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Analysis of butyltin compounds in *Crassostrea gigas* and *Tapes philippinarum* on the korean coast

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한국연안의 참굴 및 바지락 속에 존재하는 부틸주석화합물의 분석

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요 약: IMO (International Maritime Organization)와 한국은 2003년을 기점으로 하여 TBT의 사용을 전면 금지 하였다. 이 금지를 통하여 몇몇 나라에서는 해양환경(바닷물, 퇴적물, 굴)에서 TBT의 오염도가 차츰 감소되고 있는 것을 확인 하였다. 본 연구에서는 2003년 규제 이후 TBT의 오염도를 확인 하기 위하여 *Crassostrea gigas*(굴) 및 *Tapes philippinarum*(바지락)을 선택 하여 부틸주석화합물(butyltin compounds)의 농도를 측정하였다. *Crassostrea gigas* 및 *Tapes philippinarum* 가 생산되는 농장 및 주요 TBT 오염이 예상되는 지점에서 시료를 직접 채취하여 농도를 측정하였다. 각각 측정된 농도는 *Crassostrea gigas* 의 경우 MBT, DBT와 TBT가 각각 N.D. -N.D., N.D. -N.D., N.D. -60.21 ng Sn g^{-1} (wet wt)으로 측정 되었다. *Tapes philippinarum*의 경우 MBT, DBT와 TBT가 N.D. -12.27, N.D. -45.95, N.D.-7.30 ng Sn g^{-1} (wet wt)로 각각 측정이 되었다. 본 연구에서는 TBT 오염도 감소를 확인 하기 위하여 1994년도에 측정된 동일 지역의 *Crassostrea gigas*의 부틸주석 화합물의 농도 대하여 비교조사를 해보았다. 조사결과 비교대상 7개 지점 샘플 중에 모든 샘플에서 TBT가 감소 추세를 보이는 것으로 나타났다. 이 중 4개 지점 샘플은 butyltin compounds가 검출한계 이하로 측정이 되었다. *Tapes philippinarum* 의 경우도 외국 연구자료와 비교하여 볼 때 높지 않은 것으로 나타났다.

Abstract: Tributyltin (TBT) use has been prohibited by the IMO (International Maritime Organization), as well as Korea, since 2003. This prohibition has affected a gradual reduction in TBT use in maritime environs (seawater, sediment, and biosamples) in several countries. In this study, *Crassostrea gigas* (oyster) and *Tapes philippinarum* (manila clam) were chosen and measured for butyltin compound concentrations to verify TBT pollution levels since its 2003 ban. Specimens were taken directly from farms for concentration measurement, where *Crassostrea gigas* and *Tapes philippinarum* are cultivated, as well as prevalent locations predicted to have been polluted. Concentrations of MBT, DBT, and TBT of *Crassostrea gigas* were measured to be N.D

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-N.D., N.D. -N.D., and N.D. -60.21 ng Sn g^{-1} (wet wt), respectively. Regarding *Tapes philippinarum*, MBT, DBT, and TBT were N.D. -12.27 ng Sn g^{-1} (wet wt), N.D. -45.95 ng Sn g^{-1} (wet wt), and N.D.-7.30 ng Sn g^{-1} (wet wt), respectively. For this research, a correlative study was conducted to determine butyltin compound concentration in *Crassostrea gigas* at the locations where measurements were made in 1994 to determine to what extent TBT pollution level decreased. A decrease in all 7 sites was observed. In 4 of the 7 samples, butyltin compounds were measured to be below the detection limit, while that of the *Tapes philippinarum* did not appear high compared to foreign research data.

Key words : Tributyltin(TBT); Dibutyltin(DBT); Monobutyltin(MBT); Crassostrea gigas, *Tapes philippinarum*

1. Introduction

Over the past few decades, TBT use has contributed to unfavorable effects in marine ecosystems, as well as many problems worldwide, such as intersex,[1] imposex,[2] and population reduction.[3] Since the discovery of imposex in France in 1970,[4] many researchers globally began inspecting the coasts of each country and discovered its occurrence.[5-7] Subsequent reports have stated that TBT can infiltrate the human body through polluted marine food or water and may pose a threat to marine ecosystems and human life.[8-10] Thus, the IMO prohibited use of TBT on all ships as of 2003.

Types of marine food that Koreans consume regularly and in large amounts are *Crassostrea gigas* (oyster) and *Tapes philippinarum* (manila clam). These two species were targeted for analysis and predicted decrease in TBT concentration since the ban in 2003 was enacted.

Many nations monitor the change in concentrations of butyltin compounds. Germany was the first developed country to ban TBT on ships smaller that 25 m in 1989 and subsequently analyzed several species of fish from 1994 to 2003 to observe the effect of the prohibition on TBT concentration. Fish sampled at Elbe, Prossen contained a TBT level of 68 ng Sn g^{-1} (wet wt) in 1994 that had decreased to 14 ng Sn g^{-1} (wet wt) in 2003.[11] China, a neighboring country of Korea, measured the total butyltin compounds (ΣBTs) in mollusks in the Bohai sea from 2002 to 2005 and observed no decreasing trends from 2002 to 2004; however, there was a decrease in 2005.[12] Decreases in the TBT pollution level in *Crassostrea gigas* suggests that human consumption of these is free from TBT.

Youn *et al*. (1994) in our laboratory collected *Crassostrea gigas* samples from 7 sites in Korea and presented research concerning pollution levels caused by butyltin compounds.[13] In this research, MBT (monobutyltin compound) concentrations ranged between 16.1 and 51.9 ng Sn g^{-1} (wet wt), DBT (dibutyltin compound) between 22.9 and 73.3 ng Sn g^{-1} (wet wt), and TBT (tributyltin compound) between 22.2 and 52.3 ng Sn g^{-1} (wet wt). From these measurements, the decrease in concentration was correlated with the prohibition of TBT use since 2003. Sonication,[14] microwave,[15] and shacking methods[13] are typical methods for extracting *Crassostrea gigas*. Herein, the microwave extraction method was employed in this research with GC-FPD analysis.

In the study, the target is verification of the distribution of butyltin compound concentration and pollution levels since the ban in 2003 for *Crassostrea gigas* and *Tapes philippinarum* on the coasts of Korea.

2. Experimental

2.1. *Crassostrea gigas* and *Tapes philippinarum* Sampling

Samples of *Crassostrea gigas* and *Tapes philippinarum* were collected at farms, where available, or sampled at natural locations in the event that farms were not available (*Table* 1). Locations of *Crassostrea gigas* where measurements were made in 1994 (7 sites) were referred to for oyster sampling locations

Table 1. Location of sampling points

	Location		*Crassostrea gigas*			*Tapes philippinarum*
	Latitude °N	Longitude °E	Kind	Latitude °N	Longitude °E	Kind
Incheon	37° 31' 58.99"	126° 31' 58.99"	W	37° 31' 27.82"	126° 34' 17.62"	W
Jebu	37° 10' 33.12"	126° 37' 31.8"	W	37° 31' 27.82"	126° 34' 17.62"	W
Seosan	36° 54' 42.98"	126° 20' 26.74"	C			
Taean	36° 50' 24.14"	126° 9' 55.48"	C	36° 44' 13.88"	126° 7' 53.3"	C
Muan	34° 59' 37.19"	126° 7' 52.48"	C			
Haenam	34° 29' 52.62"	128° 27' 21.32"	C			
Goheung	34° 26' 26.88"	127° 13' 10.2"	C			
Yeosu	34° 44' 59.49"	127° 39' 48.31"	C			
Suncheon	34° 49' 36.67"	127° 24' 39.97"	C	34° 50' 40.98"	127° 28' 11.88"	C
Gwangyang	34° 57' 3.23"	127° 43' 34.54"	C	34° 55' 44.96"	127° 41' 57.47"	W
Sacheon	34° 58' 4.56"	127° 59' 11.11"	C			
Gosung	34° 55' 31.16"	128° 18' 20.02"	C			
Tongyeong	34° 51' 28.49"	128° 33' 1.52"	C			
Masan	35° 6' 46.25"	128° 36' 3.55"	W			
Kunsan				35° 49' 6.22"	126° 24' 57.21"	W

Bold: Measured in 1994
C: Culture
W: Wildness

to verify the decrease in TBT pollution levels, and 8 with additional sites selected to determine the overall distribution on the Korean coasts. In reference to the manila clam, 6 Korean representative sampling sites were chosen. All samples were collected from 19th August 2006 to 25th August 2006 and stored in plastic bags at -20 °C.

2.2. Chemicals

HPLC grade methanol was obtained from J.T Baker. Dibutyltin dichloride (DBT, 96%) and tributyltin chloride (TBT, 96%) were purchased from Aldrich Chemical, Inc., triethyltin chloride (TET, 98%) from Merck, and monobutyltin trichloride (MBT, 95%) from Johnson Mattery Alfa Products. These standard materials were used without further purification. Methanolic stock solutions containing 1,000 ppm of tin were prepared monthly in a 25-mL volumetric flask and stored at 4 °C. Working solutions were prepared daily from this stock solution. Sodium tetraethylborate was obtained from Aldrich. Solutions were prepared daily by dissolving 0.2 g of the sample in 10 mL of deionized water. HPLC grade methanol and hexane were obtained from Fisher.

2.3. Extraction methods

The samples (*Crassostrea gigas* and *Tapes philippinarum*, 1.0 g wet weight), in 20 mL of concentrated acetic acid, and triethyltin chloride (TET, 100 ng/g) as the internal standard, were transferred to a Teflon container specific to a CEM microwave system (MARS5). Each sample was irradiated at 600 W and 100 °C for 4 min. The sample was then cooled to room temperature and centrifuged for 5 min at 4000 rpm. The upper transparent solution was then transferred to a 100-mL volumetric flask, whereupon 25 mL of aqueous NaOH (20 g/50 mL), 1.0 mL of sodium tetraethylborate, and 1.0 mL of *n*-hexane were added. After vigorous stirring of the solution for 1 h, the agitation was stopped and the organic phase transferred into a vial for injection into the GC-FPD.

2.4. Instrument Measurements

For the butyltin compounds, a Hewlett Packard 5890 II Gas Chromatography (GC) equipped with a split/splitless injector, fused silica capillary column (Ultra-1, 50 m, 0.32 mm, *i.d.*, film thickness 0.52 μm), and a flame photometry detector were used with a 610 nm cut off interference filter at 250 °C,

employing respective hydrogen and air flow rates of 45 and 35 psi/cm^2. The temperature of the injection port was set to 220 °C with helium (20 psi/cm^2) as the carrier gas, with a splitless mode for 90 s. The column temperature was programmed to remain at 60 °C for 2 min and heated to 300 °C at a ramp of 30 °C/min

3. Results and Discussion

3.1. Recovery rates and limits of detection

For recovery analysis, 500 ng Sn g^{-1} of the butyltin compounds were spiked in the samples. Replicate analyses of the spiked matrices (n=3) revealed adequate precision with good recovery and repeatability. The mean recoveries (RSD) of *Crassostrea gigas* were: 96.98±1.00% for MBT; 92.75±0.33% for DBT; 91.17±3.63% for TBT. The statistical detection limits (3 of blank) are also shown in *Table* 2. The respective detection limits for mono-, di-, and tributyltin were 5.80, 4.03, and 5.26 ng Sn g^{-1}. The mean recoveries (RSD) of *Tapes philippinarum* were: 112.27±5.89% for MBT; 102.87±4.89% for DBT; 83.53±4.80% for TBT. *Fig.* 1. contains the chromatogram of the *Crassostrea gigas* and *Tapes philippinarum* collected from Sacheon, Incheon and the standard solution, respectively. The retention times were: 10.200 min for the internal standard, TET; 11.827 min for MBT; 13.507 min for DBT; 15.120 min for TBT, respectively.

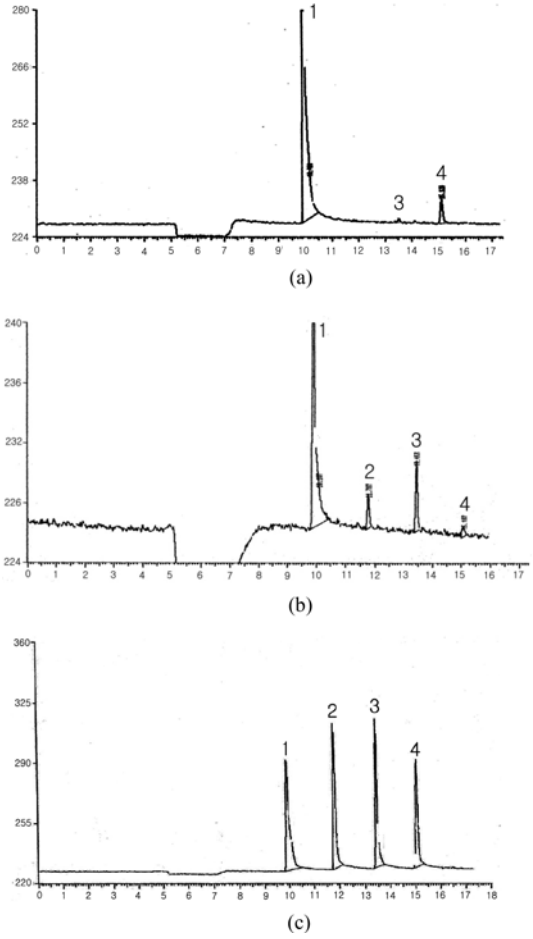

Fig. 1. GC-FPD chromatogram of samples (Sacheon (A), Incheon (B)) and standard (C) exhibiting the presence of: (1) TET (Internal standard, 100 ng Sn g^{-1}); (2) MBT (3) DBT (4) TBT

3.2. Concentration of butyltin compounds in *crassostrea gigas* and *tapes philippinarum*

Concentration of butyltin compounds in *Crassostrea gigas* and *Tapes philippinarum* are shown in *Table* 3 and *Table* 4. The investigation indicated that the *Crassostrea gigas* harvested at 7 sites among the 15 (Goheung, Taean, Suncheon, Muan, Yeosu, Gosung,

Table 2. Recovery and limit of detection of selected booster biocides from spiked *Crassostrea gigas* and *Tapes philippinarum* samples

Analyte	Butyltin compounds	Mean Recovery (%)	RSD% (n = 3)	LOD (ng Sn g^{-1})
Crassostrea gigas (500 ng Sn g^{-1})	MBT	96.98	1.00	5.80
	DBT	92.75	0.33	4.03
	TBT	91.17	3.63	5.26
Tapes philippinarum (500 ng Sn g^{-1})	MBT	112.27	5.89	5.25
	DBT	102.87	4.89	4.76
	TBT	83.53	4.80	5.75

Table 3. Summarized data on the concentration of butyltin compounds in *Crassostrea gigas* taken in Korea (ng Sn g^{-1} (wet wt))

	MBT	DBT	TBT	ΣBTs
Incheon	N.D.	N.D.	60.21	60.21
Jebu	N.D.	N.D.	13.88	13.88
Seosan	N.D.	N.D.	13.34	13.32
Taean	N.D.	N.D.	N.D.	N.D.
Muan	N.D.	N.D.	N.D.	N.D.
Jindo	N.D.	N.D.	39.72	39.72
Haenam	N.D.	N.D.	N.D.	N.D.
Goheung	N.D.	N.D.	N.D.	N.D.
Yeosu	N.D.	N.D.	N.D.	N.D.
Suncheon	N.D.	N.D.	N.D.	N.D.
Gwangyang	N.D.	N.D.	20.32	20.32
Sacheon	N.D.	N.D.	37.90	37.90
Gosung	N.D.	N.D.	N.D.	N.D.
Tongyeong	N.D.	N.D.	17.82	17.82
Masan	N.D.	N.D.	37.46	37.46

Table 4. Summarized data on the concentration of butyltin compounds in *Tapes philippinarum* taken in Korea (ng Sn g^{-1} (wet wt))

	MBT	DBT	TBT	ΣBTs
Incheon	14.73	30.47	7.30	52.50
Jebu	N.D.	5.94	N.D.	5.94
Taean	12.27	7.41	N.D.	19.68
Kunsan	6.30	45.95	N.D.	52.25
Suncheon	N.D.	9.98	7.23	17.21
Gwangyang	N.D.	N.D.	N.D.	N.D.

Haenam) contained butyltin compounds (MBT, DBT, TBT) below the detection limit (46.7%). At the other 8 sites, only TBT was detected, ranging from 13.88 to 60.21 ng Sn g^{-1} (wet wt). The measurement was highest in Incheon, 60.21 ng Sn g^{-1} (wet wt), a consequence of the international port that bears much marine traffic. MBT and DBT were below detection limits at all sites. As shown in *Fig.* 1, DBT was detected by chromatography, yet was below the detection limit, thus, MBT and DBT were distributed at low concentrations.

According to Ebdon *et al.*, the measured concentrations of DBT and MBT below the detection limit due to the varying concentration ratios of butyltin compounds in various types of shellfish were also affected by the time of year.[16] In several studies, the TBT composition ratio in *Crassostrea gigas* specimens was studied, yielding 44-82%, much higher than that of DBT or MBT. This is due to the fact that *Crassostrea gigas* has a lower metabolic capacity than other shellfish, thus containing more TBT in the body.[17,18] Researchers have reported that TBT concentrations were especially high when fat levels were elevated, which is assumed to be connected to the lipophilicity of TBT.[19] Therefore, the results of this research inform that many factors, including metabolic capacity and fat content of *Crassostrea gigas,* affect measurements below the detection limit, as well as that constant monitoring is required. *Tapes philippinarum* was measured at a total of 7 sites, and at 4 of these, its TBT concentration was below the detection limit. Butyltin compounds were detected at Incheon, Kunsan, and Suncheon. At Incheon, TBT was not detected, unlikely from *Crassostrea gigas.* MBT and DBT were measured to be 14.73 and 30.47 ng Sn g^{-1} (wet wt), respectively. At Kunsan, respective MBT and DBT levels were 45.95 and 6.30 ng Sn g^{-1} (wet wt), while in Suncheon, only 9.98 ng Sn g^{-1} (wet wt) of DBT was detected. These results appear to indicate that the ban on TBT use in 2003 was influential. According to the acceptable daily intake (ADI) for TBTO (tributyltin oxide) suggested in Japan, the level that humans can consume is 1.6 μg/kg by body weight/day (Harino *et al.*, 1998).[20] For a 60 kg adult, the acceptable intake is 96 μg, and based on this result, the TBT pollution of *Tapes philippinarum* does not appear to have a serious effect upon human intake.

3.3. Comparison of butyltin compounds in *Crassostrea Gigas* Measured in 1994 and 2006

Changes in butyltin compound concentrations measured in 1994 and 2006 are shown in *Fig.* 2. To observe the change in concentration since the 2003 TBT prohibition, *Crassostrea gigas* samples collected from the same sites as those in 1994 were examined.

As a result of the investigation in 2006, TBT concentrations showed a decreasing trend at all 7 measurement sites taken in 1994. All butyltin compounds (MBT, DBT, TBT) measured below the detection limit at Muan, Gosung, Yeosu, and Haenam,

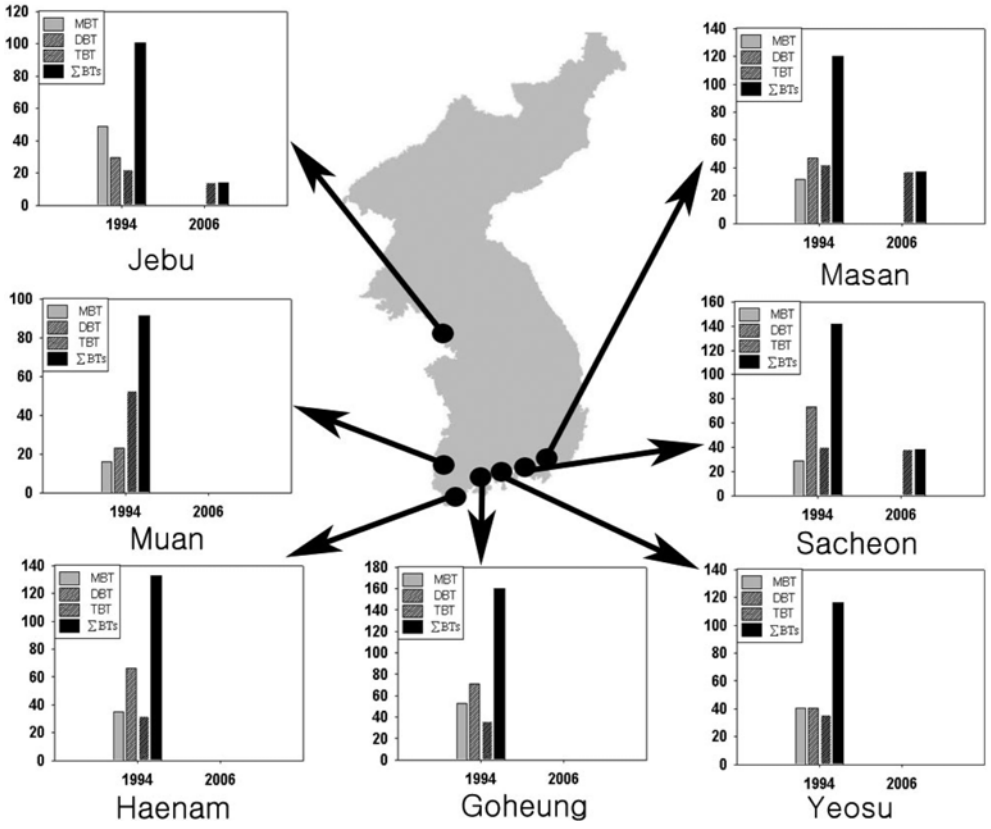

Fig. 2. Concentrations measured for butyltin compounds in *Crassostrea gigas* samples taken in 1994[13] and 2006.

thus the prohibition of 2003 had clear effects. At the remaining sites, Jebu, Suncheon, and Masan, among the total butyltin compounds, only TBT was detected. Regarding Jebu, the TBT concentration was 13.88 ng Sn g^{-1} (wet wt) in 2006, a decrease from 22.2 ng Sn g^{-1} (wet wt) in 1994. The same trend was also observed for Suncheon and Masan. The sum of MBT, DBT, and TBT (BTs) was 13.88 ng Sn g^{-1} (wet wt) at Jebu in 2006, down from 100.6 ng Sn g^{-1} (wet wt) in 1994. In Sacheon, the TBT concentration, 39.7 ng Sn g^{-1} (wet wt) in 1994, was 37.9 ng Sn g^{-1} (wet wt) in 2006, only a slight decrease, though BTs indicated an overall decreasing pattern from 141.7 ng Sn g^{-1} (wet wt) in 1994 to 39.7 ng Sn g^{-1} (wet wt) in 2006. TBT concentrations in Masan were 41.9 ng Sn g^{-1} (wet wt) in 1994 and 37.46 ng Sn g^{-1} (wet wt) in 2006, a slight drop, similar to Sacheon. However, BTs also decreased at this site, from 120.4 ng Sn g^{-1} (wet wt) in 1994 to 37.46 ng Sn g^{-1} (wet wt) in 2006. Considering that the decrease in BTs indicated a decrease in gross usage of butyltin compounds, the TBT restriction in 2003 was effective.

To grant statistical meaning to the decrease in concentration, a pair-sample t-test was conducted for TBT concentrations in *Crassostrea gigas* (1994 *versus* 2006) using SPSS 10. As a result, the significant possibility (P) was 0.016 ($P<0.05$), thus the TBT concentration has decreased since 2003.

3.4. Comparison with foreign research

The results from this experiment were compared with literature from investigations in foreign countries (*Table* 5). Data were investigated in two cases: one after 2003, a case for data after TBT use was completely banned by the IMO; the other prior to that. The data measured after 2003 indicated that the TBT concentration ranged from 5-287 ng Sn g^{-1} (wet wt) at the Otsuchi area in Japan[21] and N.D. -767 ng Sn g^{-1}

Table 5. Levels of MBT, DBT, and TBT in biosamples reported in the literature.

Case	Country	Location	MBT	DBT	TBT	ΣBTs	Analyts	Sampling site	Year	Ref.
1	China	Weiha (w.w.)	N.D.-4.9	N.D.-17.8	N.D.-19.8	0-40.1	mollusks	Large Seaports	2005	12
		Yangkou (w.w.)	N.D.-35.8	N.D.-78.5	N.D.-68.1	0-45.6	mollusks	Fishery Areas	2005	12
	Japan	Otsuchi (d.w.)	4.0-32	3-92	5.0-287	12-411	*Mytilus galloprovincialis*	Ship yard	2005	21
		Maizuru Bay (w.w.)	0.83-2.9	0.83-3.1	0.77-11	No data	*Mytilus galloprovincialis*	Industrial Area	2003	22
	Korea	West coast (d.w.)	No data	No data	N.D.-767	No data	Bivalve species	Harbor and Industrial Area	2005	23
	Spain	Estuary of river Eo (d.w.)	2.9-582.4	7.6-440.7	74.1-192.8	89.2-1215.9	Crassostrea gigas	Harbour and Ship Yard	2006	24
2	Twain	west coast (d.w.)	N.D.-N.D.	N.D.-N.D.	101.9-113	101.9-113	*Crassostrea gigas*	Mariculture	2000	25
	India	Dona Paula bay (d.w.)	No data	N.D.- 87.8	25.7-368.5	67.3-368.5	*Saccostea cucculata*	Mariculture	2000	26
		West coast (d.w.)	N.D.	45.0-88.0	124-732	169.0-732.0	*Crassostrea gigas*	Mariculture	2002	27
	USA	Oregon (w.w.)	N.D.-3.1	N.D.-N.D	N.D.-20.4	3.1-23.5	*Crassostrea gigas*	Harbour	1992-1994	28
	Portuguese	South Portuga (d.w.)	10.1	28.5	275.0	313.6	*Ruditapes decussatus*	Market	2003	29
	This Experiment	South West Coast	N.D.-N.D.	N.D.-N.D.	N.D.-60.21	N.D.-60.21	*Crassostrea gigas*	mariculture	2006	
	This Experiment	South West Coast	N.D.-12.27	N.D.-45.95	N.D.-7.30	N.D.-52.50	*Ruditapes decussatus*	mariculture	2006	

d.w.: dry weight
w.w.: wet weight

(wet wt) in Korea.[23] This is due to the fact that seashell samples were collected near ship yards, where it is thought that high concentrations of TBT on the surface of ships dissolve into seawater through sand-blasting processes. Conversely, the TBT concentration in mollusks collected at fishery areas in Yangkou, China, similar to the areas in this experiment, was measured to be N.D. -68.1 ng Sn g^{-1} (wet wt), not as high as the results at the ship yards. In particular, in this thesis, it is reported that TBT concentrations were lowered as a result of monitoring from 2001 to 2005.[12]

The pre-2003 TBT concentrations in biosamples from developed countries (USA) resulted from research aiming to determine the effect of improvement since the early ban (1995). The remaining areas indicated pollution levels at the time. In particular, Twain's *Crassostrea gigas* samples collected at mariculture were measured to have levels of MBT and DBT below the detection limit, but only TBT was measured between 101.9 and 113 ng Sn g^{-1} (wet wt), similar to the results of this research[25] and not greatly different from the concentrations measured in the USA.[28]

The results from the foreign research were similar to the samples collected at the farms, as well as related research. As shown in other research, the concentration continues to be high at ship yards, hence these areas may require more time to indicate the improvement since the TBT ban. In particular, since the prohibition in 2003, the IMO agreement did not choose to completely eliminate existing TBT on the surface of ships, but rather chose a sealer coat method, from whence TBT will continue to dissolve into the water. Thus, constant monitoring of butyltin compounds is suggested.[30]

4. Conclusions

As a result of measurement of the concentration of butyltin compounds in *Crassostrea gigas* (oyster) and *Tapes philippinarum* (manila clam) collected at main Korean sites, total butyltin compounds in *Crassostrea gigas* were measured below the detection limit at 7 of the 15 sites and TBT in *Tapes philippinarum* was below the detection limit at 4 of 6 sites. When *Crassostrea gigas* was compared with the results from 1994, all 7 investigated sites yielded a decreasing pattern of BTs and TBT concentrations. In reference to *Tapes philippinarum*, this measurement was not high compared to foreign results. Thus, as a result, the decrease in TBT in those two species was verified, but constant monitoring is still required.

References

1. J. S. Oehlmann, B. Liebe, E. Watermann, P. Stroben and U. Fioroni, *Hydrobiologia*, **378**, 199-213(1998).
2. A. C. Birchenough, N. Barnew, S. M. Evans, H. Hinz, I. Kronke and C. Moss, *Mar. Pollut. Bull*., **44**(6), 534-543 (2002).
3. C. Alzieu, *Mar. Environ. Res*., **32**, 7-17(1991).
4. S. J. M. Blaber, *Proc. Malac. Soc. Lond*., **39**, 231-233 (1970).
5. W. J. Shim, S. H. Kahng, S. H. Hong, N. S. Kim, S. K. Kim and J. H. Shim, *Mar. Environ. Res*., **49**(5), 435-451(2000).
6. D. V. Ellis and L. A. Pattisina, *Mar. Pollut. Bull*., **24**(5), 248-253(1990).
7. P. E. Gibbs, G. W. Pascoe and P. L. Burt, *J. Mar. Biol. Assoc. U.K*., **67**(3), 507-523(1987).
8. M. Azenha, and M. T. Vasconcelos, *J. Agr. Food. Chem*., **50**(9), 2713-2716(2002).
9. D. S. Forsyth and B. Jay, *Appl. Organomet. Chem*., **11** (7), 551-558 (1997).
10. L. C. Chien, T. C. Hung, K. Y. Choang, C. Y. Yeh, P. J. Meng, , M. J. Shieh and B. C. Han, *Sci. Toal. Environ*., **285**(3), 177-185(2007).
11. H. RÜdel, J. MÜller, J. Steinhanses and C. S. Kermani, *Chemosphere*, **66**(10), 1884-1894(2007).
12. R. Yang, D. Cao, Q. Zhou, Y. Wang and G. Jiang, *Environ. Int*., **34**(6), 804-810(2008).
13. C. H. Youn and D. S. Lee *J. of KSEE*., **17**(5), 421-430 (1995).
14. F. Pellizzato, E. Centanni, M. G. Marin and V. Moschino, *Sci. Total. Environ*., **332**, 89-100(2004).
15. M. Monperrus, R. C. Rodriguez, M. Doimeadios, J. Scancar, D. Amouroux and O. F. X. Donard, *Anal. Chem*., **75**(16), 4095-4102(2003).

16. L. Ebdon, K. Evans and S. Hill, *Sci. Total. Environ*., **83**, 63-83(1989).
17. R. F. Lee, A. O. Valkris and P. F. Seligman, *Environm. Sci. Technol*., **23**, 1515-1518(1989).
18. R. F. Lee, *Institute of Electrical and Electronics Engineers*, **4**, 1165-1170(1986).
19. S. N. Choi, H. K. Choi, H. Song, C. H. Oh, J. S. Park, *J. Fd. Hyg. Scfety*, **17**(3), 137-145(2002).
20. H. Harino, M. Fukushima, Y. Yamamoto, S. Kawai and N. Miyazaki, *Environmental pollutioin*., **101**, 209-214(1998).
21. H. Harino, Y. Yamamoto, S. Eguchi, S. Kawai, Y. Kurokawa, T. Arai, M. Ohji, H. Okamura, N. Miyazaki, *Arch. Environ. Contam. Toxicol.*, **52**, 179-188(2007).
22. M. Ohji, T. Arai, S. Midorikawa, H. Harino, R. Masuda and N. Miyazaki, *Water Air Soil Pollut*., **178**, 255-265 (2007).
23. M. K. Choi, H. G. Choi, H. B. Moon and G. Y. Kim, *Environ Monit Assess*., **151**, 301-310(2009).
24. M. Uveges, P. R. Gonzalez, J. I. G. Alonso, C. Medel and A. S. Fodor, *Microchem. J.*, **85**(1), 115-121(2007).
25. P. J. Meng, J. T. Wang, L. L. Liu, M. H.. Chen and T. C. Hung, *Sci. Total. Environ.*, **349**, 140-149(2005).
26. A. Garg, N.B. Bhosle Butyltin compounds in the oyster, saccostrea cucculata, from the west coast of India, Bull. Environ. Contam. Toxicol, **75**, 982-988(2005).
27. N. B. Bhosle, A. Garg, S. Jadhav, R. Harjee, S. S. Sawant, K. Venkat and A.C. Anil, *Chemosphere*, **57**(8), 897-907(2004).
28. K. Elgethun, C. Neumann and P. Blake, *Chemosphere*, **41**(7), 953-964(2000).
29. J. Diaz, R. Higuera-Ruiz, J. Elorza, A. Irabien and I. Ortiz, *Chemosphere*, **67**(3), 623-629 (2007).
30. J. S.Choi, *Haeyang susan Donghang*, **14**, 385-393(2001).