the suspension with the ultrasonication tip (1 min). This step is required to optimize the dispersion and the stability of the suspension during the analysis.

- (2) Filling of the microfluidic channel with the suspension to be analyzed.
- (3) Stabilization of the suspension (30 s) and microwave parameters acquisition.
- (4) Repetition of the steps 2 and 3.
- (5) Cleaning of the microfluidic channel with the “DiW + CMC” solution before filling with the next suspension to be analyzed.

Step 4 was repeated 4 times to perform the calculation of the average and the standard deviation of the relative permittivity of each suspension.

## 2.6. Data processing

From microwave parameters, calculations performed as in Grenier et al. [27] led to the extraction of the real and the imaginary part of the relative permittivity of the analyzed suspension vs frequency. The shape of the dielectric response of the suspensions vs the frequency of the EM field applied is shown in Fig. 2. Regardless of the presence of DWCNTs in the biological suspensions, the behavior of the real part and the imaginary part of the permittivity over frequency was characteristic of a liquid-based dielectric relaxation. The maximum value of the real permittivity was obtained for the lowest frequencies that have been studied ( $\sim 1$  GHz). The frequency increase led to a decrease in the real part of the permittivity and simultaneously a rapid rise of the imaginary part (until  $\sim 15$  GHz), followed by a gradual decrease.

Nevertheless, both the real part and the imaginary part of the permittivity of the biological suspension that are composed of DWCNTs (“Lar10”, “Lar50”, “Int10”) were higher than those of the reference suspension “Xen0” whatever the frequency of the applied EM field. Moreover, for a given frequency, the values corresponding to “Lar10” and “Lar50” were similar but higher than those of the reference suspension “Xen0” and lower than those of “Int10”. For these reasons, we have decided to use the dielectric contrast  $\Delta\epsilon_i$  (Eq. (1)) defined as the difference between the mean permittivity of

the  $i$ th biological suspension (“Lar10”, “Lar50” and “Int10” in Fig. 2) and those obtained with the reference suspension “Xen0”.  $\Delta\epsilon'_{Int10}$  and  $\Delta\epsilon''_{Int10}$  are shown respectively in the Fig. 2A and B:

$$\Delta\epsilon_i = \epsilon(\text{Sample})_i - \epsilon(\text{Xen0})_{\text{mean}} \quad (1)$$

with  $\epsilon(\text{Sample})_i$  the real or imaginary part of the permittivity for the analyzed suspension corresponding to the  $i$ th measurement, and  $\epsilon(\text{Xen0})_{\text{mean}}$  the real or imaginary part of the permittivity for the suspension taken as reference (Xen0).

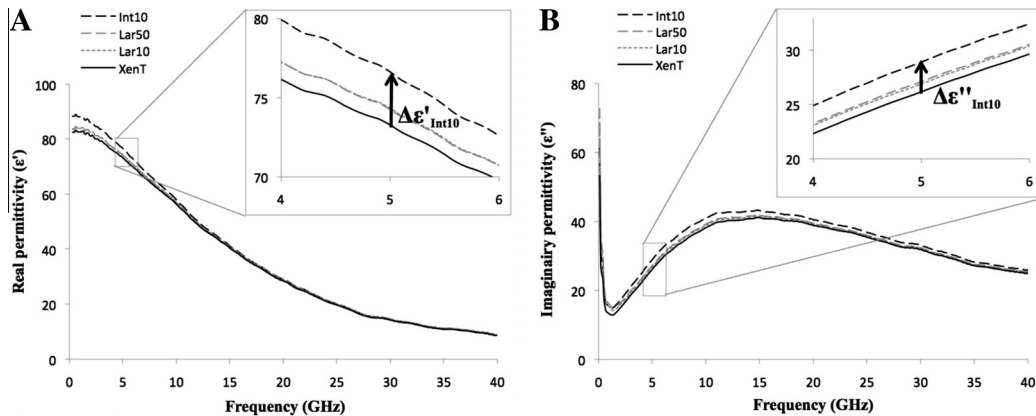
The lower the frequency, the higher the dielectric contrasts. The maximum contrast’s value was reached around 5 GHz, which justifies our choice to focus on the real part of the permittivity at this selected frequency in order to assess the dielectric contrasts  $\Delta\epsilon'_i$  (Eq. (2)):

$$\Delta\epsilon'_i = \epsilon'(\text{Sample})_i - \epsilon'(\text{Xen0})_{\text{mean}} \quad (2)$$

with  $\epsilon'(\text{Sample})_i$  the real part of the permittivity for the suspension analyzed corresponding to the  $i$ th measurement, and  $\epsilon'(\text{Xen0})_{\text{mean}}$  the real part of the permittivity for the suspension taken as reference (Xen0).

## 2.7. Indirect calculation of the DWCNT concentration from the concentration measurement of the DWCNT synthesis by-products (Co and Mo)

The DWCNTs (and more generally the CNTs) consist mainly of carbon, which is also the major chemical element of biological samples. Nevertheless, while the cobalt (Co) and molybdenum (Mo) weight content in DWCNT reaches respectively  $3.00 \pm 0.15$  wt.% and  $0.90 \pm 0.04$  wt.%, these elements are naturally present only in negligible concentrations in *Xenopus* larvae ( $<0.005$  wt.%; cf. 3.3). Assuming that the ratio C:Co:Mo in the DWCNTs is not affected by interaction with larvae, the DWCNT concentration ( $[\text{DWCNT}]_{\text{Sample}}$ ; mg DWCNTs/g dry sample) in “Xen0”, “Lar10”, “Lar50” et “Int10” samples was estimated from the Co or Mo weight content in the sample ( $\text{wt.\%}X_{\text{Sample}}$ ) and the Co or Mo weight content in the DWCNTs ( $\text{wt.\%}X_{\text{DWCNT}}$ ) (Eq. (3)). Then, the as-estimated DWCNT concentrations could be compared to the ones



**Fig. 2 – (A) Real part  $\epsilon'$  and (B) imaginary part  $\epsilon''$  of the permittivity as a function of the EM waves frequency between 40 MHz and 40 GHz. Dielectric measurements of the reference biological suspension “Xen0” and the biological suspensions “Lar10”, “Lar50” and “Int10” with unknown DWCNT concentration. (A)  $\Delta\epsilon'_{Int10}$ : dielectric contrast between the real permittivity of “Xen0” and “Int10”. (B)  $\Delta\epsilon''_{Int10}$ : dielectric contrast between the imaginary permittivity of “Xen0” and “Int10”.**

estimated after the dielectric measurements of these same suspensions:

$$[\text{DWCNT}_{\text{sample}}] = \frac{\text{wt.}\%X_{\text{Sample}}}{\text{wt.}\%X_{\text{DWCNT}}} \times 100 \quad (3)$$

The catalyst residues content in the biological samples was measured by AAS (measurement accuracy  $\approx \pm 10\%$ ), which is the same technique used to determine those in DWCNTs.

### 3. Results

#### 3.1. Calibration curve

According to the processed data obtained at 5 GHz after the microwave analysis of the calibration suspensions “Xen0 + DWCNT”, the contrast of the real permittivity  $\Delta\epsilon'$  was extracted for each DWCNT concentration. Mean values and standard deviations of  $\Delta\epsilon'$  of five replicate measurements performed for each CNTs concentration are presented in Fig. 3 and reveal a linear relationship between  $\Delta\epsilon'$  and the CNTs concentration. A linear regression (performed on all data points, not on mean values (Fig. S1)) allows to define the calibration curve of the technique with a slope of 1.704 mL suspension/mg DWCNTs and a coefficient of determination  $R^2$  of 0.982. Thus, the value of the sensitivity  $S$  (Eq. (4)) could be used to predict the unknown DWCNT concentration in the biological suspensions.

$$S = \frac{\partial\Delta\epsilon'_{5\text{GHz}}}{\partial[\text{DWCNT}]} = 1.704 \text{ mL suspension / mg DWCNTs} \quad (4)$$

with  $S$  the sensitivity (mL suspension/mg DWCNTs) corresponding to the slope of the linear regression of the calibration curve, which describes the trend of the real permittivity at 5 GHz ( $\delta\Delta\epsilon'_{5\text{GHz}}$ ) over the DWCNT concentration range ( $\delta[\text{DWCNT}]$ , mg DWCNTs/mL suspension).

#### 3.2. Detection limit and accuracy of the measurement technique

In order to make an evaluation of the detection limit and the accuracy of our DWCNT quantification technique by HF measurements, we computed the root-mean-square deviation (RMSD) between measured dielectric contrast values and the proposed linear model for each DWCNT concentration. The 95% confidence interval ( $CI_{95\%}$ ) for DWCNT concentration prediction was calculated from these values by Eq. (5):

$$CI_{95\%}(\text{DWCNT conc.}) = \frac{\text{RMSD}(\Delta\epsilon)}{S} \times t \quad (5)$$

where  $CI_{95\%}(\text{DWCNT conc.})$  is the 95% confidence interval for DWCNT concentration prediction,  $\text{RMSD}(\Delta\epsilon)$  the root mean square deviation of the dielectric contrast relatively to the proposed model,  $S$  the calibration curve slope ( $S = 1.704 \text{ mg/mL}$ ) and  $t$  the student's  $t$ -distribution (considering the two sided critical regions) with a degree of freedom of 4 as 5 measurements for each concentration were performed to establish the model (in our case:  $t = 2.7765$ ).

As we expected a limit of detection in the range of tenth of mg/mL, values of 95% confidence interval for DWCNT

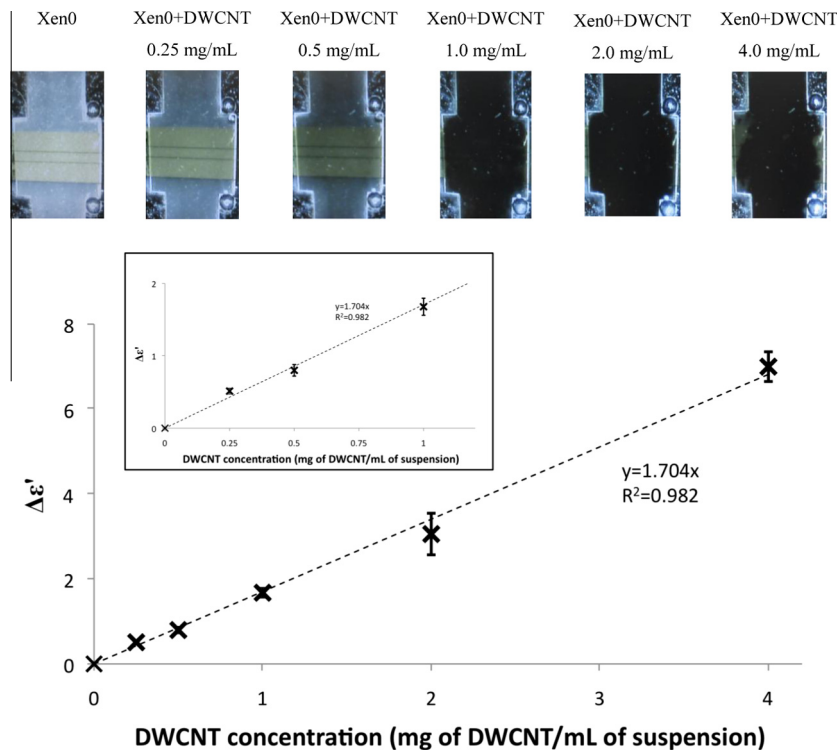


Fig. 3 – Calibration curve generated from the dielectric measurement of “Xen0 + DWCNT” suspensions with known DWCNT concentration. The photographs were taken during their measurements. The insert shows the calibration curve for DWCNT concentration up to 1 mg/mL. The actual volume under examination is 0.13  $\mu\text{L}$ , so the maximum amount of CNT on this graph (4 mg/mL) is only 0.52  $\mu\text{g}$ . (A color version of this figure can be viewed online.)



concentration of 0.25, 0.5 and 1 mg/mL (resp.) were considered to estimate the limit of detection. Eq. (5) gives the  $CI_{95\%}$  values of 0.138, 0.142 and 0.164 mg/mL for the three concentrations respectively. The limit of detection (LoD) of the proposed technique can confidently be set around 0.15 mg/mL (corresponding to ca. 0.02  $\mu$ g of CNTs in the measurement chamber of 0.13  $\mu$ L).

### 3.3. Validation of the proposed calibration model and its accuracy

In order to validate the proposed calibration model and its accuracy of our DWCNT quantification technique by HF measurements, five biological suspensions “Blind1”, “Blind2”, “Blind3”, “Blind4” and “Blind5”, composed of a known DWCNT concentration, were blind analyzed. The predicted DWCNT concentration (mg DWCNTs/mL suspension) was extracted from the mean contrast of the real permittivity ( $\Delta\epsilon'_{\text{moy}}$ ; Table 1) and the sensitivity  $S$  calculated previously. The results are compared to the DWCNT nominal concentrations in Table 1.

The estimated DWCNT concentrations feature a maximum standard deviation of 0.15 mg/mL which corresponds to the previously calculated accuracy of the technique. Moreover, the mean values of the estimated DWCNT concentration for “Blind3”, “Blind4” and “Blind5” differ from their nominal values by only few tenths of  $\mu$ g/mL, which points out the potentialities of performing repetitive measurements to improve the technique accuracy. Nevertheless, the estimated DWCNT concentration for “Blind1” and “Blind2” do not adequately reflect the nominal concentrations, as these two blind concentrations are below the detection limit. Taken together these results validate that the minimum DWCNT concentration detectable ( $=\text{LoD}$ ) is effectively around 0.15 mg/mL of analyzed sample.

### 3.4. Comparison between the DWCNT concentration estimated from dielectric measurements and those obtained from quantitative chemical analysis of catalytic residues (Co and Mo)

DWCNT concentrations in suspensions prepared from “Lar10” and “Lar50”, which correspond to samples of entire larvae exposed respectively to 10 and 50 mg DWCNT/L, but also in suspension prepared from “Int10”, which corresponds to intestines of larvae exposed to 10 mg DWCNTs/L, were determined by dielectric measurements. In order to check the reliability of the results, DWCNT concentrations were in

addition estimated from the residual catalyst content (Co and Mo) in these same samples. All the results are given in Table 2.

First of all, the quantification of Co and Mo content in the reference sample “Xen0” confirmed the hypothesis that these chemical elements are not present, or in negligible amounts, in larvae non-exposed to DWCNTs. Indeed, their concentrations were found to be lower than the analytical detection limit ( $<50$  ppm).

Then, we observed that whatever the technique involved, the DWCNT concentration in “Lar10” was not significantly different from those in “Lar50”, whereas the DWCNT concentration is about 3.5 times higher in “Int10” compared to the two former samples. These results on the one hand indicate that, on the contrary of what was expected, DWCNT concentration of “Lar50” is not significantly higher than those of “Lar10” and on the other hand confirm the dilution effect related to the nature of the sample (intestine vs. entire larvae).

Finally, we observed that the DWCNT concentration predicted in “Lar10”, “Lar50” and “Int10” by dielectric measurements were on average 1.6 and 2.7 times higher than those estimated from the quantification of respectively Co and Mo. Moreover, the Co:Mo ratio in “Lar10”, “Lar50” and “Int10” reached 5.6:1 (mean value) while it was estimated to be 3.3:1 in the DWCNTs and these chemical elements were found in trace levels in the biological matrix. The alteration of this ratio affects the estimation of the DWCNT concentration from the Co or Mo quantification. Indeed, the DWCNT concentrations estimated in “Lar10”, “Lar50” and “Int10” from the Co concentrations were on average 1.7 times higher than those estimated from the Mo concentrations. These observations and their effects on the interpretation of DWCNT concentration determined by dielectric measurements are discussed in the next section.

## 4. Discussion

We show in this work for the first time that the quantitative analysis of CNTs can be performed from the direct use of dielectric parameters, and thus not an indirect effect of the microwaves (temperature increase). This is of course not a completely new concept because we already successfully demonstrated it earlier with the same DWNTs [25], but this is still the first time that this direct approach is demonstrated in such complex environments. The analysis of biological matrices free (“Xen0”) or not (“Lar10”, “Lar50” and “Int10”) from DWCNTs revealed a predictable dielectric frequency

**Table 1 – Results of the blind test analysis. The DWCNT concentrations (mg DWCNT/mL suspension) estimated from the mean dielectric contrasts ( $\Delta\epsilon'_{\text{mean}}$ ) are compared to the nominal DWCNT concentrations.**

	Nominal DWCNT concentration (mg/mL)	$\Delta\epsilon'_{\text{mean}}$ ( $\pm$ standard deviation)	Estimated DWCNT concentration (mean $\pm$ standard deviation; mg/mL)
Blind1	0.06	$-0.014 \pm 0.243$	$-0.01 \pm 0.14$
Blind2	0.13	$0.052 \pm 0.043$	$0.03 \pm 0.03$
Blind3	0.5	$0.802 \pm 0.222$	$0.47 \pm 0.13$
Blind4	0.8	$1.482 \pm 0.262$	$0.87 \pm 0.15$
Blind5	1	$1.677 \pm 0.125$	$0.99 \pm 0.07$

**Table 2 – DWCNT concentrations (mg DWCNT/g dry sample) estimated after the analysis of the biological suspensions (intestine or entire larvae) with unknown DWCNT concentration. The DWCNT concentrations estimated from the mean dielectric contrasts ( $\Delta\epsilon'_{\text{mean}}$ ) are compared to those estimated from the quantitative analysis of metallic by-products (%m  $X_{\text{Ech}}$  with X : Co or Mo,%m). Xen0 is exempted of DWCNT and was used as the reference suspension.**

	Microwave measurements		Chemical analysis	
	$\Delta\epsilon'_{\text{mean}}$ ( $\pm$ standard deviation)	DWCNT concentration (mean $\pm$ standard deviation; mg/g)	%m $X_{\text{Ech}}$ ( $\pm$ standard deviation)	DWCNT concentration (mean $\pm$ standard deviation; mg/g)
Xen0	0.00	0.00	Co:<0.005 Mo:<0.005	0.00 0.00
Lar10	0.794 $\pm$ 0.106	46.49 $\pm$ 6.21	Co: 0.079 $\pm$ 0.008 Mo: 0.014 $\pm$ 0.001	26.38 $\pm$ 3.96 15.64 $\pm$ 2.35
Lar50	0.720 $\pm$ 0.084	42.24 $\pm$ 4.95	Co: 0.087 $\pm$ 0.009 Mo: 0.016 $\pm$ 0.002	29.05 $\pm$ 4.36 17.32 $\pm$ 2.60
Int10	2.590 $\pm$ 0.686	151.99 $\pm$ 40.24	Co: 0.29 $\pm$ 0.029 Mo: 0.049 $\pm$ 0.005	96.83 $\pm$ 14.52 54.75 $\pm$ 8.21

behavior: (i) a drop of the real part of the permittivity over the entire frequency range reveals a relaxation mechanism and (ii) a rise of the imaginary part were simultaneously observed versus frequency varying from 1 to 15 GHz, and were followed by a decrease above 15 GHz. This dielectric relaxation phenomenon could be explained by the presence of the polar water molecules in the suspensions, whose dielectric response is characterized by an important dipolar relaxation in the microwave range [27,34]. Furthermore, concerning the presence of the DWCNTs, their remarkable intrinsic dielectric properties [25,35] participate to the rise of both the real and imaginary parts of the “Lar10”, “Lar50” and “Int10” relative permittivity compared to the reference biological suspension “Xen0”. Dragoman et al. [25] also reported an increase in the effective permittivity up to 65 GHz when the sensing device was filled with DWCNT powder (synthesized in the same conditions than those used in the present work). Some researchers argue that these observed phenomena may originate from the cylindrical walls of rolled graphene composing CNTs. Indeed, their arrangement in concentric layers and their  $\pi$ -conjugated electronic structure should make them effective drivers and resistors [20,36]. More generally, the dielectric properties of CNTs should depend on their intrinsic characteristics, such as the number of walls, their aspect ratio, their chirality (leading to metallic or semi-conductor behavior), their purity (nature and concentration of the catalytic by-products), but also on the EM frequency applied (notably in the HF range) [20,24,26,34–37]. Moreover, further observations indicate that the shifts of the dielectric parameters are more pronounced around 5 GHz and justify why we have limited the data treatment to readouts recorded at 5 GHz. We have also considered only the real part of the permittivity contrasts between each analyzed suspension and the reference suspension.

The analysis of the calibration range of “Xen0 + DWCNT” suspensions (with known DWCNT concentration) highlighted a proportional increase in the real permittivity contrast at 5 GHz with the DWCNT concentration, which is consistent with the modulation of the dielectric signature by the CNT concentration reported by other authors [20,24,34,35]. For example, Decrossas et al. [35] studied the dielectric response of SWCNT and MWCNT powder with various density. On

the one hand, whatever the CNT density of the analyzed sample, both real and imaginary parts of the permittivity decreased rapidly when frequency rises until an asymptotic state. On the other hand, at a given frequency, they observed that real and imaginary parts of the permittivity rise linearly when the CNT density increases. A calibration curve was consequently determined for DWCNT concentrations up to 4 mg/mL. Replicate measurements also permit to define the detection limit of our DWCNT quantification technique. The proposed model and its accuracy were successfully validated with blind biological suspensions with a known DWCNT concentration ranging from 0.06 to 1 mg/mL. Finally the as-obtained model was used to quantify the DWCNT concentration in “Lar10”, “Lar50” and “Int10”. According to the results of the blind analysis, the accuracy as well as the detection limit were estimated around 0.15 mg DWCNTs/mL suspension, that it to say 15  $\mu$ g DWCNTs per mg of dry sample in suspension where the biological matrix concentration was 10 mg/mL. Considering the volume of the microchamber, the amount of detectable DWCNTs in the sensing area reaches only ca. 0.02  $\mu$ g. This very low detection limit makes the microfluidic and dielectric-based CNT-quantification technique described in this paper highly sensitive and efficient to detect traces of DWCNTs in *Xenopus* larvae suspensions and to estimate accurately the DWCNT concentration. For comparison, Irin et al. [38] developed a technique for quantitative detection of SWCNTs in alfalfa (*Medicago sativa*) roots by utilizing the thermal response of CNTs under microwave irradiation that was limited to the detection of SWCNT amounts of only 0.1  $\mu$ g. Improvement of detectable CNTs featured by our proposed microfluidic-based setup compared to [38] mainly originates from its scaling down of the analyzed volume around hundreds nanoliters.

From the HF-based quantitative technique, the DWCNTs concentration measured in the samples of whole dry larvae exposed to 10 mg/L and 50 mg/L reached respectively 46.5  $\pm$  6.2 mg/g and 42.2  $\pm$  4.9 mg/g. These results did not reveal any significant difference in the DWCNT concentration in spite of the fact that larvae were exposed, during the same duration (96 h), to two different DWCNT concentrations. Petersen et al. carried out 48 h-exposures with daphnia to two  $^{14}\text{C}$ -MWCNT concentration ranges (0.04–0.10–0.40 mg/L [4]

and 0.025–0.20 mg/L [12]). They reported an increase in the MWCNT concentration vs. time in these organisms, but after 24 h of exposure the CNT concentration remained stable until the end of the test. In the particular case of the daphnia exposed to the highest MWCNT concentration, 10 h were enough to reach a steady state. In this work, it is possible that the maximum DWCNT concentration in *Xenopus* larvae has been reached before the end of the 96 h-exposure, from an exposure concentration of 10 mg/L. This would explain that the DWCNT concentration measured in “Lar50” and “Lar10” was not significantly different. Besides, the ingestion of DWCNTs by larvae exposed during 12 days to 10 mg/L led to an accumulation of 152.0 mg DWCNTs/g dry intestine. Thus, this study revealed that the DWCNT concentration in “Int10” was 3.5 times higher than in “Lar10”, for the same exposure concentration of 10 mg/L, and as we suspected from visual inspections it confirmed that CNTs are concentrated in the gut of the larvae.

The high exposure concentrations used here (10 mg/L, 50 mg/L) are obviously much higher than what could be expected in the environment but still correspond to the actual concentration range at which effects are observed in laboratory exposure conditions [1]. In this work, the larvae were not placed in CNT-free medium at the end of the exposure protocol, so their intestine was still full of CNTs. The method described here would be of great interest in order to monitor the time required to fully clean the intestine. As the weight of the fresh larvae was not measured before freeze drying, it was unfortunately not possible to calculate the actual concentration of CNTs accumulated in the organisms. This work is in progress.

The direct quantitative analysis of CNTs in carbon-containing matrices, such as biological matrices, is especially challenging as the classical chemical analysis cannot be used. The indirect determination from the quantitative analysis of residual metals is an interesting alternative, which has not been used so much so far [41–43]. The results obtained from dielectric measurements were compared to the results obtained from the Co and Mo content remaining in the same samples. Whatever the measurement technique, the averaged DWCNT concentration estimated in “Lar10” and “Lar50” was not significantly different, and both of them were significantly lower than the concentration measured in “Int10”. Nevertheless we observed that the DWCNT concentration estimated in the same sample from residual Co was 1.7 times higher than the concentration estimated from residual Mo, while this approach was supposed to lead to similar results. Indeed, considering the fact that these elements exist only in trace amounts in larvae non-exposed to CNTs, the Co:Mo ratio measured in the samples of larvae exposed to DWCNTs should be identical to that of DWCNTs alone. However, it increased from 3.3 in the starting DWCNT sample to 5.6 (mean value of all the biological samples of larvae exposed to DWCNTs). Several hypotheses can be proposed to explain this observation: an incomplete mineralization of Mo or both Mo and Co and/or a possible selective dissolution of Mo in the exposure media (even though it is a mild biological environment). The latter hypothesis is still under investigation. This specific loss of Mo is not yet explained, and was not expected as Co is present in the metallic state (carbon-encapsulated

Co(0) nanoparticles) while Mo is present as Mo carbide [39], none being expected to be soluble in a biological matrix. This selective dissolution of one of the catalytic metals reveals that the direct extrapolation from the initial metal content in the starting material may not be always relevant and possibly do not constitute an absolute standard method to quantify CNTs in carbon containing matrices. Similar observations were made very recently by Schierz et al. [43] in the case of single-wall CNTs also prepared by CCVD using a Co:Mo catalyst, and where rather different Co:Mo ratios were measured in different environmental compartments. However, there is a very good correlation between dielectric measurements and elemental analysis, which show exactly the same proportionality (elemental analysis roughly corresponds to 62% (mean value) of the concentration obtained by the dielectric analysis) for the 2 methods and our results finally aim to show that the direct quantification of CNTs from catalyst residues (at least for our DWNTs with only ca. 4 wt.% of residual metals) is possible but not as straightforward as we expected, and requires some control experiments (while the microwave measurements do not).

On the contrary, the HF technique demonstrates a rather good sensitivity and accuracy, and could be used in various complex environments ranging from biological samples to contaminated soils or water. This method is thus very important for the quantitative assessment of the presence of CNTs in a real environmental matrix. We have shown earlier that similar results could be obtained in a different matrix (polymer) and that the electrical permittivity signature of CNTs could be used to get information about the amount of CNTs [25]. We confirm here that this approach seems to be applicable to various matrices, as long as they do not interfere with the response of the CNTs in the investigated HF range. Doudrick et al. [18] mentioned that when thermogravimetric methods are used, the ratio of defects in the CNTs (quantified using the Raman intensity ratio between the D and G bands) can lead to different behaviors. Indeed, it is obvious that as the number of structural defects increases (which is what the increase in  $I_D/I_G$  ratio shows), the oxidation temperature will decrease. There are many parameters likely to influence the dielectric response of CNTs, including the number of walls, the aspect ratio, the chirality, and the presence of impurities. However, we do not expect any significant relationship between the  $I_D/I_G$  ratio and the dielectric permittivity, because this is not related to any of the parameters cited earlier. It is also important to add that, although very interesting, the quantification of CNTs in complex matrices using thermogravimetric analysis (TGA) requires to take a few precautions: simple TGA measurement is not enough, and a preliminary investigation by Raman spectroscopy must be done in order to determine whether the CNTs under investigation have to be considered as “strong” or “weak” towards oxidation [18]. Also, digestion techniques must be used to degrade organic matter to prevent formation of pyrolytically generated elemental carbon, making the actual use of this method rather difficult [38]. This suggests that our approach may be more general. It is also worth mentioning that agglomeration of CNTs should not be an issue in this work. The addition of surfactant in this work was only intended to make easier the loading and circulation of fluids in the device.

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## 5. Conclusion

The purpose of this study was the development of a DWCNT quantification technique in *Xenopus* larvae after exposure. We have shown that the concentration of CNTs can be quantitatively assessed in complex biological samples and that the results are consistent with classical chemical analysis. The detection limit was estimated around 0.15 mg DWCNTs/mL suspension (the amount of detectable DWCNTs in the sensing area is only 0.02 µg), that is to say 15 µg DWCNTs/mg dry sample in suspension, which is to the best of our knowledge the highest sensitivity reported to date. We have demonstrated that in our experimental exposure conditions, the amount of CNTs accumulated in the intestine of *Xenopus* larvae reaches a plateau whatever the concentration in the exposure medium. This is an important information especially to highlight the fact that exposure at low concentration for a long period may lead in the end to similar CNTs internal bioaccumulation level than in shorter exposure at higher concentration.

Subsequently, it would be interesting to study, thanks to our HF measurement technique, the DWCNT ingestion and excretion kinetics by *Xenopus* larvae, but also the depuration ("full" excretion in DWCNT-free media), by varying parameters such as the DWCNT concentration and their initial dispersion state (with or without surfactant for example). Focusing directly on the analysis of the intestines, which is an organ that could easily be isolated and sampled on the *Xenopus* larvae, should enhance the sensitivity especially in very low exposure conditions, due to the large amount of CNTs present in this organ. Finally, it is expected that this measurement technique could be transposed to other CNTs, such as MWCNTs, but also other biologic models and specific organs, such as the liver, lungs, kidneys and spleen, which are known to be target, transit or accumulating organs for most contaminants.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.carbon.2014.09.086>.

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## REFERENCES

- [1] Mouchet F, Landois P, Puech P, Pinelli E, Flahaut E, Gauthier L. Carbon nanotube ecotoxicity in amphibians: assessment of multiwalled carbon nanotubes and comparison with double-walled carbon nanotubes. *Nanomedicine* 2010;5:963–74.
- [2] Kennedy AJ, Hull MS, Steevens JA, Dontsova KM, Chappell MA, Gunter JC, et al. Factors influencing the partitioning and toxicity of nanotubes in the aquatic environment. *Environ Toxicol Chem* 2008;27:1932–41.
- [3] Zhu X, Zhu L, Chen Y, Tian S. Acute toxicities of six manufactured nanomaterial suspensions to *Daphnia magna*. *J Nanopart Res* 2009;11:67–75.
- [4] Petersen EJ, Akkanen J, Kukkonen JVK, Weber WJ. Biological uptake and depuration of carbon nanotubes by *Daphnia magna*. *Environ Sci Technol* 2009;43:2969–75.
- [5] Li M, Huang CP. The responses of *Ceriodaphnia dubia* toward multi-walled carbon nanotubes: effect of physical-chemical treatment. *Carbon* 2011;49:1672–9.
- [6] Templeton RC, Ferguson PL, Washburn KM, Scrivens WA, Chandler GT. Life-cycle effects of single-walled carbon nanotubes (SWNTs) on an estuarine meiobenthic copepod. *Environ Sci Technol* 2006;40:7387–93.
- [7] Smith CJ, Shaw BJ, Handy RD. Toxicity of single walled carbon nanotubes to rainbow trout, (*Oncorhynchus mykiss*): respiratory toxicity, organ pathologies, and other physiological effects. *Aquat Toxicol* 2007;82:94–109.
- [8] Mouchet F, Landois P, Datsyuk V, Puech P, Pinelli E, Flahaut E, et al. International amphibian micronucleus standardized procedure (ISO 21427-1) for in vivo evaluation of double-walled carbon nanotubes toxicity and genotoxicity in water. *Environ Toxicol* 2009;26:136–45.
- [9] Bourdiol F, Mouchet F, Perrault A, Fourquaux I, Datas L, Gancet C, et al. Biocompatible polymer-assisted dispersion of multi walled carbon nanotubes in water, application to the investigation of their ecotoxicity using *Xenopus laevis* amphibian larvae. *Carbon* 2013;54:175–91.
- [10] Edgington AJ, Roberts AP, Taylor LM, Alloy MM, Reppert J, Rao AM, et al. The influence of natural organic matter on the toxicity of multiwalled carbon nanotubes. *Environ Toxicol Chem* 2010;29:2511–8.
- [11] Roberts AP, Mount AS, Seda B, Souther J, Qiao R, Lin S, et al. In vivo biomodification of lipid-coated carbon nanotubes by *Daphnia magna*. *Environ Sci Technol* 2007;41:3025–9.
- [12] Petersen EJ, Pinto RA, Mai DJ, Landrum PF, Weber WJ. Influence of polyethyleneimine graftings of multi-walled carbon nanotubes on their accumulation and elimination by and toxicity to *Daphnia magna*. *Environ Sci Technol* 2011;45:1133–8.
- [13] Larue C, Pinault M, Czarny B, Georgin D, Jaillard D, Bendjab N, et al. Quantitative evaluation of multi-walled carbon nanotube uptake in wheat and rapeseed. *J Hazard Mater* 2012;227–228:155–63.
- [14] Yang S, Guo W, Lin Y, Deng X, Wang H, Sun H, et al. Biodistribution of pristine single-walled carbon nanotubes in vivo. *J Phys Chem C* 2007;111:17761–4.
- [15] Xiang R, Hou B, Einarsson E, Zhao P, Harish S, Morimoto K, et al. Carbon atoms in ethanol do not contribute equally to formation of single-walled carbon nanotubes. *ACS Nano* 2013;7:3095–103.
- [16] Yang M, Kwon S, Kostov Y, Rasooly A, Rao G, Ghosh U. Study of the biouptake of labeled single-walled carbon nanotubes using fluorescence-based method. *Environ Chem Lett* 2011;9:235–41.
- [17] Bisesi JH, Merten J, Liu K, Parks AN, Afroz ARMN, Glenn JB, et al. Tracking and quantification of single-walled carbon nanotubes in fish using near infrared fluorescence. *Environ Sci Technol* 2014;48:1973–83.
- [18] (a) Plata DL, Reddy CM, Gschwend PM. Thermogravimetry-mass spectrometry for Carbon Nanotube Detection in Complex Mixtures. *Environ Sci Technol* 2012;46:12254–61. (b) Doudrick K, Herckes P, Westerhoff P. Detection of carbon

- nanotubes in environmental matrices using programmed thermal analysis. *Environ Sci Technol* 2012;46:12246–53.
- [19] Makeiff DA, Huber T, Saville P. Complex permittivity of polyaniline-carbon nanotube and nanofibre composites in the X-band. *PMMA Compos* 2005.
- [20] Wu J, Kong L. High microwave permittivity of multiwalled carbon nanotube composites. *Appl Phys Lett* 2004;84:4956–8.
- [21] Zhao D-L, Li X, Shen Z-M. Electromagnetic and microwave absorbing properties of multi-walled carbon nanotubes filled with Ag nanowires. *Mater Sci Eng B* 2008;150:105–10.
- [22] Che RC, Peng L-M, Duan XF, Chen Q, Liang XL. Microwave absorption enhancement and complex permittivity and permeability of Fe encapsulated within carbon nanotubes. *Adv Mater* 2004;16:401–5.
- [23] Zhao D-L, Li X, Shen Z-M. Preparation and electromagnetic and microwave absorbing properties of Fe-filled carbon nanotubes. *J Alloys Compd* 2009;471:457–60.
- [24] Qin F, Brosseau C. A review and analysis of microwave absorption in polymer composites filled with carbonaceous particles. *J Appl Phys* 2012;111. 061301:1–25.
- [25] Dragoman M, Grenier K, Dubuc D, Bary L, Fourn E, Plana R, et al. Experimental determination of microwave attenuation and electrical permittivity of double-walled carbon nanotubes. *Appl Phys Lett* 2006;88. 153108:1–3.
- [26] Han M, Deng L. High frequency properties of carbon nanotubes and their electromagnetic wave absorption properties. In: Marulanda JM, editor. *Carbon Nanotube Application of Electron Devices*. InTech; 2011.
- [27] Grenier K, Dubuc D, Poleni P-E, Kumemura M, Toshiyoshi H, Fujii T, et al. Integrated broadband microwave and microfluidic sensor dedicated to bioengineering. *IEEE Trans Microw Theory Tech* 2009;57:3246–53.
- [28] Grenier K, Dubuc D, Poleni P-E, Kumemura M, Toshiyoshi H, Fujii T, et al. Resonant based microwave biosensor for biological cells discrimination. 2010 IEEE Radio Wirel Symp RWS 2010:523–6.
- [29] Chen T, Dubuc D, Poupot M, Fournie J, Grenier K. Accurate nanoliter liquid characterization up to 40 GHz for biomedical applications: toward non-invasive living cells monitoring. *IEEE Trans Microw Theory Tech* 2012;60:4171–7.
- [30] Flahaut E, Bacsa R, Peigney A, Laurent C. Gram-scale CCVD synthesis of double-walled carbon nanotubes. *Chem Commun* 2003:1442–3.
- [31] Landois P. Synthèse, fonctionnalisation et impact sur l'environnement de nanotubes de carbone. Toulouse III: Université Paul Sabatier; 2008 (Thèse de doctorat).
- [32] Nieuwkoop PD, Faber J. *Normal tables of Xenopus laevis (Daudin)*. Amsterdam: North-Holland Publishers; 1956.
- [33] ISO International Standard. Water quality – evaluation of genotoxicity by measurement of the induction of micronuclei – Part 1: evaluation of genotoxicity using amphibian larvae. ISO 21427–1, ICS: 13.060.70, 2006.
- [34] Decrossas E, El Sabbagh MA, Hanna VF, El-Ghazaly SM. Broadband characterization of carbon nanotube networks. *Symp Electromagn Compat EMC* 2010;2010:208–11.
- [35] Decrossas E, El Sabbagh MA, Hanna VF, El-Ghazaly SM. Rigorous characterization of carbon nanotube complex permittivity over a broadband of RF frequencies. *IEEE Trans Electromagn Compat* 2012;54:81–7.
- [36] Basu R, Iannacchione GS. Dielectric response of multiwalled carbon nanotubes as a function of applied AC-electric fields. *J Appl Phys* 2008;104. 114107:1–4.
- [37] Xiao T, Yang HL, Zhang GP. The influence of carbon nanotube structure on complex permittivity and determination of filler density by microwave techniques. *J Appl Phys* 2011;110. 024902:1–5.
- [38] Irin F, Shrestha B, Cañas JE, Saed MA, Green MJ. Detection of carbon nanotubes in biological samples through microwave-induced heating. *Carbon* 2012;50:4441–9.
- [39] Flahaut E, Peigney A, Bacsa WS, Bacsa RR, Laurent Ch. CCVD synthesis of carbon nanotubes from (Mg Co, Mo)O catalysts: influence of the proportions of cobalt and molybdenum. *J Mater Chem* 2004;14:646–53.
- [40] Ayouni-Derouiche L, Gauthier L, Méjean M, Gay P, Milliand ML, Lantéri P, et al. Development of efficient digestion procedures for quantitative determination of Cobalt and Molybdenum catalyst residues in carbon nanotubes. *Carbon* 2014;80:59–67.
- [41] Plata DL, Gschwend PM, Reddy CM. Industrially synthesized single-walled carbon nanotubes: compositional data for users, environmental risk assessments, and source apportionment. *Nanotechnology* 2008;19:185706.
- [42] Reed RB, Goodwin DG, Marsh KL, Capracotta SS, Higgins CP, Fairbrother DH, et al. Detection of single walled carbon nanotubes by monitoring embedded metals. *Environ Sci: Processes Impacts* 2013;15:204–13.
- [43] Schierz A, Espinasse B, Wiesner MR, Bisesi JH, Sabo-Attwood T, Ferguson PL. Fate of single walled carbon nanotubes in wetland ecosystems. *Environ Sci: Nano* 2014. Advance article, DOI: 10.1039/C4EN00063C.