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Bioaccumulation, distribution and elimination of chlordecone in the giant freshwater prawn *Macrobrachium rosenbergii*: Field and laboratory studies

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ABSTRACT

Chlordecone is a persistent organochlorine pesticide that has been widely used in Guadeloupe (French West Indies) to control the banana weevil *Cosmopolites sordidus* from 1972 to 1993. A few years after its introduction, widespread contamination of soils, rivers, wild animals and aquatic organisms was reported. Although high chlordecone concentrations have been reported in several crustacean species, its uptake, internal distribution, and elimination in aquatic species have never been described. This study aimed at investigating the accumulation and tissue distribution of chlordecone in the giant freshwater prawn *Macrobrachium rosenbergii*, using both laboratory (30 days exposure) and field (8 months exposure) approaches. In addition, depuration in chlordecone-free water was studied. Results showed that chlordecone bioconcentration in prawns was dose-dependent and time-dependent. Moreover, females appeared to be less contaminated than males after 5 and 7 months of exposure, probably due to successive spawning leading in the elimination of chlordecone through the eggs. Chlordecone distribution in tissues of exposed prawns showed that cephalothorax organs, mainly represented by the hepatopancreas, was the most contaminated. Results also showed that chlordecone was accumulated in cuticle, up to levels of 40% of the chlordecone body burden, which could be considered as a depuration mechanism since chlordecone is eliminated with the exuviae during successive moults. Finally, this study underlined the similarity of results obtained in laboratory and field approaches, which highlights their complementarities in the chlordecone behaviour understanding in *M. rosenbergii*.

1. Introduction

The tropical climate of the French West Indies (FWI) promotes the rapid development of pests which exert significant pressure on crops, leading to the use of considerable amounts of pesticides in these regions (Bocquené and Franco, 2005). The use of organochlorine pesticides first started in the 1950's and led to widespread contamination of the environment (Coat et al., 2006). The most worrying organochlorine pesticide residue for surface water in Guadeloupe (FWI) is chlordecone ($C_{10}Cl_{10}O$; CAS number 143-50-0) (Coat et al., 2011).

Chlordecone (CLD) is an insecticide whose production started in 1966 under the trade name Kepone® (Allied Chemical Corporation, Hopewell, VA, USA) mainly for the export to tropical countries, where it was used against a wide range of pests (Dolfing et al., 2012; Sterrett and Boss, 1977). In 1975, the Hopewell accident caused acute toxicity in plant workers (i.e toxicity symptoms in the nervous system, liver and testes - Cannon et al., 1978), as well as extensive contamination of surface water, sediment and aquatic organisms (Huggett and Bender, 1980), leading to its prohibition in 1976 in the US (US ATSDR, 1995). In Guadeloupe, CLD has been commonly employed to control the banana weevil *Cosmopolites sordidus* from 1972 to 1978 (Kepone®) and from 1982 to 1993 (Curlone®) (Cabidoche et al., 2009, 2006). Due to widespread pollution of soils, rivers, wild animals and aquatic organisms (Snegaroff, 1977), CLD was finally prohibited in 1993 (Cabidoche et al., 2009). In 2009, CLD has been included in the Stockholm Convention annexe's, and its production and use were banned worldwide (UNEP, 2009).

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Nowadays, CLD is still present in soils, especially in andosols which are rich in organic matter, in the densely cultivated areas of the south of the Basse-Terre Island (Guadeloupe). It was estimated that CLD could persist for several centuries because of its resistance to degradation in the environment (Cabidoche et al., 2009). Although Fernández-Bayo et al. (2013) showed the existence of CLD-degrading microorganisms in andosols under aerobic conditions, CLD undergoes no significant or fast biotic or abiotic degradation (Dolfing et al., 2012; Levillain et al., 2012). In nitisols and ferralsols, CLD is more easily released, and progressively transferred to aquatic ecosystems by the water cycle (Coat et al., 2011). Because of its high Soil Organic Carbon Water Partitioning Coefficient (K_{oc} = 15849 L kg^{-1}), Octanol-Water Partition Coefficient (log K_{ow} = 4.5–6.0), and its affinity for lipids, CLD is persistent in the environment and accumulates in the food web (Cabidoche and Lesueur-Jannoyer, 2012; Clostre et al., 2013; Sterrett and Boss, 1977; UNEP, 2005). According to the Direction de l'Environnement, de l'Aménagement et du Logement (DEAL) of Guadeloupe, Guadeloupian rivers are contaminated by CLD at concentrations that ranged from 0.2 to 4 µg L^{-1} with a maximum of 8.6 µg L^{-1} measured in the River Grande Anse in 2003 (GREPP, 2004; InVS-Inserm, 2009).

Freshwater and coastal fishes, molluscs and crustaceans are the most contaminated organisms (Bertrand et al., 2010; Cabidoche et al., 2006; Coat et al., 2011; Dromard et al., 2016). Nevertheless, CLD has been detected in blood samples of about 70% of the Guadeloupe population (Guldner et al., 2010; Multigner et al., 2007, 2006), mainly as a result of consumption of contaminated food, seafood and root vegetables (Dubuisson et al., 2007; Guldner et al., 2010). In such a context, a French legal Maximal Residue Limit (MRL) of 20 µg of CLD per kg wet weight for food of plant and animal origin has been adopted in 2008 (DGS, 2008), causing harsh restriction in fishing and consumption of fish. Furthermore, CLD contamination was detected in farms of the tropical giant freshwater prawn *Macrobrachium rosenbergii*, an important economic resource in Guadeloupe. This had led to the closure of many aquaculture units in 2008. In addition to be accumulate in organisms, CLD can exert toxic effects as most pesticides, and could disturb critical physiological processes including those controlled by the hormonal system (Giusti et al., 2013; Guzelian, 1982; Lafontaine et al., 2016a, b; Lafontaine et al., 2017). Several studies have demonstrated that CLD could impact the crustacean development, growth and reproduction through disturbance of hormonal processes (Bookhout et al., 1980; Lafontaine et al., 2016a, b; Nimmo et al., 1977; Oberdörster and Cheek, 2001; Sanders et al., 1981; Schimmel et al., 1979; Zha et al., 2007). However, although studies showed toxic effects of pesticides and biocides in *M. rosenbergii* (Revathi and Munuswamy, 2010; Satapornvanit et al., 2009), very few investigations have been carried out on CLD, especially its uptake and depuration kinetics, as well as its distribution within prawn tissues.

Therefore, the present study aimed at investigating the bioaccumulation and tissue distribution of CLD in the giant freshwater prawn *M. rosenbergii*, (*i*) in laboratory conditions (short-term exposure) and (*ii*) in the field (*in situ*) conditions (long-term exposure). This work allowed to understand the CLD behaviour in a crustacean decapod considered as a good model for the wild *Macrobrachium* spp. living in freshwater ecosystems of these regions. In addition, remaining prawns from the long-term exposure were used to investigate CLD depuration. As it is well known that gender can interfere with chemical uptake, distribution and elimination, CLD concentrations were measured separately in males and females when sexual differentiation was possible. Besides filling a knowledge gap on the fate of CLD in tropical prawns, which may help to understand its toxic effects in these organisms, results of this study may have practical implications, for example in the biomonitoring of river contamination, or regulatory control of the safety of marketed food products.

2. Materials and methods

2.1. Tested organisms

Three-month-old post-larvae of *Macrobrachium rosenbergii* (approx. 2 g, 1.4 cm cephalothorax length) were provided by an aquaculture farm (OCEAN-SA) located at Pointe-Noire, a geographic area free of CLD contamination. Results of pre-tests, previously carried out to evaluate the presence of CLD in tissues of prawns from the hatchery, showed no contamination (concentrations below detection limit; data not shown).

2.2. Short-term chlordecone exposure: laboratory experimental design

A total of 1540 post-larvae of *M. rosenbergii* were transferred to the laboratory (UMR BOREA, University of the French West Indies and Guiana, Guadeloupe) in February 2012. Prawns were acclimated for one week in 55 glass aquaria filled with 28 L of tap water filtered through activated charcoal (i.e. 28 prawns per aquarium). Aquaria were randomly distributed in the laboratory and were under constant aeration with a 12 h light/dark photoperiod. During acclimation, the prawns were fed daily with one artificial shrimp food pellet per individual (complete food for rearing, Le Gouessant, Brittany, France).

The 10 g L^{-1} stock solution of CLD (100%, Riedel-de-Haën, Sigma-Aldrich, USA), as well as three successive dilutions (1000, 100 and 10 mg L^{-1}) were prepared in acetone (Biosolve-Chimie, France). A volume of 56 µL of each dilution and of the stock solutions was added into the 28 L of aquarium water in order to obtain four concentrations of CLD in water: 0.02, 0.2, 2 and 20 µg L^{-1}, chosen for their environmental relevance in surface waters in Guadeloupe. In parallel to the four CLD concentrations, a water control (consisted of tap water filtered through activated charcoal), and a solvent control (obtained by spiking 28 L of filtered tap water with 56 µL of acetone) were performed. Ten aquaria were used for each exposure condition, except for the solvent control where only five aquaria were used. During the 30 days of exposure, *M. rosenbergii* were fed daily with one pellet of artificial shrimp food per individual. The food pellets were entirely consumed by prawns in less than fifteen minutes, thus limiting degradation of water quality by decaying food pellets and contamination of food by CLD. Based on a pre-test designed to measure the CLD concentrations in water according to time of exposure, it was decided to renew the exposure medium every 96 h, in order to maintain the CLD concentration during the 30 days of exposure (Table S1 in Supplementary material). A water temperature of 27.6 ± 0.2 °C was maintained, and pH remained at 7.57 ± 0.03 throughout the experiment. These measured values were in accordance with optimal water temperature and pH commonly used in prawn farms (New, 2002). The mortality rate was about 4% throughout the experiment, whatever the conditions.

In order to measure internal CLD concentrations, five individuals of *M. rosenbergii* (corresponding to 5 replicates) were randomly collected in each condition, after eight durations of exposure: 6 h, 12 h, 1 day, 2 d, 4 d, 8 d, 15 d and 30 d. After sampling, the prawns were immediately frozen in liquid nitrogen and stored at −80 °C until CLD analysis (see section 2.4).

2.3. Long-term chlordecone exposure: in situ experimental design

2.3.1. Accumulation

As for the laboratory experiment, post-larvae of *M. rosenbergii* were provided by the aquaculture farm (OCEAN-SA) in March 2012. A total of 7000 post-larvae were transferred into two farming ponds. The first one, called "control site", supplied by the River Petite Plaine, was located in Pointe-Noire (North of the Basse-Terre Island, Guadeloupe) and the CLD concentration was below the limit of detection (i.e. 0.01 μg L^{-1}). The second one, called "contaminated site", supplied by the River Rivières-aux-Herbes, was located in Saint-Claude (South of the Basse-Terre Island, Guadeloupe) and the CLD concentration in the contaminated site was 0.19 ± 0.013 μg L^{-1}. The farm based in Pointe-Noire is still in operation, whereas the farm in Saint-Claude had to cease its activity because the CLD concentration in prawns was higher than the French legal Maximal Residual Limit (MRL) of 20 ng g^{-1} wet weight (DGS, 2008). During the 8-month experiment, prawns were fed daily with artificial shrimp food pellets (complete food for rearing, Le Gouessant, Brittany, France) in order to be in the same conditions than farm productions. Water temperatures of 27.6 ± 1.5 °C and 27.7 ± 1.6 °C were measured in the ponds at Pointe Noire and Saint-Claude, respectively. The pH remained at 8.47 ± 0.47 in the Pointe-Noire pond and at 8.00 ± 0.47 in the Saint-Claude pond.

Six individuals of *M. rosenbergii* (corresponding to 6 replicates) were sampled in each pond, after seven durations of exposure: 8 d, 15 d, 30 d, 3 months, 5 m, 7 m and 8 m, between March and November 2012. After sampling, the prawns were immediately frozen in liquid nitrogen and stored at −80 °C until analysis. Individual's sex could only be determined after 5 months, therefore analyses of prawns sampled at 5, 7 and 8 months of exposure were carried out by separating males and females. In addition, when females were ovigerous, eggs were also sampled to determine the CLD concentration.

2.3.2. Depuration

After 8 months of exposure in the Saint-Claude pond, remaining prawns were trasferred to laboratory and placed in aquaria filled with 28 L of tap water filtered through activated charcoal, in order to evaluate CLD depuration. Natural photoperiod was kept (12 h light/dark) and the prawns were fed daily with one artificial shrimp pellets. Eight individuals of *M. rosenbergii* (corresponding to 8 replicates) were collected after 1, 2, 4, 8, 14, 21, 35 and 45 days of depuration, immediately frozen in liquid nitrogen and stored at −80 °C until CLD analysis.

2.4. Chlordecone concentrations in M. rosenbergii

2.4.1. Chlordecone extraction from M. rosenbergii

The prawns were thawed and dissected into four parts: internal organs within the cephalothorax, mainly represented by the hepatopancreas (hereafter referred 'the hepatopancreas'), cephalothorax cuticle, abdominal muscle and abdominal cuticle. Each part was accurately weighted in order to determine, in a further step, the total CLD amount (body burden) in the whole organism and the "CLD concentration" calculated by weighing the total CLD body burden by the individual fresh weight. However, prawns sampled in the ponds after 8, 15 and 30 days of exposure were too small to dissect the four parts, therefore CLD concentrations were measured in the whole body. Moreover, eggs sampled in ovigerous females were analyzed. Finally, due to the fact that *M. rosenbergii* could ingest their own exuviae and faeces, it was not possible to perform analyses on these matrices.

CLD analysis in each sample (i.e. tissues or eggs) was performed according to the method developed by Lafontaine et al. (2016a). Briefly, a minimum weight of 200 mg of each of the four anatomical parts was freeze-dried during 20 h. Extraction of CLD was performed with a mixture of *n*-hexane:dichloromethane (90:10; v:v; Biosolve-Chimie, France) using an Accelerated Solvent Extractor (ASE200; Dionex, Thermo Scientific, USA). Before the extraction, 100 μL of a hexanic solution of PCB congener 112 (Dr. Ehrenstorfer, Germany) was added to the samples as a surrogate internal standard to obtain a final concentration of 50 pg μL^{-1}. Then, after evaporation of the extracted fat fraction, the lipid content was determined gravimetrically.

2.4.2. Sample purification

Residues were resuspended in 2 mL *n*-hexane (Biosolve-Chimie, France) and transferred to a test tube for H_2SO_4 (98%) clean-up. After, the organic phase was spiked with 5 μL nonane added as a keeper and evaporated under a gentle nitrogen stream using a Visidry evaporator (Supelco, Sigma-Aldrich, USA) before being resuspended with 45 μL *n*-hexane and 50 μL PCB 209 at 100 pg μL^{-1} (in *n*-hexane) as an injection volume internal standard (Dr. Ehrenstorfer, Germany).

In parallel with sample extractions, procedural blank and a Quality Control (QC) were carried out (one of each for a series of 22 samples). The procedural blank was obtained by ASE extraction without biological matrix, allowing controlling the extraction and the clean-up procedure. The QC was performed to control CLD recovery by using a biological matrix (here, freeze-dried *Penaeus monodon* from India; Boni©, Colruyt Group, Halle, Belgium) spiked with an acetonic solution of CLD, in order to obtain a final concentration of 2.5 ng g^{-1} wet weight for biological sample. Tissues of *P. monodon* were used in order to have a biological matrix of decapod free from CLD contamination.

2.4.3. Chromatography analysis

The purified extracts, procedural blank and QC were analyzed by high-resolution gas chromatography using a ThermoQuest Trace 2000 gas chromatograph equipped with a ^{63}Ni ECD detector (Thermo Scientific, USA) and an auto-sampler ThermoQuest AS 2000 (Thermo Scientific, USA) according to Lafontaine et al. (2016a), Lagarrigue et al. (2014) and Multigner et al. (2010). Data were recorded with Chromcard 2.8 (Fisons Instruments, Italy) software for Windows. CLD was identified based on its retention time previously determined with a linear calibration curve (1.5–200 pg μL^{-1}) established with CLD certified solutions (Riedel-de Haën, Germany). CLD concentration in each sample and in the QC was corrected by the percentage recovery of the internal surrogate PCB 112. The recovery efficiency based on CLD recovery in QC and on the recovery of the surrogate internal standard (PCB 112) ranged from 88 ± 4% to 115 ± 5% respectively, which was within the limits recommended by SANCO, i.e. range from 60 to 140% (SANTE, 2015). The limit of detection (LOD) was fixed at three times the background noise of the chromatogram (i.e. 0.02 ng g^{-1} wet weight). The limit of quantification (LOQ) was determined with freeze-dried tissues of *P. monodon* spiked with various CLD concentrations and was established at 0.06 ng g^{-1} wet weight. CLD concentrations in *M. rosenbergii* were measured in several replicates (see Sections 2.2 and 2.3) per condition and the average was calculated for each condition. CLD concentration was expressed as ng g^{-1} wet weight.

At the end of the laboratory and in-situ exposures, although all precautions have been taken, we cannot exclude that *M. rosenbergii* could have eaten their own exuviae or faeces during laboratory and *in situ* exposures, therefore the bioaccumulation factor (BAF), instead of bioconcentration factor, was calculated using the method of Arnot

and Gobas (2006). According to these authors, BAF is the ratio between CLD concentration in tissues of prawns and CLD concentration in water of exposure, including chemical exposure through the diet.

2.5. In-situ toxicokinetic model

Estimation of uptake rate constant (k_1) and depuration rate constant (k_2) was performed as indicated in the OECD 305 Fish Bioconcentration Guidelines (OECD, Test No. 305). Due to the *in-situ* experiment design, it was not possible to take into account all parameters necessary for a complete model (e.g. fecal elimination constant, gill elimination constant) as described in Arnot and Gobas (2004). We assumed that CLD bioaccumulation in organism followed a first order kinetics, expressed as (equation (1)):

$$\frac{dCMr}{dt} = k1.Cw - k2.CMr \quad (1)$$

where dC_{Mr}/dt is the change rate of CLD concentration in *M. rosenbergii* (ng g^{-1} day^{-1}), k_1 is the uptake rate constant (L g^{-1} day^{-1}), k_2 the elimination rate constant (day^{-1}), C_w is the concentration of CLD in water (g L^{-1}) and C_{Mr} is the concentration of CLD in the organism (ng g^{-1}).

With the hypothesis of a first order kinetics, the model becomes linear and its slope corresponds to the elimination rate constant. To take into account the organism growing during the experimentation, a growth correction was applied by using amounts of CLD in *M. rosenbergii* instead of the CLD concentration. The equation was (equation (2)):

$$ln\,(CLDMr) = -k2g.t + c \quad (2)$$

where, $\ln(CLD_{Mr})$ is the natural logarithm of the CLD amount in *M. rosenbergii* (ng), k_{2g} is the constant rate of elimination corrected with the organism growth, and c is the intercept which equals the natural logarithm of the CLD concentration in *M. rosenbergii* at the depuration start. Then, the estimated k_{2g} can be used to calculate the k_1 by following this equation (equation (3)):

$$CMr = Cw \cdot \frac{k1}{k2g} \cdot \left(1 - e^{-k2g.t}\right) \quad for\ 0<t<te \quad (3)$$

where t is the time (days) and t_e is the end time of the uptake period (days).

Finally, the bioaccumulation factor (BAF) could be estimated as (equation (4)):

$$BCAF = \frac{k1}{k2g} \quad (4)$$

After having determined k_{2g}, the depuration time necessary to reach the MRL (i.e. 20 ng g^{-1}) in the edible part of prawns was estimated using the followed equation (equation (5)):

$$CMr = CMr,0 \cdot e^{-k2g \cdot t} \quad (5)$$

where C_{Mr} equals 20 ng g^{-1} and $C_{Mr,0}$ equals 490 ng g^{-1} (i.e. CLD concentration in the muscle tissue at the end of the accumulation phase-see Table S3 in Supplementary data).

2.6. Statistical analysis

All data met normality and homogeneity of variance assumptions (Shapiro and Bartlett tests, $p > 0.05$). To investigate the effects of the exposure duration, exposure concentration, prawn gender and exposure site on CLD concentrations in prawn tissues, data were analyzed using a two- or three-way ANOVA. Then, Tukey HSD post-hoc tests were performed to describe significant differences. A probability value of less than 0.05 was regarded as significant. Correlations between CLD concentrations in *M. rosenbergii* and CLD concentrations in water were analyzed using the Pearson correlation coefficient. All tests were performed with STATISTICA 10 Software (StatSoft, 2012; USA).

3. Results

3.1. Laboratory chlordecone exposure

Total CLD was analyzed in prawns from water and solvent controls, and concentrations were below 10 ng g^{-1} except in control prawns exposed for 30 days (Table S2 in Supplementary data). CLD bioconcentration was significantly influenced by both the exposure concentration and the exposure duration (two-way ANOVA test, $p < 0.001$). Indeed, prawns exposed to higher CLD concentrations accumulated higher amounts of CLD (Fig. 1). Generally, whatever the exposure concentration, CLD concentration was measurable as early as 6 h of exposure. The accumulation of CLD in *M. rosenbergii* was also time-dependent in prawns exposed to 0.02 and 2 µg L^{-1}, since the longer the prawns were exposed, the higher the CLD concentration was. This observation is supported by a significant positive correlation ($r = 0.84$, $p < 0.001$, $n = 40$) between CLD concentration in *M. rosenbergii* and the concentration of exposure. Moreover, whatever the concentration of exposure to CLD, the bioaccumulation slowed down after 48 h of exposure. In fact, CLD concentrations in *M. rosenbergii* reached a plateau (Fig. 1).

BAF values, calculated at the end of the exposure, showed that bioaccumulation was the highest in *M. rosenbergii* exposed to the lowest CLD concentration (i.e. 0.02 µg L^{-1}), as compared to other conditions (Table 1).

As the CLD distribution profiles were similar for each exposure concentrations (data not shown), only the profile of the prawns exposed to 0.2 µg L^{-1} is shown (Fig. 2A and Table S3 in the Supplemental material), due to the fact that this CLD concentration is close to the CLD concentration in Saint-Claude (i.e. *in situ* experiment; see below). Results showed that after 6 h of exposure, CLD was mostly found in cephalothorax organs, mainly represented by the hepatopancreas (i.e. more than 90% of the total body burden). The same trend was observed at the other durations of exposure (i.e. from 40% to 70% of the total CLD quantity present in the hepatopancreas). At the end of the 30-days exposure, the distribution of CLD was approximately 48% in the hepatopancreas, and almost 30% in the cuticle. The amount of CLD in the cuticle of the cephalothorax and abdomen varied between 15% to more than 40% compared to others compartments.

3.2. In situ chlordecone exposure

Total CLD concentrations in *M. rosenbergii* were significantly influenced by the exposure site (i.e. Pointe-Noire vs. Saint-Claude) and

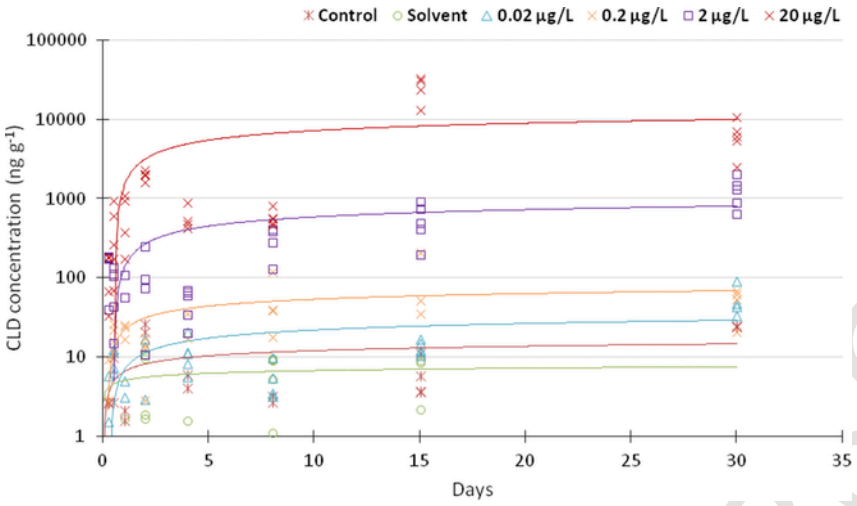

Fig. 1. Chlordecone concentrations (ng g^{-1} wet weight, mean + S.D.) measured in whole *Macrobrachium rosenbergii* exposed to four chlordecone concentrations (i.e. 0.02, 0.2, 2 and 20 µg L^{-1}) in laboratory experiment and sampled after eight exposure durations.

Table 1
Bioaccumulation factor (L Kg^{-1}) calculated at the end of experiment for the four concentrations of exposure (i.e. 0.02, 0.2, 2 and 20 µg L^{-1}) in the laboratory experiment (A) and for the contaminated pond in the *in situ* experiment (B). CLD concentrations were not measured in the water of control tanks, nor in the water of Pointe-Noire pond.

	In lab (A)				*In situ* (B)
	0.02 µg L^{-1}	0.2 µg L^{-1}	2 µg L^{-1}	20 µg L^{-1}	Saint-Claude
30 days	2708	368	642	317	564

the exposure duration (two-way ANOVA test, $p < 0.001$). An important accumulation of CLD was observed in the prawns sampled in the Saint-Claude pond throughout the experiment, while very low contaminations of CLD were measured in prawns from the Pointe-Noire pond (Fig. 3). Results obtained in Saint Claude pond showed also that CLD concentrations were significantly higher in prawns exposed for more than 90 days, than those exposed for 30 days or less.

Results of the field experiment were compared to results obtained in prawns exposed to 0.2 µg L^{-1} of CLD in the laboratory experiment, since the CLD concentration in the Saint-Claude pond was about 0.19 ± 0.013 µg L^{-1}. For the same durations of exposure (i.e. 8, 15 and 30 days), no significant differences ($p > 0.05$) were measured between CLD concentrations measured in prawns from the both exposure (Table 2). The accumulation constant rate (k_1) underlined a strong CLD accumulation at the beginning of the exposure, followed by a period of a weak CLD accumulation (i.e. low k_1 values from day 8 to day 90) and finally a stabilization observed after 90 days of exposure until the end of the experiment (Fig. 4).

3.2.1. Chlordecone concentration in anatomical compartments

The amount of CLD in the different anatomical compartments of *M. rosenbergii* exposed for 3, 5, 7 and 8 months in ponds are presented in Fig. 2B and Table S3 in the Supplemental material. Whatever the organism gender (determined after 5 month of exposure), and the duration of exposure, the accumulation of CLD in the hepatopancreas represented 50–65% of the total body burden, while it

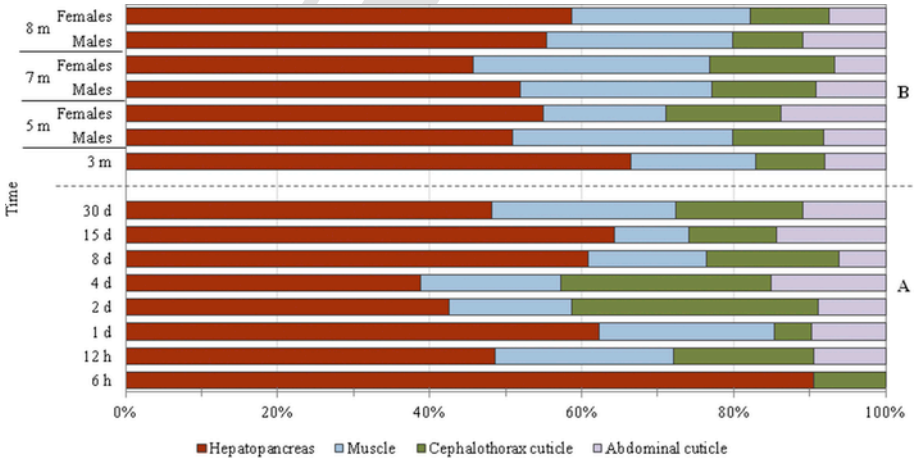

Fig. 2. Distribution of chlordecone in the four anatomical compartments (internal organs within the cephalothorax, mainly the hepatopancreas, cephalothorax cuticle, abdominal muscle and abdominal cuticle) related to CLD body burden of *M. rosenbergii*. The CLD quantity measured in each anatomical compartment were represented by a percentage of the total quantity (i.e. CLD body burden) measured in all the anatomical compartments of *M. rosenbergii.* (A) Prawns exposed to 0.2 µg L^{-1} of chlordecone during the laboratory experiment and sampled after eight exposure durations. The distribution profiles were similar for prawns exposed to 0.02, 2 and 20 µg L^{-1} of CLD. (B) Prawns exposed to chlordecone during the field experiment in the contaminated site (Saint-Claude pond) and sampled after four exposure durations.

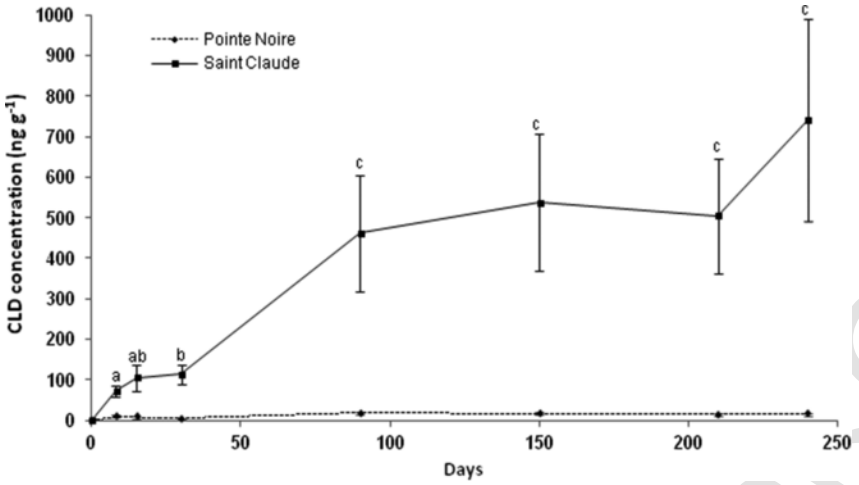

Fig. 3. Chlordecone concentrations (ng g^{-1} wet weight, mean + S.D.) measured in whole *Macrobrachium rosenbergii* sampled in the control site in Pointe-Noire and in the contaminated site in Saint-Claude after seven exposure durations. Different letters above the bars indicate significantly different values for the concentrations measured in Saint-Claude. No differences were observed in Pointe-Noire according to the duration of exposure (Tukey's HSD test, *p*-values < 0.05).

Table 2
Chlordecone concentrations (ng g^{-1} wet weight) measured in whole *Macrobrachium rosenbergii* exposed in the field and in the laboratory (0.2 µg L^{-1}) and sampled after 8, 15 and 30 days of exposure (Tukey HSD, p-value > 0.05).

	In lab	*In situ*
	0.2 µg L^{-1}	Saint-Claude
8 days	55.3 ± 10.2	72.5 ± 12.9
15 days	98.6 ± 32.5	104.9 ± 33.0
30 days	78.6 ± 34.8	112.7 ± 23.0

represented 15–25% of the total body burden, in the cephalothorax and abdomen cuticle.

3.2.2. Level of contamination according to the gender and in eggs

Total CLD concentrations were measured in males, non-ovigerous females, ovigerous females without eggs and eggs coming from ovigerous females of *M. rosenbergii* exposed in the Saint-Claude pond were presented in Fig. 5.

Males tended to be more contaminated than females except after 8 months of exposure. CLD concentrations in non-ovigerous females tended to be higher than those measured in ovigerous females sampled after 5 and 7 months of exposure. In addition, CLD concentrations measured in eggs were significantly higher than in the females that carried these eggs, except in females exposed for 8 months. Although differences were observed in CLD concentrations between males and females, the CLD distribution in the four anatomical compartments showed a similar profile whatever the gender (Fig. 2B).

3.3. Chlordecone depuration

After 8 months of CLD exposure in the Saint-Claude pond, remaining prawns were placed in the laboratory in clean water to evaluate the CLD depuration. Results showed a decrease of CLD concentration in whole *M. rosenbergii* from the 2nd day of depuration (Fig. 6). After 15 days in CLD-free water, CLD concentration in prawns was halved, and at the end of depuration experiment (i.e. 45 days), only 15% of the initial CLD concentration accumulated in prawns remained (i.e. 118 ± 32 ng g^{-1}). The estimated rate of elimination, tak-

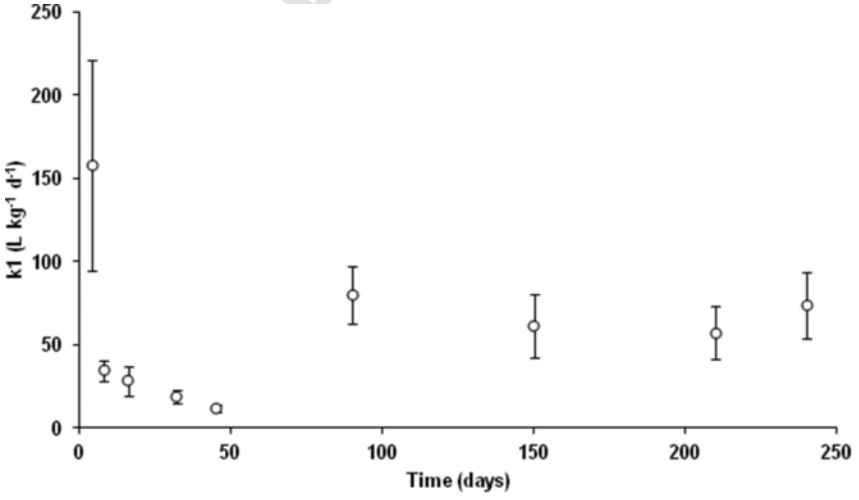

Fig. 4. Variation of the constant of accumulation rate (k_1), estimated using the constant elimination rate corrected with *M. rosenbergii* growth (k_{2g}), according to the duration of exposure.

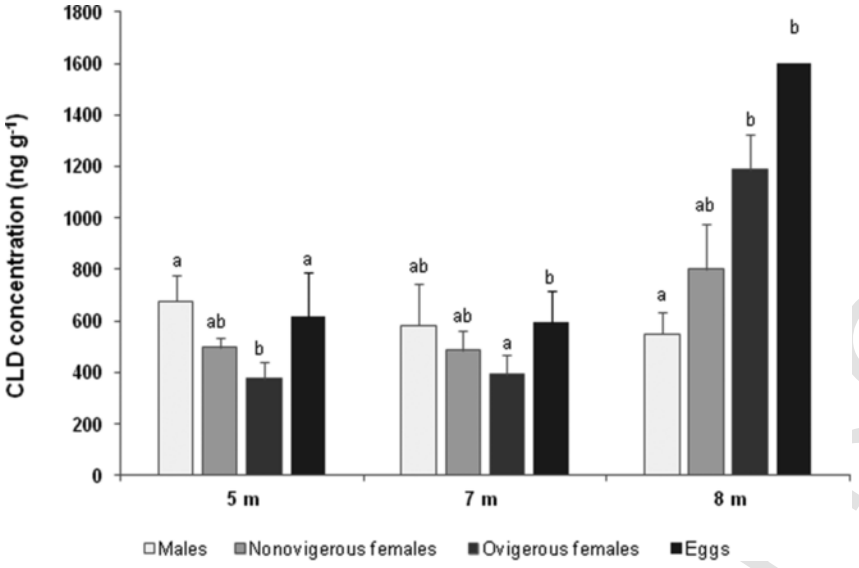

Fig. 5. Chlordecone concentrations (ng g^{-1} wet weight, mean + S.D.) measured in whole males, nonovigerous females, and ovigerous females sampled in the contaminated site in Saint-Claude after 5, 7 and 8 months of exposure. In addition, chlordecone concentration was measured in mass eggs of ovigerous females. Different letters above the bars indicate significantly different values for each sampling time (Tukey's HSD test, *p*-values < 0.05).

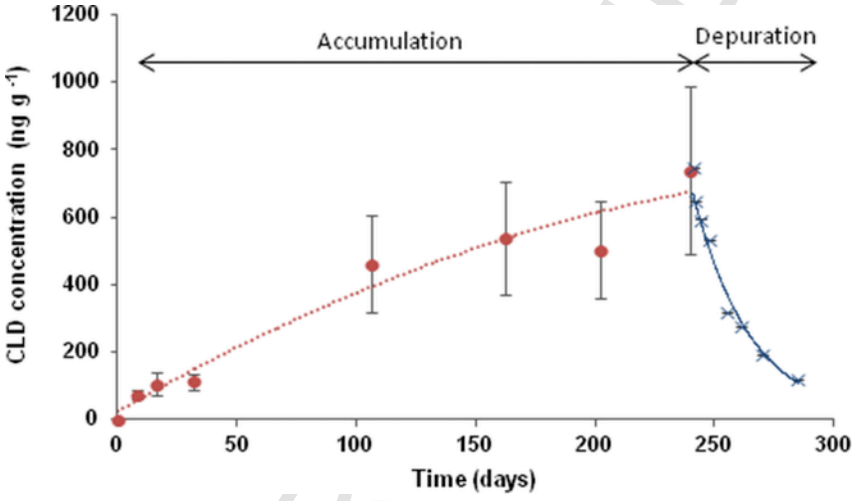

Fig. 6. Accumulation and depuration of chlordecone (ng g^{-1} wet weight ± S.D.) in whole *Macrobrachium rosenbergii*, placed in water free from contamination after 8 months (240 days) of exposure in the Saint-Claude pond.

ing into account the *M. rosenbergii* growth (k_{2g}), was equalled to 0.034 $days^{-1}$.

4. Discussion

4.1. Accumulation of chlordecone in whole M. rosenbergii

The laboratory experiment highlighted that CLD was accumulated in *M. rosenbergii* from 6 h of exposure whatever the exposure concentration. There was also a slight accumulation of CLD in control prawns, which could be explained by a cross-contamination between contaminated and control aquaria randomly placed in the room (e.g. accidental cross-contamination during sampling, cross-contamination due to bubbling aeration).

The field experiment confirmed that CLD has a high potential for bioaccumulation in aquatic organisms due to its physicochemical properties, even when concentrations are very low (US ATSDR, 1995). Our results are in agreement with previous studies highlighting CLD bioconcentration in different organisms. Bahner et al. (1977) showed the accumulation of CLD in the estuarine shrimp, *Palaemonetes pugio,* exposed to 0.026 and 0.41 µg CLD L^{-1}. Similarly, Coat et al. (2011) observed CLD bioaccumulation in tropical food web including various species of mussels, shrimps and fishes. Moreover, Monti (2007) reported CLD contamination in crustacean species from the River Rivière-aux-Herbes, which is the river that supplies the Saint-Claude pond. In this river, CLD concentrations of 2204 ng g^{-1} in *Macrobrachium carcinus*, 1134 ng g^{-1} in *Macrobrachium faustinum* and 770 ng g^{-1} in *Atya scabra* were measured. These concentrations were of the same order of magnitude as those measured in our study in *M. rosenbergii* sampled in the Saint-Claude pond. In prawns collected in the control pond of Pointe-Noire, very low concentrations were detected. This presence of CLD in control prawns could be explained by the bioaccumulation process of trace

amounts of CLD (lower than the LOQ in water, i.e. 0.01 μg L^{-1}) detected in the River Petite Plaine, supplying the Pointe-Noire farming pond (Ministère de l'Écologie du Développement durable et de l'Énergie, 2015b). However, the contamination of prawns from the control pond was not of concern since the measured concentrations were always under the MRL of 20 ng g^{-1} (DGS, 2008).

Prawns exposed to 0.02 μg L^{-1} had a higher BAF than prawns exposed to other CLD concentrations, suggesting that the bioaccumulation of CLD was the most efficient at the lowest CLD concentration in water. Therefore, this assumes that, in the field, low CLD concentrations in water could result in unexpectedly high CLD contamination in the aquatic species of the food chain. This observation was confirmed by the significant accumulation of CLD in the field experiment, and the high k_1 value mainly during the first few days of exposure.

The higher BAF in prawns from *in situ* study when compared to the BAF from the laboratory experiment could be due to ingestion of contaminated material (e.g. faeces, exuviae) which could less occurred in laboratory than in field conditions where other contaminated food sources could be present (e.g. contaminated organic matter, zooplankton).

Contaminant concentrations in exposed organisms from laboratory experiments are frequently different from those measured in the field, due to exposure conditions which can vary between both approaches (Burton et al., 2005; Hill et al., 2011; Mann et al., 2010). Results obtained from field studies are more environmentally realistic and ecologically meaningful than laboratory tests (Connon et al., 2012; Crane and Babut, 2007), but are often difficult to analyse because of several confounding factors (e.g. season, temperature, dissolved oxygen, turbidity, predation, synergistic or antagonist effects of various toxic substances in the environment) (Rotchell and Ostrander, 2003). Therefore, the relationship between laboratory and field exposures has always been the topic of ongoing debate. In our study, when comparing the same durations of exposure (i.e. 8, 15 and 30 days), it appears that prawn contamination was similar in both experiments. Therefore, the laboratory experiment reflects what takes place in the natural environment, regarding the accumulation of CLD in organisms over the short-term, and suggests that the contamination pathway is mainly via the bioconcentration process during this time.

4.2. Chlordecone in males and females M. rosenbergii

In the field experiment, results revealed that females tended to be less contaminated than males after 5 and 7 months of exposure to CLD. This finding is consistent with those of Roberts (1981) and Schimmel et al. (1979) who observed higher levels of CLD contamination in males than in females *C. sapidus*. This difference could be explained by the fact that generally, females have higher lipid content than males to ensure the reproduction, as it was already observed in other crustaceans (Gismondi et al., 2012). At 8 months of exposure, CLD concentrations strongly increased in ovigerous and non-ovigerous females than in males. This observation could be due to the cycle of ovarian maturation of females, during which lipid reserves were stored along with CLD before spawning and CLD elimination in eggs, resulting in a fluctuation of CLD concentrations. However, reproductive stages have not been studied in this work and future investigations should focus on the impact of reproductive and/or moulting stages on CLD concentrations in exposed *M. rosenbergii*.

Regarding the CLD concentrations in eggs, it appeared that eggs were more contaminated than females which carried these eggs, whatever the duration of exposure. Our results are in agreement with those of Roberts and Leggett (1980) and Roberts (1981) who observed higher CLD concentration in the egg mass than in corresponding females on the blue crab *C. sapidus* collected from the River James and Lower Chesapeake Bay (Virginia, USA), suggesting that the production of eggs was a major way of CLD depuration. The high CLD concentration in eggs could also be linked with the fact that eggs naturally have higher lipids contents than adults, and therefore lipophilic CLD may accumulate to a higher degree in eggs. Nevertheless, it cannot be excluded that high CLD concentrations in eggs could also be due to the CLD adsorption on the egg mass.

4.3. Chlordecone in the anatomical compartments of M. rosenbergii

According to our results, CLD was mainly accumulated in the hepatopancreas as early as 6 h of exposure and then, was distributed into other anatomical compartments over time. This initial storage of CLD in the hepatopancreas could be explained by its structure and binding properties. Indeed, CLD binds preferentially to serum proteins such as lipoproteins or albumin and therefore, its distribution in the whole organism is quite different compared to other organochlorines (US ATSDR, 1995; US EPA, 2009). In fact, other organochlorine pesticides such as HCB or p,p'-DDE are preferentially distributed in the adipose tissue (Gomez-Catalan et al., 1991; Soine et al., 1982). Moreover, the high proportion of CLD accumulated in hepatopancreas (~90%) in prawns exposed for 6 h showed that the main way of contamination is probably via the gills, since prawns had not been fed yet.

Results also showed that CLD was accumulated in the cuticle (i.e. the cephalothorax and abdomen cuticles). The exoskeleton of crustaceans consists of a calcified cuticle containing chitin, and during the moult cycle, specific metabolic processes occur involving an exchange of calcium between body fluids of crustaceans and their cuticle (Greenaway, 1985; Jung and Zauke, 2008). CLD could be simultaneously transferred through the ectoderm via body fluids to the cuticle (Dittman and Buchwalter, 2010). This accumulation of CLD in cuticle could be considered as a depuration mechanism, since prawns could eliminate CLD during successive moults (Dittman and Buchwalter, 2010). The amount of 15–30% of the total contamination of CLD measured in the cuticle of prawns exposed for several months could be the result this depuration mechanism (Dittman and Buchwalter, 2010; Jung and Zauke, 2008).

4.4. Depuration of chlordecone

A decrease of CLD concentrations in *M. rosenbergii* was highlighted during the depuration experiment. At the end of the experiment, only 15% of the initial concentration remained in the whole prawns. Several studies highlighted the ability of invertebrates to slowly eliminate CLD (Bahner et al., 1977; Roberts, 1981; Schimmel et al., 1979). Bahner et al. (1997) showed the presence of CLD at 1.78 μg g^{-1} (dry weight) in faeces of the oyster *Crassostrea virginica*, which were contaminated at 0.21 μg CLD g^{-1} (wet weight). Moreover, Schimmel et al. (1979) observed the loss of CLD in blue crabs *C. sapidus*, previously fed with contaminated oysters, and placed in a CLD-free environment for 28 days.

The depuration rate observed in our study (k_{2g} = 0.034 $days^{-1}$ and loss of 85% in 45 days) can be considered slow compared to other invertebrate species. For instance, Bahner et al. (1977) showed a rapid depuration of CLD from *C. virginica* at a rate of 35% loss in 24 h, while our results revealed a loss of 35% in 10 days. Moreover, according to equation (5), it was estimated that the MRL value in edible tissues of *M. rosenbergii* (i.e. 20 ng g^{-1}) could be reached after 94 days of depuration in CLD-free water with a constant water flux (to

avoid recontamination if CLD is released in water). This elimination of CLD, decreasing CLD concentration in *M. rosenbergii*, could be due to the detoxification and/or biotransformation processes. Indeed, Gaume et al. (2014) showed that CLD induced expression of enzymes involved in biotransformation and detoxification processes, such as cytochrome P450 or GST (glutathione-S-transferase), in *M. rosenbergii*. These detoxifying enzymes allow oxidation or reduction of exogenous molecules in order to increase their water solubility and facilitate their excretion by the organism (Koenig et al., 2012; Snyder, 1998). These processes occur in the hepatopancreas and shows that hepatopancreas, which is the detoxifying organ, could play an important role in the accumulation, storage and detoxification of CLD in *M. rosenbergii* (Sreeram and Menon, 2005; Zeng et al., 2010). As explained above, biotransformation of CLD is slow, and cannot explain alone the important decrease of CLD concentrations in prawn tissues (US EPA, 2009; Toppari et al., 1996). This decrease of CLD concentration could also be due to the elimination of CLD during successive moults of prawns. Indeed, prawns of *M. rosenbergii* moulted every 9–22 days, and during intermoult stage, CLD could be partly stored in their cuticle before being eliminated from organism with the old cuticle during ecdysis. Another explanation is that this decrease of CLD concentration was due to a cytosolic aldo-keto reductase enzyme (CLD–reductase, CHDR), which is responsible for the conversion of CLD to CLD alcohol in some species of mammals and thus, could increase the excretion of CLD (Molowa et al., 1986). CHDR activity was detected in the liver cytosol of rabbits, gerbils, and humans but was absent in rats, mice, hamsters, and guinea pigs (Fariss et al., 1980; Houston et al., 1981). Nonetheless, using commercial antibodies against the human AKR1C4 (aldo-keto-reductase isoform 1, family C4) and an ELISA quantification method, a CHDR has been detected and quantified in the hepatopancreas of *M. rosenbergii* (Vassaux et al., 2013). In addition, it was shown that, for the same amount of hepatic proteins, the amount of CHDR is higher in the oyster *C. gigas* than in *M. rosenbergii* (Vassaux et al., 2013), and this may explain why the depuration of CLD has been shown to be much more efficient in *C. virginica* than in crustaceans (crabs) (Schimmel et al., 1979). However, further investigations should focus on the functional characteristics of the CDHR in invertebrates and its possible role in the biotransformation of CLD in *M. rosenbergii*.

5. Conclusion

This work investigated the bioaccumulation of chlordecone in the giant freshwater prawn, *Macrobrachium rosenbergii*, using both laboratory and field approaches. Results highlighted a rapid accumulation and high storage capacity of CLD in prawn. Moreover, our results describe for the first time the tissular distribution of CLD in prawns, mainly in the hepatopancreas as early as 6 h of exposure and then, into other anatomical compartments over time. CLD was also accumulated in the cuticle, and this may result in a CLD depuration mechanism of the prawns through successive moults. A decrease of CLD concentration in prawn tissues was also highlighted in the depuration experiment, and it was estimated that under flow-through conditions in CLD-free water, the MRL value of 20 ng g^{-1} in edible tissues of *M. rosenbergii* could be reached after 94 days of depuration. However further measurement should be carried out to confirm this estimation. CLD contamination of environment in Guadeloupe is intended to last several years, decades or centuries and its rapid accumulation from water and slow depuration rate indicate that CLD may be transferred through food chain. Since prawn farming was an important economic resource in Guadeloupe and *M. rosenbergii* can be considered as a good model for the wild *Macrobrachium* spp. living in these regions, results of this study may have practical implications in e.g., the modelling of CLD contamination of freshwater *Macrobrachium* spp. in the river contamination biomonitoring or in the regulatory control of the safety of marketed food products. In addition, CLD has been found to disrupt several hormonally regulated biological processes such as reproduction or development. Further investigations are necessary in order to highlight the deleterious effects of CLD in invertebrates but also biotransformation mechanisms involved in the depuration process and the impacts of reproductive and moulting stages on bioaccumulation of xenobiotics.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.chemosphere.2017.07.099.

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