

ETAD



74I-0794-00184



FVI-94-001184
INIT 07/14/94

Ecological and Toxicological Association of the Dyestuffs Manufacturing Industry

U.S. OPERATING COMMITTEE OF ETAD

Contains No. 001

OPTS-41023

February 9, 1987

Comments by ETAD on the
ITC testing recommendations for
C.I. Disperse Blue 79
(19th ITC Report, 51 Fed. Reg. 41417, Nov 14, 1986



84940000249

These comments are submitted on behalf of the Ecological and Toxicological Association of the Dyestuffs Manufacturing Industry (ETAD), an international organization with headquarters in Basle, Switzerland, with the objective of addressing toxicological and environmental concerns about organic colorants.

The ten U.S. members of ETAD are American Hoechst*, Atlantic Industries*, BASF Corporation, Carey Industries, Ciba-Geigy Corporation*, Crompton & Knowles Corporation*, ICI Americas*, Mobay Chemical Corporation*, Morton Chemicals, and Sandoz Chemicals Corporation*.

Physical and Chemical Information

Solubility in Water

ETAD is currently collaborating with Dr. G. Baughman, University of Georgia, Athens, whose studies involve the experimental measurement of water solubility. This experimental value will be submitted when available.

Bioconcentration Factor

The recommendation that bioaccumulation studies in fish be conducted is based on the estimates of log P (4.1) and bioconcentration factor (757).

Even if current studies at the University of Georgia provide experimental confirmation of this log P estimate there are several reasons to conclude that Disperse Blue 79 will not bioconcentrate in fish to any significant extent (i.e. less than x 100).

-Studies reported by ETAD (ref.1) have included the measurement of fish bioconcentration for numerous dyes, including 23 Disperse Dyes, of which 18 were para-azo Disperse dyes. Although 10 of these dyes showed log P values of 3 or higher, none of them was found to bioaccumulate at x 100 or higher.

RECEIVED
OPPT/CTDC
94 JUL 14 AM 9:05

Bioconcentration Factor

The recommendation that bioaccumulation studies in fish be conducted is based on the estimates of log p (4.1) and bioconcentration factor (757).

Even if current studies at the University of Georgia provide experimental confirmation of this log p estimate there are several reasons to conclude that Disperse Blue 79 will not bioconcentrate in fish to any significant extent (i.e. less than x 100).

-Studies reported by ETAD (ref.1) have included the measurement of fish bioconcentration for numerous dyes, including 23 Disperse Dyes, of which 18 were mono-azo Disperse dyes. Although 10 of these dyes showed log p values of 3 or higher, none of them was found to bioaccumulate at x 100 or higher.

-Comparison of the experimental fish bioconcentration data obtained for several closely-related structural analogs of Disperse Blue 79 (Appendix I) (ref 2) permits the conclusion that significant bioconcentration of Disperse Blue 79 should not occur.

Evaluation of new substances by the Japanese authorities under the Chemical Substances Control Law places much emphasis on the bioconcentration potential of new substances which are not readily biodegradable. Numerous substances which bear a close structural resemblance to Disperse Blue 79 have been accepted by the Ministry of International Trade and Industry (MITI), which is indicative of a bioconcentration factor in fish of less than 500 the upper limit for MITI approval. A list of eleven such substances accepted by MITI is provided in Appendix II.

In view of the substantial expertise and data on fish bioconcentration available to MITI, ETAD urges the EPA to request MITI to provide the specific fish bioconcentration data for these or any other close structural analogs of Disperse Blue 79. In addition MITI should be asked to confirm that data are not available on any of the four Disperse Blue 79 analogs referred to in the ITC report. ETAD is unable to obtain these fish bioconcentration data from MITI because MITI does not disclose such information to private companies or organizations.

The lower than expected bioconcentration of similar Disperse dyes may be explicable in terms of reduced membrane diffusion rates due to the large molecular cross-section of these substances (ref 2-4). In the specific case of Disperse Blue 79 the molecular cross-sections at the lowest energy conformation (35.7 Kcal/mol) along the X, Y, Z axes are 19.7, 12.3, and 9.5 Angstrom units respectively. The maximum "folded" conformation of Disperse Blue 79 (51.2 Kcal/mol) shows X, Y, Z values of 20.8, 9.6 and 9.0 Angstrom units respectively. Furthermore, this conformation is unstable and it relaxes spontaneously to the more stable conformation with larger cross-section. Based on the experience of Opperhuizen et al. (ref 3) membrane permeation of such a large molecule is greatly hindered if indeed possible.

0 0 0 4

Exposure Information

A. Production/Use/Environmental Release

The estimate of annual production of 2 to 3 million pounds over 1980-1984, as active colorant, appears reasonable for Disperse Blue 79 and Disperse Blue 79 analogs. Our estimate of the current U.S. sales market is 1.8 million pounds of active colorant. See also comments in page 5.

Disperse Blue 79 (including DE79 analogs) is now used almost exclusively for polyester dyeing. Mr. Heath's memo (ITC ref 21) refers to the use for dyeing triacetate, but since Celanese stopped production of triacetate fiber when methylene chloride was projected to be a carcinogen, the quantity of triacetate to be processed has continually diminished and may well be approaching zero.

B. Evidence of Human Exposure

Manufacture The NOES estimate of 1450 workers at 25 plants being potentially exposed to Disperse Blue 79 is grossly misleading as a measure of the number of workers with any significant exposure. Mr. Heath's estimate of 13-26 workers at 11 sites (including manufacturing and blending) or a maximum of 236 workers seems much more reasonable. Even if all of the jobbers that have mixing equipment are added the total number of sites may reach 25, but there would only be an additional 15-30 workers.

Processing ETAD agrees that the estimate of number of workers exposed in the processing industries is reasonable.

Consumer Appendix XII of the ETAD submission to the ITC (ref 5) provided extensive experimental evidence supporting the conclusion that consumer exposure to Disperse Blue 79 from dyed fabrics is very low, due to the excellent fastness characteristics. The data presented in Table 2 of Appendix II to ref 5, relating to the extractability of four Disperse dyes from polyester textured yarns, exemplify this extremely low extractability and the last dye listed is the Br/OMe analog of Disperse Blue 79 (i.e. CAS 3618-72-2). As Disperse Blue 79 is a high-energy dye it is not anticipated that there is any significant usage in home-dyeing products.

C. Environmental exposure

The estimation of environmental exposure assumes 50% removal of the dye during passage through biological treatment plants due to adsorption on sludge solids. Current studies by the EPA, Cincinnati (G. Shaul) will provide experimental data on the extent of biodegradation and adsorptive removal which should be a sounder basis for estimating environmental exposure levels. The EPA estimation of surface water concentrations (ITC ref 22) appears to allow only for adsorptive removal of the dye in the effluent treatment plant. Such adsorptive processes also occur downstream through adsorption on river

sediments and suspended solids, effectively reducing the potential levels in drinking water supplies. In the case of substances like Disperse Blue 79 (low water solubility, finely dispersed particles) these removal processes are particularly important.

In reference 22 of the ITC report, Table 1, the estimations of surface water concentrations for the Ciba-Geigy Corporation plant at Tom's River overlook the fact that the plant effluent is not released to the river, but is piped, after treatment, for release in the ocean off-shore, which is not a source of drinking water. Furthermore, this site is being closed for the production of dyes. There appear to be further errors and the correctness of the data relating to other manufacturing sites in this tabulation should be verified.

D. Persistence - Reference is made that no protocols for the tests were provided by ETAD. It should be noted that additional information about the tests was not requested by ITC. The test procedure is shown in Appendix III.

We have also included the OECD procedure for your information. (Appendix IV.)

III. Biological effects of concern to human health.

The ITC has recommended to the EPA that Disperse Blue 79 be tested for health effects solely on the basis of potential bioavailability due to ingestion of inhaled dye-containing dust. It has recommended subchronic toxicity and absorption and disposition studies following oral administration.

The industry is willing to participate in further discussions to evaluate the need for a sub-chronic toxicity study in the light of the forthcoming data on potential exposure. ETAD questions the need for a chemical disposition study based on the available data on the hypothetical metabolite, 2-bromo-4, 6-dinitroaniline. Testing of this substance has been dropped by EPA in the context of the substituted anilines category. ETAD agrees with the ITC that no further short-term testing is required.

IV. Ecological effects of concern

The data reported in Appendices VII, VIII and IX of the ETAD ITC comments to ITC (ref 5) would appear to indicate that further acute and subchronic (short-term) effects at maximum solubility levels would provide little if any useful data. For example, Appendix VII shows an LC_0 of 200mg/l for the formulated dyestuff containing 52% Disperse Blue 79 and 48% dispersing agents. In view of the extremely low water solubility, the above LC_0 was obtained under conditions of complete saturation with excess material (98 mg/l) present in the form of micron sized particles. Even under those conditions, no deaths were observed in 48 hours.

The procedure for this testing is given as Appendix V to these comments.

ITC mentions a concern about the LC₅₀ level being over 100 times the solubility of the dye. This is because the formulated product contains the dispersants (typically sulfonated lignins) which hold the dye in dispersion.

The values reported for LC₅₀ and LC₁₀₀ could in fact be the result of the action of insoluble (dispersed) dye particles or the dispersing agents themselves on the respiratory system of the test fish. (e.g. clogging of gills).

ITC also raised a concern that the fish toxicity data related to a 48-hr test period and not to 96-hr. Additional data submitted to the EPA by an ETAD member company indicates an LC₅₀ of 42.5 mg/l in a 96-hr study in rainbow trout, generally regarded as a sensitive fish species. These data were obtained on a formulated product containing 25% of the Br/OMe analog and 75% dispersing agents.

FOCUS MEETING

Clarification of chemical identity of substances under consideration. At the Focus Meeting on December 16, 1986, it became clear that there is still some confusion over the identity of the substances which should be under consideration for testing. It is hoped that these comments will assist in clarifying the position.

-Under the Color Index nomenclature system Disperse Blue 79 applies uniquely to the substance defined by CAS No. 3956-55-6. (i.e. the Br/OEt analog).

-Of the 4 analogs referred to in the ITC report only 3 are believed to be of commercial significance.

- | | |
|--------------------------------|--------------------------|
| (1) Br/OEt (Disperse Blue 79) | CAS No. Br/OEt 3956-55-6 |
| (2) Br/OMe (p70, derivative 1) | CAS No. Br/OMe 3618-72-2 |
| (3) Cl/OMe (p70, derivative 2) | CAS No. Cl/OMe 3618-73-3 |

ETAD is currently completing a survey of the 1985 production/import of the various analogs, and this will be submitted to the EPA by February 13, 1987. Preliminary results confirm the Br/OMe analog (CAS No 3618-72-2) as the dominant analog, based on these 1985 data.

-Available health and safety data and use information on all the analogs are relevant to the assessment process and are being made available to the EPA by the ETAD member companies on a voluntary basis. It is unlikely that any further relevant health and safety data or use information would be obtained if the other analogs were subsequently to be added to the Priority List.

Estimated availability of new data from ongoing studies The EPA's attention is drawn to data, pertinent to the evaluation of Disperse Blue 79 type products, which is currently being developed.

<u>Test</u>	<u>Being Conducted by</u>	<u>Estimated Completion</u>
Water Solubility Partition coefficient (octanol/water) Sediment sorption coefficients	G. Baughman Univ. of Georgia	June, 1987
Effluent treatment plant adsorption and extent of biodegradation	G. Shaul, EPA Cincinnati	July, 1987
Occupational Exposure of textile dye color-storerroom workers	ATMI/EPA/ETAD Joint project	September, 1987
Landfill simulation	W.C. Fincher ETAD/Georgia Inst. of Technology (Atlanta)	June, 1987

Summary Assessment of ITC Testing Recommendations

Health effects

Subchronic toxicity - The need for a subchronic toxicity study should be evaluated in the light of the more comprehensive data on occupational exposure levels of color storerroom workers to dyes, and taking into account the relatively small population exposed in both manufacturing and processing plants.

Absorption and chemical disposition This test is not necessary. A fecal bacterial test should be adequate to confirm reductive cleavage of the azo group with formation of 2-bromo 4, 6-dinitro aniline as a significant metabolic pathway. The disposition of BDNA is elucidated in ref 6.

Chemical fate. Solubility in water/Biodegradation-aerobic and anerobic.

Relevant studies are currently underway.

Ecological effects

Fish acute toxicity

A study indicating a 96hr LD₅₀ of 42.5 mg/l in rainbow trout has been submitted for the Br/OMe analog.

Acute Toxicity to other organisms The fish acute toxicity is low and extensive studies support the view that aquatic animals are generally more sensitive indicators of acute toxicity than plants. Although toxicity data in other organisms may be of some interest it is unusual to seek such testing for chemicals exhibiting low acute toxicity to fish.

Fish Bioconcentration

Substantial data exist to support the conclusion that Disperse Blue 79 will not bioconcentrate in fish to any significant extent. This test recommendation is superfluous.

References

1. Anliker R, Clarke EA, Moser P. Chemosphere, 1981, 10, 263-274.
2. Anliker R and Moser P. "The limits of bioaccumulation of organic pigments in fish." Ecetox. Environ. Safety (in press).
3. Opperhuizen A et al. Chemosphere, 1985, 14, 1871-1896
4. Cobas FAPC et al. Chemosphere, 1986, 15, 1985-1986
5. ETAD, Comments submitted to ITC on draft Information Review (IR-482), August 1986.
6. Chopade H M, Matthews H B. Journal of Toxicology and Environmental Health, 1986. 17, 37-50.

Respectfully submitted,

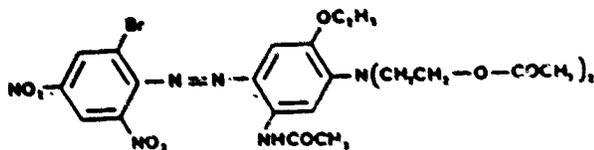
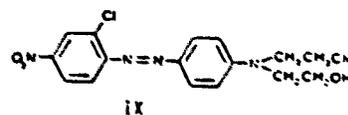
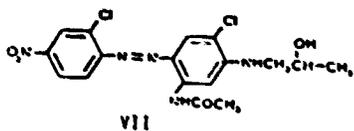
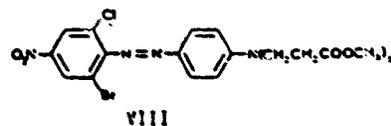
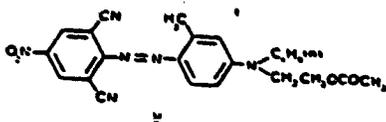
Eric A. Clarke

Eric A. Clarke
Executive Secretary

APPENDIX I

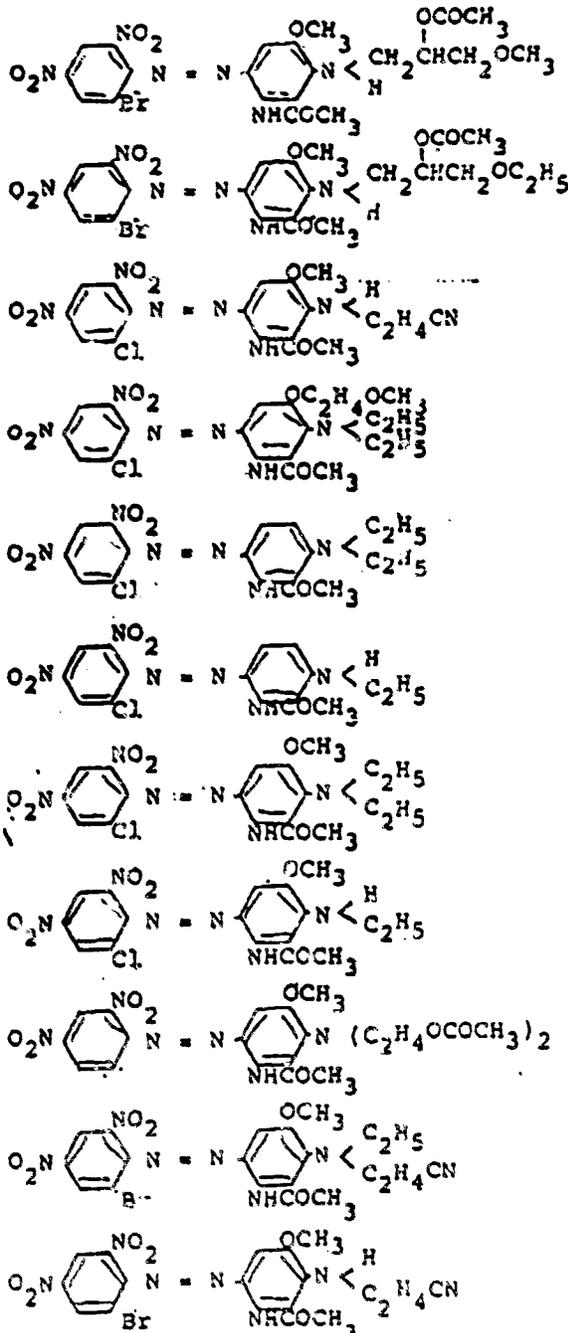
Data on partition coefficient, water solubility and fish bioconcentration of closely related structural analogs of Disperse Blue 79. (abstract from ref.2).

Compound	M.W.	m.p. (°C)	log p (exptl)	log p (calc)	water sol (calc)mg/l	log (fish bioconcentration)
V	449	148	4.0	4.65	0.8-4.7	<0.7
VII	427	170	4.0	3.54	0.37-2.8	1.76
VIII	529	117	4.1	5.35	2.8-9.2	0.70
IX	374	146	3.9	3.03	1.0-5.3	0.30
Disperse Blue 79	639	143	?	4.1	5.4	?



APPENDIX II

Chemical structures of Disperse Dyes, structurally related to Disperse Blue 79, registered in Japan under Chemical Substances Control Law: indicative of fish bioconcentration factor <500.



Testing the Biological Elimination of Products and Wastewaters in a Stability Test

1. General

The "stability test" method described below was developed so as to be able to test the degradability of products and chemical compounds in a simple and readily reproducible manner and under conditions similar to those in actual practice, based on a wastewater treatment plant.

The standard used in the test is the ratio of the COD value at the end and at the beginning of the test.¹⁾ As the determinative magnitude, the COD value has the advantage that nearly all substances can be measured with the same analytical method and, unlike substance-related analytical methods, the actual elimination is tested. Compared with the determination of biological oxygen demand, the measurement of the COD has the advantage that not only the dissimilatory but also the assimilatory processes are recorded.

The substance under investigation is used in concentrations corresponding to COD values of 300 - 1 000 mg O₂/l. Even though these concentrations may seem too high and unsimilar to those found in actual practice, they nevertheless have the advantage that

- a) the concentration of dissolved metabolism products of activated sludge (blank value), measured as the COD, is small compared with the COD value exhibited by the substance;

1) Determination of the degree of degradation by measuring the oxidizability properties was recommended by W. Janicke (Gesundheits-Ing. ____ (1968), p. 309).

- b) the scattering caused by sampling and analysis methods up to a degree of degradation of over 90% remain small compared with the COD value attributable to the substance;
- c) the test takes place under more severe conditions for the activated sludge and therefore the results are always on the safe side.

The ecology is generally not disturbed at these concentrations, although it is advisable to ensure this by toxicity tests.

Through the repeated loading of the same sludge this method offers the possibility of investigating adaptation processes which are of decisive importance for the degradation process in biological wastewater treatment plants.

The starting amount is set at 2 liters so that daily sampling is possible even over prolonged test periods, even when other analyses are required than COD determination, as for example with detergents.

2. Substrate

The product is weighed out according to a (measured) COD of approx.

Solvent: cold tap water

Fertilizer addition: 220 g urea and 89 mg $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$
per liter (COD : N : P = 100 : 5 : 1)

The pH is adjusted to 6.5 - 7.5 with either NaOH or H_2SO_4 as required.

3. Activated sludge

Activated sludge taken from an aeration basin of a (public or industrial) biological wastewater treatment plant is centrifuged at 2000 rpm for 5 min, suspended in tap water and adjusted to 2 g Tr.S./l ($\pm 20\%$).

4. Test batch

Mix the solution prepared as described in Section 2. and the suspension prepared as in Section 3. together in equal parts (preferably 1 liter each) in an open vessel and adjust the temperature to 22°C (\pm 3°C). The substrate concentration will then be 1 600 mg COD/l, or 400 mg TOC/l.

5. Control batch

For each test series, one vessel is included which contains only activated sludge (1 g Tr.S./l) and fertilizer (one half the concentration of that given in Section 2.).

6. Test conditions

Aeration is carried out by means of a magnetic or blade stirrer and additional supply of compressed air via a wadding filter and bubbler. The stirring speed should be set so that the sludge does not settle. The air supply must maintain an O₂ concentration of at least 2 mg/l.

Losses due to evaporation are to be compensated with distilled or demineralized water (at the latest before each sampling). The pH must be checked each day and corrected as necessary (see Section 2.).

7. Analysis

The elimination of the organic substance is checked by COD determination carried out each day (ASTM D 1252-58 T) or by determination of the organic carbon content (TOC). For preparing the measurement, the sample from the test and control vessel which is to be analyzed is filtered through a paper filter (Schleicher and Schüll, Blue Band No. 589) which has been washed three times with distilled water. If the filterability is poor, the solids can be eliminated beforehand by centrifuging.

8. Evaluation

The COD elimination in percent of the substance under investigation is given by the formula:

$$\frac{COD_A - COD_T - COD_K}{COD_A} \cdot 100$$

COD_A = one half COD according to Section 2. (initial value)

COD_T = COD of test batch

COD_K = COD of control batch (blank value)

The same applies to the determination according to TOC.

For determination of any adsorption onto the activated sludge, the first sample is analyzed about 3 hours after it is placed in the test vessel.

9. Time of test

The test is continued for as long as the degree of degradation remains virtually unchanged (maximum 14 days). If satisfactory degradation does not occur, it should be checked whether

- a) the substance inhibits bacteria at the concentration set. If required, the test should be repeated at a correspondingly higher dilution. However, the initial COD (COD_A) should not be below 300 mg O_2/l or the TOC not less than 120 mg/l.
- b) improvement in the degradation may be obtained by repeating the test with the adapted sludge.

**"Inherent Biodegradability:
Modified Zahn-Wellens Test"****302 B**

APPENDIX IV

1. INTRODUCTORY INFORMATION**• Prerequisite**

- Water solubility

• Guidance information

- Information on the relative proportions of the major components of the test material will be useful in interpreting the results obtained, particularly in those cases where the result lies close to the "pass level".
- Information on the toxicity of the chemical may be useful to the interpretation of low results and in the selection of appropriate test concentrations.

• Qualifying statements

The method is only applicable to those organic test materials which, at the concentration used in the test,

- are soluble in water to the extent necessary for the preparation of the test solutions,
- have negligible vapour pressure,
- are not inhibitory to bacteria,
- do not significantly adsorb on glass surfaces, and
- are not lost by foaming from the test solution.

This test has been found suitable by the OECD Expert Group "Degradation/Accumulation" for determining the inherent biodegradability of organic chemicals under aerobic conditions. It has been tested in the OECD Laboratory Intercomparison Test Programme (1978-1980).

• Recommendations

Test chemicals giving a result of greater than 20 per cent loss of DOC (dissolved organic carbon) in this test may be regarded as inherently biodegradable, whereas a result of greater than 70 per cent loss of DOC is evidence of ultimate biodegradability. The use of a compound specific analytical technique on ¹⁴C-labelled test substances may allow greater sensitivity. In these last cases a lower level may be regarded as evidence of inherent biodegradability.

Users of this Test Guideline should consult the Preface, in particular paragraphs 3, 4, 7 and 8.

• Standard documents

This Test Guideline is based on a modification of the method cited in reference 1 and in reference 2, Section 4, literature.

2. METHOD

A. INTRODUCTION, PURPOSE, SCOPE, RELEVANCE, APPLICATION AND LIMITS OF TEST

The static test is a simple, reproducible method for evaluating the ultimate biodegradability of organic substances in water by micro-organisms in an aerobic milieu.

The static method is limited to the examination of the biodegradability of water soluble, non-volatile organic compounds. The compounds to be studied are used in concentrations corresponding to DOC-values in the range of 50-400 mg/litre or COD-values in the range of 100-1000 mg/litre (DOC = dissolved organic carbon; COD = chemical oxygen demand).

These relatively high concentrations have the advantage of analytical reliability. Compounds with toxic properties may delay or inhibit the degradation process.

• Definition and units

The amount of degradation attained at the end of the test is reported as the

"Biodegradability in the Static Test":

$$D_T (\%) = 1 - \frac{(C_T - C_B)}{C_A} \cdot 100$$

D_T = biodegradation (%) at time T

C_A = initial value (DOC or COD values in the test mixture calculated from the DOC or COD values in the stock solution, [mg/l])

C_T = DOC or COD values at time of sampling, [mg/l]

C_B = DOC or COD value of the blank, [mg/l]

The degradation rates are rounded to the nearest full percent.

Percentage degradation is stated as the percentage DOC (or COD) removal of the tested substance.

• Reference substances

In some cases when investigating a new substance reference substances may be useful, however specific reference substances cannot yet be recommended.

• Principle of the test method

Activated sludge, mineral nutrients and the test material as the sole carbon source in an aqueous solution are placed together in a 1-4 litre glass vessel equipped with an agitator and an aerator. The mixture is agitated and aerated at 22°C (\pm 3°) under diffuse illumination or in a dark room for up to 28 days. The degradation process is monitored by determination of the DOC (or COD) values in the filtered solution at daily or other appropriate regular time intervals. The ratio of eliminated DOC (or COD) after each interval to the value at the start is expressed as percentage biodegradation and serves as the measure for the rate of degradation at this time. The result is plotted versus time to give the biodegradation curve.

• Quality criteria

Reproducibility

Reproducibility has been proven in ring tests. Detection of < 20 per cent; > 20 to < 70 per cent; > 70 per cent DOC-removal as required for testing inherent biodegradability is possible.

Sensitivity

The limits for sensitivity are given by: the sensitivity of the carbon analysis (0.5 - 1 mg C/l) and the COD-analysis (5-10 mg O₂/l).

Specificity

Applicable for tests with water soluble (>100 mg/l); non-volatile organic substances.

Possibility of standardisation

Standardisation is possible.

Possibility of automation

Automation of analysis is possible.

B. DESCRIPTION OF THE TEST PROCEDURE• PreparationsReagents

- Test water: drinking water with an organic-carbon content < 5 mg/l. The concentration of calcium and magnesium ions together must not exceed 2.7 mole/l; otherwise adequate dilution with deionised or distilled water is required.
- Sulfuric acid, analytic reagent (A.R.) 50 g/l
- Sodium hydroxide solution, A.R., 40 g/l
- Mineral nutrient solution: dissolve in one litre deionised water:
36.5 g ammonium chloride, NH_4Cl , A.R.
33.4 g sodium dihydrogenphosphate,
 $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$, A.R.
8.5 g potassium dihydrogenphosphate,
 KH_2PO_4 , A.R.
21.75 g di-potassium mono-hydrogenphosphate
 K_2HPO_4 , A.R.

The mixture serves both as a nutrient compound and as a buffering system.

Apparatus

- Glass vessels with a volume of 1-4 litre (e.g. cylindrical vessels)
- Agitator with a glass or metal stirrer on a suitable shaft. (The stirrer should rotate about 5 to 10 cm above the bottom of the vessel.) A magnetic stirrer with a 7-10 cm long rod can be used instead
- Glass tube of 2-4 mm inner diameter to introduce air. The opening of the tube should be about 1 cm above the bottom of the vessel

- Centrifuge (at least 2000 rpm)
- pH-meter
- O₂-measuring instrument
- Paper filters
- Membrane filtration apparatus
- Membrane filters, pore size 0.2 µm
- Analytical equipment for determining organic carbon content and chemical oxygen demand

Preparation of the inoculum

Activated sludge from a biological treatment plant is washed by (repeatedly) centrifuging or settling with testwater (above).

The activated sludge must be in an appropriate condition. Such sludge is available from a properly working sewage treatment plant. To get as many possible different species or strains of bacteria in special cases it may be preferred to make a mixture from different sources (e.g. different treatment plants, soil extracts, river waters, etc.). The mixture is to be treated as described above.

For checking the activity of the activated sludge see functional control, below.

Preparation of the test solutions

To the test vessel add 900 ml of test water 2.5 ml/litre mineral nutrient solution and activated sludge in an amount corresponding to 0.2-1.0 g/l (normally 0.2 or 1.0 g/l*) dry matter in the final mixture. Add sufficient stock solution of the test material or of the waste water to be tested that a DOC concentration of 30-400 mg/l (normally 30 or 400 mg/l*) results in the final mixture. The corresponding COD-values are 100-1000 mg/l. Make up with test water to a total volume of 1-4 litres. The total volume to be chosen is dependent on the number of samples to be taken for DOC or COD determination and the volumes necessary for the analytical procedure.

- * The lower concentrations: 0.2 g dry matter of activated sludge/litre and a DOC-concentration of 30 mg/l are introduced to make the test instructions compatible with the instructions of the ENPA-Test (Switzerland). See reference 4.

0 0 2 0

Normally a volume of 2 litres can be regarded as satisfactory.

At least one control vessel (blank) is set up to run in parallel with each test series; it contains only activated sludge and mineral nutrient solution made up with test water to the same total volume as in the test vessels.

Note: Before starting the test it is advisable to make certain with appropriate methods that no inhibition occurs at the chosen concentration of test material. Run the test with a smaller concentration if an inhibitory effect is found.

Performance of the test

The test vessels are agitated with magnetic stirrers or screw propellers under diffuse illumination or in a dark room at 22°C (\pm 3°). Aeration is accomplished by compressed air cleaned by a cotton wool strainer and a wash bottle if necessary. It must be ensured that the sludge does not settle and the oxygen concentration does not fall below 2 mg/l.

The pH-value must be checked at regular intervals (for example daily) and adjusted to pH 7-8 with NaOH or H₂SO₄, if necessary.

Losses from evaporation are made up just before each sampling with deionised or distilled water in the required amounts. The best procedure is to mark the liquid level on the vessel before starting the test and after each sampling (without aeration and stirring). The first samples are always taken 3 hours after the start of the test in order to detect adsorption of test material by the activated sludge.

The elimination of the test material is followed by DOC- or COD-determinations made daily or at some other regular interval. The samples from the test vessel and the blank are filtered through a carefully washed paper filter. The first 9 ml of test solution-filtrate are returned to the test vessel. Sludges difficult to filter may be removed previously by centrifugation. DOC and COD determinations are made at least in duplicate. The test is run for up to 28 days.

Note: Turbid remaining samples are filtered through membrane filters. The membrane filters must not release or adsorb any organic material. Otherwise they are to be purified by boiling in deionised water previously. For procedure in connection with adaptation processes see below.

Functional control

A vessel with a known substance should be run parallel with each test series in order to check the functional capacity of the activated sludge. For this purpose compounds, such as diethyleneglycol, sodiumbenzoate, and aniline, are recommended.

Adaptation

If analyses are carried out at relatively short intervals (e.g. daily), adaptation can be clearly recognized from the degradation curve (see Figure 2).

If the adaptation occurs in the final days of the test time, the test time can be prolonged until the degradation is finished.

Note: If a broader knowledge of the behaviour of the adapted sludge is needed, the same activated sludge is exposed once again to the same test material in accordance with the following procedure:

Switch off the agitator and the aerator and allow the activated sludge to settle. Draw off the supernatant liquid, fill up to 2 litres with test water, stir for 15 minutes and allow to settle again. After the supernatant liquid is drawn off again use the remaining sludge to repeat the test with the same test material in accordance with Preparation of test solutions and Performance of the test, above. The activated sludge can also be isolated by centrifuging instead of settling.

The adapted sludge may be mixed with fresh sludge to a total amount of 0.2 - 1 g dry weight/litre.

Analytical means

Normally samples are filtered through a carefully washed paper filter (for washing use deionised water).

Samples which remain are filtered through membrane filters (0.2 μ m, diameter 25 mm). Membrane filters are suitable if it is assured that they neither release nor adsorb organic compounds. Otherwise the membrane filters must be purified from soluble organic material by boiling them 3 times in deionised water. The purified filters may be stored in water.

The DOC concentration is determined twice in the sample filtrates (the first 5 ml are discarded) by means of the TOC instrument. If the filtrate cannot be analysed on the same day, it must be stored in the refrigerator until the next day. Longer storage cannot be recommended.

The COD concentration is determined in the sample filtrates with a COD analytical set up by the procedure described in reference (3), below.

3. DATA AND REPORTING

• Treatment of results

DOC and COD concentrations are determined at least in duplicate in the samples according to Performance of the Test and Analytical means, above. The degradation at the time t is calculated according to the formula (with definitions) given under Definitions and units, above.

The degradation rates are rounded to the nearest full percent. The amount of degradation attained at the end of the test is reported as the "Biodegradability in the Static Test".

Note: If complete degradation is attained before the test time is over and this result is confirmed by a second analysis on the next day, the test can be concluded.

• Test report

The test report comprises information about

- The test substance (name, structure, impurities, solubility, concentration, etc.)
- The inoculum (sampling of the inoculum, concentration, status of adaptation)
- The kind of analysis
- The toxicity evaluations
- The functional control (calibration compound)

The test results at different sampling times, are seen in the example given below.

A second part of the test report is made up by the biodegradation curve (Figures 1 and 2).

Evaluation example

Organic compound : 4-Ethoxybenzoic acid
 Theoretical test concentration : 600 mg/l
 Theoretical DOC : 390 mg/l
 Inoculum : Treatment plant of
 HOECHST AG in
 Frankfurt/M.-Höchst
 Concentration : 1 g dry material/
 litre
 Adaptation status : not adapted
 Analysis : DOC-determination
 Amount of sample : 3 ml
 Functional control : Diethyleneglycol
 Toxicity of compound : No toxic effects
 below 1000 ppm
 (Gärrührchentest)

Sampling time	Functional control			Test compound			
	DOC ^a mg/l	DOC ^a mg/l	DOC net mg/l	Degrad. %	DOC ^a mg/l	DOC net mg/l	Degrad. %
0	--	--	390	--	--	390	--
3 h	4.0	298	294	2	371.6	367.6	6
1 d	6.1	288.3	282.2	6	373.3	367.2	6
2 d	5.0	281.2	276.2	8	360.0	355.0	9
3 d	6.3	270.5	264.2	12	193.8	187.5	32
6 d	7.4	233.3	245.9	18	143.9	136.	65
7 d	11.3	212.5	201.2	33	104.5	93.2	76
8 d	7.8	142.5	134.7	33	58.9	51.1	87
9 d	7.0	35.0	28.0	91	18.1	11.1	97
10 d	18.0	37.0	19.0	94	20.0	2.0	99

^a Mean values of triplicate determinations

11024

Figure 1: Static Test - Examples of biodegradation curves

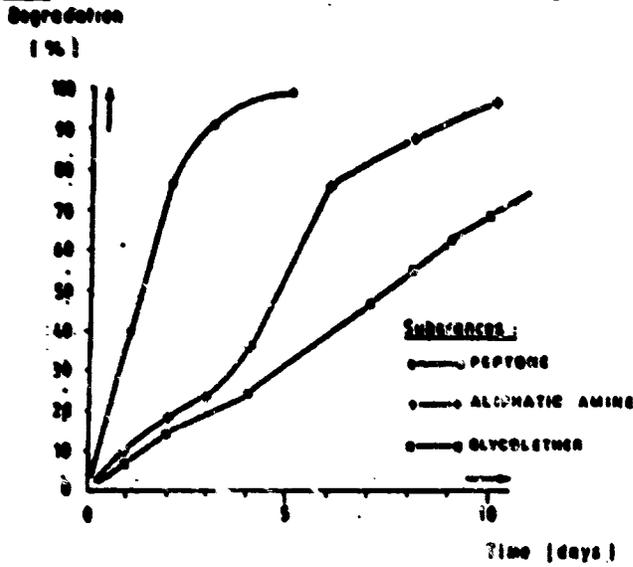
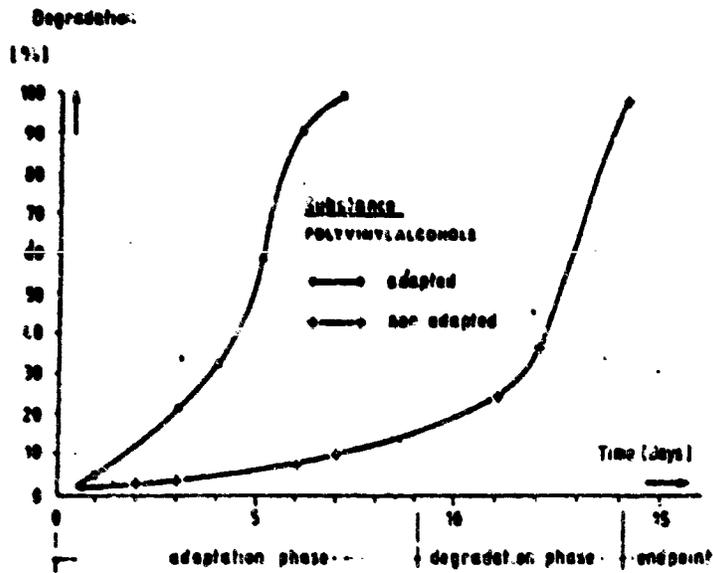


Figure 2: Static Test - Examples of sludge adaptation



• Interpretation/evaluation
of results

The degree of biodegradation attained at the end of the test after 28 days or, if complete degradation is attained in less than 28 days, at an earlier time, is reported as "inherent biodegradability in the static test (after x days)".

If the result of analysis of the first sample (3 h after starting the test) is significantly different from the theoretical value, the amount of deficient DOC is to be reported as "adsorbed by the activated sludge".

The significant points of the degradation curve are to be reported as

- adaptation-phase (days)
- degradation-phase (days)
- endpoint of degradation reached after ... days (see Figure 2, above)

4. LITERATURE

(1) R. Zahn und H. Wellens, Ein einfaches Verfahren zur Prüfung der biologischen Abbaubarkeit von Produkten und Abwasserinhaltsstoffen, in: Chemiker-Zeitung 90, p. 228-232 (1974).

(2) Umweltbundesamt: OECD-Ring-Test Programme on Detecting Biodegradability of Chemicals in Water, Berlin July 31, Test prescription No. 4, 1978.

(3) Standard Methods for the Examination of Water and Waste Water, 13th ed., p. 495-499 (No. 220), published by the American Public Health Association, 1971.

(4) W. Schefer, Bestimmung der biologischen Eliminierbarkeit von Abwasser-Inhaltsstoffen, in Forum-Stadte-Hygiene 110 (1978).

Procedure for determining
acute fish toxicity

Determination according to modified
Routine Bioassay Method from Standard Methods
for the Examination of Water and Wastewater,
12th ed. 1965.

1. Principle

The following terms are used as measurement standards of fish toxicity:

1. Median tolerance limit = $TL_m = LC_{50}$ =
Concentration of test substance at which
exactly 50% of the test animals survive
an exposure time of 48 h.

2. Toxicity limit = LC_0 Maximum concentration of test substance
at which all the test animals survive an
exposure time of 48 h.

3. Lethality limit = LC_{100} Minimum concentration of test substance
which causes all the test animals to die
within 48 h.

It is advantageous to select the test substance concentrations so that,
of two neighboring concentrations, one is slightly above and the other
slightly below the TL_m , allowing the TL_m to be found by interpolation.

A preliminary test is performed by setting up a dilution series of e.g. 1, 10, 100 ml/l or mg/l, in order to determine the necessary concentration range. The desired concentration range lies between the highest concentration at which all the fish survive after an exposure time of 24 h, and the lowest concentration at which all the fish die within the same period. In most cases this preliminary test suffices for industrial wastewaters.

In the main test, 4 - 6 concentrations should be tested within the concentration range found.

2. Equipment

2.1 Test vessels

Cylindrical glass vessels 20 cm in diameter and 16 cm high with a plastic lid and aeration device.

2.2 Acclimatization tanks

Glass or plastic aquaria equipped with continuous water inlet and outlet as well as aeration (water-jet pump).

3. Test fish

3.1 Fish

Rainbow trouts ca. 5 - 6 cm long weighing about 3 - 4 g (if obtainable; otherwise as small as possible).

3.2 Acclimatization

At least 10 - 14 days in the acclimatization tank.

Fish which have been used must not be re-used for at least 3 weeks.

Once a week the tanks should be cleaned by suctioning out waste feed and fecal matter.

Every 2 - 3 weeks the tanks must be disinfected by adding

100 ml formaldehyde 37 per tank

(final formaldehyde concentration = 0.1%).

The dissolved O_2 content must not be less than 5 mg/l.

3.3 Feeding

3 times a week (on Monday, Wednesday and Friday). The fish are not fed on the day before the test is to begin.

3.4 Fitness

During the last 4 days before beginning the test the number of dead and seriously unhealthy fish must not exceed 10% of the total; otherwise the entire lot is to be discarded.

4. Test substances

4.1 Storing

Samples of industrial wastewaters should be tested immediately. If this is not possible, they must be stored in completely full bottles with ground-glass stoppers at 0 - 4°C (refrigerator).

4.2 Dilutions

Mix the substance and the dilution water carefully. All dilutions must be made with the same sample of the substance. Stock solutions must be kept in completely full bottles with ground-glass stoppers at 0 - 4°C (refrigerator) only as long as absolutely necessary.

4.3 Indication of concentration

1. Industrial wastewaters: ml/l or % by volume
2. Substances: mg/l or ppm.

8. Test procedure

8.1 Preliminary tests

- The tests are carried out at room temperature (20 - 23°C)
- Dilution series: Generally 1, 10, 100, 1000 mg/l (for substances) or 0.1, 1, 10 ml/l (for wastewaters)
- In each test vessel, the required amount of test substance is made up to 2 liters with activated charcoal-filtered tap water and adjusted to pH 6.8 - 7.5 with either NaOH (CSL) or H₂SO₄.
- Use 2 fish per concentration.
The total weight of the fish must not exceed 4 g/l, i.e. two fish of up to 4 g weight (ca. 5 - 6 cm long) can be used per test vessel. Larger or heavier fish must be used alone.
Place the fish in the test vessel carefully. In case of "accident" the fish is not to be used.
- The amount of dissolved O₂ must not be less than 5 mg/l.
The test vessels should be aerated only lightly.
- Usually an exposure time of 24 h suffices to estimate the concentration required. If no fish dies within a 24-hour period, the test is prolonged to 48 h. An exposure time of 48 h must be maintained if the preliminary test is for determining the toxicity of industrial wastewaters.
- The desired concentration range lies between the highest concentration at which all the fish survive an exposure time of 24 h and the lowest concentration at which all the fish die within the same period.
- With each test, at least 4 fish should be tested in pure dilution water by way of control. The mortality rate of all controls should not exceed 10%.
- Dead fish are to be removed from the test immediately.
- The fish are not fed during the test.

5.2 Main tests

- Within the concentration range found, 4 - 6 concentrations should be tested. Generally a linear distribution of the concentration range such as the following is suitable:
 - 1 . 10^x mg/l
 - 2 . 10^x mg/l
 - 4 . 10^x mg/l
 - 6 . 10^x mg/l
 - 8 . 10^x mg/l
 - 10 . 10^x mg/l
- For each concentration 10 fish are used, distributed over 5 or more test vessels, depending on the weight of the fish (weight \leq 4 g/l).
- In the main test the exposure time is 48 h. When more than half the fish survive when exposed to the highest concentration for 48 h, the concentration range must be extended upwards.
- Observation during test:

Note the number of dead fish after 24 h and after 48 h; also note the number of living fish which show any symptoms.

Before the beginning and after the end of the test, measure the temperature, pH and content of dissolved oxygen.

Otherwise the same procedure applies as used in the preliminary test.

5.3 Results

The TL_m is determined by means of a graph plotted on semi-logarithmic paper:

The 2 neighboring concentrations where one is with more and the other with less than 50% surviving fish are connected by a line. The lethality limit and the toxicity limit result either from the concentrations used or from the graph by extension of the lines described above.

The following applies for evaluating the wastewaters in a test corresponding to the preliminary test:

For each concentration tested, give the number of surviving fish in each case.