Verification of the success of recent use restrictions for tributyltin by retrospective monitoring of archived biological samples from North and Baltic Seas

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INTRODUCTION

For several decades tributyltin (TBT) was used broadly as antifouling agent in coatings of ships and boats. Although the high toxicity to aquatic organisms and endocrine effects on mussels and snails were known since the 1980s, only in 2003 the use of organotin-based antifoulants within the European Union was completely banned. A previous study revealed that TBT levels in marine biota samples from North and Baltic Seas were quite constant during the 1990s while levels apparently decreased after 2003 (Rüdel et al. 2009). However, since the original method was not sensitive enough to follow the current low levels of butyl tin compounds, a follow-up study with a new method was initiated.

To this end, standardized pooled samples of eelpout (fish) and blue mussel were retrieved from the German Environmental Specimen Bank (ESB). Analysis of TBT and its potential degradation products dibutyltin (DBT) and monobutyltin (MBT) was performed by species-specific isotope dilution analysis by GC/ICP-MS (Monperrus et al. 2003) following digestion with tetramethyl ammonium hydroxide, n-hexane extraction and derivatization with tetraethyl borate.
Time series cover the periods 1985-1997, for which two different measurement methods are compared, and 2004 - 2008 for blue mussel, as well as 2006-2009 for eelpout.

ORIGIN OF THE SAMPLES

In the framework of the German ESB blue mussels (*Mytilus edulis*) are collected from North Sea sites bi-monthly and from one Baltic Sea site semi-annually. Mussel soft bodies are used for the preparation of standardized pooled samples for each year. Samples are cooled directly after collection at temperatures below -150°C. About 2 kg of mussel soft bodies are homogenized and about 200 sub-samples are prepared for long-term archiving in the ESB.

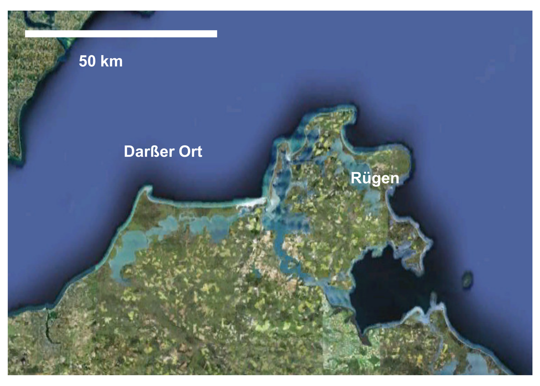

Blue mussel (*Mytilus edulis*)

Eelpout (*Zoarces viviparus*) are collected annually in June from three sampling sites. Muscle tissue from at least 100 fish are dissected and stored directly after collection at temperatures below -150°C. Preparation for long-term storage is performed as described above.


Eelpout (*Zoarces viviparus*)

Sites

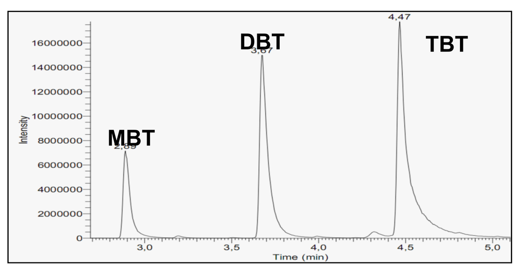
- Baltic Sea: Darßer Ort (blue mussels and eelpout)
- North Sea:
  1 Königshafen (blue mussels)
  2 Meldorf Bay (eelpout)
  3 Transect Varel-Mellum (eelpout)
  4 Eckwarderhörne/ Jadebay (blue mussels)



North Sea (left) and Baltic Sea (above) (Pictures taken from Google Earth)

ANALYTICAL METHOD

1. Weighing in of sample, addition of TMAH, addition of organotin isotope spike
2. Mixing of spiked sample followed by microwave digestion
3. After addition of buffer, ethylation and mixing of solution
4. Extraction with *n*-hexane and shaking of the solution
5. Centrifugation and collection of the supernatant
6. Separation by GC and detection by SF-ICP-MS



Chromatogram of separated butyltin species

Quality Assurance: Measurements of CRM ERM-CE477, Butyltin species (TBT, DBT, MBT) in mussel tissue (measurement uncertainty U calculated according to the NORDTEST procedure with a coverage factor of k = 2):

Recovery	MBT [%]	DBT [%]	TBT [%]
CRM, original (n = 9)	90.6 ± 8,7	91.9 ± 3,3	98.3 ± 3,6
CRM, 1:100 diluted (n = 7)	80.5 ± 9,2 (n = 3)	81.0 ± 4.1	102 ± 12

Measurement uncertainty	MBT	DBT	TBT
certified (ng/g ± U; k = 2)	1500 ± 280	1540 ± 120	2200 ± 190
measured (ng/g ± U; k = 2)	1277 ± 472 (n = 32)	1436 ± 307 (n = 32)	2235 ± 385 (n = 32)

Limit of Detection (LOD) and Limit of Quantification (LOQ) of Butyltin species in ng/g; calculation following DIN 32645 and the approach by Rodriguez Martin-Doimeadios et al. (2003):

LOD	ng/g	LOQ	ng/g
TBT	0.08	TBT	0.23
DBT	0.02	DBT	0.06
MBT	0.03	MBT	0.09

ASSESSMENT OF BODY BURDENS REGARDING POSSIBLE EFFECTS

To estimate the relevance of the detected body burdens of butyltin compounds in mussel and fish tissue two aspects were considered:

1) Comparison of TBT concentrations in mussel tissue with the environmental assessment criterion (EAC) of 2.4 ng/g fresh weight derived in the context of the OSPAR convention:
Results (Fig. 2 - 4): since 2004 (Königshafen/North Sea, Darßer Ort/Baltic Sea) respectively 2007 (Jadebay/North Sea) TBT concentrations are below the EAC.

2) Conversion of measured TBT concentrations in tissue of mussel and fish to average concentrations in water to which the organisms were exposed by applying bioconcentration factors (BCF) taken from literature. Comparison of these data to the environmental quality standard for TBT derived for the Water Framework Directive (0.2 ng/L; 2008/105/EG):
Results 2007-2009 (for BCF sources refer to Rüdel et al. 2003, 2009)
Mussel, BCF 5000 respectively 10400, estimated concentration in water: 0.1 - 0.4 ng/L
Fish (eelpout), BCF 9400 respectively 11000, estimated concentration in water: 0.1 - 0.4 ng/L
The estimated concentration in water is in the range of the environmental quality standard.

RESULTS AND DISCUSSION – METHOD COMPARISON

Fig. 1 shows a comparison of butyltin data for mussels from the period from 1985 until 1997. GC-AED-data are from Rüdel et al. (2003).

In most years TBT levels determined with both methods are similar within the range of the measurement uncertainty.

However, larger differences are detectable for DBT and MBT for which concentrations were always in the range of the LOQ of the GC-AED method.

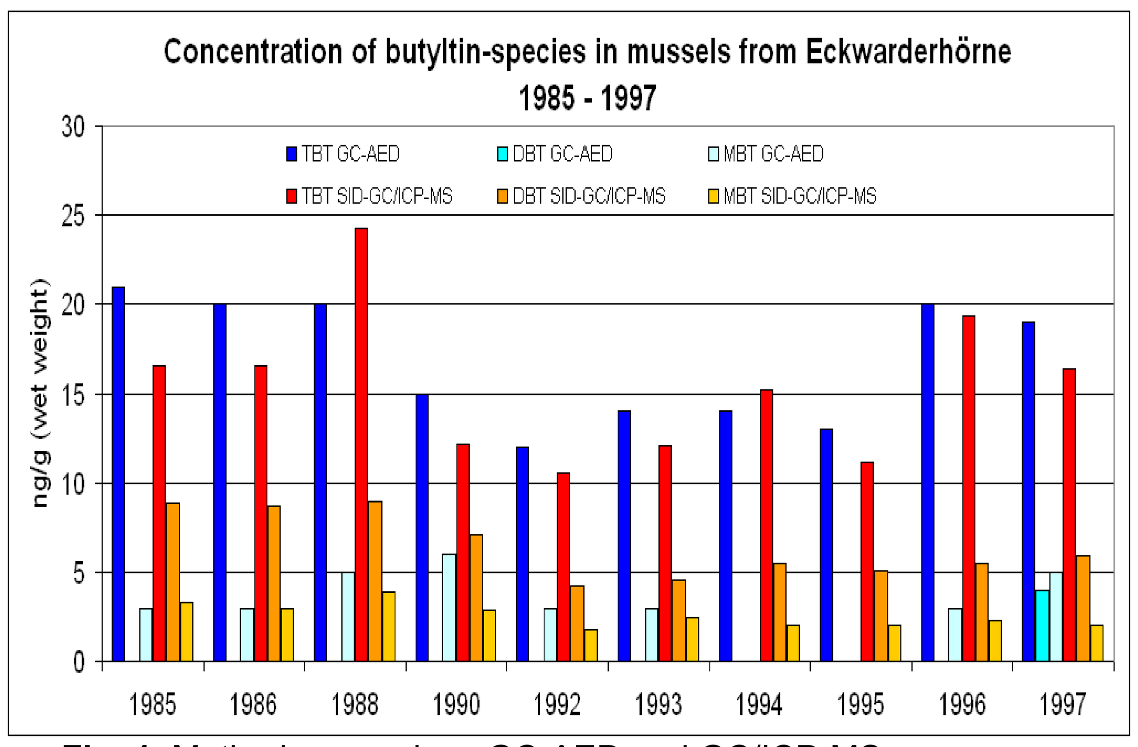


Fig. 1: Method comparison GC-AED and GC/ICP-MS.

RESULTS AND DISCUSSION – TRENDS FOR BLUE MUSSEL

In the time series for the period from 1985 to 1992 (Fig. 1) a decrease in levels of butyltin species is detectable. Then, TBT concentrations increased until 1996 by about 60 % compared to the levels in 1992. Fig. 2 - 4 exhibit decreasing concentrations of tributyl-, dibutyl- and monobutyltin species in blue mussel in recent years until 2008/2009. Concentrations are given as mean value ± standard deviations (n=2-3) in ng/g wet weight.

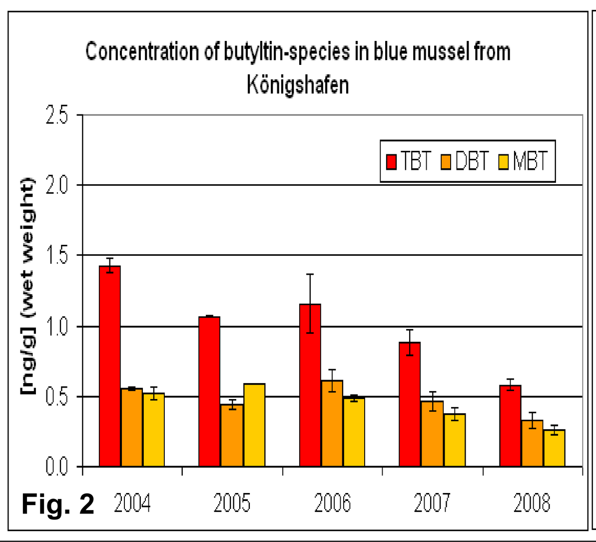


Fig. 2

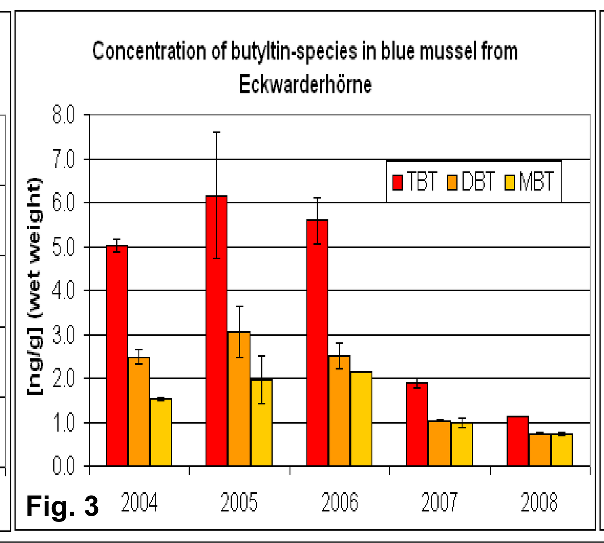


Fig. 3

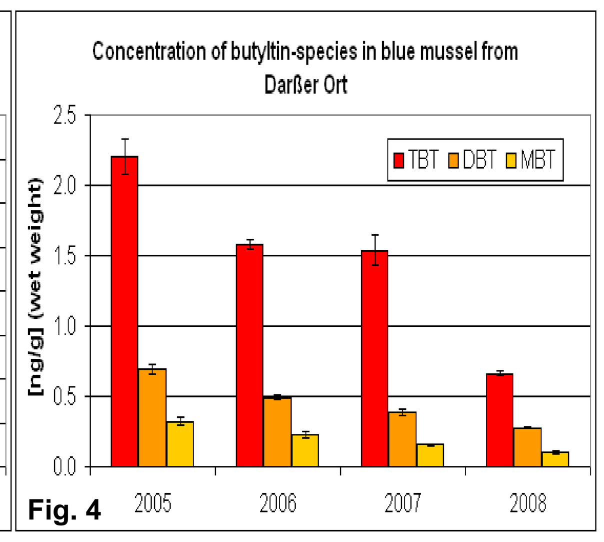


Fig. 4

RESULTS AND DISCUSSION – TRENDS FOR EELPOUT

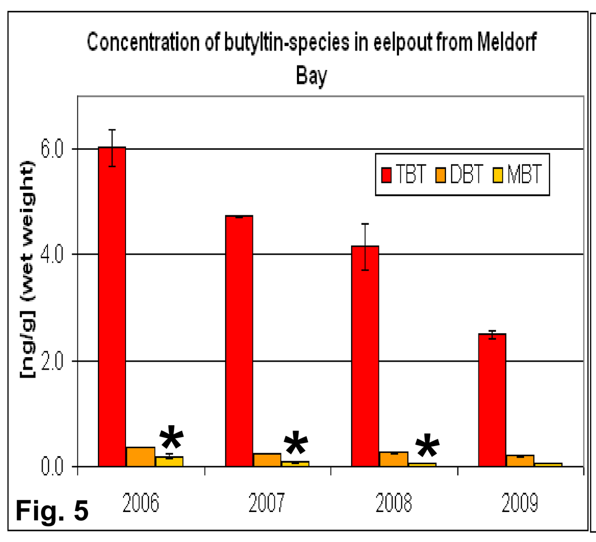


Fig. 5

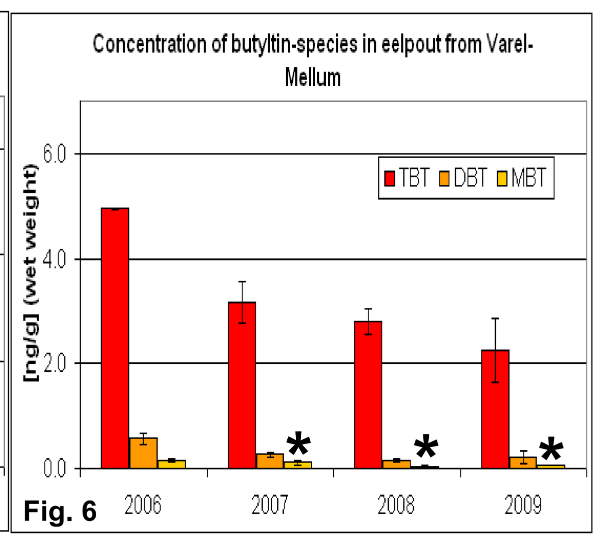


Fig. 6

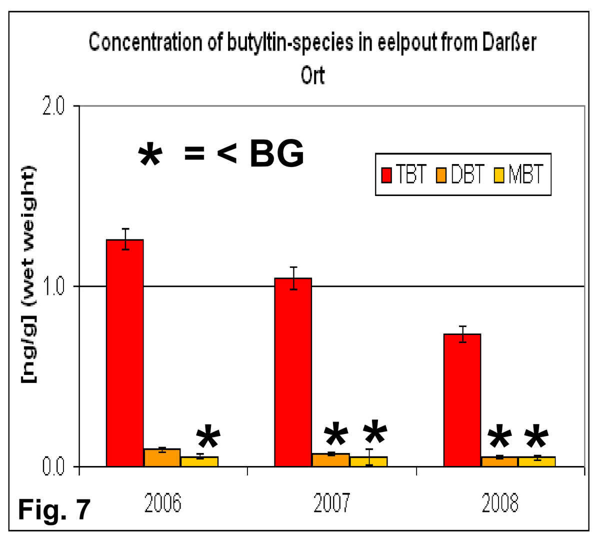


Fig. 7

Fig. 5 - 7 demonstrate decreasing concentrations of all three butyltin species in muscle tissue of eelpout in recent years. In eelpout from Meldorf Bay, Varel-Mellum and Darßer Ort the concentration of DBT and MBT are below the LOQ (*). Concentrations are given as mean values ± standard deviations (n=2-3) in ng/g wet weight.

CONCLUSIONS

- With the established SID-GC/ICP-MS method low amounts of butyltin species in biota samples can be quantitatively determined with high precision
- For North Sea biota higher concentrations of butyltin species in eelpout as compared to blue mussel were detected in most years. For the Baltic Sea concentrations in blue mussel are mostly higher than in eelpout muscle tissue
- Concentrations of TBT are decreasing in all biota samples over the last years
  in blue mussel - decrease by about 60 - 80 % from 2004/2005 till 2008
  in eelpout - decrease by about 40 - 60 % from 2006 till 2008/2009
- Banning of antifouling coatings proved to be efficient as demonstrated by the decrease of the concentration of butyltin species at the German ESB sites in North and Baltic Seas
- TBT water concentrations estimated from measured tissue concentration are still in the range of the Water Framework Directive environmental quality standard derived for TBT. Thus adverse effects towards sensitive marine organisms cannot be excluded. However, levels in mussels are below the environment assessment criterion for TBT derived by OSPAR.

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