Huizhen Li ORCID iD: 0000-0002-5200-1916

Jing You ORCID iD: 0000-0002-4006-8339

19-00257

Environmental Toxicology

Ma et al.

Running Head: Chronic toxicity of sediment-bound DDT to *Chironomus dilutes*

Full Life-cycle Toxicity Assessment of Sediment-bound DDT and Its Degradation Products on *Chironomus dilutes*

Ping Ma,[a,b,c] Huizhen Li,[b*] Jing You[b]

[a] State Key Laboratory of Organic Geochemistry, Guangzhou Institute of Geochemistry, Chinese Academy of Sciences, Guangzhou 510640, China

[b] Guangdong Key Laboratory of Environmental Pollution and Health, School of Environment, Jinan University, Guangzhou 511443, China

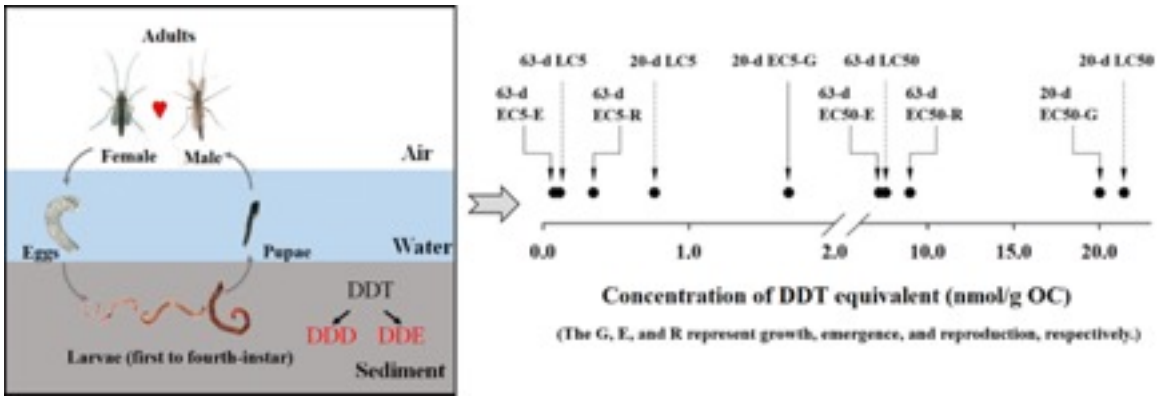
[c] University of Chinese Academy of Sciences, Beijing 100049, China

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1002/etc.4593.

(Submitted 16 April 2019; Returned for Revision 12 May 2019; Accepted 03 September 2019)

Abstract: Due to its hydrophobicity and persistence, dichlorodiphenyltrichloroethane (DDT) is ubiquitous in sediments and poses significant risk to benthic organisms. Therefore, it is imperative to evaluate long-term toxicity of DDT. However, limited information is available on its chronic toxicity to benthic invertebrates. Full life-cycle toxicity of sediment-bound DDT to *Chironomus dilutus* was assessed. Median lethal concentrations (with 95% confidence limits) of DDT and its degradation products (DDX) to *C. dilutus* were 334 (165–568), 21.4 (11.2–34.3), and 7.50 (4.61–10.6) nmol/g organic carbon (OC) after 10-, 20-, and 63-d exposure, respectively. In addition, median effect concentrations of DDX were 20.0 (15.0–25.3), 7.13 (4.10–10.5), and 8.92 (3.32–15.1) nmol/g OC for growth, emergence, and reproduction, respectively. A toxicity spectrum was established to visually summarize chronic effects of DDX to midges. In addition, DDT degraded to DDD and DDE during sediment aging, and their toxicity differed from that of the parent compound. Predicted toxic units of DDX in porewater were utilized to distinguish between toxicity from DDT and that of DDD and DDE. The results showed that DDD was the main contributor to the toxicity in *C. dilutus*. To improve the accuracy of sediment risk assessment of DDT, composition of DDX should be considered.

Graphical Abstract



Keywords: Full life-cycle toxicity assessment; DDT; Degradation products; Sediment; *Chironomus dilutus*

This article includes online-only Supplemental Data.

*Address correspondence to lihuizhen@jnu.edu.cn

Published online XXXX 2018 in Wiley Online Library (www.wileyonlinelibrary.com).

DOI: 10.1002/etc.xxxx

INTRODUCTION

Dichlorodiphenyltrichloroethane (DDT) is a legacy organochlorine pesticide and has been extensively used in the past (Xin et al. 2011). As a consequence of its persistence, DDT is still ubiquitous in the environment (Arias et al. 2011; Li et al. 2011; Bao et al. 2012), even though it has been banned for more than 30 years in most countries (Xin et

al. 2011; Matsushima 2018). As a hydrophobic compound with log K_{OW} of 6.91 (De Bruijn et al. 1989), DDT tends to accumulate in sediment, posing significant threats to the benthos and possible biomagnification in aquatic ecosystems (Matsushima 2018). Thereby, it is imperative to evaluate long-term toxicity of sediment-bound DDT to benthos, which requires effective assessment thresholds for this chemical.

Chironomus dilutus is a benthic invertebrate, which has been recommended as a model organism for sediment toxicity testing by the U.S. Environmental Protection Agency (USEPA) (2000) due to its wide distribution in freshwater ecosystems and ease of culturing in the laboratory. This species has been utilized as an indicator organism to monitor sediment risk associated with pesticides (Nowell et al. 2016). Previous studies reported that DDT caused mortality to the midges with median lethal concentrations (LC50) ranging from 0.49 to 1.25 μg/L in 10-d water-only toxicity testing (Phipps et al. 1995; Hoke et al. 1997; Ding et al. 2012). These acute LC50 values are much higher than DDT concentrations measured at contaminated sites, for example, 0.5–82 ng/L in China (Bao et al. 2012), suggesting toxic thresholds based on acute mortality have low environmental relevance. Qi et al. (2015) evaluated the toxicity of sediment samples from Tai Lake in China using *C. dilutus*. No acute mortality to the midges occurred, yet impairments in growth, emergence, and fecundity of *C. dilutus* have been frequently observed after long-term exposure to these sediments. At the same time, a variety of

pesticides have been detected in these sediments at the concentrations which would not cause acute mortality to the midges (Qi et al. 2015). In such conditions, chronic toxic thresholds of frequently detected pesticides, such as DDT, are necessary to quantitatively evaluate the contribution of individual contaminants to sediment toxicity. Unfortunately, chronic toxicity data are not available for DDT, calling for evaluating long-term toxicity of this chemical.

Degradation of DDT occurs in sediment and the organisms are actually exposed to a mixture of DDT and its degradation products, such as dichlorodiphenyldichloroethane (DDD) and dichlorodiphenyldichloroethylene (DDE). In many cases, only the concentration of parent compound is utilized as a dose metric in sediment toxicity assessment (Schuytema et al. 1989; Chung et al. 2007; Fathallah 2014), which neglects toxicity contributions of the degradation products. The total concentration of DDT *+* DDD *+* DDE (referred to as DDX), serves as a better dose metric in assessing DDT toxicity in sediment (Hoke et al. 1997; Lotufo et al. 2001a). However, LC50 values of DDT, DDD, and DDE can vary by an order of magnitude (Hoke et al. 1997), suggesting that a simple dose metric based on DDX concentration would introduce uncertainty in toxicity assessment. Therefore, it is necessary to distinguish toxicity contributions from DDT and its degradation products when developing chronic toxicity thresholds of sediment-bound DDT. The freely dissolved concentration of a chemical in sediment

porewater better reflects its toxicity than is the case for the sediment concentration (Di Toro et al. 1991), because the former reduces uncertainties in toxicity prediction by accounting for variables such as sediment aging and sediment organic carbon contents and characteristics (Xu et al. 2007).

The main objective of the present study was to assess acute (10-d) and chronic (full life-cycle) toxicity of sediment-bound DDT to *C. dilutus*. Chronic toxicity endpoints included lethal effects at 20 d and the end of midge life cycle, as well as sub-lethal effects, such as reduced growth, impaired emergence, and altered reproduction. A toxicity response spectrum including individual endpoints was established for *C. dilutus* after a life-cycle exposure to DDT spiked sediments. In the meantime, toxicity contributions of DDT and its degradation products to *C. dilutus* were quantified using toxic units (TUs) derived from chemical concentrations in sediment porewater.

MATERIALS AND METHODS

Chemicals and Reagents

As *p,p'*-DDT is the predominant isomer in the environment (Ma et al. 2001) and generally is more toxic to invertebrates than *o,p'*-DDT (Ginsburg 1947), only *p,p'*-DDT and its degradation products were evaluated in the present study. Neat compound of *p,p'*-DDT with a purity of 97% was purchased from Toronto Research Chemicals. Stock

solutions of *p,p'*-DDT, *p,p'*-DDD, and *p,p'*-DDE in methanol were purchased from O2SI Smart Solutions. Additional degradation products, 1-chloro-2,2-bis-(*p*-chlorophenyl) ethylene (*p,p'*-DDMU) and 2,2-bis*(p*-chlorophenyl)methane (*p,p'*-DDM), and the surrogate standard polychlorinated biphenyl (PCB)-67 were obtained from AccuStandard.

Concentrated H_2SO_4 and anhydrous Na_2SO_4 were bought from Guangzhou Chemical Reagent Factory (guaranteed reagent). Anhydrous Na_2SO_4 was baked for 4 h at 450 ^oC prior to use. Copper powder was purchased from Macklin and was used to remove sulfur in sediment samples. Copper powder was activated by diluted HCl, cleaned with water and a mixture of acetone and hexane (1:1, v/v) for three cycles, and stored in hexane prior to use. Diatomaceous earth was purchased from Thermo Fisher Scientific. HPLC-grade hexane and dichloromethane were obtained from Oceanpak Alexative Chemical Limited. Analytical grade acetone was obtained from Tianjin Chemical Reagent Factory and redistilled prior to use.

Sediment spiking

A control sediment was collected from a drinking water reservoir in Conghua, Guangzhou, China. The sediment was sieved through a 35-mesh sieve onsite, transported back to the laboratory and stored at 4 ^oC. The sediment showed no acute

and chronic mortality to the midges and concentrations of DDX in the sediment were lower than the reporting limits (Table S1). DDT dissolved in acetone (100 µL acetone/kg sediment) was spiked into the sediment to create a series of spiked sediments for acute (Level A1–Level A8 (LA1–LA8)) and chronic (Level 1–Level 6 (L1–L6)) toxicity tests. The spiking concentrations used for acute toxicity testing were selected based on the estimated 10-d sediment LC50 value (8745 nmol/g OC) of DDT to *C. dilutus* (Weston et al. 2004). The spiking concentrations used for chronic toxicity testing were determined by dividing the 10-d sediment LC50 values gained in the present study by an acute chronic ratio (ACR) of 25 proposed for organic chemicals (Kenaga 1982). After spiking, the sediment was thoroughly homogenized for 2 h. In order to adhere to the requirement for sediment aging (USEPA 2000) while minimizing degradation of DDT (Ding et al. 2013), the spiked sediment was aged at 4 ^{o}C in dark for only 7 days. Prior to bioassays, the sediments were homogenized again for 2 h after decanting overlying water. Measured sediment concentrations at the beginning and the end of the acute and chronic toxicity testing are shown in Supplemental Data, Tables S1 and S2, respectively.

Toxicity testing

The 10-d acute toxicity testing was performed according to the USEPA standard protocol (USEPA 2000). Detailed testing methods are shown in the Supplemental Data.

At the beginning and the end of the testing, sediment samples were collected for analyzing the concentrations of DDT and its degradation products. The measurements were performed using three replicates.

Chronic toxicity testing was performed following the standard protocols with small modifications (USEPA 2000; OECD 2010; Du et al. 2013). The test was performed using newly hatched midge larvae and the test was terminated once eggs hatched in the next generation. In brief, twenty newly hatched midge larvae within 24 h were randomly transferred into each test chamber which contained 60 g of wet sediment and 250 mL of moderately hard reconstituted water. Overlying water was renewed twice a day using an automated water delivery system. Temperature was maintained at 23 ± 1 ^{o}C and the photocycle was set at 16:8 h of light: dark during the testing. At the different life stages, the midges in a testing chamber were fed with ground fish food once a day as follows: no feeding at 1 and 2 d, 0.6 mg per chamber from 3 to 7 d, 3 mg from 8 to 12 d, and 6 mg from 13 d to the end of the bioassays (USEPA 2000; OECD 2010; Du et al. 2013). The amounts of organic carbon associated with different feeding levels per day accounted for 0.12% to 1.07% of that in the sediment, indicating negligible effects of different feeding levels on changes in sediment organic content. In addition, no residual food was found on the surface of the sediment during the chronic toxicity testing. Therefore, the effect of varying feeding levels on sediment toxicity was not considered.

Three groups were conducted for chronic bioassays at each concentration and each group was performed using three replicates. The first group was terminated at 20 d for monitoring the mortality and impaired growth of *C. dilutus*. Surviving organisms (fourth-instar larvae) were sieved from the sediment using a 500-µm sieve. After recording mortality, growth of the midges was evaluated by measuring their ash-free dry weight (AFDW). In brief, the surviving organisms per replicate were dried at 60 ^{o}C for 3 d to acquire a constant dry weight. The organisms were then heated to 550 ^{o}C and kept for 3 h to remove organic matter and the weight was measured again. The AFDW was obtained as the difference of the two measurements (Maul et al. 2008).

The second group was applied to assess the lethal and sub-lethal effects, such as emergence and reproduction of *C. dilutus* in the life cycle testing. The mortality and emergence of the midges were recorded daily since the first emergence at 26 d and ended until one week after emergence of the last midge at 56 d. Once emerged, adult females were transferred to a covered beaker containing clean reconstituted water. At least one adult male from the corresponding concentrations was added for mating. Egg laying and hatching were monitored daily. The hatchability of the eggs was calculated by dividing the eggs hatched at 7 d by the total eggs (Benoit et al. 1997).

The third group was started one week later than the previous two groups. The third group was used to provide sufficient males to mate with the females in the second

group, as the emergence of females was often one week later than the males (Benoit et al. 1997).

Sediment extraction and chemical analysis

DDT and its degradation products in sediments were extracted using an ASE 350 accelerated solvent extractor (Thermo Fisher Scientific). Approximately 2 g of freeze-dried sediment was mixed with diatomaceous earth and activated copper powder, and then were added to a 22-mL stainless-steel extraction cell. After adding 50 ng of surrogate (PCB-67), the extraction was initiated using a mixture of dichloromethane: acetone (1:1, v/v). The extraction was conducted for three cycles of 5 min each, with temperature being set at 80 ^{o}C and pressure at 1500 psi. The extracts were concentrated by a gentle flow of nitrogen and solvent exchanged to 1 mL of hexane and purified using concentrated H_2SO_4 (Wang et al. 2015). Briefly, 1 mL of concentrated H_2SO_4 was added to the extracts and the solution was shaken for 3 min and centrifuged at 2000 rpm for 5 min. The process was repeated three times and the cleaned extracts were combined, passed through a column packed with anhydrous Na_2SO_4 for removing residual water and H_2SO_4, and finally concentrated to 1 mL for instrumental analysis.

The concentrations of DDT and its degradation products, including DDD, DDE, DDM, and DDMU were quantified using an Agilent 7820A gas chromatography equipped with an electron capture detector. Additional confirmation of the analytes was performed using a Shimadzu QP-2020 GC-MS operated in electron impact ionization mode. A HP-5 column (30 m × 0.25 mm, 0.25 μm film thickness) was used to separate the analytes and helium was used as carrier gas at a flow rate of 1.2 mL/min. The initial oven temperature was set at 60 ^{o}C and kept for 1 min, increased to 240 ^{o}C at a rate of 20 ^{o}C/min and held for 6 min, and then increased to 280 ^{o}C at 30 ^{o}C/min, and finally held for 5 min. The injection volume was 1 μL in splitless injection mode. The temperature of the injector and detector was both set at 280 ^{o}C.

Data analysis

Median lethal and effective concentrations (LC50 and EC50) and the 5% lethal and effective concentrations (LC5 and EC5) with 95% confidence limits were estimated using SAS 9.4. The figures were plotted using SigmaPlot 10.0. Significant differences between the treatments and control were determined using one-way ANOVA with $p < 0.05$. Data are presented in mean ± standard deviation.

Porewater concentrations (C_{PW}) of individual DDX were calculated by dividing OC normalized sediment concentration (C_s) by partitioning coefficient of the chemical

between OC and water (K_{OC}) (Eq. 1). Then, predicted toxic units of individual DDX in porewater were calculated by dividing C_{PW} by the LC50 value for the midges in water-only tests (Eq. 2).

$$C_{PW} = \frac{C_S}{f_{OC} \times K_{OC}} \quad (1)$$

$$TU = \frac{C_{PW}}{LC50} \quad (2)$$

Where, f_{OC} represents the total organic carbon (TOC) content of the sediment (1.85 ± 0.14%), which was measured using a Vario EL III Elemental Analyzer after removing inorganic carbon with 1 mol/L HCl. The K_{OC} values of DDT, DDD, and DDE were 5.31, 5.00, and 4.82, respectively (Sabljić et al. 1995; Hornsby et al. 1996). The reported 10-d LC50 values for DDT, DDD, and DDE to the midges in water-only toxicity testing were 3.53, 0.84, and 9.97 nmol/L, respectively (Hoke et al. 1997). The 20-d LC50 values were then estimated by dividing the 10-d water-only LC50 values by an ACR of 15.6 (from 10 d to 20 d) determined in the present study, and the values were 0.23, 0.054, and 0.64 nmol/L for DDT, DDD, and DDE, respectively.

Quality assurance and quality control

A batch of quality control samples, including a method blank, a solvent blank, a matrix spike, and a matrix spike duplicate were analyzed for every 20 samples. A

calibration standard of DDT was analyzed for every 10 samples to check the instrument and ensure variations in response of each target analyte were within 20%. Thermal degradation of DDT in the GC liner was 6.7 ± 2.0%, which was acceptable for instrumental analysis. No target analytes were detected higher than the reporting limits in blank samples and the control sediments in the bioassays. Recoveries of the target analytes in the matrix spike samples and the surrogate (PCB-67) in all the samples were 89.0 ± 10.3% and 91.1 ± 11.1%, respectively.

RESULTS AND DISCUSSION

Degradation of DDT in Sediment

To check the degradation of DDT during sediment aging and bioassay periods, the concentrations of DDT and its degradation products (DDD, DDE, DDM, and DDMU) in sediment were analyzed at the beginning and the end of acute and chronic testing (Tables S1 and S2). No DDM and DDMU were detected in any of the samples, thus only the concentrations of DDT, DDD, and DDE were reported. In addition, only *p,p'*-DDTs were evaluated in the present study and toxicity assessment of *o,p*-DDTs requires further study.

Although only DDT was spiked into the sediment, approximately 30% of chemicals detected in sediment after aging (the beginning of the bioassays) were the degradation

products, mainly DDE (Tables S1 and S2). The concentrations of DDT continued to decrease during the toxicity testing. While DDT is regarded as a persistent organic pollutant, fast degradation of DDT in freshly spiked sediments has also been commonly reported (Hoke et al. 1997; Lotufo et al. 2001b; Ding et al. 2013; Wang et al. 2015). As a result, the organisms were actually exposed to a mixture of DDT, DDD, and DDE in the present study. The concentrations of DDT equivalent (DDX) were the sum concentrations of DDT, DDD, and DDE and were used as dose metrics for the bioassays instead of DDT concentrations solely. On average, the concentrations of DDT, DDD, and DDE accounted for 59.6 ± 7.1%, 22.6 ± 7.6%, and 17.8 ± 11.0%, respectively, of the total DDX concentrations in sediments during the acute and chronic toxicity testing. In other studies, the relative concentrations of DDX varied significantly across sediments, for example, the relative concentrations of DDT, DDD, and DDE were 9.28%–56.9%, 9.00%–57.0%, 18.7%–35.6%, respectively, in field-contaminated sediments (Arias et al. 2011; Li et al. 2011; Qi et al. 2015).

Sediment concentrations of DDX at 10 d were 74.7%–115% of those at 0 d, indicating slight decrease during the acute toxicity testing (Table S1). The measured concentrations of DDX at 63 d were 40.0%–64.7% of those at 0 d in chronic toxicity testing (Table S2). Because no DDM and DDMU were detected in sediment, secondary degradation of DDE and DDD was not the reason for the significant reduction of DDX

concentrations in chronic toxicity testing. Alternatively, increasing sequestration and the presence of non-extractable DDX may have increased in the sediments as a function of exposure time (Schwarzbauer et al. 2003). In addition, loss of chemicals may occur through the renewal of overlying water. Considering the change of DDX concentrations during the bioassays, arithmetic means of DDX concentrations at the beginning and the end of the acute and chronic toxicity testing were used as exposure concentrations in calculating thresholds, as suggested by Heye et al. (2016).

Acute toxicity testing

The conductivity, dissolved oxygen, pH, and temperature were 335 ± 21 µs/cm, 4.84 ± 0.55 mg/L, 7.52 ± 0.09, and 22.8 ± 0.6 ^{o}C, respectively, during the acute toxicity testing. The concentrations of ammonia were lower than 1.0 mg/L throughout the testing. All these parameters were within the acceptable criteria (USEPA 2000).

Survival of *C. dilutus* was 87.5 ± 9.6% and 90.0 ± 8.2% in negative control and solvent control, respectively, in the 10-d acute sediment toxicity testing (Figure S1). The calculated 10-d LC50 value (with 95% confidence limits) to the midges based on DDX concentrations was 334 (165–568) nmol/g OC. The LC50 value was comparable with that of methoxychlor (106 nmol/g OC) whose structure is similar to DDT (You et al. 2004). Hoke et al. (1997) reported a 10-d sediment LC50 of 7052–8463 nmol/g OC for *C. dilutus*

exposed to DDX in field-contaminated sediments receiving untreated industrial effluent with DDT (27–50 years of aging). This LC50 was one order of magnitude higher than the one found in the present study (Table 1). A plausible reason for these large difference in LC50 values is a large difference in DDX bioavailability between field-contaminated and laboratory-spiked sediments. Weston et al. (2004) estimated the 10-d sediment LC50 value of DDT to be 8745 nmol/g OC based on the relative sensitivity of DDT to *Hyalella azteca* and *C. dilutus* in water-only toxicity testing. Comparatively, the experimental acute LC50 value in the present study was approximately 20 times lower than the estimated value, suggesting that this estimated value significantly underestimated toxicity contribution of sediment-bound DDT to midges.

Chronic toxicity testing

The conductivity, dissolved oxygen, pH, and temperature were 345 ± 22 µs/cm, 4.99 ± 0.98 mg/L, 7.48 ± 0.17, and 22.6 ± 1.0 ^{o}C, respectively. The concentrations of ammonia were less than 0.4 mg/L throughout the bioassays. All these parameters were within the acceptable criteria (USEPA 2000).

Mortality. In the chronic sediment toxicity testing, survival of *C. dilutus* was 83.3 ± 7.6% and 76.0 ± 5.5% in negative control and 85.0 ± 5.0% and 73.0 ± 11.0% in solvent control at 20 and 63 d, respectively, which met the acceptable criteria of > 70% at 20 d

and > 65% at the end of the full life-cycle toxicity testing (USEPA 2000). The LC50 values of DDX to the midges were 21.4 (11.2–34.3) and 7.50 (4.61–10.6) nmol/g OC after 20- and 63-d exposure, respectively. ACR values were calculated from the acute and chronic LC50 values in the present study, which equaled to 15.6 and 44.5 for 20- and 63-d exposure, respectively. The ACRs were close to the proposed ACR value of 25 for organic pesticides (Kenaga 1982).

As shown in Figure 1, pronounced mortality was observed for the midges in the treatments L3 to L6 at 20 d and those in all treatments at 63 d. On average, mortality of the midges at 63 d was higher than that at 20 d for all treatments, but the difference was not significant ($p > 0.05$), which was likely related to the loss of adult midges as a result of the impeded pupation and emergence (Benoit et al. 1997; Cavallaro et al. 2017). During the pupation process, the contaminants re-distribute to different tissues as biochemical and physiological changes occur in the midges. As a consequence of possible transport of contaminants from lipid deposit to target sites, toxicity of the chemicals to the organisms increased, which may cause death of the midges before forming pupae (Benoit et al. 1997). In addition, some pupae failed to emerge in the present study, probably due to molt-related mortality (Cavallaro et al. 2017). The main degradation product of DDT, DDE is regarded as a potent endocrine-disrupting chemical

(Kelce et al. 1995; Matsushima 2018) and the molting of pupae might be disturbed by DDE.

Growth. Growth of the midges was evaluated by AFDW. At the end of 20-d exposure, the AFDW of the midges in the control samples was 1.23 ± 0.05 mg/midge, which met the required limit of > 0.48 mg/midge (USEPA 2000). A relatively high variability in AFDW was observed in the L1 treatment (with a relative standard deviation of 39.8%), which may have been due to strong differences in sensitivity among individual organisms. The midges in DDT spiked sediments (L3–L6) had much lower AFDW, indicating growth inhibition (Figure 1). The EC50 value of DDX for midge growth was 20.0 (15.0–25.3) nmol/g OC. The reduction of midge growth due to DDX exposure was likely associated with energy allocation within the organisms. Energy is required by organisms for their survival, growth, maintenance, and reproduction (Jager et al. 2004). More energy is allocated to resist the stress induced by pollutants, such as DDT and its degradation products, and less energy is available for growth. Sibley et al. (1997) demonstrated that a reduced growth would lead to decreased emergence and fecundity of the midges, which eventually caused damages on the population level.

Emergence. The cumulative emergence rate of *C. dilutus* was 77.0 ± 4.5% and 76.0 ± 11.4% for negative control and solvent control, respectively, in the chronic sediment toxicity testing, which met the required limit of > 70% on average (OECD 2010). As

shown in Figure 2, cumulative emergence rates of midges exposed to all DDT spiked sediments were significantly lower than controls, indicating impact of DDX on midge emergence. Midge emergence was seriously perturbed in the treatments L5 and L6 that the cumulative emergence rates could not reach 20% until the termination of the life cycle tests. The EC50 was 7.13 (4.10–10.5) nmol/g OC. Reduced growth would lead to delayed emergence of the midges. A previous study showed that the emergence of the midges might cease when AFDW was less than 0.5 mg/individual (Sibley et al. 1997). The development time was also delayed due to DDX exposure (Figure 2), for example, the times till 20% emergence of the midges were 31 and 45 d for the negative control and the treatment L4, respectively. Delayed emergence would increase the chance for midge larvae being preyed and impede the mating of the adults due to an extended time gap for the emergence of male and female midges, which in turn would induce undesirable outcomes on the population level (Forbes and Cold 2005; Heye et al. 2016) and eventually affect the structure and function of the ecosystems (Sibley et al. 1997).

Reproduction. In the present study, sex ratio (ratio of female to male), fecundity ratio (ratio of the number of females that successfully oviposited and the total number of females per replicate), the number of eggs per replicate, and hatchability of the eggs were measured as endpoints of reproduction. As shown in Figure 3A, no significant difference was observed for sex ratio of the adult midges after being exposed to

sediment-bound DDX at all treatments. Fecundity ratio of the females decreased with increasing DDX concentrations (Figure 3A). This revealed that although the midges might be emerged successfully after exposure to DDX, their fecundity was jeopardized (Schuler et al. 2007). The number of eggs per replicate was a combined effect of the number of females per replicate and the number of eggs per female (Figure 3B). The EC50 value based on the number of eggs per replicate was 8.92 (3.32–15.1) nmol/g OC. The hatchability of the eggs was 98.9 ± 1.3% in all treatments regardless of the concentrations of DDX in sediment, suggesting hatchability was not impacted by DDX exposure (Figure 3B). Similar results have been previously reported for lindane, permethrin, and benzo[a]pyrene (Taylor et al. 1993; Du et al. 2013, 2014). Insensitivity of egg hatchability was likely related to the gelatinous matrix and the chorion of the eggs which adsorbed the contaminants and reduced the diffusion of the contaminants into the eggs (Taylor et al. 1993).

Chronic toxicity thresholds

Overall, the 5% and 50% lethal and effective concentrations of individual endpoints were summarized to construct a toxicity spectrum for *C. dilutus* under a life-cycle exposure to sediment-bound DDT. As shown in Figure 4, the most sensitive endpoints to the midges were emergence and mortality at 63 d, followed by reproduction, growth at 20 d, and mortality at 20 d of the midges. The toxicity thresholds of DDT to the midges

were compared with other benthic invertebrates (Table 1). Overall, the insect *C. dilutus* is sensitivie to DDT, and the sensitivity is comparable to that of the two crustaceans *H. azteca* and *Leptocheirus plumulosus*.

DDT has been extensively applied in the last century and it is persistent in sediment, DDT is ubiquitous in the environment worldwide. For example, a previous study showed that DDX was detected in 100% of the sediment samples collected from urban waterways in Guangzhou with concentrations ranging from less than reporting limits to 1.04 nmol/g OC (Li et al. 2011). In addition, DDT equivalent concentrations in sediment ranged from 0.19 to 0.44 nmol/g OC in Tai Lake, China (Qi et al. 2015) and from 0.12 to 1.12 nmol/g OC in sediment in Argentina (Arias et al. 2011). In these studies, the concentrations of DDT equivalent in the sediment samples were all below the acute toxicity thresholds, yet some of DDX concentrations were greater than the chronic toxicity thresholds which were acquired in the present study, implying the possible chronic effects of DDX at environmentally relevant concentrations in some regions.

To assess potential risk of sediment-bound DDX, a benchmark of 5.28 ng/g dry weight (1.49 nmol/g OC) for DDX in sediment is recommended by the USEPA (2006). Compared with the chronic thresholds in the present study, this sediment benchmark value for DDX is higher than the 20- and 63-d LC5 values and the EC5 values for reproduction and emergence in chronic toxicity testing, thus the benchmark value is not

conservative as expected. This validated the need for developing chronic toxicity thresholds of DDX, which would improve the accuracy of assessing the risk of DDT in sediment.

Predicted porewater concentrations and DDX toxicity to the midges

To evaluate the uncertainty of using DDT or DDX as dose metric for sediment toxicity, the contribution of each compound to the observed toxicity was calculated. As shown in Tables 2 and S3, porewater concentrations and associated TUs (Eqs. 1 and 2) were calculated for DDT, DDD, and DDE, and their respective contributions to the observed toxicity were also estimated. Toxicity contributions of DDT, DDD, and DDE to the mortality of midges were 35.2 ± 7.0%, 52.2 ± 10.9%, and 12.6 ± 6.5%, respectively, across the acute and chronic treatments. While the concentrations of DDD in sediments were lower than the parent compound, it was the main contributor to the toxicity in midges except for the two highest concentrations in acute toxicity testing. Hoke et al. (1997) also found that DDD predominated in sediment porewater and was considered as the principal driver for the observed sediment toxicity to *C. dilutus*.

As shown in the dose-response curves of the sum TUs of DDX and the mortality of the midges (Figure S2), the 50% mortality of the midges corresponded to 0.96 (0.50–1.56) and 1.09 (0.60–1.71) TUs for DDX at 10- and 20-d toxicity tests, respectively.

Instead, if only the concentrations of DDT were used as dose metrics, 50% mortality of the midges corresponded to 0.30 (0.15–0.52) and 0.36 (0.18–0.60) TUs for DDT at 10- and 20-d toxicity tests, respectively. In theory, 50% mortality of organisms equals to one TU. The dose-response relationships confirmed that DDX concentrations were better dose metrics than just DDT concentrations. It should be noted that the use of predicted porewater concentrations only takes porewater exposure into consideration, while dietary uptake is an important exposure route for deposit feeders (Belfroid et al. 1996). Dietary uptake may change the composition of DDX in the organisms, which eventually changes the observed toxicity of DDX to *C. dilutus*. Therefore, dietary uptake should also be taken into consideration in future study.

Overall, DDT degrades (albeit slowly) as contaminated sediment aging, and benthic organisms are therefore exposed to a combination of DDT and the degradation products DDD and DDE. Using DDX as a dose metric accounts for the presence of the degradation products. However, DDT and its degradation products differ in toxicity (Hoke et al. 1997), so the use of DDX is not adequate for assessing toxicity when the relative abundance of its constituent differs among samples or changes over time. The present study's method of predicting toxicity on the basis of porewater TUs for the different chemicals is therefore better suited for toxicity assessment for DDT-contaminated field sediments.

CONCLUSIONS

The present study filled the toxicity data gaps of sediment-bound DDT to *C. dilutus*, particularly chronic toxicity data. Chronic toxicity thresholds of DDT and its degradation products were established based on life cycle testing of *C. dilutus*. The chronic toxicity assessment thresholds were valuable for more accurately assessing aquatic risk of long-term exposure to sediment-bound DDX. Predicted porewater concentrations and TUs were used to evaluate toxicity contributions of DDT and its degradation products to the observed toxicity. Although the concentration of DDT was the highest in sediment, DDD was the predominated contributor to the toxicity in midges.

Supplemental Data—The Supplemental Data are available on the Wiley Online Library at DOI: 10.1002/etc.xxxx.

Acknowledgments—We thank Y. Tong and Y. Lian for their assistance with the experiments. Financial support by the National Natural Science Foundation of China (41773101 and 41473106) and the Natural Science Foundation of Guangdong Province, China (2015A030310219, 2016A030312009 and 2014A030310002). This is contribution No. IS-**** from GIGCAS.

Disclaimer—All the data in this paper are the results of my independent research work under the guidance of my tutor. As far as we known, this paper does not contain any

research results published or written by any other individual or group except those cited in the paper.

Data Accessibility—Data, associated metadata, and calculation tools are available from the corresponding author (lihuizhen@jnu.edu.cn).

REFERENCES

Arias AH, Pereyra MT, Marcovecchio JE. 2011. Multi-year monitoring of estuarine sediments as ultimate sink for DDT, HCH, and other organochlorinated pesticides in Argentina. *Environ Monit Assess* 172:17–32.

Bao L-J, Maruya KA, Snyder SA, Zeng EY. 2012. China's water pollution by persistent organic pollutants. *Environ Pollut* 163:100–108.

Belfroid A, Sijm D, Gestel CV. 1996. Bioavailability and toxicokinetics of hydrophobic aromatic compounds in benthic and terrestrial invertebrates. *Environ Rev* 4:276–299.

Benoit DA, Sibley PK, Juenemann JL, Ankley GT. 1997. *Chironomus tentans* life-cycle test: Design and evaluation for use in assessing toxicity of contaminated sediments. *Environ Toxicol Chem* 16:1165–1176.

Cavallaro MC, Morrissey CA, Headley JV, Peru KM, Liber K. 2017. Comparative chronic toxicity of imidacloprid, clothianidin, and thiamethoxam to *Chironomus dilutus* and estimation of toxic equivalency factors. *Environ Toxicol Chem* 36:372–382.

Chung KW, Fulton MH, Scott GI. 2007. Use of the juvenile clam, *Mercenaria mercenaria*, as a sensitive indicator of aqueous and sediment toxicity. *Ecotoxicol Environ Saf* 67:333–340.

De Bruijn J, Busser F, Seinen W, Hermens J. 1989. Determination of octanol/water partition coefficients for hydrophobic organic chemicals with the "slow-stirring" method. *Environ Toxicol Chem* 8:499–512.

Di Toro DM, Zarba CS, Hansen DJ, Berry WJ, Swartz RC, Cowan CE, Pavlou SP, Allen HE, Thomas NA, Paquin PR. 1991. Technical basis for establishing sediment quality criteria for nonionic organic chemicals using equilibrium partitioning. *Environ Toxicol Chem* 10:1541–1583.

Ding Y, Landrum PF, You J, Harwood AD, Lydy MJ. 2012. Use of solid phase microextraction to estimate toxicity: Relating fiber concentrations to toxicity—part I. *Environ Toxicol Chem* 31:2159–2167.

Ding Y, Landrum PF, You J, Lydy MJ. 2013. Assessing bioavailability and toxicity of permethrin and DDT in sediment using matrix solid phase microextraction. *Ecotoxicology* 22:109–117.

Du J, Li Y, Huang ZC, You J. 2014. Chronic toxicity thresholds for sediment-associated benzo[a]pyrene in the midge (*Chironomus dilutus*). *Arch Environ Contam Toxicol* 66:370–378.

Du J, Pang J, You J. 2013. Bioavailability-based chronic toxicity measurements of permethrin to *Chironomus dilutus*. *Environ Toxicol Chem* 32:1403–1411.

Fathallah S. 2014. Toxicity of chemically spiked sediment to the carpet shell clam *Ruditapes decussatus* embryos and larvae. *Soil Sed Contam* 23:641–655.

Forbes VE, Cold A. 2005. Effects of the pyrethroid esfenvalerate on life-cycle traits and population dynamics of *Chironomus riparius*—Importance of exposure scenario. *Environ Toxicol Chem* 24:78–86.

Ginsburg JM. 1947. Comparative toxicity of DDT isomers and related compounds to mosquito larvae and fish. *Science* 105:233–234.

Heye K, Becker D, Eversloh CL, Durmaz V, Ternes TA, Oetken M, Oehlmann J. 2016. Effects of carbamazepine and two of its metabolites on the non-biting midge *Chironomus riparius* in a sediment full life cycle toxicity test. *Water Res* 98:19–27.

Hoke RA, Ankley GT, Kosian PA, Cotter AM, Vandermeiden FM, Balcer M, Phipps GL, West C, Cox JS. 1997. Equilibrium partitioning as the basis for an integrated laboratory and field assessment of the impacts of DDT, DDE and DDD in sediments. *Ecotoxicology* 6:101–125.

Hornsby AG, Wauchope RD, Herner A. 1996. Back Matter. In Hornsby, A G, Herner, A, Wauchope, R D, *Pesticide properties in the environment*, 1nd ed. Springer, New York, NY, USA, pp 26.

Jager T, Crommentuijn T, Van Gestel CA, Kooijman SA. 2004. Simultaneous modeling of multiple end points in life-cycle toxicity tests. *Environ Sci Technol* 38:2894–2900.

Kelce WR, Stone CR, Laws SC, Gray LE. 1995. Persistent DDT metabolite *p, p'*-DDE is a potent androgen receptor antagonist. *Nature* 375:581.

Kenaga EE. 1982. Predictability of chronic toxicity from acute toxicity of chemicals in fish and aquatic invertebrates. *Environ Toxicol Chem* 1:347–358.

Li H, Tyler Mehler W, Lydy MJ, You J. 2011. Occurrence and distribution of sediment-associated insecticides in urban waterways in the Pearl River Delta, China. *Chemosphere* 82:1373–1379.

Lotufo GR, Landrum PF, Gedeon ML. 2001a. Toxicity and bioaccumulation of DDT in freshwater amphipods in exposures to spiked sediments. *Environ Toxicol Chem* 20:810–825.

Lotufo G, Farrar J, Duke B, Bridges T. 2001b. DDT toxicity and critical body residue in the amphipod *Leptocheirus plumulosus* in exposures to spiked sediment. *Arch Environ Contam Toxicol* 41:142–150.

Ma M, Feng Z, Guan C, Ma Y, Xu H, Li H. 2001. DDT, PAH and PCB in sediments from the intertidal zone of the Bohai Sea and the Yellow Sea. *Mar Pollut Bull* 42:132–136.

Matsushima A. 2018. A novel action of endocrine-disrupting chemicals on wildlife; DDT and its derivatives have remained in the Environment. *Int J Mol Sci* 19:1377.

Maul JD, Brennan AA, Harwood AD, Lydy MJ. 2008. Effect of sediment-associated pyrethroids, fipronil, and metabolites on *Chironomus tentans* growth rate, body mass, condition index, immobilization, and survival. *Environ Toxicol Chem* 27:2582–2590.

Nowell LH, Norman JE, Ingersoll CG, Moran PW. 2016. Development and application of freshwater sediment-toxicity benchmarks for currently used pesticides. *Sci Total Environ* 550:835–850.

Organisation for Economic Co-operation and Development. 2010. Sediment-water chironomid life-cycle toxicity test using spiked water or spiked sediment. OECD guidelines for the testing of chemicals. Paris, French.

Phipps G, Mattson V, Ankley G. 1995. Relative sensitivity of three freshwater benthic macroinvertebrates to ten contaminants. *Arch Environ Contam Toxicol* 28:281–286.

Qi H, Ma P, Li H, You J. 2015. Assessment of sediment risk in the north end of Tai Lake, China: Integrating chemical analysis and chronic toxicity testing with *Chironomus dilutus*. *Arch Environ Contam Toxicol* 69:461–469.

Sabljić A, Güsten H, Verhaar H, Hermens J. 1995. QSAR modelling of soil sorption. Improvements and systematics of log *K*oc vs. log *K*ow correlations. *Chemosphere* 31:4489–4514.

Schuler LJ, Landrum PF, Lydy MJ. 2007. Response spectrum of pentachlorobenzene and fluoranthene for *Chironomus tentans* and *Hyalella azteca*. *Environ Toxicol Chem* 26:1248–1257.

Schuytema GS, Nebeker AV, Griffis WL, Miller CE. 1989. Effects of freezing on toxicity of sediments contaminated with DDT and endrin. *Environ Toxicol Chem* 8:883–891.

Schwarzbauer J, Ricking M, Littke R. 2003. DDT-related compounds bound to the nonextractable particulate matter in sediments of the Teltow Canal, Germany. *Environ Sci Technol* 37:488–495.

Sibley PK, Benoit DA, Ankley GT. 1997. The significance of growth in *Chironomus tentans* sediment toxicity tests: Relationship to reproduction and demographic endpoints. *Environ Toxicol Chem* 16:336–345.

Swartz RC, Cole FA, Lamberson JO, Ferraro SP, Schults DW, Deben WA, Lee Ii H, Ozretich RJ. 1994. Sediment toxicity, contamination and amphipod abundance at a DDT-and dieldrin-contaminated site in San Francisco Bay. *Environ Toxicol Chem* 13:949–962.

Taylor E, Blockwell S, Maund S, Pascoe D. 1993. Effects of lindane on the life-cycle of a freshwater macroinvertebrate *Chironomus riparius* Meigen (Insecta: Diptera). *Arch Environ Contam Toxicol* 24:145–150.

US Environmental Protection Agency. 2000. Methods for measuring the toxicity and bioaccumulation of sediment-associated contaminants with freshwater invertebrates. EPA600/R-99/064. Washington, DC.

US Environmental Protection Agency. 2006. EPA region III BTAG freshwater sediment screening benchmarks. Washington, DC.

Wang F, Pei Y, You J. 2015. Biotransformation of dichlorodiphenyltrichloroethane in the benthic polychaete, *Nereis succinea*: Quantitative estimation by analyzing the partitioning of chemicals between gut fluid and lipid. *Environ Toxicol Chem* 34:360–368.

Weston DP, You J, Lydy MJ. 2004. Distribution and toxicity of sediment-associated pesticides in agriculture-dominated water bodies of California's Central Valley. *Environ Sci Technol* 38:2752–2759.

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.

Xu Y, Spurlock F, Wang Z, Gan J. 2007. Comparison of five methods for measuring sediment toxicity of hydrophobic contaminants. *Environ Sci Technol* 41:8394–8399.

You J, Schuler L, Lydy M. 2004. Acute toxicity of sediment-sorbed endrin, methoxychlor, and endosulfan to *Hyalella azteca* and *Chironomus tentans*. *Bull Environ Contam Toxicol* 73:457–464.

Figures

Figure 1. Survival of *Chironomus dilutus* exposed to sediment-associated DDT at different concentrations in 20- and 63-d toxicity testing. Ash-free dry weight (AFDW) was also measured at 20 d. L1 to L6 represent different concentration levels of DDT equivalent in sediment. The asterisks indicate significant differences between the treatments and control ($p < 0.05$). Error bars represent standard deviations.

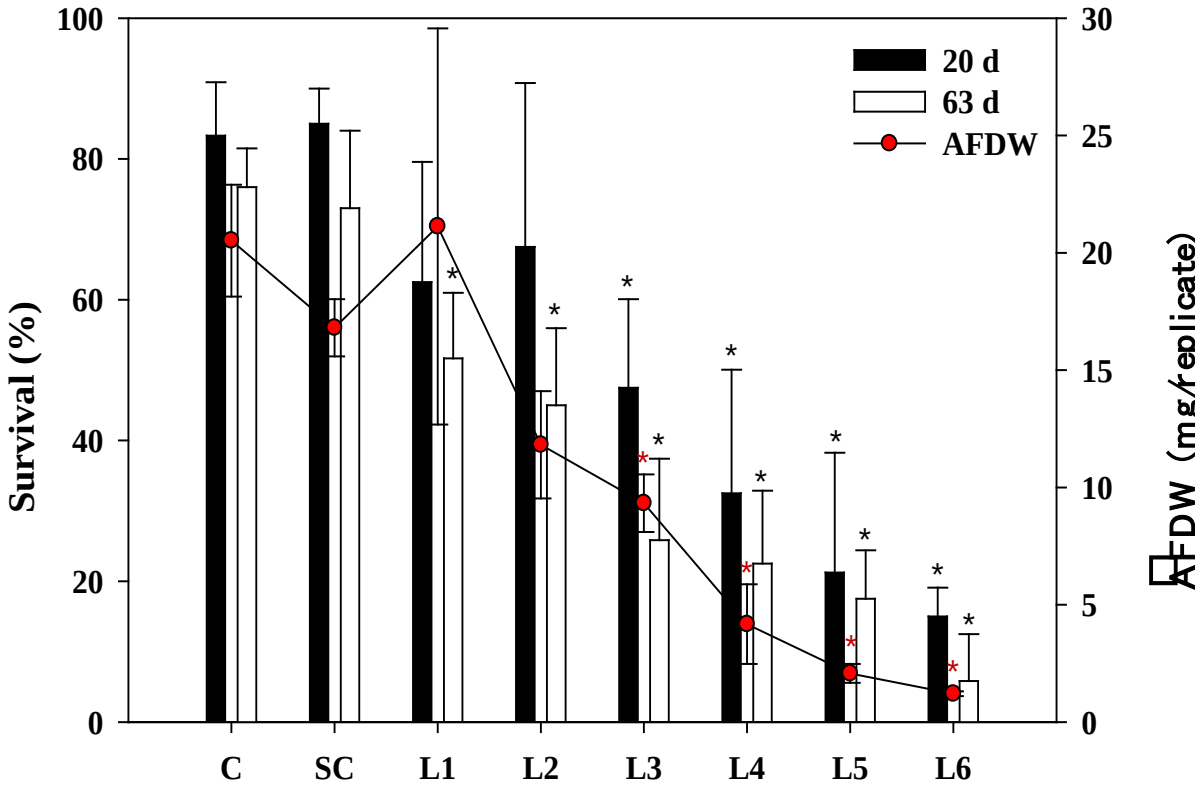


Figure 2. Emergence of *Chironomus dilutus* at different time and cumulative emergence exposed to sediment-associated DDT at different concentrations. L1 to L6 represent different concentration levels of DDT equivalent in sediment. The asterisks indicate significant differences between the treatment levels and control ($p < 0.05$). Error bars represent the standard deviations.

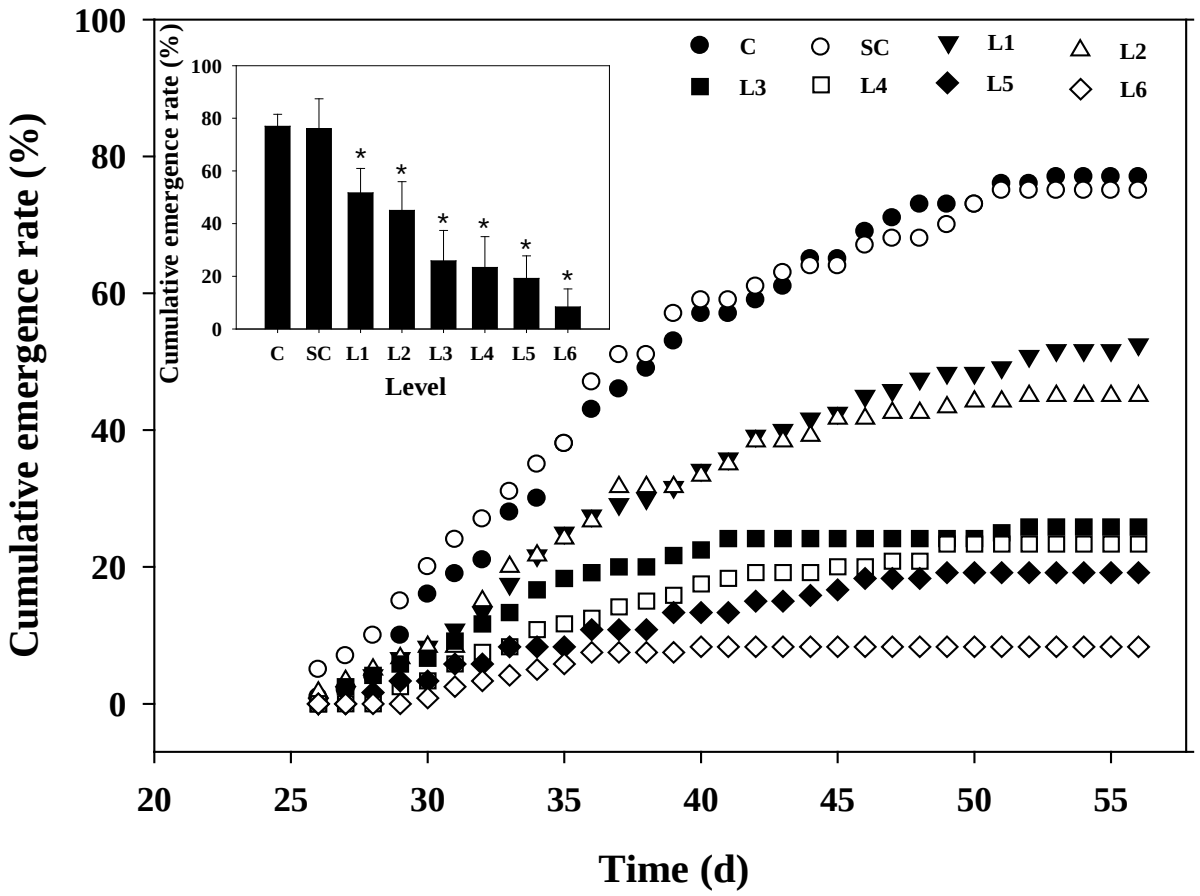


Figure 3. Impaired reproduction of *Chironomus dilutus* after exposure to sediment-associated DDT at different concentrations. The endpoints of reproduction include sex ratio of the midges and fecundity ratio of the females (A), and number of eggs per replicate and the hatchability of the eggs (B). L1 to L6 represent different concentration levels of DDT equivalent in sediment. The asterisks indicate significant differences between the treatments and control ($p < 0.05$). Error bars represent standard deviations.

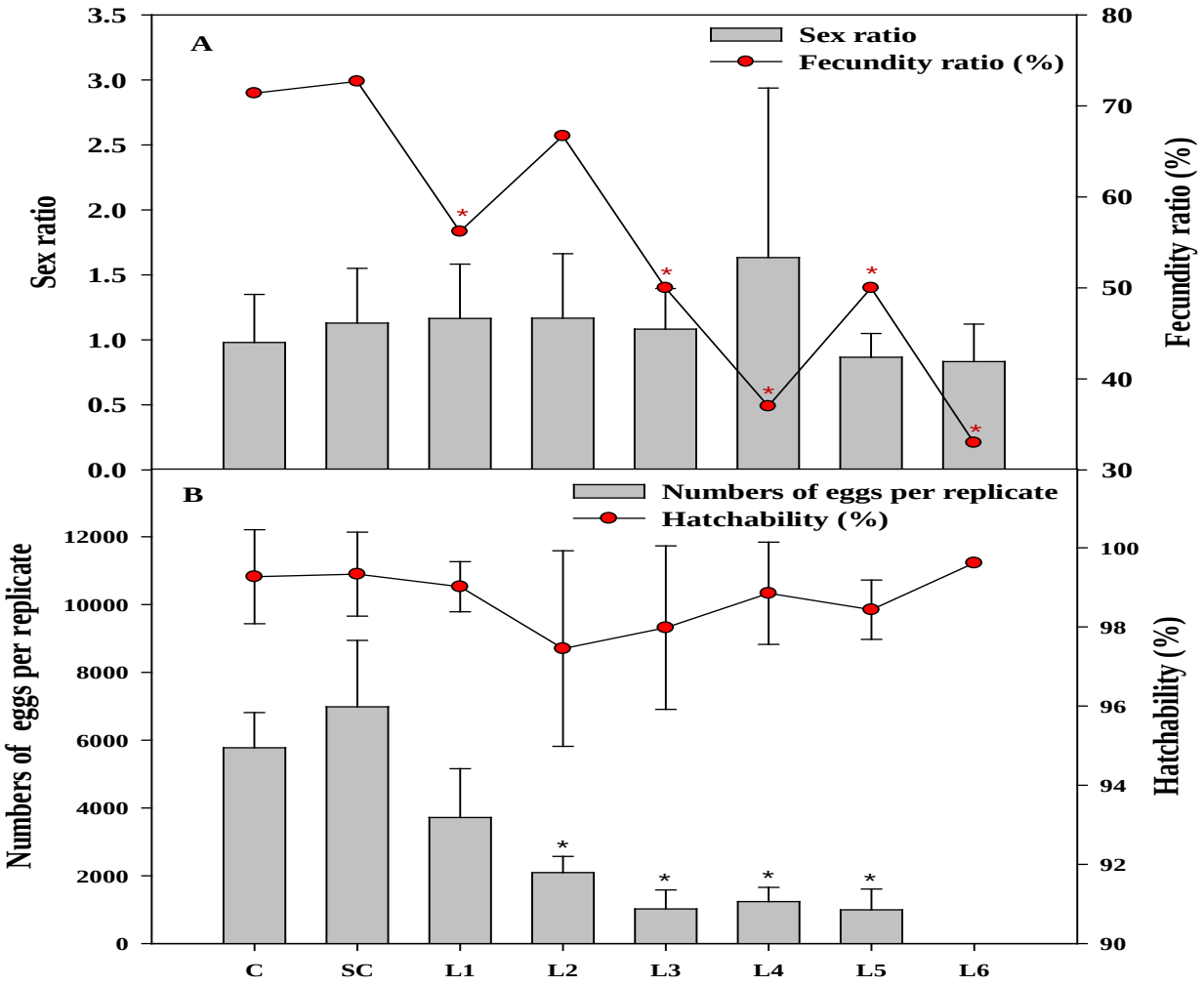
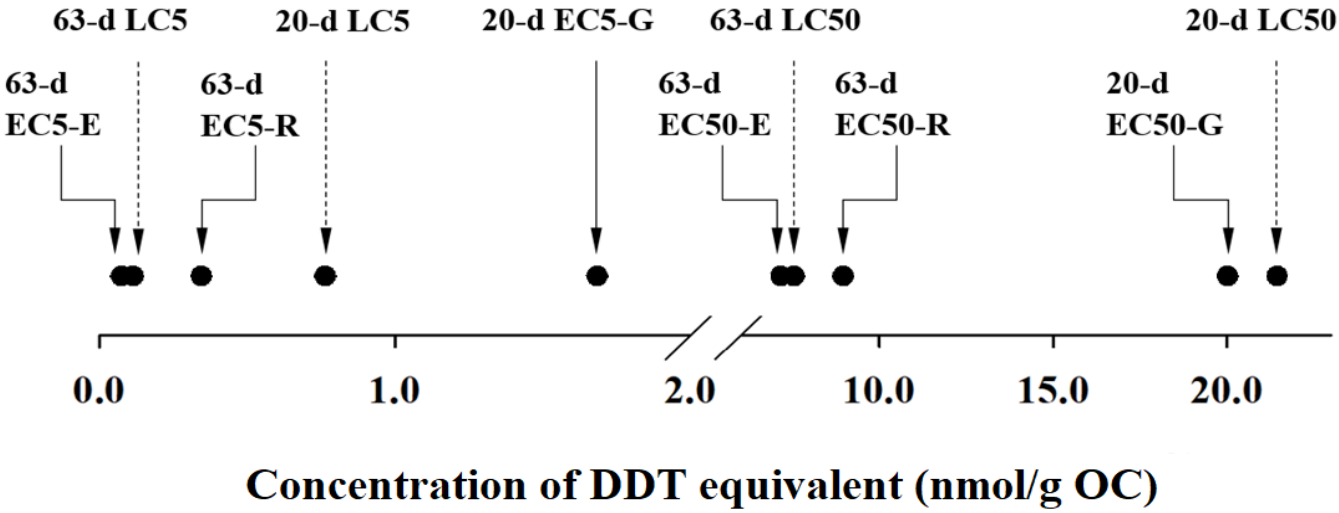


Figure 4. Toxicity response spectrum of *Chironomus dilutus* exposed to sediment-associated DDT in full life-cycle toxicity testing. The LC5 and LC50 represent 5% and 50% lethal concentrations, respectively, and EC5 and EC50 represent 5% and 50% effective concentrations, respectively. The G, E, and R represent growth, emergence, and reproduction, respectively, in chronic toxicity testing.



Graphical abstract. Full life-cycle toxicity testing of sediment-bound DDT to *Chironomus dilutus*. Chronic toxicity spectrum was established for endpoints of lethality, growth, emergence, and reproduction.

Table 1 The median lethal and effective concentrations (LC50/EC50) (with 95% confidence limits) of sediment-bound DDT to aquatic organisms

Testing organism	Organism type	Taxa group	Sediment aging time (d)	Testing period (d)	Endpoint	LC50/EC50 (nmol/g OC)	DDT% [a]	DDD% [a]	DDE% [a]	Reference
Chironomus dilutus	Fresh water	Insect	7	63	Reproduction	8.92 (3.32 – 15.1)	59.6± 7.1%	22.6± 7.6%	17.8± 11.0%	The present study
Chironomus dilutus	Fresh water	Insect	7	63	Emergence	7.13 (4.10 – 10.5)	59.6± 7.1%	22.6± 7.6%	17.8± 11.0%	The present study
Chironomus dilutus	Fresh water	Insect	7	63	Mortality	7.50 (4.61 – 10.6)	59.6± 7.1%	22.6± 7.6%	17.8± 11.0%	The present study
Chironomus dilutus	Fresh water	Insect	7	20	Growth	20.0 (15.0 – 25.3)	59.6± 7.1%	22.6± 7.6%	17.8± 11.0%	The present study
Chironomus dilutus	Fresh water	Insect	7	20	Mortality	21.4 (11.2 – 34.3)	59.6± 7.1%	22.6± 7.6%	17.8± 11.0%	The present study

Chironomus dilutus	Freshwater	Insect	7	10	Mortality	334 (165–568)	59.6± 7.1%	22.6± 7.6%	17.8± 11.0%	The present study
Chironomus dilutus	Freshwater	Insect	27–50 yr[b]	10	Mortality	7052–8463	17–100%	1.0–88%	0–63%	Hoke et al. 1997
Hyalella azteca	Freshwater	Crustacean	42	10	Mortality	395 (367–414)	Only DDT was quantified			Schuytema et al. 1989
Hyalella azteca	Freshwater	Crustacean	60	10	Mortality	517 (417–533)	85.2± 3.1%	9.0±2.3%	5.8%	Lotufo et al. 2001a
Hyalella azteca	Freshwater	Crustacean	60	28	Mortality	467 (417–483)	28.3± 6.0%	11.0± 5.0%	56.0± 5.0%	Lotufo et al. 2001a
Hyalella azteca	Freshwater	Crustacean	25–46 yr[c]	10	Mortality	7278	Not reported			Swartz et al. 1994
Leptocheirus plumulosus	Marine	Crustacean	14	10	Mortality	315 (310–320)	29–48%	24–37%	1–4%	Lotufo et al. 2001b

Leptocheirus plumulosus	Marine	Crustacean	14	28	Mortality	360 (354–365)	10–21%	44–57%	1–4%	Lotufo et al. 2001b
Diporeia spp.	Freshwater	Crustacean	60	10	Mortality	7711 (7267–8156)	89.4±8.7%	5.8±3.7%	4.8%	Lotufo et al. 2001a
Diporeia spp.	Freshwater	Crustacean	60	28	Mortality	1822 (1689–1933)	61.0±7.0%	34.3±7.6%	4.7%	Lotufo et al. 2001a
Rhepoxynius abronius	Marine	Crustacean	25–46 yr[c]	10	Mortality	2934	Not reported			Swartz et al. 1994
Eohaustorius estuarius	Marine	Crustacean	25–46 yr[c]	10	Mortality	7052	Not reported			Swartz et al. 1994
Ruditapes decussatus (Embryos)	Marine	Mollusca	1	1	Abnormal *D-shaped*	482 (316–753)[d]	Only DDT was quantified			Fathallah 2014
Ruditapes decuss	Marine	Mollusca	1	4	Mortality	1109 (835–1546)	Only DDT was quantified			Fathallah 2014

atus						[d]		
Mercenaria mercenaria	Marine	Mollusca	1	10	Mortality	2337 (1934–3345)	Only DDT was quantified	Chung et al. 2007

[a] Percentage of DDT, DDD, or DDE in the total DDX in test sediments.

[b] Field sediments which were contaminated by untreated industrial effluent with DDT 27–50 years ago.

[c] Field contaminated sediments collected from San Francisco Bay where many DDT producing companies existed 25–46 years ago.

[d] Total organic carbon was not reported in the cited paper and the toxicity data were estimated based on 1% of organic carbon.

Table 2. Mortality of *Chironomus dilutus* in chronic sediment toxicity testing (20 d), predicted porewater concentrations (C_{PW}, pmol/L), toxic units (TU) of DDT, DDD, and DDE in porewater, and their respective toxicity contributions (%) to the sum toxic units (TU_{sum}) of DDX

Level		L1	L2	L3	L4	L5	L6
Mean mortality (%)		37.5	32.5	52.5	67.5	78.8	85.0
DDT [b]	C_{PW} [a]	14.0	51.4	102	197	442	994

	TU [c]	0.06	0.22	0.44	0.86	1.92	4.32
	Contribution (%)	22.2	34.4	36.4	39.6	39.3	36.5
	C_{PW}	8.60	18.7	37.0	61.7	139	359
DDD [b]	TU	0.16	0.35	0.68	1.14	2.57	6.65
	Contribution (%)	59.3	54.7	56.2	52.5	52.7	56.2
	C_{PW}	30.3	42.1	56.6	110	248	558
DDE [b]	TU	0.05	0.07	0.09	0.17	0.39	0.87
	Contribution (%)	18.5	10.9	7.44	7.83	7.99	7.35
TU_{sum}		0.27	0.64	1.21	2.17	4.88	11.8

[a] The C_{PW} was calculated by dividing organic carbon normalized chemical concentration in sediment by partitioning coefficients of the chemical between organic carbon and water (Eq. 1).

[b] The partitioning coefficients between organic carbon and water (log *K*oc) were 5.31, 5.00, 4.82 for DDT, DDD, and DDE, respectively (Sabljić et al. 1995; Hornsby et al. 1996).

[c] The TU was calculated by dividing C_{PW} by 20-d water-only LC50 values (Eq. 2). The 20-d LC50 values for DDT, DDD, and DDE were estimated to be 0.23, 0.054, and 0.64 nmol/L, respectively, based on their 10-d water-only LC50 values (Hoke et al. 1997) and the acute chronic ratio (10 to 20 d, 15.6) determined in the present study.