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AQUATIC TOXICITY OF COMPOUNDS THAT
MAY BE CARRIED BY SHIPS (MARPOL 1973,
ANNEX II)

A PROGRESS REPORT FOR 1985

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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 1985 with 25 of such substances and several aquatic organisms. The data are summarized in Tables 1 and 2 and given in detail in Annexe A of this report.

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



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 new information that becomes available.

This report contains the results of tests on several substances carried out in the Netherlands in 1985 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

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 and R 84/59).

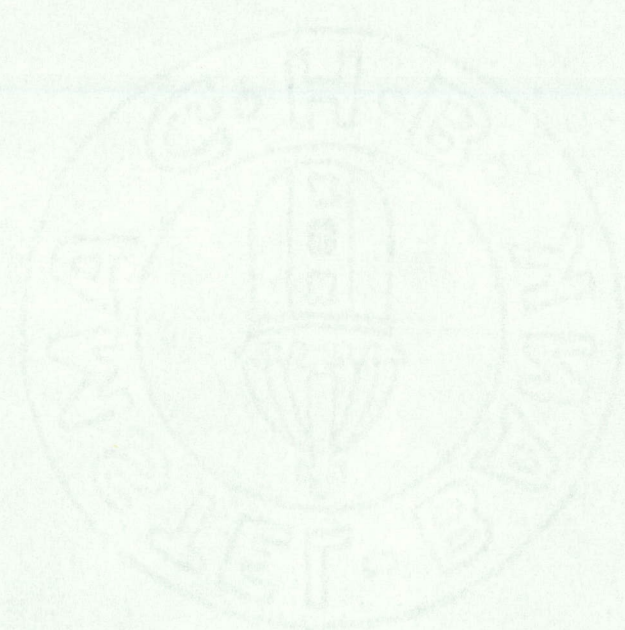
Table 1 lists the results, 96h LC50 values, for this test animal and 14 compounds.

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. Table 2 contains test results for olefins and related compounds, including fresh-water data and some data from earlier TNO reports prepared for the EHS Working Group. The data are so listed as to assist comparison.

For the same reason they are also summarized in Annexe B to this report, together with the known literature data on these and related compounds.

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

The Ministry of Transport and Public Works funded some of the fresh-water work on olefins as part of a different project, but kindly made the results available to the EHS Working Group to enable them to rate the olefins as best as possible.



2. MATERIALS AND METHODS

2.1 TEST COMPOUNDS

Sponsor supplied us with a list (Appendix 3) of compounds to be investigated. Some of these could not be obtained, and some were added at the request of the GESAMP EHS Working Group.

The actual compounds tested are listed in Tables 1 and 2.

2.2 TEST ANIMALS

Four species were used:

Chaetogammarus marinus (gammarid) (Crustaceae, 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 and R 84/59) and was the preferred test species.

Poecilia reticulata (guppy) (Pisces, Cyprinodontiformes)

The test animals were young animals, 3-4 weeks old, grown in laboratory culture in seawater.

These pelagic fishes were used to test a few chemicals in order to enable a comparison to be made between acute toxicity to, on one hand, marine crustaceans and, on the other, fishes.

Brachydanio rerio (zebra-fish) (Pisces, Cyprinoidei)

The test animals were young animals, 4-6 weeks old, born and grown in the laboratory in fresh-water (DSWL, see Appendix 5).

Daphnia magna (water flea) (Crustacea, Cladocera)

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

2.3 METHODS

The widely differing physical and chemical properties of the compounds 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 1), and this section only contains some general remarks.

The pH and oxygen concentration were monitored during the test period for all test solutions. All pH values other than 8.0 ± 0.4 , and oxygen concentrations less than 70% of saturation level are noted in the remarks sections of the appropriate data sheets.

Some rules which we followed as far as possible were:

- Volatile compounds were dissolved and tested in closed vessels.
- Sparingly soluble compounds were stirred with test medium for about 4 h to promote complete dissolution or formation of a saturated solution.
- Liquids were dosed with pipettes either directly in the seawater or as concentrated solutions in DMSO; the volumes needed were calculated from their densities; the 'concentrations tested' and the LC50-values are recorded in mg l^{-1} .

No chemical analyses of the test solutions were carried out, so the concentrations given are nominal concentrations.

We tested many of the compounds in open and in closed containers, so as to be able to evaluate any effects of the difference in the L(E)C50 values obtained.

Initially we made this comparison only for very volatile compounds, but extended it to other compounds because we had no information on possible losses by evaporation of test compound from the solutions for most compounds concerned.

The problem of whether a substance should be tested in an open or in a closed system is being studied as part of another research project (on test methodology), but the results are included in this report because they may be useful in evaluating the hazards of some compounds.

2.4 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* and 48h EC50 values for *D. magna*, when possible with their confidence intervals, are summarized in Table 1 for the compounds tested, excluding the olefins. The test results for the olefins are summarized in Table 2.

LC50 values for other experimental periods and the no observed effect concentrations are given in the separate sheets for each compound when available.

The test solutions of all compounds were almost saturated with oxygen for the duration of the tests.

Any pH values deviating from that of natural seawater controls are given on the sheets.



Table 1 Results of aquatic toxicity tests for 14 compounds (L(E)C 50 in mg.l⁻¹).

Test compound	origin	purity	96h LC50 ¹⁾ <i>C. marinus</i> open ⁵⁾ closed ⁵⁾		96h LC50 ¹⁾ <i>P. reticulata</i> (seaw) open ⁵⁾	48h EC50 <i>D. magna</i> open ⁵⁾ closed ⁵⁾		solubility in dilution water ²⁾ mg.l ⁻¹
butyl stearate	Sigma	99+%		*				< 1000 ⁴⁾
calcium hydroxide	J.T. Baker	97%			356 (206-616)			
choline chloride	Sigma	3x cryst.	abt. 500	abt. 500		> 320		≥ 1000 ³⁾
2,2-dichloroiso- propylether	Aldrich	techn.	35 ⁷⁾	abt. 40 ⁷⁾				≥ 100 ³⁾
diethyleneglycol dibutyl ether	Merck	98% (GC)	116 (80-168)	101 (67-151)				< 320 ⁴⁾
1,3-diisopropyl- benzene	Hicol B.V.	90-95%(GC) techn.	abt. 1.0	abt. 0.3				≥ 3.2 ³⁾
ethylene glycol	Riedel- de Haën AG	98%	abt. 560	abt. 900		> 1000		≥ 1000 ³⁾
methyl butyl ether								
ethyleneglycol monobutyl ether	Merck	99% (GC)	> 1000	> 1000		> 1000		≥ 1000 ³⁾
furfural	Hicol B.V.	> 99% (GC) (puriss.)	15 (13-19)	21 (17-27)		abt. 40		≥ 56 ³⁾
3-methoxy-1- butanol	Janssen chimica	99%	> 1000	> 1000		> 1000	> 1000	≥ 1000 ³⁾
propylene glycol	Sigma		> 1000			> 1000		≥ 1000 ³⁾
sodium hydroxide	J.T. Baker	98.6%			196 (144-267)			
urea	May & Baker	not less 99.5%	> 1000		> 1000			≥ 1000 ³⁾
vinyltoluene	Fluka AG	purum ⁶⁾	41	1.6		abt. 30	abt. 3	< 10 ⁴⁾

*) No mortality found within the exposure period in an 'as best as possible' saturated solution (no additives used)

1) Confidence interval in parentheses

2) Visual estimation during toxicity test

3) No undissolved test compound seen in the test solutions at concentrations ≥ value indicated

4) Undissolved test compound seen in the test solutions at concentrations ≥ value indicated

5) See section 2.3

6) abt. 60% m - + abt. 40% p-vinyltoluene

7) in µl per l

Table 2 Results of the aquatic toxicity test of olefins.

Test compound	C-atoms	Origin	Purity	96h LC50 ³⁾ mg.l ⁻¹						48h EC50 mg.l ⁻¹	
				marine animals				fresh-water		fresh-water	
				<i>C. marinus</i> (crust.)		<i>P. reticulata</i> (fish)		<i>B. rerio</i> (fish)		<i>D. magna</i> (crust.)	
				open	closed	open	closed	open	closed	open	closed
2-pentene (cis + trans)	5	Fluka AG	> 95% pract		abt. 32 ²⁾⁴⁾						
isopentene	5	Janssen chimica	99+%	abt. 60 ²⁾	abt. 30 ²⁾					84 ²⁾	abt. 30 ²⁾
1,3-pentadiene (cis)	5	Fluka AG	> 98% purum		35 ²⁾⁴⁾						
1,3-pentadiene (trans)	5	Fluka AG	> 97% purum		18 ²⁾⁴⁾						
1-hexene	6	Sigma	abt. 99%					abt. 50 ²⁾	abt. 25 ²⁾	abt. 60 ²⁾	abt. 30 ²⁾
2-methylpentene	6	Fluka AG	> 99% purum		abt. 18 ²⁾⁴⁾						
1-octene	8							> 3.2 ¹⁾ 4.8 ²⁾	> 3.2 ¹⁾ abt 6 ²⁾	abt. 7 ²⁾	abt. 6 ²⁾
2-octene (trans)	8	Sigma		0.6 ¹⁾ < 3.2 ²⁾	0.6 ¹⁾ < 3.2 ²⁾	13 ²⁾	6 ²⁾	28 ²⁾	7.5 ²⁾	abt. 8 ²⁾	abt. 6 ²⁾
1-nonene	9	Sigma	abt. 99%		0.9 ²⁾⁴⁾			> 3.2 ¹⁾ 10 ²⁾	abt 2 ¹⁾ < 3.2 ²⁾	abt. 6 ²⁾	abt. 2 ²⁾
1-undecene	11	Fluka AG	> 97% pract	< 0.32 ¹⁾ 2.0 ²⁾⁵⁾	0.3 ¹⁾ < 3.2 ²⁾	abt. 20 ²⁾	abt. 20 ²⁾				
1-dodecene	12										
1-tridecene	13	Fluka AG			* ²⁾⁴⁾		* ²⁾				
1-tetradecene	14	Fluka AG			* ²⁾⁴⁾		* ²⁾				

*) No mortality found within the exposure period in an 'as best as possible' saturated solution (no additives used)

1) with 0.1 ml.l⁻¹ of an organic solvent as carrier

2) without additives

3) for confidence intervals, see Annexe 1

4) see R85/59

5) same result obtained in 1985 as was reported in 1983

4. ADDITIONAL INFORMATION

As stated in the introduction, several hydrocarbons were tested in the intersessional period.

In addition, some novel literature data have become available. For these reasons, all available information, including literature data on paraffins, naphthenes and olefins, is summarized in Annexe B.



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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 LD₅₀ 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 LD₅₀ 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
- 0 No evidence to support one of the above ratings (+, Z, T)

Column B - Damage to living resources

<u>Ratings</u>		<u>96 hr. TLm (= 96h LC50)</u>
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

<u>Ratings</u>		<u>LD50</u> (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 amenitiesRatings

- 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 8 to BCH 13/17 and Annexe 4 to BCH 14/WP.8)

Compound	Status
butyl stearate	tested
choline chloride	"
diisopropylbenzene	"
ethylene glycol methylbutylether	"
3-methoxybutanol	"
isopentene	"
propylene glycol	"
vinyltoluene	"
furfural	"
butyl octyl phthalate	not available
diethylene glycol dibutylether	tested
ethylene glycol dibutylether	not available
2-octene	tested (as the trans isomer)
ethylene tetraacetic acid, tetrasodium salt (solution)	not available
olefins, mixture of linear chain	to be evaluated from individual com- pounds

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.

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

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

p_o is the mortality (immobilization) probability in concentration 0

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 TEST:

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.

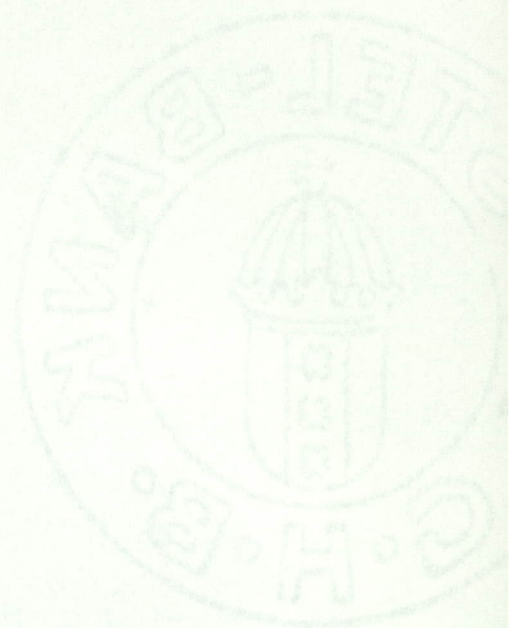
The groundwater contains several other trace elements ($\ll 1 \text{ mg.l}^{-1}$).

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 DETAILS OF THE TOXICITY TESTS



ANNEXE A

Determination of the acute aquatic toxicity of butyl stearate, Sigma, 99+%.

Test compound : butyl stearate
Concentrations tested : 0 1000 mg.l⁻¹
Preparation of test solutions : 0.5 g of test compound was weighed, added to 500 ml of seawater and stirred for 4 h in a glass-stoppered conical flask. Undissolved test compound was 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 : 10 animals in 500 ml of natural seawater, pH abt. 8, S abt. 2.8%
in a glass-stoppered conical flask.

Temperature : 15°C
Aeration : none
Food : none
Renewal of test medium : none
Test in : singular
Test duration : 96 h

Results: No mortality found during exposure period in an "as best as possible" saturated solution (no additives used).

ANNEXE A

Determination of the acute aquatic toxicity of calcium hydroxide, J.T. Baker, 97%.

Test compound : Ca(OH)_2
 Concentrations tested : 0 56 100 150 320 560 mg.l^{-1}
 Preparation of test solutions : The necessary amounts were weighted and 'dissolved' separately in 1 l of seawater. At all concentrations precipitation occurred (due to the increase of pH).
 Chemical analysis : Test solutions were not analysed for the test compound
 Test animal : *Poecilia reticulata* (guppy) (Pisces, cyprinodontiformes) young fishes 3 ± 1 week old, from a laboratory culture in natural seawater (S = 2.8%)
 Test conditions : 10 animals in 1000 ml of natural seawater, pH abt. 8, S abt. 2.8%
 in a glass beaker covered with a watch glass
 Temperature : 24°C
 Aeration : none
 Food : none
 Renewal of test medium : once a day
 Test in : singular
 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	387	218-688	56	>560
48	387	218-688	56	>560
72	387	218-688	56	>560
96	356	206-616	56	>560

'No observed effect concentration' after 96 h exposure: 56 mg.l⁻¹

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

Remarks:	conc. (mg . l ⁻¹)	pH ± 0.1 (freshly prepared solution)
	0	8.0
	56	8.7
	100	9.4
	180	9.7
	320	9.9
	560	10.0

ANNEXE A

Determination of the acute aquatic toxicity of choline chloride, Sigma, 3x crystallized

Test compound : choline chloride
 Concentrations tested : 0 320 1000 mg.l⁻¹
 Preparation of test solutions : The necessary amounts were weighted and dissolved separately in 1 l of seawater.

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 : 10 animals in 1000 ml of natural seawater,
 pH abt. 8, S abt. 2.8%
 in a glass beaker covered with a watch glass.¹⁾

Temperature : 15°C
 Aeration : none
 Food : none
 Renewal of test medium : once per 48 h
 Test in : singular¹⁾
 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	>1000		≥1000	>1000
72	abt.1000		320	>1000
96	>320<1000		320	abt.1000

'No observed effect concentration' after 96 h exposure: 320 mg.l⁻¹

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

¹⁾ in glass stoppered flasks, the results were, as expected, the same. The tests may therefore be considered to have been carried out in duplicate.

ANNEXE A

Determination of the acute aquatic toxicity of choline chloride, Sigma, 3x crystallized.

Test compound : choline chloride
 Concentrations tested : 0 1.0 10 100 320 mg.l⁻¹
 Preparation of test solutions : 1 g of test compound was weighed and dissolved in 1 l of fresh water (Appendix 5) 1.0, 10, 100 and 320 ml of this concentrated solution were diluted to 1 l with fresh water and used as test solutions.

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 : 25 animals in 1000 ml of standard fresh water (Appendix 5), pH abt. 8, hardness abt. 210 mg of CaCO₃ per l in a glass beaker, covered with a watch glass.

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

Results: No mortality found during the test period.

Time h	EC50 mg.l ⁻¹	confidence interval mg.l ⁻¹	'EC0' mg.l ⁻¹	'EC100' mg.l ⁻¹
24	>320		≥320	>320
48	>320		≥320	>320

'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 2,2-dichloroisopropylether, Aldrich, techn.

Test compound : 2,2-dichloroisopropylether
 Concentrations tested : 0 3.2 10 32 100 $\mu\text{l.l}^{-1}$
 Preparation of test solutions : The necessary amounts were pipetted, added separately to 2 l of seawater and stirred for 4 h in glass-stoppered conical flasks. Then the contents of these flasks were emptied in glass beakers and the test animals were introduced. At none of the dosed amounts test compound was visible 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 : 10 animals in 2000 ml of natural seawater,
 pH abt. 8, S abt. 2.8%
 in a glass beaker covered with a watch glass.

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

Results:

Time h	LC50 $\mu\text{l.l}^{-1}$	confidence interval $\mu\text{l.l}^{-1}$	'LC0' $\mu\text{l.l}^{-1}$	'LC100' $\mu\text{l.l}^{-1}$
24	> 100		≥ 100	> 100
48	132	66-263	32	> 100
72	57	38- 85	10	> 100
96	35	25- 51	10	100

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

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

ANNEXE A

Determination of the acute aquatic toxicity of 2,2-dichloroisopropylether, Aldrich, techn.

Test compound : 2,2-dichloroisopropylether
 Concentrations tested : 0 3.2 10 32 100 $\mu\text{l.l}^{-1}$
 Preparation of test solutions : The necessary amounts were pipetted, added separately to 2 l of seawater and stirred for 4 h in glass-stoppered conical flasks, before the test animals were introduced. At none of the dosed amounts test compound was visible 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 : 10 animals in 2000 ml of natural seawater, pH abt. 8, S abt. 2.8%
 in a glass-stoppered conical flask.

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

Results:

Time h	LC50 $\mu\text{l.l}^{-1}$	confidence interval $\mu\text{l.l}^{-1}$	'LC0' $\mu\text{l.l}^{-1}$	'LC100' $\mu\text{l.l}^{-1}$
24	abt. 110		32	> 100
48	abt. 100		32	> 100
72	abt. 60		32	100
96	abt. 40		10	100

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

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

ANNEXE A

Determination of the acute aquatic toxicity of 2,2-dichloroisopropylether, Aldrich, techn.

Test compound : 2,2-dichloroisopropylether
 Concentrations tested : 0 10 18 32 56 100 $\mu\text{l.l}^{-1}$
 Preparation of test solutions : The necessary amounts were pipetted, added separately to 2 l of fresh water (Appendix 5) and stirred for 4 h in glass-stoppered conical flasks. Then the contents of these flasks were emptied in glass beakers and the test animals were introduced. At none of the dosed amounts test compound was visible.

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

Test animal : *Brachydanio rerio* (zebra fish) (Pisces, cyprinoidei)
 young fishes 3 ± 1 cm, from a laboratory culture in standard fresh water (Appendix 5)

Test conditions : 10 animals in 2000 ml of standard fresh water (Appendix 5),
 pH abt. 8, hardness abt. 210 mg of CaCO_3 per l
 in a glass beaker, covered with a watch glass.

Temperature : 24°C
 Aeration : none
 Food : none
 Renewal of test medium : once a day
 Test in : singular
 Test duration : 96 h

Results:

Time h	LC50 $\mu\text{l.l}^{-1}$	confidence interval $\mu\text{l.l}^{-1}$	'LC0' $\mu\text{l.l}^{-1}$	'LC100' $\mu\text{l.l}^{-1}$
24	abt. 100		56	> 100
48	> 56 < 100		56	> 100
72	> 56 < 100		56	> 100
96	> 56 < 100		56	> 100

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

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

ANNEXE A

Determination of the acute aquatic toxicity of 2,2-dichloroisopropylether, Aldrich, techn.

Test compound : 2,2-dichloroisopropylether
 Concentrations tested : 0 10 18 32 56 100 $\mu\text{l.l}^{-1}$
 Preparation of test solutions : The necessary amounts were pipetted, added separately to 2 l of fresh water (Appendix 5) and stirred for 4 h in glass-stoppered conical flasks, before the test animals were introduced. At none of the dosed amounts test compound was visible.

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

Test animal : *Brachydanio rerio* (zebra fish) (Pisces, cyprinoidei)
 young fishes 3 ± 1 cm, from a laboratory culture in standard fresh water (Appendix 5)

Test conditions : 10 animals in 2000 ml of standard fresh water (Appendix 5),
 pH abt. 8, hardness abt. 210 mg of CaCO_3 per l
 in a glass-stoppered conical flask.

Temperature : 24°C
 Aeration : none
 Food : none
 Renewal of test medium : once a day
 Test in : singular
 Test duration : 96 h

Results:

Time h	LC50 $\mu\text{l.l}^{-1}$	confidence interval $\mu\text{l.l}^{-1}$	'LC0' $\mu\text{l.l}^{-1}$	'LC100' $\mu\text{l.l}^{-1}$
24	> 56 < 100		56	100
48	> 56 < 100		56	100
72	> 56 < 100		56	100
96	> 56 < 100		56	100

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

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

ANNEXE A

Determination of the acute aquatic toxicity of diethylene glycol dibutyl ether, Merck, 98% (GC).

Test compound : diethylene glycol dibutyl ether
 Concentrations tested : 0 100 180 320 560 1000 mg.l⁻¹
 Preparation of test solutions : The necessary amounts were pipetted and added separately to 1 l of seawater. At concentrations ≥ 560 mg.l⁻¹ undissolved test compound was visible in the test 'solutions'.

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

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

Test conditions : 10 animals in 1000 ml of natural seawater,
 pH abt. 8, S abt. 2.8%
 in a glass beaker covered with a watch glass.

Temperature : 15°C
 Aeration : none
 Food : none
 Renewal of test medium : once a day
 Test in : singular
 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	143	103-199	<100	320
48	134	95-188	<100	320
72	125	88-177	<100	320
96	116	80-168	<100	320

'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 diethylene glycol dibutyl ether, Merck, 98% (GC).

Test compound : diethylene glycol dibutyl ether
 Concentrations tested : 0 100 180 320 560 1000 mg.l⁻¹
 Preparation of test solutions : The necessary amounts were pipetted and added separately to 1 l of seawater. At concentrations ≥ 320 mg.l⁻¹ undissolved test compound was visible in the test 'solutions'.

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

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

Test conditions : 10 animals in 1000 ml of natural seawater,
 pH abt. 8, S abt. 2.8%
 in a glass-stoppered conical flask.

Temperature : 15°C
 Aeration : none
 Food : none
 Renewal of test medium : once a day
 Test in : singular
 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	210	157-281	<100	560
48	144	105-198	<100	560
72	109	75-158	<100	320
96	101	67-151	<100	320

'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 1,3-diisopropylbenzene, Hicol B.V., 90-95% (GC), tech.

Test compound : 1,3-diisopropylbenzene

Concentrations tested : 0 0.1 0.32 1.0 3.2 mg.l⁻¹

Preparation of test solutions : Concentrated solutions in dimethyl sulphoxide (DMSO) were prepared by weighing 0.32 g of test compound and dissolving this in 10 ml of DMSO. 3.2 ml of this solution diluted to 10 ml with DMSO gives the next concentrated solution and so on. 0.1 ml of these concentrated solutions were added to 1 l of seawater and stirred for 4 h in glass-stoppered conical flasks. Then the contents of these flasks were emptied in glass beakers and the test animals were introduced. At none of the dosed amounts test compound was 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 : 10 animals in 1000 ml of natural seawater,
pH abt. 8, S abt. 2.8%
in a glass beaker covered with a watch glass.

Temperature : 15°C

Aeration : none

Food : none

Renewal of test medium : once a day

Test in : singular

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	abt. 6.3		3.2	>3.2
48	abt. 1.8		1.0	3.2
72	abt. 1.0		0.32	3.2
96	abt. 1.0		0.32	3.2

'No observed effect concentration' after 96 h exposure: 0.32 mg.l⁻¹

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

ANNEXE A

Determination of the acute aquatic toxicity of 1,3-diisopropylbenzene, Hicol B.V., 90-95% (GC), tech.

Test compound : 1,3-diisopropylbenzene

Concentrations tested : 0 0.1 0.32 1.0 3.2 mg.l⁻¹

Preparation of test solutions : Concentrated solutions in dimethyl sulphoxide (DMSO) were prepared by weighing 0.32 g of test compound and dissolving this in 10 ml of DMSO. 3.2 ml of this solution diluted to 10 ml with DMSO gives the next concentrated solution and so on. 0.1 ml of these concentrated solutions were added to 1 l of seawater and stirred for 4 h in glass-stoppered conical flasks, before the test animals were introduced. No undissolved test compound was visible 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 : 10 animals in 1000 ml of natural seawater, pH abt. 8, S abt. 2.8%
in a glass-stoppered conical flask.

Temperature : 15°C

Aeration : none

Food : none

Renewal of test medium : once a day

Test in : singular

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		0.32	3.2
48	>0.32 <1.0		0.32	1.0
72	>0.32 <1.0		0.32	1.0
96	>0.1 <1.0		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 ethylene glycol methyl butyl ether, Riedel - De Haën AG, 98%.

Test compound : ethylene glycol methyl butyl ether
 Concentrations tested : 0 320 1000 mg.l⁻¹
 Preparation of test solutions : The necessary amounts were pipetted, added to 1 l of seawater and stirred for 4 h in glass-stoppered conical flasks. Then the contents of these flasks were emptied in glass beakers, and the test animals were introduced. At none of the dosed amounts test compound was visible 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 : 10 animals in 1000 ml of natural seawater,
 pH abt. 8, S abt. 2.8%
 in a glass beaker covered with a watch glass.

Temperature : 15°C
 Aeration : none
 Food : none
 Renewal of test medium : once a day
 Test in : singular
 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	>1000
48	>320		320	>1000
72	>320		320	>1000
96	abt.560		320	1000

'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 ethylene glycol methyl butyl ether, Riedel - De Haën AG, 98%.

Test compound : ethylene glycol methyl butyl ether
 Concentrations tested : 0 320 1000 mg.l⁻¹
 Preparation of test solutions : The necessary amounts were pipetted, added to 1 l of seawater and stirred for 4 h in glass-stoppered conical flasks, before the test animals were introduced. At none of the dosed amounts test compound was visible 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 : 10 animals in 1000 ml of natural seawater,
 pH abt. 8, S abt. 2.8%
 in a glass-stoppered conical flask.

Temperature : 15°C
 Aeration : none
 Food : none
 Renewal of test medium : once a day
 Test in : singular
 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	>1000		≥1000	>1000
72	>1000		320	>1000
96	abt.900		320	>1000

'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 ethylene glycol methyl butyl ether, Riedel - De Haën AG, 98%.

Test compound : ethylene glycol methyl butyl ether
 Concentrations tested : 0 320 1000 mg.l⁻¹
 Preparation of test solutions : 1 g of test compound was weighed and dissolved in 1 l of standard fresh water (Appendix 5). At none of the dosed amounts test compound was visible during the test period.

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 : 25 animals in 1000 ml of standard fresh water (Appendix 5), pH abt. 8, hardness abt. 210 mg of CaCO₃ per l in a glass beaker, covered with a watch glass.

Temperature : 20°C
 Aeration : none
 Food : none
 Renewal of test medium : none
 Test in : singular
 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 ethylene glycol monobutyl ether, Merck, 99% (GC).

Test compound : ethylene glycol monobutyl ether,
 Concentrations tested : 0 1000 mg.l⁻¹
 Preparation of test solutions : 1 g of test compound was weighed and dissolved in 1 l of seawater. No undissolved test compound was visible 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 : 10 animals in 1000 ml of natural seawater,
 pH abt. 8, S abt. 2.8%
 in a glass beaker covered with a watch glass.

Temperature : 15°C
 Aeration : none
 Food : none
 Renewal of test medium : once a day
 Test in : singular
 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 ethylene glycol monobutyl ether, Merck, 99% (GC).

Test compound : ethylene glycol monobutyl ether,
 Concentrations tested : 0 1000 mg.l⁻¹
 Preparation of test solutions : 1 g of test compound was weighed and dissolved in 1 l of seawater. No undissolved test compound was visible 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 : 10 animals in 1000 ml of natural seawater,
 pH abt. 8, S abt. 2.8%
 in a glass-stoppered conical flask.

Temperature : 15°C
 Aeration : none
 Food : none
 Renewal of test medium : once a day
 Test in : singular
 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 ethylene glycol monobutyl ether, Merck, 99% (GC).

Test compound : ethylene glycol monobutyl ether,
 Concentrations tested : 0 1000 mg.l⁻¹
 Preparation of test solutions : 1 g of test compound was weighed and dissolved in 1 l of standard fresh water. No undissolved test compound was visible during the test period.

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 : 25 animals in 1000 ml of standard fresh water (Appendix 5), pH abt. 8, hardness abt. 210 mg of CaCO₃ per l in a glass beaker, covered with a watch glass.

Temperature : 20°C
 Aeration : none
 Food : none
 Renewal of test medium : none
 Test in : singular
 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 furfural, Hicol B.V., >99% (GC), puriss p.a.

Test compound : furfural

Concentrations tested : 0 5.6 10 18 32 56 mg.l⁻¹

Preparation of test solutions : The necessary amounts were pipetted, added separately to 1 l of seawater and stirred for a few seconds, before introducing the test animals. At none of the dosed amounts test compound was visible 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 : 10 animals in 1000 ml of natural seawater,
pH abt. 8, S abt. 2.8%
in a glass beaker covered with a watch glass.

Temperature : 15°C
Aeration : none
Food : none
Renewal of test medium : once a day
Test in : singular
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	51	42-61	18	>56
48	24	20-29	10	56
72	19	16-23	10	32
96	15	13-19	10	32

'No observed effect concentration' after 96 h exposure: 5.6 mg.l⁻¹

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

ANNEXE A

Determination of the acute aquatic toxicity of furfural, Hicol B.V., >99% (GC), puriss p.a.

Test compound : furfural
 Concentrations tested : 0 5.6 10 18 32 56 mg.l⁻¹
 Preparation of test solutions : The necessary amounts were pipetted, added separately to 1 l of seawater and shaken for a few seconds, before introducing the test animals. At none of the dosed amounts test compound was visible 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 : 10 animals in 1000 ml of natural seawater, pH abt. 8, S abt. 2.8%
 in a glass-stoppered conical flask.

Temperature : 15°C
 Aeration : none
 Food : none
 Renewal of test medium : once a day
 Test in : singular
 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	40	32-50	10	>56
48	30	24-38	10	56
72	23	18-28	10	56
96	21	17-27	10	32

'No observed effect concentration' after 96 h exposure: <5.6 mg.l⁻¹

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

ANNEXE A

Determination of the acute aquatic toxicity of furfural, Hicol B.V., >99% (GC), puriss p.a.

Test compound : furfural

Concentrations tested : 0 5.6 10 18 32 56 mg.l⁻¹

Preparation of test solutions : The necessary amounts were weighed and added separately to 1 l of standard fresh water (Appendix 5) and stirred for a few seconds, before introducing the test animals. At none of the dosed amounts test compound was visible during the test period.

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 : 25 animals in 1000 ml of standard fresh water (Appendix 5), pH abt. 8, hardness abt. 210 mg of CaCO₃ per l in a glass beaker, covered with a watch glass.

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 <56		10	56
48	>10 <56		10	56

'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 3-methoxy-1-butanol, Janssen chimica, 99%.

Test compound : 3-methoxy-1-butanol
 Concentrations tested : 0 320 1000 mg.l⁻¹
 Preparation of test solutions : The necessary amounts were pipetted and dissolved separately in 1 l of seawater. At none of the dosed amounts test compound was visible 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 : 10 animals in 1000 ml of natural seawater,
 pH abt. 8, S abt. 2.8%
 in a glass beaker covered with a watch glass.

Temperature : 15°C
 Aeration : none
 Food : none
 Renewal of test medium : once a day
 Test in : singular
 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 3-methoxy-1-butanol, Janssen chimica, 99%.

Test compound : 3-methoxy-1-butanol
 Concentrations tested : 0 320 1000 mg.l⁻¹
 Preparation of test solutions : The necessary amounts were pipetted and dissolved separately in 1 l of seawater. At none of the dosed amounts test compound was visible 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 : 10 animals in 1000 ml of natural seawater, pH abt. 8, S abt. 2.8%
 in a glass-stoppered conical flask.

Temperature : 15°C
 Aeration : none
 Food : none
 Renewal of test medium : once a day
 Test in : singular
 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 3-methoxy-1-butanol, Janssen chimica, 99%.

Test compound : 3-methoxy-1-butanol
 Concentrations tested : 0 320 1000 mg.l⁻¹
 Preparation of test solutions : The necessary amounts were weighed and dissolved separately in 1 l of fresh water (Appendix 5). At none of the dosed amounts test compound was visible during the test period.

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 : 25 animals in 1000 ml of standard fresh water (Appendix 5), pH abt. 8, hardness abt. 210 mg of CaCO₃ per l in a glass beaker, covered with a watch glass.

Temperature : 20°C
 Aeration : none
 Food : none
 Renewal of test medium : none
 Test in : singular
 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 3-methoxy-1-butanol, Janssen chimica, 99%.

Test compound : 3-methoxy-1-butanol
 Concentrations tested : 0 320 1000 mg.l⁻¹
 Preparation of test solutions : The necessary amounts were weighed and dissolved separately in 1 l of fresh water (Appendix 5). At none of the dosed amounts test compound was visible during the test period.

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 : 25 animals in 1000 ml of standard fresh water (Appendix 5), pH abt. 8, hardness abt. 210 mg of CaCO₃ per l 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: 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 propylene glycol, Sigma.

Test compound : propylene glycol
 Concentrations tested : 0 1000 mg.l⁻¹
 Preparation of test solutions : 1 g of test compound was weighed and dissolved in 1 l of seawater. No undissolved test compound was visible 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 : 10 animals in 1000 ml of natural seawater,
 pH abt. 8, S abt. 2.8%
 in a glass beaker covered with a watch glass.

Temperature : 15°C
 Aeration : none
 Food : none
 Renewal of test medium : once a day
 Test in : singular
 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 propylene glycol, Sigma.

Test compound : propylene glycol
 Concentrations tested : 0 1000 mg.l⁻¹
 Preparation of test solutions : 1 g of test compound was weighed and dissolved in 1 l of fresh water (Appendix 5). No undissolved test compound was visible during the test period.

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 : 25 animals in 1000 ml of standard fresh water (Appendix 5), pH abt. 8, hardness abt. 210 mg of CaCO₃ per l in a glass beaker, covered with a watch glass.

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

Results: No mortality was 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 sodium hydroxide, J.T. Baker, 98.6%

Test compound : NaOH

Concentrations tested : 0 32 56 100 180 320 mg.l⁻¹

Preparation of test solutions : 10 g of test compound was weighed and dissolved in 100 ml of distilled water. 320, 560, 1000, 1800, 3200 ml of this concentrated solution were diluted to 1 l with seawater and used as test solution. Precipitation occurred at 180 mg.l⁻¹ and higher concentrations.

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

Test animal : *Poecilia reticulata* (guppy) (Pisces, cyprinodontiformes) young fishes 3 ± 1 week old, from a laboratory culture in natural seawater (S = 2.8%)

Test conditions : 10 animals in 1000 ml of natural seawater, pH abt. 8, S abt. 2.8%, in a glass-stoppered conical flask in a glass beaker covered with a watch glass

Temperature : 24°C

Aeration : none

Food : none

Renewal of test medium : once a day

Test in : singular

Test duration : 96 h

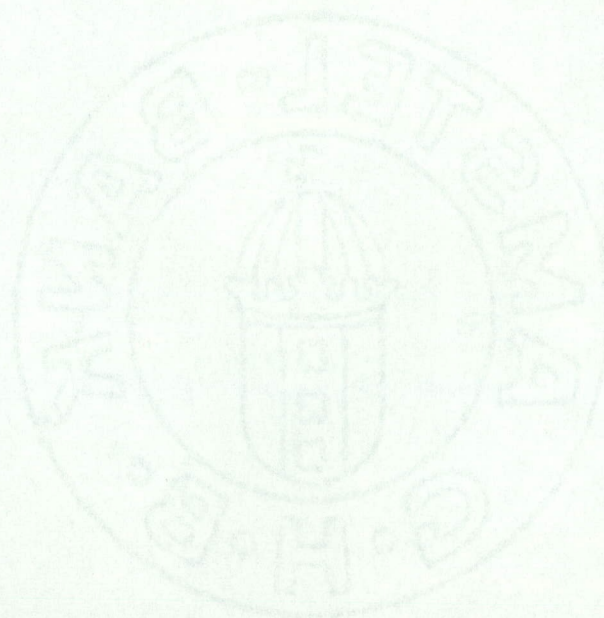
Results:

Time h	LC50 mg.l ⁻¹	confidence interval mg.l ⁻¹	'LC0' mg.l ⁻¹	'LC100' mg.l ⁻¹
24	209	153-286	56	> 320
48	196	144-267	56	> 320
72	196	144-267	56	> 320
96	196	144-267	56	> 320

'No observed effect concentration' after 96 h exposure: 56 mg.l⁻¹

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

Remarks:	conc. ($\text{mg} \cdot \text{l}^{-1}$)	pH $\pm$ 0.1 (fresh prepared solutions)
	0	8.1
	32	8.8
	56	9.1
	100	9.5
	180	9.8
	320	10.0



ANNEXE A

Determination of the acute aquatic toxicity of urea, May & Baker, not less 99.5%.

Test compound : urea

Concentrations tested : 0 1 10 100 1000 mg.l⁻¹

Preparation of test solutions : 10 g of test compound was weighed and dissolved in 100 ml of distilled water. 0.01, 0.1, 1.0 and 10 ml of this concentrated solution were diluted to 1 l with seawater and used as test solution.

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 : 10 animals in 1000 ml of natural seawater,
pH abt. 8, S abt. 2.8%
in a glass beaker covered with a watch glass.

Temperature : 15°C

Aeration : none

Food : none

Renewal of test medium : none

Test in : singular

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 urea, May & Baker, not less 99.5%.

Test compound : urea

Concentrations tested : 0 1 10 100 1000 mg.l⁻¹

Preparation of test solutions : 10 g of test compound was weighed and dissolved in 100 ml of distilled water. 0.01, 0.1, 1.0 and 10 ml of this concentrated solution were diluted to 1 l with seawater and used as test solution.

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

Test animal : *Poecilia reticulata* (guppy) (Pisces, cyprinodontiformes) young fishes 3 ± 1 week old, from a laboratory culture in natural seawater (S = 2.8%)

Test conditions : 10 animals in 1000 ml of natural seawater, pH abt. 8, S abt. 2.8%, in a glass beaker covered with a watch glass

Temperature : 24°C

Aeration : none

Food : none

Renewal of test medium : none

Test in : singular

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 vinyltoluene, Fluka AG, abt. 60% meta- + abt. 40% para-vinyltoluene, purum.

Test compound : vinyltoluene

Concentrations tested : 0 1.0 3.2 10 32 100 mg.l⁻¹

Preparation of test solutions : The necessary amounts were pipetted, added separately to 1 l of seawater in a glass beaker, and stirred for a few seconds. At concentrations ≥ 10 mg.l⁻¹ test compound was visible during the test period.

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

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

Test conditions : 10 animals in 1000 ml of natural seawater, pH abt. 8, S abt. 2.8%
in a glass beaker covered with a watch glass.

Temperature : 15°C

Aeration : none

Food : none

Renewal of test medium : once a day

Test in : singular

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	59	36-96	10	> 100
48	46	28-75	10	> 100
72	46	28-75	10	> 100
96	41	25-66	10	> 100

'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 vinyltoluene, Fluka AG, abt. 60% meta- + abt. 40% para-vinyltoluene, purum.

Test compound : vinyltoluene

Concentrations tested : 0 1.0 3.2 10 32 100 mg.l⁻¹

Preparation of test solutions : The necessary amounts were pipetted, added separately to 1 l of seawater, and stirred for a few seconds in a glass-stoppered conical flask. At concentrations ≥ 10 mg.l⁻¹ test compound was visible during the test period.

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

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

Test conditions : 10 animals in 1000 ml of natural seawater, pH abt. 8, S abt. 2.8%
in a glass-stoppered conical flask.

Temperature : 15°C

Aeration : none

Food : none

Renewal of test medium : once a day

Test in : singular

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	14.3	8.4-24.3	< 1.0	100
48	8.9	5.3-15.2	< 1.0	32
72	2.7	1.6- 4.7	< 1.0	10
96	1.6	0.9- 3.0	< 1.0	10

'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 vinyltoluene, Fluka AG, abt. 60% meta- + abt. 40% para-vinyltoluene, purum.

Test compound : vinyltoluene

Concentrations tested : 0 1.0 10 100 mg.l⁻¹

Preparation of test solutions : The necessary amounts were pipetted, added separately to 1 l of fresh water (Appendix 5) and stirred for 4 hours in a glass-stoppered conical flask. Then the contents of these flasks were emptied in beakers and the test animals introduced. No undissolved test compound was visible during the test period.

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 : 25 animals in 1000 ml of standard fresh water (Appendix 5), pH abt. 8, hardness abt. 210 mg of CaCO₃ per l in a glass beaker, covered with a watch glass.

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	>1.0<10 ¹⁾		1.0	10
48	>10<100 ¹⁾		1.0	10

¹⁾ The animals recovered during the 48 h exposure time. Probably the test compound disappeared from the open beakers.

ANNEXE A

Determination of the acute aquatic toxicity of vinyltoluene, Fluka AG, abt. 60% meta- + abt. 40% para-vinyltoluene, purum.

Test compound : vinyltoluene

Concentrations tested : 0 1.0 10 100 mg.l⁻¹

Preparation of test solutions : The necessary amounts were pipetted, added separately to 1 l of fresh water (Appendix 5) and stirred for 4 hours in a glass-stoppered conical flask before introducing the test animals. No undissolved test compound was visible during the test period.

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 : 25 animals in 1000 ml of standard fresh water (Appendix 5), pH abt. 8, hardness abt. 210 mg of CaCO₃ per l 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	>1.0<10		1.0	10
48	>1.0<10		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 isopentene, Janssen chimica, 99+%.

Test compound : isopentene
 Concentrations tested : 0 3.2 10 32 100 mg.l⁻¹
 Preparation of test solutions : The necessary amounts were pipetted, added separately to 2 l of seawater and stirred for 4 hours in glass-stoppered conical flasks. Then the contents of these flasks were emptied in glass beakers and the test animals were introduced. At none of the dosed amounts test compound was visible 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 : 10 animals in 2000 ml of natural seawater,
 pH abt. 8, S abt. 2.8%
 in a glass beaker covered with a watch glass.

Temperature : 15°C
 Aeration : none
 Food : none
 Renewal of test medium : once a day
 Test in : singular
 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	abt. 100		32	> 100
48	abt. 90		32	> 100
72	abt. 60		32	100
96	abt. 60		32	100

'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 isopentene, Janssen chimica, 99+%.

Test compound : isopentene

Concentrations tested : 0 3.2 10 32 100 mg.l⁻¹

Preparation of test solutions : The necessary amounts were pipetted, added separately to 2 l of seawater and stirred for 4 hours in glass-stoppered conical flasks, before introducing the test animals. At none of the dosed amounts test compound was visible 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 : 10 animals in 2000 ml of natural seawater, pH abt. 8, S abt. 2.8%
in a glass-stoppered conical flask.

Temperature : 15°C

Aeration : none

Food : none

Renewal of test medium : once a day

Test in : singular

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	abt. 40		10	100
48	abt. 30		10	100
72	abt. 30		10	100
96	abt. 30		10	100

'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 isopentene, Janssen chimica, 99+%.

Test compound : isopentene

Concentrations tested : 0 10 18 32 56 100 mg.l⁻¹

Preparation of test solutions : The necessary amounts were pipetted, added separately to 500 ml of fresh water (Appendix 5) and stirred for 4 h in glass-stoppered conical flasks. Then the contents of these flasks were emptied in glass beakers, before introducing the test animals. At none of the dosed amounts test compound was visible during the test period.

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 : 25 animals in 500 ml of standard fresh water (Appendix 5), pH abt. 8, hardness abt. 210 mg of CaCO₃ per l in a glass beaker, covered with a watch glass.

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
48	84	72-98	32	> 100

'No observed effect concentration' after 48 h exposure: 18 mg.l⁻¹

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

ANNEXE A

Determination of the acute aquatic toxicity of isopentene, Janssen chimica, 99+%.

Test compound : isopentene
 Concentrations tested : 0 10 18 32 56 100 mg.l⁻¹
 Preparation of test solutions : The necessary amounts were pipetted, added separately to 500 ml of fresh water (Appendix 5) and stirred for 4 h in glass-stoppered conical flasks, before introducing the test animals. At none of the dosed amounts test compound was visible during the test period.

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 : 25 animals in 500 ml of standard fresh water (Appendix 5), pH abt. 8, hardness abt. 210 mg of CaCO₃ per l 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
48	abt. 30		18	56

'No observed effect concentration' after 48 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 1-hexene, Sigma, aprox. 99%.

Test compound : 1-hexene
 Concentrations tested : 0 3.2 10 32 100 mg.l⁻¹
 Preparation of test solutions : The necessary amounts were pipetted, added separately to 1 l of fresh water (Appendix 5) and stirred for 4 h in glass-stoppered conical flasks. Then the contents of these flasks were emptied in glass beakers, before introducing the test animals. At none of the dosed amounts test compound was visible during the test period.

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

Test animal : *Brachydanio rerio* (zebra fish) (Pisces, cyprinoidei)
 young fishes 3 ± 1 cm, from a laboratory culture in standard fresh water (Appendix 5)

Test conditions : 10 animals in 1000 ml of standard fresh water (Appendix 5),
 pH abt. 8, hardness abt. 210 mg of CaCO₃ per l
 in a glass beaker, covered with a watch glass.

Temperature : 25°C
 Aeration : none
 Food : none
 Renewal of test medium : once a day
 Test in : singular
 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 <100		10	100
48	>10 <100		10	100
72	>10 <100		10	100
96	>10 <100 (abt. 50)		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 1-hexene, Sigma, aprox. 99%.

Test compound	:	1-hexene
Concentrations tested	:	0 3.2 10 32 100 mg.l ⁻¹
Preparation of test solutions	:	The necessary amounts were pipetted, added separately to 1 l of fresh water (Appendix 5) and stirred for 4 h in glass-stoppered conical flasks, before introducing the test animals. At none of the dosed amounts test compound was visible during the test period.
Chemical analysis	:	Test solutions were not analysed for the test compound
Test animal	:	<i>Brachydanio rerio</i> (zebra fish) (Pisces, cyprinoidei) young fishes 3 ± 1 cm, from a laboratory culture in standard fresh water (Appendix 5)
Test conditions	:	10 animals in 1000 ml of standard fresh water (Appendix 5), pH abt. 8, hardness abt. 210 mg of CaCO ₃ per l in a glass-stoppered conical flask.
	Temperature	: 25°C
	Aeration	: none
	Food	: none
	Renewal of test medium	: once a day
	Test in	: singular
	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 <100		10	100
48	>10 <100		10	100
72	>10 <100		10	100
96	>10 <100 (abt. 25)		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 1-hexene, Sigma, aprox. 99%.

Test compound : 1-hexene
 Concentrations tested : 0 3.2 10 32 100 mg.l⁻¹
 Preparation of test solutions : The necessary amounts were pipetted, added separately to 500 ml of fresh water (Appendix 5) and stirred for 4 hours in glass-stoppered conical flasks. Then the contents of these flasks were emptied in glass beakers and the test animals were introduced. At none of the dosed amounts test compound was visible during the test period.

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 : 25 animals in 500 ml of standard fresh water (Appendix 5), pH abt. 8, hardness abt. 210 mg of CaCO₃ per l in a glass beaker, covered with a watch glass.

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	'ECO' mg.l ⁻¹	'EC100' mg.l ⁻¹
48	>32 <100 (abt. 60)		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 1-hexene, Sigma, aprox. 99%.

Test compound : 1-hexene

Concentrations tested : 0 3.2 10 32 100 mg.l⁻¹

Preparation of test solutions : The necessary amounts were pipetted, added separately to 500 ml of fresh water (Appendix 5) and stirred for 4 hours in glass-stoppered conical flasks before introducing the test animals. At none of the dosed amounts test compound was visible during the test period.

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 : 25 animals in 500 ml of standard fresh water (Appendix 5), pH abt. 8, hardness abt. 210 mg of CaCO₃ per l 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
48	abt. 30		10	> 32

'No observed effect concentration' after 48 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 1-octene, Sigma, aprox. 99%.

Test compound	:	1-octene
Concentrations tested	:	0 0.32 1.0 3.2 mg.l ⁻¹
Preparation of test solutions	:	Three stock solutions in tert. butanol were prepared by pipetting 44.7, 140, 447 µl of test compound and dissolving these separately in 10 ml of t. butanol. 0.1 ml of these solutions were added separately to 1 l of fresh water (Appendix 5) and stirred for 4 h in glass-stoppered conical flasks. Then the contents of these flasks were emptied in glass-beakers and the test animals were introduced. At none of the dosed amounts test compound was visible during the test period.
Chemical analysis	:	Test solutions were not analysed for the test compound
Test animal	:	<i>Brachydanio rerio</i> (zebra fish) (Pisces, cyprinoidei) young fishes 3 ± 1 cm, from a laboratory culture in standard fresh water (Appendix 5)
Test conditions	:	10 animals in 1000 ml of standard fresh water (Appendix 5), pH abt. 8, hardness abt. 210 mg of CaCO ₃ per l in a glass beaker, covered with a watch glass.
	Temperature	: 24°C
	Aeration	: none
	Food	: none
	Renewal of test medium	: once a day
	Test in	: singular
	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	>3.2		≥3.2	>3.2
48	>3.2		≥3.2	>3.2
72	>3.2		≥3.2	>3.2
96	>3.2		≥3.2	>3.2

'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 1-octene, Sigma, aprox. 99%.

Test compound : 1-octene
 Concentrations tested : 0 3.2 10 32 mg.l⁻¹
 Preparation of test solutions : The necessary amounts were pipetted, added separately to 1 l of fresh water (Appendix 5) and stirred for 4 h in glass-stoppered conical flasks. Then the contents of these flasks were emptied in glass-beakers and the test animals were introduced. At none of the dosed amounts test compound was visible during the test period..

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

Test animal : *Brachydanio rerio* (zebra fish) (Pisces, cyprinoidei)
 young fishes 3 ± 1 cm, from a laboratory culture in standard fresh water (Appendix 5)

Test conditions : 10 animals in 1000 ml of standard fresh water (Appendix 5),
 pH abt. 8, hardness abt. 210 mg of CaCO₃ per l
 in a glass beaker, covered with a watch glass.

Temperature : 24°C
 Aeration : none
 Food : none
 Renewal of test medium : once a day
 Test in : singular
 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	10.0	7.5-13.3	< 3.2	32
48	4.8	3.3- 7.1	< 3.2	10
72	4.8	3.3- 7.1	< 3.2	10
96	4.8	3.3- 7.1	< 3.2	10

'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 1-octene, Sigma, aprox. 99%.

Test compound : 1-octene

Concentrations tested : 0 0.32 1.0 3.2 mg.l⁻¹

Preparation of test solutions : Three stock solutions in tert. butanol were prepared by pipetting 44.7, 140, 447 µl of test compound and dissolving these separately in 10 ml of t. butanol. 0.1 ml of these solutions were added separately to 1 l of fresh water (Appendix 5) and stirred for 4 h in glass-stoppered conical flasks before introducing the test animals. At none of the dosed amounts test compound was visible during the test period.

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

Test animal : *Brachydanio rerio* (zebra fish) (Pisces, cyprinoidei)
young fishes 3 ± 1 cm, from a laboratory culture in standard fresh water (Appendix 5)

Test conditions : 10 animals in 1000 ml of standard fresh water (Appendix 5),
pH abt. 8, hardness abt. 210 mg of CaCO₃ per l
in a glass-stoppered conical flask.

Temperature : 24°C

Aeration : none

Food : none

Renewal of test medium : once a day

Test in : singular

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	>3.2		≥3.2	>3.2
48	>3.2		≥3.2	>3.2
72	>3.2		≥3.2	>3.2
96	>3.2		≥3.2	>3.2

'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 1-octene, Sigma, aprox. 99%.

Test compound : 1-octene

Concentrations tested : 0 3.2 10 32 mg.l⁻¹

Preparation of test solutions : The necessary amounts were pipetted, added separately to 1 l of fresh water (Appendix 5) and stirred for 4 h in glass-stoppered conical flasks before introducing the test animals. At none of the dosed amounts test compound was visible during the test period.

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

Test animal : *Brachydanio rerio* (zebra fish) (Pisces, cyprinoidei) young fishes 3 ± 1 cm, from a laboratory culture in standard fresh water (Appendix 5)

Test conditions : 10 animals in 1000 ml of standard fresh water (Appendix 5), pH abt. 8, hardness abt. 210 mg of CaCO₃ per l in a glass-stoppered conical flask.

Temperature : 24°C

Aeration : none

Food : none

Renewal of test medium : once a day

Test in : singular

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	>3.2 <10		3.2	10
48	>3.2 <10		3.2	10
72	>3.2 <10		3.2	10
96	>3.2 <10 (abt. 6)		3.2	10

'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 1-octene, Sigma, aprox. 99%.

Test compound : 1-octene

Concentrations tested : 0 3.2 10 32 mg.l⁻¹

Preparation of test solutions : The necessary amounts were pipetted, added separately to 500 ml of fresh water (Appendix 5) and stirred for 4 h in glass-stoppered conical flasks. Then the contents of these flasks were emptied in glass-beakers and the test animals were introduced. At none of the dosed amounts test compound was visible during the test period.

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 : 25 animals in 500 ml of standard fresh water (Appendix 5), pH abt. 8, hardness abt. 210 mg of CaCO₃ per l in a glass beaker, covered with a watch glass.

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
48	>3.2 <10 (abt. 7)		3.2	32

'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 1-octene, Sigma, aprox. 99%.

Test compound : 1-octene
 Concentrations tested : 0 1.0 3.2 10 mg.l⁻¹
 Preparation of test solutions : The necessary amounts were pipetted, added separately to 500 ml of fresh water (Appendix 5) and stirred for 4 h in glass-stoppered conical flasks before introducing the test animals. At none of the dosed amounts test compound was visible during the test period.

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 : 25 animals in 500 ml of standard fresh water (Appendix 5), pH abt. 8, hardness abt. 210 mg of CaCO₃ per l 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
48	>3.2	<10 (abt. 6)	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 2-octene (trans), Sigma.

Test compound : 2-octene (trans)
 Concentrations tested : 0 0.32 1.0 3.2 mg.l⁻¹
 Preparation of test solutions : Three stock solutions in tert. butanol were prepared by pipetting 44.5, 139, 445 µl of test compound and dissolving these separately in 10 ml of t. butanol. 0.1 ml of these solutions were added separately to 1 l of seawater and stirred for 4 h in glass-stoppered conical flasks. Then the contents of these flasks were emptied in glass beakers and the test animals were introduced. At none of the dosed amounts test compound was visible 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 : 10 animals in 1000 ml of natural seawater, pH abt. 8, S abt. 2.8%
 in a glass beaker covered with a watch glass.

Temperature : 15°C
 Aeration : none
 Food : none
 Renewal of test medium : once a day
 Test in : singular
 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	> 3.2		1.0	> 3.2
48	1.5	1.1-2.0	0.32	3.2
72	0.7	0.5-1.1	0.32	3.2
96	0.6	0.4-0.8	0.32	1.0

'No observed effect concentration' after 96 h exposure: 0.32 mg.l⁻¹
 Effect: condition of test animals compared to the controls (visual estimation)

ANNEXE A

Determination of the acute aquatic toxicity of 2-octene (trans), Sigma.

Test compound : 2-octene (trans)
 Concentrations tested : 0 3.2 10 32 100 mg.l⁻¹
 Preparation of test solutions : The necessary amounts were pipetted, added separately to 1 l of seawater and stirred for 4 h in glass-stoppered conical flasks. Then the contents of these flasks were emptied in glass beakers and the test animals were introduced. At none of the dosed amounts test compound was visible 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 : 10 animals in 1000 ml of natural seawater,
 pH abt. 8, S abt. 2.8%
 in a glass beaker covered with a watch glass.

Temperature : 15°C
 Aeration : none
 Food : none
 Renewal of test medium : once a day
 Test in : singular
 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	(5)		< 3.2	> 100
48	< 3.2		< 3.2	≤ 3.2
72	< 3.2		< 3.2	≤ 3.2
96	< 3.2		< 3.2	≤ 3.2

'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-octene (trans), Sigma.

Test compound : 2-octene (trans)
 Concentrations tested : 0 0.32 1.0 3.2 mg.l⁻¹
 Preparation of test solutions : Three stock solutions in tert. butanol were prepared by pipetting 44.5, 139, 445 µl of test compound and dissolving these separately in 10 ml of t. butanol. 0.1 ml of these solutions were added separately to 1 l of seawater and stirred for 4 h in glass-stoppered conical flasks, before introducing the test animals. At none of the dosed amounts test compound was visible 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 : 10 animals in 1000 ml of natural seawater,
 pH abt. 8, S abt. 2.8%
 in a glass-stoppered conical flask.

Temperature : 15°C
 Aeration : none
 Food : none
 Renewal of test medium : once a day
 Test in : singular
 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	> 3.2		1.0	> 3.2
48	2.3	1.7-3.1	1.0	3.2
72	0.6	0.4-0.8	0.32	1.0
96	0.6	0.4-0.8	0.32	1.0

'No observed effect concentration' after 96 h exposure: 0.32 mg.l⁻¹
 Effect: condition of test animals compared to the controls (visual estimation)

ANNEXE A

Determination of the acute aquatic toxicity of 2-octene (trans), Sigma.

Test compound : 2-octene (trans)
 Concentrations tested : 0 3.2 10 32 100 mg.l⁻¹
 Preparation of test solutions : The necessary amounts were pipetted, added separately to 1 l of seawater and stirred for 4 h in glass-stoppered conical flasks , before introducing the test animals. At none of the dosed amounts test compound was visible 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 : 10 animals in 1000 ml of natural seawater,
 pH abt. 8, S abt. 2.8%
 in a glass-stoppered conical flask.

Temperature : 15°C
 Aeration : none
 Food : none
 Renewal of test medium : once a day
 Test in : singular
 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	abt. 6		< 3.2	32
48	abt. 3		< 3.2	10
72	< 3.2		< 3.2	≤ 3.2
96	< 3.2		< 3.2	≤ 3.2

'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-octene (trans), Sigma.

Test compound : 2-octene (trans)

Concentrations tested : 0 1.0 3.2 10 32 100 mg.l⁻¹

Preparation of test solutions : The necessary amounts were pipetted, added separately to 2 l of seawater and stirred for 4 h in glass-stoppered conical flasks. Then the contents of these flasks were emptied in glass beakers and the test animals were introduced. At none of the dosed amounts test compound was visible during the test period.

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

Test animal : *Poecilia reticulata* (guppy) (Pisces, cyprinodontiformes) young fishes 3 ± 1 week old, from a laboratory culture in natural seawater (S = 2.8%)

Test conditions : 10 animals in 2000 ml of natural seawater, pH abt. 8, S abt. 2.8%, in a glass beaker covered with a watch glass

Temperature : 24°C

Aeration : none

Food : none

Renewal of test medium : once a day

Test in : singular

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	41	25-64	3.2	100
48	20	13-32	3.2	100
72	13	8-20	3.2	100
96	13	8-20	3.2	100

'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-octene (trans), Sigma.

Test compound : 2-octene (trans)
 Concentrations tested : 0 1.0 3.2 10 32 100 mg.l⁻¹
 Preparation of test solutions : The necessary amounts were pipetted, added separately to 2 l of seawater and stirred for 4 h in glass-stoppered conical flasks, before introducing the test animals. At none of the dosed amounts test compound was visible during the test period.

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

Test animal : *Poecilia reticulata* (guppy) (Pisces, cyprinodontiformes) young fishes 3 ± 1 week old, from a laboratory culture in natural seawater (S = 2.8%)

Test conditions : 10 animals in 2000 ml of natural seawater, pH abt. 8, S abt. 2.8%, in a glass-stoppered conical flask

Temperature : 24°C
 Aeration : none
 Food : none
 Renewal of test medium : once a day
 Test in : singular
 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	9	7-12	3.2	32
48	6	4- 8	3.2	10
72	6	4- 8	3.2	10
96	6	4- 8	3.2	10

'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 2-octene (trans), Sigma.

Test compound : 2-octene (trans)

Concentrations tested : 0 18 32 56 100 mg.l⁻¹

Preparation of test solutions : The necessary amounts were pipetted, added separately to 2 l of fresh water (Appendix 5) and stirred for 4 h in glass-stoppered conical flasks. Then the contents of these flasks were emptied in glass beakers and the test animals were introduced. At none of the dosed amounts test compound was visible during the test period.

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

Test animal : *Brachydanio rerio* (zebra fish) (Pisces, cyprinoidei)
young fishes 3 ± 1 cm, from a laboratory culture in standard fresh water (Appendix 5)

Test conditions : 10 animals in 2000 ml of standard fresh water (Appendix 5),
pH abt. 8, hardness abt. 210 mg of CaCO₃ per l
in a glass beaker, covered with a watch glass.

Temperature : 24°C

Aeration : none

Food : none

Renewal of test medium : once a day

Test in : singular

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	38-60	18	100
48	34	25-40	18	56
72	30	21-34	18	56
96	28	20-32	18	56

'No observed effect concentration' after 96 h exposure: < 18 mg.l⁻¹

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

Remark: The oxygen concentration in some of the test solution was low, but it is assumed that this has not greatly influenced the test results.

ANNEXE A

Determination of the acute aquatic toxicity of 2-octene (trans), Sigma.

Test compound : 2-octene (trans)

Concentrations tested : 0 3.2 5.6 10 18 32 56 mg.l⁻¹

Preparation of test solutions : The necessary amounts were pipetted, added separately to 2 l of fresh water (Appendix 5) and stirred for 4 h in glass-stoppered conical flasks, before introducing the test animals. At none of the dosed amounts test compound was visible during the test period.

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

Test animal : *Brachydanio rerio* (zebra fish) (Pisces, cyprinoidei)
young fishes 3 ± 1 cm, from a laboratory culture in standard fresh water (Appendix 5)

Test conditions : 10 animals in 2000 ml of standard fresh water (Appendix 5),
pH abt. 8, hardness abt. 210 mg of CaCO₃ per l
in a glass-stoppered conical flask.

Temperature : 24°C

Aeration : none

Food : none

Renewal of test medium : once a day

Test in : singular

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	13-19	10	18
48	13	11-16	10	18
72	8.0	6.6-9.7	5.6	18
96	7.5	6.1-9.1	5.6	10

'No observed effect concentration' after 96 h exposure: 3.2 mg.l⁻¹

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

Remark: The oxygen concentration in some of the test solution was low, but it is assumed that this has not greatly influenced the test results.

ANNEXE A

Determination of the acute aquatic toxicity of 2-octene (trans), Sigma.

Test compound : 2-octene (trans)
 Concentrations tested : 0 3.2 10 32 mg.l⁻¹
 Preparation of test solutions : The necessary amounts were pipetted, added separately to 500 ml of fresh water (Appendix 5) and stirred for 4 h in glass-stoppered conical flasks. Then the contents of these flasks were emptied in glass beakers and the test animals were introduced. At none of the dosed amounts test compound was visible during the test period.

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 : 25 animals in 500 ml of standard fresh water (Appendix 5), pH abt. 8, hardness abt. 210 mg of CaCO₃ per l in a glass beaker, covered with a watch glass.

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
48	abt. 8		3.2	32

'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-octene (trans), Sigma.

Test compound : 2-octene (trans)
 Concentrations tested : 0 3.2 10 32 mg.l⁻¹
 Preparation of test solutions : The necessary amounts were pipetted, added separately to 500 ml of fresh water (Appendix 5) and stirred for 4 h in glass-stoppered conical flasks, before introducing the test animals. At none of the dosed amounts test compound was visible during the test period.

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 : 25 animals in 500 ml of standard fresh water (Appendix 5), pH abt. 8, hardness abt. 210 mg of CaCO₃ per l 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
48	abt. 6		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 1-nonene, Sigma, aprox. 99%.

Test compound	:	1-nonene
Concentrations tested	:	0 0.32 1.0 3.2 mg.l ⁻¹
Preparation of test solutions	:	Three stock solutions in tert. butanol were prepared by pipetting 43.9, 137, 439 µl of test compound and dissolving these separately in 10 ml of t. butanol. 0.1 ml of these solutions were added separately to 1 l of fresh water (Appendix 5) and stirred for 4 h in glass-stoppered conical flasks. Then the contents of these flasks were emptied in glass beakers and the test animals were introduced. At none of the dosed amounts test compound was visible during the test period.
Chemical analysis	:	Test solutions were not analysed for the test compound
Test animal	:	<i>Brachydanio rerio</i> (zebra fish) (Pisces, cyprinoidei) young fishes 3 ± 1 cm, from a laboratory culture in standard fresh water (Appendix 5)
Test conditions	:	10 animals in 1000 ml of standard fresh water (Appendix 5), pH abt. 8, hardness abt. 210 mg of CaCO ₃ per l in a glass beaker, covered with a watch glass.
	Temperature	: 24°C
	Aeration	: none
	Food	: none
	Renewal of test medium	: once a day
	Test in	: singular
	Test duration	: 96 h

Results: No mortality found during the test period.

Time h	LC50 mg.l ⁻¹	confidence interval mg.l ⁻¹	'LC0' mg.l ⁻¹	'LC100' mg.l ⁻¹
24	> 3.2		≥ 3.2	> 3.2
48	> 3.2		≥ 3.2	> 3.2
72	> 3.2		≥ 3.2	> 3.2
96	> 3.2		≥ 3.2	> 3.2

'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 1-nonene, Sigma, aprox. 99%.

Test compound : 1-nonene

Concentrations tested : 0 3.2 10 32 mg.l⁻¹

Preparation of test solutions : The necessary amounts were pipetted, added separately to 1 l of fresh water (Appendix 5) and stirred for 4 h in glass-stoppered conical flasks. Then the contents of these flasks were emptied in glass beakers and the test animals were introduced. At none of the dosed amounts test compound was visible during the test period.

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

Test animal : *Brachydanio rerio* (zebra fish) (Pisces, cyprinoidei) young fishes 3 ± 1 cm, from a laboratory culture in standard fresh water (Appendix 5)

Test conditions : 10 animals in 1000 ml of standard fresh water (Appendix 5), pH abt. 8, hardness abt. 210 mg of CaCO₃ per l in a glass beaker, covered with a watch glass.

Temperature : 24°C

Aeration : none

Food : none

Renewal of test medium : once a day

Test in : singular

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	abt. 30		10	> 32
48	abt. 30		10	> 32
72	11	0.8-154	3.2	32
96	10	3-35	3.2	32

'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 1-nonene, Sigma, aprox. 99%.

Test compound : 1-nonene
 Concentrations tested : 0 0.32 1.0 3.2 mg.l⁻¹
 Preparation of test solutions : Three stock solutions in tert. butanol were prepared by pipetting 43.9, 137, 439 µl of test compound and dissolving these separately in 10 ml of t. butanol. 0.1 ml of these solutions were added separately to 1 l of fresh water (Appendix 5) and stirred for 4 h in glass-stoppered conical flasks, before the test animals were introduced. At none of the dosed amounts test compound was visible during the test period.

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

Test animal : *Brachydanio rerio* (zebra fish) (Pisces, cyprinoidei)
 young fishes 3 ± 1 cm, from a laboratory culture in standard fresh water (Appendix 5)

Test conditions : 10 animals in 1000 ml of standard fresh water (Appendix 5),
 pH abt. 8, hardness abt. 210 mg of CaCO₃ per l
 in a glass-stoppered conical flask.

Temperature : 24°C
 Aeration : none
 Food : none
 Renewal of test medium : once a day
 Test in : singular
 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	> 3.2		≥ 3.2	> 3.2
48	> 1.0 < 3.2		1.0	> 3.2
72	> 1.0 < 3.2		1.0	3.2
96	> 1.0 < 3.2		1.0	3.2

'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 1-nonene, Sigma, aprox. 99%.

Test compound : 1-nonene

Concentrations tested : 0 3.2 10 32 mg.l⁻¹

Preparation of test solutions : The necessary amounts were pipetted, added separately to 1 l of fresh water (Appendix 5) and stirred for 4 h in glass-stoppered conical flasks, before the test animals were introduced. At none of the dosed amounts test compound was visible during the test period.

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

Test animal : *Brachydanio rerio* (zebra fish) (Pisces, cyprinoidei)
young fishes 3 ± 1 cm, from a laboratory culture in standard fresh water (Appendix 5)

Test conditions : 10 animals in 1000 ml of standard fresh water (Appendix 5),
pH abt. 8, hardness abt. 210 mg of CaCO₃ per l
in a glass-stoppered conical flask.

Temperature : 24°C

Aeration : none

Food : none

Renewal of test medium : once a day

Test in : singular

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	abt. 11		3.2	32
48	abt. 6		3.2	10
72	< 3.2		< 3.2	≤ 3.2
96	< 3.2		< 3.2	≤ 3.2

'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 1-nonene, Sigma, aprox. 99%.

Test compound : 1-nonene

Concentrations tested : 0 3.2 10 mg.l⁻¹

Preparation of test solutions : The necessary amounts were pipetted, added separately to 500 ml of fresh water (Appendix 5) and stirred for 4 h in glass-stoppered conical flasks. Then the contents of these flasks were emptied in glass beakers and the test animals were introduced. At none of the dosed amounts test compound was visible during the test period.

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 : 25 animals in 500 ml of standard fresh water (Appendix 5), pH abt. 8, hardness abt. 210 mg of CaCO₃ per l in a glass beaker, covered with a watch glass.

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
48	> 3.2 < 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 1-nonene, Sigma, aprox. 99%.

Test compound : 1-nonene
 Concentrations tested : 0 3.2 10 mg.l⁻¹
 Preparation of test solutions : The necessary amounts were pipetted, added separately to 500 ml of fresh water (Appendix 5) and stirred for 4 h in glass-stoppered conical flasks, before the test animals were introduced. At none of the dosed amounts test compound was visible during the test period.

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 : 25 animals in 500 ml of standard fresh water (Appendix 5), pH abt. 8, hardness abt. 210 mg of CaCO₃ per l 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
48	< 3.2 (prob. abt. 2)		< 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 1-undecene, Fluka ag, > 97%, pract.

Test compound : 1-undecene
 Concentrations tested : 0 0.32 1.0 3.2 mg.l⁻¹
 Preparation of test solutions : Three stock solutions in tert. butanol were prepared by pipetting 42.7, 133, 427 µl of test compound and dissolving these separately in 10 ml of t. butanol. 0.1 ml of these solutions were added separately to 1 l of seawater and stirred for 4 h in glass-stoppered conical flasks. Then the contents of these flasks were emptied in glass beakers and the test animals were introduced. At none of the dosed amounts test compound was visible 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 : 10 animals in 1000 ml of natural seawater, pH abt. 8, S abt. 2.8%
 in a glass beaker covered with a watch glass.

Temperature : 15°C
 Aeration : none
 Food : none
 Renewal of test medium : once a day
 Test in : triplicate
 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	> 3.2		3.2	> 3.2
48	abt. 30		< 0.32	> 3.2
72	abt. 0.2		< 0.32	> 3.2
96	< 0.32		< 0.32	> 3.2

'No observed effect concentration' after 96 h exposure: < 0.32 mg.l⁻¹
 Effect: condition of test animals compared to the controls (visual estimation)

ANNEXE A

Determination of the acute aquatic toxicity of 1-undecene, Fluka ag, > 97%, pract.

Test compound : 1-undecene
 Concentrations tested : 0 3.2 10 32 mg.l⁻¹
 Preparation of test solutions : The necessary amounts were pipetted, added separately to 1 l of seawater and stirred for 4 h in glass-stoppered conical flasks. Then the contents of these flasks were emptied in glass beakers₁ and the test animals were introduced. At 32 ml.l⁻¹ test compound was visible.

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 : 10 animals in 1000 ml of natural seawater,
 pH abt. 8, S abt. 2.8%
 in a glass beaker covered with a watch glass.

Temperature : 15°C
 Aeration : none
 Food : none
 Renewal of test medium : once a day
 Test in : triplicate
 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	> 32		< 3.2	> 32
48	15	7-31	< 3.2	> 32
72	3.4	1.4-8.4	< 3.2	32
96	2.0	0.6-7.2	< 3.2	32

'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 1-undecene, Fluka ag, > 97%, pract.

Test compound : 1-undecene

Concentrations tested : 0 0.32 1.0 3.2 mg.l⁻¹

Preparation of test solutions : Three stock solutions in tert. butanol were prepared by pipetting 42.7, 133, 427 µl of test compound and dissolving these separately in 10 ml of t. butanol. 0.1 ml of these solutions were added separately to 1 l of seawater and stirred for 4 h in glass-stoppered conical flasks, before the test animals were introduced. At none of the dosed amounts test compound was visible 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 : 10 animals in 1000 ml of natural seawater, pH abt. 8, S abt. 2.8%
in a glass-stoppered conical flask.

Temperature : 15°C

Aeration : none

Food : none

Renewal of test medium : once a day

Test in : triplicate

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	5.5	2.4-12.7	< 0.32	> 3.2
48	1.2	0.8- 1.8	< 0.32	> 3.2
72	0.6	0.4- 0.9	< 0.32	> 3.2
96	0.3	0.2- 0.5	< 0.32	3.2

'No observed effect concentration' after 96 h exposure: < 0.32 mg.l⁻¹
Effect: condition of test animals compared to the controls (visual estimation)

ANNEXE A

Determination of the acute aquatic toxicity of 1-undecene, Fluka ag, > 97%, pract.

Test compound : 1-undecene
 Concentrations tested : 0 3.2 10 32 mg.l⁻¹
 Preparation of test solutions : The necessary amounts were pipetted, added separately to 1 l of seawater and stirred for 4 h in glass-stoppered conical flasks, before the test animals were introduced. At 32 ml.l⁻¹ test compound was visible.

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 : 10 animals in 1000 ml of natural seawater, pH abt. 8, S abt. 2.8%
 in a glass-stoppered conical flask.

Temperature : 15°C
 Aeration : none
 Food : none
 Renewal of test medium : once a day
 Test in : triplicate
 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	abt. 10		< 3.2	> 32
48	abt. 6		< 3.2	32
72	< 3.2		< 3.2	10
96	< 3.2		< 3.2	3.2

'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 1-undecene, Fluka ag, > 97%, pract.

Test compound : 1-undecene

Concentrations tested : 0 3.2 10 32 100 mg.l⁻¹

Preparation of test solutions : The necessary amounts were pipetted, added separately to 2 l of seawater and stirred for 4 h in glass-stoppered conical flasks. Then the contents of these flasks were emptied in glass beakers and the test animals were introduced. At 32 and 100 mg.l⁻¹ test compound was visible.

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

Test animal : *Poecilia reticulata* (guppy) (Pisces, cyprinodontiformes) young fishes 3 ± 1 week old, from a laboratory culture in natural seawater (S = 2.8%)

Test conditions : 10 animals in 2000 ml of natural seawater, pH abt. 8, S abt. 2.8%, in a glass beaker covered with a watch glass

Temperature : 24°C
 Aeration : none
 Food : none
 Renewal of test medium : once a day
 Test in : singular
 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 < 32		10	32
48	> 10 < 32		10	32
72	> 10 < 32		10	32
96	> 10 < 32		10	32

'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 1-undecene, Fluka ag, > 97%, pract.

Test compound : 1-undecene
 Concentrations tested : 0 3.2 10 32 100 mg.l⁻¹
 Preparation of test solutions : The necessary amounts were pipetted, added separately to 2 l of seawater and stirred for 4 h in glass-stoppered conical flasks, before the test animals were introduced. At 32 and 100 ml.l⁻¹ test compound was visible.

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

Test animal : *Poecilia reticulata* (guppy) (Pisces, cyprinodontiformes) young fishes 3 ± 1 week old, from a laboratory culture in natural seawater (S = 2.8%)

Test conditions : 10 animals in 2000 ml of natural seawater, pH abt. 8, S abt. 2.8%, in a glass-stoppered conical flask

Temperature : 24°C
 Aeration : none
 Food : none
 Renewal of test medium : once a day
 Test in : singular
 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 < 32		10	100
48	> 10 < 32		10	32
72	> 10 < 32		10	32
96	> 10 < 32		10	32

'No observed effect concentration' after 96 h exposure: 10 mg.l⁻¹

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

ANNEXE B Summary of data on acute aquatic toxicity of aliphatic hydrocarbons (L(E)C50 values in mg.l^{-1} ; ~ means: about).
(test systems closed or open during exposure period as indicated)

Compound	Marine animals					Fresh-water animals						
	<i>C. marinus</i>	<i>P. reticulata</i>	<i>N. spinipes</i>	<i>C. crangon</i>	<i>C. variegatus</i>	<i>D. magna</i>			<i>L. idus</i>	<i>C. auratus</i>	<i>P. reticulata</i>	<i>B. rerio</i>
	96h LC50	96h LC50	96h LC50	48h LC50	96h LC50	24h EC50	48h EC50	48h LC50	48h LC50	24h LC50	14d LC50	96h LC50
	ref.1 ref.2	ref.1 ref.2	ref.2	ref.3	ref.4	ref.5	ref.1	ref.10	ref.6	ref.7,9	ref.8	ref.1
	closed	closed	closed	closed	open	open	closed	closed	open	open	open	closed
<u>alkanes</u>												
n-pentane C5												
n-hexane C6						>1000	~40 ⁴⁾	9.7				~40 ⁴⁾
n-heptane C7								3.9	180-4480			
octane C8								0.4	250-2940			
nonane C9												
n-decane C10					>500			0.03				
dodecane C11												
tridecane C13												
tetradecane C14												
<u>cycloalkanes</u> (naphthenes)												
cyclopentane C5								10.5				
cyclohexane C6						> 400		3.8	55-763		>84	
cycloheptane C7						> 50		1.5	204-567			
methylcyclohexane C7												
<u>alkenes</u>												
2-pentene C5		~32										
(cis + trans)												
isopentene C5	~30	35					~30					
1,3-pentadiene C5												
(cis)												
1,3-pentadiene C5		18										
(trans)												
1-hexene C6							~30					~25
2-methylpentene C6		~18										
1-heptene C7									200			
1-octene C8							~6					~6
2-octene (trans) C8	0.6	6					~6					7.5
di-isobutene C8					36 ¹⁾					3 ³⁾		
C6-C8 olefins					60							
1-nonene C9		0.9					~2					~2
nonene C9										3 ²⁾		
undecene C11	0.3	(2.0) ⁵⁾	~20									
dodecene C12		*	*	*								
tridecene C13		*	*	*								
tetradecene C14		*	*	*								
C13-C14 olefins				*								
<u>cycloalkenes</u>												
cyclohexene C6						720			39-405			
cycloheptene C7						112			56-181			
bicyclo(2,2,1)-hepta-2,5-diene- (2,5-norborna-diene) C7										46		
1,3,5-cyclohepta-triene C7										15		
1,5-cycloocta-diene C8										14		
1,5,9-cyclododecatriene C12										4		

* No mortality found within the exposure period in an 'as best as possible' saturated solution (no additives used)

1) 99% C8-compounds, of which 90% 2,2,4-trimethylpent-1-ene.

2) Nonene (C9 olefin mixture; propylene trimer; propene trimer (ref. 9))

3) Diisobutene (diisobutylene; 2,4,4-trimethyl-1-pentene+2,4,4-trimethyl-2-pentene)

4) Personal communication from author

5) Open test system!

Remark

Data of refs. 1, 2 and 10 are from tests in closed containers. The test solutions were prepared by prolonged mixing of dilution water and hydrocarbon before introduction of the test animals. The intrinsic toxicities of these hydrocarbons, as determined in the other tests mentioned, may well be estimated too low.

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Table 1 Results of aquatic toxicity tests for 14 compounds (L(E)C 50 in mg.l⁻¹).

Test compound	origin	purity	96h LC50 ¹⁾		96h LC50 ¹⁾ <i>P. reticulata</i> (seaw) open ⁵⁾	48h EC50 <i>D. magna</i>		solubility in dilution water ²⁾ mg.l ⁻¹
			<i>C. marinus</i> open ⁵⁾	closed ⁵⁾		open ⁵⁾	closed ⁵⁾	
butyl stearate	Sigma	99+%		*				< 1000 ⁴⁾
calcium hydroxide	J.T. Baker	97%			356 (206-616)			
choline chloride	Sigma	3x cryst.	abt. 500	abt. 500		> 320		≥ 1000 ³⁾
2,2-dichloroiso- propylether	Aldrich	techn.	35 ⁷⁾	abt. 40 ⁷⁾				≥ 100 ³⁾
diethyleneglycol dibutyl ether	Merck	98% (GC)	116 (80-168)	101 (67-151)				< 320 ⁴⁾
1,3-diisopropyl- benzene	Hicol B.V.	90-95%(GC) techn.	abt. 1.0	abt. 0.3				≥ 3.2 ³⁾
ethylene glycol methyl butyl ether	Riedel- de Haën AG	98%	abt. 560	abt. 900		> 1000		≥ 1000 ³⁾
ethyleneglycol monobutyl ether	Merck	99% (GC)	> 1000	> 1000		> 1000		≥ 1000 ³⁾
furfural	Hicol B.V.	> 99% (GC) (puriss.)	15 (13-19)	21 (17-27)		abt. 40		≥ 56 ³⁾
3-methoxy-1- butanol	Janssen chimica	99%	> 1000	> 1000		> 1000	> 1000	≥ 1000 ³⁾
propylene glycol	Sigma		> 1000			> 1000		≥ 1000 ³⁾
sodium hydroxide	J.T. Baker	98.6%			196 (144-267)			
urea	May & Baker	not less 99.5%	> 1000		> 1000			≥ 1000 ³⁾
vinyltoluene	Fluka AG	purum ⁶⁾	41	1.6		abt. 30	abt. 3	< 10 ⁴⁾

*) No mortality found within the exposure period in an 'as best as possible' saturated solution (no additives used)

1) Confidence interval in parentheses

2) Visual estimation during toxicity test

3) No undissolved test compound seen in the test solutions at concentrations ≥ value indicated

4) Undissolved test compound seen in the test solutions at concentrations ≥ value indicated

5) See section 2.3

6) abt. 60% m - + abt. 40% p-vinyltoluene

7) in µl per l

Table 2 Results of the aquatic toxicity test of olefins.

Test compound	C-atoms	Origin	Purity	96h LC50 ³⁾ mg.l ⁻¹						48h EC50 mg.l ⁻¹	
				marine animals				fresh-water		fresh-water	
				<i>C. marinus</i> (crust.)		<i>P. reticulata</i> (fish)		<i>B. rerio</i> (fish)		<i>D. magna</i> (crust.)	
				open	closed	open	closed	open	closed	open	closed
2-pentene (cis + trans)	5	Fluka AG	> 95% pract		abt. 32 ²⁾⁴⁾						
isopentene	5	Janssen chimica	99+%	abt. 60 ²⁾	abt. 30 ²⁾					84 ²⁾	abt. 30 ²⁾
1,3-pentadiene (cis)	5	Fluka AG	> 98% purum		35 ²⁾⁴⁾						
1,3-pentadiene (trans)	5	Fluka AG	> 97% purum		18 ²⁾⁴⁾						
1-hexene	6	Sigma	abt. 99%					abt. 50 ²⁾	abt. 25 ²⁾	abt. 60 ²⁾	abt. 30 ²⁾
2-methylpentene	6	Fluka AG	> 99% purum		abt. 18 ²⁾⁴⁾						
1-octene	8							> 3.2 ¹⁾ 4.8 ²⁾	> 3.2 ¹⁾ abt 6 ²⁾	abt. 7 ²⁾	abt. 6 ²⁾
2-octene (trans)	8	Sigma		0.6 ¹⁾ < 3.2 ²⁾	0.6 ¹⁾ < 3.2 ²⁾	13 ²⁾	6 ²⁾	28 ²⁾	7.5 ²⁾	abt. 8 ²⁾	abt. 6 ²⁾
1-nonene	9	Sigma	abt. 99%		0.9 ²⁾⁴⁾			> 3.2 ¹⁾ 10 ²⁾	abt 2 ¹⁾ < 3.2 ²⁾	abt. 6 ²⁾	abt. 2 ²⁾
1-undecene	11	Fluka AG	> 97% pract	< 0.32 ¹⁾ 2.0 ²⁾⁵⁾	0.3 ¹⁾ < 3.2 ²⁾	abt. 20 ²⁾	abt. 20 ²⁾				
1-dodecene	12										
1-tridecene	13	Fluka AG			* ²⁾⁴⁾		* ²⁾				
1-tetradecene	14	Fluka AG			* ²⁾⁴⁾		* ²⁾				

*) No mortality found within the exposure period in an 'as best as possible' saturated solution (no additives used)

1) with 0.1 ml.l⁻¹ of an organic solvent as carrier

2) without additives

3) for confidence intervals, see Annexe 1

4) see R85/59

5) same result obtained in 1985 as was reported in 1983

ANNEXE B Summary of data on acute aquatic toxicity of aliphatic hydrocarbons (L(E)C50 values in mg.l⁻¹; ~ means: about).
(test systems closed or open during exposure period as indicated)

Compound	Marine animals					Fresh-water animals						
	<i>C. marinus</i>	<i>P. reticulata</i>	<i>N. spinipes</i>	<i>C. crangon</i>	<i>C. variegatus</i>	<i>D. magna</i>			<i>L. idus</i>	<i>C. auratus</i>	<i>P. reticulata</i>	<i>B. rerio</i>
	96h LC50 ref.1 ref.2 closed	96h LC50 ref.1 ref.2 closed	96h LC50 ref.2 closed	48h LC50 ref.3 closed	96h LC50 ref.4 open	24h EC50 ref.5 open	48h EC50 ref.1 closed	48h LC50 ref.10 closed	48h LC50 ref.6 open	24h LC50 ref.7,9 open	14d LC50 ref.8 open	96h LC50 ref. 1 closed
<u>alkanes</u>												
n-pentane C5								9.7				~40 ⁴)
n-hexane C6						>1000	~40 ⁴)	3.9	180-4480			
n-heptane C7									250-2940			
octane C8								0.4				
nonane C9												
n-decane C10					>500			0.03				
dodecane C11												
tridecane C13												
tetradecane C14												
<u>cycloalkanes</u> (naphthenes)												
cyclopentane C5								10.5				
cyclohexane C6						> 400		3.8	55-763		>84	
cycloheptane C7						> 50			204-567			
methylcyclohexane C7								1.5				
<u>alkenes</u>												
2-pentene C5	~32											
(cis + trans)												
isopentene C5	~30						~30					
1,3-pentadiene C5	35											
(cis)												
1,3-pentadiene C5	18											
(trans)												
1-hexene C6							~30					~25
2-methylpentene C6	~18											
1-heptene C7									200			
1-octene C8							~6					~6
2-octene (trans) C8	0.6	6					~6					7.5
di-isobutene C8				36 ¹)							3 ³)	
C6-C8 olefins				60								
1-nonene C9	0.9						~2					~2
nonene C9											3 ²)	
undecene C11	0.3	(2.0) ⁵)	~20									
dodecene C12												
tridecene C13	*	*	*									
tetradecene C14	*	*	*									
C13-C14 olefins				*								
<u>cycloalkenes</u>												
cyclohexene C6						720			39-405			
cycloheptene C7						112			56-181			
bicyclo(2,2,1)- C7										46		
hepta-2,5-diene-												
(2,5-norborna-												
diene)												
1,3,5-cyclohepta- C7										15		
triene												
1,5-cycloocta- C8										14		
diene												
1,5,9-cyclodode- C12										4		
catriene												

* No mortality found within the exposure period in an 'as best as possible' saturated solution (no additives used)

1) 99% C8-compounds, of which 90% 2,2,4-trimethylpent-1-ene.

2) Nonene (C9 olefin mixture; propylene Trimer; propene trimer (ref. 9))

3) Diisobutene (diisobutylene; 2,4,4-trimethyl-1-pentene+2,4,4-trimethyl-2-pentene)

4) Personal communication from author

5) Open test system!

Remark

Data of refs. 1, 2 and 10 are from tests in closed containers. The test solutions were prepared by prolonged mixing of dilution water and hydrocarbon before introduction of the test animals. The intrinsic toxicities of these hydrocarbons, as determined in the other tests mentioned, may well be estimated too low.