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Trophic transfer and effects of DDT in male hornyhead turbot (*Pleuronichthys verticalis*) from Palos Verdes Superfund site, CA (USA) and comparisons to field monitoring

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Abstract

High concentrations of DDT and metabolites (Σ DDT) have been detected in sediment and the demersal flatfish hornyhead turbot (*Pleuronichthys verticalis*) collected from Palos Verdes (PV), California, USA, a site contaminated with over 100 metric tons of DDT throughout 1960s to 70s. This study was elaborated to assess the transfer of Σ DDT from PV-sediment into polychaete (*Neanthes arenaceodentata*) and hornyhead turbot, and to investigate if the genomic responses in turbot from two different laboratory exposures mimic those in turbot caught in PV (PV-turbot). Turbot fed PV-sediment-contaminated polychaete for 7 days had liver concentrations of Σ DDT similar to PV-turbot. After 28 days, Σ DDT also accumulated by livers of turbot gavaged with Σ DDT mixture. *In vitro* cell bioassays indicated significant increases of 17 β -estradiol equivalents (EEQ) in turbot as compared to the control in the 7-day study. These responses corresponded to those measured in PV-fish. Glucocorticoid receptor (GR), anti-androgen receptor (anti-AR), estrogen receptor (ER) or aryl hydrocarbon receptor (AhR) activities were also observed in PV-sediment, and PV-sediment-exposed worm. Anti-AR, AhR and GR activities were significantly higher in PV-sediment than reference sediment (San Diego, SD). Higher transcripts of hepatic VTG, ER α and ER β were found in PV-turbot than SD-turbot, but unaltered in fish exposed to sediment-contaminated worms for the 7-day study. In contrast, liver extracts from the 28-day treatment of Σ DDT showed lower EEQ but similar hepatic VTG and ER β transcripts relative to those of PV-turbot. These data indicated that trophic transfer of sediment-associated DDT in 7-day exposures corresponded to field measurements of DDT residues and *in vitro* ER bioactivities, but failed to mimic *in vivo* biological effects observed in field fish. In contrast, treatment with Σ DDT

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Supplementary material

Supplementary data associated with this article can be found in a separate document available at URL.

alone for 28 days mimicked *in vivo* biological effects of DDTs in PV fish, but did not correspond to liver concentrations or *in vitro* bioactivities.

Graphical Abstract

Keywords

bioaccumulation; DDT; cell-based bioassay; trophic transfer; estrogenic activity

1. Introduction

Approximately 20 metric tons of 1,1,1-trichloro-2, 2-bis (*p*-chloro-phenyl) ethane (DDT) was discharged to the Palos Verdes (PV) Shelf, California USA through the early 1970s (Young et al., 1975). The majority of DDT (a mixture of 80% *p,p'* and 20% *o,p'* isomers) was produced by the Montrose Chemical Corporation in Torrance, CA, and discharged in effluent from the Joint Water Pollution Control Plant onto the PV shelf (Mearns et al., 1991, Eganhouse et al., 2000). To date a 40 km² area of DDT contamination is attributed to this discharge, and is now designated the Palos Verdes Superfund Site (Stull et al. 1996, **Fig. 1**). Although technical DDT input from effluent discharge was almost non-detectable by the early 1990s (Raco-Rands and Steinberger, 2001), DDT and its metabolites (4,4'-DDT; 2,4'-DDT; 4,4'-DDE; 2,4'-DDE; 4,4'-DDD; 2,4'-DDD) (Σ DDT) in sediments on the PV shelf remain high, with mean measured concentrations of 49 mg/kg dry wt. and 43 mg/kg dry wt. during 2012 and 2013 (LACSD 2014). Due to the high hydrophobicity of several isomers of Σ DDT (K_{ow} = 5.87 for *o,p'*-DDD to 6.91 for *p,p'*-DDT), there is an increased risk of bioaccumulation. Lipid normalized concentrations of total DDTs measured in the hornyhead turbot (*Pleuronichthys verticalis*) taken from three zones in PV during 2012 and 2013 averaged 11,700 ng/g at zone 1, near the effluent outfall, to 633 ng/g in zone 3, north of PV in Santa Monica Bay (**Fig. 1**) (LACSD, 2014). Flatfish tissue concentrations of DDTs correlated highly with sediment concentrations in Southern CA (Schiff and Allen 2000, Allen et al., 2004). Therefore, understanding how DDTs bioaccumulate from sediments to fish through trophic levels is important to reduce uncertainty in ecological and human health risk assessments.

In addition to better understanding exposure assessments, it is also important to quantify biological responses of DDT contamination. Historical evaluations of the PV Superfund site have reported alterations in reproductive and endocrine metrics in several pelagic and demersal fish species (Hose et al., 1989; Spies et al., 1996). Several DDT isomers were found to bind the estrogen receptor in Japanese medaka (Chakraborty et al., 2011) and Atlantic salmon (Tollefsen et al., 2003), to alter gonadal development (Zaroogian et al., 2001), to depress plasma testosterone, and to induce vitellogenesis in juvenile male summer flounder *in vivo* (Mills et al., 2001). Induction of vitellogenin (VTG), an estrogen-dependent precursor egg yolk protein, and disruption of steroid hormones in flatfish from this site have also been noted in several studies (Schlenk et al., 2005; Rempel-Hester et al., 2009; Forsgren et al. 2012; Reyes et al. 2012). Induction of VTG in males has been shown to indicate exposure to estrogenic contaminants and potential reproductive impairment (Kidd et al., 2007). In addition to estrogenic responses, other DDT metabolites (*p,p'*-DDE) have also been identified as androgen receptor (AR) antagonists (Hotchkiss et al. 2008).

Previous work has quantified the bioavailability of Σ DDT to benthic invertebrates from several sediment samples from PV in laboratory studies (Bao et al., 2013; Jia et al., 2014). However, it is unclear whether laboratory exposures can mimic trophic transfer from benthic invertebrates to fish (Zeng and Tran 2002). The objectives of the present study were 1) To measure the uptake of Σ DDT from PV sediment to an indigenous marine polychaete worm (*Neanthes arenaceodentata*), and evaluate their transfer to hornyhead turbot (*Pleuronichthys verticalis*); 2) To investigate the transfer of estrogen, aryl hydrocarbon, glucocorticoid, and anti-androgen receptor ligands from sediments to biota; 3) To assess if the ER related mRNA responses in turbot from diet-derived or chemical-only exposures in the laboratory mimic those of turbot caught from PV; and 4) To determine the contribution of Σ DDT to the ER responses. Ultimately, the results from this integrated study will help our understanding of potential bioaccumulation and effects of Σ DDT found in the PV Superfund area on demersal fish species.

2. MATERIALS AND METHODS

2.1 Chemical and reagents

The following chemicals were purchased from AccuStandard: 1,1,1-Trichloro-2, 2-bis-(*p*-chlorophenyl)ethane (*p,p'*-DDT), 1,1,1-trichloro-2,4-bis-(*o*-chlorophenyl)ethane (*o,p'*-DDT), 1,1-dichloro-2,2-bis-(chlorophenyl) ethane (*p,p'*-DDD), 1,1-dichloro-2,2-bis-(chlorophenyl) ethylene (*p,p'*-DDE), 1,1-dichloro-2, 4-bis-(chlorophenyl) ethylene (*o,p'*-DDE), 1,1-dichloro-2,4-bis-(chlorophenyl) ethane (*o,p'*-DDD), surrogate standards PCB-67 and PCB-191, and internal standards PCB-30 and PCB-82. The performance reference compounds (PRCs) ^{13}C -*o,p'*-DDD and ^{13}C -*o,p'*-DDE and *p,p'*-DDT- d_8 , *o,p'*-DDT- d_8 , *p,p'*-DDD- d_8 , and *p,p'*-DDE- d_8 were purchased from Cambridge Isotope Laboratories (Tewksbury, MA) or C/D/N Isotopes (Pointe-Claire, Canada). Chemicals known to activate estrogen receptor (ER) (17 β -estradiol), Aryl-hydrocarbon Receptor (AhR) (PCB126), Glucocorticoid Receptor (GR) (dexamethasone) and inhibit the Androgen Receptor (anti-AR) (cyproterone acetate) were purchased at the highest purity available from Sigma-Aldrich (St. Louis, MO). The AR ligand (methyltrienolone, R1881) was purchased from

Perkin Elmer. Solvents and other chemicals were of analytical grade and purchased from Fisher Scientific. Deionized water with electrical resistivity of 18.2 M /cm was prepared using a Barnstead E-pure system.

2.2 Sample collection

A single grab sample of sediment (0–15 cm) contaminated with Σ DDT was taken from the ocean floor at site 8C (33° 41.91' N; 118° 20.14' W) along the PV Shelf off the coast of Los Angeles, California, USA, with known contamination of DDT and PCBs (Jia et al. 2014) (**Fig. 1**). Reference sediment was purchased from New Fields (Port Gamble, Washington, USA). All collected sediments were sieved through a 2-mm mesh and stored at 4 °C before use. Chemical analyses of DDT and its metabolites was performed on each triplicate samples of sediment (n = 3) as described below.

Two laboratory studies were conducted and compared to field samples in animals at the time of animals capture. The first study was a 7 day bioaccumulation study where laboratory-cultured polychaete worms (*Neanthes arenaceodentata*) were exposed to sediment for 4 days and then fed to flatfish for 7 days. Extracts from sediments, worms, and fish were evaluated for receptor ligands through cellular bioassays and chemically for DDT and its metabolites. Since previous studies found estrogenic activity in fish from this location (Spies et al. 1996), laboratory and field-collected fish were evaluated for estrogenic activity (vitellogenin and estrogen receptor expression) and showed a strong correlation between DDTs and estrogenic activity (see below). Given the relationship between estrogenic activity and DDT, the second study was a 28 day study feeding flatfish a mixture of DDT and its metabolites at concentrations measured in worm extracts (**Table 1**) (ambient and ~3X). Estrogen receptor ligands, DDT and its metabolites, and estrogenic activity was measured in the laboratory fish and again compared to field samples.

In the 7-day laboratory study, 4–6 fish per treatment were exposed to worms that were contaminated in the lab via a 4-day exposure in PV and reference sediments. Male hornyhead turbot were collected by otter trawl at a reference site off the coast of Orange County (OC) (33° 36.06' N; 118° 05.20' W), ~30 km south of site 8C on the PV Shelf. Twenty five to thirty turbot were collected during February of 2012 and 2013 at the OC site and in February 2014 at the PV site. Turbot were either sampled for blood, liver and bile or transported to the laboratory and acclimated in artificial seawater (32‰ Instant Ocean) for six months to depurate potential DDT metabolites. During acclimation and depuration, each fish was individually fed live earthworms three times per week before initiating the polychaete experiment. Liver tissues, blood plasma (3,000 × g supernatant), and bile were frozen and stored at –80 °C for subsequent chemical and biological measurements.

For the 7-day bioaccumulation study, polychaete worms (*Neanthes arenaceodentata*) 2–3 week old were purchased from Aquatic Toxic Support (Bremerton, WA) and acclimated in 32‰ artificial seawater at 20 °C for one week. Worms were fed dry algae once before sediment exposure. These worms were used only for sediment exposures (4 days-see below) and subsequently fed to fish for the 7-day exposure study

In the second study, the contribution of DDT and its metabolites were evaluated by a 28-day gavage study. Male hornyhead turbot were collected from off the coast of San Diego (SD) (32° 39.94' N; 117° 19.49' W), at the City of San Diego's wastewater monitoring reference site, approximately 150 km south of the PV site in February/March of 2014. Based on the 7-day study, turbot were acclimated with feeding as described above, and depurated of Σ DDT for one month prior to the bioaccumulation experiment. The acclimation time was less than that of OC turbot due to lower concentrations of DDTs measured in fish collected from SD determined from previous studies (Maruya et al. 2012).

2.3 Bioaccumulation

In the 7-day study, 40 worms were transferred to a 1-L beaker containing 40 g (wet wt.) sediment and 350 mL of 32‰ artificial seawater at 20 °C. The overlying water was aerated via continuous bubbling, and the water level maintained by adding artificial seawater as needed. At the end of a 4-day exposure, the worms were harvested by sieving the sediment slurry through a 100-mesh sieve. The relatively short exposure time was chosen through preliminary experiments to avoid potential toxicity effects to the test organism (Jia et al. 2014). Worms were then divided by wet wt. low (3g of worm/kg of turbot body weight) and high (10g of worm/kg turbot wt.) and fed to corresponding turbot. A subset of worms was flash frozen and stored at -80 °C for later extraction and chemical/bioassay analysis. Turbot were individually weighed, randomly divided into 8 L aquaria containing 32‰ artificial seawater, and allowed to acclimate in single aquaria for one week prior to exposure. During acclimation turbot were fed earthworms three times per week. During acclimation and exposure, 75% water exchange occurred every-other day. For the 7-day feeding exposure, turbot were fed worms daily from one of four treatments (4-6 fish per treatment), 3 g worm wet wt./kg of turbot wet wt. clean sediment (control), 3 g worm wet wt./kg of turbot wet wt. PV 8C sediment, 10 g worm wet wt./kg of turbot wet wt. clean sediment (control), 10 g worm wet wt./kg of turbot wet wt. PV 8C sediment. At the end of 7 day, fish were euthanized with MS-222, weighed, and liver and bile were collected and flash frozen and stored at -80 °C for later evaluation.

In the 28-day study, turbot were individually weighed and then individuals were randomly divided into 8 L aquaria containing 32‰ artificial seawater. Turbot were allowed to acclimate for one week prior to exposure and fed clean earthworms three times per week. During acclimation and exposure, 75% water exchange occurred every-other day. For the 28-day feeding exposure, turbot were fed clean worms three times per week in the morning and gavaged two hours later from one of the three treatments, control (saline solution), DDT metabolite solution equal to 9g worm wet wt./kg of turbot body (low DDT), 30g of worm wt./ kg of turbot body wet wt. (high DDT). DDT metabolite concentrations were based on initial 4 day exposures of worms in 8C sediment (**Table 1**). After 28 days, fish were euthanized with MS-222, weighed, and liver and bile tissue were collected and flash frozen and stored at -80 °C for later use.

2.4 Extraction and analysis of Σ DDT in sediment and tissues

Sediment and tissue samples were sonicated using acetone and dichloromethane as described in Bao et al. (2013) and Jia et al. (2014). Sample extracts were analyzed by GC-

MS in the selected ion monitoring mode for the DDT analytes, recovery surrogates and PRCs listed in 2.1. Details for these procedures can be found in **Supplemental Materials**.

2.5 Biota-sediment accumulation factor and biomagnification factor

The biota-sediment accumulation factor (BSAF) (Eq.1) is the ratio of chemical tissue concentrations with respect to chemical concentrations in sediment. BSAF is calculated using lipid normalized (tissue) and organic carbon normalized (sediment) concentrations (USEPA 2003).

$$BSAF_{\sum DDTs} = (C_t/V_f) / (C_s/TOC) \quad (\text{Eq. 1})$$

Where C_t is the concentration in the tissue, V_f is the lipid fraction in the tissue, C_s is the concentration in the sediment, and TOC is the total organic carbon content in sediment.

The biomagnification factor (BMF) is the ratio of a chemical concentration in the tissue of an organism compared to the tissue concentrations of its prey. BMFs are calculated by dividing lipid normalized chemical tissue concentrations within an organism by the lipid normalized concentration(s) of that chemical in the prey of the organism. BMFs > 1 indicate the occurrence of biomagnification (USEPA 2008).

$$BMF_{\sum DDTs} = (C_t/V_f)_{\text{predator}} / (C_t/V_f)_{\text{prey}} \quad (\text{Eq. 2})$$

2.6 Cell-based Bioassays

The estrogenic (ER) and aryl hydrocarbon receptor-like (AhR) activities in sediment and worm extracts were assessed by using the established *in vitro* LUMI-CELL™ ER (BG1Luc4E2) assay and AhR assay (GeneBLAzer CYP1A1-bla LS-180, Life Technologies, Carlsbad, CA). BG1Luc4E2 cells were obtained from Dr. Michael Denison (University of California-Davis). GeneBLAzer glucocorticoid receptor (GR) and anti-androgen receptor (AR) HEK 293T division arrested cells were purchased from Life Technologies (Carlsbad, CA) and the assays conducted at Southern California Coastal Research Project, Costa Mesa, CA. ER, AhR, and GR activities were expressed as 17β-estradiol equivalent (EEQ), aryl hydrocarbon toxicity equivalent (TEQ), and dexamethasone equivalent (DEX-Q), respectively. The anti-AR activities were expressed as the percent maximum response relative to the AR agonist methyltrienolone (R1881, 1×10^{-7} M). Due to extract limitations, only ER analyses were conducted on fish bile and liver extracts. Bioassay protocols are described in detail in **Supplementary Information**.

2.7 mRNA Expression

RNA was extracted from liver samples using TriZol reagent (Life Technologies, Carlsbad, CA), following the instruction of the manufacturer. Samples were cleaned to remove any solvent contamination by precipitating RNA with a 2 M NaCl aqueous solution, and washing the precipitate with ethanol. RNA was then dissolved in water, and stored at -80°C . RNA concentrations were measured using a Nanodrop spectrophotometer (Thermo Fischer Scientific, Wilmington, DE). Liver RNA was DNase treated (Promega, Madison, WI) prior

to cDNA creation. For each tissue sample, cDNA was synthesized from 200 ng/μL of liver RNA using the High Capacity cDNA reverse transcription kit (Life Technologies, Carlsbad, CA) following the instruction of the manufacturer. Primers were designed using the PrimerQuest software from Integrated DNA Technologies (Coralville, IA), and all primers were ordered from their site. Primer sequences are listed in **Table S1**. Gene expression was quantified using Power SYBR® Green qPCR Master Mix (Life Technologies, Carlsbad, CA) per manufacturer's instruction. RT-PCR was performed on the iCycler-MyIQ (Bio-Rad, Hercules, CA) with the following protocol: 1 cycle at (i) 95 °C for 10 min; 39 cycles at (ii) 95 °C for 1 min; (iii) 55 °C for 30 s. Following the amplification reaction, a melting curve analysis was carried out between 60 and 95 °C, fluorescence data were collected at 0.5 °C intervals. Relative qPCR expression was determined using 2^{-C_t} and normalized to the transcript levels for β-Actin, as it did not vary across treatments. Data was presented as fold-induction relative to control.

2.8 Statistics

All mRNA transcript data from the experiment were tested for normality using a Kolmogorov-Smirnov test, and homogeneity of variance using Levene's test. In cases where the qPCR data or log2 transformed data met parametric assumptions, one-way analysis of variance (ANOVA) was used to test for differences across groups, while differences between groups were determined using Tukey test. T-test was used to compare the differences of mRNA expression and bioactivities in between PV and SD reference site. For the cases in which the data did not conform to parametric assumptions, a nonparametric Kruskal-Wallis test (KW) was used to test for differences across groups, and a Dunn's test was used to determine differences among groups. To calculate EEQs, TEQs and DEX-eq dose-response curves of the reference compounds E2, PCB126 and DEX were generated by SigmaPlot software (SPSS, Chiacago, IL). For the results of *in vitro* bioactivities from laboratory exposures, one-way ANOVA followed by post hoc Tukey test was used to examine statistical differences amongst different treatment and control groups. Differences were considered significant at $p < 0.05$. All statistical analyses were conducted using PASW v.19 (SPSS, Chicago, IL).

3 Results

3.1 Accumulation of DDTs by analytical chemistry

In the field-collected PV 8C sediment, all six DDT compounds measured were detected with *p,p'*-DDE constituting 68% of ΣDDT, followed by *o,p'*-DDE at 13% (**Table 1**). Lipid normalized ΣDDT concentrations in lab-exposed worms after 4 days led to a BSAF estimate of 0.007. Of the total DDT content, approximately 74% was *p,p'*-DDE. *p,p'*-DDD made up nearly 20% of the total residue in polychaetes.

The sediment to turbot BSAF in the 7-day study was 0.006 and 0.0028 for male hornyhead turbot fed 3g and 10g of worms exposed to PV sediment/kg turbot, respectively. Accumulation of ΣDDT in the liver of male hornyhead turbot from ingestion of either 3 g or 10 g of PV sediment-exposed worm/kg turbot after the 7 days exposure provided a BMF

from worm to turbot of 0.91 and 4.09 for turbot fed 3g and 10 g of PV sediment-exposed worm/kg turbot, respectively.

Accumulation of Σ DDT was also observed in the liver of male hornyhead turbot gavaged with a DDT isomer mixture equal to 30g of PV-sediment treated worm / kg turbot (517 ± 152 ng/g dry weight) after 28 days of exposure. The Σ DDT concentration in liver of gavaged animals was lower than liver concentrations observed in animals collected from PV as well as those treated with sediment-contaminated worms in the laboratory. In accordance with the composition of the Σ DDT mixture derived from worm body burdens (**Table 1**), *p,p'*-DDE constituted approximately 46 % of the total residues followed by *p,p'*-DDD with 36% of the total. The Σ DDT concentration in turbot gavaged with the solution of Σ DDT equivalent to 9g of worm/ kg turbot was not significantly different from the control group (**Table 1**).

3.2 Cell-based in vitro bioassays

Estrogenic activity was observed in extracts from sediment collected from PV site 8C, extracts of worms exposed to PV sediment, and bile and liver of turbot samples collected from PV (**Table 2**). Liver extracts of turbot collected from PV showed significantly higher EEQ than extract of livers from fish collected from the SD reference site. AhR and GR activities measured in extracts of sediment collected from PV were significantly higher than those detected in sediments from SD. Significantly higher levels of anti-AR activities were also observed in the PV sediment extracts compared to the SD sediment extracts. In each bioassay, no significant increase was observed with ER or AhR activities in worms (**Table 2**). However, relatively high (ng/g) concentrations of Dex-eq was observed in extracts of PV sediment-exposed worms relative to PV sediments.

In the 7-day study, bile of turbot treated with 3 g of PV sediment exposed worms/kg turbot elicited significantly higher estrogenic activities (EEQs) than turbot fed control worms (**Table 2**). The bile extract of turbot treated with 10g of PV sediment exposed worms/kg turbot showed comparable EEQ to that of bile of turbot caught from PV. In the 28-day study, EEQs of liver extracts of fish treated with Σ DDT were significantly lower than that of liver extracts of turbot collected from PV (**Table 2**).

3.3 Expression of ER related mRNA

Transcript abundance of ER α and ER β was significantly upregulated in male turbot collected from PV relative to fish from SD. There was a trend toward an increase in VTG mRNA expression but significant increases were not observed. In laboratory studies, no significant treatment effects were observed with the transcripts, compared with controls, for male fish in the 7-day study (**Fig. 2b**). In the 28-day study, hepatic VTG and ER β mRNA expression were significantly upregulated in male turbot gavaged with the highest Σ DDT concentration (**Fig. 2c**). Significant correlations were observed between hepatic Σ DDT concentrations and VTG transcripts in the livers of 28 day fish treated with DDT and fish collected from PV (**Fig.3**).

4. Discussion

4.1 Bioaccumulation

Previous studies demonstrated that maximal concentrations of Σ DDTs in worms were attained after 4 days of exposure to PV 8C sediment (Bao et al., 2013). After 4 days, there was an increase in *N. arenaceodentata* mortality and a decrease in Σ DDT concentrations. The increase in mortality in *N. arenaceodentata* suggested potential acute toxicity of the sediment. Site 8C is near the Joint Water Pollution Control Plant (JCWP) outfall and contains high concentrations of Σ DDT, as well as other hydrophobic chemicals (e.g. PCBs) and metals (LACSD, 2014). In contrast, survival and growth of *N. arenaceodentata* was unaltered in earlier studies with PV sediment in either a 20-d test (Gerlinger et al., 1995) or in a 120-d test (Murdoch et al., 1997). It is unclear why the sediments from our study were more toxic, but other uncharacterized compounds may be present in the current samples relative to those in the 1990s.

BSAF values for sediment to worms were similar to those determined from Bao et al. (2013) and Jia et al. (2014), who observed a linear relationship of worms, total DDT observing BSAF values of 0.003-0.006. The calculated BMF and BSAF values in this study (4.09 and 0.007, respectively) were comparable to previous values determined from LACSD (4.06 and 0.0026, respectively) (LACSD 2014). The higher values of Σ DDTs in turbot from the fish exposed in the laboratory fed sediment-contaminated worms is consistent with biomagnification studies previously reported in the PV shelf (Zeng and Tran 2002).

4.2 Cell-based in vitro bioassays

Although PV sediments and biota have been shown to possess relatively high concentrations of DDTs and PCBs, other unknown contaminants are likely present given the proximity to the wastewater plant outfall (Maruya et al. 2012). Transactivation receptor assays have been used to assess individual chemical binding affinity in environmental matrices (Wilson et al., 2004), as well as to assess estrogenic and anti-androgenic activity in fish bile following exposure to effluent (Hill et al., 2010). Effect-based *in vitro* assays also have been used for surface and drinking water assessments (Escher et al., 2014, Mehinto et al., 2015). The role of *in vitro* effect-based assays may increase as the USEPA continues bioassay use in ToxCast High Throughput System (HTS) assessments (USEPA, 2014) and in environmental risk assessments (Doyle et al., 2014).

In the present study, *in vitro* bioassays suggested the presence of compounds able to bind to the estrogen (ER), aryl hydrocarbon (AhR) and glucocorticoid (GR) receptors, and compounds that antagonize the response of the androgen receptor (anti-AR) in organic extracts of PV sediment, worms, and fish tissues/bile. Sediment samples collected from PV elicited relatively higher estrogenic, AhR, glucocorticoid and Anti-androgenic responses than the San Diego reference site (**Table 2**). The magnitude of estrogenic, dioxin-like and glucocorticoid activities detected in the PV sediment was relatively low, averaging 1.26 ng/g EEQ, 0.38 ng/g TEQ and 21.1 pg/g DEX-q, respectively. The EEQ concentrations are similar to measured concentrations of estrone, estradiol and nonylphenol isomers (1.3 ng/g dw EEQ) in sediments from this site in 2003 (Schlenk et al. 2005). GR activities have been

observed in wastewater effluents and using effect-based fractionation, with triamcinolone acetonide, dexamethasone and prednisolone as the main contributors in a study in the Netherlands (Schriks et al. 2010). Triamcinolone Acetonide is prescribed to relieve skin inflammation, itching, dryness, and redness. Dexamethasone is used to treat inflammatory conditions such as allergies, skin conditions, ulcerative colitis, arthritis and breathing disorders. Prednisolone is a synthetic glucocorticoid used to treat a variety of inflammatory and autoimmune conditions and some cancers (drug information from MedLine Plus, <https://www.nlm.nih.gov/medlineplus/>).

Relatively high concentrations of AhR ligands were observed in PV sediments relative to SD. Likely candidates include multiple coplanar contaminants such as PCBs and PAHs (LACSD, 2011). The most recent evaluation of total PCB concentrations indicated concentrations between 2.69–4.38 µg/g in PV sediment according to LACSD (2014). However, the identities and concentrations of the congeners were not provided. Studies performed in the late 1990s, showed correlations between PAHs that were AhR ligands, coplanar PCBs and an *in vitro* AhR-driven reporting system (Anderson et al. 1999). While there were correlations between Toxicity Equivalent Factors derived from individual chemicals and the *in vitro* bioassay response, the overall contributions of either chemical class to the bioassay were not determined.

Higher anti-AR activities were also found in the PV sediment than in SD reference sediment samples (**Table 2**), which has significantly higher *p,p'*-DDE concentrations (**Table 1**). The structures of AR antagonists are diverse and include insecticides or their metabolites (e.g., *p,p'*-DDE and certain pyrethroids), herbicides, industrial contaminants, such as PCB congeners (Hotchkiss et al. 2008) and chlorphenes (Rostkowski et al. 2011). Given the high concentrations of *p,p'*-DDE in PV sediments and worms, it is likely these isomers were responsible for the activity.

Turbot exposed for 7 days to worms that were treated with PV-sediment showed significantly increased estrogenic activity as compared to laboratory controls which followed a trend toward an increase from sediments to worms (**Table 2**). In addition, the EEQs in bile extracts of laboratory fish were similar to values observed in fish collected from PV. The BMFs for worm to turbot for EEQ (calculated as a ratio) was 1.14 and 1.29 for turbot fed 10 g of PV sediment-exposed worm/kg turbot and turbot collected from PV, respectively. *p,p'*-DDT, *o,p'*-DDT, *o,p'*-DDD, *p,p'*-DDA, and *o,p'*-DDE bind and transcriptionally activate the human estrogen receptor (hER) (Chen et al., 1997). Since the bioassay values of sediment and fish correlated well with DDT residues in fish, additional exposures to a DDT mixture was conducted for 28 days. In contrast to the 7 day trophic exposure, EEQs, while dose-dependent, were significantly less than EEQs measured in bile and liver extracts from PV fish suggesting other compounds could be present in sediments. In addition to DDT and its metabolites, other potential compounds could be hydroxylated PCBs which have been shown to bind the ER (Soto et al. 1995). PCBs have been observed in PV sediments (Jia et al. 2014), but hydroxylated compounds have not been evaluated. Since ER ligands were present in extracts of sediments, worms and fish, expression of ER-dependent genes were evaluated in the liver tissues of male fish.

4.3 mRNA expression of ER regulated genes

In addition to the trend toward higher VTG mRNA in turbot from the PV site, significant induction of ER α and ER β mRNA in livers of male fish from PV relative to those from SD indicated a greater exposure to estrogenic compounds at the PV site. A significant correlation was found between hepatic VTG, ER α , ER β mRNA and Σ DDTs in field-collected male turbot indicated that DDT and its metabolites may contribute to the *in vivo* estrogenic activity observed in PV fish (**Fig. 3**). However, 7-day trophic transfer experiments with Σ DDT failed to alter hepatic VTG, ER α and ER β mRNA, even though body burden residues (and biliary EEQs) in fish were similar to those collected in the field. In the 28-day study where fish were only treated with Σ DDT by gavage, VTG and ER β mRNA were significantly upregulated in the liver of male turbot exposed, despite lower body burdens compared to the 7-day food-borne fish and fish collected from PV. In addition, even though overall Σ DDT concentrations were lower in the 28-day laboratory treated fish relative to PV fish, VTG mRNA significantly correlated with hepatic Σ DDT residues in both field and the 28 day exposure.

Several studies have shown that DDT isomers elicit estrogenic responses such as VTG production in fish (Ankley et al. 1998; Cheek et al. 2001; Leaños-Castañeda et al. 2002), but few studies have evaluated other targets. In largemouth bass treated with p'p'-DDE, VTG and ER transcripts were induced in liver (Garcia-Reyero et al. 2006). The two ER subtypes, ER α and ER β , were co-expressed in the liver of fish (Socorro et al., 2000; Menuet et al., 2002). Although they are ligand-modulated transcription factors, both subtypes bind ligands with different affinities and may display different roles in the physiological responses to estrogen (Matthews and Gustafsson, 2003). Pennie et al. (1998) found that ER β showed a greater activation than ER α when linked to the ERE from the VTG gene in transfected Cos-1 cells. Similarly, Leaños-Castañeda and Kraak (2007) concluded that VTG production is mainly mediated through ER β , implying that compounds which induce VTG may also exhibit ER β augmentation. However, competition binding assays detected no striking differences in the relative binding affinities for estrogenic chemicals and phytoestrogens, including DDT derivatives, between recombinant human ER α and ER β (Kuiper et al., 1998). The role of each ER subtype to estrogens in fish thus remains an open question.

5. Conclusions

This study relates the occurrence of sediment-associated DDTs to biologically meaningful responses in a locally abundant flatfish via trophic transfer as well as through direct DDT solution exposure. Analytical chemistry results indicate the bioaccumulation of DDT and metabolites from sediment to worm to fish. EEQs in bile of turbot from the 7-day laboratory treatments corresponded to those of field caught turbot from the DDT contaminated Palos Verdes shelf. Similarly, GR activities were higher in worms exposed to PV sediment. There was a discrepancy in ER-regulated mRNA expression in male hornyhead turbots between the 7-day (diet-derived exposure) and 28-day study (gavaged Σ DDT solution exposure), indicating occurrence of anti-estrogens within sediment-exposed worms, or pharmacokinetic factors associated with dietary exposure impaired the bioavailability of Σ DDTs. A longer duration of exposure at lower doses may be required to elicit estrogenic activities in

laboratory experiments that would allow for modeling of the exposure and effects of DDT in fish from historically contaminated sites such as Palos Verdes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

- Allen, MJ.; Groce, AK.; Noblet, JA. Distribution of contaminants above predator-risk guidelines in flatfishes on the southern California shelf in 1998.. In: Weisberg, SB.; Elmore, D., editors. Southern California Coastal Water Research Project Biennial Report 2003–2004. Southern California Coastal Water Research Project; Westminster, CA, USA: 2004. p. 149–171.
- Anderson JW, Zeng E, Jones JM. Correlation between the response of a human cell line (P450RGS) and the distribution of sediment PAHs and PCBs on the Palos Verdes Shelf, California. *Environ. Toxicol. Chem.* 1999; 18(7):1506–1510.
- Ankley G, Mihaich E, Stahl R, Tillitt D, Colborn T, McMaster S, et al. Overview of a workshop on screening methods for detecting potential (anti-) estrogenic/androgenic chemicals in wildlife. *Environ. Toxicol. Chem.* 1998; 17(1):68–87.
- Bao LJ, Jia F, Crago J, Zeng EY, Schlenk D, Gan J. Assessing bioavailability of DDT and metabolites in marine sediments using solid-phase microextraction with performance reference compounds. *Environ. Toxicol. Chem.* 2013; 32:1946–53. [PubMed: 23661411]
- Chakraborty T, Katsu Y, Zhou LY, Miyagawa S, Nagahama Y, Iguchi T. Estrogen receptors in medaka (*Oryzias latipes*) and estrogenic environmental contaminants: an *in vitro-in vivo* correlation. *J. Steroid Biochem. Mol. Biol.* 2011; 123:115–121. [PubMed: 21145966]
- Cheek AO, Brouwer TH, Carroll S, Manning S, McLachlan JA, Brouwer M. Experimental evaluation of vitellogenin as a predictive biomarker for reproductive disruption. *Environ. Health Perspect.* 2001; 109(7):681. [PubMed: 11485866]
- Chen CW, Hurd C, Vorojekina DP, Arnold SF, Notides AC. Transcriptional activation of the human estrogen receptor by DDT isomers and metabolites in yeast and MCF-7 cells. *Biochem. Pharmacol.* 1997; 53(8):1161–1172. [PubMed: 9175721]
- Doyle E, Biales A, Focazio M, Griffin D, Loftin K, Wilson V. Effect-Based Screening Methods for Water Quality Characterization Will Augment Conventional Analyte-by-Analyte Chemical Methods in Research As Well As Regulatory Monitoring. *Environ. Sci. Technol.* 2014 DOI: 10.1021/es5053254.
- Eganhouse RP, Pontolillo J, Leiker TJ. Diagenetic fate of organic contaminants on the Palos Verdes Shelf, California. *Mar. Chem.* 2000; 70:289–315.
- Escher BI, Allinson M, Altenburger R, Bain PA, Balaguer P, Busch, et al. Benchmarking organic micropollutants in wastewater, recycled water and drinking water with in vitro bioassays. *Environ. Sci. Technol.* 2014; 48:940–56.
- Forsgren KL, Bay SM, Vidal-Dorsch DE, Deng X, Lu G, Armstrong J, Gully JR, Schlenk D. Annual and seasonal evaluation of reproductive status in hornyhead turbot at municipal wastewater outfalls in the Southern California Bight. *Environ. Toxicol. Chem.* 2012; 31(12):2701–2710. [PubMed: 22987602]
- Garcia-Reyero N, Barber D, Gross T, Denslow N. Modeling of gene expression pattern alteration by p,p'-DDE and dieldrin in largemouth bass. *Mar. Environ. Res.* 2006; 62:S415–S419. [PubMed: 16707152]

- Gerlinger TV, Reish DJ, Fanizza M. Survival and growth of juvenile *Neanthes arenaceodentata* (Annelida: Polychaeta) in marine sediment taken from the vicinity of an ocean outfall. *Bull. Southern Calif. Acad. Sci.* 1995; 94:65–74.
- Hill EM, Evans KL, Horwood J, Rostkowski P, Oladapo FO, Gibson R, Shears JA, Tyler CR. Profiles and some initial identifications of (anti)androgenic compounds in fish exposed to wastewater treatment works effluents. *Environ. Sci. Technol.* 2010; 44:1137–1143. [PubMed: 20055407]
- Hose JE, Cross JN, Smith SG, Diehl D. Reproductive impairment in a fish inhabiting a contaminated coastal environment off southern California. *Environ. Pollut.* 1989; 57(2):139–148. [PubMed: 15092459]
- Hotchkiss AK, Rider CV, Blystone CR, Wilson VS, Hartig PC, Ankley GT, et al. Fifteen years after “Wingspread”—environmental endocrine disrupters and human and wildlife health: where we are today and where we need to go. *Toxicol. Sci.* 2008; 105(2):235–259. [PubMed: 18281716]
- Jia F, Bao LJ, Crago J, Schlenk D, Gan J. Use of isotope dilution method to predict bioavailability of organic pollutants in historically contaminated sediments. *Environ. Sci. Technol.* 2014; 48:7966–7973. [PubMed: 24946234]
- Kidd KA, Blanchfield PJ, Mills KH, Palace VP, Evans RE, Lazorchak JM, Flick RW. Collapse of a fish population after exposure to a synthetic estrogen. *P. Natl. Acad. Sci.* 2007; 104(21):8897–8901.
- Kuiper GG, Lemmen JG, Carlsson BO, Corton JC, Safe SH, van der Saag PT, et al. Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor β . *Endocrinology.* 1998; 139(10):4252–4263. [PubMed: 9751507]
- LACSD. Southern California coastal water research project. Technical Report 650. Jul.2011 2011.
- LACSD.. Los Angeles County Sewage District. Whittier, CA.: 2014. 2012-2013 Joint water pollution control plant biennial receiving water monitoring report..
- Leaños-Castañeda O, Van Der Kraak G. Functional characterization of estrogen receptor subtypes, ER α and ER β , mediating vitellogenin production in the liver of rainbow trout. *Toxicol. Appl. Pharm.* 2007; 224(2):116–125.
- Leaños-Castañeda O, Van Der Kraak G, Lister A, Simá-Alvarez R, Gold-Bouchot G. o, p'-DDT induction of vitellogenesis and its inhibition by tamoxifen in Nile tilapia (*Oreochromis niloticus*). *Mar. Environ. Res.* 2002; 54(3):703–707. [PubMed: 12408639]
- Maruya KA, Vidal-Dorsch DE, Bay SM, Kwon JW, Xia K, Armbrust KL. Organic contaminants of emerging concern in sediments and flatfish collected near outfalls discharging treated wastewater effluent to the Southern California Bight. *Environ. Toxicol. Chem.* 2012; 31(12):2683–2688. [PubMed: 22987513]
- Matthews J, Gustafsson JÅ. Estrogen signaling: a subtle balance between ER α and ER β . *Mol. Interv.* 2003; 3(5):281. [PubMed: 14993442]
- Mearns, AJ.; Matta, M.; Shigenaka, G.; MacDonald, D.; Buchman, M.; Harris, H.; Golas, J.; Lauenstein, G. NOAA Technical Memorandum NOS ORCA 62. U.S. Department of Commerce, National Oceanic and Atmospheric Administration, National Ocean Service; Seattle, WA.: 1991. Contaminant trends in the Southern California Bight..
- Mehinto AC, Jia A, Snyder SA, Jayasinghe BS, Denslow ND, Crago J, Schlenk D, Menzie C, Westerheide SD, Leusch FD, Maruya KA. Interlaboratory comparison of in vitro bioassays for screening of endocrine active chemicals in recycled water. *Water. Res.* 2015; 83:303–309. [PubMed: 26177482]
- Menuet A, Pelligrini E, Anglade I, Blaise O, Laudet V, Kah O, Pakdel F. Molecular characterization of three estrogen receptor forms in zebrafish: binding characteristics, transactivation properties, and tissue distributions. *Biol. Rep.* 2002; 66:881–1892.
- Mills LJ, Gutjahr-Gobell RE, Haebler RA, Horowitz DJ, Jayaraman S, Pruell RJ, McKinney RA, Gardner GR, Zarogian GE. Effects of estrogenic (o,p'-DDT; octylphenol) and anti-androgenic (p,p'-DDE) chemicals on indicators of endocrine status in juvenile male summer flounder (*Paralichthys dentatus*). *Aquat. Toxicol.* 2001; 52:57–76.
- Murdoch M, Chapman P, Johns DM, Paine MD. Chronic effects of organochlorine exposure in sediment to the marine polychaete *Neathes arenaceodentata*. *Environ. Toxicol. Chem.* 1997; 16:1494–1503.

- Soto AM, Sonnenschein C, Chung KL, Fernandez MF, Olea N, Serrano FO. The E-SCREEN assay as a tool to identify estrogens: an update on estrogenic environmental pollutants. *Environ. Health Persp.* 1995; 103(Suppl 7):113.
- Pennie WD, Aldridge TC, Brooks AN. Differential activation by xenoestrogens of ER α and ER β when linked to different response elements. *J. Endocrinol.* 1998; 158:R11–R14. [PubMed: 9846177]
- Raco-Rands, VE.; Steinberger, A. Characteristics of effluents from large municipal wastewater treatment facilities in 1997.. In: Weisberg, SB.; Hallock, D., editors. Southern California Coastal Water Research Project Annual Report 1999–2000. Southern California Coastal Water Research Project; Westminster, CA, USA: 2001. p. 28-44.
- Rempel-Hester MA, Hong H, Wang Y, Deng X, Armstrong J, Gully J, Schlenk D. Site-specific effects of 17 β -estradiol in hornyhead turbot (*Pleuronichthys verticalis*) collected from a wastewater outfall and reference location. *Environ. Res.* 2009; 109(5):552–558. [PubMed: 19286174]
- Reyes JA, Vidal-Dorsch DE, Schlenk D, Bay SM, Armstrong JL, Gully JR, Cash C, Baker M, Stebbins TD, Hardiman G, Kelley KM. Evaluation of reproductive endocrine status in hornyhead turbot sampled from southern California's urbanized coastal environments. *Environ. Toxicol. Chem.* 2012; 31(12):2689–2700. [PubMed: 22987624]
- Rostkowski P, Horwood J, Shears JA, Lange A, Oladapo FO, Besselink HT, Tyler CR, Hill EM. Bioassay-directed identification of novel antiandrogenic compounds in bile of fish exposed to wastewater effluents. *Environ. Sci. Technol.* 2011; 45:10660–10667. [PubMed: 22047186]
- Schiff K, Allen MJ. Chlorinated hydrocarbons in livers of flatfishes from the Southern California Bight. *Environ. Toxicol. Chem.* 2000; 191:1559–1565.
- Schlenk D, Sapozhnikova Y, Irwin MA, Xie L, Hwang W, Reddy S, Brownawell BJ, Armstrong J, Kelly M, Montagne DE, Kolodziej EP, Sedlak D, Snyder S. *In vivo* bioassay-guided fractionation of marine sediment extracts from the southern California bight, USA, for estrogenic activity. *Environ. Toxicol. Chem.* 2005; 24:2820–2826. [PubMed: 16398118]
- Schriks M, van Leerdam JA, van der Linden SC, van der Burg B, van Wezel AP, de Voogt P. High-resolution mass spectrometric identification and quantification of glucocorticoid compounds in various wastewaters in the Netherlands. *Environ. Sci. Technol.* 2010; 44(12):4766–4774. [PubMed: 20507090]
- Socorro S, Power DM, Olsson P-E, Canario AVM. Two estrogen receptors expressed in the teleost fish, *Sparus aurata*: cDNA cloning, characterization and tissue distribution. *J. Endocrinol.* 2000; 166:293–306. [PubMed: 10927619]
- Spies RB, Thomas P, Matsui M. Effects of DDT and PCB on reproductive endocrinology of Paralabrax clathratus in southern California. *Mar. Environ. Res.* 1996; 1(42):175–176.
- Stull JK, Swift DJP, Niedoroda AW. Contaminant dispersal on the Palos Verdes continental margin: I. Sediments and biota near a major California wastewater discharge. *Sci. Total. Environ.* 1996; 179:73–90.
- Tollefsen KE, Mathisen R, Stenersen J. Induction of vitellogenin synthesis in an Atlantic salmon (*Salmo salar*) hepatocyte culture: a sensitive in vitro bioassay for the oestrogenic and anti-oestrogenic activity of chemicals. *Biomarkers.* 2003; 5:394–407. [PubMed: 14602523]
- USEPA.. White paper on methods for assessing ecological risks of pesticides with persistent, bioaccumulative and toxic characteristics. Submitted to the FIFRA Scientific Advisory Panel for review and comment. United States Environmental Protection Agency, Environmental Fate and Effects Division; 2008.
- USEPA.. Next Generation Risk Assessment: Incorporation of recent advances in molecular, computational, and systems biology. Washington DC.: 2014. EPA/600/R-14/004
- USEPA.. Technical Support Document Volume 2: Development of National Bioaccumulation Factors. United States Environmental Protection Agency. Office of Water; Washington, DC: 2003. Methodology for Deriving Ambient Water Quality Criteria for the Protection of Human Health (2000).. EPA-822-R-03-030
- Wilson VS, Cardon MC, Thornton J, Korte JJ, Ankley GT, Welch J, Gray LE Jr, Hartig PC. Cloning and in vitro expression and characterization of the androgen receptor and isolation of estrogen receptor alpha from the fathead Minnow (*Pimephales promelas*). *Environ. Sci. Technol.* 2004; 38:6314–21. [PubMed: 15597887]

- Young DR, Mc Dermott DJ, Heesen TC, Hotchkiss DA. DDT residues in bottom sediments, crabs, and flatfish off southern California submarine outfalls. Calif. Water. Pollut. Control. Assoc. Bull. 1975; 12:62–66.
- Zaroogian G, Gardner G, Horowitz DB, Gutjahr-Gobell R, Haebler R, Mills L. Effect of 17beta-estradiol, o,p'-DDT, octylphenol and p,p'-DDE on gonadal development and liver and kidney pathology in juvenile male summer flounder (*Paralichthys dentatus*). Aquat. Toxicol. 2001; 54:101–12. [PubMed: 11451429]
- Zeng EY, Tran K. Distribution of chlorinated hydrocarbons in overlying water, sediment, polychaete, and hornyhead turbot (*Pleuronichthys verticalis*) in the coastal ocean, southern California, USA. Environ. Toxicol. Chem. 2002; 21(8):1600–1608. [PubMed: 12152759]

Highlights

DDT metabolites accumulate from Palos Verdes (PV) sediment to worm and fish.

Estrogenic activity in fish in diet-derived exposure corresponds to that in PV fish.

Upregulated ER gene in DDT-gavaged fish corresponds with those of PV fish.

Fold-change of ER-related genes correlates with DDT concentration in fish.

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Fig. 1. Palos Verdes Superfund Site and DDTs concentrations. Site 8C sediment location as well as fish sampling locations, Zones 1-3, from LACSD (2014) are labeled. Modified from Figure 4-1 in EPA (2009).

Fig. 2. mRNA of vitellogenin and estrogen receptors in livers of turbot collected from San Diego and Palos Verdes (near site 8C) (a) and in turbot fed 7days with worms exposed to PV sediments (b) and in turbot gavaged with a DDT mixture for 28-days (c). Each value represents the mean of 4-6 animals + SD. * $p < 0.05$.

Fig. 3. Relationships between ΣDDT concentration in livers and VTG mRNA in male turbot sampled from PV and SD (a) and in male turbot gavaged with ΣDDT for 28 days (b), and between ΣDDT concentrations and ERα mRNA(c) and ERβ mRNA in livers from turbot sampled from PV and SD (d).

Table 1

DDT metabolite concentrations in sediment, worm, and turbot liver tissue (ng/g). Each value represents the mean ($\pm$ SD).

		<i>o,p'</i> -DDD	<i>p,p'</i> -DDD	<i>o,p'</i> -DDE	<i>p,p'</i> -DDE	<i>o,p'</i> -DDT	<i>p,p'</i> -DDT	Σ DDT (dry wt.)	Lipid Normalized
Sediment	Reference (n=3)	0	0	0	0.23 (0.08)	0	0	0.23 (0.08)	
	8C avg. (n=3)	619 (26.5)	3,410 (957)	3,670 (228)	19,500 (1,970)	265 (108)	1,110 (383)	28,600 (2,970)	
Polychaete	Reference (n=3)	16.2 (7.43)	0.27 (0.06)	8.88 (2.62)	68.2 (2.92)	0	8.85 (0.32)	102 (1.50)	1,340
	8C avg. (n=3)	7.51 (7.51)	183 (36.4)	53.5 (0.82)	671 (89.4)	0	0	897 (144)	2,880
Turbot-field	LACSD 2012-2013 Zone 1*	284	1,910	10,400	158,000	20.4	173	170,000	11,700
7 day	San Diego (n=8)	33.2	130	904	16,700	0	16.0	17,800	1,650
	Control 3g/kg worm (n=5)	1.15 (0.36)	3.21 (1.53)	3.41 (1.02)	87.5 (15.2)	2.96 (0.58)	0.98 (0.45)	99.2 (14.9)	89.3 (14.2)
	DDT 3g/kg worm (n=5)	7.71 (2.66)	169 (66.6)	84.8 (26.1)	2,610 (920)	2.04 (0.22)	6.21 (2.03)	2,880 (1,010)	2,610 (994)
	Control 10g/kg worm (n=5)	0	7.25 (2.35)	4.84 (0.69)	214 (16.9)	0.08 (0.08)	0.52 (0.35)	227 (19.0)	1,100 (549)
28 day	DDT 10g/kg worm (n=5)	15.2 (9.65)	3,650 (67.2)	1,090 (21.9)	5,550 (358)	1.56 (1.03)	34.4 (0.79)	6,690 (308)	11,700 (1,150)
	Control (n=3)	35.2 (17.0)	53.2 (38.0)	23.4 (18.5)	51.9 (31.4)	0	0	164 (105)	1,025 (656)
	Low DDT (n=4)	36.4 (5.47)	71.5 (26.1)	36.5 (3.88)	46.7 (9.55)	0	0	191 (35.0)	1,190 (219)
	High DDT (n=3)	51.3 (19.5)	235 (81.7)	45.7 (13.2)	184 (37.2)	0	0	517 (152)	3,230 (950)

Note:

* Data taken from LACSD (2014). PV, Palos Verdes; SD, San Diego reference site; High DDT, turbot gavaged with DDT solution equal to 30 g worm wet wt./kg of turbot body for 28 days; Low DDT, turbot gavaged 9 g of worm wet wt./ kg of turbot body wet for 28 days; DDT 3g/kg worm, turbot fed with 3g of sediment-treated worm/kg of turbot body weight for 7 days; DDT 10g/kg worm, turbot fed with 10g of sediment-treated worm/kg turbot body weight for 7 days.

Table 2

Estrogen, Aryl hydrocarbon, glucocorticoid and anti-androgen receptor activities in extracts of sediment and biota.

		EEQ (ng/g)	TEQ (pg/g)	DEX-q (pg/g)	Anti-AR (%)
Field	SD sediment	0.3±0.2	0.1±0.1	9.6±1.7	64±1.2
	PV sediment	1.3±0.8	380.5±276.2 *	21.1±2.5 *	51± 7 *
7 day	Clean worm	3.0±1.7	0.6±1.1	2.0±0.05 ng/g	66±1
	8C worm	4.1±2.6	0.4±0.8	3.0±1.6 ng/g	65±1
	Control bile	0.4 ± 0.04			
	Bile 3g/kg	1.0±0.05 *			
	Bile 10g/kg	4.7±1.1			
	Control liver	1.74 ± 0.8			
28 day	Low DDT liver	13.4±4.9			
	High DDT liver	31.9±49.0			
Field	SD bile	0.23±0.03			
	PV bile	5.3±7.8			
	SD liver	3.3±0.3			
	PV liver	90.6±3.9 *			

PV, Palos Verdes; SD, San Diego reference site; EEQ, 17β-estradiol equivalent; TEQ, aryl hydrocarbon toxicity equivalent; DEX, dexamethasone equivalent; AR; anti-androgen receptor.

Each value represents the mean of 3-4 sample ± SD using dry weight normalization.

*
p < 0.05.