Estimation of PCDD/F and PCB detoxification rates in contaminated Tapes philippinarum in the Lagoon of Venice

Stefano Raccanelli[1], Roberto Pastres[2], Piero Vio[3], Maurizio Favotto[1]

[1]Consorzio I.N.C.A.
[2]University of Venice
[3]Regione Veneto

Introduction

The lagoon of Venice (Italy) is the largest Italian Lagoon, covering an area of about 550 km^2. Its average depth about is 0.7 m,, but its morphology is characterized by the presence of large shallow areas and by a network of deeper channels. As can be seen in Fig. 1, three narrow inlets connect the lagoon to the Adriatic Sea. In the last century the Industrial Zone of Porto Marghera, see Fig. 1, was converted into one of largest industrial area in Europe. As a result of the industrial activities, dioxin and dioxin-like compounds, as well as other classes of pollutants, were released into the environment. The lagoon sediment surrounding the Industrial area represented the main sink for these contaminants: in fact, concentrations higher than 2500 ng(I-TE)/kg d.w. were found in the sediment in the channels of the industrial area. The bivalve specis *Tapes Philippinarum* was introduced in the lagoon of Venice in the late 1980ies and, being more resistant to pollution than the autochtonous *Tapes decussatus*, at present can be found in large areas of the lagoon, including the highly polluted area nearby the Industrial zone. At present, *Tapes Philippinarum* is the target of an intensive fishing activity, which severely damages the lagoon sediment and the benthic community(1). The fishing of *Tapes philippinarum* is, of course, not allowed nearby the Industrial Zone, but highly polluted specimen illegally caught in this area still find their way to the market. In order to discourage the illegal fishing of this bivalve, in the year 2004, the local County Council, i.e. the Regione Veneto, prompted a detoxification experiment, aimed at assessing the effectiveness of a cultured based fishery strategy based on the removal of specimen below the marketable size from the highly polluted areas surrounding the Industrial Zone and their resettlement in the clean areas which are reserved to the rearing of *Tapes philippinarum.*.

Methods

The contaminated specimen of *Tapes philippinarum*, (length ³ 25mm= minimum marketable size) were collected in the area surrounding the Industrial Zone and brought into contact with the low contaminated sediment in the area of the South Lagoon shown in Fig. 1. The specimen, total biomass: about 50 Kg w.w., were kept in 50cm x 100cm plastic nets (food -grade), with a mesh size of 5mm, which were laid on the sediment. The biomass density in each net was approximately 500g w.w./m^2. The PCDD/F+PCB concentration in the highly contaminated sediment was 227.9+19.7 ngWHO-TE/kg d.w and the average concentration in the clam flesh was equal to 338.12+72.85 pgWHO-TE/g lipid. In contrast, the PCDD/F+PCB concentration in the South Lagoon sediment was equal to 0.26+0.017 ngWHO-TE/kg d.w. Each sediment sample reflects, in fact, the composition of ten sub-samples of the surficial sediment, first 10 cm, which were separately taken and then homogenized. The total duration of the detoxification experiment was 120 days: as one can see from Table 1, the PCDD/Fs and PCBs concentrations were determined at day 10, 20, 30, 45, 60, 90 and 120.

The concentrations of PCDD/F; PCBs; HCB in all sediment samples were calculated on a dry weight basis. The moisture content of each sediment sample was determined by drying a separate sub-sample of sediment overnight in a conventional oven at 105°C. The concentrations in the clam flesh are expressed on a fat (lipid) weight basis.

All glassware was washed with basic detergent, rinsed with distilled water, treated with a solution of ammonium persulphate 350 g/L in sulphuric acid (98%) and rinsed twice with distilled water and acetone. Subsequently, the cleaned glassware was treated with dimethyldichlorosilane 5% in toluene, rinsed twice with distilled water and acetone, heated to 300°C for 3h, and covered with aluminium foil.

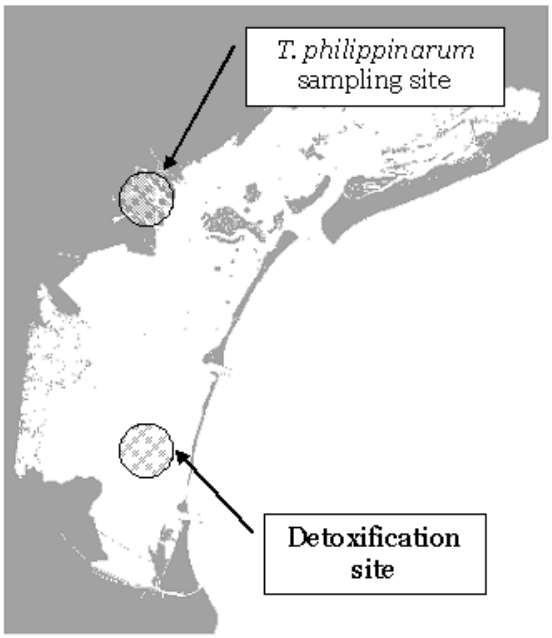

Fig. 1. Sampling and detoxification site.

The sediment samples were transported on ice and stored at 4°C. They were thoroughly mixed with a stainless steel spatula, in order to obtain 10.0±0.1 g sub-samples. The clam flesh was rinsed with distilled water,homogenised, freeze-dried, grinded and 10.0±0.1 g sub-samples were transferred to glass beker. Each sediment and clam sub-sample (10.0±0.1g) was first spiked with a series of 15 $^{13}C_{12}$-labeled 2,3,7,8 PCDD/F (EDF8999) with a series of 12 $^{13}C_{12}$-labeled PCB (EC4937), with $^{13}C_{12}$-HCB (CLM351) substituted isomers, as internal standards, mixed with Spe-ed Matrix. The extraction by ASE 200 (DIONEX Sunnyvale, CA) was performed with 50 mL of toluen at 135 °C, 2000 psi, 7 min. heat-up and 2 cycles of 10 min static time. The extracts were transferred to hexane before the clean up treatment. The extracts were transferred to hexane and then were treated with sulphuric acid (98%) and potassium hydroxide (20%) in a 100 mLseparatory funnel The extracts were firstly spiked with $^{37}C_4$-labeled 2,3,7,8 PCDD (EDF6999) and with 3 $^{13}C_{12}$-labeled PCB (EC4978) and then cleaned up using the automatic system, Power Prep (Fluid Management System Inc) with pre-packed disposable columns containing multilayer silica, alumina and carbon for PCDD/F, PCB and HCB. The HRGC/HRMS analyses were conducted using a HP 6890 plus gas chromatograph coupled to a MicromassAutospecUltima mass spectrometer operating in EI mode at 35 eV and with a resolution of 10.000 (5% valley). PCDD/F Sample injections were performed in the splitless mode on a 60 m DB5 ms column (J&W 0.25 mm ID, 0.25 µm film) and on a 60 m Rtx 200 (Restek 0.25 mm ID, 0.25 µm film) for verification. The quantitative determination of PCDDs/PCDFs was performed by an isotope dilution method using relative response factors previously obtained from five standard solutions injections (EDF 9999 Cambridge Isotope Laboratories, Woburn, MA.), as recommended by the US-EPA[(2)]. The quantitative determination of PCB-HCB was performed by an isotope dilution method using relative response factors previously obtained from five standard solutions injections (EC1668 Cambridge Isotope Laboratories, Woburn, MA.), as recommended by the US-EPA[(3)].

Results and discussion

The results of the detoxification experiment are summarized in Table 1, which shows the PCDDs and PCDFsconcentrations in the clam flesh, expressed both as pg WHO-TE/g d.w. and pg WHO-TE/g lipid, as a function of time.

time (day)	WHO-TE PCDD/F (pg/g d.w.)	WHO-TE PCDD/F (pg/g lipid)	WHO-TE PCB (pg/g d.w.)	WHO-TE PCB (pg/g lipid)

t_0= 0	3.365	338.1	0.73	72.8
t_1= 10	0.198	21.3	0.09	9.6
t_2= 20	0.136	18.0	0.05	6.6
t_3= 30	0.095	11.2	0.06	7.6
t_4= 45	0.077	12.3	0.04	6.5
t_5= 60	0.071	8.2	0.06	6.8
t_6= 90	0.048	6.4	0.05	6.8
t_7= 120	0.055	12.0	0.02	4.3
y_b	0.067	10.0	0.05	6.4

As one can see, in both cases the equivalent toxicities normalized on the lipidic fraction decrease of approximately an order of magnitude in ten days and after 30 days they do not show any systematic trend. Based on this information, one can obtain a preliminary estimation of the cumulative biological half-life of PCDD/Fs and PCBs. Assuming a first-order detoxification rate, the last five values were averaged, in order to estimate the background values, y_b, given in Tab. 1. The decay rate, k, was then estimated using the expression:

$$\ln [(y_0-y_b)/ (y_1- y_b) = -k(t_1-t_0) \quad (1)$$

in which y_0 and y_1 represent, respectively the equivalent toxicities at time t_0 and t_1. The application of eq. 1 to the equivalent toxicities normalized non the lipidic fractions yields:

$$k_{PCDD/F} = 0.34 \; k_{PCDD/F} = 0.32$$

Therefore, the biological half-lifes of PCDD/Fs and PCBs are, respectively, 2.1 day^{-1} and 2.2 day^{-1}.

As far as the estimation of the background equivalent toxicities is concerned, it can be remarked that the estimated background levels, normalized on the wet weight, compare well those given in (DiDomenico, 2003) [(4)]. The PCDD/F background TE, normalized on wet weight, is also consistent with the one estimated using the regression model presented in (Raccanelli et al. 2004) [(5)]:

$$\ln(\text{WHO-TE})_{\text{clam w.w.}} = -1.8 + 0.33 \ln (\text{WHO-TE})_{\text{sed}} \quad (2)$$

In fact, using eq. 1, one obtains y_b= 0.11, which is of the same order of magnitude of the values given in Table 1, second column.

The biological half-lifes estimated in this detoxification experiment are not in contrast with the findings presented in (DiDomenico, 2003) [(4)]: in this study a three to four fold decrease in the TE of contaminated clams was observed over thirty days. Based on this finding, it was decided to increase the sample frequency during the course of the present experiment. However, and somewhat surprisingly, even our data do not allow one to obtain statistically meaningful estimates of the biological half-lifes, since the detoxification rates appear to be extremely high.

Conclusion

The results presented in this paper show that the WHO-TE PCDD/F and the WHO-TE PCDD/F in the bivalve species *Tapes Philippinarum* undergo a very rapid decline when the specimen are brought into contact with low contaminated sediment. The estimates of the cumulative biological half-lifes of PCDD/Fs and PCBs here presented must certainly be considered as a preliminary result, which will have to checked by running further short-term detoxification experiment, in order to obtain statistically meaningful estimates. However, this findings are certainly extremely interesting, since they suggest that polluted areas of the lagoon, where the fishing of *Tapes Philippinarum* is at present now allowed but where this bivalve is abundant, could be used as nursery areas in a culture based fishery

regime.

References

1 S. Guerzoni, S. Raccanelli., “The ScikLaggon - Dioxin and other organic pollutants (POPs) in the lagoon of Venice” pp17-35, pp 47-54, pp55-70, 2004 ed. Cafoscarina, Venezia, Italy.

2 US EPA Method 1613B/94: Tetra-Through Octa-Chlorinated Dioxins and Furans by Isotope Dilution HRGC/HRMS.

3 US EPA Method 1668A/99: Chlorinated Biphenil Congeners in water, soil, sediment, and tissue by HRGC/HRMS

4 A. Di Domenico, E. Cherin, S. DeLuca,F.Ferri, M.Magliuolo, R.Miniero, “Microcontaminanti potenzialmente pericolosi presenti nei sedimenti e nei molluschi bivalvi vivi e relativa detossificazione una volta trasferiti in zone di inquinamento di fondo” Rapporto tecnico finale (revisione), Convenzione tra Regione Veneto e Istituto Superiore di Sanità, Roma 10 novembre 2003.

5. S. Raccanelli, R.Pastres, M.Favotto, P.Vio, “Correlation between POPs in sediment and edible bivalve in the Lagoon of Venice and estimation of the daily intake” Organohalogen Compound, pp1823-1826, Vol.66(2004).