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Effect of organotin compounds (TBT) used in antifouling paints on cultured marine molluscs - a literature study

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Abstract

Organotin salts, used as antifouling substances have a deleterious effect on the environment, particularly on molluscs and especially on the Japanese oyster *Crassostrea gigas*. A synthesis of the effects of tributyltin (TBT) on cultured molluscs reveals the following effects: 1) acute and chronic toxicity to the adults; 2) accumulation in the flesh of the adults; 3) perturbation of reproduction particularly fertility, mortality of offspring, and decrease in larval growth rate; 4) decrease in the growth rate of juveniles and thickening of the shell; and 5) modification of the physiology at sublethal levels.

KEYWORDS: Organotin, Marine molluscs, Oyster, *Crassostrea gigas*.

Introduction

The first indications of the effects of tributyltin (TBT) on non-target molluscs were noted on Pacific oysters on the French Atlantic coast in the proximity of a large number of pleasure craft (Alzieu et al., 1981). The main effects noted were lack of spat and shell malformation of the adult oysters (Héral et al., 1981). Subsequent experimental work revealed TBT to be the causative agent for shell thickening (Alzieu et al., 1982; Alzieu and Héral, 1984), for direct mortality of larvae (Robert and His, 1981), or indirect via the phytoplankton (His et al., 1986). Since these observations, research on the effects of TBT on molluscs increased in several countries, since it appeared that the concentrations of this contaminant in seawater were quite high near harbors all over the world. It has been confirmed that TBT can cause reproductive failure and abnormalities in shell production in the Japanese oyster (Waldock and Thain, 1983). Since then, several authors have investigated the effects on other types of cultured molluscs such as mussels (*Mytilus edulis*), oysters (*Ostrea edulis*), clams (*Ruditapes decussatus*, *Ruditapes philippinarum*), and scallops (*Pecten maximus*). Antifouling paints containing TBT act on molluscs by causing different perturbations in function of the dose and the duration of exposure:

- acute and chronic toxicity to adults;
- accumulation of TBT;
- perturbation of reproduction (fertility, mortality of larvae, and decrease in larval growth rate);
- decrease of the growth rate of juveniles and thickening of the shell of adults;
- modifications of the physiology at sublethal levels.

The characteristics of these anomalies are analyzed for the different species with the data

available in the literature.

Acute toxicity

Only recently data have become available on the acute toxicity of organotin salts on adult molluscs, showing that tributyltins dissolved in water are highly toxic. Cardwell and Sheldon (1986) reported that lethal concentrations for zooplankton are as low as 0.4µg.l^{-1}. Although, more resistant than larvae older bivalves are still highly sensitive to the antifouling agent (LC50:300µg.l^{-1}) (Table I). This difference in sensitivity can be explained, at least partially, by the fact that postlarval bivalves can close their valves when exposed to the toxicant.

Chronic toxicity

Prolonged exposure to concentrations below 48-96h LC50 for molluscs can cause mortality due to bioconcentration of the pollutant. TBT is chronically toxic for adult marine bivalves at concentrations as low as 0.2µg.l^{-1} which cause 50% mortality after 180 days exposure (Table II).

Results in Table II show a quite large variability between the different experiments, which can be due to the characteristics of the tests (flow-through or static) with eventual shortage of food or oxygen deficiency. Moreover the experiments have been conducted at different temperatures, with molluscs of different age, and in various physiological conditions.

Accumulation of organotin

Bivalves can concentrate organotins to substantial levels. The bioaccumulation factor of TBT ranges from 2 000 to 11 000 (Table III). The differences in results between the experiments are most probably due to the different characteristics of the tests, particularly the temperature which is in direct relation with the assimilation, and the level of feeding. *Crassostrea gigas* accumulates TBT more readily than *Ostrea edulis*. Depuration experiments (Waldock et al., 1983) showed that for *C. gigas* half the body burden was lost after 10-23 days.

Perturbation of reproduction

In France, perturbations in the reproduction of the cupped oyster (*Crassostrea gigas*) have been observed in Arcachon Bay (Alzieu et al., 1981; His and Robert, 1983). In England, studies have been performed on the effect of TBT antifouling paints on the reproduction (Thain et al., 1986) before restocking of flat oysters (*Ostrea edulis*). These investigations showed that reproduction failure can be caused either by perturbations of the fertility of the breeders, by the spawning of abnormal larvae, by high levels of larval mortality, or by a decreased growth rate of the larvae.

Thain et al. (1986) showed, by histological examination of the gonad, a predominance of maleness at a concentration of 0.24µg.l^{-1} of tributyltin oxide (TBTO). This can be due to a retardation of sex change from male to female during the gametogenic cycle. The same phenomenon has been demonstrated for the dog-whelk, *Nucella lapillus* (Bryan et al., 1986; Gibbs and Bryan, 1986). At a higher concentration (2.6µg.l^{-1} of TBT) little or no gonadal differentiation occurred, indicating extreme retardation of development by TBT. These experiments suggest that in order to have a normal reproduction for *Ostrea edulis*, the level of TBT must be under 0.24µg.l^{-1}.

For *Crassostrea gigas* no such detailed work has been made. His and Robert (1987) obtained a normal reproduction with oysters cultivated on experimental trays with one side painted with TBT but the main contamination of the oysters occurred too long a time (11 months) before the reproduction.

Different toxic levels have been found for various stages in the larval development (His and Robert, 1980; Robert and His, 1981). All results are expressed in µg.l^{-1} TBT.

Crassostrea gigas

20:	inhibition of segmentation;
25:	partial reduction of segmentation;
10:	absence of formation of trochophores;
3-5:	absence of veligers, malformation of trochophores;
1:	abnormal veligers, malformation of trochophores;

Table I. Acute toxicity of organotin to some commercial bivalve species

Species	Compound	Toxic concentration ($\mu g.l^{-1}$)	Exposure time (days)	Mortality (%)	References
Crassostrea gigas	TBTO	1 800	2	50	Thain (1983)
		290	4	50	
	TBTO	120	4	50	Gendron (1985)
Crassostrea virginica	TBTO	560 - 1 000	2	50	MT Chemicals Inc. (1976)
Mytilus edulis	TBTO	300	2	50	Thain (1983)
		38	4	50	

Table II. Chronic toxicity of organotin to adult oysters

Species	Compound	Toxic concentration ($\mu g.l^{-1}$)	Exposure time (days)	Mortality (%)	References
Crassostrea gigas	TBTF	2-3 (estimations)	12-40	50	Alzieu et al. (1982)
		0.2	180	50	
	TBTF	5 (estimations)	5	50	Héral et al. (1983)
		0.5	25	50	
	TBTO	1.25	21	50	Waldock et al. (1983)
	TBTO	1.6	56	20	Thain and Waldock (1983)
	TBTO	1 (non-specific method)	15	50	Gendron (1985)
		2.5	25	50	
		0.5	41	50	
		0.25	48	50	
Ostrea edulis	TBTO	1.25	21	50	Waldock et al. (1983)
		2.6	45	50	Thain and Waldock (1985)

Table III. Accumulation of organotin in commercial bivalves

Species	Compound	Concentration levels ($\mu g.l^{-1}$)	Exposure time (days)	Bioaccumulation factor	References
Crassostrea gigas	TBTF	0.2-0.5 (estimations)	80	0.10--4 (total tin,$mg.kg^{-1}$) 8 000 to 20 000	Alzieu et al. (1982)
	TBTO	0.15-1.25	22	2 000 to 6 000	Waldock et al. (1983)
	TBTO	0.15	56	11 400	Waldock and Thain (1983)
	TBTO	0.15	-	10 000	Waldock and Miller (1983)
	TBTO	-	112	0.11--1.45 (total tin,$mg.kg^{-1}$) 0.01--0.87 TBT ($mg.kg^{-1}$)	Davies et al. (1986)
	TBT	-	30	0.10	Alzieu et al. (1986)
			1984	5.50 (total tin,$mg.kg^{-1}$)	
		-	30	0.10	
			1985	3.30 (total tin, $mg.kg^{-1}$)	
	TBTO	5 (estimations)	21	2 000 to 4 000	Gendron (1985)
Ostrea edulis	TBTO	0.15-1.25	22	1 000 to 1 500	Waldock et al. (1983)
	TBTO	0.15	-	2 000	Waldock and Miller (1983)
Pecten maximus	TBTO	-	112	0.12--2.5 (total tin,$mg.kg^{-1}$) 0.05--1.86 TBT ($mg.kg^{-1}$)	Davies et al. (1986)

0.5: numerous anomalies, total mortality in 8 days

0.2: perturbation in food assimilation, total mortality after 12 days;

0.1: normal D-shaped larvae slow growth, total mortality after 12 days;

0.05: slow growth, high mortality;

0.02: no observable effect.

Mytilus galloprovincialis

50: inhibition of segmentation;

25: partial reduction of segmentation;

10: absence of formation of trochophores;

3-5: malformation of trochophores;

1: abnormal veligers, total mortality in 6 days.

Larvae of the different species are substantially (= 100 times) more sensitive than the adults (Table IV). Below concentrations of 0.5μg.l^{-1} numerous abnormal larvae are found, between 0.5 and 0.1μg.l^{-1} perturbation of the food uptake is associated with a decrease in the growth rate. TBT can also, at very low concentrations, modify the quantity of phytoplankton (Walsh et al, 1985) and nanoplankton (His et al., 1986) causing indirect mortalities for the larvae by lack of food (His et al., 1986).

Shell thickening

For several years shell deformities have been observed in Pacific oysters (*Crassostrea gigas*) in some English and French bays. Key et al. (1976) suggested that a high level of fine particles in suspension might be responsible. Alzieu et al. (1981, 1982) and Alzieu and Héral (1984) put the thickening of the shell in relation with TBT salt leaching from antifouling paints on ship hulls. In tank experiments with tributylin fluoride (TBTF) antifouling paints, at an estimated concentration of 0.2μg.l^{-1}, these authors obtained the same pronounced thickening of the upper shell valves as in a harbour, with a large amount of proteinaceous gel between the shell layers. At a higher concentration (close to 2μg.l^{-1}) mortality was high but the oyster shell was normal. Waldock and Thain (1983) confirmed with *Crassostrea gigas* spat that at low concentrations (0.15μg.l^{-1} of TBT) oysters grew less well and showed thickening of the upper shell valve. High levels (1.6μg.l^{-1}) on the contrary, resulted in no growth and no shell thickening prior to mortality, which made the authors suggest that the no-effect level for *Crassostrea gigas* must be below 0.08μg.l^{-1}. Gendron (1985) obtained the same shell thickening at a concentration of 0.05μg.l^{-1} and Lawler and Aldrich (1988) demonstrated that thickening was obvious from 0.05 to 0.01μg.l^{-1}. Recently Stephenson et al (1986) found that the mussel (*Mytilus edulis* and *Mytilus californianus*) could also suffer from malformation of the shell. After the French law banned organotins in antifouling paints Alzieu et al. (1986) observed a decrease in shell anomalies in cultured oysters in evident relation to the decrease of contamination by these compounds. Presently Minchin et al. (1987) use shell distortion of *Crassostrea gigas* to monitor the presence of organotins in a number of Irish bays in relation to scallop populations. In the same way Stephenson et al. (1986) proposed that observations on growth and thickening of Japanese oysters and mussels can be used in routine monitoring programmes to study TBT effects.

Growth rate

The first observations on shell thickening (Héral et al., 1981) showed that the formation and development of chambers with overproduction of jelly in the shell of Japanese oysters occurred three times a year during summer, resulting in the wellknown stunted shape. These authors who followed the growth of the cultured oysters during 3 years, mainly found a thickening of the shell but no observable decrease in length growth. Likewise, in the bay of Arcachon, Alzieu et al. (1986) demonstrated remaining shell chambers but without growth decrease in *Crassostrea gigas*. On the contrary, Thain and Waldock (1983) found reduced growth for the Japanese oyster even at a concentration of 0.15μg.l^{-1} tributyltin oxide (TBTO). Thain (1986) reported a series of tests on spat of *Ostrea edulis*, *Crassostrea gigas*, *Mytilus edulis*, *Ruditapes decussatus*, and *Ruditapes*

Table IV. Acute toxicity of organotin to larvae of commercial bivales

Species	Compound	Toxic concentration ($\mu g.l^{-1}$)	Exposure time (days)	Mortality (%)	References
Crassostrea virginica	TBTO	0.9	2	50	MT Chemicals Inc. (1977)
Crassostrea gigas	TBTO	1.6	2	50	Thain (1983)
Mytilus edulis	TBTO	2.3	2	50	Thain (1983)
		10	5	100	Beaumont and Budd (1984)
		1	10	100	
		0.1	15	50	

philippinarum. At 2.6µg.l^{-1} high mortality occurred for all species except for *Ruditapes philippinarium* where 90% survival was found. At a concentration of 0.2µg.l^{-1} the Manila clam and the flat oyster presented no decrease in growth showing that these species are less sensitive to TBT. *Ruditapes decussatus*, *Mytilus edulis*, and *Crassostrea gigas* on the contrary, had a reduced growth rate. At 0.2µg.l^{-1} there was no decrease in growth of *Crassostea virginica* spat; growth was decreased only at 2µg.l^{-1}. No mortality of the oysters occurred and no evidence of any shell thickening appeared in either of the treatments. Maurer et al (1985) also found that in the natural environment, the proximity of ships with antifouling paints considerably reduced the settlement efficiency and caused growth inhibition in spat of *Crassostrea gigas*. Paul and Davies (1986) confirmed the detrimental effects of TBT on Japanese oysters with decrease in growth and thickened shells. In the same experiment the TBT paint caused reduced growth as well as mortality in scallop spat (*Pecten maximus*) but without affecting the adults. A recent paper (Lawler and Aldrich, 1987) deals with the influence of TBTO at estimated concentrations from 0.01 to 0.2µg.l^{-1}, on the growth of *C. gigas*. The authors fond a significant relationship between TBTO concentrations and reduction in size increment. Shell thickening was obviously high even at a concentration of 0.01µg.l^{-1} although there was no difference in size increase with the control. These results could explain the differences between the findings of Héral et al. (1981), Alzieu et al. (1986) and those of Waldock and Thain (1983) and Thain (1986). Indeed a very low concentration of TBT (0.01µg.l^{-1}) can occur which does not (or only slightly) affect growth, but which nevertheless induces formations of chambers with shell thickening. It seems that, except for the immediate vicinity of harbors, such concentration levels have been observed in the majority of French oyster ponds.

Effects on physiology

Few studies have investigated the effects of TBT at sublethal levels. The action of TBT on the metabolism can be followed by measuring the respiration. At an estimated concentration of 5µg.l^{-1} of TBTO, Héral et al. (1983) found an immediate decrease of respiration rate of 71% for *C. gigas*. At a concentration estimated at 2µg.l^{-1} of TBTO, respiration had decreased by 18%, 8 days after the beginning of the test, and further remained at this level. After 30 days the content in ATP had decreased by 30% and mortality occurred after 50 days. Testing lower concentrations (0.01 to 0.4µg.l^{-1}) of TBT on *C. gigas*, Lawler and Aldrich (1988) found significant differences in oxygen consumption. In the same test, these authors found a significant relationship between the TBT concentration and the decrease in feeding rate. It has also been demonstrated that a low TBT level (0.05µg.l^{-1}) decreased the weight of the flesh, particularly lipids, proteins, and glycogen (Gendron, 1985).

All these results (decrease in oxygen consumption, in ATP value, in glycogen, and increase in the thickening of the shell due to modification of calcification) indicate that the physiology of the mollusc is highly perturbed even at very low concentrations of the toxicant. We have suggested (Alzieu et al., 1982) that organotins modify shell deposition by inhibiting ATP synthesis (Simkiss, 1976) and reducing the respiration rate. These inhibitions in turn decrease the concentration of the Ca-ATP chelate which reacts with HCO_3. The latter decreases in relation with the respiration producing CO_2 necessary for the formation of $CaCO_3$. Furthermore Krampitz et al. (pers. commun., 1983) demonstrated that soluble proteins isolated from jelly of malformed oyster shells were deficient in aspartic acid, in serine, and glycine, which are the main amino acids of the organic matrix binding the calcium. These perturbations in amino-acids composition could be explained by the action of TBT on proteins, binding the chains and giving, with amino acids, tin compounds (Evans and Smith, 1976). TBT moreover inhibited amino-acid transport (Singh and Bragg, 1979). All these results contribute to explain the deficiency in calcification.

Conclusion

The following synthesis table can be put forward for the effect of TBT salts at different concentrations on the Japanese oyster (*C. gigas*). All figures are given in µg.l^{-1}:

1 800: acute toxicity for adults: 50% mortality in 2 days;

200: acute toxicity for adults: 50% mortality in 4 days;

50: inhibition of segmentation of the eggs;

10: no formation of trochophora larvae;

1-5: chronic toxicity for adults: 50% mortality in 10 to 50 days;

1 to 0.05: thickening of the shell, decrease in growth rate, feeding rate and respiration for adults; perturbation of food assimilation, slow growth, and mortality in the larvae;

0.02: no effect on larva;

0.01: thickening of the shell, jelly still remaining.

All these results demonstrate that TBT is a very dangerous pollutant for oyster cultures causing damage even at very low levels. *Ostrea edulis*, *C. virginica*, and the Manila clam *Ruditapes philippinarum* are less sensitive than the Japanese oyster. Presently regulations are applied in France, England, and Germany to protect the marine environment from the effects of organotins (Champ, 1986).

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