



## Dioxin analysis in water by using a passive sampler and CALUX bioassay

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### ABSTRACT

Passive sampling of organic pollutants is a new trend in environmental monitoring and analysis. Passive samplers are being developed to overcome the drawbacks of the conventional snapshot sampling approach. The ceramic toximeter is a promising passive sampler for monitoring dioxin-contaminated surface and ground waters. It consists of an alumina cylinder lined with a thin coating of titania and a pore diameter of 0.05  $\mu\text{m}$ . The cylinder serves as a diffusion barrier limiting the analyte transport to molecular diffusion only, as well as a container for a selective trapping material of a high capacity and affinity towards the chemical(s) of concern. The cylinder is closed from both sides with PTFE caps. The ceramic toximeter was filled with activated carbon as the trapping material and has been tested in vitro for the sampling of dioxin-contaminated water. In addition, the utilization of the CALUX bioassay technique for analyzing the trapped dioxin has greatly reduced the time and costs for dioxin scanning in aqueous media. Exposure times varied between 1 and 7 days in a solution of 1.35 ng-TCDD L<sup>-1</sup> (TCDD is 2,3,7,8-tetrachlorodibenzodioxin). The mean effective molecular diffusion coefficient of TCDD in the toximeter amounts to  $11.9 \times 10^{-6} \text{ m}^2 \text{ d}^{-1}$  while the minimum concentration detectable in an aquatic system after 30 days of exposure amounts to 0.89 pg-TCDD L<sup>-1</sup>.

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### 1. Introduction

Sampling is the most important step in the monitoring of aquatic environments because it influences all following steps involved in a water pollution assessment process. The two major drawbacks of conventional snapshot sampling are the contamination risks and the sample's representativeness. The sampling vessel, devices, and storage containers are all potential sources of loss or contamination of the real analyte's concentration in the aquatic system. Since some pollutants are present only at trace levels and cannot be measured directly, the sample has to be treated and mostly pre-concentration techniques have to be applied in the laboratory. The representativeness of the samples forms a second problem when studying trace element distributions in large water masses or in fast flowing aquatic systems.

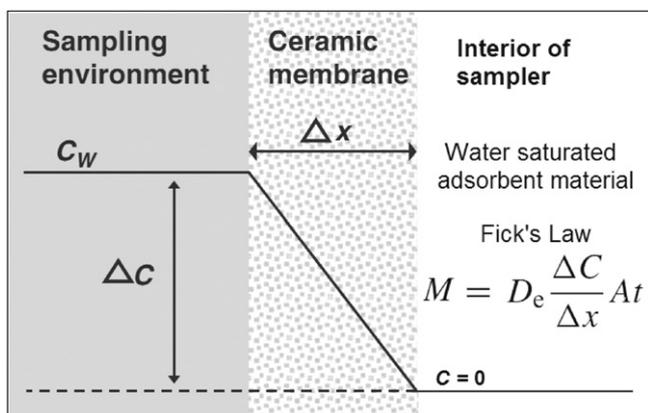
The most common sampling procedure involves the collection of an aliquot by a discrete grab or by a pumping system. Such a sample will only provide a momentary, snapshot concentration of the contaminants' level at the time and place of sampling. This means that intermediate episodic pollution events or spatial variability might be overlooked. An excellent alternative to avoid most of the

limitations related to conventional, snapshot sampling is the use of a passive sampler with or without the pre-concentration of the analyte. In "passive sampling", collecting the pollutants proceeds without the need for any power sources other than the difference in chemical potential [1]. The chemical potential difference of a substance in two media causes the analyte molecules to flow from the sampled medium to be trapped by a suitable material, known as the receiving phase, within the sampling device. This flow of analytes into the sampling device continues until equilibrium is established in the system (e.g. the technique of diffusive equilibrium in thin films or DET), or until the desired sampling period is finished (e.g. the technique of diffusive gradient in thin films or DGT). The receiving phase can be a liquid (dissolution) or a solid adsorbent (chemisorption) [2]. An important condition is that the receiving medium must act as a perfect sink, which means that it should release the trapped molecules even if the concentration of the analyte around the sampler decreases to zero. A variety of passive samplers have been developed for environmental sampling purposes. The ceramic toximeter (or dosimeter) is one of those promising passive samplers for organic pollutants comparable to the DGT technique used for inorganic compounds [3].

The DGT, a purely diffusion-controlled device, is based on a flat hydrogel layer (e.g. polyacrylamide or agarose) in the case of inorganic compounds, or a porous ceramic membrane in the shape of a cylinder in the case of hydrophobic organic compounds. In this

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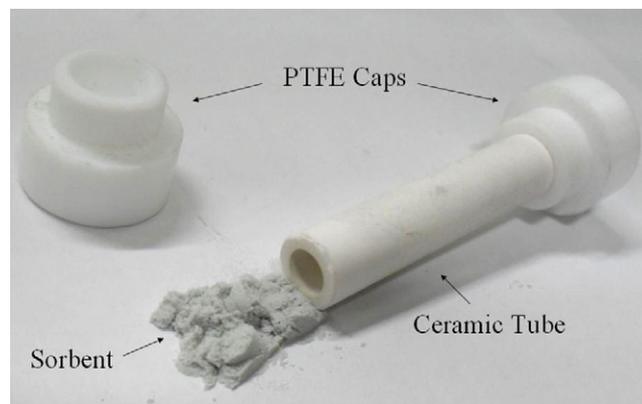


**Fig. 1.** Concentration gradient across the ceramic membrane.  $M$ , mass of chemicals adsorbed on the receiving material;  $D_e$ , effective diffusion coefficient of the chemicals in porous membrane;  $\Delta C$ , difference in concentration inside ( $C=0$ ) and outside the toximeter ( $C$ );  $\Delta x$ , thickness of the diffusion membrane;  $A$ , area of the exposed ceramic surface;  $t$ , exposure time (adapted after Weiß et al. [6]).

paper we will only discuss the latter device and compounds. The ceramic cylinder functions as a diffusion barrier as well as a container for the sorbent material. The latter can be selected depending on the compounds of interest and the time scale planned for monitoring. A suitable sorbent should have a high affinity and capacity for the uptake of the hydrophobic chemical(s) of concern, combined with an easy extraction and high recovery rates. As long as such sorbents are validated, the ceramic toximeter might suit many sampling and analytical needs. The diffusive transport of chemicals across the ceramic membrane at steady state is represented in Fig. 1 and can be described by Fick's first law of diffusion. The accumulated mass ( $M$ ) of a chemical at the end of an exposure period ( $t$ ) can be used to calculate the time-weighted average concentration ( $C_w$ ) at which this chemical was present over the entire sampling time [4,5]. In this case, calibration and frequent snapshots are not necessary [6].

Polychlorinated dibenzo- $p$ -dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) are man-made persistent compounds with a high potential for accumulating in biological tissues. They have been found in all compartments of the ecosystem, including water, although their solubility in this medium is very low [7]. However, small quantities of dioxins in the diet can cause adverse health effects. Research on animals showed that only a few ppt's can have harmful effects [8]. The most studied and most toxic congener is 2,3,7,8-tetrachlorodibenzo- $p$ -dioxin (TCDD). It has been classified as carcinogenic to humans [9] and as an endocrine disrupting agent [10]. The toxicity of different dioxins is expressed on a common basis by comparing the toxicity of the congeners to that of TCDD [11]. Traditionally, high resolution gas chromatography/mass spectrometry (HRGC/HRMS) is being used to detect the presence and concentration of dioxins. This is a complex, expensive, and time consuming method, especially for routine monitoring or large-scale scanning purposes. Alternatively, the CALUX bioassay is a rapid, less expensive, and more easily performed method of estimating toxic equivalency (TEQ). The CALUX (Chemically Activated Luciferase gene eXpression) assay is a reporter gene mammalian cell bioassay. The genetically modified cells used in the CALUX bioassay contain a stably transfected AhR-responsive firefly luciferase reporter gene, which responds to dioxin-like chemicals by the induction of luciferase in a time-, dose-, and AhR-dependent manner [12].

The aim of this research is to develop and test the ceramic toximeter as a passive sampler in combination with the CALUX bioassay for monitoring dioxin-polluted water.



**Fig. 2.** A disassembled ceramic toximeter.

## 2. Materials and methods

### 2.1. Reagents

2,3,7,8-Tetrachlorodibenzo- $p$ -dioxin (TCDD) was obtained from Campro Scientific (The Netherlands). Hexane (for dioxins and PCBs, minimum 96%), acetone (Pesti-S grade, minimum 99.9%) and toluene (for dioxins and PCBs, minimum 99.8%) were purchased from Biosolve (The Netherlands). The X-CARB was from Xenobiotic Detection Systems (USA). Dimethyl sulfoxide (DMSO) was from Merck (Germany). The mouse hepatoma H1L7.5c1 cell line used in CALUX bioassay was supplied by UC-Davis (CA, USA).

### 2.2. The ceramic passive sampler

The sampler consists of a ceramic cylinder of alumina ( $\alpha$ - $\text{Al}_2\text{O}_3$ ) internally lined with a  $5\ \mu\text{m}$  layer of titania ( $\text{TiO}_2$ ) from ATECH Innovations (Germany). The cylinder is 5 cm length, 2 mm wall thickness, 1 cm external diameter, and 30% porosity. The mean pore diameter of the alumina support is  $0.4\ \mu\text{m}$  and that of the internal titania coating is  $0.05\ \mu\text{m}$ , serving as a diffusion barrier controlling the flux of solute to molecular diffusion only. About 0.62 g of conditioned X-CARB was packed in each cylinder and small plugs of pre-cleaned glass-wool were inserted in both ends. Two caps of PTFE tightly close both ends of each cylinder (Fig. 2). The toximeters were immersed in deionized Milli-Q water under vacuum to remove the air bubbles and to ensure a full saturation of the toximeters with water.

### 2.3. Preparation of the saturated TCDD solution

A modified design of the generator column has been used to synthesize a saturated solution of the hydrophobic 2,3,7,8-tetrachlorodibenzo- $p$ -dioxin (TCDD). Using the rotatory evaporator (Rotavap), 100 g of glass-beads of 1.5 mm radius has been coated by  $60\ \mu\text{g}$  of TCDD to work as a substrate. The column consists of an external water jacket to maintain the temperature as constant as possible. The water jacket surrounds an internal helical glass column of 2 cm radius and 150 cm length. The spiral column has two controlling taps on both upper and lower openings for precise control of the flow rate of water injection. The flow rate was kept low to allow maximum contact between the water flow and the coated substrate [13]. The design of the water reservoir was made to be higher than the column assembly in order to maintain a stable water flow. After packing the TCDD-coated beads to the helix, the deionized Milli-Q water was injected into the lower opening of the spiral column at  $0.5\ \text{mL min}^{-1}$  [14]. The generated solution was collected through the upper terminal of the helix into glass bottles

for quantitative analysis by the CALUX bioassay. The concentration of this stock solution was being checked prior to each use.

#### 2.4. Clean up and conditioning of materials

The new ceramic cylinders were rinsed with tap water to remove the superficial traces, immersed in acetone, toluene, and hexane respectively to dissolve any possible organics, and rinsed with Milli-Q deionized water then dried before sintering. In a porcelain crucible, the cylinders were sintered at 850 °C for 7 h. The same clean-up procedures (except tap water rinsing) were applied between every two consecutive application cycles of the cylinders. The glassware was baked for 4 h at 450 °C to avoid any possible contamination of cell cultures by organic materials during the CALUX analysis. Silicon liners were used to avoid the direct contact between the solutions and plastic caps of vials. Both X-CARB and glass-wool were conditioned by fluxing in toluene on a shaker for 3 days at room temperature with a daily replacement of toluene by fresh amounts [15]. At the end of the conditioning period, toluene was discarded and the residues were dried in a heated vacuumed centrifuge.

#### 2.5. Testing of the toximeters

An aqueous TCDD solution of 0.00135 ng mL<sup>-1</sup> was prepared by diluting the generated stock TCDD solution by a factor of 20. Four dosimeters were immersed in 500 mL TCDD solution in a number of light-shielded glass bottles. The bottles were put on a shaker throughout the exposure periods in order to keep the solution homogeneous. The ceramic dosimeters were extracted at the time periods: 24, 48, 72, 96, 120 and 168 h to investigate the uptake of the TCDD by the sorbent with time. All experiments were carried out in duplicate or triplicate to investigate the repeatability of the results and it was also one of our interests to investigate the re-usability of the ceramic dosimeters.

#### 2.6. Extraction of the dosimeters

Each dosimeter taken out from the TCDD solution was rinsed by *n*-hexane and left to dry before extraction. The PTFE caps were carefully removed, and a metal rod was used to eject most of the X-CARB out of the dosimeter into a glass vial. The dosimeter with the remaining X-CARB sticking to its wall was also put into the glass vial. Subsequently, 25, 10, 10 and 5 mL of toluene were used to extract all X-CARB (both the ejected and wall-attached ones). The vials containing disassembled dosimeters were ultrasonicated for 15 min each time fresh toluene was added. Enough time was allowed for the suspension to settle down. The toluene extracts were pipetted into a new vial through a filter of pre-cleaned glass wool to eliminate the suspended sorbent particles, if present. When the extraction was finished, toluene was vaporized in a heated vacuumed centrifuge, and the extracted TCDD was dissolved in 5 mL of *n*-hexane. Different dilutions of TCDD/hexane solution were made to be dosed to CALUX 96-well plates for quantitative analysis.

#### 2.7. CALUX bioassay

The CALUX bioassay was performed using the sensitive mouse hepatoma H1L7.5c1 cell line [16,17]. The protocol used was previously described by Croes et al. [18] relying on the XDS method 4435 [19]. The cells were grown in cell-culture disks containing 15 mL RPMI 1640 medium, supplemented with 8% fetal calf serum (FCS) and 1% penicillin/streptomycin (Gibco, UK). The CALUX 96-well plates were prepared at a cell concentration between 55 and 65 × 10<sup>4</sup> cells mL<sup>-1</sup> and 200 μL of cell suspension was added to each well. After 24 h incubation at 37 °C with 5% CO<sub>2</sub> and 80% humidity,

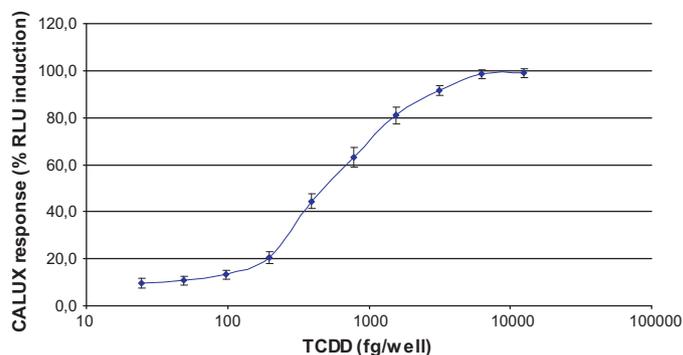


Fig. 3. A standard TCDD calibration curve.

the medium was removed. 188 μL of a standard solution or sample extract in RPMI with 1% (v/v) DMSO content were added to each well of the plate. After 20–24 h incubation, the medium was removed and the wells were rinsed with 75 μL PBS and visually inspected under the microscope for any significant defects in the cell cultures. 50 μL lysis reagent (Promega, USA) was added and the plate was shaken for 5 min. After a 10 min incubation period in the luminometer (Glomax, Promega, USA), the machine started to inject 50 μL luciferine reagent (Promega, USA) into each well (integration time 3 s, lag time 5.6 s). The measured luminescence was expressed in relative light units (RLU) which were later converted into biological equivalency value (CALUX-BEQ) by comparing the response of a given sample to a dose-response curve obtained from 2,3,7,8-TCDD standards [20,21].

#### 2.8. Statistical methods

CALUX dose–response curves for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) are typically smooth and sigmoid when the dose is plotted on a logarithmic scale (see Fig. 3). This sigmoid curve is usually fitted with a 4-parameter Hill function according to Eq. (1):

$$y_i = y_0 + \frac{m \times x_i^h}{k^h + x_i^h} + \varepsilon_i \quad (1)$$

where  $y_i$  is the % RLU induction (100 × RLU signal of the standard divided by maximum RLU signal),  $y_0$  is the intercept parameter,  $x_i$  is the TCDD concentration,  $\varepsilon_i$  is the residual term,  $m$  is the limiting value of the RLU response as TCDD concentration increases,  $k$  is the dose responding to 50% of the maximum dose response, and  $h$  is the parameter that determines sigmoid shape of the curve. A Weighted Least Squares (WLSs) technique was applied for parameter optimization as described by Elskens et al. [22].

### 3. Results and discussion

#### 3.1. Concentration of the generated TCDD solution

The saturation levels of the highly hydrophobic compound TCDD in the aqueous medium has always been a matter of divergence [23]. In addition, it is strongly dependent on temperature [13]. According to the literature, the aqueous solubility of TCDD at 25 °C is ranging between 7.91 × 10<sup>-6</sup> mg L<sup>-1</sup> [24] and 1.58 × 10<sup>-2</sup> mg L<sup>-1</sup> [25]. In our experiments, the generated TCDD aqueous solution had a concentration of 6.70 × 10<sup>-5</sup> mg L<sup>-1</sup> directly after production (August 2010). Four months later (November 2010), the concentration of the stock solution was decreased to 2.70 × 10<sup>-5</sup> mg L<sup>-1</sup> (about 2.5 times lower than the initial concentration). The concentration decrease can have a number of reasons, including sorption on the vessel's wall, volatilization or photodegradation [26]. The most probable reason in our case is the sorption on the glass wall

**Table 1**  
Blank experiments carried out on re-used cleaned ceramic tubes with and without X-CARB.

Experiment	Extract	Dilution factor ( $\mu\text{L}$ hexane)	Recovery (%)	Cylinder status
1	Empty cylinder	300	96.9	Clean
	Complete dosimeter	300	88.2	Clean
2	Complete dosimeter	300	146.2	Contaminated
3	Complete dosimeter	300	133.7	Contaminated
	Complete dosimeter	100	95.9	Clean
4	Empty cylinder	300	129.6	Contaminated
	Empty cylinder	300	167.0	Contaminated
	Complete dosimeter	300	101.0	Clean
	Complete dosimeter	300	141.7	Contaminated

because the vessels were photo-shielded throughout storage and testing periods. In addition, TCDD has a low vapor pressure of only  $5.56 \times 10^{-7}$  Pa at  $25^\circ\text{C}$  [27] which implies that the volatilization process is inconsiderable [28]. From November 2010 on, the concentration remained almost constant up to February 2011 when it was tested for the last time. This stability in concentration reflects an equilibrium state between dissolved and wall-adsorbed TCDD molecules.

### 3.2. Blank tests

With every testing cycle of the dosimeters, blank experiments were carried out to check the re-usability of cylinders and the effectiveness of cleanup procedures (Table 1). Dosimeters for blank testing were prepared similarly as the other applicable ones. They were immersed in Milli-Q deionized water for 24 h and extracted with the same procedures as the other dosimeters. The blank extracts were dissolved in 5 mL hexane. Each extract was diluted with different amounts of hexane, spiked with  $4 \mu\text{L}$  of the quality control standards and then analyzed in duplicate by CALUX. The recovery rates of some blank cylinders were higher than 100%, which means that some cylinders were still contaminated with TCDD even after the usual cleaning procedures. TCDD is thermally stable at high temperatures, even up to over  $1000^\circ\text{C}$  [29]. This requires a cleaning method relying mainly on the act of solvents rather than pyrolysis. A new method was applied to clean the previously used ceramic cylinders by immersing them in Milli-Q water for 24 h and then in toluene under vacuum. Vacuuming allows the toluene molecules to flow through the cylinder pores and to replace the air bubbles. This in turn helps in dissolving the remaining traces of TCDD. Toluene was decanted and the cylinders were vacuumed to take out toluene from the pores. Vacuuming was repeated after replacing toluene with a fresh amount. The tubes were sintered at  $850^\circ\text{C}$  for 7 h. Two empty cylinders and two complete toximeters were extracted. Each extract was dissolved in 5 mL hexane and analyzed as detailed before. The recovery rates after these new clean-up procedures still indicate some contamination of the cylinders. Therefore, we recommend discarding the ceramic cylinders after each use, a recommendation that we applied in further experiments.

### 3.3. Establishing a full dose curve for calculation of the TCDD concentration trapped on dosimeter

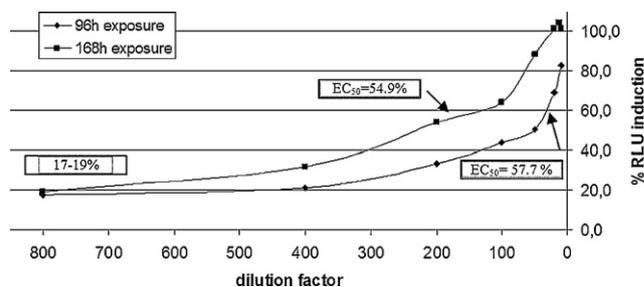
A standard dose–response curve was obtained by plotting the CALUX response (% RLU) of 10 standard TCDD solutions (in DMSO) against their concentrations (Fig. 3). With this calibration curve, we could calculate the amount of dioxin extracted from the dosimeter and the TCDD concentration in the testing solution. It is better not to use a single point estimate since this can bias the result [22]. The best way to determine the CALUX-BEQ of the 2,3,7,8-TCDD trapped

by the ceramic toximeter is by establishing a (full dose) dilution curve of the sample. For this purpose, one dilution was made and the % RLU induction is measured. Depending on this result, the choice of the other dilutions to be measured was decided.

For the ceramic tube that was immersed for 168 h in the TCDD solution, a maximum of around 100% induction was found for a dilution factor (df) of 10, while at a df of 800, the induction was close to that of the DMSO blank. The full dose curve is shown in Fig. 4 with 100% of induction at a df of 10 and a leveling off to a minimum response at a df of 800. For an exposure time of 96 h, the maximum was not yet reached at a df of 10, but the signal is higher than 80% of induction. Since the slope of the curve is still steep, it can be expected that the maximum will also be around the theoretical maximum of the calibration curve (100% RLU induction).

Depending of the shape of the dilution curve, BEQ-values from the dosimeter were assessed using one of the following methods described in [22 and reference therein]: (i) when the dose–response curves for the TCDD and the dosimeter are parallel, the EC50-values are directly generated from Hill regressions. The potency is assessed as the ratio  $[\text{EC}50]_{\text{TCDD}}$  over  $[\text{EC}50]_{\text{DOSIMETER}}$ ; (ii) when the dose–response curves for TCDD and the dosimeter are not parallel, a relative potency range is determined using various EC-TCDD to dosimeter ratios (e.g. EC20 and EC80). The spread of the potency range defined as the range between minimum and maximum potency is then used as a measure of the confidence for the result; (iii) for dose–responses curves, which do not exhibit a lower and/or upper plateau, or for which the Hill fit is inaccurate, the BEQ assessment is performed using the slope ratio method after linearization with Box–Cox transformations, i.e.  $[\text{slope}]_{\text{DOSIMETER}}$  over  $[\text{slope}]_{\text{TCDD}}$ .

In the exposure experiment of 168 h, the amount of TCDD trapped by the activated carbon in the toximeter was 0.0113 ng of TCDD in the first experiment and 0.093 ng in the second one. The 96 h exposure experiment yielded a CALUX-BEQ of 0.025 ng of TCDD. Following this approach, CALUX-BEQ values were assessed for exposure times between 1 and 7 days (Fig. 5). The average amounts of TCDD trapped by the toximeters varied between 0.018 ng (1 day of exposure) and 0.103 ng (7 days of exposure).



**Fig. 4.** Dose–response dilution curves for 96 and 168 h exposure times.

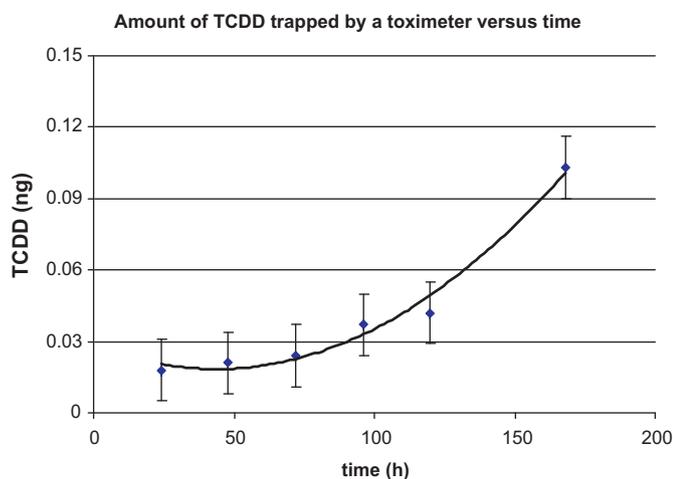


Fig. 5. Amounts of TCDD trapped by the toximeter versus time.

#### 3.4. Calculation of diffusion coefficient and sampling rate

Assuming that the transport of the TCDD molecules from the bulk solution to the interior of the ceramic dosimeter is based solely on diffusion; the accumulated mass can be described according to Fick's first law of diffusion [30]:

$$M = \frac{D_e \Delta C A t}{\Delta x} \quad (2)$$

where  $D_e$  is the effective diffusion coefficient;  $\Delta C$  is the concentration in the bulk solution and equals  $0.00135 \text{ ng L}^{-1}$  (the concentration at the sorbent is assumed to be zero);  $A$  is the area of the toximeter exposed to the bulk solution and equals  $1257 \text{ mm}^2$  (exposed height is 40 mm and diameter is 10 mm);  $t$  is the exposure time (d), and  $\Delta x$  is the wall thickness which equals 2 mm. The effective diffusion coefficient ( $D_e$ ) refers to an alteration in the diffusion rate of analytes (TCDD) in the porous ceramic membrane compared to water molecules. According to Archie's law we can write:

$$D_e = D_w \times \varepsilon^m \quad (3)$$

with  $D_w$  = the diffusion coefficient of TCDD in water with a mean value of  $5.11 \times 10^{-5} \text{ m}^2 \text{ d}^{-1}$  at  $25^\circ \text{C}$  [31]. It is inversely proportional to the concentration of the TCDD.  $\varepsilon$  is the porosity of the ceramic membrane (0.30) and  $m$  is Archie's law exponent, which ranges from 1.5 to about 2.5 from calibration experiments in the laboratory [32]. A value of 2.0 was determined and applied based on previous calibration experiments of the ceramic dosimeter [5,30].

The effective diffusion coefficients derived from our exposure experiments can now be compared to the theoretical ones calculated from Eq. (3). The diffusion coefficients ( $D_e$ ) calculated from the mean TCDD concentrations trapped by the ceramic toximeters amount to  $9.43 \times 10^{-6}$ ,  $10.9 \times 10^{-6}$ ,  $9.90 \times 10^{-6}$  and  $17.3 \times 10^{-6} \text{ m}^2 \text{ d}^{-1}$  after 72, 96, 120 and 168 h of exposure respectively. The increase in the value of  $D_e$  may be related to the decrease of the concentration of TCDD in the aqueous medium due to the continuous uptake by the dosimeters over time (Eq. (2)). The average effective diffusion coefficient equals  $11.9 \times 10^{-6} \text{ m}^2 \text{ d}^{-1}$ . The theoretical effective diffusion coefficient for a mean value of  $m$  (2.0) equals  $4.6 \times 10^{-6} \text{ m}^2 \text{ d}^{-1}$  and is about half the values we observed experimentally between 72 and 120 h of exposure.

For comparisons with active sampling methods of TCDD such as a pumped charcoal tube, we can calculate the sampling rate of our passive ceramic toximeter, expressed in a volume per time unit. The sampling rate can be calculated using the following formula:

$$R = \frac{D_e A}{\Delta x} \quad (4)$$

According to our results, sampling rates varied between 5.93 and  $10.9 \text{ mL d}^{-1}$  with an average of  $7.5 \text{ mL d}^{-1}$  at room temperature. In future field applications, temperature would be the only parameter to be measured during the sampling period because of its impact on diffusivity [33].

#### 4. Conclusion

The activated carbon (X-CARB)-filled ceramic toximeter yielded a good reliability in the laboratory as a passive sampler for TCDD-polluted water. There is no need for snap-shot sampling or calibration steps once it is validated. Its combination with the CALUX bioassay analysis reduces the time and costs. The ease of use of the toximeter as well as the few steps required for sampling and analysis makes it a promising screening technique for suspected dioxin-polluted aquatic environments as well as a monitoring tool for controlling the official tolerance levels of dioxins in an aquatic system.

Assuming a sampling rate of  $7.5 \text{ mL d}^{-1}$ , a minimum amount of 2 pg of TCDD trapped by the activated carbon in the toximeter and a sampling time of 1 month (30 days), a concentration of  $0.89 \text{ pg-TCDD L}^{-1}$  can be assessed.

This paper has been limited to PCDD/Fs, the most difficult fraction to determine. However, we have a clean-up protocol to perfectly separate PCDD/Fs from dioxin-like PCBs and from all other ligands binding to the Ah receptor [18,21,34]. In the future, a field application in contaminated sites is planned to evaluate the dosimeter for the assessment of PCDD/Fs and dioxin-like PCBs under natural environmental conditions.

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