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Organochlorine Compounds in Blue Mussels, *Mytilus edulis*, and Pacific Oysters, *Crassostrea gigas*, from Seven Sites in the Lower Saxonian Wadden Sea, Southern North Sea

Barbara Dörr · Gerd Liebezeit

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Abstract Blue mussels (*Mytilus edulis*) and Pacific oysters (*Crassostrea gigas*) collected at seven locations in the Lower Saxonian Wadden Sea in January and February 2007 were analysed for organochlorine compounds. Contaminants were present in all samples, albeit with variable amounts and composition. The highest values were found in the Jade Bay. Congener PCB 153 was the contaminant which had the highest content of all organochlorines tested (475.75–937.39 ng/g lipid). DDT was detected in one sample only while DDD and DDE were found in all samples, the latter with contents up to 351.34 ng/g lipid. No clear differentiation could be made in terms of accumulation of organochlorines for *M. edulis* and *C. gigas*. Comparison with data from 2001 to 2006 showed an increase in 2007, which may be due to the different season the samples were taken.

Keywords Persistent organic pollutants · North Sea · Blue mussel · Pacific oyster

Organochlorines (OCs) are chemicals which contain at least one covalently bonded chlorine atom. While a large number of natural chlorinated products are known (cf. Gribble 1998, 2003) this group also encompasses anthropogenic compounds such as polychlorinated biphenyls (PCBs), dichlorodiphenyltrichloroethane (DDT) and its degradation products DDD and DDE, hexachlorocyclohexane isomers, hexachlorobenzene and others. Their lipophilic nature and persistence allows them to accumulate in both biota and sediments (e.g. Cheevaporn et al. 2005), thus often leading to bioaccumulation throughout the food web (Okumura et al. 2003). In the aquatic environment OCs occur either in dissolved form or attached to particles. Based on their high affinity to organic matter, organochlorines are more often removed from the water column than kept in solution (Cheevaporn et al. 2005).

Although most of these compounds have been banned in Europe over the last decades, they are still present in the environment (Wintermyer and Cooper 2003) and thus can give rise to ecological risks. The environmental input occurs via three major pathways: (1) municipal and industrial outfalls, (2) rivers or (3) atmosphere, the latter being the most important source for the more volatile OCs. These substances can therefore be transported on a global scale and may hence be found even in remote areas (Potrykus et al. 2003).

Bivalves have been successfully and widely used as monitoring organisms (Solé et al. 2000). Their ability to concentrate lipophilic chemicals well above ambient levels enables bivalves to represent local contamination (e.g. Baršienė et al. 2006). Bivalves also are fairly resistant and insensitive to organochlorines. Due to the fact that they are feeding on suspended matter and therefore filter large quantities of water, this makes them even better monitoring organisms. Furthermore bivalves are cosmopolitan, abundant and can easily be collected thus making comparisons within and among locations possible.

In the present study, bivalves (the native species *Mytilus edulis* and the invasive species *Crassostrea gigas*) from seven locations in the Lower Saxonian Wadden Sea were analysed for organochlorine contents in order to compare

B. Dörr (✉)
Faculty 02: Biology, University of Bremen,
Bremen, Germany
e-mail: barbaradoerr@hotmail.de; bdoerr@freenet.de

G. Liebezeit
Department of Marine Chemistry, ICBM-Terramare,
Wilhelmshaven, Germany

regional levels of contamination. Furthermore these two species were sampled to investigate whether there are differences between *M. edulis* and *C. gigas*.

Materials and Methods

Samples of *M. edulis* and *C. gigas* were taken during January and February 2007 at five locations: Hafen Neuharlingersiel, Wilhelmshaven, Wilhelmshaven Nassauhafen, and Janssand, backbarrier area of Spiekeroog Island. Additional samples analysed were collected during other studies: 2004 at the Nord West Oil Terminal in the Jade Bay and 2006 at Mellumbalje (Fig. 1).

Different numbers of *M. edulis* and *C. gigas* were collected per station. Sizes ranged from 3 to 6 cm for *M. edulis* and 7–13 cm for *C. gigas* (Table 1). At most stations only one of the two bivalve species was found.

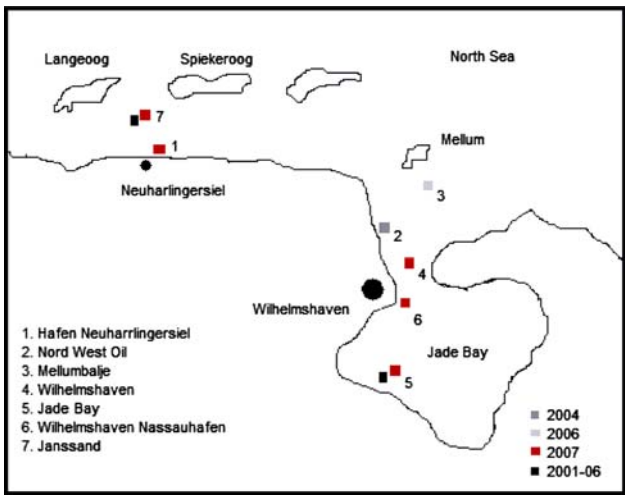

Fig. 1 Sampling locations in the Lower Saxonian Wadden Sea (including the year of sampling)

Table 1 Numbers and lengths of bivalves collected at the seven different sampling sites

Station	Species	*n*	Size (cm)
1	*M. edulis*	30	2.9–3.7
2	*M. edulis*	30	3.3–3.9
3	*C. gigas*	4	7.0–8.5
4	*M. edulis*	31	4.2–4.7
4	*C. gigas*	5	8.6–10.5
5	*M. edulis*	30	3.0–3.5
6	*C. gigas*	4	10.0–13.0
7	*M. edulis*	25	5.1–5.7
7	*C. gigas*	5	8.2–10.0

After shucking individual samples were pooled by station and species. The mussel tissues were then homogenised using an ULTRA TURRAX T25. Approximately 2 g were then spiked with an internal standard solution containing tetrabromobenzene and dried with two parts of sodium sulphate. Cell fractions and lipids were removed by eluting the samples in a column with *n*-hexane:dichloromethane (4:1), the column containing from the top sodium sulphate, silica + H_2O (10% w/w), silica + H_2SO_4 (40% w/w), sodium sulphate and glass wool. The cleaned solution was evaporated to approximately 1 ml using a rotary evaporator (Labo Rota 300 Resona, temperature 35°C, pressure ~200 hPa). After redissolution in 250 µl *i*-octane determination of organonochlorine residues was by gas chromatography/mass spectrometry. A GC-MSD 5973 Series Agilent equipped with a HT5 SGE capillary column, 25 m × 0.22 mm internal diameter, film thickness 0,1 µm was used under the following chromatographic conditions: injector temperature 80–12°C/s–340°C (3 min), column programme of temperatures: 60–160°C (40°C min^{-1}); 160–220°C (2.5°C min^{-1}); 220–280°C (10°C min^{-1}); 280°C (5 min). Helium was used as the carrier gas.

All samples were analysed in duplicate. Total lipid contents were determined gravimetrically after extraction with cyclohexane:isopropanol 1:1.

A total of 53 PCB congeners was quantitatively determined. From these only PCBs 153, 126, 28, 118, 110, and 138 will be discussed in detail in the following. The value of congener PCB 153 is frequently used as an approximation of ΣPCB as it is generally present with the highest contents and correlates well with other analysed PCBs (OSPAR 2006). PCB 126 is highly toxic while PCBs 28, 118, 110, and 138 are major components of technical mixtures. All data are normalised to lipid content. For statistical analysis Student's t-test was used.[1]

Results and Discussion

The mean lipid value of *M. edulis* (1.4% ± 0.6% lipid) was slightly higher than that of *Crassostrea gigas* (1.0% ± 0.1% lipid) but no significant difference ($p > 0.05$) could be detected for the lipid contents of *M. edulis* and *C. gigas*.

Organochlorine compounds (OCs) were detected in all samples analysed (Fig. 2). *M. edulis* of station 5 in the inner Jade Bay had the highest sum of OCs (5.2 µg/g lipid). The lowest contents were determined at stations 4 (outer Jade Bay, 2.3 µg/g lipid) and 7 (Janssand, 2.6 µg/g lipid). However, no significant difference regarding the sampling stations could be determined for the sum of OCs in both species. A direct comparison of *M. edulis* and *C. gigas*

[1] Excel, Microsoft Office 2000.

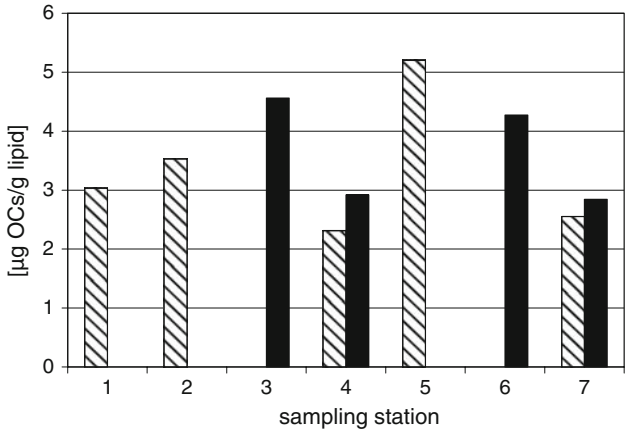

Fig. 2 Comparison of ΣOCs in *M. edulis* (*stripes*) and *C. gigas* (*black*) at the seven sampling locations

regarding the sampling station is only possible for stations 4 and 7. Both species showed no significant differences ($p > 0.05$) in contaminant levels.

At station 2 most OCs except for PCB 128 were determined. PCB 128 was also absent in all other samples. All other locations showed a greater variation in the composition of organochlorines. PCBs were detected in every sample tested. However, the amount determined varied in the different congeners. The content of PCB 153 was significantly higher ($p < 0.05$) than all other determined PCBs. Mean values in *M. edulis* were 19.6% ± 15.1% of PCB 153 of the total organochlorine concentration. Mean values for *C. gigas* were 20.1% ± 19.3% of PCB 153 regarding the total concentration of OCs. No significant difference could be determined regarding contents of PCB 153 and species. PCBs 28, 118, 110, 138, four of the major components of technical mixtures, were determined in all samples.

DDT could only been determined at station 2, while its degradation products p,p′-dichlorodiphenyldichloroethylene (DDE) and p,p′-dichlorodiphenyldichloroethane (DDD) were present in all samples (Fig. 3). The DDE contents are significantly higher ($p < 0.01$) than those of DDD. This can be shown for both species. DDE contents are also significantly higher ($p < 0.01$) than the DDT level (0.084 µg/g lipid) detected in *M. edulis* at station 2.

γ-HCH was only detected in *C. gigas* at stations 4 and 6. HCB could be found in all samples, except for *C. gigas* at station 7, all showing relatively low contents. Chlordane was determined in only two samples (in *M. edulis* at stations 2 and 5). Neither *trans*- nor *cis*-nonachlor were detected at station 5, but were found at station 2. The contents of *trans*- and *cis*-nonachlor were in the same range as chlordane at the same station but none of the three OCs showed significant levels of contamination.

For comparison with the years 2001–2006 (data provided by Niedersächsisches Landesamt für Wasserwirtschaft, Küsten- und Naturschutz – NLWKN) the data for organochlorines, in this study were re-calculated to mg/kg lipid. The NLWKN study was only based on *M. edulis* and the PCBs found differed from the OCs determined in this study. This is why a direct comparison with data of this study from 2007 was only possible for *M. edulis* at stations 5 and 7 focussing on PCBs 28, 52, 101, 118, 153, 194, DDT and its metabolites, HCB and γ-HCH. Due to a lack of data at station 5 in 2002 and 2003, only data of station 7 are presented in Fig. 4.

At station 7 data are missing for all OCs in 2002. ΣPCB (28, 52, 101, 118, 153, 194; Fig. 4) was determined in all other years, showing a pronounced increase in 2005. PCB 153 had the highest content of all OCs tested in the present study ($p < 0.05$). Levels of PCB 153 were high in the NLWKN investigation as well. The detected content of γ-HCH in 2001 is higher (6.9 mg γ-HCH/kg lipid) than in 2003 (0.8 mg γ-HCH/kg lipid) and the following years. No γ-HCH could be detected at station 7 in 2007, although samples were tested for all OCs. HCB, DDD and DDE have been detected in all years. Contents for DDT are missing in 2007. In 2005 HCB, DDT, DDD and DDE all show an enormous increase in comparison to the year before (Fig. 5).

No significant difference in accumulation of OCs could be determined in *M. edulis* and *C. gigas*. OC accumulation depends on several factors, such as different body conditions, e.g. filtration rate, lipid contents. Wehrmann et al. (2006) determined a higher filtration rate for *C. gigas* based on clearance rates. Furthermore *C. gigas* has a higher growth rate (Wehrmann et al. 2006; Damm and Neudecker

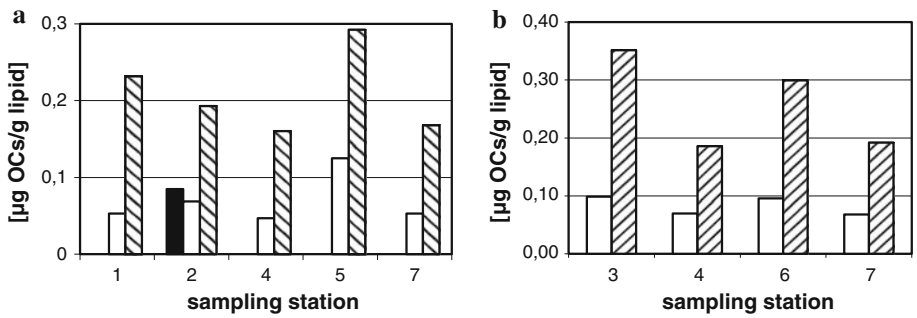

Fig. 3 Comparison of contents of DDT (*black*), DDD (*white*), and DDE (*stripes*) contents. No *M. edulis* were present at station 3 and 6, no *C. gigas* were present at station 1, 2 and 5

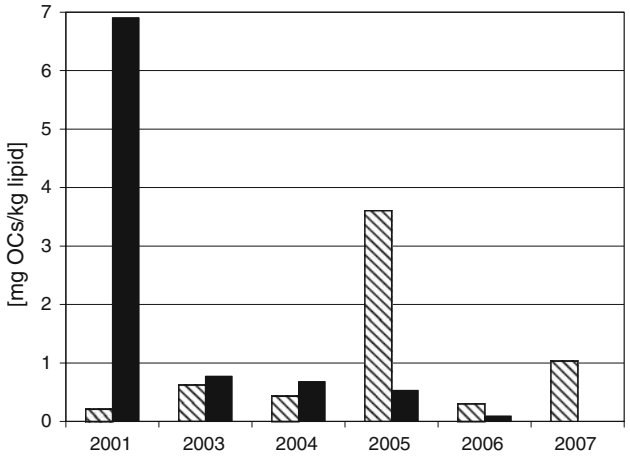

Fig. 4 Mean contents of ΣPCB (*stripes*) and γ-HCH (*black*) of M. *edulis* at station 7 between 2001 and 2007. In 2002 no samples were taken. Data of 2001–2006 is from the NLWKN, data of 2007 was determined in this study

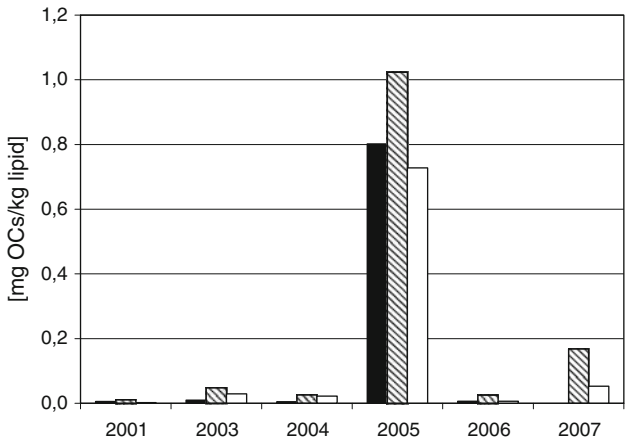

Fig. 5 Mean values of DDT (*black*), DDD (*white*), and DDE (*stripes*) of *M. edulis* at station 7 between 2001 and 2007. No data exists for 2002. Data of 2001–2006 is from the NLWKN, data of 2007 is determined in this study

2006, both indicating higher expected values of contamination for *C. gigas*.

Deslous-Paoli and Héral (1988) determined a lipid content between 6% and 20% dm for *C. gigas*, Pazikowska and Szaniawska. (1988) determined a mean lipid content of 17.3% for *M. edulis*. Additionally, the lipid content in all samples analysed in the present study ranged between 0.5% and 1.9% dm for both species, being far lower than the data mentioned above. This might be due to the sampling being done at different seasons.

According to Deslous-Paoli and Héral (1988) and Pazikowska and Szaniawska (1988) *M. edulis* for which the higher lipid content was detected should also have had the higher levels of OCs. This could not be shown in the present study. Direct comparison for stations 4 and 7 showed the trend of a higher value of contamination in case of *C. gigas*, although *C. gigas* had the lower lipid content. The present data do not allow a clear statement concerning differences in accumulation of contaminants by *M. edulis* and *C. gigas.*

Based on the fact that biologically active OCs were found in all samples, it has been shown that these substances still exist in the environment, although most of them are banned in (western) Europe since the 1970s.

The seven different locations examined exhibit variation in OC content and composition albeit not at a statistically significant level. This was surprising due to a variety of factors including topography and currents of the Jade Bay as well as suggested anthropogenic influences. The Jade-Bay exchanges its water with the North Sea by tides, freshwater input is negligible. Tidal inflow tends to occur on the western side, the outflow on the eastern side. OCs associated with particles might eventually accumulate on the western part of the Jade Bay and therefore settle down and/or are ingested by bivalves located there. The mean velocities into the Jade Bay are about 2.5–5 cm/s (www.bsh.de – Bundesamt für Schifffahrt und Hydrographie). This suggests that the flow along the western side in the outer Jade Bay and its rather narrow connection to the inner part is too fast for particle settlement. The literature on tidal flow and velocity data leads to the hypothesis that contents of OCs should be highest at station 5, which is located furthest south in the Jade Bay. This could not be proven significantly with the results given in this study. Nevertheless, the data indicate a tendency toward higher contents at station 5 and lower OC values at stations 2, 4 and 6.

At station 2 nearly all contaminants tested were found. No direct comparison with other locations can be made as the sample was already taken in 2004. Therefore, the composition could have changed until 2007. Nevertheless an explanation for these results, independent of the year, may be sought in the location itself. Station 2 is located near major industries, including Nord West Oil, chemical industries and the sewage treatment plant of Wilhelmshaven. A general source for the OCs determined may also be merchant shipping (Potrykus et al. 2003) as an economic focus of Wilhelmshaven. Involved in these port activities is the deepening of the shipping channel, which takes place several times a year (Götschenberg and Schlüter 2005). This leads to (a) resuspension of fine grained sediment and associated contaminants and (b) to clouds of fine grained material at the dumping sites. One of these is located in the inner part of Jade Bay.

No significant difference could be detected between station 7, located exterior of the shipping channel and the industrial region, and the remaining stations. A possible explanation might be a negligible impact of the

surrounding industries and the merchant shipping regarding the total amount of OCs in the Jade Bay.

PCB 153 is ortho-substituted. In comparison to meta- and para-substituted PCBs, ortho-substituted are more resistant against biodegradation (Schmidt 2001) and breakdown in general. Furthermore PCB 153 is one of the major components of technical mixtures. This suggests that the values of PCB 153 in the environment will remain high even after long residence in the environment. In addition, the association of PCB 153 with sinking particulate matter, which is taken up by bivalves, is more pronounced (up to 90% of the entire content) than that of other PCBs (Ilyina 2007). Combined with the high lipophilicity, based on the higher degree of chlorination and therefore longer half life (Witczak and Leszczynska 2006), this could explain the dominating levels of PCB 153 detected in all samples taken.

PCBs with chlorine substitutes at both, para- and meta-position are the most biochemically active ones, e.g. PCB 126 (DeVoogt et al. 1990). They are also planar. This combination makes them the most toxic PCBs. Although these PCBs were present in trace quantities in technical mixtures, e.g. flame retardants, capacitors, and heat transfer fluids, they cause a major risk to the environment (DeVoogt et al. 1990). No significant difference in PCB 126 contents could be detected in either of the two bivalve species. In terms of all PCBs tested PCB 126 did not show significant differences as could be shown for PCB 153. The low contents detected lead to the conclusion that no recent input of PCB 126 occurred in the investigation area. Trace values could be due to introduction in the environment during the use of PCB containing technical mixtures until the 1970s. Based on the long half life of PCBs, this might explain the values detected. This may also be valid for the planar PCBs 28, 110, 118, 138, as major components of technical mixtures (DeVoogt et al. 1990), which were determined in low values in all samples and not detected in significantly different concentration from one another.

DDT was detected in negligible amounts at station 2 only, indicating, that there has been no recent input in the investigation area. Half life of DDT ranges from days in the atmosphere, to 15 years in (marine) sediments, depending on temperature and region. Station 2 is located near major industries in this area, suggesting that DDT levels detected might be due to earlier contamination. Following the *Quality Status Report* 2000 the contents of DDT in biota have generally decreased due to a ban of DDT in the 1970s in Western Europe and North America. In contrast, DDE and DDD, the breakdown products of DDT, were detected in all samples, DDE showing the highest contents compared to DDT and DDD in both species ($p < 0.05$). DDT is rapidly metabolised to DDD by dechlorination and slower matabolised to DDE by dehydrochlorination or through DDD to DDE by hepatic microsomal enzymes in organisms (Sheridan 1975). Furthermore, DDD rapidly gets detoxified and secreted by organisms, DDT and DDE are stored in tissues and further metabolism and secretion is exponentially slow (Beard et al. 2000). DDE was found to be the major component detected in biota (Oslo-Paris-Commission, Quality Status Report 2000), which is also shown by the present data. In fact, the detected contents of DDD and DDE, determined in all samples are possibly due to the breakdown of DDT. One explanation for the levels detected in 2007 might be an accumulation of DDT due to former use and recent release from sediments. Based on the fact that DDE is more persistent in lipid-rich tissues than DDD (Gladen et al. 2004), the higher values of DDE detected in all samples could be explained. A general decrease of DDE and DDD could not be shown by the *Quality Status Report* 2000 over the last decades. Due to the fact that DDD is more rapidly secreted than DDE (Beard et al. 2000), the detected values of DDD lead to the conclusion that the breakdown of DDT in the bivalves tested did not occur long ago although DDT has been banned since the 1970's.

The values of γ-HCH detected at stations 4 and 6 are low. Despite not being produced commercially anymore, restricted use of γ-HCH is permitted in several countries, including Germany. Although low, the contents detected in this study might be an indicator for this restricted use. Values of HCB, chlordane, *trans*- and *cis*-nonachlor detected in this study are low, indicating no recent input in the Jade Bay.

The main problem comparing data of 2007 to those of 2001–2006 was the choice of sampling sites. A direct comparison could only be made for stations 5 and 7, with a limitation at station 5 because of missing samples from the years 2002 and 2003. Data of the NLWKN was given in mg/kg lipid and did not allow conversion into µg/g lipid. Therefore data of this study were re-calculated into mg/kg lipid.

No significant difference could be determined between the data of 2001–2006 and the present study in 2007. The slightly elevated levels of ΣPCB, DDD and DDE in 2007 may be due to the season in which the samples were taken. Samples of the years before were taken from July to September, samples of the present study were taken in January and February 2007. *M. edulis* for example strongly accumulates PCBs during winter time, leading to an elevated content of PCBs, which could be caused by a lower filtration rate (Lee et al. 1996). In early spring gametogenesis takes place leading to a mobilization of energy, a decrease in lipid content and therefore results in a mobilization of accumulated and stored contaminants (Deslous-Paoli and Héral 1988).

The major increase of OCs in 2005 could not be explained so far but possibly may be due to a change in the performing laboratory (NLWKN pers. comm. 2007).

Further research in the investigation area is necessary to understand more clearly from where OCs enter the environment and which risk they cause.

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