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THE HYDROID HYDRACTINIA ECHINATA AS A SUITABLE BIOASSAY SPECIES FOR LOW-REACTING COMPOUNDS

G.T. HOUVENAGHEL

Laboratoire d'Océanographie Biologique,
Université Libre de Bruxelles
Avenue F.D. Roosevelt, 50
B-1050 Brussels, Belgium

ABSTRACT

A bioassay with the hydroid Hydractinia echinata was developed in order to assess the toxicity and environmental impact of low-reacting compounds like organosilicons or hydrocarbons. The hydroid's response to the toxicant was measured by the changes in its feeding rate. This technique is relatively easy to undertake, rapid, accurate, and not expensive.

KEYWORDS

Marine ecotoxicology, Methods, Sessile benthos, Hydrozoa, Hydractinia echinata, Behaviour, Feeding, Oils, Silicones.

INTRODUCTION

Among the cnidarians (or coelenterates), hydroids are typical members of the coastal benthic marine communities. Their use as bioassay test-organisms was recommended by Karbe (1972) because of their high sensitivity and ease of cultivation in vitro. Further developments in hydroid bioassays mainly by Stebbing since 1976, are reviewed by this author in these

proceedings (Stebbing and Brown, 1984). The hydroid's responses to pollutants used in these tests are related to the growth of the polyp or the colony and therefore imply long-term experiments lasting up to many weeks.

In addition to the advantages with regard to their cultivation (clones) and sensitivity, hydroids have, like other cnidarians, particular histo- and ecophysiological properties which may be used in toxicity tests. Primitive organisms like cnidarians are diploblastic. Their organisation in two cell layers involves less than ten different cellular types. The nervous system is also very elementary ; sensory cells and epithelial conducting cells are connected with one another and form a nonpolarized nervous net. Therefore cnidarians do exhibit fewer and simpler behavioural and physiological functions than animals of higher phyla in which, owing to their body structure composed of more intricated tissues and organs, and their complex nervous and endocrinological systems, the physiological mechanism is numerous, interconnected, and rather complicated.

The polyp-type of organisation (like Hydra, hydroids' polyps, sea anemones, corals, etc.) is a sessile life form and the organism's behaviour is entirely concerned with its feeding activity. Observations on the feeding behaviour in cnidarians are restricted to the last century. Laboratory experiments on the feeding reaction of hydroids were started by Loomis (1955) with the freshwater polyp Hydra littoralis. Later many marine species were studied as well. Reviews of this subject are given by Lenhoff (1968), Haynes (1973), and Houvenaghel (1974). The typical food of hydroid polyps consists of zooplankton and small animals crawling over the polyps. When waiting for food the tentacles of the polyp are fully extended. If a prey hits one of the tentacles, a triggering mechanism releases a set of nematocysts hooking the foreign body with their threads and spiny shafts. In addition some types of nematocysts inject their poisonous content immediately after discharge. Thereafter, a feeding reaction occurs consisting of following steps :

1. the tentacles contract, bringing the prey towards the mouth ;
2. the mouth opens ;
3. the prey is ingested.

As soon as the prey is taken in in the gastrovascular cavity, the digestion starts. More than one prey can be treated simultaneously. As first suggested by Parker (1896), and later experimented by Loomis (1955) and others, the stimulating mechanism that elicit the feeding reaction of the

hydroid (steps 1 to 3) is the prey's body fluid leaching into the medium after transfixion of the body wall by the nematocysts. These researchers obtained typical feeding reactions in the absence of a prey and without nematocyst activity, but only by adding some body fluid of the prey to the medium or some particular chemicals as e.g reduced glutathione or its analogs, S-methyl glutathione, proline, pipecolic acid, valine, leucine, etc.. The intensity of the obtained reaction differed from one species to another and from one chemical to another. However, in many cases, feeding reactions were noticed at concentrations as low as 10^{-5} to 10^{-6}M.

The feeding reaction and behaviour is a sequential series of actions starting with a chemical stimulation by the prey which is quickly moved to the wide opening of the mouth allowing the engulfment of the food in the gastrovascular cavity within the shortest time possible. This sequence corresponds to the scheme proposed by Beck (1965) and Lindstedt (1971) describing all types of stimuli, positive and negative, affecting the hydroid's feeding reaction (Table I).

Table I. Sequential scheme of the positive and negative stimuli affecting the hydroid's feeding reaction (after Beck, 1965 and Lindstedt, 1971)

Action	Evoking stimulus	
	Positive	Negative
Orientation of the tentacles	Attractant	Repellent
	Arrestant	Repellent
Initiation of the feeding reaction	Incitant	Suppressant
Continuation of the feeding reaction	Stimulant	Deterrent
Termination of the feeding reaction		

Further feeding steps include the extracellular digestion of the food inside of the gastrovascular cavity, followed by pinocytosis and phagocytosis, and intracellular digestion by the gastrodermic cells. These steps have been described in detail for various hydroid species by Semal-Van Gansen (1954), Van de Vyver (1961), Houvenaghel (1966), Bouillon and Houvenaghel (1970), and Marfenin (1981).

Foreign bodies and chemicals present in the medium may interact with the feeding reaction by acting as one or more positive or negative stimuli. As a general rule, well-ionized chemicals with low molecular weight do act as arrestant or repellent. They induce the contraction of all tentacles and gastric column. This escape mechanism is not followed by a feeding activity even if food is present and the polyps are starved. Much less pronounced reactions are obtained when high molecular compounds, not or slightly ionized, are added to the medium. These observations actuated us to study the applicability of such feeding disfunction effects as a bioassay tool to test chronic and acute effects of pollutants at sublethal levels. The present paper describes the proposed bioassay and gives one example of application for testing a low-reacting compound.

TEST SPECIES

Almost all hydroids present similar feeding patterns only differing in the prey preference according to the shape and size of the polyps, and to some extent in the feeding rate. Therefore many hydroid species are suitable bioassay test organisms. The choice must, however, be restricted for obvious reasons, to species easy to collect, or to colonial species easy to cultivate. In addition, the advantages of cultivating clones in order to exclude genetic variability were pointed out by Stebbing (1976).

Instead of selecting a colonial thecate hydroid, which possesses small hydranths forming usually dense branched colonies, we retained an athecate (= gymnoblastic) hydroid Hydractinia echinata (Fleming, 1828) as test organism. Feeding behaviour of this species was studied by Christensen (1967) and this author reported that a great variety of small benthic or planktonic animals were actively catched as prey. This absence of selectivity permitted to use this species for experiments with different artificial foods. Hydractinia echinata was chosen after many trials with other rather large athecate hydroids currently kept in our laboratory, such as the marine species Clava squamata, the brackish water species

Cordylophora caspia, or the freshwater Hydra. The major argument in favour of the use of H. echinata in bioassays is its ease to handle and to observe. The polyps are large and displayed in the colony one next to the other. Moreover, the pattern of the colony creeping on a rather flat substrate, allows observation of large parts of the colony under dissecting binocular microscope without moving the field or focussing continuously.

H. echinata is a Bougainvillidae forming polymorphic reptant colonies covering the substrate with hydrorhiza bearing spines, fertile blastostyles bearing dense groups of nematocysts at the distal end and a little lower, three to eight gonophores. The feeding polyps, with a crown of 15 - 20 tentacles around the mouth, reach a height of 5 - 6 mm when stretched out. According to the size of the substrate (shell), the colony may reach many cm^2 consisting of hundreds of polyps. This species is very common and widely distributed in the temperate, boreal, and arctic neritic waters of the North Atlantic, the Arctic, and the Far Eastern Pacific ocean. The substrates on which they settle are usually shells, mainly gastropods, live or dead, and often those inhabited by pagurid crabs (e.g. Buccinum, Natica, Nassarius, Lora, Turritella, Littorina, Trophon) (Naumov, 1960 ; Vervoort, 1964). They are found on a variety of bottoms. Collection is easy and may be done by hand at the low water tide-level or by diving or dredging in the subtidal zones.

The laboratory tests may be undertaken with batches of Hydractinia colonies freshly collected and maintained for a short time in static or flow-through aquaria for the standardisation of the trophic state. Batches of Hydractinia may also be obtained by in vitro cultivation. This practice, similar to the techniques used for other hydroids, has been used earlier for other experimental purposes (Hauenschild, 1954 ; Christensen, 1967). The development of the hydrorhiza on microscopic slides gives rise to very flat colonies easy to observe and to handle.

THE FEEDING REACTION IN HYDRACTINIA ECHINATA

The feeding response of H. echinata to the presence of live preys (like Calanus or Artemia nauplii) has been described by Christensen (1967). Specimens that are not feeding have tentacles fully outstretched and the whole hydranth appears relaxed. On contact with the prey, the hydranth contracts ; immediately the tentacles extend while bending and swaying. When the prey does touch a tentacle, a number of nematocysts are devaginated

and pierce the prey's body. The mouth, which is closed in the non-feeding state and has a conical shape, now elongates, bending slowly while extending towards the prey. The mouth opens progressively to such an extend that the prey can be engulfed by slow gliding. The whole phenomenon lasts 20 to 30 min.

A similar reaction is elicited when non-living natural food or some artificial materials are presented to the polyp. Among the materials tested are : detritus from the seston (exuvia of crustaceans, dead copepods), particles of cotton fibres, wood, filter paper, particles of neutral red, etc. (Houvenaghel, 1966). The ability of the polyps to ingest easily many types of materials led to some experiments in which low-reacting compounds like silicones were offered during feeding in order to assess the eventual positive or negative chemical stimuli given.

As described in the literature for many other hydroids, the feeding reaction in H. echinata was also elicited when plankton extract or body fluid (obtained by crushing animals in a mortar) was administered to the test animals. This property was used for selecting a "natural" stimulant as standard stimulating agent in our feeding experiments. When artificial stimulation (glutathione, proline, etc.) was used the first steps of the feeding reaction were induced (orientation = movements of the tentacles ; and initiation of the feeding = opening of the mouth). However, the reaction was stopped at this stage, and the hydranths remained with open mouth for a long time. When natural stimulation was used, the feeding reaction was complete, with termination of the feeding when ingestion was finished. This satiation behaviour was induced by the filling of the gastrovascular cavity, as described for Hydra by Burnett et al. (1960), which was not obtained in the case of a chemical stimulation due to the absence of food particles.

DESIGN OF A FEEDING-REACTION BIOASSAY WITH HYDRACTINIA ECHINATA

MATERIALS AND METHODS

All experiments were carried out at the Biological Station of Roscoff on the English Channel coast of Brittany (France).

H. echinata colonies, growing on gastropod shells inhabited by pagurid crabs, were dredged on shallow soft bottoms in the vicinity of Roscoff. The shells were broken and the pieces carrying 50 to 150 well-developed feeding polyps were placed in an aquarium with running filtered seawater. The bioassay experiments were conducted in small dishes allowing the simultaneous observation of the entire colony under dissecting microscope. Before starting the experiment the dishes were placed in a water bath at a temperature ranging from 14.5 to 15 °C. Prior to use the test solutions were incubated in the same bath in order to avoid any thermic stimulation.

The experiments were performed as follows : a few min before starting the experiment, the dishes were placed individually under the microscope and emptied until only a small and known amount of water remained covering the polyps ; while doing this, the observation through the microscope permitted to check if the polyps remained decontracted ; test solutions were prepared with membrane-filtered seawater at the appropriate concentration to obtain the desired concentration when added to the remaining water in the dish.

During the bioassay, the behaviour of the polyps was observed and the movements registered continuously during 1 h and with regular time intervals 1afterwards if necessary. From all the movements displayed by the different parts of the body (mouth, tentacles, body column) the opening of the mouth was chosen in this study as a very characteristic indicator of the feeding reaction. This criterion was also taken by Loomis (1955) for his tests with Hydra and by Fulton (1963) for marine hydroids. The behaviour was monitored by means of a video recorder. From these data, graphs were drawn showing the chronological evolution of the behaviour. In addition other information was gathered as well, such as the filling of the gastrovascular cavity indicating the result and achievement of the feeding activity.

STANDARD STIMULATING AGENT

From preliminary experiments it was concluded that preys like copepods or Artemia nauplii are unsuitable natural stimulating agents, because such voluminous food material released irregular amounts of body fluid, partly depending on the number of nematocyst punctures. The exact amount of evoking agent could therefore not be determined and the responses to the stimulus were irregular in intensity. Therefore, preference was given to a liquid natural material as stimulating agent such as e.g. homogenized mussel tissue (Mytilus edulis). Whole mussels were ground with a warring blender for 2

min, and the homogenate diluted with freshly filtered seawater (0,45 µm) in order to obtain final concentrations of 0.005 %, 0.025 %, 0.25 % and 2.5 % (v : v).

STANDARD FEEDING REACTION RESPONSES

For each concentration of mussel homogenate the feeding reaction of the polyps was recorded for 30 s after contact. The number of hydranths reacting and the intensity of this reaction was related to the concentration applied. At the lowest concentrations (0.005 and 0.025 %) respectively only 5 and 13% of the polyps in the samples reacted. The others did not show any feeding reaction at all, or at least no typical movements were observed (contraction and extension of the column and tentacles ; opening of the mouth). At these low concentrations of stimulating agent, the polyps returned rapidly to a characteristic non-feeding state with full extension of body column and tentacles.

In the test with 0.05 % homogenate the entire population of hydranths reacted. The mouth opening did not last for a very long time and the hydranths returned progressively within a few min to a "normal" state, which was reached for the entire sample 19 min after starting the experiment (Fig. 2A).

At higher concentrations (0.25 and 2.5 %) a total reaction was obtained as well. Moreover, the mouth remained open for a longer time according to the concentration of the homogenate (Fig. 1). During these experiments ingestion of some food material was also noted giving rise to the swallowing of the gastrovascular cavity, and after a while, the endodermic cells were filled with digestive material. The ingestion of mussel material occurred within 2 min after contact when concentrations of 0.25 and 0.5 % were used. In the case of 2.50 % homogenate ingestion was practically immediate.

From this series of experiments the following conclusions were drawn :

- The feeding reaction behaviour of the polyps is started immediately after the contact with the stimulating material.
- The feeding reaction is exhibited by all the polyps in the sample when exposed to stimulating material at concentrations above a threshold level ; for mussel homogenate this threshold concentration lays between 0.025 and 0.050 %.
- The feeding reaction is more intense when higher concentrations of

stimulating material are applied.

- The feeding reaction is followed by effective ingestion and digestion of the material present.
- The ingestion, followed by digestion processes, prevents the hydranth to return immediately to a "normal" state when a new feeding reaction is possible. This return occurs after about 24 h.

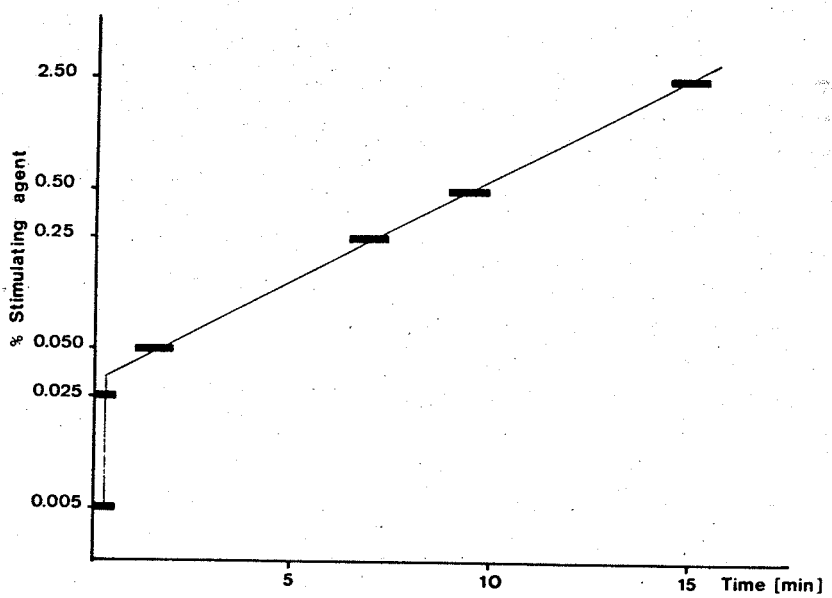

Fig. 1. Duration of the feeding reaction of Hydractinia echinata (time during which the mouth remained open in 100 % of the hydranths) in relation to the concentration of stimulating agent (SA) expressed as percentage (v : v) of mussel body fluid in seawater.

The bioassay procedure described in this paper concerns exclusively the feeding reaction displayed by the hydranths and not the digestion. In order to avoid any misleading effect or interaction when feeding reaction is accompanied by ingestion, the stimulation was limited to a concentration of 0.05 % of homogenate. At this level 100 % response (mouth opening) was obtained, and the hydranths returned to a "normal" watch state within 20 min. Fig. 2A shows the chronological evolution of this standard feeding reaction.

PRELIMINARY RESULTS WITH THE FEEDING REACTION BIOASSAY

During the non-feeding watch state, when the body and tentacles are fully outstretched, the polyps exhibit a very sensitive reaction elicited by a wide variety of substances in the medium. With polar and ionic chemicals, strong and long lasting contraction of the tentacles and the gastric column was observed. With organic substances like sugars and amino acids, feeding reactions were induced at relatively low concentrations. Organic compounds with low reactivity, which an hydrophobic, viscous, and of high molecular weight (e.g. hydrocarbons, oils, including crude oils or derivatives, silicones) were assumed to induce the same type of reaction or to interact with the normally evoked course of feeding reaction, and therefore were tested in a series of bioassays.

The feeding reaction bioassays were conducted in the following way : in one series of colonies, the test substance was applied together with the feeding reaction stimulating agent (0.05 % mussel homogenate) ; in a second series, the application of the test substance was done in the absence of any simultaneous or preceeding feeding reaction stimulation ; a third series, in which only mussel homogenate was added, was taken as a control. In each case, following parameters were recorded in function of time : the number of hydranths with open mouth, the number of hydranths with contracting tentacles, the number of hydrants with contracting body column. From these observations were deduced :

a. the time necessary to obtain the first hydranth with open mouth ;
b. the maximal number of hydranths displaying an open mouth ;
c. the time necessary to reach the maximal number of open mouths in the colony ;
d. the maximal number of hydranths with a column returning from a contracted state to a normal watch state ;
e. the time necessary to obtain the maximal return observed in d ;

f. the maximal number of hydranths with tentacles returning to an outstretched position ;
g. the time necessary to obtain the maximal return observed in f.

When the hydranths did not return rapidly to the non-feeding state, and remained contracted or blocked in one feeding state or another, the observation was continued during the next hours, up to at least 24 h which is the maximal average time required to digest food during normal feeding. This prolonged observation also included the recording of the number of hydranths with a mouth remaining open, and the number of hydranths with a body column or tentacles which remained contracted.

The typical response obtained in these bioassays is illustrated in Fig. 2 for a test with an organosilicon at a concentration of 100 ppm. In this example, it appears that hydranths' responses were induced both with and without the stimulating mussel homogenate. The response, however, was never total, and the maximal number of hydranths with open mouth was noted with some delay. Afterwards not all the hydranths returned to the normal state, even many hours after the start of the test.

Similar results could be obtained with other organosilicon compounds with different viscosities and with mixtures of low-reacting organic chemicals such as e.g. hydrocarbons.

The exact mode of action of a pollutant may be multiple and complex. Several mechanisms may interact at the level of initiating the hydroid feeding reaction : a mimetic chemical action eliciting the feeding reaction, an inhibition of the receptor mechanisms, or a negative stimulating action (repellent and suppressant). The irreversible state, when any return to the normal non-feeding state is prevented even after a long delay, may result from an inhibition of the satiety or termination of the feeding reaction, and/or from a narcotic effect on the nervous system.

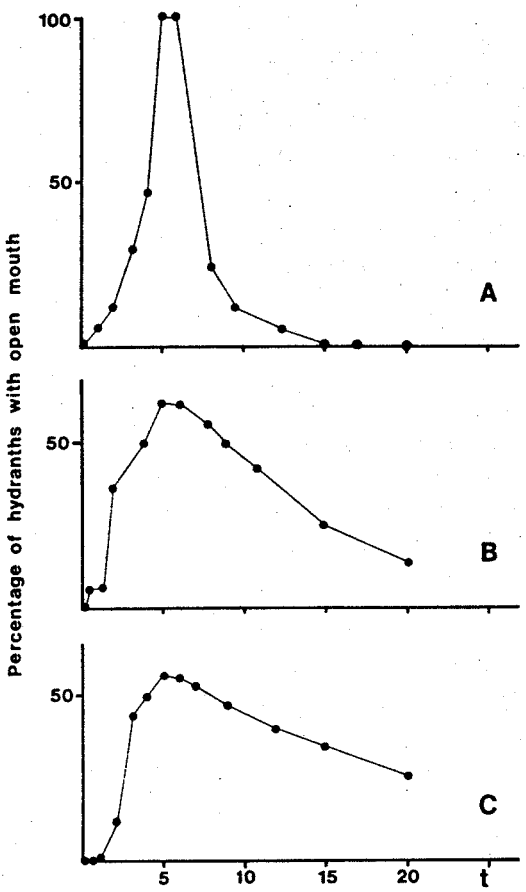

Fig. 2. The number of hydranths of Hydractinia echinata (expressed as %) exhibiting a feeding reaction (open mouth) in function of time :

A : after application of 0.05 % stimulating agent ;

B : after application of 0.05 % stimulating agent together with an organosilicon compound ;

C : after application of the organosilicon alone.

DISCUSSION AND CONCLUSIONS

The response of the hydroid H. echinata to pollutants was measured by changes in its feeding reaction steps, especially regarding the chronology, the duration, and the delay in returning to the initial non-feeding watch state. Generally, the effects measured in this study can be defined as stress symptoms according to the current definition of stress since "a physiological process is altered in such way as to render the individual (or the colony) less fit for survival" (Brett, 1958).

Stress results in a change of the rate of physiological processes. In the present bioassay this change could be measured easily and accurately which is according to Bayne (1980) a prerequisite for effective toxicity monitoring. With increasing stress the response of the hydroid progressively changed from normal feeding reaction to altered feeding behaviour shown by an increasing number of hydranths in the colony, at first in a reversible way, but later reaching an irreversible phase leading to death. The lowest stress induced the lowest response. These findings validate the use of the present bioassay for tracing any sublethal chronic or acute effect of a pollutant in the marine environment. This is particularly interesting for ecotoxicological hazard assessment since data at this level are more difficult to collect than immediate lethality. In addition to the assessment of the dose-response relationship at the sublethal level, this bioassay gives a very short-term answer thanks to the rapid response time of the test organisms (from min to a few hours). Therefore, this bioassay is a useful tool not only for general surveys and ranking of chemicals or mixtures, but also for hot-spot investigations in which an immediate answer is required.

There are a few more arguments in favour of the use of the present bioassay in applied ecotoxicology. The physiological function studied in this test is feeding which is one of the fundamental physiological and behavioural parts of an animal's life. In the present case it concerns the carnivorous feeding strategy of a typical benthic organism, based on a chemoreceptive process inducing the catch of the prey. This is totally different from most data published regarding toxic effects on the feeding behaviour of aquatic organisms which mostly deal with molluscs (bivalves) or crustaceans (copepods), both obligate filter feeders (Bayne et al., 1980). Consequently, the hydroid feeding bioassay may be considered very useful for

assessing the impact of a pollutant upon a carnivorous feeding mechanism associated with chemoreception which is a widespread trophic mechanism in the aquatic ecosystem.

Similar hydroid feeding bioassays could be developed for ecological studies in brackish or freshwater environments. Cordylophora caspia and Hydra are suitable test material for this purpose because of their similar feeding mechanisms and ease to handle comparable to that of Hydractinia. Moreover, since their hydromedusae do exhibit the same feeding behaviour as the polyps (Houvenaghel, 1966 ; Bouillon and Houvenaghel, 1970), this feeding reaction bioassay could be extented to the pelagic environment as well.

In addition to the physiological and ecological arguments in favour of the hydroid feeding reaction bioassay, there are some methodological advantages to point out :

- there are no complicated endogenous nervous or endocrinological interactions with the feeding reaction of the test animals ;
- work with an entire colony implies the same genotype and phenotype for all the polyps and an identical metabolic state ;
- smooth handling during collection ;
- easy transportation from the sea to the laboratory ;
- small size requiring only limited laboratory space ;
- limited culture and maintenance requirements ;
- easy to meet standard test conditions ;
- suitable for large series of experiments ;
- rapid response after contact with the toxicant ;
- allowing the assessment of a dose-response relationship ;
- rapid setup, when prompt hot-spot investigations are requested ;
- possibilities for record automation (time-lapse video monitoring).

In conclusion it can be said that the present hydroid feeding bioassay is easy to carry out and economical to run. It concerns a basic life function in the aquatic ecosystem (carnivorous feeding) and is fully suitable for chronic or acute ecotoxicological tests in the field or in the laboratory, in order to assess the impact of pollutants, and especially of low-reacting compounds, on the marine environment.

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