Study Title

AQUEOUS STABILITY OF 8-2 TELOMER B ALCOHOL AS A FUNCTION OF pH

Test Guideline

OECD (1981). Hydrolysis as a Function of pH. OECD Guideline for the Testing of Chemicals Method 111, adopted 12-May-1981.

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Study Completion Date

27-March-2003

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CERTIFICATION OF AUTHENTICITY

AQUEOUS STABILITY OF 8-2 TELOMER B ALCOHOL AS A FUNCTION OF pH

We, the undersigned, declare that the work described in this report was performed under our supervision, and that this report provides an accurate record of the procedures and results.

Report by:

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27-Mar-2003
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27-MAR-2003
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Study Initiation Date:
25-April-2002

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AQUEOUS STABILITY OF 8-2 TELOMER B ALCOHOL AS A FUNCTION OF PH

Author
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1.0 SUMMARY

Test System:
The aqueous stability of the test substance 8-2 Telomer B Alcohol (i.e., 8-2 TBA) in sterile aqueous solutions buffered at pH 1.2, 4.0, 7.0, and 9.0 was determined. Test systems consisted of the test substance in aqueous buffered solutions in sterilized containers. The test system was incubated in the dark at 50°C at pH 4, 7, and 9 and at 37°C at pH 1.2. An unstable test substance is indicated by a 10% or greater loss of test substance within 5 days.

Findings:
The test substance, 8-2 TBA, is stable under the conditions of the test at pH 4, 7, and 9 at 50°C and pH 1.2 at 37°C.

Conclusions:
The time in which 50% of the test substance will transform is estimated as greater than one year: $t_{½}$ >1 year.

2.0 GENERAL STUDY INFORMATION

Study Objectives
This test generates essential environmental fate information relevant to the persistence of the test substance. The aqueous stability of a substance (e.g., hydrolytic stability) is one of the most common reactions controlling abiotic degradation and is therefore one of the main potential degradation paths of substances in the environment. A procedure to determine aqueous stability rates also is important in indicating whether other testing should be performed on a parent substance or on its aqueous stability products. Aqueous stability behavior needs to be examined at pH values normally found in the environment (pH 4 to 9) at environmentally relevant temperatures (10 to 60°C). It is also sometimes desirable to determine aqueous stability of a test substance at physiologically relevant pH and temperatures, such as pH 1.2 and 37°C, respectively.

Specific objectives include:

1. To determine if a compound is stable in an aqueous environment,
2. To provide a material mass balance that accounts for greater then 90% of the initially applied test substance.
3. For unstable compounds:
   a. To determine the aqueous stability rate of the test substance in sterile buffer solutions at pH 4, 7, and 9 between 10 to 60°C and at pH 1.2 and 37°C,

b. To identify and follow the formation and decline of the aqueous stability product(s) of the test substance if formed,

c. To determine a first-order aqueous stability rate constant and half-life of the test substance (if rate constant and half-life of major aqueous stability products cannot be determined, then a aqueous stability study with the aqueous stability product(s) will be conducted exactly as the parent study).

Test System Justification

The test system is outlined by OECD Guideline 111 and was requested by the sponsor.

Study Personnel

E.I. du Pont de Nemours and Company

- Central Research and Development – Corporate Center for Engineering Research - Environmental and Microbiological Sciences & Engineering
- Haskell Laboratory for Health and Environmental Sciences

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Study Execution Dates

Study Initiation Date:	25-April-2002
Experimental Start Date:	15-July-2002
Experimental Completion Date:	9-September-2002
Study Completion Date:	27-March-2003

3.0 MATERIALS AND METHODS

3.1 Test Guidelines

The aqueous stability of the test substance 8-2 TBA in sterile aqueous solutions buffered at pH 1.2, 4.0, 7.0, and 9.0 was determined. Test systems consisted of the test substance in aqueous buffered solutions in sterilized containers made of non-adsorbing materials. The test systems were incubated in the dark at 50°C at pH 4, 7, and 9 and 37°C at pH 1.2. Day 0 and Day 5 samples were analyzed for the concentration of the test substance. If less than 10% of the test substance degrades in 5 days ($t_{½}$ is greater than 1 year at 25°C), the test substance is considered stable and no additional testing for aqueous stability is performed.

If degradation of the test substance had occurred at pH 4, 7, and/or 9 and 50°C, aqueous stability would also be performed at two additional temperatures separated by at least 15°C (e.g., 20°C and 35°C) at the pH values at which the test substance was shown to be unstable. To test for first-order behavior, each test vessel would have been analyzed in time intervals that provided a minimum of six-spaced data points normally between 20% and 70% of aqueous stability of the test substance. Test solution would have been analyzed for the concentration of the test substance and its transformation product(s). The test substance concentration would be plotted against the sampling intervals to determine its degradation rate constant and half-lives at each pH.

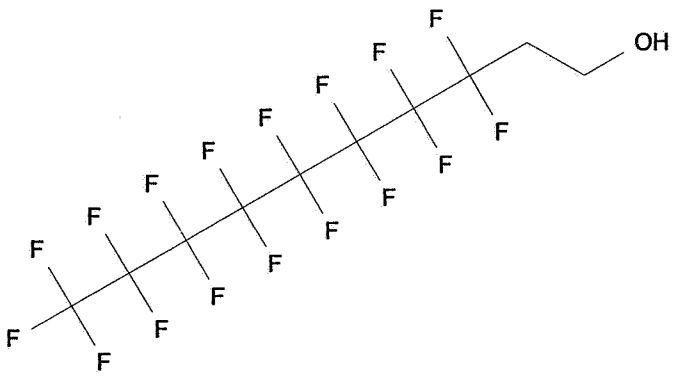
3.2 Test System

3.2.1 Chemical System

3.2.1.1 Test Substance

Name:	8-2 Telomer B Alcohol
Synonym:	(Perfluorooctyl)ethanol, 8-2 TBA
Active substance(s):	8-2 Telomer B Alcohol, 99%
Molecular Weight:	464.12 g $mole^{-1}$
CAS Name:	1-Decanol, 3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-heptadecafluoro-
CAS Number(s):	678-39-7

Structure:



H Number:	H-24691
Lot Number:	P.00/001
EMSE Sample Number:	E93386-63
Concentration of a.s., nominal:	99%
Concentration of a.s., analyzed:	99.2%
Certificate of Analysis Date:	13-Sept-2001
Date Received:	26-Mar-2002
Supplier:	Clariant Co.
Solubility at 25°C:	~140 µg L^{-1}
Vapor pressure:	0.023 mm Hg
Stability:	Stable at ambient room temperature
Appearance/Color:	White solid
Storage Conditions:	Room temperature; keep tightly closed
Safety Precautions:	Wear lab coat, protective gloves, and safety glasses

3.2.1.2 Reference Substance

None

3.2.1.3 Test Vehicle

Solutions buffered at pH 1.2, 4, 7, and 9 were added to glass test vessels containing 8-2 TBA L^{-1} methanol to attain an initial concentration of 150 µg 8-2 TBA L^{-1}. For test solutions with a volume of 4.5 mL, 33.8 µL of 20 mg 8-2 TBA L^{-1} methanol was used to attain a concentration of 150 µg 8-2 TBA L^{-1}. For test solutions with a volume of 3.0 mL, 22.5 µL of 20 mg 8-2 TBA L^{-1} methanol was used to attain a concentration of 150 µg 8-2 TBA L^{-1}.

3.2.1.4 Application Information

The test substance in methanol was mixed with pH 1.2, 4, 7, or 9 buffer solutions to attain a concentration of 150 µg 8-2 TBA L^{-1} buffer solution. The amount of methanol in the final test solution was less than 1% by volume.

3.2.2 Test System

3.2.2.1 Test Units

Different types of test vessels were used to reduce or eliminate the amount of test substance lost during the test period. Test vessels used to conduct this study were either 10 mL borosilicate glass vials with aluminum-lined crimp caps (pH 1.2, 7, and 9 samples) or 7 mL borosilicate glass vials with aluminum-lined screw caps (pH 4). All test vessels were gravity sterilized by autoclaving for 20 minutes at 121°C.

3.2.2.2 Test Conditions

Test solutions buffered at pH 4, 7, and 9 were held at 50 ± 1°C for 5 days in the dark by covering the test vessels using the lid of the shaking water bath. The test conditions were replicated 4 times at each pH and time. Test solutions buffered at pH 1.2 were held at 37 ± 1°C for 5 days in the dark by covering the test vessels using the lid of the shaking water bath. This test condition was also replicated 4 times.

3.3 Test Conduct

Sterile buffer concentrations of 0.05 M were used. After preparation, each solution was filter sterilized by passing through a 0.2-mm filter.

3.3.1 Buffer Solutions

3.3.1.1 pH 1.2 Buffer:

250 mL of 0.200 M potassium chloride (KCl) and 475 mL of 0.200 N HCl were diluted to 1.000 L with water. Final pH adjustments were made with 1.0 N HCl or NaOH as necessary.

3.3.1.2 pH 4 Buffer:

500 mL of 0.100 M potassium hydrogen phthalate [$KH(C_8H_4O_4)$] and 4.00 mL of 0.100 N sodium hydroxide (NaOH) were diluted to 1.000 L with water. No final pH adjustment was necessary.

3.3.1.3 pH 7 Buffer:

500 mL of 0.100 M potassium dihydrogen phosphate (KH_2PO_4) and 296 mL of 0.100 M sodium hydroxide were diluted to 1.000 L with water. Final pH adjustments were made with 5N HCl.

3.3.1.4 pH 9 Buffer:

500 mL 0.050 M boric acid (H_2BO_2) and 213 mL of 0.100 M NaOH were diluted to 1.000 L with water. Final pH adjustments were made with 5N HCl.

3.3.2 *Preliminary Test*

A preliminary test was performed on the test substance at 50°C at pH 4.0, 7.0, and 9.0 and at 37°C and pH 1.2.

3.3.3 *Aqueous Stability of an Unstable Substance*

The test substance is stable; therefore, testing for the aqueous stability of an unstable substance was not performed.

3.3.4 *Sample Collection and Storage*

3.3.4.1 *Sampling Intervals*

Day 0 samples were frozen immediately after the addition of the test substance until extracted and analyzed. Day 5 samples were shaken at 37°C (buffer pH 1.2) or 50°C buffer pH 4, 7, and 9) for 5 days, at which time they were frozen until extracted and analyzed.

3.3.4.2 *Test Solution Storage*

Test solutions were stored at approximately -20°C.

3.4 ***Parameters Observed***

3.4.1 *Analysis of Test Systems*

3.4.1.1 *Sterility Measurements*

Sterility was determined on Day 5 samples of each of the test systems. Sterile trypticase soy broth (TSB) medium was used to check the sterility of each sample. At sampling, 0.5 mL of the test solution was aseptically added to a culture tube containing 4.5 mL of TSB medium. The TSB medium was incubated 3 to 4 days in darkness at 30°C. The presence or absence of cloudiness as compared to sterile TSB medium controls determined sterility.

3.4.1.2 *pH Measurement*

Test solution pH was measured and documented at Day 0 and at the end of the experiment.

3.4.1.3 *Temperature Measurements*

The temperature of the test system was monitored and recorded daily throughout the course of the study.

3.4.2 *Analytical Methods for Test Substance and Products*

3.4.2.1 *Analysis of Test Substance*

Sample preparation:

The GC/MS analysis of 8-2 TBA samples involved use of an internal standard and surrogate spiked into each sample to trace the extraction recovery. The 1-Octanol, 3,3,4,4,5,5,6,6,7,8,8,8-dodecafluoro-7-(trifluoromethyl)- (CAS# 20015-46-7, 98%, Oakwood Products, West Columbia, SC), referred to as C9-iso, was used as the surrogate. The 1-Decanol, 3,3,4,4,5,5,6,6,7,7,8,8,9,10,10,10-hexadecafluoro-9-(trifluoromethyl)- (CAS# 31200-98-3, 98%, Oakwood Products, West Columbia, SC), referred to as C11-iso, was used

as the internal standard. The 1-Decanol, 3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-heptadecafluoro- (CAS# 678-39-7, 97.6%, Oakwood Products, West Columbia, SC), referred to as 8-2 TBA, was used as the analytical standard of 8-2 TBA. Stock solutions at approximately 1000 μg mL^{-1} of internal standard and surrogate were prepared in methanol and stored refrigerated. The stocks were diluted to appropriate concentration with methanol before use for each set of samples analyzed.

The pH 1.2, 7, and 9 samples (4.5-mL test solution volume) in crimp-capped 10-mL glass vials were spiked with 13.5 μL of 50 μg C9-iso mL^{-1} methanol by injecting the solution into the closed vial through the vial septum using a syringe. Then, 2.25-mL propane, 2-methoxy-2-methyl- [CAS# 1634-04-4 (methyl t-butyl ether, referred to as MTBE] was introduced into the vial using a syringe. The contents of the vial were vortexed for 15 min using a multi-tube vortexer. The vial was opened and 1 mL of the MTBE layer was sampled to a glass GC vial (1.7 mL), spiked with 6 μL of a 50 μg C11-iso mL^{-1} methanol, crimped, and subjected to GC/MS analysis. The calibration standards were prepared by spiking appropriate volumes of 50 μg mL^{-1} methanol of C9-iso, C11-iso, 8-2 TBA to 1-mL MTBE. The calibration curves were constructed using peak area of signal obtained for ion m/z: 95.

The pH 4 samples (3-mL test solution volume) required a different preparation procedure because the presence of phthalates in the sample buffer interfered with the analysis of the test substance. The pH 4 samples in 7 mL glass vials with screw caps were opened and 9 μL of 50-μg C9-iso mL^{-1} methanol was added to the vial using a syringe, followed by 1.5 mL of hexane (CAS# 110-54-3). The contents of the vial were vortexed for 15 min using a multi-tube vortexer. The SPE column (Isolute Si, 100 mg /10mL, Jones Chromatography) was conditioned by cleaning it with 2 mL of isopropanol (IPA) and conditioning with 2 mL hexane. The hexane extract was loaded onto the conditioned SPE column. After loading the hexane extract, the column was washed with 2 mL of hexane and the analytes were eluted with 1 mL IPA. No drying of the column was applied after the hexane wash. A 0.5 mL aliquot of the IPA extract was sampled to a glass GC vial (1.7 mL), spiked with 3 μL of 50 μg C11-iso mL^{-1} methanol, crimped, and subjected to GC/MS analysis. The calibration standards for the pH 4 samples were prepared by spiking appropriate volumes of 50 μg mL^{-1} methanol of C9-iso, C11-iso, 8-2 TBA to 1 mL of matrix matched IPA. The matrix matched IPA was prepared by subjecting the blank pH 4 buffer to the SPE procedure, the same way as the samples. The IPA eluate was used to prepare the matrix matched calibration standards. The calibration curves were constructed using peak area of signal obtained for ion m/z: 95.

Instrumentation and conditions:

GC/MS system:	HP 6890 Plus GC (Agilent, Wilmington, DE USA), HP 5973 Mass Selective Detector (Agilent), MPS2-MultiPurposeSampler (Gerstal, Baltimore, MD USA)	
Column:	DB-5MS, 30 m x 0.25 mm, 1 μm film (Agilent, Wilmington, DE USA)	
Temp. ramp:	Initial:	80°C for 2 min 20°C/min to 120°C 50°C/min to 300°C and hold for 3 min
Flow rate:		1.0 mL/min; He; constant flow mode
Split:		5:1
Inlet temp.:		250°C
Injection volume:		2 μL
MSD transfer line temp.:		280°C
SIM ions monitored:		m/z: 31, 95, 131

Retention time:	8-2 TBA:	4.93 min
	C9-iso:	4.60 min
	C11-iso:	5.38 min

3.5 Statistical Analysis

Paired t-test tests were used to determine if the mean test substance concentrations in paired Day 0 and Day 5 buffer solutions are the same (JMP, 1995).

3.6 Protocol Deviations

- None

4.0 RESULTS AND DISCUSSION

Under the conditions of the test at pH 1.2 and 37°C and at pH 4, 7, and 9 and 50°C, 8-2 TBA is stable (Table 1). Tables in Appendix A (Tables A-1, A-2, A-3, and A-4) contain the analytical results of the aqueous stability study of the test substance that are summarized in Table 1.

Stability of the test substance, 8-2 TBA:

- At pH 1.2, 8-2 TBA concentration after five days at 37°C was 195 ± 8.00 μg L^{-1}, compared to 183 ± 10.9 μg L^{-1}at the start of the test (Day 0). The difference between the Day 5 and Day 0 concentrations of the test substance was +6.7%, indicating that 8-2 TBA is stable at this pH. The difference between Day 5 and Day 0 was not statistically significant (p = 0.05).
- At pH 4, 8-2 TBA concentration after five days at 50°C was 143 ± 10.0 μg L^{-1}, compared to 156 ± 4.22 μg L^{-1}at the start of the test (Day 0). The difference between the Day 5 and Day 0 concentrations of the test substance was -8.7%, indicating that 8-2 TBA is stable at this pH. The difference between Day 5 and Day 0 was not statistically significant (p = 0.05).
- At pH 7, 8-2 TBA concentration after five days at 50°C was 181 ± 12.6 μg L^{-1}, compared to 199 ± 9.12 μg L^{-1} at the start of the test (Day 0). The difference between the Day 5 and 0 concentrations of the test substance was -9.6%, indicating that 8-2 TBA is stable at this pH. The difference between Day 5 and Day 0 was not statistically significant (p = 0.05).
- At pH 9, 8-2 TBA concentration after five days at 50°C was 175 ± 10.5 μg L^{-1}, compared to 169 ± 5.99 μg L^{-1} at the start of the test (Day 0). The difference between the Day 5 and Day 0 concentrations of the test substance was +3.8%, indicating that 8-2 TBA is stable at this pH. The difference between Day 5 and Day 0 was not statistically significant (p = 0.05).

Experimental conditions – pH:

- The pH 1.2 test solutions were pH 1.11 and 1.54 at days 0 and 5, respectively, a variation of more 0.1 units (Table 2). This had no significant effect on the outcome of the test because the compound was stable.
- The pH 4 test solutions were pH 4.02 and 4.12 at days 0 and 5, respectively; a variation of more 0.1 units (Table 2). This had no significant effect on the outcome of the test because the compound was stable.

- The pH 7 test solutions were pH 7.03 and 7.04 at days 0 and 5, respectively; and the pH 9 test solutions were pH 9.02 and 8.97 at days 0 and 5, respectively (Table 2). The pH of the test solutions at these 2-pH levels did not change significantly (pH change less than 0.1 unit) during the test.

Experimental conditions – temperature:

- The temperature of the shaking water bath used to maintain the pH 1.2 test solution at a constant temperature did not fluctuate by a total of more than 0.2°C at 37°C (Table 3). The average temperature of the shaking water bath was 37.1°C and the standard deviation was 0.1°C (n = 6).
- The temperature of the shaking water bath used to maintain the pH 4 test solution at a constant temperature did not fluctuate by more than 0.1°C at 50°C during the 5 days of the test (Table 4). The average temperature of the shaking water bath was 50.0°C and the standard deviation was 0.05°C (n = 6).
- The temperature of the shaking water bath used to maintain the pH 7 and 9 test solutions at a constant temperature did not fluctuate by more than 0.1°C at 50°C during the 5 days of the test (Table 5). The average temperature of the shaking water bath was 50.0°C and the standard deviation was 0.1°C (n = 6).

Experimental conditions – sterility:

- The sterility check solutions for the control, pH 1.2 at 37°C, and pH 7 and 9 at 50°C were sterile at the end of the test, as indicated by the lack of bacteria growth in the Trypticase Soy Broth (TSB) medium (Table 6).
- The sterility check solution for the pH 4 at 50°C was not sterile as indicated by a slightly cloudiness of the TSB medium after the incubation period. (Table 6). This did not affect the outcome of the study because the test substance was stable at this pH and temperature (Table 1).

Trial studies:

Prior to the definitive studies, several trial studies were performed. The results of these trial studies are found in Appendix B (Tables B-1, B-2, B-3, and B-4). The information below summarizes these results.

- Table B-1 lists the results of a pilot experiment that was conducted to demonstrate that the experimental design, implementation, and subsequent analysis of the test substance was adequate to determine its aqueous stability. This study was conducted at pH 4 and room temperature for Day 0 only.
- Table B-2 lists the results of the aqueous stability of the test substance at pH 1.2 and 50°C. The test substance was shown to be stable at this pH and temperature after 5 days. The study was repeated because it should have been conducted at a pH of 1.2 and 37°C. Both demonstrate that the test substance is stable (Tables 1 and A-1).
- Table B-3 lists the results of the aqueous stability of the test substance at pH 7 and 50°C on Day 0 and Day 5. This test was repeated using different test vessels because of the large standard deviation of the average test substance concentration at Day 5 (standard deviation of 39.8 $\mu g\ L^{-1}$ for Day 5 compared to 6.25 $\mu g\ L^{-1}$ for Day 0), which appears to be due to the loss of the test substance in two or more of the Day 5 replications. The repeated study demonstrated that the test substance is stable at pH 7 (Tables 1 and A-3).

- Table B-4 lists the results of the aqueous stability of the test substance at pH 9 and 50°C on Day 0 and Day 5. This test was repeated using different test vessels because of the large standard deviation of the average test substance concentration at Day 5 (standard deviation of 42.9 $\mu g\ L^{-1}$ for Day 5 compared to 11.5 $\mu g\ L^{-1}$ for Day 0), which appears to be due to the loss of the test substance in two of the Day 5 replications. The repeated study demonstrated that the test substance is stable at pH 9 (Tables 1 and A-3).

5.0 CONCLUSIONS

- The time in which 50% of the test substance will transform in water at environmentally relevant pH ranges and temperatures is greater than one year ($t_{½}$ >1 year), because >90% of the test substance added at Day 0 was recovered after 5 days at pH 1.2 and 37°C and pH 4.0, 7.0, and 9.0 and 50°C.

6.0 RETENTION OF RECORDS

For the periods demanded by GLP guidelines and specific country requirements, study documents and materials will be stored in the archives of the test facility, including but not limited to:

- study protocol;
- any protocol and/or report amendments or addenda or SOP deviations;
- all raw data;
- one original signed copy of the final report;
- laboratory-specific or site-specific raw data such as personnel files, instrument, equipment, refrigerator, and/or freezer raw data.

Documents and materials are archived according to the principles of Good Laboratory Practice in the organization of the testing facility.

7.0 DISPOSAL OF TEST SUBSTANCE

After issuance of the final report, the remaining test substance will be stored at the DuPont EMSE laboratory until its expiration date and then destroyed by incineration, unless other arrangements are made between the submitter and the Test Facility.

8.0 REFERENCES

8.1 OECD Guidelines for the Testing of Chemicals, Section 1, Method 111, Hydrolysis as a Function of pH.

8.2 Environmental Protection Agency. 1989. Good Laboratory Practice Standards, 40 CFR, Part 160, Final Rule. EPA, Washington, DC.

8.3 JMP. 1995. JMP® Statistical and Graphics Guide. Version 3 of JMP. SAS Institute Inc. Cary, NC USA.

TABLE 1
CONCENTRATION OF 8-2 TBA AT PH 1.2 AT 37°C AND AT PH 4, 7, AND 9 AT 50°C AFTER 0 AND 5 DAYS

pH	Time	Date	8-2 TBA Average Concentration	Difference between Day 5 and Day 0†
	Day		*ug L^{-1}*	*%*
1.2	0	21-Aug-2002	183 ± 10.9‡	
1.2	5	26-Aug-2002	195 ± 8.00	+6.4
4	0	24-Jul-2002	156 ± 4.22	
4	5	29-Jul-2002	143 ± 10.0	-8.7
7	0	21-Aug-2002	199 ± 9.12	
7	5	26-Aug-2002	181 ± 12.6	-9.5
9	0	21-Aug-2002	169 ± 5.99	
9	5	26-Aug-2002	175 ± 10.5	+3.5

† Difference between Day 5 and Day 0, % = $(C_5 - C_0)/((C_5 + C_0)/2) \times 100$, where:
C_0 = 8-2 TBA concentration at Day 0
C_5 = 8-2 TBA concentration at Day 5

‡ Mean ± standard deviation; n = 4.

TABLE 2
pH OF BUFFER SOLUTIONS CONTAINING 8-2 TBA AT DAYS 0 AND 5

Time	Date	Nominal pH	Measured pH	Nominal temperature	Temperature of pH measurement
Day		*S.U.†*	*S.U.*	*°C*	*°C*
0	21-Aug-2002	1.2	1.11	Room temperature	22.5
5	26-Aug-2002	1.2	1.54	37	37.1
0	24-Jul-2002	4	4.02	Room temperature	23.0
5	29-Jul-2002	4	4.12	50	50.0
0	21-Aug-2002	7	7.03	Room temperature	22.5
5	26-Aug-2002	7	7.04	50	50.0
0	21-Aug-2002	9	9.02	Room temperature	22.5
5	26-Aug-2002	9	8.97	50	50.0

† S.U. = Standard Units

TABLE 3
DAILY TEMPERATURE READINGS OF SHAKING WATER BATH FOR AQUEOUS STABILITY OF 8-2 TBA CONDUCTED AT PH 1.2

Time	Date	Temperature
Day		*°C*
0	21-Aug-2002	37.1
1	22-Aug-2002	37.0
2	23-Aug-2002	37.2
3	24-Aug-2002	37.0
4	25-Aug-2002	37.1
5	26-Aug-2002	37.1
Average, n = 6		37.1
Standard Deviation		0.1

TABLE 4
DAILY TEMPERATURE READINGS OF SHAKING WATER BATH FOR AQUEOUS STABILITY OF 8-2 TBA CONDUCTED AT PH 4

Time	Date	Temperature
Day		°C
0	24-Jul-2002	49.9
1	25-Jul-2002	50.0
2	26-Jul-2002	49.9
3	27-Jul-2002	49.9
4	28-Jul-2002	50.0
5	29-Jul-2002	50.0
Average, n = 6		50.0
Standard Deviation		0.05

TABLE 5
DAILY TEMPERATURE READINGS OF SHAKING WATER BATH FOR AQUEOUS STABILITY OF 8-2 TBA CONDUCTED AT PH 7 AND 9

Time	Date	Temperature
Day		°C
0	21-Aug-2002	49.9
1	22-Aug-2002	50.0
2	23-Aug-2002	50.1
3	24-Aug-2002	49.9
4	25-Aug-2002	50.0
5	26-Aug-2002	50.0
Average, n = 6		50.0
Standard Deviation		0.1

TABLE 6
STERILITY OF TEST SOLUTIONS AT DAY 5 IN STERILE TRYPTICASE SOY BROTH (TSB) MEDIUM

Treatment	Result	Conclusion
Control	Clear	Sterile
Buffer 1.2 pH, 37°C, Day 5	Clear	Sterile
Buffer 4 pH, 50°C, Day 5	Cloudy	Non-sterile
Buffer 7 pH, 50°C, Day 5	Clear	Sterile
Buffer 9 pH, 50°C, Day 5	Clear	Sterile

† Sterile indicates that no bacteria were observed in 4.5 mL of TSB containing 0.5 mL test solution after 3 to 4 days of incubation in the dark at 30°C.

TABLE A-1
ANALYTICAL RESULTS OF THE AQUEOUS STABILITY OF 8-2 TBA, PH 1.2, 37°C, DAYS 0 (21-AUG-2002) AND 5 (26-AUG-2002)

No	pH	Time	Rep	Test Substance	Test Substance Analytical Replication Average	Difference Between Analytical Replications†
		day		*ug L^{-1}*	*ug L^{-1}*	*%*
1	1.2	0	1	171	175	4.0
2	1.2	0	1	178		
3	1.2	0	2	201	197	4.6
4	1.2	0	2	192		
5	1.2	0	3	190	186	4.0
6	1.2	0	3	183		
7	1.2	0	4	171	174	2.9
8	1.2	0	4	176		
Average					183	
Standard Deviation					10.9	
9	1.2	5	1	200	200	0.0
10	1.2	5	1	200		
11	1.2	5	2	186	189	4.0
12	1.2	5	2	193		
13	1.2	5	3	204	204	0.2
14	1.2	5	3	205		
15	1.2	5	4	203	188	15
16	1.2	5	4	174		
Average					195	
Standard Deviation					8.00	

† Calculated by dividing the absolute difference of the two analytical replications by their average value, times 100.

TABLE A-2
ANALYTICAL RESULTS OF THE AQUEOUS STABILITY OF 8-2 TBA, PH 4.0, 50°C, DAYS 0 (24-JUL-2002) AND 5 (29-JUL-2002)

No	pH	Time	Rep	Test Substance	Test Substance Analytical Replication Average	Difference Between Analytical Replications†
		day		*ug L^{-1}*	*ug L^{-1}*	*%*
1	4	0	1	162	162	0.2
2	4	0	1	162		
3	4	0	2	156	157	0.4
4	4	0	2	157		
5	4	0	3	150	153	3.7
6	4	0	3	156		
7	4	0	4	155	153	3.1
8	4	0	4	151		
Average					156	
Standard Deviation					4.22	
9	4	5	1	128	130	2.6
10	4	5	1	132		
11	4	5	2	155	154	1.3
12	4	5	2	153		
13	4	5	3	143	143	0.2
14	4	5	3	143		
15	4	5	4	145	145	0.0
16	4	5	4	145		
Average					143	
Standard Deviation					10.0	

† Calculated by dividing the absolute difference of the two analytical replications by their average value, times 100.

TABLE A-3
ANALYTICAL RESULTS OF THE AQUEOUS STABILITY OF 8-2 TBA, pH 7.0, 50°C, DAYS 0 (21-AUG-2002) AND 5 (26-AUG-2002)

No	pH	Time	Rep	Test Substance	Test Substance Analytical Replication Average	Difference Between Analytical Replications†
		day		*ug L^{-1}*	*ug L^{-1}*	*%*
1	7	0	1	202	199	2.3
2	7	0	1	197		
3	7	0	2	195	195	0.3
4	7	0	2	196		
5	7	0	3	201	190	12
6	7	0	3	179		
7	7	0	4	211	211	0.7
8	7	0	4	212		
Average					199	
Standard Deviation					9.12	
9	7	5	1	195	192	3.1
10	7	5	1	189		
11	7	5	2	169	166	3.6
12	7	5	2	163		
13	7	5	3	178	175	3.4
14	7	5	3	172		
15	7	5	4	202	191	12
16	7	5	4	180		
Average					181	
Standard Deviation					12.6	

† Calculated by dividing the absolute difference of the two analytical replications by their average value, times 100.

TABLE A-4
ANALYTICAL RESULTS OF THE AQUEOUS STABILITY OF 8-2 TBA, pH 9.0, 50°C, DAYS 0 (21-AUG-2002) AND 5 (26-AUG-2002)

No	pH	Time	Rep	Test Substance	Test Substance Analytical Replication Average	Difference Between Analytical Replications†
		day		*ug L^{-1}*	*ug L^{-1}*	*%*
1	9	0	1	185	176	10
2	9	0	1	167		
3	9	0	2	168	170	2.6
4	9	0	2	173		
5	9	0	3	159	162	3.7
6	9	0	3	165		
7	9	0	4	163	167	4.5
8	9	0	4	171		
Average					169	
Standard Deviation					5.99	
9	9	5	1	172	182	12
10	9	5	1	193		
11	9	5	2	173	179	6.7
12	9	5	2	185		
13	9	5	3	160	160	0.6
14	9	5	3	159		
15	9	5	4	169	180	13
16	9	5	4	191		
Average					175	
Standard Deviation					10.5	

† Calculated by dividing the absolute difference of the two analytical replications by their average value, times 100.

TABLE B-1
ANALYTICAL RESULTS OF THE AQUEOUS STABILITY OF 8-2 TBA FOR PILOT EXPERIMENT CONDUCTED AT pH 4 AND ROOM TEMPERATURE, DAY 0 ONLY (16-JUL-2002)†

No	Rep	Test Substance	Test Substance Analytical Replication Average	Difference Between Analytical Replications‡
		$ug\ L^{-1}$	$ug\ L^{-1}$	%
1	1	106	112	10
2	1	117		
3	2	104	108	8.2
4	2	113		
5	3	109	110	2.4
6	3	111		
7	4	102	113	19
8	4	123		
9	5	114	110	7.3
10	5	106		
11	6	125	117	13
12	6	109		
13	7	103	106	6.9
14	7	110		
15	8	118	115	4.9
16	8	112		
17	9	114	115	3.2
18	9	117		
19	10	100	94.1	12
20	10	88.4		

† Experiment performed to assess the adequacy of the test methods and analytical procedure. It was performed using pH 4 buffer at room temperature, Day 0 samples only. Test vessels were 7 mL glass scintillation vials with aluminum-lined screw caps. 22.5 µL of a 20 mg 8-2 TBA L^{-1} methanol solution was added to each test vessel with 3 mL of the pH 4 buffer solution. After capping, the samples were vortex-mixed for 10 s. Samples were frozen until analyzed.

‡ Calculated by dividing the absolute difference of the two analytical replications by their average value, times 100.

TABLE B-1 (CONT'D)

No	Rep	Test Substance	Test Substance Analytical Replication Average	Difference Between Analytical Replications‡
		ug L^{-1}	*ug L^{-1}*	*%*
21	11	108	106	3.6
22	11	104		
23	12	120	103	35
24	12	84.9		
25	13	117	119	4.4
26	13	122		
27	14	101	108	12
28	14	115		
29	15	112	108	7.4
30	15	104		
31	16	106	114	13
32	16	121		
Average			110	
Standard Deviation			6.09	

† Experiment performed to assess the adequacy of the test methods and analytical procedure. It was performed using pH 4 buffer at room temperature, Day 0 samples only. Test vessels were 7 mL glass scintillation vials with aluminum-lined screw caps. 22.5 µL of a 20 mg 8-2 TBA L^{-1} methanol solution was added to each test vessel with 3 mL of the pH 4 buffer solution. After capping, the samples were vortex-mixed for 10 s. Samples were frozen until analyzed.

‡ Calculated by dividing the absolute difference of the two analytical replications by their average value, times 100.

TABLE B-2
ANALYTICAL RESULTS OF THE AQUEOUS STABILITY OF 8-2 TBA, PH 1.2, 50°C, DAYS 0 (31-JUL-2002) AND 5 (5-AUG-2002)†

No	pH	Time	Rep‡	Test Substance	Test Substance Analytical Replication Average	Difference Between Analytical Replications¶
		day		*Ug L^{-1}*	*ug L^{-1}*	*%*
1	1.2	0	1	136	137	1.2
2	1.2	0	1	138		
3	1.2	0	2	117	119	2.0
4	1.2	0	2	120		
5	1.2	0	3	157	150	8.7
6	1.2	0	3	144		
7	1.2	0	3	152		1.6
8	1.2	0	3	149		
	1.2		4	114	110	8.8
	1.2		4	105		
Average					129	
Standard Deviation					18.4	
9	1.2	5	1	143	141	3.8
10	1.2	5	1	138		
11	1.2	5	2	140	134	9.0
12	1.2	5	2	128		
13	1.2	5	3	102	102	NA§
14	1.2	5	4	165	164	0.8
15	1.2	5	4	163		
Average					135	
Standard Deviation					25.6	

† Test was performed at 50°C instead of 37°C. Experiment was performed using pH 1.2 buffer at 50°C. Test vessels were 7 mL glass scintillation vials with aluminum-lined screw caps. 22.5 µL of a 20 mg 8-2 TBA L^{-1} methanol solution was added to each test vessel with 3 mL of the pH 1.2 buffer solution. After capping, the samples were vortex-mixed for 10 s. Samples were frozen until analyzed.

‡ Day 0, replicate 3 measured four times; Day 5 replicate 3 measured once.

¶ Calculated by dividing the absolute difference of the two analytical replications by their average value, times 100.

§ NA = Not applicable; only one analytical measurement of sample.

TABLE B-3
ANALYTICAL RESULTS OF THE AQUEOUS STABILITY OF 8-2 TBA, PH 7.0, 50°C, DAYS 0 (31-JUL-2002) AND 5 (5-AUG-2002)†

No	pH	Time	Rep	Test Substance	Test Substance Analytical Replication Average	Difference Between Analytical Replications‡
		day		*ug L^{-1}*	*ug L^{-1}*	*%*
1	7	0	1	103	104	1.6
2	7	0	1	104		
3	7	0	2	114	111	5.7
4	7	0	2	108		
5	7	0	3	98.0	95.7	4.9
6	7	0	3	93.3		
7	7	0	4	102	102	1.3
8	7	0	4	101		
Average					103	
Standard Deviation					6.25	
9	7	5	1	58.7	57.2	5.3
10	7	5	1	55.7		
11	7	5	2	24.6	24.6	0.3
12	7	5	2	24.7		
13	7	5	3			ND¶
14	7	5	3			
15	7	5	4	96.7	91.7	11
16	7	5	4	86.7		
Average					43.4	
Standard Deviation					39.8	

† Test was repeated because of the variation in replicate results of test substance concentration at Day 5. Experiment was performed using pH 7 buffer at 50°C. Test vessels were 7 mL glass scintillation vials with aluminum-lined screw caps. 22.5 µL of a 20 mg 8-2 TBA L^{-1} methanol solution was added to each test vessel with 3 mL of the pH 1.2 buffer solution. After capping, the samples were vortex-mixed for 10 s. Samples were frozen until analyzed.

‡ Calculated by dividing the absolute difference of the two analytical replications by their average value, times 100.

¶ ND = Not determined; samples were lost prior to analysis

TABLE B-4
ANALYTICAL RESULTS OF THE AQUEOUS STABILITY OF 8-2 TBA, pH 9.0, 50°C, DAYS 0 (31-JUL-2002) AND 5 (5-AUG-2002)†

No	pH	Time	Rep	Test Substance	Test Substance Analytical Replication Average	Difference Between Analytical Replications‡
		day		*ug L^{-1}*	*ug L^{-1}*	*%*
1	9	0	1	162	162	0.6
2	9	0	1	163		
3	9	0	2	149	142	9.6
4	9	0	2	135		
5	9	0	3	153	150	4.4
6	9	0	3	147		
7	9	0	4	132	136	5.2
8	9	0	4	139		
Average					147	
Standard Deviation					11.5	
9	9	5	1	150	143	9.8
10	9	5	1	136		
11	9	5	2	71.3	80.8	24
12	9	5	2	90.3		
13	9	5	3	59.7	59.0	2.3
14	9	5	3	58.3		
15	9	5	4	131	142	15
16	9	5	4	152		
Average					106	
Standard Deviation					42.9	

† Test was repeated because of the variation in replicate results of test substance concentration at Day 5. Experiment was performed using pH 9 buffer at 50°C. Test vessels were 7 mL glass scintillation vials with aluminum-lined screw caps. 22.5 µL of a 20 mg 8-2 TBA L^{-1} methanol solution was added to each test vessel with 3 mL of the pH 1.2 buffer solution. After capping, the samples were vortex-mixed for 10 s. Samples were frozen until analyzed.

‡ Calculated by dividing the absolute difference of the two analytical replications by their average value, times 100.

APPENDIX C
CERTIFICATE OF TEST SUBSTANCE ANALYSIS

Clariant GmbH, Werk Gendorf
CQG / Qualitätsmanagement
D-84504 Burgkirchen
Fax: ++49 8679 / 2676

Clariant

Inspection certificate
according to EN10204-3.1B

Date: 13.11.2001
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Our consignment
Material : 1-Decanol, 3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,
10-heptadecafluoro
Material-no. : 104705
Batch No. : 200/001

On the batch, of which the consignment is a part, the following values were
determined. They conform to the agreed product specification.

Inspection characteristic/-method	Specification	Result
C8-Perfluoralkylethanol Capillary GC	>= 97,5	99,3%[a/a]
Sum Perfluoroalkylethanol Capillary GC	>= 98,0	99,9%[a/a]
Sum Perfluoralkylbutanol Capillary GC	<=1,0	< 0,1%[a/a]
Sum Perfluoralkylethene Capillary GC	<=0,5	< 0,1%[a/a]
Water content Karl Fischer DIN 51777	<=0,2	< 0,1%
N-Methylpyrrolidone content Capillary GC	<=0,2	< 0,1%

The above particulars do not release the customer from the obligation to carry out an
inspection of goods received.

Ms. Baumgärtner - Worksinspector