**Hazard/Risk Assessment**

**SPATIALLY DISTRIBUTED ECOLOGICAL RISK FOR FISH OF A COASTAL FOOD WEB EXPOSED TO DIOXINS**

CHRISTIAN MICHELETTI,† TOMAS LOVATO,‡ ANDREA CRITTO,† ROBERTO PASTRES,‡ and ANTONIO MARCOMINI*†

†University Cà Foscari of Venice, Department of Environmental Sciences, Calle Larga S. Marta 2137, 30123, Venezia, Italy
‡University Cà Foscari of Venice, Department of Physical Chemistry, Calle Larga S. Marta 2137, 30123, Venezia, Italy

(Received 27 February 2007; Accepted 4 October 2007)

Abstract—The ecological risk posed by 2,3,7,8-polychlorodibenzo-p-dioxins and furans (PCDD/Fs) and dioxin-like polychlorobiphenyl (PCB) congeners to five edible fish species of the aquatic food web of Venice Lagoon, Italy, was estimated by applying a state-of-the-art kinetic bioaccumulation model. Site-specific data were used to define a representative food web. The experimental data set for model validation and application included PCB and PCDD/F congener concentrations in sediments, in water, and in five organisms (both invertebrates and fish). The spatial distribution of risk was evaluated by dividing the lagoon into six homogeneous areas, and for each area, sediment, water, and organism dioxins concentrations were calculated. The bioaccumulation model was calibrated for both nonmetabolizing and metabolizing congeners, the metabolic elimination rates of which were estimated. The calibrated model was applied to the six areas of the lagoon to estimate the fish predicted exposure concentration as 2,3,7,8-tetrachlorodibenzo-p-dioxin toxicity equivalents from sediment concentration. Internal no-effect concentrations were calculated for each fish species from literature data. Risk was estimated by applying the hazard quotient (HQ) approach, obtaining the ecological risk for each fish species on the basis of 90 and 99% protection levels, in each of the six lagoon areas. The sediment dioxins concentration does not pose a significant risk to the selected fish species at the 90% protection target (HQ < 1), whereas risk is significant (HQ > 1) at the 99% protection target. Risk results were higher near the Porto Marghera industrial district, Italy, and in lagoon zones characterized by a low water-exchange rate and freshwater basin inputs.

Keywords—Ecological risk assessment Polychlorobiphenyls Polychlorodibenzo-p-dioxin/furans Bioaccumulation

**INTRODUCTION**

Polychlorodibenzo-p-dioxins and furans (PCDD/Fs) and polychlorobiphenyls (PCBs) are anthropogenic, persistent organic pollutants that are amenable to bioaccumulation in the tissues of living organisms. Moreover, PCDD/Fs and PCBs can biomagnify through the food web [1], raising the issue of adverse effects to aquatic organisms at higher levels of the food web [2], such as fish. Ecological risk assessment (ERA) [3] can be applied to predict the potential adverse effects of bioaccumulated pollutants to organisms by comparing the exposure concentration to an effect concentration.

The pollutant concentration in an organism’s tissues can be obtained by experimental measurements. Often, however, experimental data are insufficient or not available for economic or technical reasons [4] and, thus, are not suitable to characterize all investigated environmental media as well as all key species composing the food web under consideration. Several authors [5] have demonstrated that bioaccumulation models can be an effective alternative to predict the bioaccumulation of pollutants. To obtain a reliable estimation, however, models need a site-specific calibration [4]. Bioaccumulation of PCBs and PCDD/Fs was extensively investigated and modeled for individual aquatic species, whereas relatively few investigations have been reported concerning the bioaccumulation of PCBs and PCDD/Fs in selected food webs [6–8]. To estimate the effects of mixtures of bioaccumulative pollutants, McCarty and Mackay [9] proposed an approach based on the critical body burden (CBB) concept. The CBB is defined as the highest tissue concentration corresponding to no observed adverse effects. Recently, methods to estimate CBB concentrations were proposed [10], and an open database of residue-effect data (http://el.erdc.usace.army.mil/ered/) [11] was developed. Rarely, however, has the CBB approach been reported as a tool for performing an ERA [12–14].

Within the present work, the CBB approach was applied to Venice Lagoon (i.e., a coastal lagoon), Italy, to estimate the effect concentrations for five species of edible fish exposed to dioxins (i.e., PCDD/F and dioxin-like PCB congeners) stored in sediments. Venice Lagoon is a well-known case study, and a detailed description of environmental characteristics and anthropogenic stressors has been reported elsewhere [13,14]. Previous studies estimating the ecological risk posed by dioxins in Venice Lagoon have been published. In particular, a screening ERA based on 2,3,7,8-PCDD/F experimental tissue concentrations in fish, shellfish, and piscivorous wildlife in Venice Lagoon [12] has been reported. No spatial variability of contaminant concentrations was included, however, and only a few selected organisms were considered. More recently, another study estimating the ecological risk for a limited set of organisms on the basis of PCB and 2,3,7,8-PCDD/F congener experimental tissue concentrations was published [14]. That study, however, took into account only two fish species and was based on a small number of sampling stations (i.e., impeding the spatial representativeness of risk), and the uncertainty associated with effect assessment was not considered.

For the purposes of the present study, it is important to stress the uneven spatial distribution of the contamination...
[15,16], which depends on the distance and the typology of point and nonpoint sources, sediment characteristics (e.g., fine fraction), morphology of lagoon habitats, and lagoon hydrodynamics [17–19]. Moreover, mobility and bioavailability of toxic pollutants increased as a result of increased resuspension rate of the fine particle–bound contaminants caused by the fishing activity [20], raising the concern about its potential ecological risk to the aquatic food web. Sediment contamination and pollutant redistribution also can have an economic impact, potentially hampering fish and shellfish farming, with the production of clams being actually at the top in Europe [21].

The objective of the present study was to perform a spatial ERA for fish exposed to dioxin-like PCBs and 2,3,7,8-PCDD/Fs, both directly from the first 15 cm of sediments and through the lagoon food web. The selected food web included five representative lagoon fish species, differentiated by age (i.e., juveniles as well as adults) and by size (i.e., body wt). A state-of-the-art, calibrated bioaccumulation model [22] and literature tissue residue-effect concentrations [23] were applied to estimate bioaccumulation and to assess effects for the fish species.

MATERIALS AND METHODS

The risk assessment was performed following the ERA procedure proposed by the U.S. Environmental Protection Agency [3] using the hazard quotient (HQ) approach [24] (i.e., ratio of predicted exposure concentration [PEC] to predicted no-effect concentration [PNEC]). The PEC was obtained by applying a calibrated and validated state-of-the-art bioaccumulation model [22], estimating PCDD/F and PCB congener concentrations in aquatic organism tissues. The PNEC was estimated according to the tissue residue approach from literature benchmarks [23] as the internal effect concentration of 2,3,7,8-substituted PCDD/F congeners (Table 1) recorded in sediment, water, and organisms (Table S1 of the Experimental database). All the homogeneous lagoon areas, including samples); and in the southern lagoon near the city of Chioggia (four samples). All the homogeneous lagoon areas, including Malamocco and the western lagoon, are identified in Figure 1.

The risk assessment was performed following the ERA procedure proposed by the U.S. Environmental Protection Agency [3] using the hazard quotient (HQ) approach [24] (i.e., ratio of predicted exposure concentration [PEC] to predicted no-effect concentration [PNEC]). The PEC was obtained by applying a calibrated and validated state-of-the-art bioaccumulation model [22], estimating PCDD/F and PCB congener concentrations in aquatic organism tissues. The PNEC was estimated according to the tissue residue approach from literature benchmarks [23] as the internal effect concentration of 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD), taking into account the uncertainty in effect estimation. Finally, the ecological risk for five selected fish species was assessed by comparing the PEC expressed as 2,3,7,8-TCDD toxicity equivalents (TEQs) [25] against the corresponding PNEC.

**Experimental characterization**

**Experimental database.** The database used in the present study consisted of selected 2,3,7,8-substituted PCDD/F congeners (Table 1) recorded in sediment, water, and organisms according to up-to-date analytical methods [15,16]. The database contained concentrations of PCDD/Fs and PCBs in 95 sediment samples, in tissue of five typical lagoon organisms (Mytilus galloprovincialis, six samples; Tapes philippinarum, 23 samples; Carcinus mediterraneus, nine samples; Chelon labrosus, six samples; and Zosterisessor ophiocephalus, eight samples), and in 16 water samples. The experimental data as well as the sampling point location have been reported previously by Micheletti et al. [14].

The sediment sampling sites were homogeneously distributed over the whole lagoon. The water samples were collected in the northern lagoon (three sampling stations); the central lagoon, including the navigable channels (five samples) and the channels within Porto Marghera industrial district (three samples); and in the southern lagoon near the city of Chioggia (four samples). All the homogeneous lagoon areas, including Malamocco and the western lagoon, are identified in Figure 1.

*For an unknown data distribution, the 95% upper confidence limit of the mean - the limit above which this value is unlikely to occur [26]. This choice allows one to deal with uncertainty and variability of the experimental data set without excessively conservative assumptions that may result in overestimation of risk. The organisms and sediment concentrations were reported for each subarea as 2,3,7,8-TCDD TEQs by applying the toxic equivalency factors proposed by the World Health Organization [25].

**Bioaccumulation model for Venice Lagoon.** The exposure of the organisms was estimated using the mechanistic food-web bioaccumulation model recently proposed by Arnot and Gobas [22], who presented several improvements compared to the previous version proposed by Gobas [7]. In fact, the
model includes an enhanced estimation of chemical concentrations in algae, phytoplankton, and zooplankton and a set of allometric relationships that allow estimation of species-specific parameters for a wide range of aquatic organisms. Furthermore, mechanistic formulations for prediction of the gastrointestinal magnification of chemical concentrations are included.

At steady state, the overall uptake and loss rates are equal; therefore, the model mass-balance equation for a given chemical reads is

$$ C_{BJ} = \frac{k_1 C_{WD} + k_0 C_{D,m}}{k_2 + k_E + k_G + k_M} $$

where $C_{BJ}$ (g/kg wet wt), $C_{WD}$ (g/L), and $C_{D,m}$ (g/kg wet wt) represent, respectively, the estimated concentrations of a chemical in biota (B) included in the food web, the dissolved concentration in water, and in organism $m$ of the diet. The remaining parameters characterize the uptake and loss routes: $k_1$ (L/kg·d) and $k_0$ (kg/kg·d) are, respectively, the uptake rates through respiration and through diet, and $k_2$, $k_E$, $k_G$, and $k_M$ (all 1/d) represent the respiration, excretion, growth dilution, and biotransformation rates, respectively. The $C_{WD}$ was estimated using the equation proposed by Gobas [7], based on the partitioning between the suspended organic matter and the total water concentration:

$$ C_{WD} = \frac{C_{WT}}{1 + \left(\frac{K_{OW} \cdot MOM}{d_{OM}}\right)} $$

where $C_{WD}$ is measured in g/L, $C_{WT}$ is the total concentration in water (g/L), $K_{OW}$ is the octanol–water partitioning constant, MOM is the organic matter concentration in water (kg/L), and $d_{OM}$ is the organic matter density (kg/L).

The model was calibrated and validated using as input the sediment and water concentration data [15,16]. The MOM was estimated by the product of the suspended solids concentration in water (kg/L; as reported by Micheletti et al. [14]) by the organic carbon fraction in suspended solids, which was assumed to be identical to the organic carbon fraction in sediment. The $K_{OW}$ values of the examined compounds are reported in Table 1, whereas the density of the organic matter was assumed to be 0.9 kg/L [7].

The $C_{WT}$ data, which was used for the model verification and calibration, were reported previously by Micheletti et al. [14]. Unfortunately, the $C_{WT}$ data sets for the sampling stations...
included in the western lagoon and Porto Marghera areas were not available. Therefore, to apply the bioaccumulation model to the whole lagoon for risk assessment, $C_{\text{WT}}$ was estimated for each lagoon area by applying the following equations:

$$C_{\text{WT}} = C_s K_{\text{BSW}}$$

where $C_s$ (g/kg dry wt) is the concentration in sediments. The partition coefficient between bottom sediment and water ($K_{\text{BSW}}$) was estimated according to the equation of Seth et al. [28]:

$$K_{\text{BSW}} = \phi_{\text{BS}} \cdot 0.35 \cdot K_{\text{OW}} \cdot d_{\text{BS}}$$

where $\phi_{\text{BS}}$ is the organic carbon fraction in bottom sediments and $d_{\text{BS}}$ (kg/L) represents the bottom sediment density.

A site-specific food web was selected to apply the model (Eqn. 1) based on the results presented in literature [29–33]. The food web includes three planktonic, nine benthonic, and six nektonic organisms, which are represented in Figure 2.

Organisms belonging to the lower trophic levels (i.e., microzooplankton and macroinvertebrates) were grouped on the basis of common habitat and feeding similarities. In some instances, juveniles and adults were considered as different compartments to account for the differences in their metabolism, internal tissues composition, and feeding habits. The diet matrix (Table S2 [http://dx.doi.org/10.1897/074-1692.S1]) was defined on the basis of the previously cited literature. Organism- or group-specific parameters, such as the gill ventilation rate or the feeding efficiency, were estimated using the allometric equations proposed by Arnot and Gobas [22]. Parameters concerning the environmental conditions and biota are reported in Tables S3 and S4 (http://dx.doi.org/10.1897/074-1692.S1), respectively.

Bioaccumulation model verification, calibration, and validation. The model [22] was verified (i.e., used for predicting the bioaccumulation) for nonmetabolizing PCBs (Table 1) and was calibrated and validated for metabolizing PCBs and PCDD/Fs using available field data for $T$. philippinarum, $C$. mediterraneus, $C$. labrosus, and $Z$. ophiocephalus. Therefore, the data set was divided into a calibration set (i.e., data collected in the northern lagoon, central lagoon, and Chioggia areas in Fig. 1) and a validation set (i.e., data collected in the western lagoon area). Median sediment, water, and biota concentrations were used as input data. The logarithm of the overall model bias ($MB_o$) [22] was taken as a measure of the performance of the model and of its tendency to over- or underestimate the bioaccumulation factors (BAFs). The logarithm of $MB_o$ can be interpreted as the mean of the difference between the predicted and observed logarithms of the BAFs for all chemicals in all species, with the ideal fit-to-data being the zero value. The criterion for accepting or rejecting the model setup and calibration was assessed by testing whether the log $MB_o$ was significantly different from zero as determined with a Student’s $t$ test ($\alpha = 0.05$).

In the evaluation of model performance, the $MB_o$ was expressed on an exponential scale to permit a straightforward evaluation of model behavior with respect to the observed data. In other words, a $MB_o$ of greater than one indicates a general tendency of the model to overestimate the BAFs.

Estimation of PECs through the numerical model. The validated numerical model was applied to estimate the bioaccumulation of dioxin-like PCBs and PCDD/Fs in the aquatic food web for each of the six areas of Venice Lagoon (northern lagoon, Porto Marghera, central lagoon, Malamocco, Chioggia, and western lagoon) (Fig. 1). The input parameters used for the model application were those reported in Tables S1 to S5 (http://dx.doi.org/10.1897/074-1692.S1).

The PEC for the organisms included in the food web was estimated from the bioaccumulation of individual congeners in organism tissues. This was estimated by the model, expressed as 2,3,7,8-TCDD TEQs, and then summed to obtain the PEC. Next, the PEC was compared with the effect concentration in fish tissues estimated for the 2,3,7,8-TCDD.

Effects characterization

The PNEC of the dioxin-like PCBs and PCDD/Fs in fish tissues was estimated according to the CBB approach [9] as the internal no-effect concentration for 2,3,7,8-TCDD (INEC$_{\text{TCDD}}$). The INEC$_{\text{TCDD}}$ was estimated using residue-effect benchmarks for fish recently reported in the open literature by Steevens et al. [23], which proposed tissue effect concentrations in terms of ng 2,3,7,8-TCDD/g lipid in fish, defining two values corresponding to 90 and 99% of fish species. These data were estimated using a species-sensitivity distribution obtained from 26 studies concerning 10 fish species (mainly freshwater salmonids) that are considered to be more sensitive than other species to dioxin-like chemicals [23]. According to this range of tissue effect concentrations, two INEC$_{\text{TCDD}}$ values were estimated by multiplying the effect concentration protecting 90% (INEC$_{\text{90}}^{\text{TCDD}}$) and 99%
(INEC<sub>TCD</sub>) of the fish community [23] by g lipid/kg fish body weight as calculated from the data reported in Table S4 (http://dx.doi.org/10.1897/074-1692.51). Therefore, the ecological risk for fish included in the food-web model was estimated using both estimated INE<sub>TCD</sub> values. The application of two INE<sub>TCD</sub> values permitted inclusion in the risk estimation of the uncertainty involved in the extrapolation of effect concentrations for the aquatic community from experimental effect data for few species [23]. Because the considered species are closely related and are more sensitive than other fish to the effects of dioxin, however, there could be a very conservative estimation of PNEC, especially at the 99% protection level. This aspect should be considered when evaluating the risk assessment results.

**Risk characterization**

Results obtained from exposure and effects characterization were used during the last phase of the ERA procedure to estimate the risk for fish included in the food web. In the present study, ecological risk was estimated using the HQ approach [24]. The HQ was calculated by comparing the exposure concentration (i.e., PEC) in TEQ and the INE<sub>TCD</sub> according to the following equation:

\[
HQ = \frac{PEC}{INEC_{TCD}}
\]

where INE<sub>TCD</sub> represents the tissue pollutant concentration correlated with no adverse effects. When PEC is greater than INE<sub>TCD</sub> (i.e., HQ > 1), an adverse effect is likely to occur. The HQ allows a relative risk comparison between different areas and for different assessment endpoints.

**RESULTS AND DISCUSSION**

Ecological risk for five fish species of Venice Lagoon was estimated by comparing modeled tissue concentration of dioxin-like PCBs and PCDD/Fs expressed as 2,3,7,8-TCDD by an internal effect concentration for 2,3,7,8-TCDD estimated on the basis of literature CBB data [9]. The bioaccumulation model was first calibrated and validated using data collected in three lagoon areas and then applied to all six selected lagoon areas to estimate the exposure of fish.

**Exposure characterization**

To assess the bioaccumulation of PCB and PCDD/F congeners into the aquatic food web of Venice Lagoon, the state-of-the-art bioaccumulation model proposed by Arnot and Gobas [22] was applied to verify the goodness of fit for non-metabolizing PCB congeners. At the same time, it was calibrated and validated for metabolizing PCB and PCDD/F congeners by estimating the metabolizing rate constant. The bioaccumulation model was then applied to estimate the PEC for fish and, thus, the ecological risk in the six selected lagoon zones.

**Verification and validation of the selected bioaccumulation model**

The model verification and validation was carried out in two steps using the data available for three areas: The northern lagoon, the central lagoon, and Chioggia (Fig. 1). In the first step, the model was verified (i.e., used for predicting bioaccumulation of the nonmetabolized PCBs) (Table 1); in this case, the biotransformation rates \(k_M\) were set to zero in Equation 1. The results of the first step are shown in Figure 3, which displays the predicted BAFs versus the observed ones for each PCB congener and for *T. philippinarum*, *M. galloprovincialis*, *C. mediterraneus*, *C. labrosus*, and *Z. ophiocephalus* (i.e., \(n\) in the legend of Fig. 2). The continuous line represents the optimal fit, whereas the dotted lines identify the 95% confidence limit, estimated according to method described by Gobas et al. [34]. The dashed lines indicate the region in which predictions and observations differ by less than one order of magnitude. The model shows a general tendency to underestimate the observations, because MB \(0.86\) and \(0.72\), respectively. Instead, the model underestimates the BAFs for *C. mediterraneus* and *C. labrosus*, but the model bias values of 0.86 and 0.72, respectively, are still acceptable [22]. The logarithm of the predicted BAF ranged between 3.9 and 6.4. According to acceptability criterion, the results were not significantly different from the observations, and the verification step confirmed the suitability of the adopted model parameterization to reproduce bioaccumulation of PCBs in the Venice Lagoon ecosystem.

In the second step, the model was calibrated for metabolizing non-ortho-PCBs congeners, dioxins, and furans: \(k_M\) values were estimated using the observations for *T. philippinarum*, *C. mediterraneus*, *C. labrosus*, and *Z. ophiocephalus* in the calibration areas represented by the northern lagoon, the central lagoon, and Chioggia. Because specific values for \(k_M\) in fish and invertebrates were not available, biotransformation rates were estimated by minimizing the residual error, which equals \(|C_{\text{predicted}} - C_{\text{observed}}|\) [35], and assuming as the maximum acceptable \(k_M = 0.5/d\) [36], whereas the rate was set to zero in the remaining compartments for which experimental data were not available. The calibration of the parameters was conducted separately for each of the three areas, adopting as final...
rates the average of the three estimates (Table S5 [http://dx.doi.org/10.1897/074-1692.S1]).

The results obtained from the calibration are shown in Figure 4. The predicted log BAFs ranged from 3.3 to 5.9, whereas the MB0 was 1.01. The MB0 indicates that the model tends to overestimate the field data, but the statistical difference of the mean was not significant. In particular, this tendency was more pronounced for C. labrosus (MB1.4) and for Z. ophiocephalus (MB = 1.37). Overall, the performance of the model was still good, with 99% of data falling within the factor-10 confidence bounds.

Subsequently, the model was validated by comparing the predicted and observed BAFs of the western lagoon (Fig. 1) area for all congeners. The results for nonmetabolized and metabolized chemicals are shown in Figures 5 and 6 and are not dissimilar to those reported in Figure 4 (i.e., representing a good model performance).

As expected, in both groups, the MB0 was less accurate than that obtained during the verification and calibration steps. The results for nonmetabolized PCBs (MB0 = 0.7) were underestimating the observations, whereas for metabolized chemicals, the MB0 of 1.17 indicated a slight overestimation. Nevertheless, the model still satisfied the acceptability criteria, because differences between observed and predicted BAFs were not statistically significant. For the two sets of compounds, only 4 and 7%, respectively, of log BAFs were beyond the one-order-of-magnitude region. The predicted log BAFs ranged between 3.6 and 5.8 for nonmetabolized PCBs and between 3.5 and 5.6 for metabolized PCBs.

**Application of the bioaccumulation model**

Application of the calibrated Arnot and Gobas [22] bioaccumulation model permitted estimation of the PEC for the mixture of PCDD/F and dioxin-like PCB congeners in the fish tissues. Because the capability of fish to biotransform PCDD/Fs has been widely recognized [37], it adopted a background value for the metabolizing rate constant (kM = 0.01; i.e., the minimum obtained from calibration) to include such parameterization for the remaining fish species.

Table 2 reports the results expressed as 2,3,7,8-TCDD TEQs for the bioaccumulation estimated in fish tissues for the sum of dioxin-like PCBs and PCDD/Fs in the six investigated lagoon areas (Fig. 1). The lagoon areas with the highest bioaccumulation were Porto Marghera (located near the chemical industrial district), Malamocco, and the central lagoon. The
estimated concentrations were in the range of $7.3 \times 10^{-4}$ and $4.76 \times 10^{-2} \mu g TEQ/kg wet weight. In the northern lagoon, Chioggia, and the western lagoon, the estimated TEQ concentrations were between $8.38 \times 10^{-4}$ and $6.37 \times 10^{-2} \mu g TEQ/kg wet weight. The highest concentration was estimated for Atherina boyeri.

The TEQ tissue concentrations estimated by the Arnout and Gobas model [22] were used as the PEC to be compared with the INECTCDD according to Equation 6.

**Effects characterization**

As described in *Materials and Methods*, two INECTCDD values at two protection targets (i.e., 90 and 99% of the fish community) [23] were estimated. Thus, the ecological risk was estimated by comparing the PEC with both the INECTCDD and the INECTCDD to estimate the risk with regard to the two levels of fish community protection. Steevens et al. [23] reported 2.3,7,8-TCDD residue-effect concentrations protecting 90 and 99% of the fish community of 6.99 $\times 10^{-4}$ and 5.7 $\times 10^{-5}$ μg TCDD/g lipid, respectively. To estimate the INECs in terms of μg TCDD/kg body weight, the residue-effect concentrations were normalized by the lipid content in fish tissues per kilogram of body weight (on a wet-wt basis) (Table 3). The resulting INECs for fish species included in the food-web model are reported in Table 3. The INECTCDD concentration was included between 3.5 $\times 10^{-2}$ μg TCDD/kg body weight for Nektom carnivorous benthic feeders and 9.35 $\times 10^{-2}$ μg TCDD/kg body weight for *D. labrax*. The INECTCDD values were between 3.9 $\times 10^{-3}$ and 7.63 $\times 10^{-3}$ μg TCDD/kg body weight for *C. labrosus* and *D. labrax*, respectively, resulting in values one order of magnitude lower than the INECTCDD. These results reflect the difference between the 90 and 99% 2.3,7,8-TCDD residue-effect concentrations, resulting in a high uncertainty regarding the PNEC estimation.

**Risk characterization**

The ecological risk from the bioaccumulation of the dioxin-like PCB and PCDD/F congeners mixture was estimated using the HQ approach [24] for fish included in the aquatic food web applied in the bioaccumulation model. Accordingly, the HQ was calculated for each lagoon subarea (i.e., northern lagoon, central lagoon, Porto Marghera, Malamocco, Chioggia, and western lagoon) by comparing the modeled bioaccumulation (i.e., the PEC) for fish with both the INECTCDD and the INECTCDD (Eqn. 5).

The HQs obtained for each lagoon zone (Table 4) were calculated according to the following equation:

$$HQ_i = \frac{TEQ_i (PCDD/Fs + dioxin-like PCBs)}{NECTCDD}$$

where $i$ is the lagoon subarea ($1 \leq i \leq 6$) and $j$ is the trophic group ($1 \leq j \leq 9$).

The hazard posed by the PCBs and PCDD/Fs mixture generally decreases from the lowest level of the fish of the food web (i.e., *C. labosus* juveniles and adults) to the highest level (i.e., *D. labrax* juveniles and adults). The ecological risk estimated for fish included in the food-web model using the INECTCDD was in the range 0.02 to 1.2, with a negligible risk for fish (i.e., HQ < 1 means the absence of potential adverse effects for the considered species) according to the HQ results obtained by Micheletti et al. [14]. On the contrary, the risk estimation on the basis of the INECTCDD resulted in HQs of greater than one in all selected lagoon areas.

In general, the highest values were obtained for small fish (i.e., *A. boyeri*) and for juveniles (i.e., *C. labosus*, *Sparus aurata*, and *D. labrax*), both of which were characterized by a body weight of less than 4 g. These results can be explained

<table>
<thead>
<tr>
<th>Fish species</th>
<th>Lipid content (g lipid/kg body wet wt)</th>
<th>Residue-effect concentration, 90% (μg TCDD/g lipid)</th>
<th>Residue-effect concentration, 99% (μg TCDD/g lipid)</th>
<th>INECTCDD (μg TCDD/kg body wt)</th>
<th>INECTCDD (μg TCDD/kg body wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Chelon labrosus</em> juveniles</td>
<td>68</td>
<td>6.99 $\times 10^{-4}$</td>
<td>5.7 $\times 10^{-4}$</td>
<td>4.75 $\times 10^{-2}$</td>
<td>4.75 $\times 10^{-2}$</td>
</tr>
<tr>
<td><em>Chelon labrosus</em></td>
<td>68</td>
<td>6.99 $\times 10^{-4}$</td>
<td>5.7 $\times 10^{-4}$</td>
<td>4.75 $\times 10^{-2}$</td>
<td>4.75 $\times 10^{-2}$</td>
</tr>
<tr>
<td><em>Atherina boyeri</em></td>
<td>68</td>
<td>6.99 $\times 10^{-4}$</td>
<td>5.7 $\times 10^{-4}$</td>
<td>4.75 $\times 10^{-2}$</td>
<td>4.75 $\times 10^{-2}$</td>
</tr>
<tr>
<td><em>Zosterisessor ophiocephalus</em></td>
<td>80</td>
<td>6.99 $\times 10^{-4}$</td>
<td>5.7 $\times 10^{-4}$</td>
<td>4.75 $\times 10^{-2}$</td>
<td>4.75 $\times 10^{-2}$</td>
</tr>
<tr>
<td>Nektom carnivorous benthic feeders</td>
<td>50</td>
<td>6.99 $\times 10^{-4}$</td>
<td>5.7 $\times 10^{-4}$</td>
<td>4.75 $\times 10^{-2}$</td>
<td>4.75 $\times 10^{-2}$</td>
</tr>
<tr>
<td><em>Sparus aurata</em> juveniles</td>
<td>97</td>
<td>6.99 $\times 10^{-4}$</td>
<td>5.7 $\times 10^{-4}$</td>
<td>4.75 $\times 10^{-2}$</td>
<td>4.75 $\times 10^{-2}$</td>
</tr>
<tr>
<td><em>Sparus aurata</em></td>
<td>97</td>
<td>6.99 $\times 10^{-4}$</td>
<td>5.7 $\times 10^{-4}$</td>
<td>4.75 $\times 10^{-2}$</td>
<td>4.75 $\times 10^{-2}$</td>
</tr>
<tr>
<td><em>Dicentrarcus labrax</em> juveniles</td>
<td>133.8</td>
<td>6.99 $\times 10^{-4}$</td>
<td>5.7 $\times 10^{-4}$</td>
<td>4.75 $\times 10^{-2}$</td>
<td>4.75 $\times 10^{-2}$</td>
</tr>
<tr>
<td><em>Dicentrarcus labrax</em></td>
<td>133.8</td>
<td>6.99 $\times 10^{-4}$</td>
<td>5.7 $\times 10^{-4}$</td>
<td>4.75 $\times 10^{-2}$</td>
<td>4.75 $\times 10^{-2}$</td>
</tr>
</tbody>
</table>
by the diet of the juvenile fish, which includes mainly zooplankton and small benthic organisms rather than other fish, and by the role of body weight in the bioaccumulation model [22], which is used to estimate the growth elimination rate constant (i.e., \( k_\beta \)), meaning that a lower body weight causes a relative increase of the pollutant tissue concentration.

As far as ranking the lagoon areas according to estimated risk, Porto Marghera, Malamocco, and the western lagoon were the most potentially affected areas, with HQs (based on INEC\(_{99}\)) ranging from 0.6 (for C. labrosus adults) to 14.1 (for C. labrosus juveniles) and 14.7 (for Nektom carnivorous benthic feeders). Porto Marghera and Malamocco are directly affected by past emissions from the Porto Marghera industrial district [16] as well as by the actual pollutants emissions [38], resulting in extended areas of contaminated sediments [16].

The western lagoon is a confined area with a water residence time of between 20 and 40 d [39] that receives freshwater inputs [40] and is characterized by sediments with a high content of organic carbon [16]. All these factors caused localized hot spots with high concentrations of persistent pollutants [16] and, thus, an increased bioaccumulation potential through the food web from the detritivorous and filter-feeders benthic organisms to predator fish species. In Chioggia, the central lagoon, and the northern lagoon, HQs (based on INEC\(_{99}\)) in the range of 2.2 to 5 for small fish and juveniles and of 0.2 to 2.3 for adult fish were estimated, ranking these areas as having an intermediate hazard condition. Chioggia was the area with the lowest risk to fish, having the lowest HQ. Chioggia and the central lagoon are characterized by a higher exchange between sea and lagoon (water residence time, 15–20 d [39]), whereas the central lagoon also is characterized by an erosion process started in the year 1990, transferring contaminated sediments from the lagoon to the sea and, thus, reducing the concentration of pollutants [41]. The northern lagoon is a confined area with a water residence time of more than 60 d, but it is less affected by anthropogenic activities and receives a low freshwater input from the drainage basin.

The present study carried out a site-specific analysis, taking into account a representative food web (including a significant number of benthic and fish species), the spatial distribution of sediment contamination in the lagoon, and the age and size differentiation. Comparison of the present results with previous results reported in the literature [12,14] for Venice Lagoon, however, confirmed a negligible risk to the fish community because of exposure to dioxin-like PCBs and PCDD/Fs, especially taking into account the 90% protection level for the fish population.

The use of two protection thresholds (i.e., 90 and 99% of fish population) allowed the present study to take into account the uncertainty of effect estimation into a preliminary risk assessment and to support the risk management process (i.e., identification of risk acceptability) by estimating risk in relation to the level of aquatic life protection. The potential risk to fish that is obtained by applying the INEC\(_{99}\) needs to be evaluated further by means of experimental measurements and lines of evidence (e.g., bioaccumulation measurements and toxicity bioassays) to address the uncertainty related to estimation of the INEC\(_{99}\).

### Supporting Information

Table S1. Estimated 95% upper confidence limits of sediment contamination (ng/kg dry wt) for the six areas of Venice Lagoon, Italy. CDD = chlorodibenzo-p-dioxin; CDF = chlorodibenzofuran; PCB = polychlorinated biphenyls.

Table S2. Diet composition (% volume) for Venice Lagoon, Italy. Note that suspended solids are grouped with sediment. Juv = juvenile.

Table S3. Sediment and water parameters for Venice Lagoon, Italy. OC = organic carbon.

Table S4. Biota parameterization for biomass, lipid fraction (LF), nonlipidic organic matter (NLOM) fraction, and water fraction (WF). Juv = juvenile.

Table S5. Estimated values for biotransformation parameter, \( k_M \) (1/d). CDD = chlorodibenzo-p-dioxin; CDF = chlorodibenzofuran; PCB = polychlorinated biphenyls. All found at DOI: 10.1897/07-162.S1 (18 KB PDF).

---

Table 4. Hazard quotients (HQs) estimated for fish included in the food web model in the six selected lagoon areas by using both the internal no-effect concentration (INEC\(_{99}\)) and INEC\(_{99}\) estimated from the tissue effect concentration protecting 90 and 99% of the fish community [23].

<table>
<thead>
<tr>
<th>HQ</th>
<th>Northern lagoon</th>
<th>Central lagoon</th>
<th>Porto Marghera</th>
<th>Malamocco</th>
<th>Chioggia</th>
<th>Western lagoon</th>
</tr>
</thead>
<tbody>
<tr>
<td>INEC(_{99})</td>
<td>Chelon labrosus juveniles</td>
<td>0.4</td>
<td>0.4</td>
<td>0.9</td>
<td>1.0</td>
<td>0.4</td>
</tr>
<tr>
<td>Chelon labrosus</td>
<td>0.02</td>
<td>0.02</td>
<td>0.05</td>
<td>0.05</td>
<td>0.02</td>
<td>0.1</td>
</tr>
<tr>
<td>Atherina boyeri</td>
<td>0.3</td>
<td>0.3</td>
<td>0.7</td>
<td>0.8</td>
<td>0.3</td>
<td>1.0</td>
</tr>
<tr>
<td>Zosterisessor ophiocephalus</td>
<td>0.02</td>
<td>0.01</td>
<td>0.03</td>
<td>0.04</td>
<td>0.01</td>
<td>0.04</td>
</tr>
<tr>
<td>Nektom carnivorous benthic feeders</td>
<td>0.4</td>
<td>0.4</td>
<td>0.8</td>
<td>1.0</td>
<td>0.4</td>
<td>1.2</td>
</tr>
<tr>
<td>Sparus aurata juveniles</td>
<td>0.2</td>
<td>0.2</td>
<td>0.5</td>
<td>0.5</td>
<td>0.2</td>
<td>0.6</td>
</tr>
<tr>
<td>Sparus aurata</td>
<td>0.1</td>
<td>0.1</td>
<td>0.2</td>
<td>0.3</td>
<td>0.1</td>
<td>0.3</td>
</tr>
<tr>
<td>Dicentrarchus labrax juveniles</td>
<td>0.2</td>
<td>0.2</td>
<td>0.5</td>
<td>0.5</td>
<td>0.2</td>
<td>0.6</td>
</tr>
<tr>
<td>Dicentrarchus labrax</td>
<td>0.2</td>
<td>0.2</td>
<td>0.4</td>
<td>0.4</td>
<td>0.2</td>
<td>0.5</td>
</tr>
</tbody>
</table>

*HQ values greater than one are presented in italic.*
Acknowledgement—We are thankful to the Venice Water Authority–Consortorio Venezia Nuova for providing the experimental data and to the Consortium for the Co-ordination of the Research in the Lagoon of Venice, which partly supported the research. A special thanks to Simone Libralato and Elena Semenzin for their technical support.

REFERENCES
42. Mackay D, Biwu WY, Ma KC. 1992. Illustrated Handbook of Physical-Chemical Properties and Environmental Fate for Organic Compounds. Lewis, Boca Raton, FL, USA.