Ecotoxicology and Environmental Safety 171 (2019) 1–11

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Environmental characterization of 4,4′-dichlorobenzophenone in surface waters from Macao and Hong Kong coastal areas (Pearl River Delta) and its toxicity on two biological models: *Artemia salina* and *Daphnia magna*

Lucia Ivorra[a,*], Patricia G. Cardoso[b], Shek Kiu Chan[a], Karen Tagulao[a,1], Catarina Cruzeiro[c,d,1]

[a] Institute of Science and Environment, ISE—University of Saint Joseph, Macao
[b] CIIMAR/CIMAR — Interdisciplinary Centre for Marine and Environmental Research, University of Porto, Matosinhos, Portugal
[c] Department of Life Sciences, CFE—Centre for Functional Ecology, University of Coimbra, Coimbra, Portugal
[d] Helmholtz Zentrum München, German Research Centre for Environmental Health, GmbH, Research Unit Comparative Microbiome Analysis, Ingolstaedter Landstrasse 1, 85764 Neuherberg, Germany

ARTICLE INFO

Keywords:
Organochlorine pesticides
Dicofol
DDT
Metabolite
LC_{50}
EC_{50}

ABSTRACT

The Pearl River Delta (PRD) is one of the areas with higher environmental concentration of organochlorine pesticides (OCPs), being DDT one of the most abundant. In this work, 4,4′-dichlorobenzophenone (4,4′-DCBP), a common metabolite of dicofol (DDT related) and DDT, was quantified in surface waters of Hong Kong and Macao, together with the analysis of physicochemical and nutrients parameters. Hong Kong presented higher 4,4′-DCBP mean levels (12.50 ng/L) than Macao (4.05 ng/L), which may be due to the use of dicofol as a pesticide and DDT as antifouling-paint for ships. The region presented a possible eutrophication state due to the high nutrients' concentration. For the first time, toxicity evaluation of this metabolite in *Artemia salina* and *Daphnia magna* was done, in order to compute valid EC_{50}s and theoretically evaluate the risk in the PRD. The toxicity results (EC_{50} = 0.27 mg/L for *A. salina; and* EC_{50} = 0.17 mg/L and LC_{50} = 0.26 mg/L for *D. magna*), together with the 4,4′-DCBP levels quantified, indicated a low environmental risk.

1. Introduction

Pesticides contamination in coastal areas has become an important worldwide problem since the 1950s, due to the agriculture system, runoff from treated plants and soil, atmospheric exchange or sewage discharge (Scholtz et al., 2002; Guan et al., 2009; Özkara et al., 2016). These chemicals persist in the environment, affecting surface water quality and having an ecotoxicological effect on aquatic flora, fauna and human health (Miyamoto et al., 1990; Ongley, 1996; Lozowicka et al., 2014; Skretteberg et al., 2015). Organochlorine pesticides (OCPs) are a group of persistent organic pollutants (POPs) used in agriculture worldwide, mainly from the 1950s to the 1980s, and are characterized for their stable chemical structure that allow them to accumulate, persist and biomagnify in the environment for decades (Dimond and Owen, 1996; Nakata et al., 2002; Carvalho, 2017). Because of their characteristics, OCPs are common pollutants in the marine environment (Luo et al., 2004) and have been a worldwide concern due to their reported toxic effects on humans and wildlife (Guan et al., 2009; Mrema et al., 2013); Especially in China, considered as the largest producer and consumer of pesticides in the world, 80% of the pesticides produced before 1983 (year of the official ban) were OCPs (Grung et al., 2015).

The Pearl River Delta (PRD) in South China is considered one of the areas with high environmental concentrations of pesticides due to fast industrial and agricultural development in the region (Tieyu et al., 2005; Guo et al., 2009). During the last two decades, overfishing and pollution problems (Duan et al., 2009) also compromised the water and air quality in the area (Fu et al., 2003; Guan et al., 2009). One of the most common OCPs present in the PRD waters, is dichlorodiphenyltrichloroethane (Grung et al., 2015), also known as DDT, a pesticide that has been widely used for pest control and mosquito abatement prior to the global ban in the 1970s and 1980s (Guo et al., 2009). Previous studies suggested that DDTs concentration levels in the PRD have remained considerably high despite China's official ban in 1983 (Fu et al., 2003; Guo et al., 2009). Zhang et al. (2002), found no sign of declining concentrations of DDTs in sediment cores collected from the PRD during the summer of 1997, with mean values ranging from 2.5 to 22.7 ng/g DW. A more recent study, carried on between December 2009 and March 2010, showed average levels of 18.4 ng/g

* Corresponding author.
E-mail address: 201600297@usj.edu.mo (L. Ivorra).
[1] Both authors contributed equally to this work; joint last authors.

https://doi.org/10.1016/j.ecoenv.2018.12.054
Received 2 August 2018; Received in revised form 12 December 2018; Accepted 18 December 2018

DW (Wei et al., 2015). In addition, sediment samples from Hong Kong and Macao, collected during 2007 showed alarming concentrations (i.e. 76–7 350 and 967–5 810 ng/g DW, respectively), which are 3.8-fold higher than the concentrations found in previous years in the PRD (Lin et al., 2009). The amount of DDTs detected in this environment may be associated to a historical contamination where excessive soil runoff enhanced by the large-scale land modifications and regional flooding might have contributed to the transport of OCPs from soil to the sedimentary system (Zhang et al., 2002; Guo et al., 2009). However, some authors have indicated the existence of currently fresh inputs or unknown sources of DDTs, which may also contribute to these environmental levels (Qiu et al., 2005; Wang et al., 2007), and can be targeted by different DDT isomers ratios. If the ratio between DDT and the sum of its metabolites (DDT/(DDE + DDD)) is higher than 1, and the ratio between DDT isomers (o,p′-DDT/p,p′-DDT) ranges between 1.3 and 9.3, often indicates a new source of DDT pollution, which can be explained by the use of technical dicofol, as mentioned by Fu et al. (2003) and Qui et al. (2005), respectively.

The annual average production of DDTs was about 6 000 t from 1988 to 2002, and nearly 80% of that was used to produce dicofol, a pesticide responsible for some of the new inputs of DDT in the environment (Qiu et al., 2005). According to Guo et al. (2008), the amount of dicofol used in China was almost 9 000 t between 1988 and 2002, and in 2003, an average of more than 14 t of dicofol was applied in the PRD region. Zheng et al. (2016), detected dicofol as the most frequent OCPs in water and sediment samples from Jiulang River (North East China). Since dicofol is produced from technical DDT — through a pathway including chlorination followed by hydrolysis to form the final product — its molecular structure is similar and it is associated with the same concerns as DDT and its metabolites (Fujii et al., 2011). It is estimated that 93% of 4,4′-DDT is converted to 4,4′-dicofol while only 37% of 2,4′-DDT is converted to 2,4′-dicofol (Qiu et al., 2005), with 4,4′-conformation as the main isomer present in the final product, and therefore the 4,4′-dichlorobenzophenone (4,4′-DCBP) as the main breakdown isomer present after the degradation of dicofol (Thiel et al., 2011). Owing to the instability and easy degradation of dicofol in water — when exposed to a higher pH, light or higher temperature — 4,4′-DCBP is the main expected form in surface waters (Fujii et al., 2011; Thiel et al., 2011; Yin et al., 2017). Moreover, it has also been reported that degradation of the main metabolites of 4,4′-DDT can contribute to the levels of 4,4′-DCBP in the environment (Purnomo et al., 2008; Ricking and Schwarzbauer, 2012).

To our knowledge, no previous studies have focused on the environmental characterization of 4,4′-DCBP in PRD; and its toxicity effects. This study may be considered as the first work in which these topics will be addressed, using two biological models, *Artemia salina* and *Daphnia magna*. The crustaceans *A. salina* (brine shrimp) and *D. magna* (water flea) are two invertebrate models that have been widely used for ecotoxicological studies in saline and freshwater environments, respectively (Cleuvers, 2003; Favilla et al., 2006). The life cycle of *A. salina* begins by hatching of dormant inactive cysts (0.2–0.3 mm), into free-swimming nauplii (0.45 mm; instar II/III), in a period of 24–36 h and after being rehydrated in salty water. The larvae are very adaptive to a wide range of salinities (5–250) and temperatures (6–35 °C), having a short life cycle (3–5 weeks to reach adult life) or a high adaptability to adverse environmental conditions (Lu et al., 2012). The life cycle of *D. magna* begins by hatching of dormant inactive eggs (ephippia). The eggs develop in about 3 days into neonates, which can then be used immediately for the toxicity test. The measurement endpoints generally evaluated for this animal model are the 48 h-LC_{50} (for survival), and the 48 h-EC_{50} (for immobility) (Jonczyk and Gilron, 2005). All these characteristics, make them appropriate models for short toxicological tests with low costs in routine and research practices (Cruzeiro et al., 2017; Lu et al., 2012).

Due to the limited knowledge in the potential environmental occurrence and toxicological effects of 4,4′-DCBP, the main goals of the present work were: a) to optimize and validate an analytical GC-MS/MS method to analyse 4,4′-DCBP in water samples; and for the first time b) conduct an environmental characterization of the 4,4′-DCBP levels in surface waters collected from the east and west mouth of the PRD, Hong Kong and Macao, respectively; and c) evaluate the toxicity of 4,4′-DCBP on two aquatic species, *A. salina* and *D. magna*, considered as ideal biological models for determination of LC_{50} and EC_{50}.

2. Materials and methods

2.1. Study area

The Pearl River Delta (PRD), embraced by Hong Kong S.A.R. and Macao S.A.R., is located in southern China (112°00'∼115°25'E and 22°30'∼23°45'N) (Duan et al., 2009). It has a land area of approximately 40,000 km^2 (Guo et al., 2008) and includes the third largest river (331.9×10^9 m^3/yr) in China and the largest river system flowing into the South China Sea (SCS) (Zhao, 1990).

The PRD belongs to the subtropical climatic zone, characterized by high precipitations (1 600–2 200 mm, annually), mild temperature all year around (19.5–22.3 °C) and humidity ranging from high to low, during summer and winter, respectively (Guo et al., 2009). Flood periods occur for at least three months in the summer, and 80% of the total flow befalls between April and September (Chen et al., 2004), where April is considered as a transition month.

Due to the population rise in this region, the amount of sewage discharge increased to around 14% from 2.61 billion m^3 to 2.97 billion m^3, only between 2005 and 2015 (Liu et al., 2018).

2.2. Water collection and quality measurements

Water samples were collected during the transition season (April) and middle of the flood season, also named as wet season (June) of 2017. A total of 10 sampling locations, distributed around Hong Kong (HK1-HK5) and Macao coastal areas (M1-M5), were sampled (Fig. 1). At each site and sampling occasion, water samples (2 L) were collected during low tide (LT) and high tide (HT) into pre-rinsed amber glass bottles for quantification of 4,4′-DCBP, nutrients and psychochemical parameters. For 4,4′-DCBP quantification, all samples (0.5 L) were filtered (0.45 μm glass fibre filter; Sartorious, Germany) and acidified to pH 5 with acetic acid (CH_3COOH; Sigma-Aldrich, USA) prior to extraction for a higher sample stability. During transport and after filtering, water samples were kept at 4 °C in the dark, for a maximum period of 24 h. Details about the chemicals and the reference standards are described in the Supplementary material. Nutrients analysis (i.e. dissolved inorganic nitrogen (DIN, mg/L) and dissolved inorganic phosphorous (DIP, mg/L)), were measured in the laboratory, with a photometer device from Palintest (YSI 9500 photometer, UK). Physicochemical parameters, such as temperature (T, °C), dissolved oxygen (DO, %), total dissolved solids (TDS, g/L), pH, and salinity were measured in situ (using a portable meter (YSI pro plus, USA)), while Chlorophyll a (Chl-a, mg/m^3) and total suspended solids (TSS, g/L) were quantified in the laboratory. Chl-a, was quantified by filtering 500 mL of water, through a Whatman GF/C glass fibre filter, following the protocol of Parsons et al. (1985); and TSS were quantified using 200 mL of the water samples following the protocol described by APHA (1995).

2.3. Water sample pre-concentration (SPE)

In the final and optimized protocol, cartridges were conditioned sequentially with 5 mL of methanol (MeOH), followed by 5 mL of ultrapure water, at a flow rate of 1–2 mL/min. Then, water samples (500 mL) spiked with the surrogate, were loaded into SPE cartridges at a constant flow rate of 5 mL/min and then allowed to dry. Subsequently, the samples were eluted with 2.5 mL of ethyl acetate,

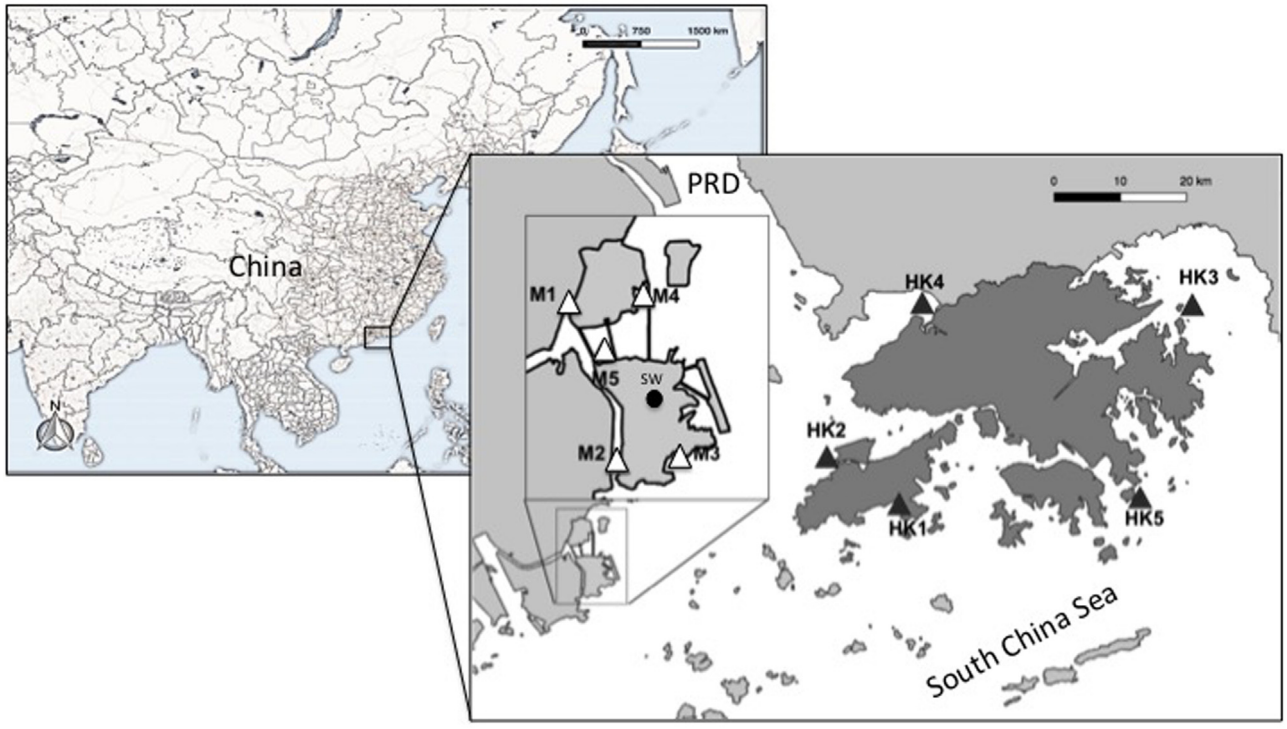

Fig. 1. Map of the Pearl River Delta (PRD) region and the distribution of the sampling sites in Hong Kong (HK1 to HK5) and Macao (M1 to M5); SW indicates the location were the spring water was collected. QGIS 2.18 Desktop, version 2.18.15.

followed by 2.5 mL of dichloromethane and 2.5 mL more of a 1:1 mix of dichloromethane and ethyl acetate (v/v), at a rate of 1 mL/min. The extracts were evaporated to dryness, under N_2 stream (99.995%) and then reconstituted into 200 µL of MeOH. The method optimization is fully described in the Supplementary material.

2.4. Instrumental methods, quality assurance and quality control procedures

Analyses were carried out using a gas chromatograph (Trace 1310 GC, Thermo Scientific), coupled with a triple quadrupole mass spectrometer detector (TSQ 8000 EVO, Thermo Scientific), an autosampler (Thermo ScientificTriPlus™) and a Trace Pesticides column (TR-pesticides II, 30 m × 0.25 mm × 0.25 µm). Column oven temperatures were programmed for a 14 min period using several ramps: a) from 75 °C with an initial equilibrium time of 3 min to b) 180 °C at 30 °C/min until c) 280 °C at 5 °C/min, where the temperature was maintained for 1 min. The injector port temperature was set to 250 °C, and both ion source and MS transfer line were at 280 °C. Helium (99.999% purity) was used as carrier gas and was maintained at a constant flow rate of 1.3 mL/min. Sample injection (1 µL) was in the split-less mode (4 mm straight liner, 453A1925), using a 50 mm long needle.

The performance of the method was checked daily, using method blanks (solvent controls), quality controls (two-fold higher than the limit of quantification), fortified samples spiked with both surrogates, and using, weekly, new calibration curves. The limits of detection (LODs) and quantification (LOQs) were defined as LOD = 3.3 α/S and LOQ = 10 α/S; here, α is the standard deviation slope and S is the average slope of the calibration curves. Linearity, precision, accuracy, and recoveries were evaluated following the criteria established by SANCO/825/00 rev 8.1 (SANCO, 2010) (more details in Supplementary material).

2.5. Biological assay

2.5.1. Artemia salina acute toxicity test

The hatching and the standard operational procedure for *Artemia* toxicity screening test for estuarine and marine waters followed the Artoxkit M protocol from the company Microbiotest (Artoxkit M, Microbiotest), which is based on the ASTM standard Guide E1440-91 (ASTM American Society for Testing and Materials, 1987). Dry cysts (Ocean Nutrition, batch number: ONG01805) were incubated in artificial salty water (35 Sea Salt), previously aerated, at 25 °C and 3000–4000 lx (light intensity). Thirty-six hours later, groups of 10 free-swimming nauplii (animals in instar II and III) were randomly transferred into 2 mL glass beakers and placed in a 24-multiwell plate to a final volume of 1 mL/well. This test was performed in four independent replicates, using one plate with three wells per treatment, and one plate for the standard toxicant reference ($K_2Cr_2O_7$). Animals were exposed during 24 h (maintained in the dark), at 25 °C. The concentrations used for 4,4′-DCBP were 0, 0.019, 0.039, 0.078, 0.156, 0.312, 0.625, 1.25 and 2.5 mg/L (in consideration of its max. solubility in MeOH); and for the $K_2Cr_2O_7$ were 0, 10, 18, 32, 56 and 100 mg/L according to the standard operational procedure of the protocol. The saline control (just saltwater) and the solvent control (0.1% of MeOH) were included in all four plates in triplicates. The same procedure was repeated in three different days. Toxicity was analysed by counting the dead nauplii (no movement in 10 s of observation), using a binocular stereomicroscope (6.5 x of magnification).

In addition, sub-lethal effects in swimming behaviour (*i.e.* displacement (cm) and speed (cm/s)), were also analysed in order to determine the EC_{50} for this compound. For this purpose, the same range of concentrations (mg/L) were used. A total of 192 videos (four animals per treatment, per replica) of 50 s duration each, were recorded and analysed using the UMAtracker software, version 0.1. For both cases, the plate results were valid if mortality of the control group was below 10%.

2.5.2. Daphnia magna acute toxicity test

The test was done using the DaphTox F magna™ kit procedure (MicroBioTests, 2006; Kit number DM232; Batch number DM140217), which is based on the OECD guideline 202. Briefly, pre-rinsed ephippia were incubated in a standard freshwater solution (ISO 6341) at 21 °C, for 72 h with a continuous light exposure of 6000 lx. Afterwards, the hatched animals were collected and fed, with spirulina, for a period of 2 h before the subsequent test exposure. This test was performed in four independent replicates, using one plate with three wells per treatment. Five daphnia neonates were placed per 10 mL well, and stored in the dark at 21 °C, for 48 h. Eight different concentrations (ranging from 0.0195 to 2.5 mg/L, as in the *Artemia* assay), plus the control and solvent control (0.1% MeOH), were tested. After 48 h exposure, mortality of four different plates and same sub-lethal effects (following the same procedure as in *A. salina*) were analysed. For the analysis of the mortality, the number of dead neonates was recorded; and for the analysis of the swimming behaviour, a total of 160 videos (four animals per treatment, per replica), of 50 s duration each, were recorded and analysed using the same software mentioned above. The plate results were valid if mortality rate of the control group was below 10%. To validate the assay, an additional control was performed submitting the animals to different $K_2Cr_2O_7$ concentrations (0.32, 0.56, 1, 1.8, and 3.2 mg/L), as it was described in the protocol.

2.6. Data analysis

Method validation: results represented in Fig. S1 and S2, and Table S2 (Supplementary material) are expressed as mean ± standard deviation of the mean (SD). Statistical analyses were done with the software Prism 6 version 6.0c. After checking assumptions of normality (Kolmogorov-Smirnov test) and homogeneity of variances (Levene's test), data sets were analysed by one-way analysis of variance (ANOVA) with post-hoc comparison via Tukey's test. Logarithmic transformations were applied when assumptions were not accomplished.

Environmental data: water samples were analysed for 4,4′-DCBP and only concentrations above LOQs were used for posterior analyses (data in Table S3). For graphical representation, data were organized according to sampling site (n = 10) and tide (n = 2; Fig. 2) and expressed as final quantified environmental concentrations (ng/L). Normality and homoscedasticity were assessed and differences between sampling sites and tides were analysed using two-way ANOVA, with post hoc comparison via Tukey's test. For the physicochemical parameters and nutrients, Kruskal-Wallis and Mann-Whitney tests were applied, and correlations were explored using Spearman correlation

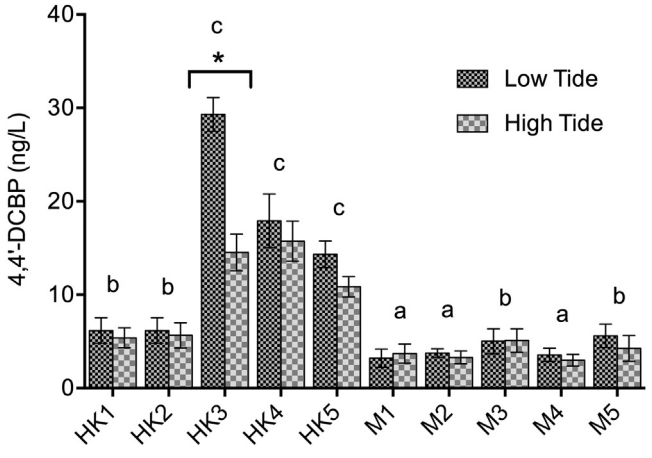

Fig. 2. 4,4′-DCBP levels (ng/L) in Hong Kong (HK1-HK5) and Macau (M1-M5) during high and low tide. Different letters indicate significant differences between sites, while the upper asterisk indicates significant difference between low and high tide for the site HK3. Data are presented as means ± (SD).

(Fig. 3). In addition, heatmap was performed in order to observe how samples were grouped (through cluster analysis), using Euclidean distance and the R software (heatmap.2, version 3.5.0) (Fig. 4).

Toxicity tests: for determination of LC_{50} and EC_{50}, mortality and abnormal swimming behaviour data was verified for outliers using Rout (alpha = 0.5%). Data was treated as: % = (4,4′-DCBP/Solvent Control)*100 before logarithmical transformation and data normalization; then, a non-linear regression was applied (Fig. 5c and d, 6c and d, 7b). Mortality rates, for *D. magna* and *A. salina*, were also analysed and presented as mortality (%) vs. toxic concentration (μg/L) (Fig. 7a). Considering that the solvent control group is the appropriate control group for comparisons with treated groups (OECD, 2006) and no significant differences were observed between control and solvent control, the "solvent only method" (Green, 2014) was the one followed to do all the comparisons. One-way ANOVA followed by Dunnett's post-hoc test, were performed to study differences between the different concentrations and the solvent control.

Risk assessment: a simplified theoretical model approach was used, to predict the environmental hazard of 4,4′-DCBP detected levels, as suggested by Backhaus and Faust (2012). The EC_{50} (mg/L) values used in this theoretical approach were the ones obtained in the acute-toxicity tests with *D. magna* and *A. salina*. The Predicted No-Effect Concentrations (PNECs) was calculated by the ratio between the EC_{50} (mg/L) levels and an assessment factor (AF) of 100, as: PNEC = EC_{50}/AF; the assessment factor was stabilised according to the Water Framework Directive (2000/60/EC, European Commission 2000), and considering that only the EC_{50} data from one trophic level was used in this approach. Then, the risk quotient (RQ) was calculated as the ratio between measured environmental concentration (MEC) and PNEC: RQ = MEC/PNEC. If the RQ was higher than 1, indicates high risk, if $0.1 < RQ < 1$, medium risk and if $RQ < 0.1$, low risk.

3. Results

3.1. Method validation

Eight nominal calibration standards mixtures, with concentrations ranging from 3 to 400 ng/L and a fixed surrogate concentration of 50 ng/L, were spiked in the spring water matrix (salinity ca. 20). The calibration curves proved to have good fits with r^2 ranging from 0.986 to 0.999 and a final LOD and LOQ of 0.272 and 0.824 ng/L were obtained, respectively. Considering the three studied concentrations (2LOQ = 1.60 ng/L, 20LOQ = 16.48 ng/l and 100LOQ = 82.40 ng/L), all validation criteria presented successful ranges established by SANCO/825/00 rev 8.1 (SANCO, 2010). The final recovery rates ranged from 72.13% to 121.24%, while precision results (0.67–13.95%) were always below the maximum established, and accuracy ranged from 72.74% to 116.00%, demonstrating high robustness during the extraction process (detailed data in Table S2).

3.2. Water quality

4,4′-DCBP levels measured in Macao and HK coastal areas presented a different spatial pattern during the transition season (Fig. 2). Significant differences were found between Macao and HK sites (2-Way ANOVA, $F_{(9,20)} = 166.5$, $p < 0.05$). Namely, sites HK3, HK4 and HK5, presented significantly higher levels of contamination, with a range of 10.85–29.87 ng/L, than the remaining ones. Intermediate levels of 4,4′-DCBP (6.17–4.26 ng/L) were detected in both coastal areas, specifically in HK1 and HK2 (corresponding to Lantau island) and M3 and M5. The lowest levels of 4,4′-DCBP were detected in Macao, encompassing M1, M2 and M4, ranging from 3.77 to 2.98 ng/L.

Comparing tides, no significant differences ($p > 0.05$) were observed between high and low tide for all sampling sites, except for HK3 (2-Way ANOVA, $F_{(1,20)} = 28.11$, $p < 0.05$). However, a significant interaction was found between tides and sites (2-Way ANOVA, $F_{(9,20)}$

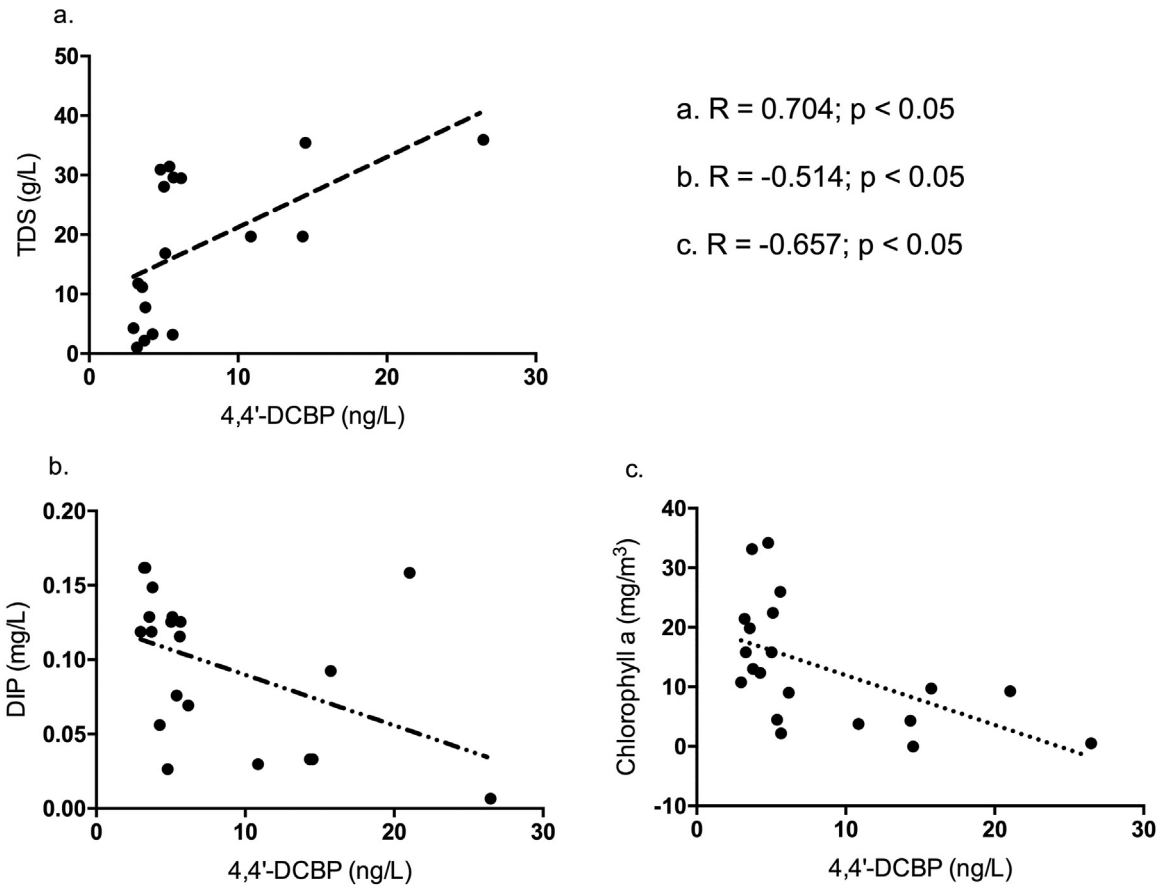

Fig. 3. Spearman correlations of 4,4′-DCBP levels during transition season vs. (a) TSS, (b) DIP and (c) Chl-a.

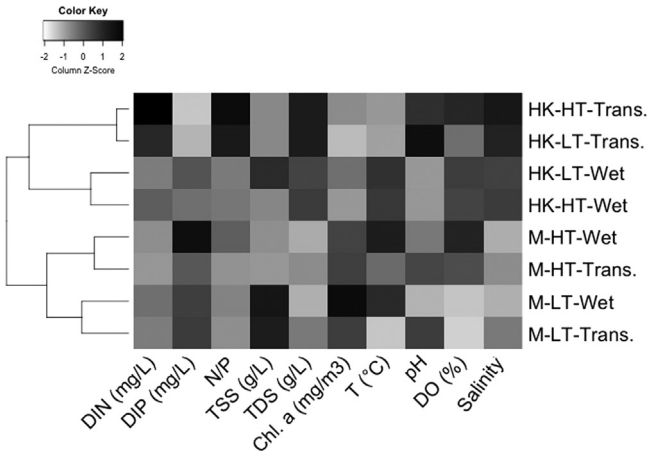

Fig. 4. Heat map representing the physicochemical parameters and nutrients quantified in the surface waters of Macao and Hong Kong during wet and transition (Trans.) season. Scale is indicated by the color key; the higher the value the darker the color.

= 4.73, $p < 0.05$) indicating an influence of the tide in the levels of 4,4′-DCBP detected only in HK3.

Regarding the wet season campaign, 95% of the obtained values were below LOQ, except for M1-LT, presenting similar concentrations (≈ 2.80 ng/L) to the ones detected in the transition season but no significant differences were found between them (*t*-test, $p > 0.05$). Detailed data can be consulted in Table S3.

Nutrients and physicochemical data for the different sampling sites are summarised on Fig. 4 and Tables S4a and S4b. Higher DIN levels were recorded during the transition season, mainly for HK1-HT (16.43 mg/L), HK2-LT (14.73 mg/L), and HK2-HT (13.19 mg/L). Significant DIN differences were found between transition (5.52 mg/L) and wet (2.21 mg/L) seasons (Mann-Whitney, U = 21, $p < 0.05$) but only for Hong Kong waters. Significant differences were also found between Macao (0.14 mg/L) and Hong Kong (0.09 mg/L) coastal areas for DIP levels (Mann-Whitney, U = 126.5, $p < 0.05$), where higher values were observed during wet season and especially in M4-HT (0.59 mg/L) and M1-LT (0.22 mg/L). For the N/P ratio, higher levels were found in Hong Kong waters during the transition season, registering the highest ratios for HK2-HT (1106.47), HK3-LT (662.94), and HK2-LT (429.73).

Concerning the physicochemical parameters, higher TSS levels were found in Macao in M5-LT, during wet season (0.76 g/L) and in M5-LT, during transition season (0.40 g/L). Only for Macao coastal waters, significant differences were observed between tides (0.26 g/L and 0.05 g/L for LT and HT, respectively; Mann-Whitney, U = 17.50, $p < 0.05$). Macao waters also presented higher Chl-a levels (Table S4a), mainly during wet season for M4-LT and M1-LT (49.66 and 44.72 mg/m^3, respectively). In addition, during the transition season, significantly higher levels were observed in Macao (19.06 mg/m^3) than in Hong Kong (7.74 mg/m^3; Mann-Whitney, U = 10, $p < 0.05$).

Higher TDS levels were found in HK3 during transition and in HK4 during wet season (35.68 and 33.95 g/L, respectively). Significant differences were found between Hong Kong and Macao locations, for both seasons (Kruskal-Wallis, H = 24.52, $p < 0.05$). Concerning DO (%) levels, most of the sampling locations presented acceptable levels (78.52–151.3%) according to the 75/440/EEC Directive, which establishes a minimum of 70% DO for surface waters (European Economic Commission, 1975). However, locations like HK2 (except for HT transition season), HK4-LT, M1 (except for HT wet season), and M4-LT (during transition season) presented levels below the optimum established. Average temperature levels in both coastal waters were 23.86 °C

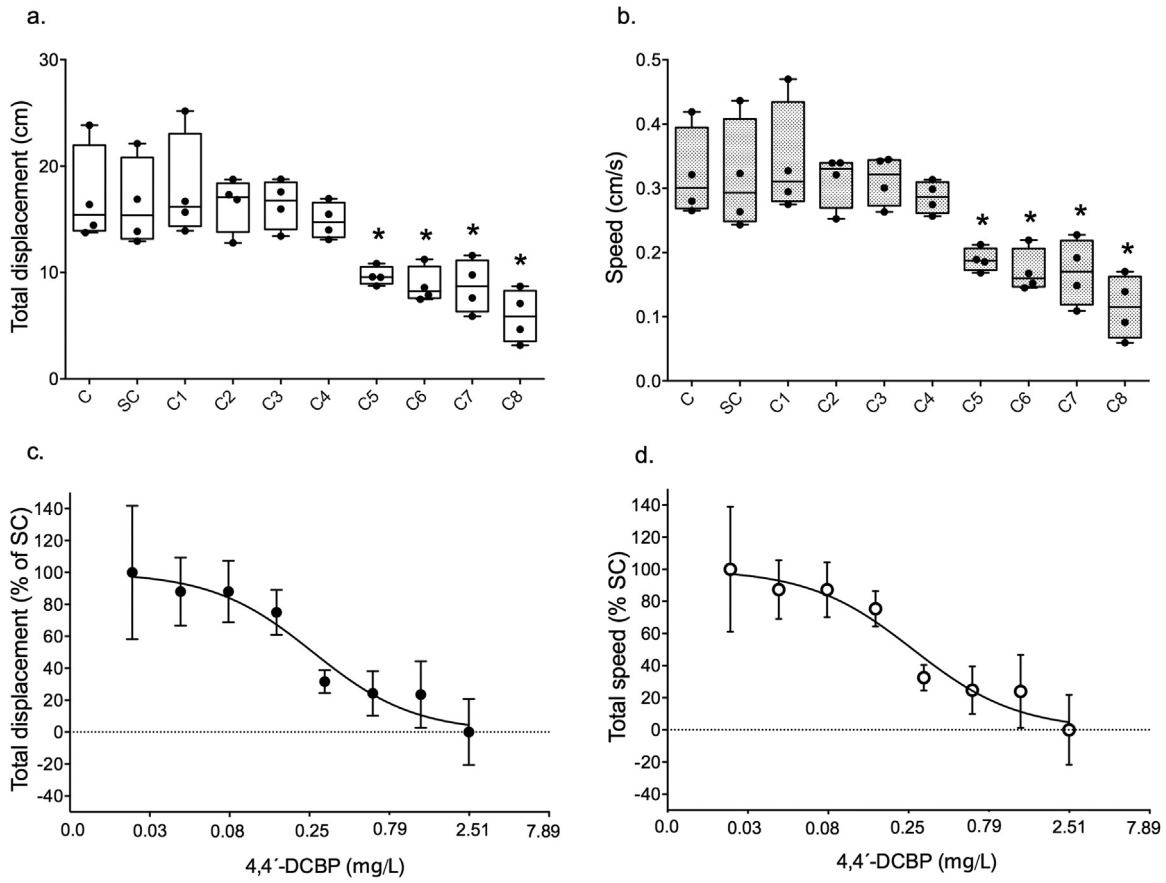

Fig. 5. Total displacement and speed of *A. salina* (n = 4 plates) after 24 h exposure to different 4,4′-DCBP concentrations: C = control, SC = solvent control, C1 = 0.019 mg/L, C2 = 0.039 mg/L, C3 = 0.078 mg/L, C4 = 0.156 mg/L, C5 = 0.315 mg/L, C6 = 0.625 mg/L, C7 = 1.25 mg/L and C8 = 2.5 mg/L. The figures a) and b) represent box and whisker plots of the total displacement (cm) and speed (cm/s), respectively. The horizontal line within the box indicates the median, boundaries of the box indicate the 25th- and 75th -percentile, and the whiskers indicate the highest and lowest values of the results. Upper asterisk indicates significant differences among the treatments and the SC. The figures c) and d) represent a dose-response experiment considering displacement (%) and d) speed (%), respectively. The values at each concentration were calculated as follows: % = (4,4′-DCBP/Solvent Control)*100, and vertical bars represent ± (SD).

and 29.46 °C for transition and wet season, respectively. Salinity ranged from 3 to 35 for all the locations, except for M1 (< 3). As can be observed in the cluster analysis (Fig. 4), physicochemical and nutrients distribution showed a different pattern between Hong Kong and Macao coastal waters. Within Hong Kong territory, differences between seasons were stronger than between tides, while in Macao differences between tides were stronger than between seasons.

3.3. Toxicity assays

3.3.1. Artemia salina

Acute toxicity tests using *A. salina* (24 h exposure) were performed in order to obtain the mortality rate (LC_{50}) and the sub-lethal effects (EC_{50}). An average LC_{50} of 48.46 mg/L was computed for the positive control ($K_2Cr_2O_7$), which is in accordance with the ARC-test range (28–64 mg/L; Vanhaecke andPersoone, 1984). Considering the effects of this metabolite in the swimming behaviour, a final average 24 h-EC_{50} value of 0.26 mg/L and 0.27 mg/L, were obtained for total displacement and speed, respectively (Fig. 5). Significant differences were also found between SC and C5-C8 concentrations (0.315 and 2.5 mg/L; respectively) for total displacement (Fig. 5a) and speed (Fig. 5b) analyses (One-way ANOVA, $F_{(8)} = 9.878$, $p < 0.05$ and One-way-ANOVA, $F_{(8)} = 9.927$, $p < 0.05$, respectively).

3.3.2. Daphnia magna

Lethal and sub-lethal effects of 4,4′-DCBP were studied using an acute test in *D. magna* (48 h exposure). Considering the effects of this metabolite in the swimming behaviour, a final average 48 h-EC_{50} value of 0.17 mg/L, was obtained (Fig. 6). Significant differences were also found between C8 (2.5 mg/L) and SC for total displacement (Fig. 6a) and speed (Fig. 6b) analysis (One-way ANOVA, $F_{(8)} = 5.165$, $p < 0.05$, and $F_{(8)} = 5.356$, $p < 0.05$ respectively). Regarding the mortality rate, a good fit line response was achieved ($r^2 = 0.79$), reaching an average 48 h-LC_{50} of 0.26 mg/L (Fig. 7).

4. Discussion

4.1. Method validation

The method validated in the present study, for the analysis of 4,4′-DCBP in surface waters, accomplished all the criteria (i.e., evaluation of linearity, accuracy, precision and recoveries) established by SANCO/825/00 rev.8.1 (SANCO, 2010), and the stability of the extracts showed ideal recovery rates, in the first 48 h, demonstrating the importance of analysing all the extracted samples within this period. Due to the lack of pesticide-free coastal water, a spring water source from Coloane (SW, Fig. 1) was used as a matrix for the method validation with a previous addition of aquarium reef salt to simulate the average salinity conditions of the selected coastal areas (ca. 20); this matrix demonstrated to be valid for 4,4′-DCBP extraction and quantification method. With this optimized method, it was possible to quantify the metabolite at very low range of concentrations (0.8–50 ng/L) in different surface coastal waters with a wide range of salinities (0.19–36.07), confirming its robustness.

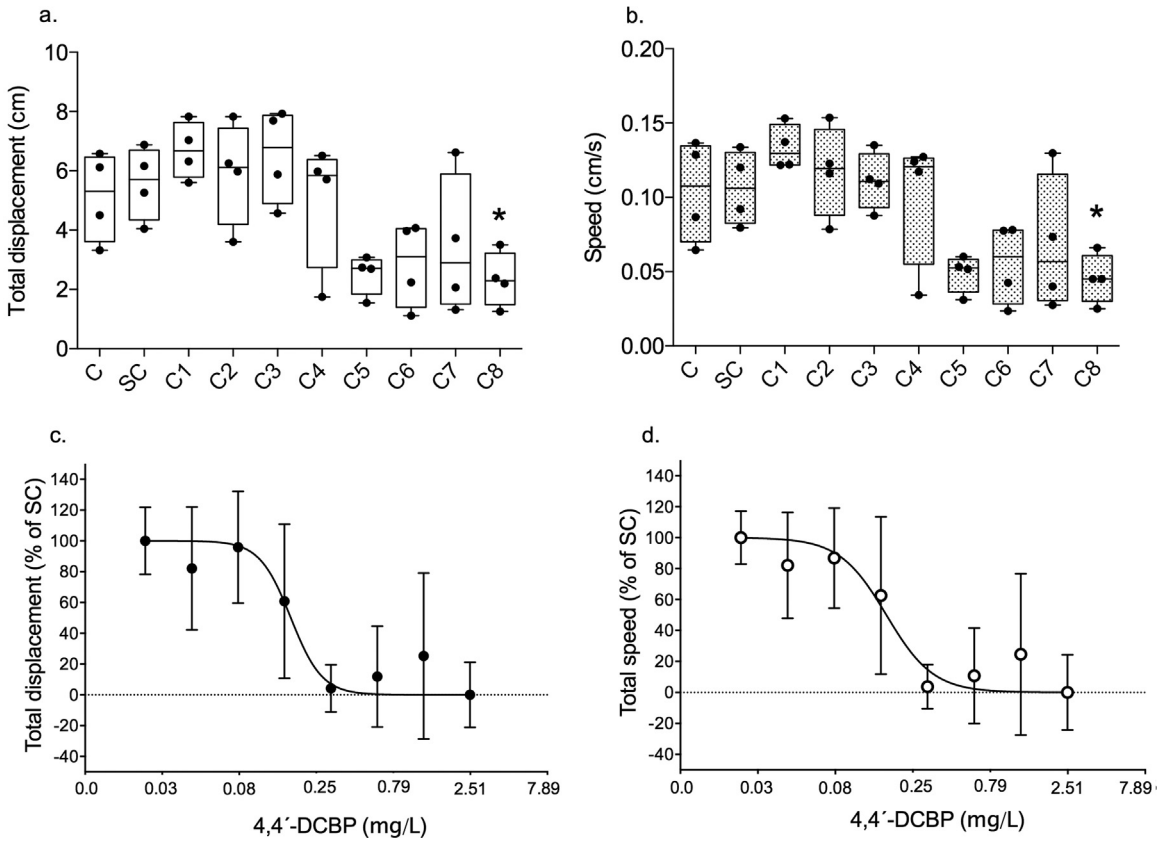

Fig. 6. *D. magna* (n = 4 plates) total displacement and speed after 48 h exposure to different 4,4′-DCBP concentrations; C = control, SC = solvent control, C1 = 0.019 mg/L, C2 = 0.039 mg/L, C3 = 0.078 mg/L, C4 = 0.156 mg/L, C5 = 0.315 mg/L, C6 = 0.625 mg/L, C7 = 1.25 mg/L and C8 = 2.5 mg/L. The figures a) and b) represent box and whisker plots of the total displacement (cm) and speed (cm/s), respectively. The horizontal line within the box indicates the median, boundaries of the box indicate the 25th- and 75th -percentile, and the whiskers indicate the highest and lowest values of the results. Upper asterisk indicates significant differences among the treatments and the SC. The figures c) and d) represent a dose-response experiment considering displacement (%) and d) speed (%), respectively. The values at each concentration were calculated as follows: %= (4,4′-DCBP/Solvent Control)*100, and vertical bars represent ± (SD).

4.2. Water quality

The average 4,4′-DCBP values obtained for HK were similar to the ones reported in the mouth of the Yongdingxin River (16.75 ng/L), north of China (Wan et al., 2005), while the levels quantified in Macao (4.05 ng/L) were comparable with the ones found in the River Elbe (3.8 ng/L), Germany (Federal Environmental Agency, 2008). The strong and frequent rainy periods commonly observed during wet season, may have contributed to the vestigial concentrations found due to dilution effects. This temporal pattern was also observed by Yang et al. (2012) when several OCPs (including DDTs) were detected from Guangzhou (Pearl River Delta) from March to August 2005. Also, Zheng et al. (2016) observed a similar seasonal pattern for dicofol in Jiulong River (South China). No further information regarding 4,4′-DCBP has been

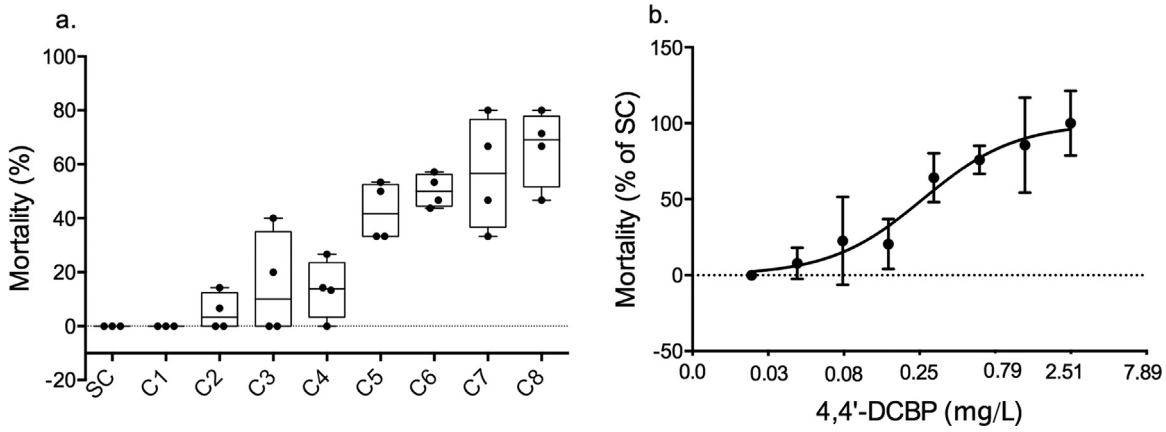

Fig. 7. *D. magna* (n = 4 plates) mortality rates (%) after 48 h exposure to different 4,4′-DCBP concentrations; C = control, SC = solvent control, C1 = 0.019 mg/L, C2 = 0.039 mg/L, C3 = 0.078 mg/L, C4 = 0.156 mg/L, C5 = 0.315 mg/L, C6 = 0.625 mg/L, C7 = 1.25 mg/L and C8 = 2.5 mg/L. The figures a) represents box and whisker plots of the mortality rate (%). The horizontal line within the box indicates the median, boundaries of the box indicate the 25th- and 75th -percentile, and the whiskers indicate the highest and lowest values of the results. The figure b) represents a dose-response experiment considering the mortality. The values at each concentration were calculated as follows: %= (4,4′-DCBP/Solvent Control)*100, and vertical bars represent ± (SD).

reported in other countries, which once more shows the importance of this study.

The highest 4,4′-DCBP concentrations found in Hong Kong (mainly in HK3, HK4 and HK5), may be due to an extensive use of the main precursor pesticides, like dicofol, DDT, chloropropylate and chlorobenzilate, in the area. Although, chloropropylate and chlorobenzilate have also been reported as possible sources (Knowles and Ahmad, 1971; Yin et al., 2017), information regarding its use in China is scarce and only some residues of those pesticides have been described, in tea leaves from Indonesia (1968) and India (1969; Bartsch et al., 1971). Therefore and considering the usage and detected levels of dicofol and DDT in China (Tieyu et al., 2005; Grung et al., 2015; Zheng et al., 2016), these pesticides can be considered as the main precursors of 4,4′-DCBP in this aquatic environment.

Since 1950s, DDT began to serve as efficient additive for antifouling, which are considered as a potential regional source of DDT in the PRD (Guo et al., 2008; Xin et al., 2011). The shipping and fishery industry — that use those paints to prevent the adhesion of sea organisms — could explain in part, the higher levels of 4,4′-DCBP detected in Hong Kong. Air samples analysed by Wang et al. (2007), showed higher levels of DDT in Hok Sui (corresponding to our HK3) and Tap Mui (corresponding to our HK5), when compared to other areas of Hong Kong. Other studies done in this region also reported higher DDTs levels in Hong Kong (0.8–5.6 ng/L) when compared to Macao water samples (0.48–2.8 ng/L)(Luo et al., 2004; Wurl et al., 2006).

Dicofol, other potential source of DCBP since late 1950s, has been mainly used as a pesticide in southern China, including provinces such as Guangdong, Guangxi and Fujian (World Health Organization, 1996; Qiu et al., 2005). Due to its biomagnification potential in terrestrial environments (DT_{50} 30–60 days) and its susceptibility for hydrolysis with increasing pH (DT_{50} pH 5 = 47–85 days; DT_{50} pH 7 = 8–64 h; Reregistration Eligibility Decision (RED) Dicofol, 1998; OSPAR Commission, 2002), degradation of dicofol to 4,4′-DCBP can occur during its transport from land to coastal areas. While its usage is not allowed in Europe, and although with a decline from 27% (in 1999) to less than 8% after 2008 (Yang et al., 2008; Li et al., 2015), dicofol is still being used in China, especially to control mites, like *Tetranychus cinnabarinus*, *Tetranychus viennensi* and *Phyllocoptruta oleivora* on cotton, citrus and apple trees (United Nations Environmetal Programme, 2016). This could explain the higher concentrations of dicofol reported in surface waters (64.66 ng/L) from the Jiulong River (China; Zheng et al., 2016), when compared to rivers from Greece (< 0.1 ng/L) and United States (2.5 ng/L) (OSPAR Commission, 2002). As reported by the Stockholm Convention on Persistent Organic Pollutants, the current use of dicofol in Asia is below 1000 t/y (United Nations Environmetal Programme, 2016) and Hong Kong is one of the locations where dicofol can still be used, as it is indicated by the Agriculture, Fisheries and Conservation Department of Hong Kong Government (The Government of the Hong Kong Special Administrative Region, 2006). According to the European 2013/39/EU Directive, dicofol has a limit of 0.32 ng/L for surface waters (European Union, 2013). It is expected that 4,4′-DCBP, as the main and the most persistent metabolite of dicofol, will be present in the environment. All samples above LOQ presented higher 4,4′-DCBP levels than the established limit for dicofol (precursor).

Average DIN concentrations of 1.58 mg/L and 3.89 mg/L were obtained, for Macao and Hong Kong, respectively, presenting higher DIN values than the ones reported by Zhang et al., (2014, 2017) (i.e. 0.42–1.36 mg/L and 0.07–0.14 mg/L) for PRD water samples collected during 2005–2007. The DIN levels detected were also higher than the values reported in the Marine Water Quality report for Hong Kong (Marine Water Quality in Hong Kong, 2016). However, similar values to the ones observed in our study were quantified by Chen et al. (2012) for water samples from Yuqiao reservoir (north of China; 1.21–5.22 mg/L), between 1989 and 2007. The DIN levels obtained for Hong Kong and Macao were considerably higher than the ones reported by the European Environmental Agency (2012) for Mediterranean and Baltic seas, and thus could become a potential threat for the trophic state of the PRD. A similar pattern, regarding the significant difference observed between seasons in Hong Kong samples (wet = 2.21 mg/L; transition = 5.52 mg/L), was also observed by Zhang et al. (2014) in surface waters from PRD. The decrease in DIN levels generally in spring and summer (corresponding to wet season) was probably attributed to the decreasing trend of Pearl River runoff downstream (Zhang et al., 2014).

DIP average levels found in Macao water samples, presented values above the limit established by the Environmental Protection Agency (U.S. Environmental Protection Agency, 1988; 0.1 mg/L for surface waters). In addition, during wet season and as a consequence of an increase in the precipitations rate, a worst scenario regarding DIP levels was observed. Overall, the average DIP levels obtained in this study for both sampling campaigns (0.14 mg/L for Macao and 0.09 mg/L for Hong Kong) were higher than the maximum levels (0.04 mg/L) reported by Li et al. (2017) in PRD water samples (in 2015–2016) and higher than the levels reported in the Hong Kong water quality report (Marine Water Quality in Hong Kong, 2016).

Almroth and Skogen (2010), classified the southeastern part of the North Sea (DIN = 0.19 mg/L; DIP = 0.02 mg/L), Kattegat (DIN = 0.08 mg/L; DIP = 0.05 mg/L), Gulf of Riga (DIN = 0.14 mg/L; DIP = 0.09 mg/L), and Golf of Finland (DIN = 0.05 mg/L; DIP = 0.05 mg/L) as problematic areas regarding eutrophication; Cardoso et al. (2010) reported levels of DIN < 1 mg/L and DIP < 0.12 mg/L in the Mondego River (Portugal), during eutrophication period. The levels obtained in our study for Macao (DIN = 1.6 mg/L; DIP = 0.14 mg/L), and Hong Kong (DIN = 3.87 mg/L; DIP = 0.09 mg/L), are similar or higher than those ones, suggesting some signs of eutrophication in these areas too.

Regarding the N/P ratio, higher levels were found in Hong Kong waters during the transition period, mainly in HK2-HT (1106.5), HK3-LT (662.9), followed then by HK2-LT (428.7). The N/P ratio in a water body indicates which element will be the limiting factor, and consequently which one has to be controlled in order to reduce a possible increase in algae population (algal bloom) (Eutrophication and Health, 2002). The majority of our sampling locations (77.5%) presented a N/P ratio greater than the N-limitation boundary (16N:1P; Redfield, 1934), suggesting that DIP is the limiting factor for Macao and Hong Kong coastal areas. These results are in agreement with those from the Mondego estuary (Portugal) during the eutrophication period (Cardoso et al., 2010), which reinforces the idea about the eutrophic state of HK and Macao coastal waters.

Overall, the heatmap analysis (Fig. 4), showed distinct patterns between Hong Kong and Macao surface waters. Higher levels of DIN and TDS were quantified in Hong Kong, while DIP and Chl-a showed higher values in Macao waters.

In addition, significant correlations ($p < 0.05$; Fig. 3) were found between the quantified 4,4′-DCBP levels during the transition season and physicochemical parameters, like TDS, DIP and Chl-a. A positive correlation was observed between 4,4′-DCBP vs. TDS levels ($R = 0.704$, $p < 0.05$), which can be explained by the high hydrophobicity (octanol-water partition, log K_{ow} = 4.01; Han et al., 2011) of 4,4′-DCBP. Considering the theoretical log K_{ow} = 4.44, calculated through ECOSAR version 1.11 (EPISuite Kowwin v1.68 Estimate), it is expected that a substantial fraction of this metabolite will be adsorbed to suspended sediment rather than the water column (Reregistration Eligibility Decision (RED) Dicofol, 1998)., In our study, higher levels were detected in waters with higher TDS amounts, which may have an implication in the flora and fauna of the area.

On the other hand, significant negative correlations were observed for 4,4′-DCBP vs. DIP and 4,4′-DCBP vs. Chl-a ($p < 0.05$; $R = -0.514$ and $R = -0.657$, respectively). These results may be due the lower precipitation levels (≈ 137.9 mm) observed during the transition season (the only data used for these correlations), which can influence DIP and Chl-a levels, leading to negative correlations in both cases.

However, for the first correlation (4,4′-DCBP vs. DIP), other external factors may had contributed to the DIP levels found, like different sources of phosphates (i.e. detergents, fertilizers, and organophosphate pesticides), since this metabolite lacks a phosphate group. Considering the second correlation, our results also showed that locations with higher 4,4′-DCBP levels presented lower Chl-a levels, indicating a possible negative effect on algal population or biomass in the area. *In vitro* studies, using the organochlorine endosulfan (at ranges of 0.001–0.05 mg/L), showed the negative effect of the pesticide on microalgae population growth, through the reduction of chlorophyll content (Ebenezer and Ki, 2014; Sinha et al., 2015). Further in vitro studies should be conducted with 4,4′-DCBP to provide better insights on its effect on other important aquatic organisms, like microalgae.

4.3. Toxicity assays

For both animal models, the reference test ($K_2Cr_2O_7$) results were in accordance with the ARC-test range (28–64 mg/L; Vanhaecke and Persoone, 1984) and with ISO 6341 range (0.6–2.1 mg/L, after 24 h; Persoone et al., 2009), defined for *A. salina* and *D. magna*, respectively, indicating a normal resistance to this compound, thus allowing to compare these data to other published assays.

For *A. salina,* average 24 h-EC_{50} of 0.27 mg/L was successfully obtained, considering results from both swimming behaviour parameters. However, it was not possible to compute a mortality dose response because of the maximum solubility limit of the compound (2.5 mg/L in MeOH). For *D. magna*, we were able to compute both 48 h-LC_{50} and 48 h-EC_{50} values, where the average 48 h-LC_{50} value (0.26 mg/L; Fig. 7) was higher than the average 48 h-EC_{50} value (0.17 mg/L). However, and as shown by the extensive databases on acute effects of chemicals on Daphnids and in our own results, LC_{50}s and EC_{50}s, do not differ markedly and this probably explains why the Commission of the European Communities in the section on acute toxicity testing for *Daphnia,* in Directive 92/69/EEC, specifies that “the Directive requirement for the LC_{50} for *Daphnia* is considered to be fulfilled by the determination of the EC_{50} as described in this method” (EEC, 1992).

D. magna 48 h-EC_{50} of DDT and dicofol, were fixed as 0.005 mg/L and 0.14 mg/L, respectively (FOOTPRINT PPDB), indicating a higher toxicity of the original forms in comparison with this metabolite. Considering all locations and both toxicity levels obtained for both animal models, a RQ < 0.1 was calculated, indicating that the quantified 4,4′-DCBP values in the surface waters of PRD mouth, represent a potential low environmental risk. However, there are still several reasons to consider these levels a concern to the environment. As it was mentioned before, 4,4′-DCBP has a high log $K_{OW,}$ which may lead to bioaccumulation and biomagnification processes contaminating all the edible fauna, which directly or indirectly can constitute a risk to human health. Moreover, concentrations of this metabolite were already registered in bird eggs, showing a capacity of direct transmission to offspring (United Nations Environmetal Programme, 2016). *In vitro* studies also showed the antagonistic effect of 4,4′-DCBP towards the androgen receptor (with concentration range of 2.5 mg/L-25 g/L), leading to possible endocrine disrupting effects in wild life population (Thiel et al., 2011). All these facts alert us to potential risks that may affect the ecosystem and future generations.

5. Final remarks

With the successfully validated method, the metabolite 4,4′-DCBP was detected in surface waters from Macao and Hong Kong coastal areas, mainly during the transition period, demonstrating a seasonal pattern; also DIN and TDS levels were higher during this period in comparison with the wet season. During the wet season, and due to a higher river discharge, 4,4′-DCBP levels were below the LOQ, and an increase in DIP, TSS and Chl-a values was also observed. This demonstrates the importance of temporal samplings to characterize the current status of an ecosystem. Overall, levels of DIN and DIP were higher than in other water systems (as the Baltic sea), which reveals signs of lower water quality in this region.

Due to the lack of information regarding 4,4′-DCBP, toxicological tests (using two well-established models) were done in parallel with the monitoring campaign, obtaining EC_{50}s values for both species; this data is important to further apply theoretical approaches and evaluate the possible impact of the concentrations found in surface waters. In this case, a RQ < 0.1 was obtained indicating a low potential risk.

As final remarks, this work highlights the importance of studying metabolites and the need of analyse different matrices and trophic levels (since metabolites are very stable in the organic matter content), assess the toxicological effect in additional biological models (like algae and cell line cultures), and evaluate theoretically the potential effects of this metabolite in the ecosystem and in human health risk through the ingestion of contaminated edible species (bioaccumulation and biomagnification effect through trophic levels).

Acknowledgements

This work was financially supported by Fundo para o Desenvolvimento das Ciências e da Tecnologia (FDCT 117/2014/A3). The author PG Cardoso was supported by the Portuguese Science and Technology Foundation (FCT), through an FCT investigator contract (IF/01506/2014) and C Cruzeiro was supported by a Postdoctoral fellowship from FCT (SFRH/BPD/120558/2016). We are indebted to Susana Wong Un Man for the help in most of the sample collection from Hong Kong and Macao. We also thank Alex Lebel for his assistance during our field campaigns in Macao.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2018.12.054.

References

Agriculture, Fisheries and Conservation Department. The government of The Hong Kong Special Administrative Region. 〈http://www.afcd.gov.hk/english/quarantine/qua_pesticide/qua_pes_pes/qua_pes_part2.html〉.

APHA, 1995. Standard Methods for the Examination of Water and Wastewater, 19th ed. American Public Health Association.

ASTM American Society for Testing and Materials, 1987. Standard guide for conducting renewal life-cycle toxicity tests with *D. magna*. Annual Book of ASTM Standards, vol. E 1193. ASTM, Philadelphia, pp. 765–781.

Almroth, E., Skogen, M.D., 2010. A North Sea and Baltic Sea model ensemble eutrophication assessment. Ambio 39, 59–69. https://doi.org/10.1007/s13280-009-0006-7.

Backhaus, T., Faust, M., 2012. Predictive environmental risk assessment of chemical mixtures: a conceptual framework. Environ. Sci. Technol. 46, 2564–2573. https://doi.org/10.1021/es2034125.

Bartsch, E., Eberle, D., Ramsteiner, K., Tomann, A., Spindler, M., 1971. The carbinole acaricides: chlorobenzilate and chloropropylate. Residue Rev. 39, 1–93.

Carvalho, F.P., 2017. Pesticides, environment, and food safety. Food Energy Secur. 6, 48–60. https://doi.org/10.1002/fes3.108.

Chen, J.C., Heinke, G.W., Jiang Zhou, M., 2004. The Pearl River Estuary pollution project (PREPP). Cont. Shelf Res. 24, 1739–1744. https://doi.org/10.1016/j.csr.2004.06.004.

Chen, Y.Y., Zhang, C., Gao, X.P., Wang, L.Y., 2012. Long-term variations of water quality in a reservoir in China. Water Sci. Technol. 65, 1454–1460. https://doi.org/10.2166/wst.2012.034.

Cleuvers, M., 2003. Aquatic ecotoxicity of pharmaceuticals including the assessment of combination effects. Toxicol. Lett. 142, 185–194. https://doi.org/10.1016/S0378-4274(03)00068-7.

Cruzeiro, C., Amaral, S., Rocha, E., Rocha, M.J., 2017. Determination of 54 pesticides in waters of the Iberian Douro River estuary and risk assessment of environmentally relevant mixtures using theoretical approaches and *Artemia salina* and *Daphnia magna* bioassays. Ecotoxicol. Environ. Saf. 145, 126–134. https://doi.org/10.1016/j.ecoenv.2017.07.010.

Dimond, J.B., Owen, R.B., 1996. Long-term residue of DDT compounds in forest soils in Maine. Environ. Pollut. 92, 227–230. https://doi.org/10.1016/0269-7491(95)00059-3.

Duan, L.J., Li, S.Y., Liu, Y., Jiang, T., Failler, P., 2009. Ocean & coastal management a trophic model of the Pearl River Delta coastal ecosystem. Ocean Coast. Manag. 1–9.

https://doi.org/10.1016/j.ocecoaman.2009.04.005.
Ebenezer, V., Ki, J.S., 2014. Quantification of toxic effects of the organochlorine insecticide endosulfan on marine green algae, diatom and dinoflagellate. Indian J. Mar. Sci. 43, 393–399.
EEC, 1992. Directive 92/69/EEC, Official Journal of the EEC L 383 A: Methods for the determination of Ecotoxicity. C.2. Acute toxicity for *Daphnia*.
European Commission, 2000. Common implementation strategy for the water framework directive (2000/60/EC). Tech. Guid. Deriving Environ. Qual. Stand. https://doi.org/10.2779/43816.
European Commission Directorate General Health and Consumer Protection, 2010. Guidance document on pesticides residue analytical methods. In: Directorate General Health and Consumer Protection. p. 27; SANCO/825/00 rev 8.1.
European Environmental Agency, 2012. 〈https://www.eea.europa.eu/data-and-maps/indicators/nutrients-in-transitional-coastal-and-3/assessment〉.
European Union, 2013. Directive 2013/39/EU of the European Parliament and of the Council. Priority substances in the field of water policy.
European Economic Commission, 1975. Official Journal of the European Community (75/440/EEC). No.L 194/26.
Eutrophication and Health, 2002. Algal blooms, red tides, green tides, fish kills, inedible shellfish, blue algae and public health threats. What is the common link? ISBN 92–894-4413-4. 〈http://europa.eu.int〉.
Favilla, M., Macchia, L., Gallo, A., Altomare, C., 2006. Toxicity assessment of metabolites of fungal biocontrol agents using two different (*Artemia salina* and *Daphnia magna*) invertebrate bioassays. Food Chem. Toxicol. 44, 1922–1931. https://doi.org/10.1016/j.fct.2006.06.024.
Federal Environmental Agency, 2008. Identification of organic compounds in the North and Baltic seas. Environmental research of the federal ministry of the environment, nature conservation and nuclear safety; 〈https://www.umweltbundesamt.de/sites/default/files/medien/publikation/long/3509.pdf〉.
Fu, J., Mai, B., Sheng, G., Zhang, G., Wang, X., Peng, P., Xiao, X., Ran, R., Cheng, F., Peng, X., Wang, Z., Tang, U.W., 2003. Persistent organic pollutants in environment of the Pearl River Delta, China: an overview. Chemosphere 52, 1411–1422. https://doi.org/10.1016/S0045-6535(03)00477-6.
Fujii, Y., Haraguchi, K., Harada, K.H., Hitomi, T., Inoue, K., Itoh, Y., Watanabe, T., Takenaka, K., Uehara, S., Yang, H.R., Kim, M.Y., Moon, C.S., Kim, H.S., Wang, P., Liu, A., Hung, N.N., Koizumi, A., 2011. Detection of dicofol and related pesticides in human breast milk from China, Korea and Japan. Chemosphere 82, 25–31. https://doi.org/10.1016/j.chemosphere.2010.10.036.
Green, J.W., 2014. Power and control choice in aquatic experiments with solvents. Ecotoxicol. Environ. Saf. 102https://doi.org/10.1016/j.ecoenv.2014.01.024. (1420–146).
Grung, M., Lin, Y., Zhang, H., Steen, A.O., Huang, J., Zhang, G., Larssen, T., 2015. Pesticide levels and environmental risk in aquatic environments in China – a review. Environ. Int. 81, 87–97. https://doi.org/10.1016/j.envint.2015.04.013.
Guan, Y.F., Wang, J.Z., Ni, H.G., Zeng, E.Y., 2009. Organochlorine pesticides and polychlorinated biphenyls in riverine runoff of the Pearl River Delta, China: assessment of mass loading, input source and environmental fate. Environ. Pollut. 157, 618–624. https://doi.org/10.1016/j.envpol.2008.08.011.
Guo, L., Qiu, Y., Zhang, G., Zheng, G.J., Lam, P.K.S., Li, X., 2008. Levels and bioaccumulation of organochlorine pesticides (OCPs) and polybrominated diphenyl ethers (PBDEs) in fishes from the Pearl River estuary and Daya Bay, South China. Environ. Pollut. 152, 604–611. https://doi.org/10.1016/j.envpol.2007.06.067.
Guo, Y., Yu, H.Y., Zeng, E.Y., 2009. Occurrence, source diagnosis, and biological effect assessment of DDT and its metabolites in various environmental compartments of the Pearl River Delta, South China: a review. Environ. Pollut. 157, 1753–1763. https://doi.org/10.1016/j.envpol.2008.12.026.
Han, S. ying, Qiao, J. qin, Zhang, Y. yang, Yang, L. li, Lian, H. zhen, Ge, X., Chen, H. yuan, 2011. Determination of n-octanol/water partition coefficient for DDT-related compounds by RP-HPLC with a novel dual-point retention time correction. Chemosphere 83, 131–136. https://doi.org/10.1016/j.chemosphere.2011.01.013.
Jonczyk, E., Gilron, G.U.Y., 2005. 10. Acute and chronic toxicity testing with *Daphnia* Sp. Test 1, 337–393. https://doi.org/10.1007/1-4020-3120-3_11.
Knowles, C.O., Ahmad, S., 1971. Comparative metabolism of chlorobenzilate, chloropropylate, and bromopropylate acaricides by rat hepatic enzymes. Can. J. Physiol. Pharmacol. 49, 590–597.
Li, L., Liu, J., Hu, J., 2015. Global inventory, long-range transport and environmental distribution of dicofol. Environ. Sci. Technol. 49, 212–222. https://doi.org/10.1021/es502092x.
Li, R., Xu, J., Li, X., Shi, Z., Harrison, P.J., 2017. Spatiotemporal variability in phosphorus species in the Pearl River Estuary: influence of the River Discharge. Sci. Rep. 7, 1–13. https://doi.org/10.1038/s41598-017-13924-w.
Lin, T., Hu, Z., Zhang, G., Li, X., Xu, W., Tang, J., Li, J., 2009. Levels and mass burden of DDTs in sediments from fishing harbors: the importance of DDT-containing antifouling paint to the coastal environment of China. Environ. Sci. Technol. 43, 8033–8038. https://doi.org/10.1021/es901827b.
Liu, B., Peng, S., Liao, Y., Long, W., 2018. The causes and impacts of water resources crises in the Pearl River Delta. J. Clean. Pollut. 177, 413–425. https://doi.org/10.1016/j.jclepro.2017.12.203.
Lozowicka, B., Kaczynski, P., Paritova, A.Y., Kuzembekova, G.B., Abzhalieva, A.B., Sarsembayeva, N.B., Alihan, K., 2014. Pesticide residues in grain from Kazakhstan and potential health risks associated with exposure to detected pesticides. Food Chem. Toxicol. 64, 238–248. https://doi.org/10.1016/j.fct.2013.11.038.
Lu, Y., Xu, X., Li, T., Xu, Y., Wu, X., 2012. The use of a brine shrimp (*Artemia salina*) bioassay to assess the water quality in Hangzhou Section of Beijing-Hangzhou Grand Canal. Bull. Environ. Contam. Toxicol. 88, 472–476. https://doi.org/10.1007/s00128-011-0498-2.
Luo, X., Mai, B., Yang, Q., Fu, J., Sheng, G., Wang, Z., 2004. Polycyclic aromatic hydrocarbons (PAHs) and organochlorine pesticides in water columns from the Pearl River and the Macao harbor in the Pearl River Delta in South China. Mar. Pollut. Bull. 48, 1102–1115. https://doi.org/10.1007/s11356-014-3100-8.
Marine Water Quality in Hong Kong in, 2016. Environmental Protection Department. The government of the Hong Kong Special Administrative Region. 〈http://wqrc.epd.gov.hk/pdf/water-quality/annual-report/MarineReport2016eng.pdf〉.
Miyamoto, J., Mikami, N., Takimoto, Y., 1990. Environmental Fate 26. Williams AB. Levels and Distribution of Chlorinated of Pesticides. Wiley, Chichester, England, pp. 123–147.
Mrema, E.J., Rubino, F.M., Brambilla, G., Moretto, A., Tsatsakis, A.M., Colosio, C., 2013. Persistent organochlorinated pesticides and mechanisms of their toxicity. Toxicology 307, 74–88. https://doi.org/10.1016/j.tox.2012.11.015.
Nakata, H., Kawazoe, M., Arizono, K., Abe, S., Kitano, T., Shimada, H., Li, W., Ding, X., 2002. Organochlorine pesticides and polychlorinated biphenyl residues in foodstuffs and human tissues from China: status of contamination, historical trend, and human dietary exposure. Arch. Environ. Contam. Toxicol. 43, 473–480. https://doi.org/10.1007/s00244-002-1254-8.
OECD, 2006. Current Approaches in the Statistical Analysis of Ecotoxicity Data: A Guidance to Application. Organisation for Economic Co-operation and Development. Report No. EVV/JM/MONO(2006)18 number 54, pp. 1–147.
Ongley E.D., 1996. Control of water pollution from agriculture. Food and Agriculture Organization of the United Nations. Rome M-56ISBN 92-5-103875-9.
OSPAR Commission, 2002. OSPAR Background Document on Dicofol. Dicofol in drinking-water Draft background document for development of WHO Guidelines for Drinking-water Quality.
Özkara, A., Akyil, D., Konuk, M., 2016. Pesticides, Environmental Pollution, and Health. Environ. Heal. Risk - Hazard. Factors Living Species. https://doi.org/10.5772/63094.
Parsons, T.R., Maita, Y., Lally, C.M., 1985. Pigments. A Manual of Chemical and Biological Methods for Seawater Analysis. Pergamon Press, Oxford, pp. 101–104.
Persoone, G., Baudo, R., Cotman, M., Blaise, C., Thompson, K.C., Moreira-Santos, M., Vollat, B., Törökne, A., Han, T., 2009. Review on the acute *Daphnia magna* toxicity test – evaluation of the sensitivity and the precision of assays performed with organisms from laboratory cultures or hatched from dormant eggs. Knowl. Manag. Aquat. Ecosyst. 01. https://doi.org/10.1051/kmae/2009012.
Purnomo, A.S., Kamei, I., Kondo, R., 2008. Degradation of 1,1,1-trichloro-2,2-bis (4-chlorophenyl) ethane (DDT) by brown-rot fungi. J. Biosci. Bioeng. 105, 614–621. https://doi.org/10.1263/jbb.105.614.
PPDB: the pesticides properties data base: 〈https://sitem.herts.ac.uk/aeru/ppdb/en/〉.
Qiu, X., Zhu, T., Yao, B., Hu, J., Hu, S., 2005. Contribution of dicofol to the current DDT pollution in China. Environ. Sci. Technol. 39, 4385–4390. https://doi.org/10.1021/es050342a.
Redfield, A.C., 1934. On the proportions of organic derivatives in a sea water and their relation to the composition of plankton. In: Daniel, R.J. (Ed.), James Johnstone Memorial Volume. University Press of Liverpool, Liverpool, pp. 177–192.
Reregistration Eligibility Decision (RED) Dicofol, 1998. Prevention, Pesticides and Toxic Substances (7508 C). United States Environmental Protection Agency (EPA738-R-98-018, November 1998).
Ricking, M., Schwarzbauer, J., 2012. DDT isomers and metabolites in the environment: an overview. Environ. Chem. Lett. 10, 317–323. https://doi.org/10.1007/s10311-012-0358-2.
Scholtz, M.T., Voldner, E., McMillan, A.C., Van Heyst, B.J., 2002. A pesticide emission model (PEM) part I: model development. Atmos. Environ. 36, 5005–5013. https://doi.org/10.1016/S1352-2310(02)00570-8.
Sinha, V.S., Kumar, N., Pathak, R.N., 2015. Effect of Chemical Pesticides on Chlorophyll Content of Vicia faba L. J. Chem. Chem. Sci. 5, 1–4.
Skretteberg, L.G., Lyrån, B., Holen, B., Jansson, A., Fohgelberg, P., Siivinen, K., Andersen, J.H., Jensen, B.H., 2015. Pesticide residues in food of plant origin from Southeast Asia – a Nordic project. Food Control 51, 225–235. https://doi.org/10.1016/j.foodcont.2014.11.008.
Thiel, A., Guth, S., Böhm, S., Eisenbrand, G., 2011. Dicofol degradation to p,p′-dichlorobenzophenone – a potential antiandrogen. Toxicology 282, 88–93. https://doi.org/10.1016/j.tox.2011.01.016.
Tieyu, W., Yonglong, L., Hong, Z., Yajuan, S., 2005. Contamination of persistent organic pollutants (POPs) and relevant management in China. Environ. Int. 31, 813–821. https://doi.org/10.1016/j.envint.2005.05.043.
United Nations Environmetal Programme, 2016. Stockholm Convention on Persistent Organic Pollutants. Persistent Organic Pollutants Review Committee. UNEP/POPS/POPRC.12/2.
U.S. Environmental Protection Agency, 1988. Phosphorus - water quality standards criteria summaries: a compilation of state and federal criteria. EPA 440-5-88-012. U.S. Environmental Protection Agency. Office of Water Regulations and Standards, Washington, D.C.
Vanhaecke, P., Persoone, G., 1984. The ARC-Test, a standardized short-term routine toxicity test with *Artemia* nauplii. Methodology and evaluation. Ecotoxicol. Test. Mar. Environ. 2, 143–157.
Wan, Y., Hu, J., Liu, J., An, W., Tao, S., Jia, Z., 2005. Fate of DDT-related compounds in Bohai Bay and its adjacent Haihe Basin, North China. J. Mar. Pollut. Bull. 50, 439–445. https://doi.org/10.1016/j.marpolbul.2004.11.037.
Wang, J., Guo, L., Li, J., Zhang, G., Lee, C.S.L., Li, X., Jones, K.C., Xiang, Y., Zhong, L., 2007. Passive air sampling of DDT, chlordane and HCB in the Pearl River Delta, South China: implications to regional sources. J. Environ. Monit. 9, 582–588. https://doi.org/10.1039/b700798a.
Wei, Y., Bao, L., Wu, C., He, Z., Zeng, E.Y., 2015. Assessing the effects of urbanization on the environment with soil legacy and current-use insecticides: a case study in the Pearl River Delta, China. J. Sci. Total Environ. 514, 409–417.

World Health Organization, 1996. Promotion of Chemical Safety Unit & Food and Agriculture Organization of the United Nations, WHO/FAO data sheet on pesticides. no.81, Dicofol.

Wurl, O., Obbard, J.P., Lam, P.K.S., 2006. Distribution of organochlorines in the dissolved and suspended phase of the sea-surface microlayer and seawater in Hong Kong, China. Mar. Pollut. Bull. 52, 768–777. https://doi.org/10.1016/j.marpolbul.2005.11.024.

Xin, J., Liu, X., Liu, W., Jiang, L., Wang, J., Niu, J., 2011. Production and use of DDT containing antifouling paint resulted in high DDTs residue in three paint factory sites and two shipyard sites, China. Chemosphere 84, 342–347. https://doi.org/10.1016/j.chemosphere.2011.04.005.

Yang, G., Ma, L., Xu, D., Liu, L., Jia, H., Chen, Y., Zhang, Y., Chai, Z., 2012. Temporal variations of organochlorine pesticides in precipitation in Beijing, China. Atmos. Environ. 61, 614–619. https://doi.org/10.1016/j.atmosenv.2012.08.026.

Yang, X., Wang, S., Bian, Y., Chen, F., Yu, G., Gu, C., Jiang, X., 2008. Dicofol application resulted in high DDTs residue in cotton fields from northern Jiangsu province, China. J. Hazard. Mater. 150, 92–98. https://doi.org/10.1016/j.jhazmat.2007.04.076.

Yin, G., Athanassiadis, I., Bergman, Å., Zhou, Y., Qiu, Y., Asplund, L., 2017. A refined method for analysis of 4,4′-dicofol and 4,4′-dichlorobenzophenone. J. Environ. Sci. Pollut. Res. Int. 24 (15). https://doi.org/10.1007/s11356-017-8956-y.

Zhang, C., Zhang, W., Liu, H., 2017. A quantitative assessment of the contributions of climatic indicators to changes in nutrients and oxygen levels in a shallow reservoir in China. ⟨http://dx.doi.org/10.1007/s00704-017-2165-y⟩.

Zhang, G., Parker, A., House, A., Mai, B., Li, X., Kang, Y., Wang, Z., 2002. Sedimentary records of DDT and HCH in the Pearl River Delta, South China. J. Environ. Sci. Tech. 36, 3671–3677.

Zhang, L., Wang, L., Yin, K., Lü, Y., Yang, Y., Huang, X., 2014. Spatial and seasonal variations of nutrients in sediment profiles and their sediment-water fluxes in the Pearl River Estuary, Southern China. J. Earth Sci. 25, 197–206. https://doi.org/10.1007/s12583-014-0413-y.

Zhao, H.T., 1990. Pearl River Estuary Evolvement. Ocean Publisher, Beijing, pp. 1–11.

Zheng, S., Chen, B., Qiu, X., Chen, M., Ma, Z., Yu, X., 2016. Distribution and risk assessment of 82 pesticides in Jiulong River and estuary in South China. Chemosphere 144, 1177–1192. https://doi.org/10.1016/j.chemosphere.2015.09.050.