Testing methods to assess both the efficacy and ecotoxicity of antifouling coatings.

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Introduction

➢ Since 1998, the European Biocidal Products Directive (BPD; 98/8/EC), applied to 23 type products (TP) for specific uses, including TP21 the « antifouling products » (AF), regulates biocidal products before they are placed on the market. On one hand, the efficacy of active substances and their formulations must be demonstrated for their specific employment ; on the other hand their non-impact on workers neither users and environment must be proved and specifically against non-target species.

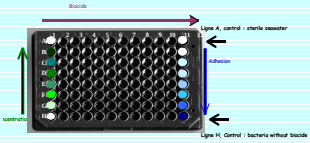
➢ The potential toxicity of actives substances as well as paints or their elutriates is assessed *in vitro* on six trophic levels following marine TGD (Technical Guide Document) recommendations (2003): bacteria, phytoplankton, crustacean, mollusc, echinoderm and fish. The potential impact of AF on the modification of the condition index of oysters (10 to 12 months exposure) is observed *in situ*.

➢ The efficacy of active substances and paints is also tested both *in vitro* and *in situ*.

Efficacy assessment

Laboratory studies

Laboratory tests (*in vitro* tests) are performed on the biocide substance **and** on simple formulations during short periods of time. Settlement bioassays are widely used tools for the screening of antifouling products and for preliminary screening of biocide. These assays include the settlement of bacteria, algae spores, diatoms, barnacles, bivalves... Of course, an untreated control must be included.
These data are generally not sufficient to predict effectiveness in service under real conditions of immersion in seawater but give information on the organisms adhesion inhibition.



A marine bacterial adhesion microplate test using the DAPI fluorescent dye: a new method to screen antifouling agents

(C. Leroy, C. Delbarre-Ladrat, J.-F. Ghillebaert, M.J Rochet, C.Compère and D. Combes Letters in Applied Microbiology, 2007)

In service monitoring or tests "patch on ship" or in service monitoring of aquaculture nets and cages

➢Dynamic immersion (representing a ship at sea) : the n/o Gwen Drez boat, as well as the fouling settlement on oyster cages exposed in tidal zones.

The n/o Gwen Drez boat

Patches on ship

Cages at the start of exposure and fouling 4 months later

Simulated field tests

➢ Tested coatings are painted on panels immersed in natural seawater on stationary rafts over one or several fouling seasons. A minimum of one year of exposure is recommended. The tested coupons should be replicated at least on three different panels on the structure to allow reliable analysis of data. The coupons are examined periodically by visual inspection for settlement of fouling organisms and for film integrity.

➢ In order to define the efficacy of an antifouling product, it is essential to introduce a reference coating of known performance (positive control) and a negative control (non-coated panel). The negative control is essential to obtain information on the fouling pressure in the test site.

➢Information concerning the physico-chemical parameters of the site test must be given (temperature, tides, depth of immersion, tropical, sub-tropical or temperate zone, salinity...).

➢ The efficacy assessment must include the identification of the type of fouling (biofilm, algae, barnacles, ascidians, tubeworms...). Efficacy results must be expressed as percent of coverage versus the references, or using a rating system to quantify the degree of fouling. A pass or fail criteria is necessary to define the relative efficacy level of the tested product against the references.

Test panels immersed in field test

Static seawater immersion (representing a ship in port)
Immersion at Ste Anne du Portzic (Brest) and Tahiti

The type of fouling will vary as a function of the geographic location of the test sites and the time of the year. Simulated field tests in different locations are strongly recommended.

Ecotoxicity assessment

➢ The assessment of the potential toxicity of a.s. (a.s. = active substance) or biocidal product (b.p. = paint elutriate) is perfomed ***in vitro*** using six marine trophic levels.

➢ The impact on adults oysters is evaluated following their growth and condition index and possible modification of shell density, when exposed 10 to 12 months in painted cages.

Bacteria the same technique used for efficacy evaluation with determination of the LC_{50} for a.s. and b.p.

Phytoplankton : the ISO 10253 norm is applied to determine the EC_{50} & NOEC on growth inhibition on *Skeletonema costatum* or *Phaedactylumtricornutum* after 72h exposition

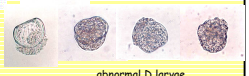
Crustacean : the LC_{50} is determined following the ISO 14669 norm, using the copepod *Acartia tonsa* exposed 48 hours.

Skeletonema costatum

Acartia tonsa

Mollusc : the exposition of embryonic development, using the Afnor XP T 90 382 norm, indicates the teratogenic effect with obtention of abnormal D larvae when exposed 24h at 24°C (*Crassostrea gigas*) or 48h at 20°C (*Mytilus edulis*)

normal D larvae



abnormal D larvae

Echinoderm : spermiotoxic or embryotoxicity effects could be determine on sea urchin (*Paracentrotus lividus* or *Sphærechinus granularis)*

fertilization membrane

P. lividus

Fish : the exposition of *Dicentrarchus labrax* juveniles 96 h permit to determine the LC_{50} following the Afnor T 90-305 Norm.

➢ The ecotoxicity assays permit to determine the PNEC (Predicted Non Effect Concentration) of the AF a. s. and b. p. which could be compared to the PEC (Predicted Environmental Concentration) obtained by the measured of lixiviation rate. The PEC/PNEC will give then the environmental risk characterization of the a.s. as well as the b.p. In order to protect the most sensitive species, in each specific ecosystem, the risk assessment could be evaluated using the safety factors proposed by the TGD (2003).

Conclusion

➢ Standard protocols for the assessment of efficacy and ecotoxicity of antifouling substances are proposed within the framework of the Ecopaint and Paintclean projects which are financed by the French Industry Ministry and labellised by the "Pôle Mer".

➢ The use of bioassays on the 6 trophic levels, will permit to characterize the environmental risk as well as the risk assessment of the active substances and the biocidal products.

➢ The proposed standard protocols including laboratory studies, simulated tests and "patch on ship" in different geographical locations gives a complete information on the efficacy of paints.

➢ The use of these two protocols should be applied when new active substances and paints are proposed as TP21 .

Assessment protocols applied on the two antifouling projects «Paintclean & Ecopaint» labellized by the French « Pôle mer »

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