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Shell Oil Company



One Shell Plaza
P.O. Box 4320
Houston, Texas 77210

FYI-0794 001002



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February 7, 1984

Tetrahydrothiophene 1,1-dioxide

Mr. Martin Greif
Executive Secretary
TSCA Interagency Testing Committee
Environmental Protection Agency (TS-792)
401 M. Street, S.W.
Washington, D.C. 20460

Confidential

Dear Mr. Greif:

This letter is in response to our telephone conversation of February 2. The proprietary reports sent by Shell to you on January 12, 1984, were submitted as public information; confidential material was submitted under cover of "TSCA-CBI". I trust this letter is adequate for your needs.

Very truly yours,

J P Sepesi

J. P. Sepesi, Manager
Product Safety & Compliance
Oil & Chemical Products, HS&E

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DETERMINATION OF ACUTE TOXICITY TO FISH
OF SHELL CHEMICALS. I.

by

A.L. BRIDIÉ, M. WINTER and C.J.M. WOLFF

I.N. 79116

Code 50070920

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Koninklijke/Shell-Laboratorium, Amsterdam
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Koninklijke / Shell-Laboratorium, Amsterdam

Shell Research B.V.



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Deze ref.: EC-1416

Dear Sirs,

The harmfulness of chemical compounds in the environment is determined by a large number of factors, many of which need further study and investigation. Of these, the acute toxicity to fish is a property which has a direct bearing on the production and application of chemicals.

We are currently carrying out a series of tests to assess the acute toxicity to fish and the (bio)chemical oxygen demand (BOD and COD) of the industrial chemicals marketed by Shell. The results and experience obtained during the routine bioassays so far are the subject of the attached Research Report AMCR.0095.73, "Determination of acute toxicity to fish of Shell chemicals" I, by A.L. Bridié, M. Winter and C.J.M. Wolff.

The work on this subject is being continued and more results will be reported in due course.

Yours faithfully,

KONINKLIJKE/SHELL-LABORATORIUM, AMSTERDAM

A handwritten signature in dark ink, appearing to read 'S. Herzberg', is written over the typed name.

(S. Herzberg)

AMGR.0095.73

DETERMINATION OF ACUTE TOXICITY TO FISH
OF SHELL CHEMICALS. I.

by

A.L. Bridié, M. Winter and C.J.M. Wolff

I.N. 79116

Code 50070620

Approved by: S. Herzberg

SUMMARY
=====

We have measured the acute toxicity to fish of a number of Shell chemicals in water solution. This report describes the methods used and presents the results obtained so far.

March 1973

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DETERMINATION OF ACUTE TOXICITY TO FISH
OF SHELL CHEMICALS. I.

I. INTRODUCTION

"With an ever increasing variety of manufacturing processes using chemicals and discharging waste products to rivers and streams, the protection of fish life from deleterious pollution is an important consideration".

This quotation from the ASTM Manual on Water (3rd Ed., p. 223) fairly accurately reflects the sense of responsibility in the Group on this subject. As a result CMFE/4 commissioned KSLA to measure a number of environmental properties of the industrial chemicals currently marketed by Shell. For a description of the scope and application of the toxicity test used we further cite the ASTM Manual.

"ASTM Method D 1345¹, "Test for Evaluating Acute Toxicity of Industrial Waste Water to Fresh-Water Fishes" is a bio-assay procedure applicable for use as a guide in evaluating acute toxicity of wastes to fresh-water fishes, and in estimating safe concentrations of such industrial waste discharges. It is a batch method which evaluates the acute toxicity of industrial wastes and ... of pure chemical compounds in water solution as well. Acute toxicity is defined as any direct lethal action of pollution to fresh-water fishes that is demonstrable within 96 h or less. Test fish are exposed to dilutions of the sample being evaluated for specified periods, and median tolerance limits are calculated. This test does not evaluate long-range toxic effects such as substances that may interfere seriously with growth and reproduction." (unquote)

The various procedures which we used were essentially those prescribed in the ASTM Method D 1345. Further experimental details and considerations are given in a number of appendices to this report.

The chemicals tested for toxicity to fish are listed in the "Shell Industrie Chemica-len Gids" issued by Shell Nederland Chemie B.V. Of the approximately 240 products mentioned in that guide, some 120 have been tested so far.

II. RESULTS

We performed our experiments in all-glass aquaria with 25 l of test solution and 6 or 10 goldfish. We determined median tolerance limit (TL_m) values, the median tolerance limit being the concentration at which 50% of the animals survive 24-h or 96-h tests. The various conditions are summarized in Table I. (The reasons for selection of these test conditions are given in Appendix VI.)

TABLE I
SUMMARY OF TEST CONDITIONS

Category	Duration, h	Number of fish	Tm, mg/l	Description	Reported in Table No.
1	24	10	> 5000	-	II
2	24	10	500-5000	toxic	III
3	24 + 96	10	< 500	toxic	IV
4	24	6	< 5000	toxic and volatile	V
5	96	10	-	low solubility	VI

The results of the tests are given in Tables II to VI. The products marked with an asterisk caused a precipitate to form when dissolved in tap water (see Appendix II).

TABLE II

CHEMICALS WITH 24-h TLM > 5000 mg/l

Acetone
3-Chloro-1,2-propanediol
Diacetone alcohol
Diethanolamine (at pH 7.0)*
Diethylene glycol
Ditto Special Grade
Lisopropanolamine (at pH 7.0)*
"DIOXITOL"
Dipropylene glycol
Glycerin
Glycerin- α -allyl ether
Hexylene glycol
Isopropyl alcohol
Ditto denatured
Ditto/C plus
Isopropyl "OXITOL"
Methyl "DIOXITOL"
Methyl ethyl ketone
Methyl "OXITOL"
Mixed isopropanolamines (at pH 7.0)*
Monoethanolamine (at pH 7.0)*
Monoethylene glycol
Ditto Fibre Nitration Grade
Monoisopropanolamine (at pH 7.0)*
Monopropylene glycol
"OXITOL"
Polyethylene glycol 200
Ditto 300
Ditto 400
Ditto 555 M
Ditto 600
Ditto 800
Ditto 1000
Ditto 1500
Ditto 4000
Ditto 4000 F
Ditto 4000 P
Ditto 6000
Polypropylene glycol 400
Ditto 750
Tertiary butyl alcohol
Triethanolamine commercial (at pH 10.3)*
Ditto 85 % (at pH 7.0)*
Ditto 98 % (at pH 9.9)*
Triethylene glycol
Trimethylene glycol
"TRIOXITOL"

TABLE III

CHEMICALS WITH TL_m 500-5000 mg/l

Chemical	24-h TL _m mg/l
1-Butyl alcohol	2600
n-Butyl alcohol	1900
sec-Butyl alcohol	4300
Butyl "DIOXITOL"	2700
Butyl "OXITOL"	1650
1,3-Dichloro-2-propanol	680
Diethanolamine (at pH 9.7)*	830
Diethyl ketone	1225
Diisopropanolamine (at pH 10.2)*	1100
2,3-Epoxypropyl trimethyl ammonium chloride	5000
Mesityl oxide	540
4-Methoxy-4-methyl-2-pentanone	3800
Mixed isopropanolamines (at pH 10.1)*	900
Polypropylene glycol 2000	1000
Sulfolane	4800
Triethanolamine 85 % (at pH 10.3)*	3500
"VERSATIC" % (at pH 7.0)	4500

TABLE IV

CHEMICALS WITH TLM < 500 mg/l

Chemical	24-h TLM mg/l	96-h TLM mg/l
Acrylamide	460	155
N-n-Butyl imidazole*	30	30
3-Chloro-1-propanol	170	50
Diallylamine (at pH 7.0)*	110	20
Ditto (at pH 9.4)*	16	7
"DOBANIC" acid (at pH 7.0)*	7	5
Ditto (at pH 6.0)*	5	5
"IONOX" 100	9	9
Monoallylamine (at pH 7.0)*	60	27
Ditto (at pH 9.5)*	6	6
Monoethanolamine (at pH 10.1)*	190	170
Monoisopropanoamine (at pH 9.9)*	220	210
"NONIDET" A10	16	16
Ditto G2C	15	14
Ditto P40	8	7
Ditto P80	15	15
Pentaerythritol triallyl ether	100	70
"TEEPOL" 610	29	28
Ditto CH31	49	45
Ditto CH53	23	22
"VERSATIC" 5 (at pH 5.4)	400	375
Ditto 911	80	80
3,5-Xylenol	34	22

TABLE V
TOXIC AND VOLATILE CHEMICALS

Chemical	24-h TL ₀₁ mg/l
Allyl alcohol	1
Allyl bromide	0.8
Allyl chloride	10
ASA-3 Antistatic additive	18
Benzene	46
o-Bromonitrobenzene	46
1-Chloro-3-bromopropane	75
1,3,5-Cycloheptatriene	15
1,3-Dichloropropane	160
Diisopropyl ether	380
Epichlorohydrin	23
Ethyl amyl ketone	80
2-Ethyl hexyl glycidyl ether	14
Isononyl alcohol	16
"LINEVOL" 79	9
Ditto 911	3
Methallyl chloride	14
Methyl isobutyl carbinol	360
Methyl isobutyl ketone	460
"OCTYLOL"	17
Phenol	46
Phorone	60
Propylene oxide	165
Styrene	26
Toluene	58
o-Xylene	13
m-Xylene	16
p-Xylene	18

TABLE VII

CHEMICALS STILL TO BE TESTED

Acrolein
Allyl glycidyl ether
Amylene dimer
Bicyclo-(2,2,1)-hepta-2,5-diene
"BONDOLAN" A
Ditto M
Butene 55
p-tert-Butylbenzoic acid
p-tert-Butyltoluene
1,5,9-Cyclododecatriene
1,5-Cyclooctadiene
Diisobutylene
"DOBANE" 55
Ditto 83
Ditto JM
n-Dodecyl succinic anhydride
"DUREX" 78 HP
Ditto 27 UK
Ditto 719 UK
Ditto 726 UK
Ethylene oxide
Hydraulic brake fluid SAE J 1703 type 401 °F
Ditto type 450 °F
Methyl "OKITOL" acetate
Nonenyl succinic anhydride
"NONILET" A50
"OKITOL" 10
Ditto 60
Ditto RD 167
"OKITOL" acetate
Pentene
Petroleum sulfonate USL
Ditto ISI
Phenyl glycidyl ether
Propane sulfone
Propylene trimer
"SHELLFLEX" 214 BG
Ditto 724 BG
Ditto 212 HF
Ditto 210 HP
Ditto 451 HP
"SHELL" smootolie JS (woollen oil)
"TEEPOL" GC 56
Thioxanthone
69 Hydrocarbon solvents

Amsterdam, March 1973
JYW/wvr/483

TABLE VI

CHEMICALS WITH LOW SOLUBILITY

(The saturated aqueous solutions of the products mentioned were found to be non-toxic at the concentrations shown)

Chemical	Concentration, mg/l
"DOBANOL" 23	2.4
Ditto 25	0.8
Ditto 45	0.7
"IONOL" CP	0.4
"IONOX" 330	1.2
Ditto 901	1.3

III. FURTHER WORK

Table VII shows the chemical compounds mentioned in the "Shell Industrie Chemicalien Gids" but not yet tested for acute toxicity to fish.

0 0 1 6

REFERENCES

1. ASTM Standards, Part 23, Water; Atmospheric Analysis, 1972.
2. Handbuch der Frischwasser- und Abwasserbiologie, H. Liebmann, 2. Auflage, 1962, Publ. R. Oldenbourg, Munich.
3. Q.H. Pickering and C. Henderson, Acute toxicity of some important petrochemicals to fish, J. Wat. Poll. Control. Fed., 8 (1966) 1419/29.
4. J.B. Sprague, Measurement of pollutant toxicity to fish. II. Utilizing and applying bioassay results. Wat. Res. 4 (1970) 3/32.
5. Standard Methods for the Examination of Water and Wastewater, 13th Ed., 1970, Publ. jointly by APHA, AWWA and WPCF. Am. Public Health Ass., Inc., 1790 Broadway, New York N.Y. 10019.

APPENDIX I

APPARATUS

We perform our routine bioassays as static tests in aquaria of 42 x 28 x 28 cm. We fill these tanks with 25 l of a test solution to a height of 25 cm. The solution is not changed or replenished during the tests which we continue for 4 days. Before the actual tests we carry out exploratory tests with two test animals in 5-l glass beakers. The results of these tests reveal the "critical concentration range" at which the actual tests must take place.

We keep our test containers in a thermostatted room. The temperature is maintained at 20 °C and is measured continuously; deviations do not exceed 1 °C.

The window of this room is equipped with blinds to filter direct sunlight and keep down the growth of algae in the aquaria.

APPENDIX II

QUALITY OF THE WATER USED

No doubt the most desirable dilution water is the actual water into which a discharge may be expected. However, since our investigation does not deal with a discharge of waste water but merely with measuring the toxicity of chemical compounds, this stipulation is not applicable. We therefore used the tap water from the town supply system. The minerals content of this water is given in Table VIII.

TABLE VIII

AVERAGE MINERALS CONTENT OF TAP WATER AT KSLA

chloride (Cl ⁻)	65	mg/l
nitrite (NO ₂ ⁻)	0	"
nitrate (NO ₃ ⁻)	4	"
sulfate (SO ₄ ⁻²)	35	"
hydrocarbonate (HCO ₃ ⁻)	25	"
silicic acid (SiO ₂)	25	"
ammonium (NH ₄ ⁺)	0	"
iron (Fe)	0.05	"
manganese (Mn)	0	"
calcium (Ca ⁺⁺)	100	"
magnesium (Mg ⁺⁺)	8	"
alkali (as Na ⁺)	30	"
phosphate (PO ₄ ⁻³)	0.15	"
pH	7.8	

During the period in which the fish were in quarantine before the tests and throughout most of the tests this tap water was used. In some cases, however, we could not use this relatively hard water for making up the concentrated (often saturated) stock solutions of the chemicals because of signs of a precipitate (most likely Ca/Mg salts) starting to develop, but we found that we could use demineralized water. The compounds which caused a precipitate are marked to this effect in the chapter "Results".

In other cases we encountered appreciable bacterial growth in the stock solutions, which we overcame by using freshly distilled water. This does not support microbial growth, in contrast to demineralized water, which may contain a great many bacteria.

Since we have found evidence that entirely salt-free test solutions are harmful and may even kill fish, we add 250 mg/l of a mixture of sodium chloride, nitrate and sulfate which is approximately isosmotic to the tap water used.

APPENDIX III

DISSOLVED OXYGEN CONTENT AND pH

The laboratory drinking-water mains pressure is maintained at 6 kg/cm². The temperature of the water varies, depending on the season, from about 10 to 20 °C. As a result the tap water is usually supersaturated with air and oxygen when it is poured into the test aquaria. After its temperature has been raised to 20 °C and aeration has been applied for a few hours, the amount of dissolved oxygen reaches a constant value of 9-10 mg/l, which corresponds to a saturation level of between 90 and 100 %.

When demineralized or distilled water is used the solutions are aerated for 2¹/₂ h before the test is started so as to ensure equilibrium of the dissolved oxygen and carbon dioxide concentrations.

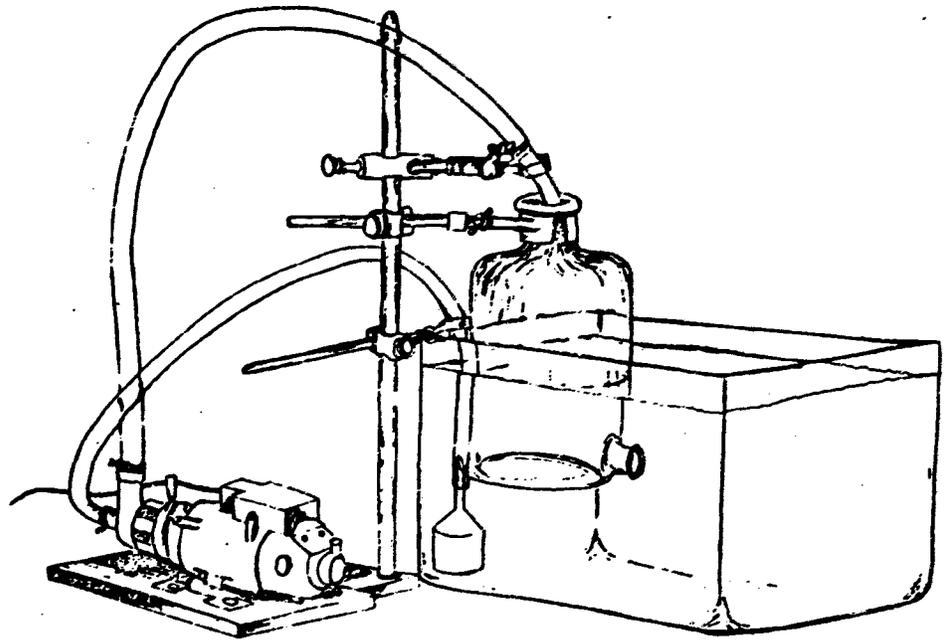
We measure the oxygen content of the water with a Beckman "Model 778 Process Oxygen Analyzer".

During our tests we do not allow the oxygen content of the water to drop below 6 mg/l. When 10 test animals swim in a tank with 25 l water for longer than one day it is always necessary to aerate the solution. We do this by bubbling air through from the laboratory's compressed-air line. This air is filtered through a fritted-steel filter and in our experience it is sufficiently clean for the purpose. We disperse the air at the very low rate of 10 l/h through a P3 filter-stick.

During tests on solutions of volatile compounds we suppress evaporation by filling the tanks to the brim and covering them with a pane of glass. In this case aeration cannot be applied and consequently we have to conduct tests of shorter duration and with fewer fish. This is further explained in Appendix VI: "Duration of tests and number of fish".

Another special category of compounds is detergents. Aeration causes foaming of detergent solutions and a consequent drop in the solute concentration. We have solved this problem with a film aerator, which consists of small pump, an aspirator bottle and a Büchner funnel (Fig. III-1). The bottle is mounted inside the aquarium with its outlet a few centimeters below the surface of the water. The pump draws water in at about 100 l/h through the funnel (to protect the fish) and returns it through tubing, the end of which is fixed in the neck at the top of the bottle. From there the water flows down as a film on the inner surface of the bottle, taking up oxygen from the air without foaming.

The pH of every solution is measured before a test is started. If the pH is outside the range 6-8, it is corrected to 7.0 with NaOH or H₂SO₄. If acidic or basic chemicals are being measured, the compounds are tested in two different forms: (1) corrected to pH 7 as indicated above and (2) at the pH which is the result of the presence of the compound concerned at the given concentration. In Tables II to IV the latter pH is given in brackets after the name of the chemical. Of course it remains unknown if in the second case the test animals die as a result of the toxicity of the chemical or the harmful pH of the solution.



APPENDIX IV

PREPARATION OF SOLUTIONS AND CHECKING CONCENTRATIONS

Chemicals which were readily soluble in water and which were measured at a relatively high concentration, were poured or fed in some other way into an aquarium containing 25 l water from a bottle or glass beaker which was then weighed back.

Lower concentrations of the same compounds were simply diluted from aqueous stock solutions. In both cases the aeration applied gave sufficient mixing.

Chemicals which did not dissolve so readily in water and with a specific mass < 1 were placed in a glass bottle on top of 10 l distilled water. The bottle was then stowed away in the dark to prevent photooxidation or growth of algae and its contents were gently stirred with a magnetic stirrer for 14 days. This saturated solution was then carefully syphoned into an aquarium for testing and further diluted if necessary.

Hard-to-dissolve chemicals which were heavier than water were, in principle, treated in the same way. But even the gentlest stirring appeared to disperse coarse as well as fine particles or droplets of the compound in water. It was sometimes difficult to ensure that after settling of the dispersed material the supernatant liquid was a clear solution. In several cases it was necessary to filter the solution carefully before testing.

The concentration of the chemical compounds in solution was determined before and after each test by means of a Beckman Model 915 TOC Analyzer.

In our experience this TOC test is an accurate and reliable means of assessing organic solute concentrations and it again worked well in the course of our test programme.

At the lower solute concentrations (< 5 mg/l) where the TOC method was no longer sufficiently accurate, a water sample was extracted with a suitable solvent and the extract subjected to GLC analysis. The original solute concentrations were deduced from the results.

In a very few cases the compound under test was present at a concentration as low as 1 or 2 mg/l, which meant that TOC determination was impossible. If these chemicals were also too highly water-soluble to be extracted with a solvent and thus also impossible to determine by chromatography, a solution was prepared by weighing as carefully as possible and the calculated concentration was adopted in good faith.

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APPENDIX V

TEST ANIMALS

With regard to the choice of the kind of fish to be used as test animals the ASTM method states that "they should be species which are common in unpolluted portions of the body of water receiving the waste to be tested, or at least species which are known to inhabit similar waters in the same major watershed".

As we have already said in Appendix II in relation to the "Quality of the water used", we must state that this is not feasible in our programme in which the type of fish in a receiving body of water which remains unknown cannot be specified.

The ASTM method then continues by saying in section 9.2 that "although any fish species which suits the purpose of the investigation may be used, species belonging to any of the following widely distributed and important families are particularly recommended: Centrarchidae, Salmonidae, Cyprinidae (exclusive of carp and goldfish) and Catostomidae". The method further says that members of these families "should be selected unless there is a good reason for making a different choice".

The ASTM method does not explain why carp and goldfish are excluded from the recommendation. Nor do other distinguished authors like Liebmann², Pickering³ or Sprague⁴ exclude the use of goldfish. On the contrary, Pickering and Henderson³ report an investigation into the toxicity of petrochemicals in which they compared the use of fathead minnows (*Pimephales promelas*), bluegills (*Lepomis macrochirus*), goldfish (*Carassius auratus*) and guppies (*Lebistes reticulatus*). They found that (96-h TLM determined in static tests "bluegills were generally the most sensitive". "Fathead minnows were the second most sensitive species". "Goldfish and guppies were slightly more resistant to the petrochemicals, with the guppy being the most resistant of the four species". These investigators further concluded that "There is a remarkably small variation in the sensitivity of these four species to these petrochemicals".

Therefore, when we started our investigation, we deliberately decided to choose goldfish because that species is: (1) of sufficient sensitivity, (2) available in large numbers (we used more than 5000 test animals in less than 2 years) and (3) of constant quality. During the past two years we have found that these three desiderata are satisfied by our goldfish, *Carassius auratus* L. The animals are imported from a hatchery in Hongkong and transported by air in large plastic bags. Sufficient availability of oxygen is ensured during transport.

When we started this work we had some trouble finding an importer who could supply animals in batches of several hundred per week and of sufficiently low mortality. We now find that the firm Oprel, Hoogvliet, The Netherlands, meets our requirements. The death rate of our test fish is on average 3 percent in a fortnight and is rarely higher than 5 percent (the ASTM method requires < 10 percent within four days). When our 5% limit is exceeded we discard a whole consignment. This is systematically the case in and immediately after the spawning season, May-June. In the period we buy no fish and carry out no tests.

The average length of the specimens is 6.2 cm (stand. dev. 0.7 cm); the average weight 3.3 g (stand. dev. 1.0 g). From this figure we calculate a load of 1.3 g fish per l water. This is within the limits of 1-2 g/l as set in the ASTM method. We comply with all the requirements mentioned in the ASTM method D 1345 with respect to origin, stock-keeping, acclimatizing, feeding and transfer of the test fish. No animal is used more than once, even in scouting tests.

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APPENDIX VI

DURATION OF TESTS AND NUMBER OF FISH

The ASTM method defines ACUTE TOXICITY as "any direct lethal action of pollution to fresh-water fishes that is demonstrable within 96 h or less". The degree of acute toxicity is expressed as the Median Tolerance Limit, TLM, the concentration of pollutants at which 50 percent of the test animals are able to survive for a specified period of exposure. In a note it is added that the exposure period may be 24, 48 or 96 h. The 24-h TLM and the 48-h TLM generally should be determined whenever the toxicity is sufficiently pronounced to permit their determination.

In our program we chose the duration of the tests and the number of fish exposed according to what may be called rather arbitrary considerations and came to the following classification in 5 categories.

Category 1

Compounds not toxic at 5000 mg/l. Since at still higher concentrations factors other than "acute toxicity" may determine fish mortality, we did not carry out tests on solutions > 5000 mg/l and did not continue the tests at 5000 mg/l for longer than 24 h.

Category 2

The TLM of compounds which appeared to be toxic in the range 500-5000 mg/l was determined, but again tests were discontinued after one day to avoid the inclusion of factors other than "acute toxicity" in the measurements.

Category 3

Compounds with a TLM lower than 500 mg/l were subjected to 96-h and 24-h tests.

Category 4

Solutions of volatile compounds could not be aerated without serious evaporation losses. Because of the limited availability of dissolved oxygen in that case, tests were carried out with six animals and for a 24-h period only.

Category 5

In this group we report compounds with limited solubility in water which were found to be non-toxic in saturated solution. These products were tested for a 96-h period.

APPENDIX VI

CALCULATION/ESTIMATION OF TL_m

A TL_m reported may be a concentration at which 50 percent survival has actually been observed in a test or it may be a value obtained by interpolation, based on observed percentages of test animals surviving at concentrations lethal to more than half and to less than half of the test subjects.

The derivation of the median value by interpolation involves merely plotting the experimental data on semilogarithmic coordinate paper, with test concentrations plotted on the logarithmic scale and survival percentages on the arithmetic scale. Then a straight line is drawn between the two points representing survival percentages at two successive concentrations of the test series which have proved lethal to, respectively, more than half and less than half of the test animals. A perpendicular drawn from the point at which this line intersects the 50-percent survival line to the concentration ordinate indicates the TL_m concentration. This method is referred to as straight-line graphical interpolation. (This description is adopted from APHA's "Standard Methods"⁵.)

On the basis of the experience collected during our test programme we tend to classify the reproducibility of the test as very reasonable. On the other hand we certainly agree with the statement in ASTM D 1345 that due to the large number of variables encountered in such tests, no limits for precision and accuracy can be given.