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TNO-report

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Aquatic toxicity of compounds that may be carried by ships (Marpol 1973, Annex II)

– A progress report for 1986 –

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SUMMARY

The GESAMP Working Group on the Evaluation of Hazards of Substances Carried by Ships is continually searching for information on substances for which relevant data are still missing. This report contains data gathered by the Netherlands from acute aquatic toxicity tests carried out in the laboratory during 1986 with 9 such substances and several aquatic organisms. The data are summarized in Table 1 and given in detail in Annexe A of this report.

This work was funded by the Ministry of Housing, Physical Planning and Environment as part of Research Project R 779.

As part of a reseach project on development of test methods for sparingly soluble, volatile substances, commissioned by the TNO Division of Technology for Society, 16 paraffins were tested. Since the acute aquatic toxicity of such industrial compounds is of interest for the GESAMP Working Group, the results of these tests are included in this report.
It turned out to be extremely difficult to test paraffins properly. In spite of all efforts to prepare solutions of the correct concentrations, these were mostly far lower than intended. As a result, the acute toxicity of sparingly soluble volatile compounds is likely to be underestimated if their concentrations in the test solutions are not determined by chemical analysis.
The hazard such compounds pose in a natural environment also depends on their residence time. This factor, however, is irrelevant in the determination of LC50-values, which presupposes constant and known concentrations of toxicants.

1. INTRODUCTION

The International Conference on Marine Pollution (Marpol) held in 1973 decided that substances (other than oil) carried by ships should be categorized according to their hazards to the environment (including man) when released in the sea.
Guidelines for this categorization were given in Appendix 1 to the 'Final act of the conference', and are collected in Appendix 1 of this report. For details on the conference, see the 'Final act' (ref. 1).
In 1972 a GESAMP[1]) Working Group on the Evaluation of Hazards of Substances Carried by Ships (EHS Working Group) was set up for the purpose of providing IMO[2]) with the information it needs to place substances in their proper pollution categories. Its recent activities have been summarized by Portmann (ref. 2). A report about its activities was published in 1982 (ref. 3).
All governments and industries concerned have been and are being urged to supply the GESAMP Working Group with information on the properties of such substances (other than oil) carried by ships as cannot be properly classified for lack of information.
As is set forth in ref. 2 and 3, the information needed covers five groups of properties of substances, viz. Bioaccumulation; Damage to living resources; Hazard to human health on oral intake; Hazard to human health on skin contact and inhalation, and Reduction of amenities. In the 'Hazard profiles' compiled by the EHS Working Group, these properties are rated by symbols in columns A, B, C, D and E, respectively. Ratings and their symbols are defined in Appendix 2. Hazard profiles, whether complete or not, of about 1500 substances are collected in a 'composite list', (e.g. Annexe 6 to ref. 3) which is updated with any information that becomes available.

This report contains the results of tests on several substances carried out in the Netherlands in 1986 and the beginning of 1987 for the purpose of providing the EHS Working Group with information enabling it to rate substances in column B.
Ratings in column B - Damage to living resources - are preferably based on 96h LC50 values determined in tests with marine crustaceans or fishes (ref. 3); as reported earlier, the preferred test animals at this laboratory

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1) Group of Experts on Scientific Aspects of Marine Pollution
2) International Maritime Organization

are young specimens of the marine crustacean *Chaetogammarus marinus* (cf. reports CL 82/14, R 83/15, R 84/59 and R 85/217).

Table 1 lists the results, 96h LC50 values, for this test animal and 9 compounds.
C. marinus being in short supply, we carried out the preceding screening tests with the freshwater animal *Daphnia magna*. The 48h EC50 values for this animal are given in Table 1 as additional information. *Mysidopsis bahia* being a widely used test animal, the compounds were also tested on this animal, to compare the results with those for *C. marinus*.

Table 2 contains test results for n-, iso-, and cycloparaffins with several test animals. These results originate from a project with a fresh-water crustacean, *Daphnia magna*. To make them more valuable for the purpose of the GESAMP Working Group and to assist comparison, the compounds were also tested on the marine crustaceans *C. marinus* and *M. bahia*.

2. MATERIALS AND METHODS

2.1 TEST COMPOUNDS

Of the list (Appendix 3) of compounds supplied by the Ministry, some could not be obtained.
The actual compounds tested are listed in Table 1.
The paraffins investigated were chosed by the author, and are listed in Table 2.

2.2 TEST ANIMALS

Three species were used:

Chaetogammarus marinus (gammarid) (Crustacea, Amphipoda)

C. marinus occurs widely in the tidal regions around the North Sea, and is readily grown in aquarium systems if provided with Fucus spec. as food and with shelter for concealment. The test animals were young animals, about 5 mm long, grown in a laboratory culture in seawater.
C. marinus was used during earlier experiments for the same purpose (see reports CL 82/14, R 83/15, R 84/59 and R 85/217) and was the preferred test species.

Mysidopsis bahia (mysid shrimp) (Crustacea, Mysidacea)

Being native to the subtropics, *M. bahia* does not occur in the North Sea, and has to be grown and tested at temperatures higher than those prevailing in the North Sea. For this reason it is not popular as a test animal in this country. It is, however, a much used test animal in the U.S., also for regulatory pruposes.
It is readily grown in aquarium systems if fed (daily) with live *Artemia nauplii*. The mysid shrimps were grown and tested in natural seawater at 20°C and a salinity of 2.8%.
The animals used in the tests were about 4 weeks old and about 6 mm long.

Daphnia magna (water flea) (Crustacea, Cladocera)

The test animals were less than 24h old, born from adults aged some three weeks. Daphnia is being continuously cultured in the laboratory under standaridized conditions.

2.3 METHODS

2.3.1 Introductory remarks

The widely differing physical and chemical properties of the compounds of Table 1 prevented us from using the same test protocol for all of them.
A summary test protocol with the relevant details is therefore given for each test compound separately in its data sheet (Annexe A).

The compounds to be tested fell into two groups:

- compounds of reasonable solubility in water and relatively low volatility; most compounds of Table 1 belonged to this group;
- compounds of low solubility in water; most of these were also highly volatile. All paraffins tested belonged to this group, together with a few of those of Table 1.

2.3.2 Method for water-soluble compounds of low volatility

These compounds pose no special problems. The necessary amount(s) of the compounds are accurately weighed out or pipetted, and dissolved in the desired volume of dilution water, either DSWL (see Appendix 5) for tests with *Daphnia magna*, or filtered natural seawater (for test with the gammarids or mysid shrimps). In this way all test solutions can be prepared separately, or the most concentrated solution to be tested is prepared in this way and that solution is accurately diluted with the dilution water to give the less concentrated solutions. This method is called 'normal dosage' in this report.
If no volatilization losses are to be expected, the tests can be performed in glass beakers covered with watch glasses and, if necessary, slightly aerated.
The tests with *Daphnia magna* were of the static type, with an exposure time of 48h; those with the two marine crustaceans were semistatic, with daily

renewal of the test medium and an exposure time of 96h. Since mysid shrimp and gammarids are rather cannibalistic, most of the tests with them were carried out with only one test animal per container (10 animals per test concentration). This set-up is less time consuming and excludes interactions between animals. (The idea of one test animal per container was first developped in the EC for the reproduction tests with *Daphnia magna*, but appeared to be useful for other test systems as well). Scintillation vials were in this case used as small, simple, inexpensive and disposable testing vessels that can be closed with caps. Since the procedure prescribes that a testing vessel should at least be loosely covered, we carried out most tests with mysids and gammarids in closed scintillation flasks regardless of the volatility of the chemical being tested.
The pH and oxygen concentration were monitored during the test period for all test solutions.
Details on numbers of animals, volumes of solutions, shape of test containers, aeration, renewal times, temperature, exposure time, duplicates, concentrations of test compound, and the results are given in the data sheets in Annexe A.

2.3.3 Methods for slightly water soluble pure compounds, usually of high volatility and with a density lower than that of (sea) water

These compounds are difficult to test, because they do not readily form aqueous solutions of their theoretical solubility, and easily escape from their solutions when these are exposed to the air.
The compounds in question float on water and may evaporate even before they have dissolved.
Their solubilities in water are not always known, and some overdosage may be needed to obtain saturated solutions. Any undissolved compound has to be removed before the animals are introduced. However, even when an excess is removed very carefully, a surface film containing a high concentration of the compound is sometimes formed during the test. Such surface films will trap some species of test animals, and kill them through over exposure. Such unrealistic mortality renders the test invalid.
The test methods described below have been developed to overcome these problems as far as possible. They involve exposure of animals to constant concentrations, less than or equal to their solubilities in water, of sparingly soluble compounds (in this case mainly paraffins).

All test solutions were prepared separately by addition of the nominal amount of compound to dilution water. This procedure differs from the normal procedure of preparing a concentrated stock solution of known concentration of a pure compound and diluting it. The normal procedure is unsuitable for slightly soluble and highly volatile pure compounds, because their solubilities are not always known with sufficient accuracy and are difficult to reach, and because they are easily lost by evaporation from their solutions. Great care should be taken in preparing the solutions. For this purpose we used two methods:

Method no. 1 was used for the more soluble, shorter-chain paraffins, and for two other compounds listed in Table 1 (alkylate gasoline and 2-methyl-2-propene-1-ol). This method is called 'via WSF' in Table 1 (WSF = water soluble fraction), a terminology normally used for mixtures of compounds, which in this case only holds for the alkylate gasoline.

Method no. 2 was used for the less soluble, longer-chain paraffins (C_{11} and above).

n-Nonane and n-decane were tested on *D. magna* by both methods, but on the marine crustaceans only by method no. 2.

Method no. 1

The compound to be dissolved is mixed for 24 h in various ratios with the aid of a magnetic stirrer with the medium (seawater for the marine crustacean or fresh water for *D. magna*) in a conical flask almost completely filled, and allowed to stand for a further 24 hrs, to allow for separation of excess of compound. The aqueous solutions are than drained off through the stopcock (Fig. 1).

Shorter times than 2 x 24 hrs may suffice, or may even be better, but these times were taken for uniformity's sake and for lack of time to optimize them. For the higher parraffins, however, a separation time of 4 hrs was accidentally found to yield much higher concentrations in the aqueous phase, and was therefore used, again without optimization of this period, to estimate their toxicities as given in Table 2.

Method no. 2

A large excess of the compound (either 100 or 1000 $mg.l^{-1}$) is stirred with water for 24 hrs, and the resulting solution is allowed to stand for 4 or 24 hrs (depending on the nature of the compound) during which the excess

separates, leaving behind a saturated aqueous phase, or so it is hoped. The aqueous solution is then drained off through the stopcock (Fig. 1). The solutions so prepared were only used for the purpose of showing that a saturated solution was not acutely toxic.

For both methods the test vessels were almost completely filled with solutions by running the latter through the stop cock at the bottom of each mixing flask (Fig. 1). In addition One-hundred ml aliquots were in the same way run into glass-stoppered 100 ml bottles for analysis of those solutions whose concentration had to be determined.
The tests on paraffins with daphnids were conducted (sometimes in duplicate) in 250 ml conical flasks closed with glass stoppers, almost completely filled with the test solution and with 25 daphnids per flask. The mobile and immobile daphnids were counted after 48 hrs and, where appropriate, a sample of the solution was then taken for analysis.
The tests on paraffins with gammarids and mysid shrimps were conducted in closed scintillation vials with a volume of about 20 ml, each containing only one animal, and almost completely filled with test solution. Ten vials were used for each test solution. Animals surviving after 24h were transferred to fresh test solutions prepared in the same way. This procedure was repeated to add up to a total duration of 96h. Samples of solution were taken for analysis, where appropriate, after 24 hrs.
The tests with alkylate gasoline and 2-methyl-2-propene-1-ol were conducted as described in the appropriate sheets of Annexe A.
The pH and oxygen concentrations of all test solutions were monitored.

Results are quoted only for these tests in which a normal dose-response curve was obtained. For the paraffins the concentrations in water were measured and the results are in addition only quoted when these concentrations were below or equal to their theoretical aqueous solubility and were initially closest to the concentrations that could be expected from the dosage and theoretical water solubility.

Most of the tests were conducted two or three times. The results were as a rule consistent, and were pooled for calculation of EC50 and LC50 values. Repeating the tests was considered necessary because, even though the results were consistent, they were less reproducible than those of tests of non-volatile, stable compounds.

The concentrations tested are given in Annexe A for the compounds listed in Table 1; for the paraffins they are listed in Table 3.

2.4 CHEMICAL ANALYSIS OF PARAFFINS

The components of the paraffins in the aqueous phase were determined as follows: 100 ml samples were taken in glass bottles, two ml of carbon disulphide were added. The resulting mixture was shaken for about 3 min. After separation the solvent layer was drawn off and analyzed by gas chromatography with an apolar capillary column and flame ionization as detection; the temperature programme was: 5 min at 40°C followed by a rise of 2°C/min up to 160°C.
Identification was based on retention time.

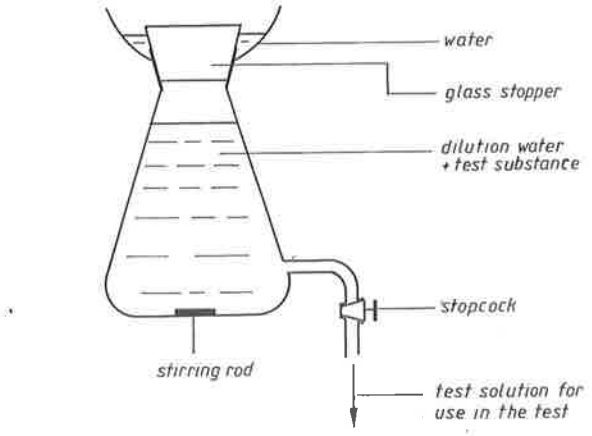

Fig. 1. Vessel for preparation of test solution.

2.5 TREATMENT OF THE RESULTS

LC50 values

The effect of a test substance on the mortality of animals is expressed by a quantity denoted as LC50 (= Lethal Concentration, 50%), i.e. the exposure concentration of the substance which would prove lethal to 50% of an infinite population of the exposed animals. The LC50 is qualified according to duration of exposure.

The LC50 values and their confidence interval were calculated by means of a parametric model developed by Kooijman (ref. 4). A summary of this method is given in Appendix 4.

If the concentration-effect relation is not suitable for exact calculations (the number of concentrations causing partial mortality being too small), the LC50 can only be given as being greater than the highest concentration causing no mortality and/or smaller than the lowest concentration causing 100% mortality. For the sake of simplicity, some Tables list the median concentration between those two preceded by 'about'.

EC50 values

For Daphnia immobilization instead of mortality is usually taken as the test criterion, and the effect is expressed as EC50 (= Effective Concentration, 50%) (Appendix 4).

NOEC values

The 'no observed effect concentrations' (NOEC values) are the highest concentrations tested showing no effects (defined below) throughout the exposure time.

The NOEC values were estimated by comparing effects on mortality and swimming activity of the exposed animals with those of the control animals (blanks).

3. RESULTS

The results, expressed as the 96h LC50 values for *C. marinus* or *M. bahia* and 48h EC50 values for *D. magna*, when possible with their confidence intervals, are summarized in Table 1 for the compounds tested, excluding the paraffins.
LC50 values for other experimental periods and the no observed effect concentrations are given in the separate sheets for each compound when available.
Any pH values deviating more than 0.5 from those of the natural seawater controls are given on the sheets (see Annexe A).
The oxygen concentrations were >50% of the saturation levels and are not supposed to have influenced the test results.

Tables 2 and 3 list the results of the tests with paraffins.
Table 2 summarizes the 48h EC50 and 96h LC50 values based on dosed amounts as well as calculated via the initial concentrations as determined by chemical analysis. These LC50 or EC50 values are indicated as L(I)C50 or E(I)C50. The results of the chemical analyses are summarized in Table 3. Only a few test concentrations were determined. Accordingly, the L(I)C50 and E(I)C50 values are only rough estimates. The result of the chemical analysis of a concentration of paraffins closest to the EC50 or LC50 value based on dosed amounts was used to calculate the corresponding E(I)C50 or L(I)C50.
The pH values of the test solutions were about the same as those of the controls (7.5-8.3) and are not supposed to have influenced the test results.
Most test solutions were almost saturated with oxygen; in a few cases the oxygen concentration dropped, but it was always greater than 6.5 $mg.l^{-1}$ and it is not supposed to have influenced the test results.

Table 1 Results of the aquatic toxicity tests for 9 compounds for which column B ratings are lacking L(E)C50 values, $mg.l^{-1}$, based on dosed amounts (~ means about).

Compound	Origin	Purity	Normal dosage	Via WSF	Special dosage	Open or closed test vessel	48h EC50 *D. magna*	96 LC50 *C. mar.*	96 LC50 *N. bahia*	Proposed column B rating
2-methyl-3-butyn-2-ol or methylbutynol	Aldrich Chemie	GC ca. 98%	x			closed	~1800		436	1
			x			open		359		
methyl heptyl ketone (= 2-nonanone)	Ega-Chemie	GC ca. 99%			x[1]	closed	~ 10	7.5	4.5	3
alkylate gasoline for aviation	B.V. Handelslab. v.h. 'Dr. A. Verwey', Rotterdam			x		closed	-	4.2	1.8	3
alpha, alpha, alpha-tris (hydroxymethyl)-methylamine or 2-amino-2-hydroxymethyl 1,3-propanediol	Aldrich Chemie	99,8 + %	x			open	~1000			1
			x			closed*		1311	888	
N,N-dimethylacetamide	Ega-Chemie	GC 99%	x			open	>1000	>1000		1
			x			closed*			966	
dimethyl succinate (Bersteinsauerdi-methylester)	Aldrich Chemie	ca. 97%	x			open	>1000	331		1
			x			closed*			110	
2-methyl-2-propene-1-ol	Aldrich Chemie	ca. 98%	x			open	37			
				x		open	23			
			x			open		341		
				x		closed		<<10		4
					x[2]	closed			0.5	
					x[1]	closed			<<1	
sodium bromate	Aldrich Chemie	99 + %	x			open	~ 560	302		1
						closed*			~240	
dimethyl glutarate	Aldrich Chemie	98%	x			open	~ 56	21		
			x			closed*			6.0	3
					x[2]	closed*			8.4	

* Not closed because we thought volatility to be a major problem but because of ease of handling the small test vessels.

[1]) The dosed amounts were stirred for 4h with the dilution water, just prior to introduction of the test animals (no period for seperation, the test compound was dissolved at the dosed levels within 4h).

[2]) Same as [1]), but stirred for 1h.

Table 2 Results of aquatic toxicity tests with paraffins expressed in EC50 or LC50 values (confidence interval in brackets).

Test compound			based on dosed amounts, $mg.l^{-1}$			based on initial concentrations as determined by GC, $mg.l^{-1}$			Numbers of test series carried out	
C	Name	S[1]) $mg.l^{-1}$	48h EC50 *D. magna* (fresh water)	96h LC50 *C. marinus* (seawater)	96h LC50 *N. bahia* (seawater)	48h E(I)C50 *D. magna* (fresh water)	96h L(I)C50 *C. marinus* (seawater)	96h L(I)C50 *N. bahia* (seawater)	*D. magna*	*C. mar.* & *N. bahia*
5	pentane	38	9.1 8.5 - 9.7	10.5 9.5 - 11.6	10.2 9.3 - 11.2	2.7	3.6	3.6	4	3
	isopentane		~4.2	~10	~10	2.3	3.2	3.2	2	2
6	hexane (mixed isomers)	≥9.5	3.2 (3.0 - 3.4)			1.1			4	
	n-hexane	9.5	13.4 (8.5 - 21.1)	~2.4	~2.4	2.1	0.4	0.4	1	1
	isohexane (2-methylpentane)	~13	~4.2	~4.2	~4.2	0.8	0.8	0.8	3	1
	cyclohexane	55	~2.4	3.1 (0.1 - 7.8)	3.1 (1.0 - 9.8)	0.9	2.2	2.2	3	1
7	n-heptane	2.7	3.9 (3.7 - 4.2)	3.1 (1.0 - 9.4)	2.1 (1.7 - 2.5)	1.5	0.2	0.1	4	1
	cycloheptane		0.74	~1.4	~1.4	0.2	1.3	1.3	4	1
8	n-octane	0.66	~S	~S	~S	0.3	0.3	0.3	1	5
	iso-octane (2,3,4-trimethyl-pentane)		~2.4	5.4 (4.3 - 6.7)	2.4	0.4	0.9	0.4	2	1
9	n-nonane	~ 0.2	~S	~S	>S	0.2	0.2	*	6	3
10	n-decane	0.050	>S	>S	>S	*	*	*	6	2
11	n-undecane		>S	>S	>S	*	*	*		1
12	n-dodecane	0.004	>S	>S	>S	*	*	*		1
13	n-tridecane		>S	>S	>S	*	*	*		1
14	n-tetradecane	0.002	>S	>S	>S	*	*	*		1

~ means: 'about'

* means: acute toxicity not reached within the aqueous solubility.

[1]) S (solubility in water) according to ref. 5 and 6.

Table 3 Survey of tests carried out, including results of concentrations tested, checked by chemical analysis for the paraffins.

C	Test compound	S[1]) mg.l^{-1}	Preparation test solutions)	Test animal concerned	Nominal conc. analysed mg.l^{-1}	Initial conc. at start of test mg.l^{-1}	Conc. in test medium after 24h/48h mg.l^{-1}	Conc. range tested with a factor of 1.8 (mg.l^{-1})	Only 100 and/or 1000 mg.l^{-1} tested
5	n-pentane	38	24h stir. + 24h sep.	daphnia	10	3.0	2.4	3.2 - 32	
					18	5.0	-		
			24h stir. + 24h sep.	gam. + mys.	3.2	0.6	0.2	1.0 - 56	
					10	3.6	2.0		
					10	2.7	1.2		
	isopentane		24h stir. + 24h sep.	daphnia	1,8	1,0	0.64	1.0 - 10	
					10	5.6	-		
				gam. + mys.	3.2	0.8	0.4	1.0 - 18	
					10	3.2	1.5		
6	hexane mixed isom.	≧9.5	24h stir. + 24h sep.	daphnia	3.2	1.1	0.9	1.8 - 18	
					18	6.5	-		
	n-hexane	9.5	24h stir. + 24h sep.	daphnia	3.2	0.5	-	1.0 - 10	
			24h stir. + 24h sep.	gam. + mysis	3.2	0.4	<0.005	1.0 - 10	
	isohexane	~13	24h stir. + 24h sep.	daphnia	1.8	0.3	0.2	1.0 - 10	
					10	3.4	-		
			24h stir. + 24h sep.	gamm. + mysis	3.2	0.6	0.3	1.0 - 10	
	cyclohexane	55	24h stir. + 24h sep.	daphnia	1.8	0.7	0.6	1.0 - 10	
					10	7.2	-		
			24h stir. + 24h sep.	gam. + mysis	1.8	1.3	0.9	1.0 - 10	
7	n-heptane	2.7	24h stir. + 24h sep.	daphnia	0.32	0.04	-	0.32- 18	
					1.0	0.04	-		
					3.2	0.5	-		
					5.6	2.1	1.7		
					10	2.2	-		
			24h stir. + 24h sep.	gam. + mysis	0.32	0.003	-	0.32- 10	
					1.0	0.07	-		
					3.2	0.2	-		
	cycloheptane		24h stir. + 24h sep.	daphnia	0.32	0.1	0.08	0.18- 5.6	
					1.0	0.7	0.6		
			24h stir. + 24h sep.	gam. + mysis	1.0	0.9	0.5	0.56- 5.6	

Table 3 Survey of tests carried out, including results of concentrations tested, checked by chemical analysis for the paraffins (continued)

C	Test compound	S[1]) mg.l^{-1}	Preparation test solutions[2])	Test animal concerned	Nominal conc. analysed mg.l^{-1}	Initial conc. at start of test mg.l^{-1}	Conc. in test medium after 24h/48h mg.l^{-1}	Conc. range tested with a factor of 1.8 (mg.l^{-1})	Only 100 and/or 1000 mg.l^{-1} tested
8	n-octane	0.66	24h stir. + 24h sep.	daphnia	-	-	-	1.0 - 5.6	
			24h stir. + 24h sep.	gam. + mysis	3.2	<0.005	-	0.32- 10	
					100	<0.005	-		x
					1000	<0.005	-		x
			24h stir. + 4h sep.	gam. + mysis	3.2	0.3	<0.005	0.32- 10	
					100	0.3	-		x
					1000	0.3	-		x
	iso-octane		24h stir. + 24h sep.	daphnia	-	-	-	1.0 - 10	
			24h stir. + 24h sep.	gam. + mysis	1.8	0.3	0.1	1.0 - 10	
9	n-nonane	~0.2	24h stir. + 24h sep.	daphnia	10	0.2	0.004	0.56- 18	
					100	0.1	-		x
			24h stir. + 24h sep.	gam. + mysis	100	<0.005	-		x
					1000	<0.005	-		x
			24h stir. + 4h sep.	gam. + mysis	100	0.1	-		x
					1000	0.1	-		x
10	n-decane	0.050	24h stir. + 4h sep.	daphnia	0.56	<0.003	-	0.56-100	
					1.0	<0.003	-		
					100	<0.003	-		x
					1000	<0.003	-		x
			24h stir. + 24h sep.	mysis + gam.	100	<0.005	-		x
					1000	<0.005	-		x
			24h stir + 4h sep.	mysis + gam.	100	<0.005	-		x
					1000	<0.005	-		x
11	n-undecane		24h stir. + 24h sep.	daphnia	1000	<0.005	-		x
			24h stir. + 4h sep.	mysis + gam.	1000	<0.005	-		x
12	n-dodecane	0.004	24h stir. + 4h sep.	mysis + gam.	1000	<0.005	-		x
13	n-tridecane		24h stir. + 4h sep.	mysis + gam.	1000	<0.005	-		x
14	n-tetradecane	0.002	24h stir. + 4h sep.	mysis + gam.	1000	<0.005	-		x

[1]) S (solubility in water) according to ref. 5 and 6.

[2]) 'stir' means stirring and 'sep' means separation; see section 2.3.3

4. DISCUSSION

4.1 COMPOUNDS LISTED IN TABLE 1

Of the compounds listed in Table 1, those of low volatility and sufficiently water solubility could be tested without difficulty. They readily gave aqueous solutions of suitable concentrations and could in principle be tested in open vessels (methylbutynol; 2-amino-2-hydoxymethyl 1,3-propanediol; N,N-dimethylacetamide and sodium bromate).
Dimethyl succinate and dimethyl glutarate could also be dosed directly. They may, however, have undergone some (unavoidable) hydrolysis, although no appreciable drop of pH was noted.
2-Nonanone was found to be only slightly soluble in water, and the amounts dosed were stirred with dilution water for 4h before the test animals were introduced. It had to be tested in closed vessels to avoid loss by evaporation.
Because of its high volatility and low water solubility, alkylate gasoline was difficult to handle. Test solutions were prepared via the 'Water Soluble Fraction' (WSF) method described in section § 2.3.3, and the test was carried out in closed vessels.
We experienced problems in testing only with 2-methyl-2-propene-1-ol, the physical properties of which were unknown, and which appeared to be less soluble than expected. It was far more toxic when tested in closed vessels than in open ones. For alcohols, the difference between these two methods usually is no more than a factor of about 10.
What has been said below (sect. 4.2) about the possible underestimation of the acute aquatic toxicity of sparingly soluble, volatile substances such as paraffins may also apply to the alkylate gasoline and 2-methyl-2-propene-1-ol.

4.2 PARAFFINS

Clearly, the actual concentrations of the paraffins were always lower than those expected from the dosed amounts. During the 24h or 48h exposure periods without renewal, these concentrations became even lower. LC50 or EC50 values based on dosed amounts therefore underestimate the toxicity of the paraffins. Only a few of the concentrations tested could be determined by chemical analysis. The results of those determinations nearest to the

LC50 value (based on dosed amounts) were used to calculate L(I)C50 values based in the initial concentrations supposed to have been present. Since the initial concentrations fell during the test, the L(I)C50 values calculated in this manner may underestimate the toxicity still further. It is not known whether the desired concentrations of paraffins, particularly those corresponding to their specified water solubilities, were reached at all or whether they were lost, e.g. during the filling of the flasks. For n-octane and n-nonane, however, it was shown that higher concentrations, corresponding to their solubilities, were reached after 24h of stirring followed by 4h rather than 24h of separation. The solutions that had stood for 4h were toxic to the animals, whereas those that had stood for 24h were harmless!
These two compounds were acutely toxic at about their maximum solubilities in (sea)water, these being 0.6 and 0.2 $mg.l^{-1}$, respectively, corresponding to a '4' in column B. Saturated solutions in (sea)water of decane and higher paraffins were harmless to the test animals after 24h of stirring with a large overdose of paraffin (1 g per l) and a short separation period. These compounds can, with reasonable certainty, be assumed to be not acutely toxic ('0' in colomn B) because they are not soluble enough in (sea)water. The ratings for the pentanes, hexanes and heptanes are questionable. On basis of dosed amounts, a '3' in column B would be appropriate, but on basis of the probable concentrations during testing, n- and iso-hexane and n-heptane would have to be rated '4' in column B.
The cyclic compounds, which are more soluble than their normal or iso analogues, were somewhat less toxic, as is to be expected from their higher solubility.
The results with the paraffins are in accordance with those of Bobra et al. (ref. 5).

The 'Hazard in a natural environment' of such compounds will of course depend on their residence time as well.
Such factors, however, are no part of the determination of LC50-values, which have to be based on constant and known concentration during testing.

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APPENDIX 1

GUIDELINES FOR THE CATEGORIZATION OF NOXIOUS LIQUID SUBSTANCES

Category A — Substances which are bioaccumulated and liable to produce a hazard to aquatic life or human health; or which are highly toxic to aquatic life (as expressed by a Hazard Rating 4, defined by a TLm less than 1 ppm); and additionally certain substances which are moderately toxic to aquatic life (as expressed by a Hazard Rating 3, defined by a TLm of 1 or more, but less than 10 ppm) when particular weight is given to additional factors in the hazard profile or to special characteristics of the substance.

Category B — Substances which are bioaccumulated with a short retention of the order of one week or less; or which are liable to produce tainting of the sea food; or which are moderately toxic to aquatic life (as expressed by a Hazard Rating 3, defined by a TLm of 1 ppm or more, but less than 10 ppm); and additionally certain substances which are slightly toxic to aquatic life (as expressed by a Hazard Rating 2, defined by a TLm of 10 ppm or more, but less than 100 ppm) when particular weight is given to additional factors in the hazard profile or to special characteristics of the substance.

Category C — Substances which are slightly toxic to aquatic life (as expressed by a Hazard Rating 2, defined by a TLm of 10 or more, but less than 100 ppm); and additionally certain substances which are practically non-toxic to aquatic life (as expressed by a Hazard Rating 1, defined by a TLm of 100 ppm or more, but less than 1,000 ppm) when particular weight is given to additional factors in the hazard profile or to special characteristics of the substance.

Category D — Substances which are practically non-toxic to aquatic life, (as expressed by a Hazard Rating 1, defined by a TLm of 100 ppm or more, but less than 1,000 ppm); or causing deposits blanketing the seafloor with a high biochemical oxygen demand (BOD); or highly hazardous to human health, with an LD_{50} of less than 5 mg/kg; or produce moderate reduction of amenities because of persistency, smell or poisonous or irritant characteristics, possibly interfering with use of beaches; or moderately hazardous to human health, with an LD_{50} of 5 mg/kg or more, but less than 50 mg/kg and produce slight reduction of amenities.

Other Liquid Substances (for the purposes of Regulation 4 of this Annex) — Substances other than those categorized in Categories A, B, C and D above.

APPENDIX 2

ABBREVIATED LEGEND TO THE HAZARD PROFILES

Column A - Bioaccumulation

+	Bioaccumulated to significant extent and known to produce a hazard to aquatic life or human health
Z	Bioaccumulated with attendant risk to aquatic organisms or human health, however with short retention of the order of one week or less
T	Bioaccumulated, liable to produce tainting of seafood
O	No evidence to support one of the above ratings (+, Z, T)

Column B - Damage to living resources

Ratings		96 hr. TLm (= 96h LC50)
4	Highly toxic	less than 1 mg/l
3	Moderately toxic	1-10 mg/l
2	Slightly toxic	10-100 mg/l
1	Practically non-toxic	100-1000 mg/l
0	Non-hazardous	greater than 1000 mg/l
D	Substance likely to blanket the sea-bed	
BOD	Substance with oxygen demand.	

Column C - Hazard to human health, oral intake

Ratings		LD50 (laboratory mammal)
4	Highly hazardous	less than 5 mg/kg
3	Moderately hazardous	5-50 mg/kg
2	Slightly hazardous	50-500 mg/kg
1	Practically non-hazardous	500-5000 mg/kg
0	Non-hazardous	greater than 5000 mg/kg

Column D - Hazard to human health, skin contact and inhalation

II	Hazardous
I	Slightly hazardous
0	Non-hazardous

Column E - Reduction of amenities

Ratings

XXX Highly objectionable because of persistency, smell or poisonous or irritant characteristics; as a result beaches liable to be closed; also used when there is clear evidence that the substance is a human carcinogen.

XX Moderately objectionable because of the above characteristics, but short-term effects leading only to temporary interference with use of beaches; also used when there is credible scientific evidence that the substance is an animal carcinogen or when the substance causes another serious health effect, but where there is no clear evidence to indicate that the material has caused cancer in humans.

X Slightly objectionable, non interference with use of beaches.

0 No problem.

Other Symbols

Ratings in brackets, ( ), indicate insufficient data available to the GESAMP experts on specific substances, hence extrapolation was required.

NA Not applicable (e.g. if gases)

\- Indicates data was not available to the GESAMP Working Group.

Note: The descriptive terms such as highly toxic, non-hazardous etc., were used by the original panel for the purposes of the 1973 International Conference on Marine Pollution. They have no particular significance in terms of hazard posed outside the particular circumstances addressed by that Conference and IMCO Sub-Committees, i.e. marine pollution as a consequence of discharges or spillages from ships.

APPENDIX 3

LIST OF COMPOUNDS RECOMMENDED BY SPONSOR FOR TESTING ON AQUATIC TOXICITY

(taken from Annexe 7 to BCH 15/WP5 and the list 'Substances known or proposed to be moved in bulk but not yet evaluated by GESAMP or profiles incomplete' from 21 May 1986 by M.D. Morrisette).

Compound	Status
Diethylene glycol monophenyl ether	not available
N,N-Dimethylaniline styrenic solution	not available
Methylbutynol	tested
Methyl heptyl ketone (= 2-nonanone)	tested
Alkylate gasoline for aviation	tested
2-amino-2-hydroxymethyl-1,3-propanediol	tested
N,N-dimethylacetamide	tested
Dimethyl succinate	tested
2-methyl-2-propene-1-ol (Methyl allyl alcohol)	tested
Sodium bromate	tested
Dimethyl glutarate	tested

APPENDIX 4

ESTIMATION OF THE L(E)C50 AND ITS CONFIDENCE INTERVAL

At a given time, the mortality (immobilization) probability of an individual is supposed to be logistically related to the logarithm of the concentration, i.e.

$$p_i = \frac{1 + p_o e_i}{1 + e_i}$$, where $e_i = \exp\{(\alpha - c_i)/\beta\}$, and

p_i is the mortality (immobilization) probability in the i^{th} concentration

p_o is the mortality (immobilization) probability in concentration o

α is the logarithm of the L(E)C50

β is a parameter inversely proportional to the maximum gradient of the dose response function

c_i is the logarithm of the i^{th} concentration.

The parameters p_o, α and β are estimated from the counts by means of the maximum likelihood method; i.e. the parameter values to be selected maximize the probability of the counts as a function of the three parameters. The variance-covariance matrix of the parameters is estimated by the inverse of the information matrix. The L(E)C50 is now given by

$\exp\{\hat{\alpha} + \frac{1}{2} \text{var}(\hat{\alpha})\}$, and the 95% confidence limits by

$\exp\{\hat{\alpha} \pm 2 \text{var}^{\frac{1}{2}}(\hat{\alpha})\}$.

APPENDIX 5

COMPOSITION OF THE SYNTHETIC MEDIUM (DSWL) USED IN THE TESTS WITH *DAPHNIA MAGNA*

Na^+	1.19	mmol.l^{-1}
K^+	0.20	"
Ca^{2+}	1.36	"
Mg^{2+}	0.73	"
Cl^-	2.72	"
SO_4^{2-}	0.73	"
HCO_3^-	1.39	"

This medium is prepared by addition of several salts to groundwater from a locality near Linschoten.
The groundwater contains several other trace elements ($<<$ 1 mg.l^{-1}).
Media prepared from it have proved to be suitable for growing several species of water organisms.
The equilibrium pH of the medium, after aeration, should be 8.3 - 8.5, but usually is slightly less, namely 8.0 - 8.2.
The hardness, expressed as $CaCO_3$, is about 210 mg.l^{-1}.

ANNEXE A

Determination of the acute aquatic toxicity of 2-methyl-3-butyn-2-ol or methylbutynol, Aldrich Chemie, about 98% (GC).

Test compound : 2-methyl-3-butyn-2-ol or methylbutynol

Concentrations tested : 0 1000 3200 $mg.l^{-1}$

Preparation of test solutions : The necessary amounts were pipetted and added separately with stirring to 1 l of standard fresh water (Appendix 5) in conical flasks. Then the 250 ml test vessels were filled with these solutions and the test animals were introduced. Apparently, all dosed amounts were soluble, no undissolved compound being visible during the test.

Chemical analysis : Test solutions were not analysed for the test compound

Test animal : *Daphnia magna* (Crustacea, Cladocera) less than 24 h old at the start of the test, from a laboratory culture in standard fresh water (Appendix 5).

Test conditions : Per test concentration: 25 animals in 250 ml of test solution, in a glass-stoppered conical flask.

Temperature	:	20°C
Aeration	:	none
Food	:	none
Renewal of test medium	:	none
Test in	:	singular
Test duration	:	48 h

Results:

Time h	EC50 $mg.l^{-1}$	confidence interval $mg.l^{-1}$	'EC0' $mg.l^{-1}$	'EC100' $mg.l^{-1}$
24	>1000 <3200		1000	3200
48	>1000 <3200		1000	3200

'No observed effect concentration' after 48 h exposure: 1000 $mg.l^{-1}$

Effect: condition of test animals compared to the controls (visual estimation)

ANNEXE A

Determination of the acute aquatic toxicity of 2-methyl-3-butyn-2-ol or methylbutynol, Aldrich Chemie, about 98% (GC).

Test compound : 2-methyl-3-butyn-2-ol or methylbutynol

Concentrations tested : 0 100 320 1000 $mg.l^{-1}$

Preparation of test solutions : The necessary amounts were pipetted and added separately with stirring to 1 l of natural seawater in glass beakers and the test animals were introduced. Apparently, all dosed amounts were soluble, no undissolved compound being visible during the test.

Chemical analysis : Test solutions were not analysed for the test compound

Test animal : *Chaetogammarus marinus* (Crustacea, Amphipoda) Length 5±1 mm, from a laboratory culture in natural seawater (S = 2.8%).

Test conditions : Per test concentration: 10 animals in 1000 ml of test solution in a glass beaker, covered with a watch glass.

Temperature	:	15°C
Aeration	:	none
Food	:	some Fucus spec.
Renewal of test medium	:	once a day
Test in	:	duplicate
Test duration	:	96 h

Results:

Time h	LC50 $mg.l^{-1}$	confidence interval $mg.l^{-1}$	'LC0' $mg.l^{-1}$	'LC100' $mg.l^{-1}$
24	1045		320	>1000
48	968		320	>1000
72	913		320	>1000
96	359		100	1000

'No observed effect concentration' after 96 h exposure: 100 $mg.l^{-1}$

Effect: condition of test animals compared to the controls (visual estimation)

ANNEXE A

Determination of the acute aquatic toxicity of 2-methyl-3-butyn-2-ol or methylbutynol, Aldrich Chemie, about 98% (GC).

Test compound : 2-methyl-3-butyn-2-ol or methylbutynol

Concentrations tested : 0 100 180 320 560 1000 $mg.l^{-1}$

Preparation of test solutions : The necessary amounts were pipetted and added separately with stirring to 1 l of natural seawater in glass beakers. Then the 20 ml test vessels were filled with these solutions and the test animals were introduced. Apparently, all dosed amounts were soluble, no undissolved compound being visible during the test.

Chemical analysis : Test solutions were not analysed for the test compound

Test animal : *Mysidopsis bahia* (Crustacea, Mysidacea) about four weeks old at the start of the test (6±1 mm), from a laboratory culture in natural seawater (S = 2.8%)

Test conditions : Per test concentration: 10 animals each in about 20 ml of test solution in a scintillation flask, closed with a screw-cap.

Temperature	:	20°C
Aeration	:	none
Food	:	*Artemia nauplii*
Renewal of test medium	:	once a day
Test duration	:	96 h

Results:

Time h	LC50 $mg.l^{-1}$	confidence interval $mg.l^{-1}$	'LC0' $mg.l^{-1}$	'LC100' $mg.l^{-1}$
24	3043		560	>1000
48	692	476 - 1006	320	>1000
72	461	331 - 642	100	>1000
96	436	314 - 607	100	>1000

'No observed effect concentration' after 48 h exposure: 100 $mg.l^{-1}$

Effect: condition of test animals compared to the controls (visual estimation)

ANNEXE A

Determination of the acute aquatic toxicity of methyl heptyl ketone (2-nonanone) , Aldrich Chemie, about 99% (GC).

Test compound : methyl heptyl ketone

Concentrations tested : 0 1.0 3.2 10 $mg.l^{-1}$

Preparation of test solutions : The necessary amounts were pipetted, added separately to 2.3 l of standard fresh water (Appendix 5) and stirred for 24 h in glass-stoppered conical-flasks with a tap. The stops were surrounded with water in a glass-collar. Then these flasks were settled down for 24 h. Then the water-phase was drawn off into 250 ml test vessels before the test animals were introduced. Apparently, all dosed amounts were soluble, no undissolved compound being visible during the test.

Chemical analysis : Test solutions were not analysed for the test compound

Test animal : *Daphnia magna* (Crustacea, Cladocera) less than 24 h old at the start of the test, from a laboratory culture in standard fresh water (Appendix 5)

Test conditions : Per test concentration: 25 animals in 250 ml of test solution, in a glass-stoppered conical flask.

Temperature	:	20°C
Aeration	:	none
Food	:	none
Renewal of test medium	:	none
Test in	:	singular
Test duration	:	48 h

Results:

Time h	EC50 $mg.l^{-1}$	confidence interval $mg.l^{-1}$	'EC0' $mg.l^{-1}$	'EC100' $mg.l^{-1}$
24	>10		≧10	>10
48	about 10		> 3.2	>10

'No observed effect concentration' after 48 h exposure: 3.2 $mg.l^{-1}$

Effect: condition of test animals compared to the controls (visual estimation)

ANNEXE A

Determination of the acute aquatic toxicity of methyl heptyl ketone (2-nonanone), Aldrich Chemie, about 99% (GC).

Test compound : methyl heptyl ketone

Concentrations tested : 0 1.8 3.2 5.6 10 18 $mg.l^{-1}$

Preparation of test solutions : The necessary amounts were pipetted, added separately to 2.3 l of natural seawater and stirred for 4 h in glass-stoppered conical-flasks with a tap. The stops were surrounded with water in a glass-collar. Then the water-phase was drawn off into 20 ml test vessels and the test animals were introduced. Apparently, all dosed amounts were soluble, no undissolved compound being visible during the test.

Chemical analysis : Test solutions were not analysed for the test compound

Test animal : *Chaetogammarus marinus* (Crustacea, Amphipoda)
Length 5±1 mm, from a laboratory culture in natural seawater (S = 2.8%).

Test conditions : Per test concentration: 10 animals each in about 20 ml of test solution in a scintillation flask, closed with a screw-cap.

Temperature	:	15°C
Aeration	:	none
Food	:	none
Renewal of test medium	:	once a day
Test duration	:	96 h

Results:

Time h	LC50 $mg.l^{-1}$	confidence interval $mg.l^{-1}$	'LC0' $mg.l^{-1}$	'LC100' $mg.l^{-1}$
24	21.7	13.9 - 33.8	10	>18
48	13.3	10.7 - 16.4	10	18
72	8.9	7.3 - 10.9	3.2	18
96	7.5	6.1 - 9.2	3.2	18

'No observed effect concentration' after 48 h exposure: 3.2 $mg.l^{-1}$

Effect: condition of test animals compared to the controls (visual estimation)

ANNEXE A

Determination of the acute aquatic toxicity of methyl heptyl ketone (2-nonanone), Ega Chemie, about 99% (GC).

Test compound : methyl heptyl ketone

Concentrations tested : 0 1.0 1.8 3.2 5.6 10 $mg.l^{-1}$

Preparation of test solutions : The necessary amounts were pipetted, added separately to 2.3 l of natural seawater and stirred for 4 h in glass-stoppered conical-flasks with a tap. The stops were surrounded with water in a glass-collar. Then the water-phase was drawn off into 20 ml test vessels and the test animals were introduced. Apparently, all dosed amounts were soluble, no undissolved compound being visible during the test.

Chemical analysis : Test solutions were not analysed for the test compound

Test animal : *Mysidopsis bahia* (Crustacea, Mysidacea) about four weeks old at the start of the test (6±1 mm), from a laboratory culture in natural seawater (S = 2.8%)

Test conditions : Per test concentration: 10 animals each in about 20 ml of test solution in a scintillation flask, closed with a screw-cap.

Temperature	:	20°C
Aeration	:	none
Food	:	*Artemia nauplii*
Renewal of test medium	:	once a day
Test duration	:	96 h

Results:

Time h	LC50 $mg.l^{-1}$	confidence interval $mg.l^{-1}$	'LC0' $mg.l^{-1}$	'LC100' $mg.l^{-1}$
24	>10	-	≧10	>10
48	14.0	6.8 - 28.8	5.6	>10
72	6.9	5.2 - 9.1	3.2	>10
96	4.5	3.5 - 5.9	1.0	>10

'No observed effect concentration' after 48 h exposure: 1.0 $mg.l^{-1}$

Effect: condition of test animals compared to the controls (visual estimation)

ANNEXE A

Determination of the acute aquatic toxicity of alkylate gasoline for aviation, B.V. Handelslaboratorium v.h. 'Dr. A. Verwey', Rotterdam.

Test compound : alkylate gasoline for aviation

Concentrations tested : 0 0.56 1.0 1.8 3.2 5.6 $mg.l^{-1}$

Preparation of test solutions : The necessary amounts were pipetted, added separately to 2.3 l of natural seawater and stirred for 24 h in glass-stoppered conical-flasks with a tap. The stops were surrounded with water in a glass-collar. Then these flasks were settled down for 24 h. Then the water-phase was drawn off into 20 ml test vessels and the test animals were introduced. Apperently, all dosed amounts were soluble, no undissolved compound being visible during the test.

Chemical analysis : Test solutions were not analysed for the test compound

Test animal : *Chaetogammarus marinus* (Crustacea, Amphipoda)
Length 5±1 mm, from a laboratory culture in natural seawater (S = 2.8%)

Test conditions : Per test concentration: 10 animals each in about 20 ml of test solution in a scintillation flask, closed with a screw-cap.

Temperature	:	15°C
Aeration	:	none
Food	:	none
Renewal of test medium	:	once a day
Test duration	:	96 h

Results:

Time h	LC50 $mg.l^{-1}$	confidence interval $mg.l^{-1}$	'LC0' $mg.l^{-1}$	'LC100' $mg.l^{-1}$
24	7.9	3.5 - 17.6	1.0	>5.6
48	5.9	3.5 - 10.0	1.0	>5.6
72			1.0	>5.6
96	4.2	2.9 - 6.3	1.0	>5.6

'No observed effect concentration' after 48 h exposure: 1.0 $mg.l^{-1}$

Effect: condition of test animals compared to the controls (visual estimation)

ANNEXE A

Determination of the acute aquatic toxicity of alkylate gasoline for aviation, B.V. Handelslaboratorium v.h. 'Dr. A. Verwey', Rotterdam.

Test compound : alkylate gasoline for aviation

Concentrations tested : 0 0.56 1.0 1.8 3.2 5.6 $mg.l^{-1}$

Preparation of test solutions : The necessary amounts were pipetted, added separately to 2.3 l of natural seawater and stirred for 24 h in glass-stoppered conical-flasks with a tap. The stops were surrounded with water in a glass-collar. Then these flasks were settled down for 24 h. Then the water-phase was drawn off into 20 ml test vessels and the test animals were introduced. Apparently, all dosed amounts were soluble, no undissolved compound being visible during the test.

Chemical analysis : Test solutions were not analysed for the test compound

Test animal : *Mysidopsis bahia* (Crustacea, Mysidacea) about four weeks old at the start of the test (6±1 mm), from a laboratory culture in natural seawater (S = 2.8%)

Test conditions : Per test concentration: 10 animals each in about 20 ml of test solution in a scintillation flask, closed with a screw-cap.

Temperature	:	20°C
Aeration	:	none
Food	:	*Artemia nauplii*
Renewal of test medium	:	once a day
Test duration	:	96 h

Results:

Time h	LC50 $mg.l^{-1}$	confidence interval $mg.l^{-1}$	'LC0' $mg.l^{-1}$	'LC100' $mg.l^{-1}$
24	4.3	3.4 - 5.5	3.2	5.6
48	2.0	1.6 - 2.5	0.56	5.6
72			0.56	3.2
96	1.8	1.4 - 2.3	<0.56	3.2

'No observed effect concentration' after 48 h exposure: < 0.56 $mg.l^{-1}$

Effect: condition of test animals compared to the controls (visual estimation)

ANNEXE A

Determination of the acute aquatic toxicity of alpha, alpha, alpha-tris (hydroxymethyl)-methyl-amine or 2-amino-2-hydroxymethyl 1,3-propanediol, Aldrich Chemie, 99,8 + %.

Test compound : alpha, alpha, alpha-tris (hydroxymethyl)-methyl-amine or 2-amino-2-hydroxymethyl 1,3-propanediol

Concentrations tested : 0 1.0 3.2 10 32 100 320 1000 $mg.l^{-1}$

Preparation of test solutions : 2 g of test compound was weighed and dissolved in 2 l of standard fresh water (Appendix 5). From this solution 1.0, 3.2, 10, 32, 100, 320 and 1000 ml were diluted to 1000 ml with fresh water. Then the 250 ml test vessels were filled with these solutions and the test animals were introduced. Apparently, all dosed amounts were soluble, no undissolved compound being visible during the test.

Chemical analysis : Test solutions were not analysed for the test compound

Test animal : *Daphnia magna* (Crustacea, Cladocera) less than 24 h old at the start of the test, from a laboratory culture in standard fresh water (Appendix 5)

Test conditions : Per test concentration: 10 animals in 250 ml of test solution in a glass beaker, covered with a watch glass.

Temperature	:	20°C
Aeration	:	none
Food	:	none
Renewal of test medium	:	none
Test in	:	duplicate
Test duration	:	48 h

Results:

Time h	EC50 $mg.l^{-1}$	confidence interval $mg.l^{-1}$	'EC0' $mg.l^{-1}$	'EC100' $mg.l^{-1}$
24	>320		320	>1000
48	~1000		320	>1000

'No observed effect concentration' after 48 h exposure: 320 $mg.l^{-1}$

Effect: condition of test animals compared to the controls (visual estimation)

Remarks:	Conc. ($mg.l^{-1}$)	pH (t = 0 h)
	0	7.8
	1.0	7.8
	3.2	7.8
	10	7.9
	32	8.1
	100	8.5
	320	8.8
	1000	9.1

ANNEXE A

Determination of the acute aquatic toxicity of alpha, alpha, alpha-tris (hydroxymethyl)-methyl-amine or 2-amino-2-hydroxymethyl 1,3-propanediol, Aldrich Chemie, 99,8 + %.

Test compound : alpha, alpha, alpha-tris (hydroxymethyl)-methyl-amine or 2-amino-2-hydroxymethyl 1,3-propanediol

Concentrations tested : 0 560 1000 1800 3200 $mg.l^{-1}$

Preparation of test solutions : 1.6 g of test compound was weighed and dissolved in 500 ml of natural seawater. From this solution 45, 80, 140, and 250 ml were diluted to 250 ml with seawater. Then the 20 ml test vessels were filled with these solutions and the test animals were introduced. Apparently, all dosed amounts were soluble, no undissolved compound being visible during the test.

Chemical analysis : Test solutions were not analysed for the test compound

Test animal : *Chaetogammarus marinus* (Crustacea, Amphipoda)
Length 5±1 mm, from a laboratory culture in natural seawater (S = 2.8%)

Test conditions : Per test concentration: 10 animals each in about 20 ml of test solution in a scintillation flask, closed with a screw-cap.

Temperature	:	15°C
Aeration	:	none
Food	:	none
Renewal of test medium	:	once a day
Test duration	:	96 h

Results:

Time h	LC50 $mg.l^{-1}$	confidence interval $mg.l^{-1}$	'LC0' $mg.l^{-1}$	'LC100' $mg.l^{-1}$
24	4130	2208 - 7725	1800	>3200
48	2916	2365 - 3596	1000	>3200
72	1685	1379 - 2059	1000	3200
96	1311	1062 - 1619	560	3200

'No observed effect concentration' after 96 h exposure: 560 $mg.l^{-1}$
Effect: condition of test animals compared to the controls (visual estimation)

Remarks:	Conc. ($mg.l^{-1}$)	pH (t = 0 h)
	0	8.1
	560	9.1
	1000	9.2
	1800	9.4
	3200	9.6

ANNEXE A

Determination of the acute aquatic toxicity of alpha, alpha, alpha-tris (hydroxymethyl)-methyl-amine or 2-amino-2-hydroxymethyl 1,3-propanediol, Aldrich Chemie, 99,8 + %.

Test compound : alpha, alpha, alpha-tris (hydroxymethyl)-methyl-amine or 2-amino-2-hydroxymethyl 1,3-propanediol

Concentrations tested : 0 560 1000 1800 3200 $mg.l^{-1}$

Preparation of test solutions : 1.6 g of test compound was weighed and dissolved in 500 ml of natural seawater. From this solution 45, 80, 140, and 250 ml were diluted to 250 ml with seawater. Then the 20 ml test vessels were filled with these solutions and the test animals were introduced. Apparently, all dosed amounts were soluble, no undissolved compound being visible during the test.

Chemical analysis : Test solutions were not analysed for the test compound

Test animal : *Mysidopsis bahia* (Crustacea, Mysidacea) about four weeks old at the start of the test, (6±1 mm), from a laboratory culture in natural seawater (S = 2.8%)

Test conditions : Per test concentration: 10 animals each in about 20 ml of test solution in a scintillation flask, closed with a screw-cap.

Temperature	:	20°C
Aeration	:	none
Food	:	*Artemia nauplii*
Renewal of test medium	:	once a day
Test duration	:	96 h

Results:

Time h	LC50 $mg.l^{-1}$	confidence interval $mg.l^{-1}$	'LC0' $mg.l^{-1}$	'LC100' $mg.l^{-1}$
24	2335	1881 - 2900	560	>3200
48	1447	1169 - 1792	560	3200
72	1148	930 - 1416	560	1800
96	888	719 - 1095	560	1800

'No observed effect concentration' after 96 h exposure: 560 $mg.l^{-1}$
Effect: condition of test animals compared to the controls (visual estimation)

Remarks:	Conc. ($mg.l^{-1}$)	pH (t = 0 h)
	0	7.9
	560	8.9
	1000	9.1
	1800	9.3
	3200	9.5

ANNEXE A

Determination of the acute aquatic toxicity of N,N-dimethylacetamide, Ega Chemie, 99% (GC).

Test compound : N,N-dimethylacetamide

Concentrations tested : 0 1.0 3.2 10 32 100 320 1000 $mg.l^{-1}$

Preparation of test solutions : The necessary amounts were pipetted and added separately with stirring to 1 l of standard fresh water (Appendix 5) in a glass beaker. Then the 250 ml test vessels were filled with these solutions and the test animals were introduced. Apparently, all dosed amounts were soluble, no undissolved compound being visible during the test.

Chemical analysis : Test solutions were not analysed for the test compound

Test animal : *Daphnia magna* (Crustacea, Cladocera) less than 24 h old at the start of the test, from a laboratory culture in standard fresh water (Appendix 5).

Test conditions : Per test concentration: 10 animals in 250 ml of test solution in a glass beaker, covered with a watch glass.

Temperature	:	20°C
Aeration	:	none
Food	:	none
Renewal of test medium	:	none
Test in	:	duplicate
Test duration	:	48 h

Results: No mortality found during the exposure period

Time h	EC50 $mg.l^{-1}$	confidence interval $mg.l^{-1}$	'EC0' $mg.l^{-1}$	'EC100' $mg.l^{-1}$
24	>1000		≧1000	>1000
48	>1000		≧1000	>1000

'No observed effect concentration' after 48 h exposure: ≧ 1000 $mg.l^{-1}$

Effect: condition of test animals compared to the controls (visual estimation)

ANNEXE A

Determination of the acute aquatic toxicity of N,N-dimethylacetamide, Ega Chemie, 99% (GC).

Test compound : N,N-dimethylacetamide

Concentrations tested : 0 320 1000 $mg.l^{-1}$

Preparation of test solutions : The necessary amounts were pipetted and added separately with stirring to 1 l of natural seawater in glass beakers before the test animals were introduced. Apparently, all dosed amounts were soluble, no undissolved compound being visible during the test.

Chemical analysis : Test solutions were not analysed for the test compound

Test animal : *Chaetogammarus marinus* (Crustacea, Amphipoda)
Length 5±1 mm, from a laboratory culture in natural seawater (S = 2.8%).

Test conditions : Per test concentration: 10 animals in 1000 ml of test solution in a glass beaker covered with a watch glass.

Temperature	:	15°C
Aeration	:	none
Food	:	some Fucus spec.
Renewal of test medium	:	once a day
Test in	:	duplicate
Test duration	:	96 h

Results: No mortality found during the exposure period

Time h	LC50 $mg.l^{-1}$	confidence interval $mg.l^{-1}$	'LC0' $mg.l^{-1}$	'LC100' $mg.l^{-1}$
24	>1000		≧1000	>1000
48	>1000		≧1000	>1000
72	>1000		≧1000	>1000
96	>1000		≧1000	>1000

'No observed effect concentration' after 96 h exposure: ≧ 1000 $mg.l^{-1}$

Effect: condition of test animals compared to the controls (visual estimation)

ANNEXE A

Determination of the acute aquatic toxicity of N,N-dimethylacetamide, Ega Chemie, 99% (GC).

Test compound : N,N-dimethylacetamide

Concentrations tested : 0 320 560 1000 1800 $mg.l^{-1}$

Preparation of test solutions : The necessary amounts were pipetted and added separately with stirring to 250 ml of natural seawater in glass beakers. Then the 20 ml test vessels were filled with these solutions and the test animals were introduced. Apparently, all dosed amounts were soluble, no undissolved compound being visible during the test.

Chemical analysis : Test solutions were not analysed for the test compound

Test animal : *Mysidopsis bahia* (Crustacea, Mysidacea) about four weeks old at the start of the test (6±1 mm), from a laboratory culture in natural seawater (S = 2.8%).

Test conditions : Per test concentration: 10 animals each in about 20 ml of test solution in a scintillation flask closed with a screw-cap.

Temperature	:	20°C
Aeration	:	none
Food	:	*Artemia nauplii*
Renewal of test medium	:	once a day
Test duration	:	96 h

Results: No mortality found during the exposure period

Time h	LC50 $mg.l^{-1}$	confidence interval $mg.l^{-1}$	'LC0' $mg.l^{-1}$	'LC100' $mg.l^{-1}$
24	3367	1121 - 10111	1000	>1800
48	1794	1302 - 2472	560	>1800
72	1198	942 - 1525	560	>1800
96	966	758 - 1231	320	>1800

'No observed effect concentration' after 96 h exposure: 320 $mg.l^{-1}$

Effect: condition of test animals compared to the controls (visual estimation)

ANNEXE A

Determination of the acute aquatic toxicity of dimethyl succinate, Aldrich Chemie, about 97%.

Test compound : dimethyl succinate

Concentrations tested : 0 1.0 3.2 10 32 100 320 1000 $mg.l^{-1}$

Preparation of test solutions : 2 g of test compound was weighed and dissolved in 2 l of standard fresh water (Appendix 5). From this solution 1.0, 3.2, 10, 32, 100, 320 and 1000 ml were diluted to 1000 ml with fresh water. Then the 250 ml test vessels were filled with this solutions and the test animals were introduced. Apparently, all dosed amounts were soluble, no undissolved compound being visible during the test.

Chemical analysis : Test solutions were not analysed for the test compound

Test animal : *Daphnia magna* (Crustacea, Cladocera) less than 24 h old at the start of the test, from a laboratory culture in standard fresh water (Appendix 5).

Test conditions : Per test concentration: 10 animals in 250 ml of test solution in a glass beaker, covered with a watch glass.

Temperature	:	20°C
Aeration	:	none
Food	:	none
Renewal of test medium	:	none
Test in	:	duplicate
Test duration	:	48 h

Results: No mortality found during the exposure period

Time h	EC50 $mg.l^{-1}$	confidence interval $mg.l^{-1}$	'EC0' $mg.l^{-1}$	'EC100' $mg.l^{-1}$
24	>1000		≧1000	>1000
48	>1000		≧1000	>1000

'No observed effect concentration' after 48 h exposure: 320 $mg.l^{-1}$

Effect: condition of test animals compared to the controls (visual estimation)

ANNEXE A

Determination of the acute aquatic toxicity of dimethyl succinate, Aldrich Chemie, about 97%.

Test compound : dimethyl succinate

Concentrations tested : 0 100 320 1000 $mg.l^{-1}$

Preparation of test solutions : 3 g of test compound was weighed and dissolved in 3 l of natural seawater. From this solution 100, 320 and 1000 ml were diluted to 1000 ml with seawater and the test animals were introduced. Apparently, all dosed amounts were soluble, no undissolved compound being visible during the test.

Chemical analysis : Test solutions were not analysed for the test compound

Test animal : *Chaetogammarus marinus* (Crustacea, Amphipoda)
Length 5±1 mm, from a laboratory culture in natural seawater (S = 2.8%).

Test conditions : Per test concentration: 10 animals in 1000 ml of test solution in a glass beaker, covered with a watch glass.

Temperature	:	15°C
Aeration	:	none
Food	:	some Fucus spec.
Renewal of test medium	:	once a day
Test in	:	duplicate
Test duration	:	96 h

Results:

Time h	LC50 $mg.l^{-1}$	confidence interval $mg.l^{-1}$	'LC0' $mg.l^{-1}$	'LC100' $mg.l^{-1}$
24	993	877 - 1124	320	>1000
48	548	385 - 781	320	>1000
72	402	279 - 580	100	1000
96	331	285 - 384	100	1000

'No observed effect concentration' after 96 h exposure: 100 $mg.l^{-1}$

Effect: condition of test animals compared to the controls (visual estimation)

ANNEXE A

Determination of the acute aquatic toxicity of dimethyl succinate, Aldrich Chemie, about 97%.

Test compound : dimethyl succinate

Concentrations tested : 0 18 32 56 100 180 $mg.l^{-1}$

Preparation of test solutions : 90 mg of test compound was weighed and dissolved in 500 ml of natural seawater. From this solution 25, 45, 80, 140 and 250 ml were diluted to 250 ml with seawater. Then the 20 ml test vessels were filled with these solutions and the test animals were introduced. Apparently, all dosed amounts were soluble, no undissolved compound being visible during the test.

Chemical analysis : Test solutions were not analysed for the test compound

Test animal : *Mysidopsis bahia* (Crustacea, Mysidacea) about four weeks old at the start of the test, (6±1 mm), from a laboratory culture in natural seawater (S = 2.8%).

Test conditions : Per test concentration: 10 animals each in about 20 ml of test solution in a scintillation flask, closed with a screw-cap.

Temperature	:	20°C
Aeration	:	none
Food	:	*Artemia nauplii*
Renewal of test medium	:	once a day
Test duration	:	96 h

Results:

Time h	LC50 $mg.l^{-1}$	confidence interval $mg.l^{-1}$	'LC0' $mg.l^{-1}$	'LC100' $mg.l^{-1}$
24	>180		≧180	>180
48	197	151 - 257	100	>180
72	155	127 - 188	100	>180
96	110	91 - 133	32	180

'No observed effect concentration' after 96 h exposure: 32 $mg.l^{-1}$

Effect: condition of test animals compared to the controls (visual estimation)

ANNEXE A

Determination of the acute aquatic toxicity of 2-methyl-2-propene-1-ol (methylallylalcohol), Aldrich Chemie, 98% (GC).

Test compound : 2-methyl-2-propene-1-ol

Concentrations tested : 0 1.0 3.2 10 32 100 320 1000 $mg.l^{-1}$

Preparation of test solutions : For concentrations 1.0 to 32 $mg.l^{-1}$ the necessary amounts were pipetted and added separately with stirring to 1 l of standard fresh water (Appendix 5) in glass beakers. Then the 250 ml test vessels were filled with these solutions and the test animals were introduced. At concentrations ≧100 $mg.l^{-1}$ undissolved test compound was visible in the test 'solutions' during the test period. These concentrations were made by pipetting and adding the necesary amounts separately with stirring to 250 ml fresh water in glass beakers before introducing the test animals.

Chemical analysis : Test solutions were not analysed for the test compound

Test animal : *Daphnia magna* (Crustacea, Cladocera) less than 24 h old at the start of the test, from a laboraoty culture in standard fresh water (Appendix 5).

Test conditions : Per test concentration: 10 animals in 250 ml of test solution in a glass beaker, covered with a watch glass.

Temperature	:	20°C
Aeration	:	none
Food	:	none
Renewal of test medium	:	none
Test in	:	duplicate
Test duration	:	48 h

Results:

Time h	EC50 $mg.l^{-1}$	confidence interval $mg.l^{-1}$	'EC0' $mg.l^{-1}$	'EC100' $mg.l^{-1}$
24	131	98 - 175	32	320
48	37	28 - 49	3.2	320

'No observed effect concentration' after 96 h exposure: 3.2 $mg.l^{-1}$

Effect: condition of test animals compared to the controls (visual estimation)

ANNEXE A

Determination of the acute aquatic toxicity of 2-methyl-2-propene-1-ol (methylallylalcohol), Aldrich Chemie, 98% (GC).

Test compound : 2-methyl-2-propene-1-ol

Concentrations tested : 0 3.2 10 32 100 320 1000 $mg.l^{-1}$

Preparation of test solutions : The necessary amounts were pipetted and added separately to 2.3 l of standard fresh water (Appendix 5) and stirred for 24 h in glass-stoppered conical-flasks with a tap. The stops were surrounded with water in a glass-colar. Then these flasks were settled down for 24 h. Then the water-phase was drawn off into 250 ml test vessels and the test animals were introduced. Apparently, all dosed amounts were soluble, no undissolved compound being visible during the test.

Chemical analysis : Test solutions were not analysed for the test compound

Test animal : *Daphnia magna* (Crustacea, Cladocera) less than 24 h old at the start of the test, from a laboraoty culture in standard fresh water (Appendix 5).

Test conditions : Per test concentration: 10 animals in 250 ml of test solution in a glass beaker, covered with a watch glass.

Temperature	:	20°C
Aeration	:	none
Food	:	none
Renewal of test medium	:	none
Test in	:	duplicate
Test duration	:	48 h

Results:

Time h	EC50 $mg.l^{-1}$	confidence interval $mg.l^{-1}$	'EC0' $mg.l^{-1}$	'EC100' $mg.l^{-1}$
24	39	30 - 50	10	100
48	23	17 - 30	3.2	100

'No observed effect concentration' after 48 h exposure: 3.2 $mg.l^{-1}$

Effect: condition of test animals compared to the controls (visual estimation)

ANNEXE A

Determination of the acute aquatic toxicity of 2-methyl-2-propene-1-ol (methylallylalcohol), Aldrich Chemie, 98% (GC).

Test compound : 2-methyl-2-propene-1-ol

Concentrations tested : 0 3.2 10 32 100 320 $mg.l^{-1}$

Preparation of test solutions : The necessary amounts were pipetted and added separately with stirring to 1 l of natural seawater in glass-beakers, before the test animals were introduced. At concentrations ≧32 $mg.l^{-1}$ undissolved test compound was visible in the test 'solutions' during the test period.

Chemical analysis : Test solutions were not analysed for the test compound

Test animal : *Chaetogammarus marinus* (Crustacea, Amphipoda)
Length 5±1 mm, from a laboratory culture in natural seawater (S = 2.8%).

Test conditions : Per test concentration: 10 animals in 1000 ml of test solution in a glass beaker covered with a watch glass.

Temperature	:	15°C
Aeration	:	none
Food	:	some Fucus spec.
Renewal of test medium	:	once a day
Test in	:	duplicate
Test duration	:	96 h

Results:

Time h	LC50 $mg.l^{-1}$	confidence interval $mg.l^{-1}$	'LC0' $mg.l^{-1}$	'LC100' $mg.l^{-1}$
24	>320		≧320	>320
48	>320		≧320	>320
72	>320		10	>320
96	341	141 - 828	10	>320

'No observed effect concentration' after 96 h exposure: 10 $mg.l^{-1}$

Effect: condition of test animals compared to the controls (visual estimation)

ANNEXE A

Determination of the acute aquatic toxicity of 2-methyl-2-propene-1-ol (methylallylalcohol), Aldrich Chemie, 98% (GC).

Test compound : 2-methyl-2-propene-1-ol

Concentrations tested : 0 10 32 100 320 1000 $mg.l^{-1}$

Preparation of test solutions : The necessary amounts were pipetted, added separately to 2.3 l of natural seawater and stirred for 24 h in glass-stoppered conical-flasks with a tap. The stops were surrounded with water in a glass-collar. Then these flasks were settled down for 24 h. Then the water-phase was drawn off into 20 ml test vessels and the test animals were introduced. Apparently, all dosed amounts were soluble, no undissolved compound being visible during the test.

Chemical analysis : Test solutions were not analysed for the test compound

Test animal : *Chaetogammarus marinus* (Crustacea, Amphipoda)
Length 5±1 mm, from a laboratory culture in natural seawater (S = 2.8%).

Test conditions : Per test concentration: 10 animals each in about 20 ml of test solution in a scintillation flask, covered with a screw-cap.

Temperature	:	15°C
Aeration	:	none
Food	:	some Fucus spec.
Renewal of test medium	:	once a day
Test duration	:	96 h

Results:

Time h	LC50 $mg.l^{-1}$	confidence interval $mg.l^{-1}$	'LC0' $mg.l^{-1}$	'LC100' $mg.l^{-1}$
24	251		32	1000
48	29		<10	1000
72	<10		<10	<10
96	<10		<10	<10

'No observed effect concentration' after 96 h exposure: <10 $mg.l^{-1}$

Effect: condition of test animals compared to the controls (visual estimation)

ANNEXE A

Determination of the acute aquatic toxicity of 2-methyl-2-propene-1-ol (methylallylalcohol), Aldrich Chemie, 98% (GC).

Test compound : 2-methyl-2-propene-1-ol

Concentrations tested : 0 0.1 0.32 1.0 3.2 10 $mg.l^{-1}$

Preparation of test solutions : 10 mg (11.7 µl) was pipetted and added to 1 l of natural seawater and stirred for 1 h in a glass stoppered conical flask. From this solution 2.5, 8, 25, 80 and 250 ml were diluted to 250 ml with seawater. Then the 20 ml test vessels were filled with these solutions and the test animals were introduced. Apparently, all dosed amounts were soluble, no undissolved compound being visible during the test.

Chemical analysis : Test solutions were not analysed for the test compound

Test animal : *Mysidopsis bahia* (Crustacea, Mysidacea) about four weeks old at the start of the test (6±1 mm), from a laboratory culture in natural seawater (S = 2.8%).

Test conditions : Per test concentration: 10 animals each in about 20 ml of test solution in a scintillation flask, covered with a screw-cap.

Temperature	:	20°C
Aeration	:	none
Food	:	*Artemia nauplii*
Renewal of test medium	:	once a day
Test duration	:	96 h

Results:

Time h	LC50 $mg.l^{-1}$	confidence interval $mg.l^{-1}$	'LC0' $mg.l^{-1}$	'LC100' $mg.l^{-1}$
24	15.5	5.7 - 42.7	1.0	>10
48	1.3	0.8 - 2.0	0.1	3.2
72			0.1	1.0
96	0.5	0.3 - 0.8	0.1	1.0

'No observed effect concentration' after 96 h exposure: 0.1 $mg.l^{-1}$

Effect: condition of test animals compared to the controls (visual estimation)

ANNEXE A

Determination of the acute aquatic toxicity of 2-methyl-2-propene-1-ol (methylallylalcohol), Aldrich Chemie, 98% (GC).

Test compound : 2-methyl-2-propene-1-ol

Concentrations tested : 0 1.0 3.2 10 32 100 $mg.l^{-1}$

Preparation of test solutions : The necessary amounts were pipetted, added to 2.3 l of natural seawater and stirred for 4 h in glass-stoppered conical-flasks with a tap. The stops were surrounded with water in a glass-collar. Then the water-phase was drawn off into 20 ml test vessels and the test animals were introduced. Apparently, all dosed amounts were soluble, no undissolved compound being visible during the test.

Chemical analysis : Test solutions were not analysed for the test compound

Test animal : *Mysidopsis bahia* (Crustacea, Mysidacea) about four weeks old at the start of the test (6±1 mm), from a laboratory culture in natural seawater (S = 2.8%).

Test conditions : Per test concentration: 10 animals each in about 20 ml of test solution in a scintillation flask, closed with a screw-cap.

Temperature	:	20°C
Aeration	:	none
Food	:	*Artemia nauplii*
Renewal of test medium	:	once a day
Test duration	:	96 h

Results:

Time h	LC50 $mg.l^{-1}$	confidence interval $mg.l^{-1}$	'LC0' $mg.l^{-1}$	'LC100' $mg.l^{-1}$
24	>1.0 <3.2		1.0	3.2
48	<1.0		<1.0	≦1.0
72	<1.0		<1.0	≦1.0
96	<1.0		<1.0	≦1.0

'No observed effect concentration' after 96 h exposure: <1.0 $mg.l^{-1}$

Effect: condition of test animals compared to the controls (visual estimation)

ANNEXE A

Determination of the acute aquatic toxicity of sodium bromate, Aldrich Chemie, 99 + %.

Test compound : sodium bromate

Concentrations tested : 0 1.0 3.2 10 32 100 320 1000 $mg.l^{-1}$

Preparation of test solutions : 2 g of test compound was weighed and dissolved in 2 l of standard fresh water (Appendix 5). From this solution 1.0, 3.2, 10, 32, 100, 320 and 1000 ml were diluted to 1000 ml with fresh water in glass beakers. Then the 250 ml test vessels were filled with these solutions and the test animals were introduced. Apparently, all dosed amounts were soluble, no undissolved compound being visible during the test.

Chemical analysis : Test solutions were not analysed for the test compound

Test animal : *Daphnia magna* (Crustacea, Cladocera) less than 24 h old at the start of the test, from a laboratory culture in standard fresh water (Appendix 5).

Test conditions : Per test concentration: 10 animals in 250 ml of test solution in a glass beaker, covered with a watch glass.

Temperature	:	20°C
Aeration	:	none
Food	:	none
Renewal of test medium	:	none
Test in	:	duplicate
Test duration	:	48 h

Results:

Time h	EC50 $mg.l^{-1}$	confidence interval $mg.l^{-1}$	'EC0' $mg.l^{-1}$	'EC100' $mg.l^{-1}$
24	>1000		≧1000	>1000
48	>100 <1000		100	1000

'No observed effect concentration' after 48 h exposure: 100 $mg.l^{-1}$

Effect: condition of test animals compared to the controls (visual estimation)

ANNEXE A

Determination of the acute aquatic toxicity of sodium bromate, Aldrich Chemie, 99 + %.

Test compound : sodium bromate

Concentrations tested : 0 32 100 320 1000 $mg.l^{-1}$

Preparation of test solutions : 3 g of test compound was weighed and dissolved in 3 l of natural seawater. From this solution 32, 100, 320 and 1000 ml were diluted to 1000 ml with seawater in glass beakers. Then the 250 ml test vessels were filled with these solutions and the test animals were introduced. Apparently, all dosed amounts were soluble, no undissolved compound being visible during the test.

Chemical analysis : Test solutions were not analysed for the test compound

Test animal : *Chaetogammarus marinus* (Crustacea, Amphipoda)
Length 5±1 mm, from a laboratory culture in natural seawater (S = 2.8%).

Test conditions : Per test concentration: 10 animals in 1000 ml of test solution in a glass beaker covered with a watch glass

Temperature	:	15°C
Aeration	:	none
Food	:	some Fucus spec.
Renewal of test medium	:	once a day
Test in	:	duplicate
Test duration	:	96 h

Results:

Time h	LC50 $mg.l^{-1}$	confidence interval $mg.l^{-1}$	'LC0' $mg.l^{-1}$	'LC100' $mg.l^{-1}$
24	>1000		≧1000	>1000
48	902	735 - 1105	320	>1000
72	512	390 - 673	100	1000
96	302	249 - 366	100	1000

'No observed effect concentration' after 96 h exposure: 100 $mg.l^{-1}$

Effect: condition of test animals compared to the controls (visual estimation)

ANNEXE A

Determination of the acute aquatic toxicity of sodium bromate, Aldrich Chemie, 99 + %.

Test compound : sodium bromate

Concentrations tested : 0 180 320 560 1000 $mg.l^{-1}$

Preparation of test solutions : 500 mg of test compound was weighed and dissolved in 500 ml of natural seawater. From this solution 45, 80, 140 and 250 ml were diluted to 250 ml with seawater in glass beakers. Then the 20 ml test vessels were filled with these solutions and the test animals were introduced. Apparently, all dosed amounts were soluble, no undissolved compound being visible during the test.

Chemical analysis : Test solutions were not analysed for the test compound

Test animal : *Musidopsis bahia* (Crustacea, Mysidacea) about four weeks old at the start of the test (6±1 mm), from a laboratory culture in natural seawater (S = 2.8%).

Test conditions : Per test concentration: 10 animals each in about 20 ml of test solution in a scintillation flask, closed with a screw cap.

Temperature	:	20°C
Aeration	:	none
Food	:	*Artemia nauplii*
Renewal of test medium	:	once a day
Test duration	:	96 h

Results:

Time h	LC50 $mg.l^{-1}$	confidence interval $mg.l^{-1}$	'LC0' $mg.l^{-1}$	'LC100' $mg.l^{-1}$
24	>560		560	>1000
48	>180 <560		180	560
72	>180 <560		180	560
96	>180 <320		180	320

'No observed effect concentration' after 96 h exposure: 180 $mg.l^{-1}$

Effect: condition of test animals compared to the controls (visual estimation)

ANNEXE A

Determination of the acute aquatic toxicity of dimethyl glutarate, Aldrich Chemie, 98%.

Test compound : dimethyl glutarate

Concentrations tested : 0 1.0 3.2 10 32 100 320 $mg.l^{-1}$

Preparation of test solutions : The necessary amounts were pipetted and added separately with stirring to 1 l of standard fresh water (Appendix 5) in glass beakers, before the test animals were introduced. Apparently, all dosed amounts were soluble, no undissolved compound being visible during the test.

Chemical analysis : Test solutions were not analysed for the test compound

Test animal : *Daphnia magna* (Crustacea, Cladocera) less than 24 h old at the start of the test, from a laboratory culture in standard fresh water (Appendix 5).

Test conditions : Per test concentration: 10 animals in 250 ml of test solution in a glass beaker, covered with a watch glass.

Temperature	:	20°C
Aeration	:	none
Food	:	none
Renewal of test medium	:	none
Test in	:	duplicate
Test duration	:	48 h

Results:

Time h	EC50 $mg.l^{-1}$	confidence interval $mg.l^{-1}$	'EC0' $mg.l^{-1}$	'EC100' $mg.l^{-1}$
24	>32 <320		32	320
48	>32 <100		32	100

'No observed effect concentration' after 48 h exposure: 32 $mg.l^{-1}$

Effect: condition of test animals compared to the controls (visual estimation)

ANNEXE A

Determination of the acute aquatic toxicity of dimethyl glutarate, Aldrich Chemie, 98%.

Test compound : dimethyl glutarate

Concentrations tested : 0 10 32 100 $mg.l^{-1}$

Preparation of test solutions : The necessary amounts were pipetted and added separately with stirring to 1 l of natural seawater in glass beakers, before the test animals were introduced. Apparently, all dosed amounts were soluble, no undissolved compound being visible during the test.

Chemical analysis : Test solutions were not analysed for the test compound

Test animal : *Chaetogammarus marinus* (Crustacea, Amphipoda)
Length 5±1 mm, from a laboratory culture in natural seawater (S = 2.8%).

Test conditions : Per test concentration: 10 animals in 1000 ml of test solution in a glass beaker, covered with a watch glass.

Temperature	:	15°C
Aeration	:	none
Food	:	some Fucus spec.
Renewal of test medium	:	once a day
Test in	:	duplicate
Test duration	:	96 h

Results:

Time h	LC50 $mg.l^{-1}$	confidence interval $mg.l^{-1}$	'LC0' $mg.l^{-1}$	'LC100' $mg.l^{-1}$
24	178	55 - 573	32	>100
48	51	38 - 67	10	>100
72	29	22 - 38	<10	100
96	21	16 - 28	<10	100

'No observed effect concentration' after 96 h exposure: <10 $mg.l^{-1}$

Effect: condition of test animals compared to the controls (visual estimation)

ANNEXE A

Determination of the acute aquatic toxicity of dimethyl glutarate, Aldrich Chemie, 98%.

Test compound : dimethyl glutarate

Concentrations tested : 0 10 18 32 56 100 $mg.l^{-1}$

Preparation of test solutions : The necessary amounts were pipetted and added separately with stirring to 1 l of natural seawater in glass beakers. Then the 20 ml test vessels were filled with these solutions and the test animals were introduced. Apparently, all dosed amounts were soluble, no undissolved compound being visible during the test.

Chemical analysis : Test solutions were not analysed for the test compound

Test animal : *Mysidopsis bahia* (Crustacea, Mysidacea) about four weeks old at the start of the test (6±1 mm), from a laboratory culture in natural seawater (S = 2.8%).

Test conditions : Per test concentration: 10 animals each in about 20 ml of test solution in a scintillation flask, closed with a screw-cap.

Temperature	:	20°C
Aeration	:	none
Food	:	*Artemia nauplii*
Renewal of test medium	:	once a day
Test duration	:	96 h

Results:

Time h	LC50 $mg.l^{-1}$	confidence interval $mg.l^{-1}$	'LC0' $mg.l^{-1}$	'LC100' $mg.l^{-1}$
24	43.8	32.3 - 59.4	18	>100
48	11.6	7.8 - 17.1	<10	32
72	7.9	4.2 - 14.9	<10	18
96	6.0	2.1 - 17.3	<10	18

'No observed effect concentration' after 96 h exposure: <10 $mg.l^{-1}$

Effect: condition of test animals compared to the controls (visual estimation)

ANNEXE A

Determination of the acute aquatic toxicity of dimethyl glutarate, Aldrich Chemie, 98%.

Test compound : dimethyl glutarate

Concentrations tested : 0 3.2 5.6 10 18 32 $mg.l^{-1}$

Preparation of test solutions : The necessary amounts were pipetted and added separately to 1 l of natural seawater and stirred for 1 h in glass-stoppered conical flasks. Then the 20 ml test vessels were filled with these solutions and the test animals were introduced. Apparently, all dosed amounts were soluble, no undissolved compound being visible during the test.

Chemical analysis : Test solutions were not analysed for the test compound

Test animal : *Mysidopsis bahia* (Crustacea, Mysidacea) about four weeks old at the start of the test (6±1 mm), from a laboratory culture in natural seawater (S = 2.8%).

Test conditions : Per test concentration: 10 animals each in about 20 ml of test solution in a scintillation flask, covered with a screw-cap.

Temperature	:	20°C
Aeration	:	none
Food	:	*Artemia nauplii*
Renewal of test medium	:	once a day
Test duration	:	96 h

Results:

Time h	LC50 $mg.l^{-1}$	confidence interval $mg.l^{-1}$	'LC0' $mg.l^{-1}$	'LC100' $mg.l^{-1}$
24	42.0	21.7 - 81.4	18	>32
48	15.2	12.3 - 18.7	10	32
72	8.4	6.8 - 10.4	3.2	18
96	8.4	6.8 - 10.4	3.2	18

'No observed effect concentration' after 96 h exposure: 3.2 $mg.l^{-1}$

Effect: condition of test animals compared to the controls (visual estimation)

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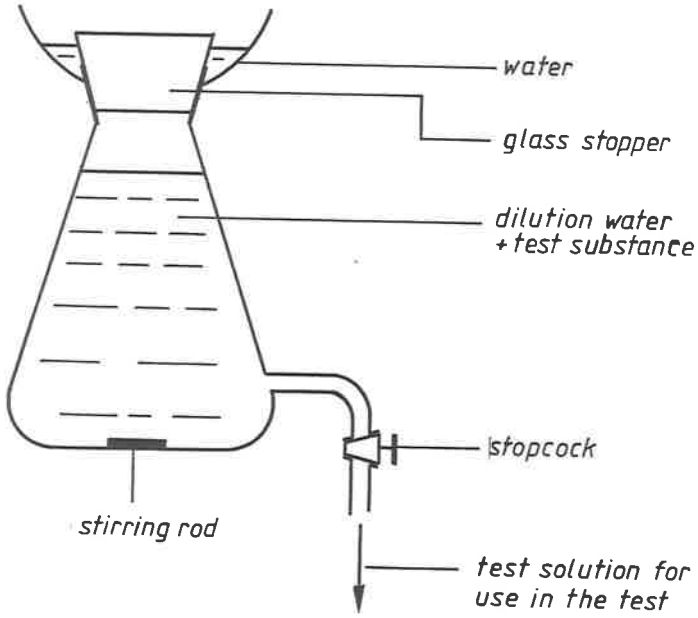
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glass stopper
dilution water
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stirring rod
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use in the test