Helgoländer wiss. Meeresunters. 20, 655–675 (1970)

The importance of avoiding chemical contamination for a successful cultivation of marine organisms*

M. BERNHARD and A. ZATTERA

Laboratorio per lo studio della contaminazione radioattiva del mare, CNEN-Euratom; Fiascherino, La Spezia, Italy

KURZFASSUNG: **Die Bedeutung der Vermeidung chemischer Kontamination für eine erfolgreiche Kultivierung mariner Organismen.** Etwa 80 chemisch verschiedene Substanzen und Materialien wurden hinsichtlich einer möglichen toxischen Wirkung an mehreren Phytoplanktonarten, dem Copepoden *Euterpina acutifrons* und den Larven des Seeigels *Arbacia lixula* geprüft. Es zeigte sich, daß die Phytoplankter empfindlichere Testorganismen sind als *Euterpina acutifrons* und die Larven von *Arbacia lixula*. Auch konnte zwischen den verschiedenen Phytoplanktonarten erhebliche Unterschiede in der Reaktion festgestellt werden. Detergentien erwiesen sich nur in relativ hohen Konzentrationen als toxisch. Viele Substanzen, die eine mannigfache Anwendung im Laboratorium finden, wie zum Beispiel roter und schwarzer Gummi oder Polyvinylchlorid, sind ausgesprochen toxisch und sollten nicht für Geräte benutzt werden, die zur Probeentnahme lebender Organismen dienen. Einige Substanzen, die sich zunächst als nicht toxisch erwiesen hatten, wurden toxisch, nachdem sie bei 0,5 atm 20 min in dem Kulturmedium sterilisiert wurden. Es konnte jedoch nachgewiesen werden, daß Polyäthylene (naturfarben), Gabraster (mit Katalysatoren), Perspex, Silicongummi, Algoflon (Teflon), Tygon, Silikon SE 1201 und Kautschuk (für Stöpsel) auch nach dem Sterilisieren optimales Wachstum der Testorganismen zulassen. Da aber Firmen oft die Zusammensetzung ihrer Materialien ändern, müssen die einzelnen Lieferungen immer wieder auf Toxizität geprüft werden. Nur so kann verhindert werden, daß Wasserschöpfer, Planktonnetze und andere Geräte, die zur Probenentnahme von lebenden Organismen benutzt werden, die Organismen nicht bereits negativ beeinflussen, bevor das eigentliche Experiment angesetzt worden ist. Das gleiche gilt natürlich auch für Materialien, die zur Konstruktion der Kulturgefäße und -apparate benutzt werden.

INTRODUCTION

In the past, great efforts have been made to design suitable culture media, isolation techniques and elaborate culture conditions, but often the possibility that the organisms may be poisoned prior to the start of the actual experiment has not been taken into consideration. In this paper all materials are religiously tested which come into contact with marine organisms from the moment of collection to actual experimentation. The results show that, on many occasions, the outcome of the experiments is seriously impaired by the toxicity of materials used during sampling, storage and experimentation.

* Contribution under "Association Contract CNEN-EURATOM".

Table 1

Details of the products tested as provided by the supplying firms

Denomination of materials	Commercial names	Firms	Raw materials	Additions	Use
Algoflon	Algoflon	Montedison Milano	Poly-tetra-fluorethylene	No dyes	Laboratory items, tubes, construction material
Araldit	Araldit	CIBA – Milano	Constituents CY 208, CY 248, HY 965 and HY 966		Construction material
CY 208	Araldit CY 208	CIBA – Milano	long chain Epoxy resin	No information from the firm	Component of Araldit
CY 248	Araldit CY 248	CIBA – Milano	Epoxy resin	No information from the firm	Component of Araldit
HY 965	Araldit HY 965	CIBA – Milano	No information from the firm	No information from the firm	Hardener for Araldit
HY 966	Araldit HY 966	CIBA – Milano	No information from the firm	No information from the firm	Hardener for Araldit
Aluminium foil	Alluminio in fogli per uso domestico	Soc. Italiana Lav. alluminio Alessandria	Aluminium	No information from the firm	For covering laboratory items
Autobucato	Autobucato tipo speciale	Prodotti per l'Industria Serica Porlezza-Como	Heavy duty detergent. No further information	No information from the firm	Detergent
Ava	Ava heavy duty detergent	Mira Lanza Genova	No information from the firm	No information from the firm	Detergent
Alconox	Alconox	Alconox Inc. N.Y. – USA	Bleaching and complexing agents	No information from the firm	Detergent
"Balco"	Balco	Bally Chimie Svizzera	Rubber Caoutchouc as basic material	Hardener	Glue for nets

Caoutchouc for stoppers (Natural)	Versilic stoppers	Pasi Torino (Manifacture Gen. de Caotchoux Verneret-Ivrey Seine)	Pure rubber latex	No dyes	Stoppers for glassware
Caoutchouc for tubes (Natural)	Versilic tubes	Pasi Torino (Manifacture Gen. de Caotchoux Verneret-Ivrey Seine)	Pure rubber latex	No dyes	Tubes for connecting different apparatus
Cyclohexanone	Cicloesanone Depurato	Carlo Erba Milano	Cyclohexanone	No information from the firm	Glue for tygon and vipla
Decon 75	Decon 75	Medical Development Ltd Mercanton House Sussex England	No information from the firm	No information from the firm	Detergent
Ertalon white	Ertalon	Angst & Pfister Milano	Special polyamide	No information from the firm	Construction material
Ertalon black	Ertalon	Angst & Pfister Milano	Special polyamide	No information from the firm	Construction material
Ebanite 3012	Ebanite 3012	Tamburini Milano	Pure rubber	Filler: Kaolin, zinc oxide and calcium carbonate, etc.	Construction material
Ebanite 3019	Ebanite 3019	Tamburini Milano	Pure rubber	Filler: Kaolin, zinc oxide and calcium carbonate, etc.	Construction material
Eltex	Eltex	Solvay Milano	Polyethylene at high density	No dyes	Laboratory items
Flotronics membranes	Flotronics membranes FM 47	Selos Flotronics Spring House Pennsylvania USA	Silver	No information from the firm	Inorganic microfilter

Table 1 (continuation)

Denomination of materials	Commercial names	Firms	Raw materials	Additions	Use
Gabraster	Gabraster 1701	Montedison Milano	Polyester resin	Catalyzer	Construction material, used for covering materials, shielding of instruments, wooden walls; glassfibre reinforced plastics, used e.g. in high speed samplers (Delfino), "air-bubble-lift" covering of the hull and cabin walls research vessels "Odalisca".
"F" Catalyzer	Catalizzatore F	Montedison-Milano	Methylethylketone peroxide in dimethyl phthalate	No dyes	Catalyzer for Gabraster
Gloves "Erista special"	Erista special (oilproof-acidproof)	Il Politene Milano	No information from the firm	No information from the firm	Gloves
Gloves "Glovyl" 328	Glovyl 328	Il Politene Milano	No information from the firm	No information from the firm	Gloves
Gloves "Kachele"	Kachele	Kartell Milano	Plastified polyvinyl chloride	No information from the firm	Gloves
Gloves "Lux"	Surgeon gloves of pure rubber art. "300"	Soc. Ital. Hatù Bologna	Smoked natural rubber type R SS 1	Ingredients for the vulcanization No dyes	Gloves
Gloves "Marigold"	Marigold	Allen Rubber Lydney England	No information from the firm	No information from the firm	Gloves
Gloves "Nimble finger"	"Nimble finger"	The Pioneer Rubber Co. Willard, USA	No information from the firm	No information from the firm	Gloves
Gloves "Stanzoil Red"	"Stanzoil Red"	The Pioneer Rubber Co. Willard, USA	No information from the firm	No information from the firm	Gloves

Makrolon	Makrolon	Bayer Leverkusen West Germany	Polycarbonate of bisphenole	No information from the firm	Construction material, petriplates
Montivel	Montivel	Montedison Milano	Polyester film	No information from the firm	Film for covering
Membranefilter	Sartorius Membranefilter	Membranfilter Göttingen West Germany	Compounds of cellulose acetates	No information from the firm	For filter
Moplen I	Made for our use only	Ferrari Parma	Regenerated polypropylene	No dyes	Construction material for sampler
Moplen II (for stoppers)	No commercial name used only for stoppers in Laboratory equipments	Bicassa Milano	Isotattic polypropylene	No information from the firm	Stoppers
Moplen III (Semi finished products)	Moplen	Montedison Milano	Isotattic polypropylene	No information from the firm	Construction material
Neoprene	Neoprene	SIRSI Metallizator Varese	Polymer of Chloroprene	No information from the firm	Shielding, electrical cables, membrane pump
Nylon I (for bar)	Sniamid ASn	Energon Milano	Pure polyamidic resin	No dyes and fillers	Construction material
Nylon II (transparent film)	Sniamid ASn	Energon Milano	Pure polyamidic resin	No dyes	Bags for samplers and storage of samplers
Nylon III	Nylon FN	Snia Viscosa Milano	Pure polyamidic resin	No information from the firm	For screw caps
Omo	Omo	Lever Gibbs Milano	No information from the firm	No information from the firm	Detergent
Polypropylene cable*		Montedison Milano	Polypropylene	No information from the firm	For hydrographic wire
Polyuretani	Polyuretan	Bayer West Germany	Polyuretane resin	No information from the firm	Construction material

Table 1 (continuation)

Denomination of materials	Commercial names	Firms	Raw materials	Additions	Use
Paint Vinilver finish	Vinilver finish	Lavorazione Materiali Plastici Torino	No information from the firm	No information from the firm	Paint
Paint Vinilver primer	Vinilver primer	Lavorazione Materiali Plastici Torino	No information from the firm	No information from the firm	Paint
PVC, red	None	Montedison Milano	Polyvinyl chloride	No information from the firm	Construction material (messenger and ZoBell apparatus)
PVC, white	None	Montedison Milano	Polyvinyl chloride	No dyes	Construction material (messenger and ZoBell apparatus)
Polypropylene screw caps	None	Bio Tech. New York	No information from the firm	No information from the firm	Screw caps
Perspex	Perspex	Adreani Milano (I.C.I.)	Polymer of methyl-methacrylate	The coloured plates contain different type of pigments	Construction material (van Dorn samplers)
Polyethylene, black	Polytene	Montedison Milano	Polyethylene at low density	No information from the firm	Laboratory items, bottle
Polyethylene, white	Polytene	Montedison Milano	Polyethylene at low density	No dyes	Laboratory items, bottle, funnels etc.
Rubber band	None	Moroni Gomma Milano	Natural raw rubber		Elastic bend for closure device of Van Dorn and ZoBell sampler
Rubber, black	None	Moroni Gomma Milano	Natural raw rubber	With addition of sulphur for vulcanization	Stoppers

Rubber, black reinforced for tubes	TT 5	Rapisarda Milano	No information from the firm	No information from the firm	Tubes
Rubber, brown	None	Moroni Gomma Milano	Natural raw rubber	An addition of sulphur for vulcanization. No information about the nature of colours	Seals, washers
Rubber, clear	None	Moroni Gomma Milano	Natural rubber latex	An addition of sulphur for vulcanization. Colourless	Seals, washers
Rubber vacuum tubing without lining	Type Sumatra	Pirelli Milano	Natural rubber	Various contents	For vacuum tubes
Rubber, white I (for stoppers)	None	Carlo Erba Genova	Natural rubber	Various contents	Stoppers
Rubber, white II	None	Pirelli Milano	Natural rubber	Various contents	Spheres in hydraulic valves
Sicodur 'C' red 10/10	Sicodur C	Mazzucchelli Varese	Polyvinyl chloride	No information from the firm	Construction material
Sicodur 'C' red 30/10	Sicodur C	Mazzucchelli Varese	Polyvinyl chloride	No information from the firm	Construction material
Silicon SE 1201 translucent	Silicone SE 1201 translucent	General Elect. USA	Silic. organic compounds	No information from the firm	Glue for nets
Seitz filter apparatus:					
Filter plate	None	Seitz-Werke Kreuznach Rheinland	Cullulose and amiants fibres	No information from the firm	Components of Seitz filter apparatus
Grey rubber seal	None	Seitz-Werke Kreuznach Rheinland	Caoutchouc Rubber latex	No information from the firm	Components of Seitz filter apparatus
Light-brown rubber seal	None	Seitz-Werke Kreuznach Rheinland	Caoutchouc Rubber latex	No information from the firm	Components of Seitz filter apparatus

Table 1 (continuation)

Denomination of materials	Commercial names	Firms	Raw materials	Additions	Use
Silicone artificial rubber	Made for our use only	POSA Milano	Silicone polymer	No dyes	For vacuum tubes and for ZoBell samplers
Silicone Paint	RP/632 type	Ripi Genova	Silicone based paint	Silicone and natural caoutchouc with graphite added	Paint
Silicone rings	Rings R	Le Joint Française Paris	Silicone polymer	No information from the firm	Washers and seals
Stainless steel Avesta 832 SK	Avesta 832 SK	Lerici Milano (Avesta SpA)	Stainless steel	No dyes	Construction material
Tensol cement n. 7 (component A)	T. Cement n. 7 (component A)	Adreani Milano (I.C.I.)	Partially poly-merized methacrylate	No dyes	Glue for perspex
Tensol Cement no 7 (Component B)	Tensol cement no 7 Component B	I.C.I. England	Dilute solution of benzoyl peroxide in dimethyl phthalate	No information from the firm	Catalyzer for Tensol Cement no 7 comp. A
Vipla I (for smooth tubes)	Zinnia tubes	Pirelli Milano	Plastified polyvinyl chloride	Plasticizers: dibutyl phthalate, diisobutyl phthalate chlorinated paraffin Stabilizer: Pb stearate, Aril-phosphite, Ba-Cd salt of fatty acids	Tubes
Vipla, Kristall	Vipla Kristall flexiclor	Cattaneo Varese	Plastified polyvinyl chloride	No information from the firm	Sheets, construction material
Vipla tubes	Tygon type	U.S. Stoneware Ohio USA	Plastified polyvinyl chloride	No information from the firm	Tubes

Vipla, smooth transparent	None	Pirelli Milano	Plastified polyvinyl chloride	No information from the firm	Seals and washers
Vulkollan	Vulkollan Bayer	Angst & Pfister Milano	Synthetic elastomer belonging to polyuretane group	Produced with Bayer process: polyaddition of polyester and diisocyanate compounds.	Construction material
Vipla II	Plastified PVC	Pinza La Spezia	No information from the firm	No information from the firm	Tubes, employed in membrane pump
Vipla, clear ruled	None	Pinza La Spezia	Plastified polyvinyl chloride	No information from the firm	Tubes, employed in membrane pump
7 X	7 X	Limbro Chemical Co New Haven Connecticut-USA	Anionic compounds with special solvent in buffered solution with phosphatidic complex	No information from the firm	Detergent

* Breaking strength
500 kg for 6 mm in ∅
1380 kg for 12 mm in ∅

Table 2

Toxicity of gloves used for handling glassware and other laboratory items. ++++ normal growth rate; +++ slightly inhibited growth rate; ++ 50 % normal growth rate; + no growth, cells alive; 0 no growth, cells dead

Gloves	*Leptocylindrus danicus* EtRSW	*Prorocentrum micans* EtRSW	*Chaetoceros danicus* EtRSW	*Coccolithus huxleyi* EtRSW
Lux	++++	++++	++++	++++
Glovyl 328 (heavy duty gloves)	++++			
Marigold	0			
Pirelli	0			
Erista special	0			
Stanzoil red		0		
Nimble fingers yellow		0		
Kachele clear		0		

MATERIAL AND METHODS

In Table 1 are listed all products tested, together with their uses in our laboratory. These products include nylon used for plankton nets and "Balco paste" and "Silicon SE 1201" used to glue nylon tissue; polypropylene used for hydrographic cables; perspex used for constructing VAN DORN samplers; PVC employed for constructing messengers; membranefilters; various components of the SEITZ filter apparatus used for preparing sterile culture media; and various kinds of tubing employed in continuous cultures or running seawater systems. Among the substances tested were the highly toxic common red rubber tubing and the toxic Vipla (plastified polyvinylchloride) tubing which have gained equally wide distribution in modern laboratories.

Furthermore, widely used plastics such as polyethylene, artificial silicone rubber, and detergents for washing glassware, as well as gloves used for handling the glassware, have been considered. The biological activity of a paint and its effectiveness to cover a lead weight has also been studied.

The phytoplankton species *Leptocylindrus danicus, Chaetoceros danicus, C. curvisetum, Coccolithus huxleyi, Phaeodactylum tricornutum* and *Prorocentrum micans,* and the copepod *Euterpina acutifrons* from our culture collection were used in the tests.

Natural seawater (SW) collected at station A off the coast of La Spezia with the "air-bubble-lift", which had then been "aged" for one month in the dark at room temperature, enriched with 0.2 g $NaHCO_3$, 0.02 g $NaHPO_4$ $12H_2O$ and 0.1 g $NaNO_3$ per liter (RSW), and the same enriched seawater with an addition of 50 ml soil extract (EtRSW) per liter were used as culture media for phytoplankton. The soil extract was prepared by autoclaving (0.5 atm, 20 min) 10 g of dry soil (collected at an undisturbed location) together with 10 ml of quarz-distilled water and then filtered whilst still hot.

The sea urchin *(Arbacia lixula)* larvae originated from eggs and spermatozoa obtained from sea urchins collected in the Gulf of La Spezia near the Fiascherino laboratory. *Arbacia lixula* larvae were chosen since, in earlier work, these larvae had been shown to be very sensitive to contaminations (BERNHARD 1955). The culture media of the sea urchin tests were RSW-medium and RSW-medium with an addition of 10^{-4} mol EDTA (VRSW). Several pairs of sea urchins were used to obtain fertilized eggs, but only batches of those pairs which showed more than 90 % membrane elevation were used in the tests (BERNHARD 1955). The pH of all media was brought to a value of 8.1 ± 0.1 with diluted NaOH. All tests were carried out in a culture room at 18^0 C under artificial illumination (fluorescent lamps; 12^h).

Experiments with *Euterpina acutifrons* were carried out in RSW-medium and the copepods were fed with a mixture of *Platymonas suecica,* (β_2) *Dicrateria sp.* (β_3) and *Platymonas sp.* (β_{43}). The phytoplankton tests were conducted in test tubes and the tests with *Euterpina acutifrons* and *Arbacia lixula* larvae in Boveri dishes. Before testing, all materials were thoroughly washed with "Omo", one of the detergents which have been proved non-toxic (see below), and rinsed repeatedly with deionised double quarz distilled water.

The glassware was washed and handled with "Marigold" gloves although the material of which "Marigold" gloves are made proved to be toxic when suspended in algae cultures.

No suitable non-toxic gloves are available to date (Tab. 2). The materials were washed and handled with "Lux" surgeons' gloves which allow growth of *Leptocylindrus danicus*, but which are too slippery and delicate to handle wet glassware (Ignis neutro Murano or Jena 20). All materials were tested at least 3 replicates. After vigorous cleaning, the materials were added to the culture medium and remained in the culture vessel during the experiment. In some tests the materials were autoclaved in the culture media (0.5 atm, 20 min). These materials were autoclaved twice successively in new medium and the medium of the second autoclaving was used in the tests without the materials. Obviously, some materials did not withstand autoclaving.

RESULTS

Effects on phytoplankton

Table 3 presents results obtained in previous experiments (BERNHARD et al. 1966) and in new experiments. In these experiments the materials, after thorough cleaning, were added to the alga medium and remained in the culture vessel during the experiment. All products were tested at least three times. Four different estimations of growth rates were made; the mean of these estimations is listed in the table. The tests were carried out in natural enriched seawater (RSW) and in natural enriched seawater plus soil extract (EtRSW), in order to test whether heavy metal contamination may be compensated by chelation of the soil extract.

The materials are listed in increasing order of inhibitory effects, ranging from materials which showed no effects at all to the very widely used rubbers and plastified polyvinyl chlorides which are highly toxic. Several components of plastic materials were tested individually. The results (Tab. 4) show that single components are much more toxic than the final material. That means careful cleaning of plastic materials is necessary to avoid toxicity effects from their components.

In other experiments the test materials were autoclaved. These were materials which are either normally autoclaved or which are used so widely that they should be tested under more stringent conditions. The tests were carried out in the medium which remained after the second autoclaving. The autoclaved materials had been removed before the tests. Again, the materials are listed according to their relative toxicity (Tab. 5).

As can be seen, the original Seitz filtration apparatus should not be used for preparing media, as several components of this apparatus are toxic. They must be substituted by non-toxic materials.

Test tubes with screw caps are very useful for phytoplankton work. Since bakelite caps are toxic (personal communication of Dr. PROVASOLI) two types of screw caps have been tested. Polypropylene caps are non-toxic. Nylon screw caps seem to lose toxicity with successive sterilisations; this phenomenon may be due to leaching of substances from the nylon.

Table 3

Compatibility of marine algae with various materials. (Based on new information and data from BERNHARD et al. 1966). Degree of toxicity expressed in the same way as in legend to Table 2

Materials	*Leptocylindrus danicus* RSW*	*Leptocylindrus danicus* EtRSW**	*Chaetoceros danicus* RSW	*Chaetoceros danicus* EtRSW	*Chaetoceros curvisetum* EtRSW	*Coccolithus huxleyi* EtRSW	*Prorocentrum micans* RSW	*Prorocentrum micans* EtRSW
Algoflon (similar to Teflon)	++++	++++				++++		
Perspex	++++		++++	++++		++++		
Tensol cement no. 7	++++		++++	++++		++++		
Polythene, natural color	++++		++++	++++		++++		
Silicon artificial rubber		++++	++++	++++		++++		
Caoutchouc for stoppers (Natural)	++++	++++			++++	++++		
Nylon I (for bar)	++++	++++	++++	++++	++++			
Moplen I	++++	++++	++++	++++	++++			
PVC, red	++++	++++	++++	++++	++++	++++		
Sicodur "C" red 10/10	++++	++++		++++	++++	++++		
Polypropylene for cable	++++	++++				++++	++++	++++
Ertalon white		++++				++++		++++
Ertalon black		++++				++++		++++
Makralon		++++				++++		++++
Aluminium foil						++++		++++
Polyurethane								++++
Silicon SE 1201 translucent								++++
Tygon glued with cyclohexanone								++++
Montivel	++++	+++		++++	++++	++++		
PVC, white	++++	++++	+++	++++	++++			
Stainless Steel Avesta 832 SK	+++	++++	+++	++++	++++	++++		
Paint Vinilver finish	++++	+++	+++	++++	++++	++++		

Paint Vinilver primer	++++	+++	+++	+++	++++	++++		
Sicodur "C" red 30/10	++++	++++	++++	+++	++	++++		
Moplen II (for stoppers)	++++	++++	++	+++	++++	++++		
Moplen III (semifinished products)	++++		+++	++++		+++		
Polythene, black	+++		+++	++++		++++		
Nylon II (transparent film)	++++		+++	++++		++++		
Caoutchouc for tubes (Natural)	++++	+++	+++	++++	++++	++		
Vipla Kristall	+++	+++	++	+++	++++	++		
Vulkollan								++
Balco		0				++		0
Ebonit 3012	++++	++++	++	+++	++++	0		
Lead weight covered with Vinilver primer + finish	+++	+	++	++	++++	++		
Rubber, black	0	0	+++	++++	0	++++		
Ebonit 3019	0	++++	++++	0	++	0		
Rubber vacuum tubing without lining	0	++++	++	++++	0	0		
Rubber, brown	0	+++	+++	+++	0	0		
Rubber, clear	0	++++	+++	0	0	++		
Rubber, white I (for stoppers)	0	+++		0	0	0		
Vipla I (for smooth tubes)	0	++	+	0	0	+		
Vipla II (for large tubes employed in membrane pump pipe)	0	0	0	0	0	++		
Flotronics membrane								0
Araldit	0						0	
Neoprene								0
Rubber, white II (for spheres)	0	+	0	0	0	0		
Rubber, black reinforced for tubes	0	0	0	0	0	0		
Vipla clear ruled		0		0		0		

* RSW = Natural enriched seawater; ** EtRSW = Natural enriched seawater + soil extract

Table 4

Toxicity of some constituents of plastic materials. For degrees of toxicity consult legend to Table 2. Because of the low solubility of the components Araldit, Tensol Cement no 7 (component A) and Gabraster, one drop of material (0.0135–0.0211 g) was added to 100 ml seawater medium and left overnight. Then the medium was diluted with new medium according to the dilution rates given in the table. All tests were carried out 3 replicates

Materials	*Coccolithus huxleyi* RSW 1/1	1/4	1/16	1/64	1/256	1/1024	*Leptocylindrus danicus* EtRSW 1/1	1/4	1/16	1/64	1/256	1/1024
HY 966 for Araldit	0	0	++	++	++	++	0	0	+	+	+	++
CY 248 for Araldit	0	0	0	++	++	+++	0	0	+	+	+	+
CY 208 for Araldit	0	++	+++	+++	+++	+++	0	++++	++++	++++	++++	++++
HY 965 for Araldit	0	0	0	+	++	++	0	0	0	++	+++	+++
Tensol cement no 7 (component A)	0	0	++	+++	+++	++++	0	++	++	+	++	+++
Gabraster	0	++	++	++++	++++	++++	0	0	++	+++	++++	++++
Concentration % v/v	0.01	0.04	0.002	0.001			0.1	0.04	0.002	0.001		
Tensol cement no 7 (component B)	0	+	+	++			0	+	++	++++		
Concentration % v/v	10	4	2	1			10	4	2	1		
"F" Catalyzer for Gabraster	0	+	++	++			0	++	++++	++++		

Table 5

Compatibility of 4 phytoplankton species with various materials, after autoclavation (0.5 atm 20 min). For degrees of toxicity consult legend to Table 2. (Based on new information and data from BERNHARD et al. 1966)

Materials	*Leptocylindrus danicus*		*Chaetoceros danicus*		*Prorocentrum micans*		*Coccolithus huxleyi*	
	RSW	EtRSW	RSW	EtRSW	RSW	EtRSW	RSW	EtRSW
Polyethylene natural colour	++++	++++	++++	++++	++++	++++	++++	++++
Gabraster + "F catalyzer"	++++	++++			++++	++++	++++	++++
Perspex	++++	++++	++++	++++	++++	++++	++++	++++
Silicone artificial rubber	++++	++++	++++	++++	++++	++++	++++	++++
Algoflon	++++	++++	++++	++++	++++	+++	++++	++++
Tygon	++++	++++			++++	++++	++++	++++
Silicone SE 1201 translucent						++++		
Caoutchouc for stoppers	++++	+++	++++	++++	++++	++++	+++	+++
Moplen	++++	++++	++	++	++	++++	+++	++
Makrolon		++++				++++		++
PVC, Red	++++	++++	+++	++++	++++	++++	0	++++
Membranefilter	++++	++++			++++	++++	0	0
Nylon I	++++	++	++	0	+++	+	++	++
Araldit					0	0		
Flotronics membrane						0		
Polypropylene screw caps								
1⁰ sterilization	++++	+++			++++	++++	++++	++++
2⁰ sterilization	++++	++++			++++	++++	++++	++++
5⁰ sterilization	++++	+++			++++	++++	++++	++++
Nylon screw caps type III								
1⁰ sterilization	0	0			0	++++	++++	++++
3⁰ sterilization	++++	0			++++	++++	++++	++++
5⁰ sterilization	++++	++			+++	++++	++++	++++
Seitz filter apparatus								
filter plates	0	0			0	+++	++++	++++
grey rubber seal	+++	++			++++	++++	++++	++++
light brown rubber seal	+	0			+	0	0	0
Tensol cement no 7 (component A + component B)	0	0	0	0	++	++	0	0

Table 6

Toxicity of zinc, copper, chromium and cobalt for *Phaeodactylum tricornutum* and *Coccolithus huxleyi*. For degrees of toxicity consult legend to Table 2.

$$F = \frac{\text{Tolerance concentration}}{\text{Concentration in natural seawater}}$$

Compounds	M/l	*Phaeodactylum tricornutum* RSW	*Coccolithus* RSW *huxleyi*	M/l in NSW×10^{-6}	F
$ZnSO_4 7H_2O$	3.06.10^{-6}	++++	++++	0.05–0.1	
	6.12 „	++++	+++		30
	12.24 „	++++	+		
	15.29 „	+++	+		
	30.58 „	+++			
	76.47 „	++			
	152.9 „	+			
$CuSO_4 5H_2O$	0.16.10^{-6}	++++	++++	0.05	
	0.32 „	++++	++++		16
	0.80 „	++++	++++		
	1.57 „	+++	+++		
	3.14 „	++	+		
	6.28 „	+			
	12.56 „	0			
	15.7 „	0			
$[CrCl_2(H_2O)_4]$ $Cl 2H_2O$	1.92.10^{-6}	++++	++++	0.0009	150,000
	3.84 „	++++	++++		
	7.68 „	++++	++++		
	15.36 „	++++	++++		
	19.2 „	++++	+++		
	38.4 „	++++	++		
	76.8 „	+++	++		
$Co(NO_3)_2 6H_2O$	33.93.10^{-6}	++++	++++	0.0016	20,000
	67.86 „	+++	+++		
	135.72 „	++	++		
	169.65 „	++	++		
	339.30 „	+	00		

Heavy metal toxicity has been blamed for the inhibition of growth and development in marine organisms; it was, therefore, interesting to test the sensitivity of some phytoplankton species to heavy metals. Some preliminary results are given in Table 6. *Phaeodactylum tricornutum* and *Coccolithus huxleyi* showed marked differences in their sensitivity to zinc, but practically no difference was noticed in the sensitivity of the two algae to copper, chromium and cobalt. Comparing the critical test concentration at which an effect can be noticed with the normal concentration of these ions in the seawater, we noted that the normal concentration of copper in seawater is only about 15 times lower than the maximum test concentrations tolerated, the corresponding value for zinc amounts to about 30, for chromium 150,000 and for cobalt 20,000. Therefore, small additions of copper and zinc can easily exceed the tolerance concentration of the organisms for these ions.

Table 7

Effect of various detergents on *Arbacia lixula* larvae and three phytoplankton species. For degrees of toxicity consult legend to Table 2. (Based on new information and data from BERNHARD et al. 1966)

Detergent	mg/l	*Arbacia lixula* RSW**	*Leptocylindrus danicus* RSW	*Coccolithus huxleyi* RSW	*Prorocentrum micans* RSW
Autobucato	1	5.3	++	++++	
	0.5	5.6	+++	++++	
	0.1	6.0	+++	++++	
	0.01	5.8	+++	++++	
	0.001	5.9	+++	++++	
OMO	1	1.2	++++	++	
	0.5	5.8	++++	+++	
	0.1	5.9	+++	++++	
	0.01	6.0	++++	++++	
	0.001	6.0	++++	++++	
AVA	1	1.8	++	++	
	0.5	4.8	+++	+++	
	0.1	5.8	+++	++++	
	0.01	5.8	+++	++++	
	0.001	6.0	+++	++++	
7×*	1				++++
	0.5				++++
	0.1				++++
	0.01				++++
	0.001				++++
Alconox	1		++++	++++	++++
	0.5		++++	++++	++++
	0.1		++++	++++	++++
	0.01		++++	++++	++++
	0.001		++++	++++	++++
Decon 75*	1		++++	++++	++++
	0.5		++++	++++	++++
	0.1		++++	++++	++++
	0.01		++++	++++	++++
	0.001		++++	++++	++++

* 7× and Decon 75 had been added as liquids, therefore their concentrations are expressed as 10^{-3} ml detergent/l seawater
** Developmental stage reached. 6: maximum developmental stage. 0: larvae died

Finally, 6 detergents have been tested. As can be seen from Table 7 the detergents have slight or no effect in the concentrations at which they normally remain on the surface of glassware; however, higher concentrations should be avoided.

Effects on adults of *Euterpina acutifrons* and sea urchin larvae

In addition to the tests with phytoplankton species, several materials have also been tested in regard to their effects on adult *Euterpina acutifrons* and larvae of *Arbacia lixula*. The results obtained with *Euterpina acutifrons* are shown in Table 8.

Table 8

Effect of various plastic materials on the survival time of *Euterpina acutifrons*. (After NEUNES, personal communication)

Materials	Survival time in days
Tygon tubes	14.7 – 17.9 – 29.7
Perspex	29.4
Polythene white for bottles	20.7 – 29.4
Caoutchouc versilic for tubing	17.8 – 20.6 – 27.6
Caoutchouc for stoppers	17.1 – 17.8 – 27.3
Stripe	13.8 – 23.7
Silicone rubber for vac. tubing	16.4 – 19.6 – 22.8
Red rubber for vacuum tubing	0 – 17.1
Vipla zinnia	0 – 3.0 – 16.9
Vipla gemma	2.1 – 3.6 – 4.5
Control	20.5 – 24.6 – 28.2 – 28.8

Table 9

Compatibility of *Arbacia lixula* larvae with various materials. (After BERNHARD et al. 1966)

Materials	Mean developmental stage* VRSW	RSW
PVC, red	6.0	5.8
Rubber band II, elastic for VAN DORN sampler	6.0	5.8
Vipla, red	6.0	5.2
Vipla, blue	6.0	5.2
PVC, green	5.6	5.3
Perspex transparent	5.7	5.8
Tensol cement no 7 (components A + B)	5.4	5.8
Vipla, clear ruled	4.9	4.7
Vipla, green	4.8	5.3
Rubber vacuum tube without lining	4.7	4.8
Perspex, white	4.6	4.4
Rubber band I, elastic for VAN DORN sampler	0.0	0.0

* see note in Table 7

Certain materials reduce considerably the survival time of this copepod. The strongest inhibition, similar to that in the phytoplankton experiments, is caused by Vipla and rubber.

The tests with sea urchin larvae were carried out in RSW and VRSW in order to see if the EDTA would chelate eventually released heavy metals (Tab. 9). No significant difference between the two media was, however, observed; therefore, it seems unlikely that the inhibition observed is due to heavy metal poisoning. The most striking effect was obtained from the rubber band I used to trigger the VAN DORN Sampler. This rubber band was later substituted by rubber band II. The influence of detergents on the larvae development was negative for very high concentrations only. Low concentrations seem to favour the development (Tab. 7).

CONCLUSION

The few data from literature (e. g. DYER & RICHARDSON 1952, ROBERTSON 1968) and results obtained on phytoplankton, *Euterpina acutifrons* and larvae of the sea urchin *Arbacia lixula* have led our laboratory to take special precautions when working with living organisms.

VAN DORN and ZoBELL samplers and plankton nets have been constructed of entirely non-toxic materials. The VAN DORN sampler was built using Perspex tubing for the body and white polyethylene funnels for the closing cups. In constructing the ZoBBELL sampler we used red PVC for the apparatus itself and artificial silicone rubber for the bulb and the tubing.

The triggering device of both samplers is made of Perspex or PVC, nylon thread, stainless steel with water tight covering of polyethylene tubing, and a rubber band of the II type. Clamps to fasten the samplers onto the polyethylene hydrographic cable are made of Perspex or PVC. The messengers are white PVC cylinders filled with lead.

Incidently, the commonly used bulb of red and black rubber and the rubber strings to close some VAN DORN samplers and the rubber bulb of the original ZoBELL sampler are highly toxic.

The lead weights are covered with watertight polyethylene sheets or with glass reinforced Gabraster. The plankton nets are constructed from stainless steel rings covered with watertight polyethylene tubing. The cloth is nylon glued with non-toxic Silicon SE 1201 instead of "Balco" glue. The bucket and the screws of the bucket window are PVC or perspex; the ropes are made of polypropylene or nylon.

Collecting large amounts of seawater for culture purpose also presents problems. Metal pumps obviously cannot be used. The plastic membrane pumps purchased by our laboratory had a Neoprene membrane which proved to be toxic and thus, had to be substituted by an artificial silicone rubber membrane. This membrane, however, had a very short life span. At present, we are utilising either a PVC centrifugal pump, or a peristaltic pump, or an "air-bubble-lift" (BERNHARD & MACCHI 1966) with tygon tubing.

All non-sterile samples are stored in white polyethylene bottles; sterile samples are kept in glassware, usually closed with non-toxic screw caps. All work at sea is done with plastic cables of a 6 and 12 mm diameter polypropylene woven thread. Formerly, nylon cables were used. These cables have great advantages over the usual metal cable. Apart from metal contamination of the sample, they need no maintenance, because they do not rust; they do not cut the hands of the operator; they are practically weightless in seawater and, therefore, the winches do not need to be so powerful when working at great depth. Finally, they are much less expensive. The only precautions to be taken are to avoid sharp edges, if metal clamps have to be used.

It may interest persons who are hesitant to use plastic hydrographic cable to hear that since 1959 in all our work (biological and non-biological) we have used polypropylene (and formerly, nylon) cables as hydrographic wire even when working at depths greater than 2,000 m. In all these years we have only once lost a series of high speed plankton samplers.

Further interesting information concerns our 18 m ship. Its wooden hull was covered with Gabraster and more than three years' experience have shown that it is much easier to keep the hull clean; in the long run, the use of Gabraster instead of paint has made maintenance more economical. Gabraster is non-toxic and, therefore, no toxic anti-fouling substances can leach into the surrounding seawater.

Continuous culture experiments (ZATTERA 1966) carried out in our laboratory have shown that it is extremely difficult to clean plastics like nylon and PVC once they have been dirtied in the workshop. After several trials, we settled for an all-glass vessel, rotating back and forth, and closed with a glass stopper. The flowing system consisted of tygon tubing and a peristaltic pump. The avoidance of toxic materials has certainly facilitated the cultivation of marine organisms in our laboratory. Our culture collection contains, at present, more than 100 strains of known and unknown flagellate species. So far, only our attempts to start unicellular cultures of *Ceratium* sp. failed.

Table 10

Adsorption or release of ionic zinc. (After TORTI & PAPPUCI, personal communication)

Materials	Adsorption $\mu g\ Zn/cm^2$ $\times 10^{-2}$	Release $\mu g\ Zn/cm^2$ $\times 10^{-2}$
Silicon rubber (stoppers)	1.10	
Moplen (beakers)	1.60	
Polythene, white bottle	1.77	
Perspex (sheets)	1.84	
Polythene (beakers)	1.87	
Vipla (sheets)	2.19 (Pb)	
Silicon rubber (sheets)	2.28	
Algoflon (sheets)	2.59 (Pb)	
Rubber for VAN DORN bottle	3.96	
PVC white		0.25 (Pb Cu)
Polythene grey bottle		0.29
Electric cable (ext. cover)		6.37 (Pb)
Gabraster + glass wool		8.46 (Pb)
PVC red		9.38 (Pb)
Rubber para (sheets)		3335

Zn^{++} in the normal seawater used: 2.85 $\mu g/l$

How careful one has to be when working with different materials, is also illustrated by an experiment on the zinc concentration of natural seawater to which various materials had been added (Tab. 10). Some materials absorb zinc from, others release zinc into the seawater.

SUMMARY

1. Using 6 phytoplankton species and/or the copepod *Euterpina acutifrons* or larvae of the sea urchin *Arbacia lixula* the potential inhibitory effects of chemicals released from some 70 different materials (mainly plastics) have been tested. In addition, the effects of 6 detergents have been examined.

2. Several materials, such as natural rubbers and polyvinyl chlorides, are highly toxic and should never be used when experimenting with living marine organisms.
3. Teflon (Algoflon), Perspex, Polyethylene, Tygon, Polypropylene, Polycarbonates (Makrolon) and Polyester (Gabraster) have been shown to be non-toxic and are, therefore, suitable for use in cultivation of marine organisms. Some materials had slightly negative effects on the organisms tested and should, therefore, be used only if no alternatives are available.
4. Some suggestions are advanced on how to construct non-toxic samplers and laboratory equipment used for experiments with marine organisms.

LITERATURE CITED

BERNHARD, M., 1955. Die Kultur von Seeigellarven (*Arbacia lixula* L.) in künstlichem und natürlichem Meerwasser mit Hilfe von Ionenaustauschsubstanzen und Komplexbildner. *Pubbl. Staz. zool. Napoli* **29**, 80–95.

— & MACCHI, G., 1966. Application and possibilities of automatic chemical analysis in oceanography [Orig.-T.: Anwendung und Möglichkeiten der automatischen chemischen Analyse mit dem Auto-Analyzer in der Ozeanographie]. *In:* Automation in analytical chemistry [Automation in der analytischen Chemie. Internationales Technicon Symposium, Frankfurt, Okt. 1965. U. d. Schriftltg. von D. I. Schmidt. Technicon GmbH, Frankfurt, 83–93.]. Medical Inc., New York, 255–259.

— ZATTERA, A. & FILESI, P., 1966. Suitability of various substances for use in the culture of marine organisms. *Pubbl. Staz. zool. Napoli* **35**, 89–104.

DYER, D. L. & RICHARDSON, D. E., 1962. Materials of construction in algae culture. *Appl. Microbiol.* **10**, 129–131.

ROBERTSON, D. E., 1968. Role of contamination in trace element analysis of sea water. *Analyt. Chem.* **40**, 1067–1072.

ZATTERA, A., 1966. Colture continue di *Phaeodactylum tricornutum* e *Prorocentrum micans*. Tesi di Laurea in Scienze biologiche, Univ. di Parma.

First author's address: Dr. M. BERNHARD
Laboratorio per lo studio della
contaminazione radioattiva del mare
CNEN-Euratom
Fiascherino, I 19030, La Spezia, Italy