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Contains...

THE PROCTER & GAMBLE COMPANY

IVORYDALE TECHNICAL CENTER

5299 SPRING GROVE AVENUE, CINCINNATI, OHIO 45217 1987

04940000235

June 27, 1988

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

REC'D
JUN 28 1988
R.H. Brink

SUBJECT: OPTS-40017; FRL-3332-9; ITC Request for Information on Tertiary Amines (53 FR 5466; 2/24/88)

Dear Mr. Brink:

The Procter & Gamble Company and its subsidiaries are making this submission in response to the Interagency Testing Committee's (ITC) request for information regarding certain tertiary amines. We are making this submission as a major manufacturer of tertiary amines that may be subject to Section 4(a) rulemaking involving alkyl dimethylamines. Our specific production is considered confidential business information and has been so indicated by brackets. In addition, a sanitized version of this response is attached for public use.

We manufacture and process four of the listed tertiary amines:

- 1-dodecylamine, N,N-dimethyl- (CAS number 112-18-5),
- 1-hexadecylamine, N,N-dimethyl- (CAS number 112-69-6),
- 1-tetradecylamine, N,N-dimethyl- (CAS number 112-75-4), and
- 1-octadecylamine, N,N-dimethyl- (CAS number 124-28-7)

as components of mixtures of tertiary amines or as single materials. We estimate that our manufacture and consumption of these materials accounts approximately [] of these alkyl dimethylamines used in the U.S.

As part of this submission we have provided material safety data sheets, current production and usage information, toxicology studies, and environmental safety information.

A key factor regarding the human and environmental safety of alkyl dimethylamines is that they are used predominately as chemical intermediates. All of P&G's production is used to produce derivative chemicals. Consequently, both minimal human exposure and environmental releases are associated with these tertiary amines.

From the standpoint of human safety considerations, P&G manufactures and consumes tertiary amines at only two sites. We further limit human exposures to these intermediates by producing, handling, transporting, and reacting them in closed systems. Activities such as occasional sampling which are conducted outside these closed systems are executed using defined safe practices which

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THE PROCTER & GAMBLE COMPANY

Mr. Robert Brink, Executive Secretary
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include the use of appropriate protective equipment such as gloves, goggles, and protective clothing. The low vapor pressure characteristic of these materials also reduces potential human exposures.

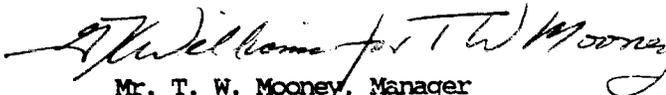
Several factors that minimize human exposures to alkyl dimethylamines, such as their use as chemical intermediates, the use of closed reaction, transporting, handling systems, and the materials low vapor pressure, etc., also serve to reduce environmental exposure to alkyl dimethylamines. Furthermore, environmental safety testing shows that any alkyl dimethylamines which may reach wastewater or surface waters would be rapidly and extensively mineralized.

Because of the very limited human and environmental exposures to tertiary amines, we believe additional safety testing of these materials under Section 4 of the Toxic Substances Control Act is not warranted.

Attached to this cover letter is more detailed information regarding alkyl dimethylamines. We appreciate the opportunity to provide you with information regarding these tertiary amines and hope you find this submission helpful. We recognize that as the ITC reviews this submission and information from other sources, additional questions may arise. We would be more than glad to answer questions or provide additional perspectives regarding tertiary amines. Dr. Clifford Ivy at (513) 530-3551 can be contacted with further questions regarding tertiary amines and can provide additional information or will put you in contact with other appropriate people.

Sincerely,

THE PROCTER & GAMBLE COMPANY


Mr. T. W. Mooney, Manager
Technical Government Relations

GKW/es
Attachments

PRODUCTION AND USAGE OF ALKYL DIMETHYLAMINES

U.S. annual production of alkyl dimethylamines is approximately [] million pounds. Alkyl dimethylamines are used mainly as a chemical intermediate to produce various amine derivatives. A minor fraction of these materials is used in industrial applications, i.e. corrosion inhibitors.

The Procter & Gamble Manufacturing Company produces approximately [] million pounds of alkyl dimethylamines on an annual basis. The majority of this production, about [] million pounds, is used by the Company as a chemical intermediate. The remaining [] million pounds we produce are used by our industrial customers at their sites as chemical intermediates.

FROCTER & GAMBLE'S ANNUAL TERTIARY AMINE PRODUCTION

<u>Brand Name</u>	<u>Type</u>	<u>CAS #</u>	<u>Annual Volume</u> <u>MM lbs.</u>
AT-1280	80% C12*	67700-98-5	[]
AT-1295	95% C12	112-18-15	[]
AT-1214	70% C12 30% C14	112-18-15 112-75-14	[]
AT-1495	95% C14	112-75-4	[]
AT-1695	95% C16	112-69-6	[]
AT-1695A	95% C16	112-69-6	[]
AT-1895A	95% C18	124-28-7	[]

* Contains C₁₀ - C₁₆ dimethylamines.

HUMAN EXPOSURE TO ALKYL DIMETHYLAMINES

Alkyl dimethylamines present very little risk to humans because there is only minimum potential for human exposure to these materials. There are several important factors which limit human exposure to alkyl dimethylamines.

Alkyl dimethylamines are predominately used as chemical intermediates to produce amine derivatives. Because of this, there is no broad scale human exposure to this class of materials and the only potential for human exposure resides in the workplace. Workplace exposure is limited by various manufacturing practices which are discussed below.

Modern manufacturing practices are designed to minimize the number of workers involved, and because of this there is only a small number of people potentially exposed to alkyl dimethylamines. For example, the Procter & Gamble Manufacturing Company manufactures approximately [] million pounds of alkyl dimethylamine annually. This manufacture takes place at only one site and involves a total of only [] employees. The Company also internally processes approximately [] million pounds of one alkyl dimethylamine it produces. There are only [] employees involved in this total operation. Our belief is that other U.S. operations involving alkyl dimethylamines would employ comparable numbers of workers. The number of people exposed to this class of material is, therefore, very limited.

In addition to there being only a very limited number of people exposed to alkyl dimethyl amines in the workplace, there are additional factors which control the levels of these amines to which these workers may be potentially exposed. Alkyl dimethylamines are manufactured in closed processing conditions and do not come into contact with workers under normal circumstances. Nonroutine tasks are performed following specified safe practices, including the use of appropriate protective equipment (gloves, impervious clothing, goggles). Inhalation is not a likely route of entry due to the low volatility of the materials.

The corrosive nature of alkyl dimethylamines necessitates that protective garments (safety gloves and glasses) be used in their handling. Thus, essentially no worker exposure is expected. Any worker exposure would likely be accidental under these working conditions. However, it is expected that any material accidentally contacting the skin or eyes would be quickly rinsed off, thus resulting in minimal exposure. Our workplace experience has shown no short-term or chronic effects associated with exposure to these materials other than irritation. Furthermore, most processing conditions used to react alkyl dimethylamines to produce amine derivatives require closed systems which further safeguard any human contact.

In summary, the C12 to C18 alkyl dimethylamines are considered to be safe under present conditions of industrial production and use. The likelihood of these materials to pose a risk to human health is essentially nonexistent with current limited exposure.

TOXICOLOGICAL STUDIES FOR ALKYL DIMETHYLAMINES

This summarizes the results of safety studies on commercial alkyl dimethylamines sponsored by the Procter & Gamble Company. Unless otherwise specified, the test materials used in the safety studies were commercial samples of the materials described by CAS #67700-98-5, 112-18-5, 112-75-4, 112-69-6, and 124-28-7 (C10-16 alkyl dimethylamines, dimethyldodecanamine, dimethyltetradecanamine, dimethylhexadecanamine, and dimethyloctadecanamine, respectively). Copies of the study reports are attached.

Overall Summary

Acute safety studies have been conducted with several commercial alkyl dimethylamines. Undiluted alkyl dimethylamines can be corrosive to the skin. In general, they produced severe skin and eye irritation under nonrinse conditions. No delayed hypersensitive reactions were noted with a 25% solution of alkyl dimethylamines in guinea pigs.

Acute Toxicity

Oral: (V1847-45, V1847-158) C10-C16 alkyl dimethylamines and C14-C18 alkyl dimethylamines exhibited oral LD50's of 1.1 and 1.0 g/Kg respectively in albino rats.

Ocular: (V1926-19, V2000-7, V2586-15) Undiluted samples of C10-C16 or tallow alkyl dimethylamine were placed into the conjunctival sac of one of the rabbit's eyes at a dose level of 100 mg/animal. The untreated eye served as a control. The eyes were examined for irritation at different time intervals, up to a maximum of 35 days, following treatment. Instillation of 100 mg of alkyl dimethylamine in the rabbit's eye produced severe eye irritation under nonrinse conditions. The severity of the response decreased with rinsing.

Dermal Irritation: (V1244-70, V1928-27) Undiluted samples of C10-C16 or tallow alkyl dimethylamines were applied to the clipped, intact and abraded skin of rabbits (closed patch) for 24 hours. The test sites were evaluated 30 minutes and 48 hours after patch removal. In addition, a study (V2236-165) with undiluted and diluted material was conducted. These alkyl dimethylamines produced severe to corrosive skin response in rabbits.

Undiluted samples of dimethyl-N-hexadecylamine were studied (Report 191-1221) under DOT test conditions in rabbits. The material was found to be corrosive under these experimental conditions.

Sensitization: (V1724-22), a 25% aqueous alkyl benzene sulfonate solution of C10-C16 alkyl dimethylamines was applied to the clipped backs of guinea pigs for 6 hours, once a week for 4 weeks. No sensitization was noted in this study. (V1724-34), a similar study with tallow dimethylamine was conducted and also showed no sensitization.

ENVIRONMENTAL FATE AND EFFECTS TESTING WITH TERTIARY AMINES

This document summarizes the available environmental fate and effects testing for alkyl dimethylamines, in particular, stearyl dimethylamine CAS# 124-28-7. Final reports discussing the results of these studies are attached for review. Environmental fate and effects data for CAS# 112-18-5, 112-69-6, 112-75-4, and 121-44-8 are not available. However, due to structural analogies and similar environmental chemistries of these substances relative to CAS# 124-28-7, similar environmental fate and effects are predicted for these related amines.

Usage and Environmental Fate

As discussed previously, tertiary amines (alkyl dimethylamines) are manufactured by P&G, and are used strictly as chemical intermediates for the production of amine derivatives. Therefore, only very limited fugitive amounts of these intermediates are expected to be released to the environment.

Biodegradation of CAS# 124-28-7

Screening level and definitive (radiolabeled) biodegradation studies (Attachment I) have demonstrated that CAS# 124-28-7 is effectively and rapidly mineralized in laboratory test systems. In a screening level study, unrealistically high (10 and 20 mg/l) test concentrations of CAS# 124-28-7 were required to monitor CO₂ production above background levels. Glucose (10 ppm) was used as a positive control. The mineralization for CAS# 124-28-7 was similar to or greater than those of glucose, demonstrating the ready biodegradability and lack of interference at these excess levels to the microflora. Biodegradability was confirmed using radiolabeled techniques and realistic trace level concentrations (0.2 and 2.0 mg/l). The indigenous microflora in sludge rapidly and extensively mineralized the CAS# 124-28-7. Greater than 80% of the amine was converted to ¹⁴CO₂ with a half-life of 17 to 20 hours. The residual ¹⁴C-activity was associated with the biomass or dissolved in solution. Additionally, the kinetics of mineralization and distribution of ¹⁴C-material for CAS# 124-28-7 were similar to those of readily degradable stearic acid, demonstrating the biodegradable nature of this amine. Since the shorter chain length amines (C12 - C16 amines) are mineralized by the same biochemical mechanisms (1), we would expect the extent and rate of degradation for these amines to be similar.

Toxicity of CAS# 124-28-7

The toxicity of CAS# 124-28-7 to sewage microflora, and fresh water and marine organisms are summarized in Attachment II. CAS# 124-28-7 is innocuous to activated sludge and digester sludge microflora even when tested at unrealistically high concentrations (10-1,000 ppm). These data support the speculation that CAS# 124-28-7 was not toxic to the microflora in the screening level biodegradation study where high test concentrations were used.

CAS# 124-28-7 is toxic to fresh water and marine species when tested in "clean" water laboratory medium and filtered sea water, respectively. However, when tested in more environmentally relevant systems such as natural waters containing sediments, the toxicity of CAS# 124-28-7 is reduced. For example, LC50 values for *Daphnia magna* and blue gill in natural river waters are approximately an order of magnitude higher than those in clean water systems. Although no actual physical/chemical laboratory tests are available to explain the mechanism of this toxicity reduction, sorption of the amine onto solids or complexation with anions or other organics in the natural water may be major factors in altering the speciation and reducing the toxicity of the amine.

Discussion

Alkyl dimethylamines (CAS #'s 124-28-7, 112-18-5, 112-69-6, 112-75-4 and 121-44-8) are used only as chemical intermediates by P&G. Therefore little, if any, of these intermediate amines are expected to be released to the environment. However, if these amines were released to the environment, the available fate data demonstrates that CAS# 124-28-7 should be effectively biodegraded during typical wastewater treatment processes. Any trace levels of the amine that would be subsequently released to surface waters from wastewater treatment facilities would be expected to continually biodegrade in natural surface waters. These fate data are supported by biodegradation mechanisms for amines (including primary, secondary, and tertiary amines) which are well characterized in the literature. Primary amines are most readily biodegradable, while biodegradation kinetics for higher amines may be influenced by branching and chain length effects (2). If branching occurs on the alkyl chain or if the chain length is increased, rates of biodegradation may be slower than without these changes. Based on the fate data for CAS# 124-28-7, this tertiary amine is effectively mineralized in lab systems and in batch activated sludge. Although the enzymes for amine degradation are common among a variety of microflora (1), biodegradation is also expected to be an effective removal mechanism by indigenous microflora in the environment. The rate and extent of biodegradation for the shorter chain length C12 to C16 alkyl dimethylamines are expected to be equal to or greater than the C18 (stearyl) dimethylamine.

Stearyl dimethylamine, if present in surface waters, is expected to be sorbed to sediment solids, or complexed with anions and natural organics commonly found in fresh or marine waters. The sorption and chemical complexation mechanisms are expected to reduce the toxicity of this amine. The toxicities of the shorter amines are expected to be similar to that of the C18 alkyl dimethylamine, and are also expected to be mitigated by physical/chemical and biological processes in the environment.

(1) Meikle R. W. 1972. Decomposition: Qualitative Relationships. In C. A. I. Gorms, ed. Organic Chemicals in the Environment. Vol. 1, 145-251.

CONCLUSIONS

Since alkyl dimethylamines (CAS #'s 124-28-7, 112-18-5, 112-69-6, 112-75-4 and 121-44-8) are used by Procter & Gamble in the production of amine derivatives only, no direct release of these materials to the environment is expected.

C10 through C18 alkyl dimethylamines, if released to the environment, are expected to be rapidly and extensively mineralized by indigenous microflora.

C10 through C18 alkyl dimethylamines are expected to be sorbed to solids or complexed to anionic materials commonly found in the environment and should not occur as free amines. The aquatic toxicity of sorbed or complexed alkyl dimethylamines (CAS #'s 124-28-7, 112-18-5, 112-69-6, 112-75-4 and 121-44-8) are reduced relative to the amine under realistic conditions in natural surface waters.

Attachment I

Biodegradability of Tertiary Amine
(CAS# 124-28-7)

<u>Type Study</u>	<u>Concentration</u> (mg/l)	<u>Length Incubation</u> (days)	<u>CO₂</u> (%)
<u>Screening Level</u>			
CAS# 124-28-7	10	40	118
	20	40	51
Glucose control	10	40	43

¹⁴C-Biodegradation - Definitive Study (7 day)

<u>Treatment</u>	<u>Conc.</u> (ppm)	<u>¹⁴CO₂</u> (%)	<u>k₁</u> (h ⁻¹)	<u>t_{1/2}</u> (hours)	<u>Mass Balance</u> (%)
CAS# 124-28-7	0.2	91.1	0.99	16.8	105.8
	2.0	79.4	0.85	19.6	96.0
Stearic acid (control)	0.2	61.3	0.93	17.9	116.9
	2.0	68.9	1.28	13.0	92.8

Attachment II

Toxicity of Tertiary Amines
(CAS# 124-28-7)Toxicity to Sewage Organisms

<u>Inoculum</u>	<u>Highest Conc.</u>	<u>Result</u>	<u>Conclusion</u>
Activated Sludge	10 ppm	No effect	Non-toxic
Digester Sludge	1,000 ppm	No-effect	Non-toxic

Toxicity to Aquatic Organisms (Lab Water Systems)

<u>Species</u>	<u>Result</u>
Blue gill	96hr-LC50 = 0.30 mg/l
<u>Daphnia magna</u>	48hr-LC50 = 0.042 mg/l
Algae	
<u>Selenastrum capricornutum</u> :	5 day algistatic 0.029 mg/l 5 day algicidal >0.032 mg/l
<u>Microcystis aeruginosa</u>	5 day algistatic 0.11 mg/l 5 day algicidal 0.16 mg/l

Toxicity to Aquatic Organisms (Natural River Waters)

Blue gill	96hr-LC50 = 2.1 mg/l
<u>Daphnia magna</u>	48hr-LC50 = 0.35 mg/l

Toxicity to Marine Organisms (Filtered Sea Water)

Sheepshead minnow	96hr-LC50 = 8.8 mg/l
Mysid shrimp	96hr-LC50 = 0.074 mg/l

ENVIRONMENTAL SAFETY TEST SUMMARY REVIEW

Test Material Name: (monotallow dimethyl amine)

Test Substance Identification Number: B0793.01 Suffix #: .01

Type of Study: CO₂ Production Test Report #: 85-0245-11

Name of Originator: Division:

Contains No. 2

Laboratory Involved: Mill Top

Date Report Written: 05/02/85 Date Received by Operations Section: 05/06/85

This report has been received and found in agreement with the Protocol and there appear to be no inaccuracies in the numerical data or written portions with the following exceptions:

Logistics Reviewer

Date 8/1/85

This report has been reviewed for scientific quality and is summarized with the following comments: Test results indicate that this material is biodegradable, but, due to operator error, they cannot be reliably quoted. This material was shown to be biodegradable using ¹⁴C radiolabeled sludge method

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Principal Investigator:

Date 8/1/85

Date 9/11/86

File approved for entry:

Date 9-10-86

Entered into:

Date 9-22-86 By _____

Microfilming Completed: Date _____

By _____

Copy returned to: _____

Date _____

TEST SUMMARY SHEET

A. Keywords _____

B. Text: (Note - tabulations must be limited to 71 characters in width)

Test results not summarized for data entry for the following reasons:
1) Glucose TCDs only reached 43.1 %;
2) Inconsistent % TCDs between test material concentrations;
See report.

C. Project Status Sheet Code No. R0793.01.01

D. Test Type: CO2P CO2 Production
KEYWORD

E. Test Material: ODRG

F. Title/Date/ESD File No. CO2 Production Test on R0793.01/5-2-85

G. Test Location: Hill Top H. Lab Project No.: RS-1245-11

I. Date: YY' MM' DD

K. Sponsor's Principal Invest. (sign) _____ (print) _____

M. TSIN: R0793.01

N. Date Work Done: 2/2/85 - 4/2/85

TEST SUBST. ICE CHARACTERIZATION REPORT (TICR)

Test Substance Identification Number (40-21): **20193-21**

Principal Investigator:

Product or Ingredients

Physical Description: White Solid/Semi solid Solubility: HK
 Recommended Storage Conditions: Room Temperature Expiration Date: 2 1 year
 Hazards (i.e. flammability, toxic gases): Non-Hazardous
 Dept. of Transportation Hazard Classification: Non-Hazardous CAS No. (a): 124-28-7

Formulation Composition (b)

Component (c)	Mol. Wt.	Nominal Level (X by Wt.)	Acceptable Range	Stock		Lot Number (RX-Ref.)
				Code No.	Supplier	
Di-Nonyl Stearyl Amine	297	100.00		HK		4059-S-666334

- (a) Include CAS number(s) for the three most major components of a formulation or for single chemical products. Footnote to the material with which the respective number is associated.
- (b) If information requested is not known, then the symbol HK will be entered.
- (c) Chemical names which are inconveniently long may be abbreviated in tables but should be listed in full in referenced footnotes. Non-chemical names, such as Tergitol 15-S-0 or Yellow Dye #10, may not be acceptable but should be provided with the responsible toxicologist. Nondefinitive identification (e.g. Arquad, DC-base) is not acceptable.

The above information provided by:

12/18/91
(Date)

The above information reviewed and accepted by:

Principal Investigator:

[Signature]

TEST SUBSTANCE CHARACTERIZATION REPORT (TSCR)

Side 2 of 2

Test Substance Identification Number (TSIN):

Analyzed Composition

<u>Date Submitted</u>	<u>Submitter Code</u>	<u>Analysis Code/Analysis</u>	<u>Estimated Value</u>	<u>Measured Value</u>	<u>Testing Laboratory</u>
-----------------------	-----------------------	-------------------------------	------------------------	-----------------------	---------------------------

Side 2

Analytical Information Verified By:

(Signature)

Date:

This test substance is suitable for animal (non-clinical) safety testing.

Principle Investigator:

(Signature)

Date:

This test substance is suitable for human (clinical) safety testing.

Principle Investigator:

(Signature)

Date:



Hill Top Research, Inc.

P.O. Box 42501, Cincinnati, Ohio 45242 (513) 831-3114

Ref.: 85-0245-11

Date: April 18, 1985

CO₂ PRODUCTION TEST ON B0793.01

For: The Procter and Gamble Company

PURPOSE

To determine the rate and extent of CO₂ production of the test material.

TEST MATERIAL

One sample of test material, identified as B0793.01 was received from The Procter & Gamble Company on January 29, 1985 for use in this study. The Sponsor provided the following information regarding the test material.

Color	Milky
Form	Liquid/Gel
Percent Active	100%
Solubility	Insoluble (Solubility of 5-10% in Isopropyl alcohol)
Theoretical TOC	0.81 mg TOC/mg active
TOC ₂	2.963 mg CO ₂ /mg active
Storage Conditions	Ambient
Expiration Date	In progress

B0793.01 is insoluble in water, therefore the test material was added directly to provide concentrations of 10 mg/l and 20 mg/l. On February 20, 1985, a 1000 mg active/l H₂O stock solution of glucose was prepared by adding 0.1012g of anhydrous dextrose to approximately 50 ml of deionized water and bringing to a final volume of 100 ml with deionized water.

The total organic carbon level of the test material stock solution (at 1000 mg active/l) was determined to be 0.400mg carbon/mg active. This analysis was performed on February 21, 1985, according to SOP 11-BIOL-20-0011A. The stock solution was refrigerated throughout the study.



STUDY CONTRIBUTORS

The following members of Hill Top Research, Inc. contributed to the conduct and reporting of this study:

<u>Name</u>	<u>Title</u>	<u>Function</u>
Bolly Beyeradoerfer	Technician, Biodegradation	Conduct of Study
Nina Re	Technician, Biodegradation	Conduct of Study
Jean Weingartner	Technician, Biodegradation	Conduct of Study
Gayla Apking	Technician, Biodegradation	Conduct of Study
Warren H. Pence, B.S.	Group Section Head, Biodegradation	Study Director & Reporting of Study

TEST SCHEDULE

The study was started on February 21, 1985, and finished on April 2, 1985.

PROCEDURE

A. Test Method:

The test was performed according to the protocol entitled CO₂ Production Test on B0793.01 which was provided by _____ Principal Investigator for the Sponsor. The protocol effective date was December 21, 1984.

The Investigator's Standard Operating Procedures which were followed include:

11-BIOL-20-0012A	Suspended Solids Analysis
11-BIOL-16-0160A	pH Analysis
11-BIOL-20-0011A	DOC/SOC Analysis
11-BIOL-20-0015A	DOC/SOC Sample Preparation and Centrifugation
11-BIOL-20-0017A	Procedure for Titration of 0.024 N Barium Hydroxide
11-BIOL-20-0018A	Operation of the Carbon Dioxide Evolution Test System
11-BIOL-20-0027A	Total Bacterial Count

Four 4-liter Erlenmeyer flasks were set up and prepared as follows for use in this test:

Flask #5 Designated as a Blank Control and received inoculum only.
 Flask #6 Designated as a Glucose Control and received 40 ml of a 1000 mg active/l glucose stock solution in order to achieve a final concentration of 20 mg/l glucose.
 Flask #7 Received 0.0421 grams of B0793.01 in order to achieve a final concentration of 20 mg/l.
 Flask #8 Received 0.0203 grams of B0793.01 in order to achieve a final concentration of 10 mg/l.

All flasks received a 1% acclimated inoculum (20 ml/flask) and the final volume of each flask, including the mineral salts medium, was 2000 ml.

B. Inoculum

The inoculum used in this test was obtained from two Semi-Continuous Activated Sludge Units, prepared as per protocol. The sludge was incrementally acclimated to the test material (4ppm - 20ppm) for five days, then maintained at 20 ppm for eight additional days. The units were also fed synthetic sewage and tap water during the acclimation period. SCAS units were fed and maintained according to SOP No. 11-BIOL-20-0014A. The inoculum contained an estimated count of 6.3×10^5 organisms/ml as determined by standard plate count.

C. Temperature Range

The temperature ranged from 20-23°C during the course of the study. On one day the temperature dropped to 16°C due to maintenance turning off the furnace. This deviation did not affect the integrity of the study.

MODIFICATIONS TO THE PROTOCOL

The study was extended to Test Day 40 due to additional CO₂ production in the test flasks beyond the standard 25 day test period.

RESULTS

The cumulative percent TCO₂ values for each test flask are presented in the Table of Results.

The results of final soluble organic carbon analysis are presented below:

<u>Flask Content</u>	<u>SOC-Value (mg/l)</u>
Blank	5.0
Glucose (20 mg/l)	3.2
B0793.01 (20 mg/l)	4.6
B0793.01 (mg/l)	2.5

The glucose control produced a below normal percent TCO₂ over the test period of 43.1%. The Study Director feels that this low value was probably caused by a technician error in adding only 20 ml of 1000 ppm glucose stock solution instead of 40 ml. It is the opinion of the Study Director that this occurrence does not invalidate the results of the biodegradability of B0793.01.

RETENTION OF DATA

Copies of all data pertaining to this study are retained in the archives under Reference No. 85-0245-11 according to SOP No. 11-BIOL-0001A.

REGULATORY COMPLIANCE AND REPORT APPROVAL

Statement of Compliance

This study was performed in compliance to EPA Good Laboratory Practices (40 CFR 792).

HILL TOP RESEARCH, INC.

Warren H. Pence
Warren H. Pence, B.S.
Study Director

5-2-85
Date

Approved for: HILL TOP RESEARCH, INC.

By: Warren H. Pence 5-2-85
Warren H. Pence, B.S. Date
Study Director
Biodegradation Section

By: Gayle K. Mulberry 5/2/85
Gayle K. Mulberry, M.S. Date
Director of Technical Services
Microbiological Services Division

By: L. Lea Johnston 5-2-85
L. Lea Johnston, M.A. Date
Director, Quality Assurance

CO2 PRODUCTION TEST
SUMMARY SHEET I

PROJECT NUMBER: 85-0215
SAMPLE IDENTIFICATION: B079

DATE	DAY	M. STP. HCl/100 ML Ba(OH)2			Ba(OH)2 CONTROL			CONVULATIVE MG CO2 BLANK CORRECTED			CONVULATIVE PERCENT TC02			CONVULATIVE MG CO2 PER LITER
		BLANK	GLUCOSE	LA*	10mg/L	20mg/L	40.0	44.0	GLUCOSE	10mg/L	20mg/L	GLUCOSE	10mg/L	
2/24	3	40.8	32.1	38.0	40.9	42.8	44.0	9.6	0.0	-0.1	16.4	0.0	0.0	1.8
2/26	6	41.9	38.6	38.0	42.8	44.0	44.0	13.2	4.3	-1.1	22.5	7.1	-0.8	3.0
3/1	8	41.8	37.6	37.5	39.3	43.6	43.6	17.8	9.0	1.7	30.3	14.9	1.4	4.0
3/5	12	41.9	38.0	37.8	34.4	43.5	43.5	22.1	13.5	10.0	37.6	22.4	8.0	4.9
3/12	18	41.2	38.9	31.1	15.5	43.8	43.8	24.6	24.6	38.3	42.1	40.8	30.6	6.4
3/15	22	41.5	41.3	29.2	32.2	44.5	44.5	24.8	48.1	48.5	42.4	63.2	38.8	8.1
3/19	26	42.1	41.7	33.5	36.4	43.4	43.4	25.2	57.6	54.8	43.1	79.6	43.8	8.8
3/25	32	39.9	40.3	30.6	36.2	43.8	43.8	24.1	67.8	58.9	41.2	95.9	47.1	11.0
3/29	36	40.7	41.5	35.1	39.6	43.6	43.6	23.2	74.0	60.1	39.7	106.2	48.1	12.6
4/1	39	41.0	39.2	36.7	40.2	42.4	42.4	25.2	78.7	61.0	43.1	114.0	48.8	13.4
4/2	40	42.2	42.2	40.0	40.2	42.8	42.8	25.2	81.1	63.2	43.1	118.0	50.6	13.8

*LA = Laboratory Accident: Sample contained H2O instead of Ba(OH)2, any CO2 that would have passed through the scrubber would have been captured by the second or third scrubber bottle.

Ref.: 85-0245-11

Date: April 18, 1985

STUDY REVIEW RECORD

REFERENCE: 85-0245-11
DATE OF STUDY INITIATION: February 8, 1985
DATE OF STUDY COMPLETION: April 2, 1985
STUDY DIRECTOR: Warren H. Pence, B.S.

DISTRIBUTION OF REMAINING TEST MATERIAL: (Check appropriate box)

Unused sample was returned to the sponsor on April 17, 1985.

LOCATION OF RAW DATA: Archives of Hill Top Research, Inc.
LOCATION OF FINAL REPORT: Archives of Hill Top Research, Inc.

QUALITY ASSURANCE UNIT STATEMENT

Date(s) Study Inspected: 2/21/85, 3/5/85, 4/1/85
Date Report Reviewed: 5/1/85, 5/11/85 ^{initial} 29.5.85
Date(s) Findings Reported to Management: 2/26/85, 3/11/85, 4/4/85
Date(s) Findings Reported to Study Director: 2/26/85, 3/11/85, 4/4/85

C. R. Woodman
Quality Assurance Auditor
C. R. Woodman

W. H. Pence
Quality Assurance Director

5-2-85

5-2-85



THE PROCTER & GAMBLE COMPANY

MIAMI VALLEY LABORATORIES

P. O. BOX 39175
CINCINNATI, OHIO 45247

January 24, 1985

Mr. James R. Agin
Mill Top Research, Inc.
Miamiville, OH 45147

Dear Mr. Agin:

This is to authorize you to carry out the following study according to the attached protocol, and in conformance with the stipulations of our current Laboratory Services Agreement.

Protocol: CO₂ Production Test on B0793.01

Date: 12/21/84

Sponsor's Principal Investigator:

Notice: The stipulations of this protocol are to be implemented in conformance with EPA Good Laboratory Practice Regulations (40 CFR, Part 792).

Please have the Study Director approve and complete both copies of the attached protocol by adding your study or project number, estimated starting and reporting dates, and date the test material was received. Retain one copy for your files and return the other to me.

Please return all unused portions of the test material to the Sponsor's Principal Investigator at the following address:

The Procter & Gamble Company
Sharon Woods Technical Center
11520 Reed Hartman Hwy.
Cincinnati, OH 45241

We understand the estimated cost for this study is \$1,180. This is based on your standard cost of \$950 for the CO₂ test, plus an additional charge of \$230 for the SCAS acclimation. Invoices are also to be sent to the Principal Investigator.

Matters involving the scientific aspects of the work can be handled directly with the Sponsor's Principal Investigator. Feel free to contact me at (513) 245-2120, if you have any other questions or concerns.

Sincerely,

THE PROCTER & GAMBLE COMPANY

Approved:

SUPPLEMENTAL INSTRUCTIONS

DATE: 4/4/85

PROJECT NO.: 85-0245-11
PAGE:

SUPERVISOR: WP

TYPE OF PROJECT

CO₂ Production Test
CLIENT: Procter & Gamble Co.
CHARGES AUTHORIZED BY:
EFFECT ON BUDGET: ADD \$310.00

LETTER OF:
SUBTRACT \$

VERBALLY ON: 4/1/85

SAMPLES AND DESCRIPTIONS	LOT NO.	DATE RECEIVED
B0793.01		See Sample Sheet

NEW INSTRUCTIONS:

Protocol Modification #1

Test was extended to test day 39 due to increased CO₂ Production from flasks containing test material. The test was terminated as per protocol under authorization from Principal Investigator.

APPROVED BY:

Warren H. Pence
Warren H. Pence, B.S.
Study Director

4-19-85
Date

5/3/85
Date

Principal Investigator

ACCOUNTING

Two weeks of additional testing at \$155.00/wk. = \$310.00

PREPARED BY: WP, JA, GKM TYPED BY: bms

1180
310
1490 ✓

PROTOCOLSPONSOR: The Procter & Gamble Company, Cincinnati, Ohio

HM Top Research, Inc.

LABORATORY: Cincinnati, OH 45242TITLE: CO₂ Production Test on B0793.01OBJECTIVE: To determine the rate and extent of the ultimate biodegradation of the test material.JUSTIFICATION FOR TEST SYSTEM: Most of our products are disposed of through wastewater treatment systems where microorganisms can biodegrade the organic product components. Since activated sludge is a common wastewater treatment process and contains a variety of microbial species, it has been chosen to provide the inoculum for this test.TEST MATERIAL:Sample Code B0793.01 Color Milky Form Liquid/Gel% Active 100% Density --- Solubility 3-10% in Isopropyl AlcoholExpiration Date in progress Other Sample melts & clarifies at 150°TCO₂ 2.963 mg CO₂/mg active Theoretical TOC 0.81 ^{as per instruction for 10%} mg TOC/mg active

Storage Conditions - Ambient.

Safe Handling Precautions - Avoid skin and eye contact - rinse with large amount of water. Material is "Not TOCA Chemical" and safety data has not been completed. SEE SECTION 5 ^{Strong pink color, prepare stock with care and of possible flammability as per instructions for 10%}

The Sponsor accepts full responsibility for appropriate characterization and stability verification of this test material.

TEST MATERIAL PREPARATION/ADDITION:

(check one and indicate test concentrations)

[] For compounds soluble or dispersible in water, prepare a stock solution or homogeneous suspension at 1,000 µg/L active ingredient by weight/volume dilution with ASTM Type II water or equivalent. Determine the total organic carbon (TOC) concentration in the stock solution following laboratory's standard operating procedures. If the measured TOC is not within 15% of theoretical, contact the Principal Investigator before initiating the study. Store the stock solution under refrigeration for a maximum of three days prior to test initiation. If greater than three days elapses, another stock solution should be prepared. At test initiation, determine the pH of an aliquot of the stock solution. If outside the range of 4.0-10.0, adjust the pH of the stock solution to 7.0 (+1.0) with HCl or NaOH. To avoid contaminating the stock solution, aliquots taken for pH measurements should be discarded. Add the appropriate volume of the stock solution to the respective flasks to obtain ___ and ___ µg active/L test concentrations.

¹Standard Specification for Reagent Water", ASTM Committee D-19 on Water, ASTM Designation D1193-74, June 27, 1974.

11125

[X] For compounds insoluble in water, add the appropriate amount of test material directly to the respective flasks on a [X] weight (analytical balance) or [] volume (microliter syringe) basis to obtain 10 and 20 mg active/L test concentrations. Initial pH determinations are not required.

GLUCOSE PREPARATION/ADDITION:

Prepare a stock solution of standard reagent-grade glucose at 1,000 mg/L in ASTM Type II water or equivalent. Determine the TOC concentration of the stock solution. The criteria for acceptable use and storage requirements are the same as those given for the preparation/addition of soluble compounds. Initial pH determinations are not required. Add 40 ml of the stock solution to the respective flask to obtain 20 mg/L glucose.

TEST ORGANISMS: (check one; for acclimated inoculum, indicate test concentration.)

- [X] Acclimated inoculum obtained on day 14 from SCAS units tested at 20 mg/L active ingredient. Increase active level incrementally over first five days to reach max. of 20 mg/l. *Add material by direct weighing 20 for instructions. 10/2/85*
- [] Unacclimated inoculum obtained from SCAS units that have not received any test material. These units should be maintained following laboratory's standard operating procedures.

The procedure for preparation of the inoculum is as follows:

Equal volumes of mixed liquor are collected from duplicate SCAS units and composited. The mixed liquor is then homogenized at room temperature for approximately two minutes at medium speed in a Waring blender or equivalent. This homogenized sample is poured into a beaker and allowed to settle for 15-30 minutes before the supernatant is carefully decanted. Carryover of sludge solids should be avoided since this may significantly increase background carbon and endogenous CO₂ production. Sufficient volumes of mixed liquor should be collected and treated at one time to provide enough inoculum for all flasks. Inoculum is used on the same day of preparation.

CO₂ Scrubbing Apparatus:

For a series of 12 flasks or less:

Five 1-liter plastic bottles filled with 700 ml 10 N NaOH

One 1-liter Erlenmeyer flask filled with 700 ml 0.024 N Ba(OH)₂ to serve as a CO₂ indicator trap

One empty 1-liter Erlenmeyer flask to prevent liquid carryover

These containers are connected in series with Tygon tubing to a pressurized air source (~5 psi) and air is sparged through the scrubbing solution at a constant rate. The flow rate is adjusted to insure that all test flasks are receiving CO₂-free air (see Test Procedure 7, page 3).

CO₂ PRODUCTION APPARATUS:

Erlenmeyer flasks are connected by tubing to the CO₂-free air source. Each flask is also connected by tubing to a series of three 4-oz. "French squares" to serve as Ba(OH)₂ traps. The flasks and traps are to be equipped with 2-hole rubber stoppers with solid plastic or glass tubing inserted to force the air through the test media and Ba(OH)₂ solutions.

TEST PROCEDURE

1. Testing is conducted in 4-L Erlenmeyer flasks. The final volume of medium + test material + inoculum in each flask is 2 liters.
2. The test medium is modified BOD water which contains, per liter of distilled water, the following standard BOD reagent solutions:
 - 1 ml of standard magnesium sulfate solution (Fisher #30-M-109 or equivalent)
 - 1 ml of standard calcium chloride solution (Fisher #30-C-10 or equivalent)
 - 2 ml of standard phosphate buffer (Fisher #30-P-341 or equivalent)
 - 4 ml of standard ferric chloride solution (Fisher #30-F-97 or equivalent)
 - 1 ml of a 4% (w/v) solution of $(\text{NH}_4)_2\text{SO}_4$.
3. Flasks containing the appropriate amount of test medium are aerated overnight with CO_2 -free air to purge the system of carbon dioxide.
4. After the aeration period, three CO_2 absorber bottles are filled with 100 ml of 0.024N $\text{Ba}(\text{OH})_2$ and connected in series to the exit air line of each flask. The $\text{Ba}(\text{OH})_2$ solution should be filtered through E&D 617 filter paper, or equivalent, before use. All bottles are to be filled from one $\text{Ba}(\text{OH})_2$ solution.
5. Test material is added to two of the four flasks to achieve the concentrations specified on page 1 or 2 under Test Material Preparation/Addition. The third flask receives no test material (blank) and the last receives glucose from the 1000 mg/L stock solution to achieve the final concentration of 20 mg/L.
6. Each flask receives a 1X inoculum (10 ml/L) of the activated sludge preparation. A bacterial plate count is performed on the sludge preparation following laboratory's standard operating procedures to ascertain viability of the test organisms. Plates are incubated at test temperature. The sludge preparation is thoroughly mixed before taking aliquots for inoculation and enumeration.
7. The test is started by aerating the headspace of each flask at 50-100 ml/min (2-4 bubbles/sec in the $\text{Ba}(\text{OH})_2$ traps). The CO_2 produced in each carboy reacts with the $\text{Ba}(\text{OH})_2$ and precipitates as BaCO_3 . The amount of CO_2 produced is determined by titrating the remaining $\text{Ba}(\text{OH})_2$ with 0.05N standardized HCl. Periodically, (every 2 or 3 days or before BaCO_3 is observed in the second trap) the CO_2 absorber nearest the flask is removed for titration.

The remaining two absorbers are each moved one place closer to the test flask, and a new absorber with 100 ml of 0.024N $\text{Ba}(\text{OH})_2$ is placed at the far end of the series. With every change of traps, an extra 100 ml of $\text{Ba}(\text{OH})_2$ solution is titrated to allow CO_2 production from the blank test flask (no test material) to be monitored. All respective absorbers within a test are titrated on the same day. All respective absorber bottles are to be filled from one $\text{Ba}(\text{OH})_2$ solution.
8. The test is conducted for 25 days. If CO_2 production in either the test flasks or the glucose flask does not reach a plateau by this time, contact the Principal Investigator before terminating the study. If CO_2 production in the glucose flask does not plateau at >70% of theoretical, the acceptability of the study should be discussed in the final report.

9. After CO_2 production reaches a plateau, the pH of each flask is measured and 1 ml of concentrated HCl is added to each flask to drive off inorganic carbonate. The flasks are aerated overnight and the final titration is made the following day.
10. Titrations of the $\text{Ba}(\text{OH})_2$ solution are made after removing the bottles closest to the flasks. The entire bottle is emptied into a 400 ml beaker and titrated to a phenolphthalein end point with standardized 0.05N HCl (Fisher #CS-126-1 or equivalent) from a 50 ml burett. Back titration with standard 0.05N NaOH (Fisher #Se-8-278 or equivalent) from a 5 ml burett is performed if over-titration occurs. The amount of base used in the back-titration is subtracted from the volume of acid titrated to get a corrected figure.
11. After acidification and overnight aeration, the amount of soluble organic carbon remaining in each flask is determined following laboratory's standard operating procedure.
12. Temperature must not fall below 20°C , exceed 28°C , or vary more than 4°C during the test period as measured in a flask containing 2 liters of water in close proximity to the test. Flasks should not be in direct sunlight and room lighting should only be on during daily maintenance. Flasks are agitated on a rotary platform shaker at 110 ± 10 rpm for the duration of the test.

CALCULATIONS - STATISTICAL ANALYSIS

1. Determine the amount of CO_2 produced by the test material and glucose for each day of titration by the following equation:

$$\text{mg CO}_2 = \text{ml titrant for blank trap} - \text{ml titrant for experimental trap} \times 1.1$$
2. Determine the cumulative mg CO_2 produced for both test flasks and the glucose at each titration day.
3. Determine the cumulative \bar{X} of theoretical CO_2 (TCO_2 given on page 1) for each titration day by the following equation:

$$\bar{X} \text{ TCO}_2 = \frac{\text{cumulative mg CO}_2 \text{ produced}}{(\text{mg material added}) (\text{TCO}_2)} \times 100$$

4. Computer analysis - The cumulative \bar{X} TCO_2 values obtained on the respective days are analyzed by a non-linear regression analysis on the form:

$$y = a(1 - e^{-b(x-c)}) \text{ for } x > c \text{ or } 0 \text{ for } x \leq c$$

where

- a = asymptote of curve ($\bar{X} \text{TCO}_2$)
- b = rate constant (day^{-1})
- c = lag time before CO_2 production occurs (day)
- x = days
- y = cumulative $\bar{X} \text{TCO}_2$

The constants a, b and c, along with their associated 95% confidence intervals are generated for each concentration of test material and for the glucose control. Computer plots of the cumulative $\bar{X} \text{TCO}_2$ vs. time data are also made for each test material concentration and the glucose control.

Labeling

The label shown below for AT-1280 is an example of the caution statement used for all Procter & Gamble alkyl dimethylamines.

P&G FATTY AMINES

AT-1280

C10-16 Alkyldimethylamine

DANGER: CAUSES CHEMICAL BURNS

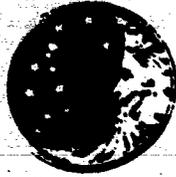
Do not get in eyes, on skin or clothing. Wash thoroughly after handling.

First Aid: Eyes - Immediately flush with plenty of water. Get medical attention.

Skin - Wash thoroughly with soap and water. Remove contaminated clothing. Wash before reuse. Discard contaminated shoes.

Made in U.S.A. by Procter & Gamble, Cincinnati, Ohio 45228

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PROCTER & GAMBLE
 INDUSTRIAL CHEMICALS DIVISION
 P.O. BOX 599, CINCINNATI, OHIO 45201

MATERIAL SAFETY DATA SHEET

SECTION I		ISSUE DATE: June 1, 1984
EMERGENCY TELEPHONE NUMBERS PROCTER & GAMBLE OPERATOR (513) 562-1100 (24 Hour Emergency Service)		
CHEMICAL FAMILY C10-16 Alkyldimethylamine	FORMULA $CH_3-(CH_2)_n-N \begin{matrix} CH_3 \\ \\ CH_3 \end{matrix}$	
CAS NO. 67700-98-5 (Amines, C10-16-alkyldimethyl)	n = 9 to 15	
TRADE NAMES AT-1280 ALKYLDIMETHYLAMINE	MOLECULAR WEIGHT 220 (Average)	

SECTION II PHYSICAL DATA			
BOILING POINT (°F) @ 760mm Hg	Greater Than 400°F	SPECIFIC GRAVITY (H ₂ O = 1)	0.79
VAPOR PRESSURE (mm Hg) 100°F	Less Than 1 mm Hg	PERCENT VOLATILE BY VOLUME (3)	Not Known
VAPOR DENSITY (AIR = 1)	Not Known	EVAPORATION RATE (Standard Conditions = 1)	Not Known
SOLUBILITY IN WATER	Very Slight		
APPEARANCE AND ODOR	Colorless to yellow liquid; Clear to slightly cloudy; Fishy odor.		

SECTION III FLAMMABILITY AND EXPLOSIVITY DATA			
FLASH POINT (METHOD USED)	235°F (FMCC)	FLAMMABLE LIMITS Not Applicable	EXPLOSIVE LIMITS (Lower) (Upper)
EXTINGUISHING MEDIA	Foam, CO ₂ , Dry Chemicals		Not Applicable
SPECIAL FIRE FIGHTING PROCEDURES	Protective Clothing		
UNUSUAL FIRE AND EXPLOSION HAZARDS	None		

SECTION IV HEALTH AND SAFETY DATA	
THRESHOLD LIMIT VALUE	Not Established
EFFECTS OF OVEREXPOSURE	Can cause severe eye or skin irritation. Prolonged exposure to eyes or skin can cause severe chemical burns.

EMERGENCY AND FIRST AID PROCEDURES
 FOR EYES, Rinse immediately with copious amounts of water and obtain medical attention. For skin, wash thoroughly with soap and water, then rinse with water. Immediately remove any contaminated clothing. Wash clothing before reuse. Contaminated shoes should be discarded.

Data supplied to be used only in connection with occupational safety and health.

SECTION V REACTIVITY DATA			
STABILITY	UNSTABLE		CONDITIONS TO AVOID
	STABLE	X	
INCOMPATIBILITY (Materials to avoid)			
HAZARDOUS DECOMPOSITION PRODUCTS			
HAZARDOUS POLYMERIZATION	MAY OCCUR		CONDITIONS TO AVOID
	WILL NOT OCCUR	X	

SECTION VI SPILL OR LEAK PROCEDURES	
STEPS TO BE TAKEN IN CASE MATERIAL IS RELEASED OR SPILLED	
Small Spills: Absorb on inert material and dispose	
Large Spills. Contain spill and remove for disposal	
Appropriate protective clothing should be used during cleanup	
WASTE DISPOSAL METHOD	
Dispose of as corrosive material waste by methods which are permitted by Federal, State and Local agencies	

SECTION VII SPECIAL PROTECTION INFORMATION		
RESPIRATORY PROTECTION (Specify type)		
Exposure to contaminated atmosphere—NIOSH/MSHA approved respirator for organic vapor		
VENTILATION	LOCAL EXHAUST	SPECIAL
	MECHANICAL (General)	OTHER
	Acceptable	
		EYE PROTECTION
		Goggles
PROTECTIVE GLOVES		
Rubber		
OTHER PROTECTIVE EQUIPMENT		
Boots, eye wash fountain, safety shower		

SECTION VIII SPECIAL PRECAUTIONS	
PRECAUTIONS TO BE TAKEN IN HANDLING AND STORING	
Avoid contact. Store in closed containers.	
OTHER PRECAUTIONS	
Product is a "Corrosive Material" to skin by DOT definition	

IMPORTANT NOTE: The technical product information and suggestions for use, while believed to be accurate and reliable, are given without guarantee or warranty of any kind, expressed or implied. Purchaser assumes all risk of acting on this information, or any advice or assistance that may be provided by Procter & Gamble representatives. Individual requirements vary and each purchaser is urged to perform their own tests, experiments and investigations in their use of Procter & Gamble products and for purposes of determining compliance with applicable Federal, State and local laws and regulations.

Nothing contained herein shall be construed as a recommendation to use any product in conflict with existing patents covering any material or use. Moreover, no license is to be implied under any Procter & Gamble patents relating to uses of the above-described chemicals other than



PROCTER & GAMBLE
INDUSTRIAL CHEMICALS DIVISION
P.O. BOX 500, CINCINNATI, OHIO 45201

MATERIAL SAFETY DATA SHEET

PROCTER & GAMBLE OPERATOR (613) 585-1100 (24 Hour Emergency Service)

SECTION I IDENTIFY		ISSUE DATE
Trade Name	AT-1214, AT-1214LT	11/86
Chemical Identity	1-Dodecanamine, N,N-dimethyl 1-Tetradecanamine, N,N-dimethyl	CAS Number 112-18-5 112-75-4

SECTION II PHYSICAL DATA			
BOILING POINT	@760 mm Hg	Greater than 400°F	SPECIFIC GRAVITY (4/4)
			@70°F/70°F
			0.3
VAPOR PRESSURE	100°F	Less than 1 mm Hg	PERCENT VOLATILE BY VOLUME (%)
			Not Known
VAPOR DENSITY (AIR=1)		Not Known	EVAPORATION RATE (HEXANE = 1)
			Not Known
SOLUBILITY IN WATER		Very Slight	
APPEARANCE AND ODOR		Colorless to yellow liquid, clear to slightly cloudy, fishy odor	

SECTION III FLAMMABILITY AND EXPLOSION DATA		
FLASH POINT (METHOD)	235°F (PMCC)	EXPLOSIVE LIMITS (LOWER) (UPPER)
		Not Applicable
EXTINGUISHING MEDIA	Foam, CO ₂ , Dry Chemicals	
SPECIAL FIRE FIGHTING PROCEDURES	Protective Clothing	
ORIGINAL FIRE AND EXPLOSION HAZARD	None	

SECTION IV HEALTH AND SAFETY DATA	
THRESHOLD LIMIT VALUE	Not Established
EFFECTS OF OVEREXPOSURE	Can cause severe eye or skin irritation. Prolonged exposure to eyes or skin can cause severe chemical burns
EMERGENCY AND FIRST AID PROCEDURES	For eyes, rinse immediately with copious amounts of water and obtain medical attention. For skin, wash thoroughly with soap and water, then rinse with water. Immediately remove contaminated clothing. Wash clothing before reuse. Contaminated shoes should be discarded.

SECTION V REACTIVITY DATA			
STABILITY	UNSTABLE		CONDITIONS TO AVOID
	STABLE	X	
INCOMPATIBILITY (Reactive to water) Copper and copper alloys			
HAZARDOUS DECOMPOSITION PRODUCTS Thermal decomposition to oxides of carbon and nitrogen			
HAZARDOUS POLYMERIZATION	MAY OCCUR		CONDITIONS TO AVOID
	WILL NOT OCCUR	X	

SECTION VI SPILL OR LEAK PROCEDURES	
STEPS TO BE TAKEN IN CASE MATERIAL IS RELEASED OR SPILLED	
Small Spills: Absorb on inert material and dispose	
Large Spills: Contain spill and remove for disposal	
Appropriate protective clothing should be used during cleanup.	
WASTE DISPOSAL METHOD	
Dispose of as corrosive material waste by methods which are permitted by Federal, State and Local agencies	

SECTION VII PROTECTION INFORMATION			
RESPIRATORY PROTECTION Exposure to contaminated atmosphere: Use NIOSH/MSHA approved organic vapor respirator			
VENTILATION	LOCAL EXHAUST	SPECIAL	
	Mechanical (General)	Acceptable	OTHER
EYE PROTECTION	Goggles	PROTECTIVE GLOVES	Rubber
OTHER PROTECTIVE EQUIPMENT Boots, eye wash fountain, safety shower, protective clothing			

SECTION VIII PRECAUTIONS	
HANDLING AND STORAGE	Avoid contact. Store in closed containers
LABELLING	DANGER: Causes chemical burns Do not get in eyes, on skin or on clothing Wash thoroughly after handling
OTHER PRECAUTIONS: Product is a "Corrosive Material" to skin by DOT definition	

The submission of this MSDS may be required by law but this is not an assertion that this substance is hazardous when used in accordance with proper safety practices and normal handling procedures.



PROCTER & GAMBLE
INDUSTRIAL CHEMICALS DIVISION
P.O. BOX 500, CINCINNATI, OHIO 45201

MATERIAL SAFETY DATA SHEET

PROCTER & GAMBLE OPERATOR (512) 686-1100 (24 Hour Emergency Service)

SECTION I IDENTIFY		REVISION DATE
Trade Name	AT-1295, AT-1295LT	11/86
Chemical Identity	1-Dodecanamine, N,N-dimethyl	CAS Number 112-18-5

SECTION II PHYSICAL DATA			
BOILING POINT @ 760 mm Hg	Greater than 400°F	SPECIFIC GRAVITY (d ₄ ²⁰) @ 70°F/70°F	0.79
VAPOR PRESSURE 100°F	Less than 1 mm Hg	PERCENT VOLATILE BY VOLUME (%)	Not Known
VAPOR DENSITY (AIR=1)	Not Known	EVAPORATION RATE (d ₄ ²⁰ = 1)	Not Known
SOLUBILITY IN WATER	Very Slight		
APPEARANCE AND ODOR	Colorless to yellow liquid; clear to slightly cloudy; fishy odor		

SECTION III FLAMMABILITY AND EXPLOSION DATA	
FLASH POINT (METHOD)	230°F (FMCC)
EXTINGUISHING MEDIA	Foam, CO ₂ , Dry Chemicals
SPECIAL FIRE FIGHTING PROCEDURES	Protective Clothing
ORIGINAL FIRE AND EXPLOSION HAZARDS	None

SECTION IV HEALTH AND SAFETY DATA	
THRESHOLD LIMIT VALUE	Not established
EFFECTS OF OVEREXPOSURE	Can cause severe eye or skin irritation. Prolonged exposure to eyes or skin can cause severe chemical burns.
EMERGENCY AND FIRST AID PROCEDURES	For eyes, rinse immediately with copious amounts of water and obtain medical attention. For skin, wash thoroughly with soap and water, then rinse with water. Immediately remove any contaminated clothing. Wash clothing before reuse. Contaminated shoes should be discarded.

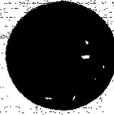
SECTION V REACTIVITY DATA			
STABILITY	UNSTABLE		CONDITIONS TO AVOID
	STABLE	X	
INCOMPATIBILITY (reacts to avoid) Copper and copper alloys			
HAZARDOUS DECOMPOSITION PRODUCTS Thermal decomposition to oxides of carbon and nitrogen			
HAZARDOUS POLYMERIZATION	MAY OCCUR		CONDITIONS TO AVOID
	WILL NOT OCCUR	X	

SECTION VI SPILL OR LEAK PROCEDURES	
STEPS TO BE TAKEN IN CASE MATERIAL IS RELEASED OR SPILLED	
Small Spills: Absorb on inert material and dispose	
Large Spills: Contain spill and remove for disposal	
Appropriate protective clothing should be used during cleanup	
WASTE DISPOSAL METHOD	
Dispose of as corrosive material waste by methods which are permitted by Federal, State and Local agencies	

SECTION VII PROTECTION INFORMATION			
RESPIRATORY PROTECTION Exposure to contaminated atmosphere: Use NIOSH/MSHA approved organic vapor respirator			
VENTILATION	LOCAL EXHAUST		SPECIAL
	MECHANICAL (Remote)	Acceptable	OTHER
EYE PROTECTION	Goggles	PROTECTIVE GLOVES	Rubber
OTHER PROTECTIVE EQUIPMENT Boots, eye wash fountain, safety shower, protective clothing			

SECTION VIII PRECAUTIONS	
HANDLING AND STORAGE	Avoid contact. Store in closed containers.
LABELLING	DANGER: Causes chemical burns Do not get in eyes, on skin or on clothing Wash thoroughly after handling
OTHER PRECAUTIONS	Product is a "Corrosive Material" to skin by DOT definition

The submission of this MSDS may be required by law but this is not an assertion that this substance is hazardous when used in accordance with proper safety practices and normal handling procedures.



PROCTER & GAMBLE
 INDUSTRIAL CHEMICALS DIVISION
 P.O. BOX 500, CINCINNATI, OHIO 45201

MATERIAL SAFETY DATA SHEET

PROCTER & GAMBLE OPERATOR (313) 588-1100 (24 Hour Emergency Service)

SECTION I IDENTITY		ISSUE DATE
Trade Name	AT-1495, AT-1495LT	11/86
Chemical Identity	1-tetradecanamine, N, N-dimethyl	CAS Number 112-75-4

SECTION II PHYSICAL DATA			
BOILING POINT @ 760 mm Hg	Greater than 400°F	SPECIFIC GRAVITY (d ₄ ²⁰) 70°F/70°F	0.80
VAPOR PRESSURE 100°F	Less Than 1 mm Hg	PERCENT VOLATILE BY VOLUME (%)	Not Known
VAPOR DENSITY (AIR=1)	Not Known	EVAPORATION RATE (NBP@20)	Not Known
SOLUBILITY IN WATER	Very Slight		
APPEARANCE AND ODOR	Colorless to yellow liquid; Clear to slightly cloudy; Fishy odor.		

SECTION III FLAMMABILITY AND EXPLOSION DATA	
FLASH POINT (METHOD)	270°F (FMCC)
EXTINGUISHING MEDIA	Foam, CO ₂ , Dry Chemicals
SPECIAL FIRE FIGHTING PROCEDURES	Protective Clothing
USUAL FIRE AND EXPLOSION HAZARDS	None

SECTION IV HEALTH AND SAFETY DATA	
THRESHOLD LIMIT VALUE	Not Established
EFFECTS OF OVEREXPOSURE	Can cause severe eye or skin irritation. Prolonged exposure to eyes or skin can cause severe chemical burns.
EMERGENCY AND FIRST AID PROCEDURES	For eyes, rinse immediately with copious amounts of water and obtain medical attention. For skin, wash thoroughly with soap and water, then rinse with water. Immediately remove any contaminated clothing. Wash clothing before reuse. Contaminated shoes should be discarded.

SECTION V REACTIVITY DATA

STABILITY	UNSTABLE		CONDITIONS TO AVOID
	STABLE		
INCOMPATIBILITY (Reactivity to water) Copper and copper alloys.			
HAZARDOUS DECOMPOSITION PRODUCTS Thermal decomposition to oxides of carbon and nitrogen.			
HAZARDOUS POLYMERIZATION	MAY OCCUR		CONDITIONS TO AVOID
	WILL NOT OCCUR	X	

SECTION VI SPILL OR LEAK PROCEDURES

STEPS TO BE TAKEN IN CASE MATERIAL IS RELEASED OR SPILLED

Small spills: Absorb on inert material and dispose.

Large spills: Contain spill and remove for disposal.

Appropriate protective clothing should be used during cleanup.

WASTE DISPOSAL METHOD

Dispose waste by methods which are permitted by Federal, State and Local agencies.

SECTION VII PROTECTION INFORMATION

RESPIRATORY PROTECTION
Exposure to contaminated atmosphere—NIOSH/MSHA approved respirator for organic vapor.

VENTILATION	LOCAL EXHAUST	SPECIAL
	MECHANICAL (hood)	OTHER

MECHANICAL (hood) **Acceptable**

EYE PROTECTION **Goggles** PROTECTIVE GLOVES **Rubber**

OTHER PROTECTIVE EQUIPMENT **Boots, eye wash fountain, safety shower**

SECTION VIII PRECAUTIONS

HANDLING AND STORAGE **Store in steel or glass lined vessels.**

LABELLING **DANGER: CAUSES CHEMICAL BURNS -- DO NOT GET IN EYES OR SKIN OR CLOTHING. WASH THOROUGHLY AFTER HANDLING.**

Product is a "Corrosive Material" to skin by DOT definition.

The submission of this MSDS may be required by law but this is not an assertion that this substance is hazardous when used in accordance with proper safety practices and normal handling procedures.



PROCTER & GAMBLE
INDUSTRIAL CHEMICALS DIVISION
P.O. BOX 800, CINCINNATI, OHIO 45201

MATERIAL SAFETY DATA SHEET

PROCTER & GAMBLE OPERATOR (612) 686-1100 (24 Hour Emergency Service)

SECTION I IDENTITY	
Trade Name	AT-1695A
Chemical Identity	1-Hexadecanamine, N,N-dimethyl
Issue Date	11/86
CAS Number	112-69-6

SECTION II PHYSICAL DATA				
Boiling Point	2760 mm Hg	626°F	Specific Gravity (d ₄ ²⁰)	0.8
Vapor Pressure	@100°F	Less than 0.1 mm Hg	Percent Volatile by Volume (%)	Not Known
Vapor Density (air=1)		Not Known	Evaporation Rate (air=1)	Not Known
Solubility in Water		Very Slight		
Appearance and Odor	Colorless to yellow liquid; fishy odor			

SECTION III FLAMMABILITY AND EXPLOSION DATA	
Flash Point (Method)	280°F FHCC
Explosive Limits (Lower)	Not Applicable
Extinguishing Media	Foam, CO ₂ , Dry Chemicals
Special Fire Fighting Precautions	Protective Clothing
Usual Fire and Explosion Hazards	None

SECTION IV HEALTH AND SAFETY DATA	
Threshold Limit Value	Not Established
Effects of Overexposure	Causes chemical burns to eyes and skin. May cause delayed skin burns.
Emergency and First Aid Procedures	For eyes, rinse immediately with copious amounts of water and obtain medical attention. For skin, wash thoroughly with soap and water, then rinse with water. Immediately remove any contaminated clothing. Wash clothing before reuse. Contaminated shoes should be discarded.

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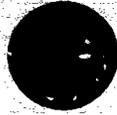
SECTION V REACTIVITY DATA			
STABILITY	UNSTABLE		CONDITIONS TO AVOID
	STABLE	X	
INCOMPATIBILITY (Materials to avoid) Copper and copper alloys			
HAZARDOUS DECOMPOSITION PRODUCTS Thermal decomposition to oxides of carbon and nitrogen			
HAZARDOUS POLYMERIZATION	MAY OCCUR		CONDITIONS TO AVOID
	WILL NOT OCCUR	X	

SECTION VI SPILL OR LEAK PROCEDURES	
STEPS TO BE TAKEN IN CASE MATERIAL IS RELEASED OR SPILLED	
Small Spills: Absorb on inert material and dispose.	
Large Spills: Contain spill and remove for disposal. Appropriate protective clothing should be used during cleanup.	
WASTE DISPOSAL METHOD	
Dispose waste by methods which are permitted by Federal, State, and Local agencies.	

SECTION VII PROTECTION INFORMATION		
RESPIRATORY PROTECTION	Exposure to contaminated atmosphere; use NIOSH/MSHA organic vapor respirator.	
VENTILATION	LOCAL EXHAUST	SPECIAL
	MECHANICAL (General)	OTHER
EYE PROTECTION	Goggles	PROTECTIVE GLOVES Rubber
OTHER PROTECTIVE EQUIPMENT	Boots, eye wash fountain, safety shower, protective clothing	

SECTION VIII PRECAUTIONS	
HANDLING AND STORAGE	Store in steel or glass lined vessels
LABELLING	DANGER: Causes Chemical Burns
	Do not get in eyes or skin or clothing. Wash thoroughly after handling.
	Product is a DOT "Corrosive Material" to skin.

The submission of this MSDS may be required by law but this is not an assertion that this substance is hazardous when used in accordance with proper safety practices and normal handling procedures.



PROCTER & GAMBLE
 INDUSTRIAL CHEMICALS DIVISION
 P.O. BOX 500, CINCINNATI, OHIO 45201

MATERIAL SAFETY DATA SHEET

PROCTER & GAMBLE OPERATOR (513) 685-1100 (24 Hour Emergency Service)

SECTION I IDENTITY

ISSUE DATE 11/86

Trade Name

AT-1695, AT-1695LT

Chemical Identity

CAS Number

1-Hexadecanamine, N,N-dimethyl

112-69-6

SECTION II PHYSICAL DATA

BOILING POINT	@760 mm Hg	626°F	SPECIFIC GRAVITY (4/20-1)	0.3
VAPOR PRESSURE	@100°F	Less than 0.1 mm Hg	PERCENT VOLATILE BY VOLUME (%)	Not Known
VAPOR DENSITY (AIR=1)		Not Known	EVAPORATION RATE (HEXANE=1)	Not Known
SOLUBILITY IN WATER		Very Slight		
APPEARANCE AND ODOR	Colorless to yellow liquid; fishy odor			

SECTION III FLAMMABILITY AND EXPLOSION DATA

FLASH POINT (METHOD)	280°F FMCC	EXPLOSION LIMITS (LOWER) (UPPER)	Not Applicable
EXTINGUISHING MEDIA	Foam, CO ₂ , Dry Chemicals		
SPECIAL FIRE FIGHTING PROCEDURES	Protective Clothing		
UNUSUAL FIRE AND EXPLOSION HAZARDS	None		

SECTION IV HEALTH AND SAFETY DATA

THRESHOLD LIMIT VALUE	Not Established
EFFECTS OF OVEREXPOSURE	Causes chemical burns to eyes and skin. May cause delayed skin burns.
EMERGENCY AND FIRST AID PROCEDURES	For eyes, rinse immediately with copious amounts of water and obtain medical attention. For skin, wash thoroughly with soap and water, then rinse with water. Immediately remove any contaminated clothing. Wash clothing before reuse. Contaminated shoes should be discarded.

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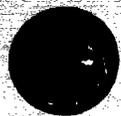
SECTION V REACTIVITY DATA			
STABILITY	UNSTABLE		CONDITIONS TO AVOID
	STABLE	X	
INCOMPATIBILITY (BASED ON OTHERS) Copper and copper alloys			
HAZARDOUS DECOMPOSITION PRODUCTS Thermal decomposition to oxides of carbon and nitrogen			
HAZARDOUS POLYMERIZATION	MAY OCCUR		CONDITIONS TO AVOID
	WILL NOT OCCUR	X	

SECTION VI SPILL OR LEAK PROCEDURES	
STEPS TO BE TAKEN IN CASE MATERIAL IS RELEASED OR SPILLED	
Small Spills: Absorb on inert material and dispose.	
Large Spills: Contain spill and remove for disposal. Appropriate protective clothing should be used during cleanup.	
WASTE DISPOSAL METHOD	
Dispose waste by methods which are permitted by Federal, State, and Local agencies.	

SECTION VII PROTECTION INFORMATION		
RESPIRATORY PROTECTION	Exposure to contaminated atmosphere; use NIOSH/MSHA organic vapor respirator.	
VENTILATION	LOCAL EXHAUST	SPECIAL
	Acceptable	OTHER
EYE PROTECTION	Goggles	PROTECTIVE GLOVES Rubber
OTHER PROTECTIVE EQUIPMENT	Boots, eye wash fountain, safety shower, protective clothing	

SECTION VIII PRECAUTIONS	
HANDLING AND STORAGE	Store in steel or glass lined vessels
LABELLING	DANGER: Causes Chemical Burns
	Do not get in eyes or skin or clothing. Wash thoroughly after handling.
	PRODUCT IS A DOT "CORROSIVE MATERIAL" TO SKIN.

The submission of this MSDS may be required by law but this is not an assertion that this substance is hazardous when used in accordance with proper safety practices and normal handling procedures.



PROCTER & GAMBLE
INDUSTRIAL CHEMICALS DIVISION
P.O. BOX 599, CINCINNATI, OHIO 45201

MATERIAL SAFETY DATA SHEET

PROCTER & GAMBLE OPERATOR (612) 622-1100 (24 Hour Emergency Service)

SECTION I IDENTITY	
Trade Name	AT-1895A
Issue Date	11/86
Chemical Identity	CAS Number
1-Octadecanamine, N,N-dimethyl	124-28-7

SECTION II PHYSICAL DATA			
Boiling Point	656°F	Specific Gravity (d ₄ ²⁰)	0.8
2760 mm Hg		Percent Volatile by Volume (%)	Not Known
Vapor Pressure	Less than 0.1 mm Hg	Evaporation Rate (d ₄ ²⁰ = 1)	Not Known
2100°F			
Vapor Density (air = 1)	Not Known		
Solubility in Water	Very Slight		
Appearance and Odor	Colorless to yellow liquid; fishy odor		

SECTION III FLAMMABILITY AND EXPLOSION DATA	
Flash Point (Method)	Explosive Limits
311°F FNCC	Lower Upper Not Applicable
Extinguishing Media	
Foam, CO ₂ , Dry Chemicals	
Special Fire Fighting Procedures	
Protective Clothing	
General Fire and Explosion Hazards	
None	

SECTION IV HEALTH AND SAFETY DATA	
Threshold Limit Value	Not Established
Effects of Overexposure	Causes chemical burns to eyes and skin. May cause delayed skin burns.
Emergency and First Aid Procedures	For eyes, rinse immediately with copious amounts of water and obtain medical attention. For skin, wash thoroughly with soap and water, then rinse with water. Immediately remove any contaminated clothing. Wash clothing before reuse. Contaminated shoes should be discarded.

SECTION V REACTIVITY DATA			
STABILITY	UNSTABLE		CONDITIONS TO AVOID
	STABLE	X	
INCOMPATIBILITY (REAGENTS TO AVOID) Copper and copper alloys			
HAZARDOUS DECOMPOSITION PRODUCTS Thermal decomposition to oxides of carbon and nitrogen			
HAZARDOUS POLYMERIZATION	MAY OCCUR		CONDITIONS TO AVOID
	WILL NOT OCCUR	X	

SECTION VI SPILL OR LEAK PROCEDURES	
STEPS TO BE TAKEN IN CASE MATERIAL IS RELEASED OR SPILLED	
Small Spills: Absorb on inert material and dispose.	
Large Spills: Contain spill and remove for disposal. Appropriate protective clothing should be used during cleanup.	
WASTE DISPOSAL METHOD	
Dispose waste by methods which are permitted by Federal, State, and Local agencies.	

SECTION VII PROTECTION INFORMATION		
RESPIRATORY PROTECTION	Exposure to contaminated atmosphere: Use NIOSH/MSHA organic vapor RESPIRATOR.	
VENTILATION	LOCAL EXHAUST	SPECIAL
	MECHANICAL (Remote)	OTHER
	Acceptable	
EYE PROTECTION	Goggles	PROTECTIVE GLOVES Rubber
OTHER PROTECTIVE EQUIPMENT	Boots, eye wash fountain, safety shower, protective clothing	

SECTION VIII PRECAUTIONS	
HANDLING AND STORAGE	Store in steel or glass lined vessels
LABELLING	DANGER: Causes Chemical Burns
	Do not get in eyes or skin or clothing. Wash thoroughly after handling.
	Product is a DOT "Corrosive Material" to skin.

The submission of this MSDS may be required by law but this is not an assertion that this substance is hazardous when used in accordance with proper safety practices and normal handling procedures.

Procter & Gamble Amine No. 42 is identified as Amines, C10-16 Alkyldimethyl(CAS# 67700-98-5) under current TSCA nomenclature. It is also known by the following names:

AT-1280
C12-C14 Dimethylamine

Contains No. 42

Typical composition of Amines, C10-16 Alkyldimethyl(CAS# 67700-98-5) is:

Before 1970:

C12 Dimethylamine	66%
C14 Dimethylamine	22
C16 Dimethylamine	12

After 1970

C10 Dimethylamine	0.5%
C12 Dimethylamine	80
C14 Dimethylamine	16
C16 Dimethylamine	3.5

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RESEARCH AND DEVELOPMENT DEPARTMENT MEMORANDUM
Research Division

V. R. King

December 8, 1967

Rabbit Skin Irritation - Closed Patch Study
(V2235-165)
(MS 7133 & 7134)

New Zealand Rabbits were subjected to a 24-hour closed patch irritation study. Group treatment information is contained in the following table.

Group	Test Material	Concentration	Number of Animals	
			Abraded	Non-abraded
A	97% Active	Undiluted	3	3
	97% Active	30%	3	3
	97% Active	3%	3	3
B	97% Active	Undiluted	3	3
	97% Active	30%	3	3
	97% Active	3%	3	3

One-third of the animals were sacrificed 48 hours after the patches were removed, and skin sections were taken for histological examination. The remaining animals were sacrificed and skin sections were taken when the treated areas appeared grossly to be healed. Time intervals for apparent healing ranged from three to five weeks.

Microscopic irritation observed 48 hours after patch removal consisted chiefly of necrotic sloughing of the epidermis, accompanied by a massive infiltration of acute inflammatory cells. Extensive pustulation was also noted, but this may have been a secondary response resulting from bacterial infection. The inflammatory response extended very deeply into the dermal layer, especially in the severely affected animals. Abrasion of the skin did not appreciably alter the extent of damage.

The type of damage observed microscopically after apparent healing had taken place was of a different nature. A thick layer of what appeared to be young fibrous tissue was found beneath the epidermis. This corresponded both in position and thickness to the acute inflammatory cell infiltration noted after 48 hours. Acute inflammation of this tissue was not uncommon, and frequently extended deeply into the dermis. Biopsy was also noted in the deep dermal layer, and in many animals hair follicles were either missing completely, or badly damaged.

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Results of the microscopic examination are summarized in the following table.

<u>Group</u>	<u>Concentration</u>	<u>Irritation - 48 Hours After Treatment (Numerical)*</u>		<u>Damage - After Apparent Healing (Numerical)*</u>	
A	Undiluted	Severe	(4)	Severe	(4)
	30%	Severe	(4)	Severe	(4)
	3%	Severe	(4)	Mod.-Severe	(3)
B	Undiluted	Severe	(4)	Severe	(4)
	30%	Severe	(4)	Severe	(4)
	3%	Severe	(4)	Mod.-Severe	(3)

* Numerical rating based on a 0-4 scale. 0 represents normal skin, while 4 represents extensive and severe irritation.

W. R. King

Copies To: MWL Library File
J. E. Weaver

Subjects
Rabbit Skin Irritation - Closed Patch Study

Product Identification

See attached sheet.

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Product Identification (V236-165)

(Amine No. 42) ca 97% active (X30-913, UBX-3582)



where:

66% C₁₂

25% C₁₄

12% C₁₆

(ca 97% active) (X30-915, UBX-3584)

(8678) (X30-917, UBX-3598)

Procter & Gamble Amine No. 42 is identified as Amines, C10-16 Alkyldimethyl(CAS# 67700-98-5) under current TSCA nomenclature. It is also known by the following names:

AT-1280
C12-C14 Dimethylamine

Typical composition of Amines, C10-16 Alkyldimethyl(CAS# 67700-98-5) is:

Before 1970:

C12 Dimethylamine	66%
C14 Dimethylamine	22
C16 Dimethylamine	12

Contains No CBI

After 1970

C10 Dimethylamine	0.5%
C12 Dimethylamine	80
C14 Dimethylamine	16
C16 Dimethylamine	3.5

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RESEARCH AND DEVELOPMENT DEPARTMENT MEMORANDUM
Research Division

G. Nixon

October 9, 1967

Rabbit Skin Irritation (#V2236-165)
(RIS 913 & 915)

Material(s) Tested

Reasons for Testing Mildness evaluation of these materials has been requested.

<u>Test Material</u>	Mean Score (<u>3 Rabbits/Sample</u>)				<u>Primary Irritation Index**</u>
	<u>3 Abraded Sites*</u>		<u>3 Intact Sites*</u>		
	<u>Hyth.</u>	<u>Mean</u>	<u>Hyth.</u>	<u>Mean</u>	
Undiluted (Amino) Sample Basis (XSB-913, UBX-3582)	4.0	4.0	4.0	4.0	8.0,+
30% (Amino) Sample Basis (XSB-913, UBX-3582)	4.0	4.0	4.0	4.0	8.0,+
3% (Amino) Sample Basis (XSB-913, UBX-3582)	4.0	4.0	4.0	4.0	8.0,0
Undiluted (Amino) Sample Basis (XSB-915, UBX-3584)	4.0	4.0	4.0	4.0	8.0,+
30% (Amino) Sample Basis (XSB-915, UBX-3584)	4.0	4.0	4.0	4.0	8.0,+
3% (Amino) Sample Basis (XSB-915, UBX-3584)	4.0	4.0	4.0	4.0	8.0,+

* Average of 24 and 72 hour scores.
 ** 1-2 = Mild; 3-5 = Moderate; 5 or above = Severe
 + = Necrosis
 0 = Necrosis on two animals only.

0051

Remarks All of the above test solutions elicited maximum primary irritation scores, with necrosis occurring in all groups. At 72 hours, one animal from each test group was sacrificed and skin samples were taken from the patch areas for histologic examination. Skin sections from the remaining animals will be taken after healing has occurred.

G. Winca

Copies To: NPL Library File
J. E. Weaver

Subjects
Rabbit Skin Irritation

Product Identification

(Amine No. 42) ca 97% active
(XEB-913, UEM-3582)

(ca 97% active)
(XEB-915, UEM-3584)

Surfactants
Granular Detergents

2 - 2
- 3
- 3

where: 2 - 66% C₁₂
2 - C₁₄
1 - C₁₆

Procter & Gamble Amine No. 42 is identified as Amines, C10-16 Alkyldimethyl(CAS# 67700-98-5) under current TSCA nomenclature. It is also known by the following names:

AT-1280
C12-C14 Dimethylamine

Contains No. 42

Typical composition of Amines, C10-16 Alkyldimethyl(CAS# 67700-98-5) is:

Before 1970:

C12 Dimethylamine	66%
C14 Dimethylamine	22
C16 Dimethylamine	12

After 1970

C10 Dimethylamine	0.5%
C12 Dimethylamine	80
C14 Dimethylamine	16
C16 Dimethylamine	3.5

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RESEARCH AND DEVELOPMENT DEPARTMENT MEMORANDUM
Research Division

E. A. Neumann

November 15, 1967

Eye Irritation (# V286-15)
(MS #733)

Material(s) Tested

Reasons for Testing Safety evaluation requested prior to further development.

<u>Test Material</u>	<u>Conc. (%) & Treatment*</u>	<u>MAG**</u>	<u>Cornaeas Involved</u>	<u>Eyes Normal in Indicated No. of Days</u>
(Amine #42) ca 97% active (XU-913, UX-3982)	Undiluted (NR)	32.6	3/3	2 in 14; 1 not in 35
	Undiluted (R)	6.0	0/3	1 in 2; 2 in 14
	Undiluted (NR)	19.0	1/3	3 in 14
ca 97% active (XU-915, UX-3984)	Undiluted (R)	11.3	0/3	1 in 7; 2 in 14

* (NR) No Rinse (R) Rinse (MAG) Max. Avg. Score ** Not Related to Treatment

Remarks (NR) produced diffuse to discrete areas of corneal translucency and severe conjunctivitis that persisted in one eye after 35 days. (NR) produced diffuse areas of corneal translucency and/or severe conjunctivitis that persisted in one eye for 14 days. Rinsing reduced the ocular involvement so that the damage was limited to moderate to severe conjunctivitis that cleared within 14 days.

Copies To: NPL Library File
J. E. Weaver

E. A. Neumann

Subjects
Eye Irritation

0054

MVP

Eye Irritation Scores of Individual Rabbits

Test Material	Treatment	No.	1 Hr.	1 Day	2 Days	3 Days	4 Days	7 Days	14 Days	21 Days	28 Days	35 Days
(Amino #42) ca 97% active	Undiluted	192	14	6	6	12	8	8	0	0	0	0
	(M)	193	30ci	6	6	10	10	6	0	0	0	0
		194	37ci	37ci	37ci	61ci	64ci	60ci	63ci	63ci	63ci	62ci
ca 97% active	Undiluted	206	6	14	6	12	10	8	0	0	0	0
	(M)	196	6	2	0	2	2	8	0	0	0	0
		197	6	2	2	2	2	8	0	0	0	0
ca 97% active	Undiluted	243	14	33ci	31ci	19c	14	12	0	0	0	0
	(M)	244	12	8	8	8	8	8	0	0	0	0
		245	10	12	10	10	10	6	0	0	0	0
ca 97% active	Undiluted	246	8	12	14	8	10	10	0	0	0	0
	(M)	247	8	12	8	6	8	6	0	0	0	0
		249	10	10	8	6	4	0	0	0	0	0

c = Corneal Involvement.
 i = Transient, slight deepening of the folds or rugae.
 f = Iritis
 * = Penicils

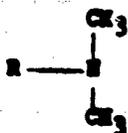
Product Identification

See attached sheet.

14 15 15 15

Product Identification (V506-15)

(Amine #2) ca 97% active (200-913, URE-3702)



where:

R = 66% C₁₂

28% C₁₄

12% C₁₆

Procter & Gamble Amine No. 42 is identified as Amines, C10-16 Alkyldimethyl(CAS# 67700-98-5) under current TSCA nomenclature. It is also known by the following names:

AT-1280
C12-C14 Dimethylamine

Typical composition of Amines, C10-16 Alkyldimethyl(CAS# 67700-98-5) is:

Before 1970:

C12 Dimethylamine	66%
C14 Dimethylamine	22
C16 Dimethylamine	12

After 1970

C10 Dimethylamine	0.5%
C12 Dimethylamine	80
C14 Dimethylamine	16
C16 Dimethylamine	3.5

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RESEARCH AND DEVELOPMENT DEPARTMENT MEMORANDUM
Research Division

S. V. Bushler

May 1, 1964

Guinea Pig Sensitization with Alkyl Amines

(V1723-22)

Alkyl dichlorohydrin amines (DCPAA) show initial promise as durable textile softeners or antistatic agents. The alkyl glycidyl amines (GMA) are likely to be formed in situ during the application of alkyl dichlorohydrin amines to textiles. Safety data was requested on these materials prior to laboratory evaluation of their efficacy.

Freester & Gamble Amine No. 42 is a tertiary amine, and this class of amines is known to cause skin burns. This particular amine is ready for test marketing.

A standard guinea pig closed patch procedure was performed. The first application of undiluted materials, however, produced sufficient irritation that subsequent patching was done with 50% solutions, except for the tertiary amine which was tested at 25%. In addition patching was delayed because of the extensive irritation so that a total of 5 patches of CE-GMA, CE-DCPAA, and Tallon-DCPAA, and 4 patches of the tertiary amine were applied at weekly intervals.

The results at challenge are indicated below:

<u>Test Material</u>	<u>No. Positive/No. Tested</u>
50% CE-GMA	0/10
50% CE-DCPAA	10/10
50% Tallon-DCPAA	10/10
25% F&G Amine No. 42	0/10

Some primary irritation was seen at challenge with all of the materials, but the increase in intensity of the reactions to the alkyl dichlorohydrin amines was sufficient to implicate them as sensitizers.

Additional experiments at lower concentrations (5%) are planned in an attempt to establish threshold values.

This work was done as requested on B73 Nos. 297, 313, and 314.

S. V. Bushler

jdk

Copies To: L. W. Beck; NYL Library File
J. P. Griffith

1972-72

Subjects

Human Pig Sensitisation
Alkyl Dichlorohydrin Amine
Alkyl glycidyl Amine
Tertiary Amine
CN-DGA
CN-DCPAA
Tallow-DCPAA
PAG Amine No. 42

Product Identification

UDK-1520 - Alkyl bis-(3-chloro-2-hydroxy-
propyl amine)(CN-DCPAA)
(XMG-670)
UDK-1524 - Alkyl bis-(3-chloro-2-hydroxy-
propyl amine)(Tallow-DCPAA)
(XMG-683)
UDK-1521 - Coconut bis-(2,3 epoxy propyl)
amine (CN-DGA)(XMG-671)
UDK-1597 - PAG Amine No. 42 (XMG-813)
(XMG-619)
C₁₂, C₁₄ dimethylamine,
53305

Procter & Gamble Amine No. 42 is identified as Amines, C10-16 Alkyldimethyl(CAS# 67700-98-5) under current TSCA nomenclature. It is also known by the following names:

AT-1280
C12-C14 Dimethylamine

Typical composition of Amines, C10-16 Alkyldimethyl(CAS# 67700-98-5) is:

Before 1970:

C12 Dimethylamine	66%
C14 Dimethylamine	22
C16 Dimethylamine	12

After 1970

C10 Dimethylamine	0.5%
C12 Dimethylamine	80
C14 Dimethylamine	16
C16 Dimethylamine	3.5

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RESEARCH AND DEVELOPMENT DEPARTMENT MEMORANDUM
Research Division

E. A. Newmann

March 4, 1966

Rabbit Skin Irritation (# VL200-70)

Material(s) Tested P80 Amine No. 42.

Reasons for Testing Safety evaluation requested prior to test marketing (STS 4297).

<u>Test Material</u>	<u>Intact</u>	<u>Abraded</u>	<u>Primary Irritation Index*</u>
P80 Amine No. 42 (100%) (IND-619, UIR-1597)	6.1	7.8	6.95

* 0-2 = mild; 2-5 = moderate; 6 or above = severe

Remarks P80 Amine No. 42 produced rather severe skin damage.

Copies To: L. J. Beck; MVL Library File
J. P. Griffith

E. A. Newmann

Subjects
Rabbit skin irritation
P80 Amine No. 42

Product Identification
P80 Amine No. 42 (IND-619)

53305 C₁₂, C₁₄ dimethylamine.

Procter & Gamble Amine No. 42 is identified as Amines, C10-16 Alkyldimethyl(CAS# 67700-98-5) under current TSCA nomenclature. It is also known by the following names:

AT-1280
C12-C14 Dimethylamine

Typical composition of Amines, C10-16 Alkyldimethyl(CAS# 67700-98-5) is:

Before 1970:

Contains No. 42

C12 Dimethylamine	66%
C14 Dimethylamine	22
C16 Dimethylamine	12

After 1970

C10 Dimethylamine	0.5%
C12 Dimethylamine	80
C14 Dimethylamine	16
C16 Dimethylamine	3.5

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RESEARCH AND DEVELOPMENT DEPARTMENT MEMORANDUM
Research Division

E. A. Humann

March 25, 1960

Eye Irritation (S V1926-19)

Material(s) Tested P40 Amino No. 42.

Reasons for Testing Safety evaluation requested prior to test marketing (STS #397).

<u>Test Material</u>	<u>Concn. (%) & Treatment*</u>	<u>MMS**</u>	<u>Cornaeas Involved</u>	<u>Eyes Normal in Indicated No. of Days</u>
P40 Amino No. 42 (XIB-619, XIB-1977)	100% (NR)	59.3	3/3	3 in 14
	100% (R)	16.6	0/3	1 in 2; 2 in 9

* (NR) No Rinse (R) Rinse (MMS) Max. Avg. Score ** Not Related to Treatment

Remarks Catalyst P40 Amino No. 42 produced diffuse to discrete areas of corneal trans-
lucency which cleared within fourteen days. There was a rather severe inflammatory
response of the palpebral mucosa which produced scar tissue and could not be scored.
Rinsing greatly reduced the eye irritancy so that only slight to moderate conjunctivitis
was produced.

Copies To: L. W. Beck; NVL Library File
J. F. Griffith

E. A. Humann

Subjects
Eye Irritation
P40 Amino No. 42

10069

Eye Irritation Scores of Individual Rabbits

Test Material	Product No.	1 Eye	2 Eyes	3 Days	4 Days	7 Days	10 Days
10065 (M)	169	70	39	41	42	400	000
	168	60	20	20	20	0	000
	167	60	30	10	10	10	000
10066 (R)	170	6	0	0	0	10	0
	171	6	6	0	0	10	0
	172	10	10	10	10	6	0

* Scores on palpebral mucosa which could not be scored.

Product Identification

MS Amine No. 42 (M-613)

Case A, C₁, C₂, C₃ directly/Amine, 53395

0 0 6 4

Procter & Gamble Amine No. 42 is identified as Amines, C10-16 Alkyldimethyl(CAS# 67700-98-5) under current TSCA nomenclature. It is also known by the following names:

AT-1280

C12-C14 Dimethylamine

Typical composition of Amines, C10-16 Alkyldimethyl(CAS# 67700-98-5) is:

Before 1970:

C12 Dimethylamine	66%
C14 Dimethylamine	22
C16 Dimethylamine	12

Contains No CBI

After 1970

C10 Dimethylamine	0.5%
C12 Dimethylamine	80
C14 Dimethylamine	16
C16 Dimethylamine	3.5

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RESEARCH AND DEVELOPMENT DIVISION
Research Division

E. V. Dushler

March 2, 1967

Acute Oral Toxicity (9 VLS-45)
(MS 507)

Material (s) Tested F&D Amine No. 42.

Species: Charles River CD Rat (200-300 gm).

<u>Test Material</u>	<u>Conc.</u>	<u>Dose Level</u>	<u>Dead/Total</u>	<u>LD₅₀</u>	<u>Conf. Limits</u>
F&D Amine No. 42 (KMS-619, UMR-1977)	100%	0.95 ml/kg	2/10	1.11 ml/kg	1.12-1.78
		1.35 ml/kg	5/10		
		1.85 ml/kg	7/10		
		2.60 ml/kg	10/10		

Remarks The acute oral LD₅₀ values of other amine derivatives range from 1.30-1.60
g/kg.

Copies To: L. W. Beck; NPL Library File
J. P. Griffith

E. V. Dushler

Subjects
Acute Oral Toxicity
F&D Amine No. 42

Product Identification

F&D Amine No. 42 (KMS-619)
C₁₂, C₁₄ dimethyl-
amine, 3195

KV #210

Procter & Gamble's Dimethyl-N-Hexadecylamine(J0207.01) is identified as 1-Hexadecanamine, N, N-dimethyl (C18H39N) (CAS# 112-69-6) under current TSCA nomenclature. It is also known by the following names:

Hexadecyldimethylamine
Cetyldimethylamine
Dimethylcetylamine
AT-1695

Contains No. 001

Typical composition is:

C14 Dimethylamine	0.1 %
C16 Dimethylamine	97.0
C18 Dimethylamine	2.7
non-amine material	0.2

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BIOLOGICAL SAFETY TEST SUMMARY REVIEW

Test Material: Dimethyl-N-Hexadecylamine

Corp. H&ES Sample Code No.: J0207.01

Contract Laboratory: IR&DC

Type of Study: Skin Corrosivity (DOT Procedure with Options)

Report No: 191-1221

Requester: H. F. Epp Division: IB Authorization #: IBTS 150

Date Report Written: 03-24-86 Date Rec'd by Operations Section: 03-31-86

This report has been reviewed and found in agreement with the Protocol and there appear to be no inaccuracies in the numerical data or written portions with the following exceptions:

Deviation from protocol is included in the report.

H&ES Operations Section Monitor:

H. A. Danner

Date: *4/18/86*

This report has been reviewed for scientific quality and is summarized, with comments (if any) as follows:

Dimethyl-N-Hexadecylamine was corrosive to skin under the test conditions. Study is acceptable and valid, even with an additional observation.

Divisional Toxicologist:

H. F. Epp
H. F. Epp

Date: *4/29/86*

This report is approved for microfilming and entry into P&G Toxicology Files

Corp. H&ES Liaison:

W. R. King
W. R. King

Date: *5/6/85*

Return to Corp. H&ES Operations Section, Room 2S179, MVL

Entered into Safety Data System:

Date: *5-9-86*

By:

ML Brown

Microfilming Completed:

Date: *7-9-86*

By:

ML Brown

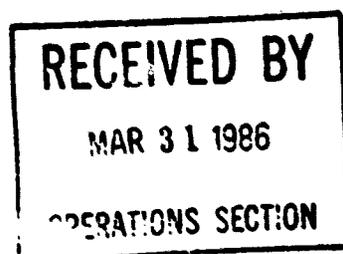


**International Research
and Development Corporation**

MATTAWAN, MICHIGAN, U.S.A. 49071 TELEPHONE (616) 668-3336

SPONSOR: The Procter and Gamble Company
SUBJECT: Skin Corrosivity
(DOT Procedure with Options)
DED NO: IBTS 150
TSIN: J0207.01
REPORT NO.: 191-1221
DATE OF SUBMISSION: March 24, 1986

191-1221



"credence through research"

Study No. 191-1221.
Sponsor Ref: IBTS 150

Skin Corrosivity
(DOT Procedure with Options)

Report of a biological test performed at:
International Research and Development Corporation
Mattawan, Michigan 49071

Deviation From
Protocol:
See notation below.

During the period:
February 27, 1986 to March 3, 1986
According to the attached protocol
(P&G NO. C9A)

Issue Date: March 1, 1982

<u>Test Substance(s) (TSIN)</u>	<u>Color</u>	<u>Physical Form</u>	<u>Storage Conditions</u>
J0207.01	cloudy	liquid	room temperature

Sponsor's Divisional Toxicologist: Harry F. Epp

Source and Strain of Animals Used: Hazleton Dutchland, Denver, Pennsylvania
New Zealand White rabbits

Concentration and Amount of Test Substance Dosed:
Administered undiluted as received, 0.5 ml per test site
(intact).

RESULTS

Group Average Dermal Irritation Scores*

<u>Test Article</u>	<u>Observation</u>			
	<u>Time</u>	<u>Skin</u>	<u>Erythema</u>	<u>Edema</u>
J0207.01	4.5 HOUR	INTACT	2.3	0.8

*Average Score = The sum of one site on six rabbits divided by six.
Individual rabbit scores are presented in Table 1.
Corrosion was observed at the test sites of all six rabbits at the 48-hour
observation interval. The results are presented in Table 2.

Note: The rabbits were not sacrificed following the 48-hour observation, as per
protocol, in order to confirm the presence of corrosion. The rabbits were examined
on March 3, 1986 when the Study Director observed that, although all animals exhibited
maximum erythema and edema, only three of the six rabbits had definitive corrosion.
There were no further evaluations conducted and the rabbits were sacrificed immediately.

Technical Supervisory Staff,

Acute Toxicology and Special Studies:

**Stephen W. Allen, B.S.
Group Supervisor**

**Linda L. Fleetwood, B.A.
Unit Supervisor**

PREPARED BY:

Brian T. Walker
**Brian T. Walker, B.S.
Report Writer
Acute Toxicology and
Special Studies**

3-18-86
Date

REVIEWED BY:

Dale E. Johnson
**Dale E. Johnson, Pharm.D., Ph.D.
Associate Director, Division
of Toxicology**

3/18/86
Date

STUDY DIRECTOR STATEMENT

The methods used in IRDC Study Number 191-1221 followed the experimental criteria specified in the protocol.

To the best of my knowledge, there were no significant deviations from the Good Laboratory Practice Regulations which affected the quality or integrity of this study. This study was conducted in conformance with the Good Laboratory Practice Regulations. This report accurately reflects the raw data obtained during the performance of this study.

All data including the final study report are stored in the International Research and Development Corporation Archives, Mattawan, Michigan.

James R. Myer
**James R. Myer, B.S.
Manager of Acute Toxicology and
Special Studies
Study Director**

3/21/86.
Date

Individual Rabbit Scores

TABLE 1.

J0207.01

0.5 ml/test site

Individual Test Site Scores

<u>Animal No.</u>	<u>Observation</u>		<u>ERYTHEMA</u>	<u>EDEMA</u>
	<u>Time</u>		<u>II</u>	<u>II</u>
<u>Site # and Skin Treatment</u>				
28857 M	HOURL	4	NA	NA
	HOURL	4.5	2	0
	HOURL	48	NA	NA
28893 M	HOURL	4	NA	NA
	HOURL	4.5	3	1
	HOURL	48	NA	NA
28905 M	HOURL	4	NA	NA
	HOURL	4.5	2	1
	HOURL	48	NA	NA
28852 F	HOURL	4	NA	NA
	HOURL	4.5	2	1
	HOURL	48	NA	NA

I = Intact

NA = Not applicable

Individual Rabbit Scores

TABLE 1 CONT.
0207.01
.3 ml/test site

Individual Test Site Scores

<u>Animal No.</u>	<u>Observation Time</u>	<u>ERYTHEMA</u>		<u>EDEMA</u>
		<u>Site #</u>	<u>and Skin Treatment</u>	<u>II</u>
28461 F	HOOR 4	NA		NA
	HOOR 4.5	2		1
	HOOR 48	NA		NA
28484 F	HOOR 4	NA		NA
	HOOR 4.5	3		1
	HOOR 48	NA		NA

I = Intact
 NA = Not applicable

191-1221

TABLE 2.
 J0207.01
 0.5 ml/test site

Rabbit Skin Irritation
 OTHER FINDINGS

Site # and Skin Treatment

Animal No.	Observation		
	Time		
28857 M	4		<u>II</u>
	4.5		
	48		c
28893 M	4		<u>II</u>
	4.5		
	48		c
28905 M	4		<u>II</u>
	4.5		
	48		c
28852 F	4		<u>II</u>
	4.5		
	48		c
28861 F	4		<u>II</u>
	4.5		
	48		c
28888 F	4		<u>II</u>
	4.5		
	48		c

c - Corrosion
 I - Intact

INTERNATIONAL RESEARCH AND DEVELOPMENT CORPORATION

PROTOCOL REVISION OR CLARIFICATION

Protocol Sheet No. 1

(ISIR# J0207.01)
Study No. 191-1221 (DRD# IBTS 150)

TITLE: SKIN CORROSIVITY (DOT PROCEDURE WITH OPTIONS)

Page 1 of 2

<u>ITEM</u>	<u>JUSTIFICATION</u>
1	Study initiation.
2	Identification of the test article.
3	Identification of the source and age of the test animals.
4	Clarification of statistical analysis.
5	Animal selection.
6	Identification of diet used.

<u>ITEM</u>	<u>PROTOCOL REVISION OR CLARIFICATION</u>
1	Conduct study in accordance with the attached protocol. The letter of authorization is considered part of the protocol.
2	The test article is identified as J0207.01, IRDC 8835.
3	The source of the rabbits will be Hazleton Dutchland, Denver, Pennsylvania. The animals will be 5-7 months of age at study initiation.
4	No statistical analyses are required on this study, therefore, none will be performed.

Study Director James R. Myer, B.S.

James R. Myer
Signature

2/25/86
Date

INTERNATIONAL RESEARCH AND DEVELOPMENT CORPORATION

PROTOCOL REVISION OR CLARIFICATION

Protocol Sheet No. 1 (TSIN# J0207.01)
Study No. 191-1221 (DSD# IBTS 150)

TITLE: SKIN CORROSION (DOT PROCEDURE WITH OPTIONS)

Page 2 of 2

ITEM

PROTOCOL REVISION OR CLARIFICATION

5

Acceptable animals will be placed on study with the aid of a computer-generated random number table.

6

The diet used in the study is Certified Rabbit Chow® #5322, Balston Purina Company.

Study Director James R. Myer, B.S.

James R. Myer
Signature

2/25/86
Date

191-1221



THE PROCTER & GAMBLE COMPANY

MIAMI VALLEY LABORATORIES

P. O. BOX 39175
CINCINNATI, OHIO 45247

February 18, 1986

Dr. Dale Johnson
International Research
& Development Corporation
500 North Main Street
Mattawan, MI 49071

Dear Dr. Johnson:

This is to authorize you to carry out the following study according to the attached protocol, and in agreement with the stipulations of our current Laboratory Services Agreement.

- Notice:**
- 1) This study is not expected to be submitted to a regulatory agency. The stipulations of this protocol are to be implemented in conformance with the Good Laboratory Practice Regulations (21 CFR, Part 58) with the following exceptions:
 - a) The study should not be listed on the Test Facility's master list of regulated studies.
 - b) If two or more test substances appear on the protocol, it may be conducted as a single study, resulting in a single final report.
 - c) There is no need to audit an in-life phase of this study.
 - 2) The final report will be inspected by the Test Facility's QAU. Sufficient data should be made a part of each report to allow the Sponsor to check the reported results against the raw data.
 - 3) Documentation of the derivation, characterization, and stability testing of the test substance(s) will be the responsibility of the Sponsor.

Test:	Skin Corrosivity (Dot Procedure with Options)		
Protocol No.:	C9A	Issue Date:	March 1, 1982
Test Substance No.:	JO207.01	Doc. Req. No.:	IBTS 150
Physical Form:	Liquid		

Three copies of the final report are needed by April 16, 1986, and are to be sent to my attention at the above address.

0 0 7 8

THE PROCTER & GAMBLE COMPANY

Dr. Dale Johnson
IRDC
February 18, 1986
Page 2

Matters involving the scientific aspects of the work can be handled directly with the Sponsor's Divisional Toxicologist. All unused samples are to be returned to the Divisional Toxicologist at the following address (the cost of shipment should be included in the study cost):

Mr. Harry F. Epp Telephone No. (513) 530-3331
The Procter & Gamble Company
Sharon Woods Technical Center
11520 Reed Hartman Hwy.
Cincinnati, OH 45241

Complete both copies of the attached protocol by adding your study number, proposed start and completion dates, and have the Study Director sign and date them. The Study Director should define the start and completion dates on the protocol. Retain one copy and return one copy (which includes the study cost) to me along with a letter stating that you agree to do the work specified in the attached protocol. In addition, if you cannot meet the report dates, please let me know.

The Invoice for this study should be sent to Mr. Harry F. Epp at his address listed previously.

Sincerely,

THE PROCTER & GAMBLE COMPANY
Research & Development Department



H. A. Derner
Human & Environmental Safety Division

Approved: 

W. E. Winters, Ph.D.
Director, Human & Environmental Safety Division

nh
Attachments
cc: Study File
Harry F. Epp

0079

PROTOCOL NO. C9A

Skin Corrosivity (DOT Procedure with Options)

Issue Date: March 1, 1982
Supersedes Issue Dated: September 1, 1981

Test Substance Identification Number (TSIN) # J0207.01

Divisional Request Document Number (DRD) # IBTS #150

Sponsor: The Procter & Gamble Company
Cincinnati, Ohio

Testing Facility:
(To be filled in by
Operations Section)

International Research
& Development Corporation
Mattawan, MI 49071

Study # 191-1221
(To be filled in by
Testing Facility)

Purpose:

To determine the skin corrosivity of a test substance when this substance is tested using the Department of Transportation (DOT) Procedure. (49 CFR 173.240)

Justification for
Selection of Test
System:

Required by DOT Procedure. (49 CFR 173.240)

Route of Administration
of Test Substance and
Reason for Choice:

Patch placed on intact skin clipped free of hair.
Required by DOT Procedure. (49 CFR 173.240)

Diet and/or Water
Analyses Required:

None (no known contaminants expected which would interfere with this study)

Records to be
Maintained:

All records that would be required to reconstruct the study and demonstrate adherence to protocol.

PROTOCOL NO. C9A (Cont'd)Skin Corrosivity (DOT Procedure with Options)

Issue Date: March 1, 1982

<u>Test Substance(s)</u>	<u>DDP</u>	<u>Description</u>		<u>Expiration</u>
<u>TSIN #</u>	<u>Number</u>	<u>Color</u>	<u>Physical Form</u>	<u>Date</u>
J0207.01	IBTS #150	Water White	Liquid	2/87

Storage Conditions: (Check one)

Room temperature Refrigerator Freezer
 Other

Hazards: (Check one)

None known. Take ordinary precautions in handling.
 As follows: Avoid eye and skin contact. If contact occurs, wash thoroughly with soap and water immediately, and get medical attention. Effect may be delayed.

Special Instructions: (Check one)

None
 As follows:

Animals:

Each test group will consist of six (6) New Zealand albino rabbits of either sex weighing \geq 2.0 kg.

Animal Care:

Follow the approved Standard Operating Procedures of the Test Facility. (Acclimation period must be a minimum of seven (7) days.)

Environmental Conditions:

Follow the approved Standard Operating Procedures of the Test Facility.

Animal Identification:

Follow the approved Standard Operating Procedures of the Test Facility.

PROTOCOL NO. C9A (Cont'd)Skin Corrosivity (DOT Procedure with Options)

Issue Date: March 1, 1982

Site Preparation:

Clip the back of each animal with a small animal clipper. Leave the test site on each animal intact.

Dose Preparation:

Test Group(s): (Check appropriate box)

- Dose test substance undiluted
 Dose as a freshly prepared _____ % (w/w) solution/suspension of test substance in _____
 Dose as a freshly prepared _____ % (w/v) solution/suspension of test substance in _____
 Dose per Special Instructions (see page 2)

Control Group

A control group should be ; should not be included in this study. If included, the control substance _____ should be tested concurrently with the test substance at a dosage level of _____.

Note

A concentration analysis of the test substance - vehicle mixture(s) will ; will not be required.

If a concentration analysis is required:

- Prepare a sufficient quantity of the test substance - vehicle mixture(s) so that a portion can be returned to the Sponsor's Divisional Toxicologist. Store solution/mixture at room temperature; refrigerator; freezer; other _____

Shipping Instructions

* RETURN UNUSED SAMPLE.

Send approximately * _____ ml. Send frozen; under ambient conditions; other _____

- Analyze the test substance - vehicle mixture(s) for test substance concentration using the analytical method in Appendix _____.

0082

PROTOCOL NO. C9A (Cont'd)

Skin Corrosivity (DOT Procedure with Options)

Issue Date: March 1, 1982

Dosing Instructions:

Introduce 0.5 ml (in the case of liquids) or 0.5 gm (in the case of solids and semisolids) of the substance to be tested under a square surgical gauze patch measuring 1 inch x 1 inch and two single layers thick. Secure patches in place with adhesive tape by placing tape around the border of the patch. Wrap the entire trunk of each animal with impervious material such as rubberized cloth. (The impervious wrap should be applied in such a manner that the hand can easily be placed between the wrap and the animal's back.) Restrain the animal to prevent it from removing wrapping.

Observations:

After 4 hours of exposure, remove the animal from the restrainer, remove the patches and evaluate the skin sites for corrosion. Readings are again made at the end of a total of 48 hours (44 hours after first reading). Corrosion is considered to have resulted if the substance in contact with the rabbit skin has caused destruction or irreversible alteration of the tissue. Tissue destruction is considered to have occurred if, at any of the readings, there is ulceration or necrosis. Tissue destruction does not include merely sloughing of the epidermis, or erythema, edema, or fissuring.

At study termination, surviving animals should be removed from study following the Standard Operating Procedures of the Test Facility.

Protocol Changes:

If it becomes necessary to change the approved protocol, verbal agreement to make this change should be made between the Study Director and the Sponsor. As soon as practical, this change and the reasons for it should be put in writing and signed by both the Study Director and the Sponsor's Divisional Toxicologist. This document is then attached to the protocol as an addendum.

Possible Options to the DOT Procedure:

Check the appropriate box(es) for any options desired and fill in any blank spaces.

1. Immediately following the initial reading for corrosion, all test sites will be thoroughly washed with Tepid Water to remove any residual test substance.

PROTOCOL NO. C9A (Cont'd)Skin Corrosivity (DOT Procedure with Options)

Issue Date: March 1, 1982

Possible Options to the DOT Procedure (Cont'd):

2. Approximately 30 minutes after the removal of the patches, evaluate the test sites using the grading scale below.

Primary Irritation Scoring

(a) Erythema and Eschar Formation

No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate-to-severe erythema	3
Severe erythema (heat redness) to slight eschar formation (injuries in depth)	4
Highest possible erythema score	4

(b) Edema Formation

No edema	0
Very slight edema (barely perceptible)	1
Slight edema (edges of area well defined by definite raising)	2
Moderate edema (raised approximately 1 mm)	3
Severe edema (raised more than 1 mm and extending beyond area of exposure)	4
Highest possible edema score	4

Re-evaluate the test sites for these parameters 48 hours after initial application.

3. If the test substance is judged to be corrosive at either reading, no primary irritation evaluation should be made. If the test substance is corrosive at the initial reading, the study is terminated.
4. If the skin reaction is questionable, regarding corrosivity at 48 hours, then the study should be continued by maintaining the test animals with questionable reactions until the reactions can be properly classified.

0 0 8 4

PROTOCOL NO. C9A (Cont'd)

Skin Corrosivity (DOT Procedure with Options)

Issue Date: March 1, 1982

Report:

Report should include how study was conducted, dates of initiation and termination, the skin evaluations at 4 and 48 hours, and whether the test substance was or was not corrosive. According to the DOT Rules and Regulations* (46 FR 49906) at least two (2) animals out of six (6) must show corrosivity of the material to be considered corrosive. If animal 2 is checked, the study report should include erythema and edema scores for each animal at each observation time. The Primary Irritation Index equals the average of all the erythema scores plus the average of all the edema scores. Correlate the Primary Irritation Index with the following descriptive terms and include in the final report:

- 0 = Nonirritating
- 0.1-2 = Mildly irritating
- >2-5 = Moderately irritating
- >5-6 = Moderately to severely irritating
- >6-8 = Severely irritating

Report all skin reactions that are not covered by the above erythema and edema grading scale.

This report shall conform to all requirements outlined in Section 58.185, Subpart J, Good Laboratory Practices Regulations.

Sponsor: HARRY F. ENN *Harry F. Enn*
 Divisional Toxicologist

Date Approved by Sponsor's Divisional Toxicologist February 12, 1986

Proposed Starting Date: 2/27/86

Defined as day of dosing

Proposed Completion Date: 3/01/86

Defined as tentative date of last observation

)To be completed
)by the Test
)Facility

Study Director: James R. Myer

Date: 2/25/86
 James R. Myer, B.S.

Study Cost: _____

0085

International Research and Development Corporation

QUALITY ASSURANCE STATEMENT

Study Title: Skin Corrosivity (Dot Procedure with Options)

Test Article: J0207.01

This report has been reviewed by the International Research and Development Corporation Quality Assurance Department in accordance with the United States Food and Drug Administration Good Laboratory Practice Regulations of June 20, 1979.

An inspection of the protocol for this study was conducted on February 27, 1986. A randomly sampled phase of the conduct of the study was inspected on February 28, 1986. Findings resulting from inspections, from a data audit, and from a review of the report were reported to management and the Study Director on March 12, 1986.

Approved And
Submitted By:

Margery J. Wirth
Margery J. Wirth, B.S.
Acting Director of Quality Assurance

3/20/86
Date

191-1221

"credence through research"

0086

30

The Procter and Gamble Company

Cincinnati, OH

Contains No. 100

Biodegradation of Radiolabeled Substances
in Wastewater

B0876.01 = CAS RN 124-28-7

Study E85-018

RECEIVED
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10/27/88

Study Director

Quality Assurance

TEST SUBSTANCE CHARACTERIZATION REPORT (TSCR)

Test Substance Identification Number (TSIN):

Safety Test Request Number:

Principal Investigator:

TSIN B087601

Product or Ingredient: (methyl - ¹⁴C-Octadecyl-dimethylamine)

Brand Notebook Ref:

Physical Description: in solution (ether) Solubility: low sol. in water pH: _____

Recommended Storage Conditions: _____ Expiration Date: in progress

Hazards (i.e. flammability, toxic gases): radioactive

Dept. of Transportation Hazard Classification: non-hazardous CAS No. (a): [124-28-7]

Formulated Composition (b)

Component (c)	Mol. Wt.	Nominal Level (X by Wt.)	Acceptable Range	Stock Code No.	Supplier
(methyl- ¹⁴ C octadecyl)	265				Wizard Labs.

1000 ug
100 ug/mg
100 ug/1000 ug
100 ug/1000 ug

Specific activity 3.0 uCi/umol; 10 uCi/g

- (a) Include CAS number(s) for the three most major components of a formulation or for single chemical products. Footnote to the material with which the respective number is associated.
- (b) If information requested is not known, then the symbol NK will be entered.
- (c) Chemical names which are inconveniently long may be abbreviated in tables but should be listed in full in referenced footnotes. Non-chemical names, such as Tergitol 15-S-O or Yellow Dye #10, may not be acceptable but should be previewed with the responsible toxicologist. Nondefinitive identification (e.g. Arquad, BC-base) is not acceptable.

The above information provided by:

1/7/86
(Date)

The above information reviewed and accepted by:

Principal Investigator:

1/7/86
(Date)

BIODEGRADATION OF RADIOLABELED SUBSTANCES IN WASTEWATER

1. Experimental Protocol

See attached Study Plan (E85-018), Standard Test Method and Study Plan/Protocol Amendment (E85-018).

2. Test Materials

B0876.01 = ^{14}C -CAS RN 124-28-7

Note octadecyldimethylamine

3. Project Information

Activated sludge was obtained from the Colerain Heights Wastewater Treatment Plant, Colerain Township, Ohio. Laboratory testing was conducted at the Environmental Safety Department laboratories of Procter & Gamble, Cincinnati, Ohio.

Samples were taken upon initiation of the experiment and at 0.5, 1, 2, 3 and 7 days post-initiation.

Testing was begun on 7/22/85 and completed 8/4/85.

4. Results

The results of the study are presented in Tables 1-5 and are summarized in Tables 6 and 7 and in Figures 1 and 2.

1-15-85

Table 1

Test Compound Distribution (0.5 day)

<u>Chemical</u>	<u>Concentration</u> (ppm)	<u>Average</u> <u>Mass Bal.</u>	<u>Average</u> <u>% CO₂ (direct)</u>	<u>Average</u> <u>% Filter</u>	<u>Average</u> <u>% Solution</u>
-----------------	-------------------------------	------------------------------------	--	-----------------------------------	-------------------------------------

8076.01	0.2	108	46	29	9
---------	-----	-----	----	----	---

Table 2

Test Compound Distribution (1 day)

<u>Chemical</u>	<u>Concentration</u> (ppm)	<u>Average</u> <u>Mass</u> <u>Balance</u>	<u>Average</u> <u>% CO₂</u> <u>Direct</u>	<u>Average</u> <u>% CO₂</u> <u>Indirect</u>	<u>Average</u> <u>%</u> <u>Filter</u>	<u>Average</u> <u>%</u> <u>Solution</u>
B0876.01	0.2	94.89 ± 23.13	47.60 ± 8.05	41.85 ± 9.22	21.79 ± 3.12	14.55 ± 3.11
"	2	87.94 ± 11.26	42.63 ± 5.94	47.50 ± 6.82	20.07 ± 1.81	12.36 ± 4.21

Table 3

Test Compound Distribution (2 days)

<u>Chemical</u>	<u>Concentration</u> (ppm)	<u>Average</u> <u>Mass</u> <u>Balance</u>	<u>Average</u> <u>% CO₂</u> <u>Direct</u>	<u>Average</u> <u>% CO₂</u> <u>Indirect</u>	<u>Average</u> <u>%</u> <u>Filter</u>	<u>Average</u> <u>%</u> <u>Solution</u>
B0876.01	0.2	101.65 ± 11.33	70.59 ± 8.20	44.91 ± 16.98	16.27 ± 4.13	22.66 ± 8.71
"	2	91.20 ± 6.19	58.88 ± 4.67	47.25 ± 5.12	18.02 ± 1.15	16.71 ± 2.88

Table 4

Test Compound Distribution (3 days)

<u>Chemical</u>	<u>Concentration</u> (ppm)	<u>Average</u> <u>Mass</u> <u>Balance</u>	<u>Average</u> <u>% CO₂</u> <u>Direct</u>	<u>Average</u> <u>% CO₂</u> <u>Indirect</u>	<u>Average</u> <u>%</u> <u>Filter</u>	<u>Average</u> <u>%</u> <u>Solution</u>
B0876.01	0.2	107.38 ± 12.97	80.36 ± 12.76	51.99 ± 7.87	14.38 ± 3.06	19.26 ± 3.12
"	2	100.85 ± 9.71	69.61 ± 7.44	53.81 ± 4.45	13.71 ± 1.72	18.77 ± 1.01

0043

Table 5

Test Compound Distribution (7 days)

<u>Chemical</u>	<u>Concentration</u> (ppm)	<u>Average</u> <u>Mass</u> <u>Balance</u>	<u>Average</u> <u>% CO₂</u> <u>Direct</u>	<u>Average</u> <u>% CO₂</u> <u>Indirect</u>	<u>Average</u> <u>%</u> <u>Filter</u>	<u>Average</u> <u>%</u> <u>Solution</u>
B0876.01	0.2	117.15 ± 18.39	91.13 ± 18.02	63.64 ± 6.42	9.91 ± 2.04	16.54 ± 2.39
"	2	105.75 ± 9.73	79.43 ± 8.42	67.65 ± 3.13	10.00 ± 1.36	12.35 ± 0.77

Table 6

Results Summary: Biodegradation
of Radiolabeled Substances in Wastewater

<u>Material</u>	<u>Conc. (ppm)</u>	<u>Final % ¹⁴CO₂^a</u>	<u>Rate Const. (day⁻¹)^a</u>	<u>Half-Life (hours)</u>	<u>Corr. Coef.</u>	<u>Avg. Mass Balance Over Test Period^a</u>
B0876.01	0.2	91.1 ± 18.0	0.99 ± 0.06	16.8	0.947	105.8% ± 8.3
B0876.01	2.0	79.4 ± 8.4	0.85 ± 0.06	19.6	0.974	96.0% ± 7.3

^a ± values represent standard deviations from triplicate flasks.

System Biomass/Dissolved Oxygen (average over 7 days; three replicate controls)

Solids: 4009 ± 222 mg/L
 Cell Concentration: 4.79 ± 0.6 × 10¹⁰ cells/L
 Dissolved Oxygen: 8.0 ± 0.3 ppm

Glucose Turnover Time (average of 7 days; three replicate controls)

1.8 ± 0.8 h

Incubation Temperature: 20° ± 2°C

Table 7

Average Total % Removal Based on ¹⁴C in Sludge Filtrate

<u>Test Substance</u>	<u>Concentration</u> (ppm)	<u>0.5 day</u>	<u>1 day</u>	<u>2 days</u>	<u>3 days</u>	<u>7 days</u>
B0876.01	0.2	91	85.4	77.3	80.7	83.5
"	2.0	93	87.6	83.3	81.2	87.7

0046

Figure 1.

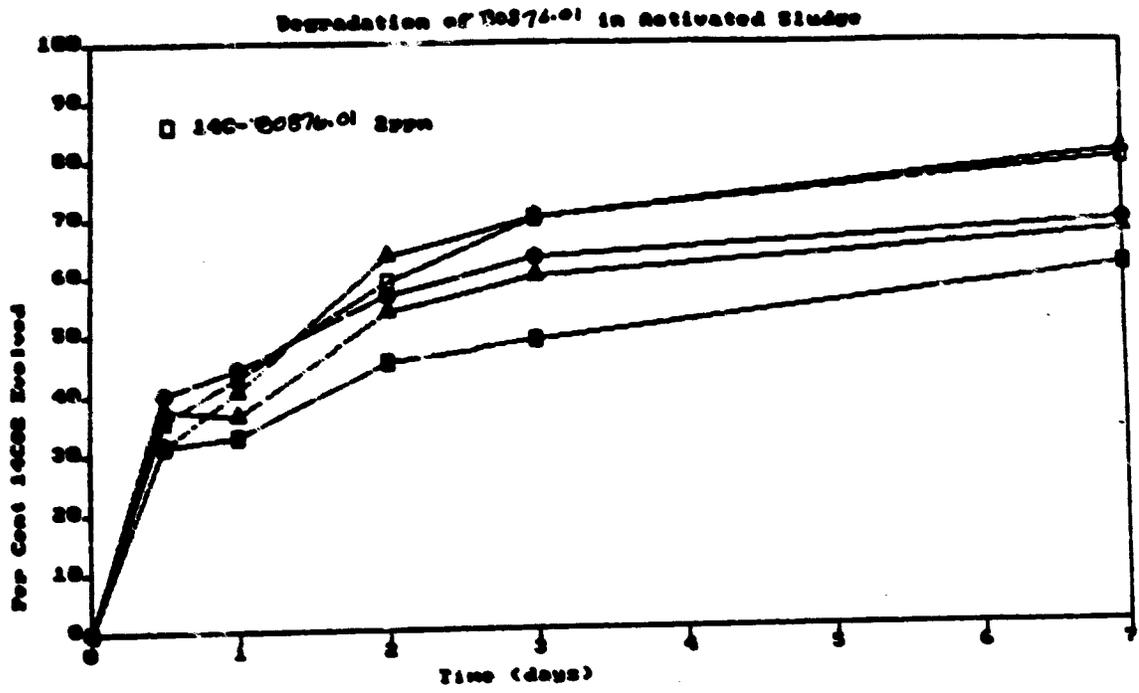
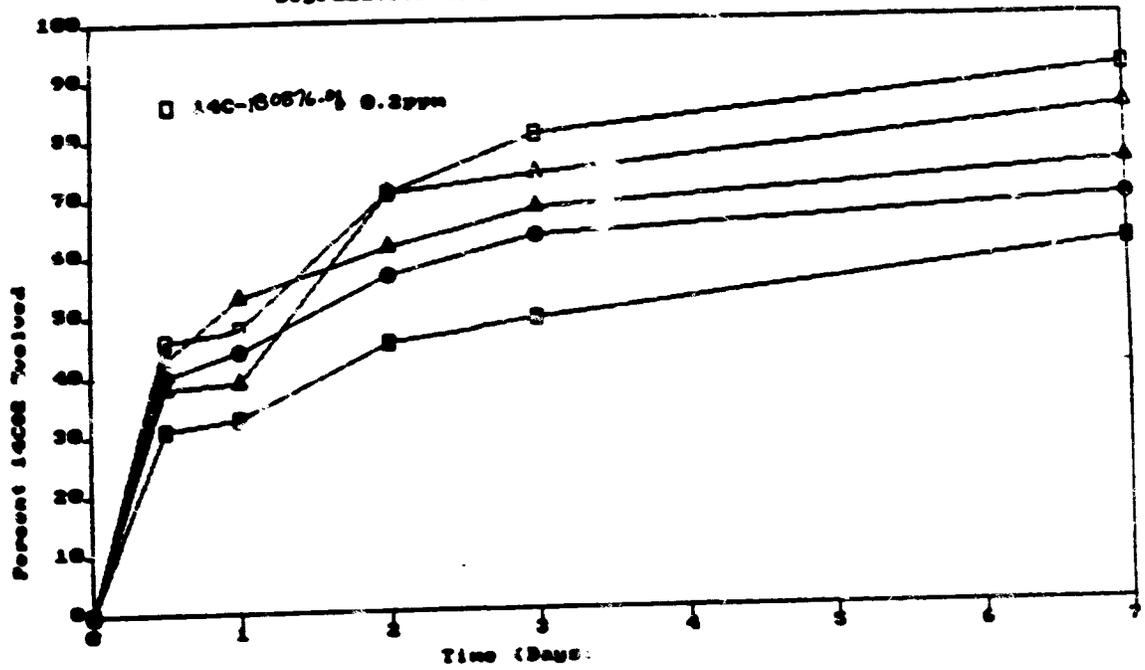


Figure 2.

Degradation of 30876.01 in Activated Sludge



Study #E85-018

STUDY PLAN

Title: Environmental Study to Determine the Biodegradability of ^{14}C -B0876.01
in Activated Sludge.

Purpose: To determine the rate and extent of removal and biodegradation of ^{14}C -labeled
B0876.01 in activated sludge collected from a wastewater
treatment plant.

Sponsor: The Procter and Gamble Company

Proposed Dates: Test Initiation - August, 1985
Test Completion - August, 1985

Test Facilities: Activated sludge will be obtained from the Colerain Heights Wastewater
Treatment Plant (WTP), Springdale Road, Colerain Township, Ohio. The laboratory portion
of the study will be conducted in the Procter and
Gamble, Cincinnati, Ohio.

Study Substances:

Observe normal precautions when handling all substances. Observe radiochemical safety
precautions when handling all ^{14}C -labeled substances. This study will include but not
be limited to the following substances:

Test Substances: See attached for detailed information.

^{14}C -Labeled

<u>TSIN #</u>	<u>BFO #</u>	<u>Spec. Act. (uCi/mg)</u>	<u>Physical Form</u>	<u>Description</u>	<u>Storage</u>	<u>Exp. Date</u>	<u>Hazard</u>
80876.01	5756	10	solid dissolved in ether	colorless	Refrig.	Not Given	None Known

Test System: The test system will be the natural population of microorganism in the activated sludge collected from the Colerain Heights WWT².

Test System Justification: This system was chosen based on expected exposure to activated sludge in a typical waste water treatment situation.

Test System Identification: The test system will be identified by Acridine Orange Direct Count (AODC)^{1,2} and Total Nonfilterable Residue (Total Suspended Matter)³.

Test System Nutrient: The nutrient used for the study will consist of a synthetic feed prepared according to the attached test method, Biodegradation of Radiolabeled Substances in Wastewater. This synthetic feed is prepared using reagent grade compounds and is not expected to contain contaminants known to be capable of interfering with the purpose or conduct of the study.

The solvent High Quality Water (HQW) used to solubilize the test and control substances is not expected to contain any contaminants.

Experimental Design: See attached test method Biodegradation of Radiolabeled Substances in Wastewaters.

Route of Administration: The test substance will be administered by means of high quality water (HQW). The test substance would naturally enter a treatment plant dissolved in the influent water. HQW is used to minimize contamination of organisms that may biodegrade the test substance prior to being placed in the study.

Test Dosing: The test substance and control substance will be added directly to the activated sludge units as HQW solutions. The total volume added will be equalized by the addition of HQW. The control units will contain an equal volume of HQW addition with none of the substances.

Test concentration based on the % active of the substances will be:

<u>BFO #</u>	<u>Concentration (ug/l)</u>
5610	200
5610	2,000
5751	200
5751	2,000
5752	200
5752	2,000
5608	200
2898	200

Absorption: A method for determining the degree of absorption of the test substance by the test system is not applicable to this study.

Type and Frequency of Analysis: The type and frequency of analysis will include but not be limited to those defined in the attached test method Biodegradation of Radiolabeled Substances in Wastewater.

Statistical Calculations:

Calculations will include but not be limited to those defined in the attached test method Biodegradation of Radiolabeled Substances in Wastewater.

Records to be Maintained:

All records necessary to reconstruct the study and demonstrate adherence to the study plan.

Changes:

If it becomes necessary to change the approved protocol, verbal agreement to make this change should be made between the Study Director and the Principal Investigator. As soon as practical, this change and the reasons for it should be put in writing and signed by both the Study Director and the Principal Investigator. This document is then attached to the protocol as an addendum.

Reporting:

1. Identification of test material by sample code, specific activity, color, form and date received.
2. Test material concentrations and procedures followed for preparation and addition.
3. Reference to the Protocol (title, author and date) and addenda if made, test methods, and any analytical or standard operating procedures used.
4. Any protocol deviations and their implications.
5. Reference to laboratory notebook or other file containing raw data.
6. Starting and ending dates for study.
7. All NOECs and first effect levels.

References

1. Hobbie, J. E., R. Daley and S. Jasper. 1977. Use of Nucleopore filters for the counting of bacteria by fluorescence microscopy. *Appl. Environ. Microbiol.* **33**: 1225-1228.
2. Kirshman, D. and R. Mitchell. 1982. Contribution of particle-bound bacteria to total microheterotrophic activity in five ponds and two marshes. *Appl. Environ. Microbiol.* **43**:200-209.
3. APHA, AWWA, AND WPCF. Standard Method for the Examination of Water and Wastewater. 15th Ed. Washington, D.C.: APHA, 1980.

209D Total Nonfilterable Residue Dried at 103°-105°C (Total Suspended Matter).

Principal Investigator/Study Director

8/22/85
Date

N. E. Jellman
Quality Assurance

8/22/85
Date

MPBSP2/tlc
4/30/85
Revised: 8/20/85

AMENDMENT
To Study Plan and Protocol for
FAS-AIA

PROTOCOL: Biodegradation of Radiolabeled Substances in Wastewater

STUDY PLAN: Environmental Study to Determine the Biodegradability of
14C-B0876-01
in Activated Sludge.

CHANGES:

Reporting:

- 1) Test material sample code, specific activity, color, and form reported in TSCRs and study plan. Date received will not be reported.
- 2) Test material concentrations and procedures followed for preparation attached. Method of addition is described in study plan.
- 7) NOECs and first level effects will not be determined and therefore not reported.

Apparatus/Equipment:

- A) CO₂ scrubbing apparatus--one train used for the 27-flask test. The scrubbing train consists of the following series of bottles: (1) empty, (5) KOH, (1) HQW, and (1) empty.

Chemicals/Reagents/Materials:

- A) Laboratory chemicals--add conc. acid (HCl or H₂SO₄)

Test Organisms:

- 1) Sludge not passed through a 2mm sieve prior to use.

Test Procedures:

- 4) Serum vials will be acidified with 200ul of 2N HCl, unless otherwise noted.
- 5) Units not required to be fed at all sample times. Feeding may vary as solids levels, frequency of sampling, changes in dissolved oxygen or pH indicate that feeding is or is not needed.

Dry weight determinations will be as per standard methods with the exception of weighing the dried filters only once.

5) Flasks will be incubated at 20 ± 2 degrees C.

Calculations/Interpretations:

2) Calculations--change 1/200 for base traps to 1/100 for base traps.

Appendix I:

Test Medium: Sludge not homogenized in a blender.

Test Conduct:

1) Order of addition changed to the following: add radiolabeled glucose or amino acid mixture--reaction is started by the addition of the sample water.

These changes are made to clarify the intent of the protocol. They do not affect the integrity of the data.

Study Director / 3/4/82
Date

A. H. Klaber / 3-2-12-86
Quality Assurance Date

PROCTER & KAMBER COMPANY
BIOLOGICAL SAFETY TESTING STANDARDS
ENVIRONMENTAL SAFETY DEPARTMENT

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ISSUE DATE: 01/01/70

SUPERSEDES: NEW

VIII. STANDARD TEST METHODS

8. Biodegradability

10. Biodegradation of Radiolabeled Substrates in Wastewater

Principle

Various concentrations of carbon-14 (^{14}C) labeled test substances are added to wastewater maintained under aerobic conditions. The conversion of radiolabel to $^{14}\text{CO}_2$ is measured as the ^{14}C -substrate is degraded by the microorganisms present in the wastewater sample.

Scope and Application

Under aerobic conditions, microorganisms metabolize organic substances to produce carbon dioxide (CO_2), water and oxidized inorganic end products. The measurement of CO_2 evolution forms the basis of several biodegradability screening tests to assess the biodegradation potential of xenobiotic organic substances (1-3). In screening tests, nonspecific (gravimetric, titrimetric) techniques are used to measure CO_2 production. This allows a wide range of substances to be tested, but severely limits control of experimental variables. For example, the concentration of test substance must be high (ppm level) since the nonspecific analytical procedures are not highly sensitive. The test substance must be present, as the sole carbon source in a dilute synthetic medium to minimize background interferences, and an artificial inoculum, generally sewage or soil, must be added to provide a source of degradative microorganisms. These experimental conditions do not simulate natural aquatic ecosystems, where substance concentrations are low (ppb level) and a variety of nutrient conditions and microbial species exist (4). The present method is designed to more accurately reflect actual environmental systems by using wastewater samples and more realistic test concentrations in the ppb range.

Safety Precautions

Use adequate safety precautions when handling radioactive substances with relatively unknown toxicological properties.

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ISSUE DATE: NOV 3 1985

B. Biodegradability

SUPERSEDES: NEW

10. Biodegradation of Radiolabeled Substrates in Wastewater
(Cont'd.)

Apparatus/Equipment

a. CO₂ Scrubbing Apparatus

- For a series of flasks:
- Five 1-liter plastic bottles filled with 700 ml 9N NaOH or 9N KOH.
- One 1-liter plastic bottle filled with 700 ml HQW.
- Two empty 1-liter bottles to prevent liquid carryover.
- The bottles are connected in series (1 empty, 5 base filled, 1 HQW, 1 empty) with Tygon tubing to a pressurized air source (~5 psi). Air is sparged through the scrubbing solution at a constant rate sufficient to provide ~50 cc/min to the headspace of test flasks.

b. Test Apparatus

For a series of 12 flasks (adjust as needed):

- Flexible tubing (Tygon or equivalent)
- 12 - 2 liter Erlenmeyer flasks
- 12 - #10 3 hole stoppers
- 36 - #4 2 hole stoppers
- ~150 - 2 ml polystyrene disposable pipets
- 12 - 20 cc disposable syringes w/ Luer-Lok tips (Becton-Dickinson #5661)
- 36 - 4 oz. French Square bottles
- Serum vials (Wheaton-Fisher No. 06406H) with towers (Kontes, K882-320) for KOH wick
- Millipore 12 - place filter manifold (Millipore Corp. #XX2702350)
- Whatman (40) ashless filter paper (1 x 15 cm strips)
- Vacuum pump capable of ~30 in. Hg
- Repeating syringe - 5 ml capacity
- Galvan GA-6 Metrical[®] Membrane filters (Fisher #09-730- (Fisher #09730-22)
- Kimble borosilicate glass scintillation vials (Fisher #03-337-4) or equivalent

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VIII. STANDARD TEST METHODS

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3. Biodegradability

SUPERSEDES: NEW

10. Biodegradation of Radiolabeled Substrates in Wastewater

Apparatus/Equipment

b. Test Apparatus (Cont'd.)

- Inlet ports of Erlenmeyer flasks are connected by Tygon tubing to the CO₂-free air source. Exit ports are connected by tubing to one empty French square followed by three French squares containing 100 ml of 1.5N KOH. The CO₂-free air supply to each flask must be regulated to insure the proper flow rate (~50 cc/min). Care must be taken to insure that aerosols of the scrubbing solution or back flows do not reach the test flasks and sterilize the contents. Flasks are fitted with a sampling tube to which a 20 ml disposable syringe is attached.

c. Other Equipment

- Rotary platform shaker with speed control
- Liquid Scintillation Counter (LSC)
- Analytical balance
- Micropipettes
- Continuous temperature recording device
- Laboratory glassware
- Combustion furnace

Chemicals/Reagents/Materials

a. Laboratory Chemicals

- Reagent grade NaOH
- Reagent grade KOH
- Concentrated acid (HCl or H₂SO₄)
- Reagent grade alcohol (Fisher A962)
- Scintanized toluene (Fisher T-313)
- Scintillation surfactant (Fisher S-570)
- Spectrafluor PPO-POPOP (Amersham #190651)
- Cab-O-Sil fumed silica - Grade M-5 (Cabot Industries)

Scintillation cocktails for this study are made and used as follows:

1) Primary Cocktail Solution (PCS)

3.3 gallons toluene + 800 ml PPO-POPOP mix for ~5 hours.

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SUPERSEDES: NEW

B. Biodegradability

10. Biodegradation of Radiolabeled Substrates in Wastewater

Chemicals/Reagents/Materials

a. Laboratory Chemicals (Cont'd.)

2) 3A Cocktail

1-liter PCS + 1 liter reagent grade alcohol. Used for 1 ml aqueous sample or 1 ml ethanol or methanol (no other organic solvents): 20 mls 3A to 1 ml sample.

3) Scintillation Surfactant Cocktail

1-liter PCS + 1-liter Scintillation surfactant. Used to count 10 ml aqueous samples: 10 mls Scintillation surfactant to 10 mls H₂O.

4) Cab-O-Sil Cocktail

Weigh 68 g Cab-O-Sil fused silica into a 2 liter filter flask. Add 1-liter PCS and shake well. Then add 1-liter reagent alcohol in four 250 ml aliquots shaking well after each addition. Stir on magnetic mixer until well mixed (15-30 min.). Used for base samples: 20 mls Cab-O-Sil to 1 ml base.

b. Test Substances

Basic physical/chemical data regarding impurities, solvent and percent active, etc. are specified by the principal investigator.

Stock solutions of ¹⁴C-labeled test substances are made in an appropriate solvent at a concentration such that µg/l concentrations can be accurately added to test flasks. Stock solutions are stored in the dark under refrigeration. Aliquots of the stock are counted by liquid scintillation techniques to verify concentrations for spiking. Generally speaking, the pH of stock solutions need not be adjusted since only small quantities (µl) are added to test flasks. For relatively high specific activity substances (i.e. >1 µCi/mg), unlabeled test substance is to be added along with radiolabeled material if test concentrations exceed 1 mg/l.

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VIII. STANDARD TEST METHODS

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B. Biodegradability

SUPERSEDES: NEW

10. Biodegradation of Radiolabeled Substrates in Wastewater
(Cont'd.)

Test Organisms

Testing is performed using the indigenous microbial population present in the wastewater media. There are several different types of wastewater that may be used. The following are some examples of those available and suggested preparations:

a. Activated Sludge from a WTP

The activated sludge should be collected fresh the day the study begins from a municipal WTP receiving primarily domestic sewage. Prior to use, the activated sludge is passed through a 2 mm sieve and adjusted to 2000-3000 mg/l mixed liquor suspended solids (MLSS) as determined by non-filterable residue analysis (12). The sludge is also characterized for pH. If necessary, the sludge should be stored under aeration for no more than 48 hrs. prior to set up and fed synthetic feed daily.

b. Activated Sludge from a Laboratory Maintained Unit

Testing may also be performed using activated sludge from the laboratory maintained continuous or semicontinuous activated sludge units. The laboratory sludge could be pre-exposed and acclimated to the test material for a period of time, if desired.

c. Trickling Filter Bacteria

Trickling Filter Solids are the natural bacteria colonized on rocks in the trickling filter of the WTP. The rocks are taken from the rock bed of the trickling filter at least two rock layers below the surface. Dilution water is trickling filter effluent, collected from the secondary/final clarifier, prior to chlorination. It is stored at $4^{\circ}\text{C} \pm 2^{\circ}\text{C}$ and brought to $22^{\circ} \pm 4^{\circ}\text{C}$ prior to use.

Trickling filter rocks with attached bacteria are collected the same day as the dilution water. The rocks are placed in secondary effluent, returned to ESD, and stored at $4^{\circ}\text{C} \pm 2^{\circ}\text{C}$ until needed. When needed, the rocks are removed from storage, taken out of the effluent and scraped with a wire mesh scouring pad to remove the attached bacteria. The

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B. Biodegradability

SUPERSEDES: NEW

10. Biodegradation of Radiolabeled Substrates in Wastewater

Test Organisms

c. Trickling Filter Bacteria (Cont'd.)

bacteria are suspended in 200 mls of dilution water and blended for 1 minute. This suspension is then diluted to a specific volume, based on biomass as determined by Total Suspended Matter at 103-105°C (6), in the range of 1000 ± 200 mg/l.

All bacterial systems will be identified using a variation of the acridine orange direct count procedure (AODC) outlined by Hobbie, et. al (12) (See Appendix II).

Test Procedure

Testing is conducted in 2-liter Erlenmeyer flasks containing 1 l of the waste water. Tests are typically conducted in multiples of 12 units at various concentrations, depending on the specific activity of the ¹⁴C-substrate. Generally, the test substance is tested at the lowest concentration possible (based on specific activity) and at 10-fold increments of this concentration. A minimum of 1000 dpm's per 2 mls of water must be present initially to allow accurate LS counting of samples as biodegradation proceeds. For a test substance with a specific activity of 10 μCi/mg, the lowest initial concentration which can be accurately tested is about 25 ppb, e.g.

$$10 \mu\text{Ci/mg} = 2.2 \times 10^7 \text{ dpm/mg}$$

$$= 2.2 \times 10^4 \text{ dpm}/\mu\text{g}$$

$$25 \mu\text{g/l} = 5.5 \times 10^5 \text{ dpm/l}$$

$$= 1100 \text{ dpm}/2 \text{ ml } \underline{\text{initial concentration}}$$

Short of concentration, specific requirements for test design and the type of variables to be tested are highly dependent on the nature of the test substance itself. A water soluble non-sorptive organic would typically be tested at 1x, 10x and 100x concentrations. Specific requirements around test design and the type of variables to be tested are specified by the Principle Investigator. However, a typical activated sludge biodegradation study for a water-soluble, non-sorptive organic substance, with a specific activity of 10 μCi/mg is given below for illustrative purposes:

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VIII. STANDARD TEST METHODS

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B. Biodegradability

SUPERSEDES: NEW

10. Biodegradation of Radiolabeled Substrates in Wastewater
Test Procedure (Cont'd.)

<u>Concentration</u>	<u>Replicates</u>
0 ppb unsupplemented control	3
25 ppb	3
250 ppb	3
2500 ppb	3
250 ppb positive control	3
250 ppb negative control	3

- 1) Two-liter Erlenmeyer flasks containing 1L of wastewater are spiked with the appropriate concentration of ¹⁴C-labeled substrate. Flasks are incubated with shaking (100-150 rpm) for 30 minutes and then triplicate 2 ml zero-time samples are withdrawn by syringe. The 2 ml samples plus 8 ml HW are immediately placed in 10 Mls of Scintillation surfactant cocktail and counted by liquid scintillation techniques to verify concentrations. The number of dpm in the triplicate samples must agree within 10% of each other and within 15% of the theoretical dpm calculated from triplicate counts of the stock solution. If >10% variation occurs in the triplicate zero times, additional triplicate samples are taken to verify test concentrations. If >15% difference occurs between zero-time dpm and stock solution dpm, both are reanalyzed to determine if respiking is necessary. Accurate estimates of the initial concentration are very important since all subsequent calculations are based on the analyzed zero-time values.

Experience with insoluble and highly sorptive test substances has shown distribution equilibration may not have been reached in 30 min. In this case, zero-time concentrations can be determined by LSC on the stock solution used for spiking, or by using the activated sludge results at each sampling time plus the base trap.

- 2) Sampling intervals will vary depending on the rate of biodegradation of the test substances and the results from previous sampling periods. Typically, for a rapidly degraded substance, samples will be taken at 0.5, 1, 2, 3, 5, 7, 10, 14, 21, 28 days. For more slowly degraded substances, weekly sampling intervals may be sufficient. The sampling intervals must be

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B. Biodegradability

10. Biodegradation of Radiolabeled Substrates in Wastewater
Test Procedure (Cont'd.)

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SUPERSEDES: NEW

sufficient to define any acclimation phase occurring for biodegradation, the rate of change of biodegradation occurring over time, and the plateau region where biodegradation decreases. Data points encompassing these three regions are necessary for accurate non-linear regression analysis of biodegradation data (see calculations/interpretations).

- 3) At various intervals, 1 ml samples are withdrawn from all external base traps. The sample from the first trap is counted in 20 ml of Cab-O-Sil cocktail and the remaining samples are wasted. As the study progresses, it is advantageous to submit samples from the second and/or third basetraps to check for carryover. If >2-3% of the counts are present in the second/third traps, include these traps in further sampling. At the same time the base traps are sampled, 10 ml water samples are withdrawn by syringe: 2 ml plus 8 ml of HQW are counted in 10 ml of Scintillation Surfactant cocktail and 2 ml are filtered through a 0.45 μ m membrane filter. The filters are washed once with 2 ml HQW, removed from the filter manifold and counted in 17 ml 3A Toluene cocktail to quantitate radioactivity in microbial biomass.
- 4) The remaining 6 ml of sample are added to a serum vial with a tower housing a filter paper wick saturated with 200 μ l 1.5N KOH. The sealed serum vials are acidified via injection of 200 μ l conc. acid to release radioactivity trapped as $H_2^{14}CO_3$ and/or $H_{14}CO_3$. The vials are then incubated at room temperature for 24 hours.

After 24 hours, 2 ml of the acidified sample are mixed with 8 ml HQW and this is counted in 10 ml Scintillation surfactant. The wick is counted in 17 ml 3A Toluene cocktail. After appropriate volume corrections, the total number of dpm is fractionated into volatile ($^{14}CO_2$) and non-volatile (soluble) categories.

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B. Biodegradability

SUPERSEDES: NEW

10. Biodegradation of Radiolabeled Substrates in Wastewater
Test Procedure (Cont'd.)

- 5) At each sampling (but prior to feeding, see 6 below), dissolved oxygen (D.O.) will be measured in all unsupplemented controls by using a D.O. meter.

Two ten ml samples shall be withdrawn from each unsupplemented control. One 10 ml aliquot will be used to determine mixed liquor suspended solids (MLSS). The remaining aliquot will be used for mixed amino acid (MAA) and/or glucose turnover time determination (see Appendix I.) and Acridine Orange Direct Counts (AODC) (see Appendix II.).

- 6) Units will be fed at each sampling using a synthetic feed of the following composition:

- Peptone	16.0 g
- Beef extract	11.0 g
- Urea	3.0 g
- NaCl	0.7 g
- $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$	0.4 g
- $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	0.2 g
- FeSO_4	0.0 g
- Tap Water	100 ml

Two ml of this feed will be added to each flask at each sampling period to maintain desired solids levels. Changes in the amount fed (0 ml to >2 ml) will be made to maintain solids of ~2500 mg/l in activated sludge and ~1000 mg/l in trickling filter samples.

- 7) Flasks are incubated at $20 \pm 2^\circ\text{C}$ on a rotary platform shaker (75-100 rpm) until degradation reaches plateau values. Testing is terminated by acidifying flasks with 10 ml of conc. acid.
- 8) After acidification, flasks are incubated with shading and aeration for 24-48 hours. Triplicate 5 ml mixed liquor samples are then withdrawn and added to 5 ml of HCl in scintillation vials containing 10 ml of Scintillation surfactant cocktail. If 5 ml samples yield too much quench, count 2 ml + 8 ml HCl as described. Duplicate 1 ml samples are withdrawn from all external base traps and counted in 20 ml of Cab-O-Sil cocktail.

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B. Biodegradability

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10. Biodegradation of Radiolabeled Substrates in Wastewater
Test Procedure (Cont'd.)

- 9) After appropriate volume corrections, the mass balance of radioactivity is determined for all test flasks (see calculations/interpretations).

Calculations/Interpretations

1) Amount of $^{14}\text{CO}_2$ produced

For water-soluble, filterable ^{14}C -test substances, the amount of $^{14}\text{CO}_2$ produced can be determined by one of the following formulas:

$$\% \text{ } ^{14}\text{CO}_2 = 100 - \frac{\text{N.V.} + \text{F.}}{\text{O times}} \times 100 \quad (1)$$

Where: N.V. = counts in non-volatile serum vial water sample

F. = counts on filter

O times = number of counts in solution immediately after spiking

or:

$$\% \text{ } ^{14}\text{CO}_2 = \frac{1.67V(w) + V(b)}{5 \text{ (O times)}} \quad (2)$$

Where: V(w) = volatile counts trapped in the serum vial wick

V(b) = volatile counts trapped in KOH flow-through traps

In equation (1) $^{14}\text{CO}_2$ production is calculated by difference after subtracting the amount of radiolabel present in solution and in biomass from that added initially. In equation (2), $^{14}\text{CO}_2$ production is measured directly by counting the amount of $^{14}\text{CO}_2$ trapped in external base traps and in solution as $\text{H}_2^{14}\text{CO}_3$, H^{14}CO_3 . Equation (1) is most useful for water soluble substances since it is not affected by the amount of $^{14}\text{CO}_2$ present in the headspace of test flasks. Equation (2) suffers from the headspace limitation, but it must be used for non-filterable

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10. Biodegradation of Radiolabeled Substrates in Wastewater
Calculations/Interpretations (Cont'd.)

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substances since non-volatile dpms cannot be determined. The method of determining evolved $^{14}\text{CO}_2$, therefore, depends on the nature of the test substance, and is specified by the Principal Investigator.

In addition to $^{14}\text{CO}_2$ production, the amount of radioactivity incorporated into biomass and remaining free in solution is determined for filterable substances, as follows:

$$\% \text{ } ^{14}\text{C in biomass} = \frac{F}{O \text{ times}} \times 100 \quad (3)$$

$$\% \text{ } ^{14}\text{C in solution} = \frac{NV-F}{O \text{ times}} \times 100 \quad (4)$$

In cases where specific gas chromatography (GC), high performance liquid chromatography (HPLC) or thin-layer chromatography (TLC) methods are available, non-volatile samples can also be fractionated to determine the distribution of radiolabel in parent substance and in metabolic intermediates.

2) Calculations

All calculations regarding $\% \text{ } ^{14}\text{CO}_2$, $\% \text{ } ^{14}\text{C}$ in biomass and $\% \text{ } ^{14}\text{C}$ in solution are done on a 10 ml basis. Corrections for volume between flasks and external base traps (V_b) are not necessary since the same ratio of sample size/total volume is used, i.e. 10/1000 for shake flasks, 1/100 for base traps. The NV dpms from the serum vials are multiplied by 1.5 to correct for the 2 ml sample size counted (on a 10 ml basis). The V_w dpms in the vials are multiplied by 1.67 to correct the 6 ml sample size counted to a 10 ml basis. The O times in equations 1 and 5 are multiplied by 5 to correct the 2 ml sample counted to a 10 ml basis.

The percent mass balance (MB) at any particular sampling point is given by the following formula:

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10. Biodegradation of Radiolabeled Substrates in Wastewater
Calculations/Interpretations (Cont'd.)

$$\% \text{ MB} = \frac{NV + \bar{V}_{(b)}}{5 \text{ (mean 0 times)}} \times 100 \quad (5)$$

Equations 1, 3, and 4 are used to calculate the distribution of radioactivity during the study and the mass balance is arbitrarily set at 100%. The final mass balance at the end study (after acidification) is then calculated as follows:

$$\% \text{ MB} = \frac{2 \bar{NV} + \bar{V}_{(b)}}{5 \text{ (mean 0 times)}} \times 100 \quad (6)$$

Where: \bar{NV} = Average of final triplicate acidified wastewater samples

(Correction factor = 2 if 5 mls removed)
(Correction factor = 5 if 2 mls removed)

$\bar{V}_{(b)}$ = Average sum of final duplicate values of all three base traps

3) Computer Modeling

Product curves for percent CO_2 production as a function of time are analyzed by the following equation, which is a generalized form of the logistics function first described by Richards (9), and modified for use in degradation studies by Larson (10):

$$\text{CO}_2 = a(1 - e^{-k_1 t})^{-1/n} \quad (7)$$

Where: y = biodegradation (% $^{14}\text{CO}_2$)

t = time (days)

a = extent of degradation (% of theoretical CO_2)

k_1 = rate constant (day^{-1})

n = constant

b = coordinate scaling factor

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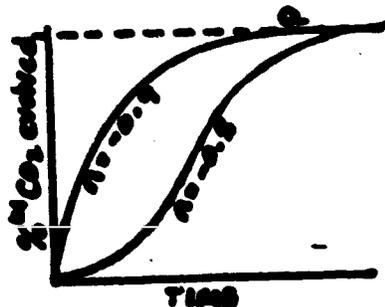
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The logistic function has been used extensively to describe microbial growth kinetics in batch systems, and is used here to estimate parameters for the extent of degradation (a) and the first order rate constant for degradation (k_1), at various substrate concentrations. All parameter estimates are obtained by least squares analysis using iterative techniques and a nonlinear computer program, as previously described (4). Analogous decay curves for removal of ^{14}C -activity from solution can be utilized if desired (11).

The constants a and k_1 , along with their associated 95% confidence intervals, are generated for each concentration of test substance. The values from replicate flasks are averaged and plots of biodegradation vs. time are made by computer. Graphical representations of equation (7) are given below.



Presentation of Results

- a. Identification of test substance by sample code, specific activity, color, form and date received.
- b. Procedures followed for test substance preparation and addition.
- c. Reference to the Protocol (title, author and date), Test Methods, and any analytical or standard operating procedures used.

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B. Biodegradability

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10. Biodegradation of Radiolabeled Substrates in Wastewater
Presentation of Results (Cont'd.)

- d. Reference to laboratory notebook or other file containing raw data.
- e. Starting and ending dates of study.
- f. LSC of stock solution, \pm standard deviation.
- g. Temperature range recorded during test period.
- h. Cells/ml, organic carbon concentration, water hardness and suspended solids, where determined.
- i. Constants generated by the nonlinear regression analysis and the computer plots of cumulative $^{14}\text{CO}_2$ or time.
- j. Mean mass balance in %, \pm standard deviation across replicate units, for each test substance and treatment tested.
- k. Type of wastewater and source used.
- l. Distribution of ^{14}C -activity (% of 0 time) in NV + V(b) for soluble, filterable test substances at each sampling period; distribution of ^{14}C -activity in V(w) + V(b) for insoluble, non-filterables at each sampling period; results for reference substance(s).

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Biodegradation of Radiolabeled Substrates in Wastewater

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2. Gledhill, W. E. 1975. Screening Test for Assessment of Ultimate Biodegradability: Linear Alkylbenzene Sulfonates. Appl. Microbiol. 30: 922-929.
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5. Procter & Gamble Environmental Safety Department - Technical Procedures, IX, A-1, August 19, 1977.
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1. Biodegradability

SUPERSEDES: NEW

Biodegradation of Radiolabeled Substrates in Wastewater
References (Cont'd.)

11. Larson, R. J. and L. M. Games. 1981. Biodegradation of Linear Alcohol Ethoxylates in Natural Waters. Environ. Sci. Technol. 15: 1488-1493.
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ENVIRONMENTAL SAFETY TEST SUMMARY REVIEW

Test Material: CAS RN = 124-28-7

Test Substance Identification Number: B0793.02

Contract Laboratory: Springborn Bionomics

Report #: BW-86-12-2189

Type of Study: Acute Marine Fish Toxicity

Name of Originator:

Date Report Written: 1-9-87

Date Rec'd

1-12-87

This report has been reviewed and found in agreement with the Protocol and there appear to be no inaccuracies in the numerical data or written portions with the following exceptions:

None

Date: 4/15/87

This report has been reviewed for scientific quality and is summarized, with comments (if any), as follows:

Date: 1/21/87

Date: 1/20/87

Date: 1/31/87

es: _____

Microfilming Completed by _____

Date: _____

TEST SUMMARY SHEET

A. 1

B. Text; (Note - tabulations must be limited to 71 characters in width)

Test Type: Acute Marine Fish Toxicity - Static

Test Protocol: Static Acute Marine Fish Toxicity Study of B0793.02 21 July 1986

Species: Sheepshead minnow Dilution Water: Filtered seawater
Gyrinocheilus awaous

Length: 30(23 - 36) mm Salinity: 32 ‰

Height: 0.67(0.22 - 0.64) gm pH: 8.0

Source: Lot # 86445 Test Temperature: 22 degrees C

Results: 96 hr LC50 (95% C.I.): 8.8 (4.4 - 11) mg/L

Control Mortality: 0%

Behavior Observations: Some fish were at the surface of the test solution: 36, 60 mg/L at 24 hours; 8.0 mg/L at 72 and 96 hours.

Reference Response (96 hr LC50): 1.2 (0.67 - 1.8) mg/L sodium lauryl sulfate

Material Solubilization: Material added via stock solution; direct weighing. Solvent used - water; IPA

A foam was present on the surface of all test concentrations throughout the exposure period.

Comments: All results are based on nominal concentrations of the active ingredient tested as 100% active

C. Project Status Sheet Code No. _____

D. Test Type: ACOF Acute Marine Fish Toxicity - Static

E. Test Material: CAS RN = 124-28-7

F. Title/Date: Acute Toxicity of B0793.02 to Sheepshead minnow (Gyrinocheilus awaous)/1/6/87

G. Test Locations: Springboro Biotech H. Lab Project No.: 1011.0286.6185.500

I. Date: ___/___/___
YY MM DD

K. Sponsor's Principal Invest. (sign) _____ (print)

M. TSIN: B0793.02

N. Date Work Done: 30 September - 4 October 1986

bg:TSACOF

ACUTE TOXICITY OF B0793.02
TO SHEEPSHEAD MINNOW
(Cyprinodon variegatus)

TOXICITY TEST REPORT
SUBMITTED TO
THE PROCTER & GAMBLE COMPANY
CINCINNATI, OHIO

REPORT #EW-86-12-2189
STUDY #1011.0986.6184.500

Springborn Bionomics, Inc.
790 Main Street
Wareham, Massachusetts 02571
December 1986

RECEIVED BY

1-1-86

SUMMARY

96-Hour Static Acute (LC50) Test with Sheepshead Minnow

**Springborn Bionomics, Inc.
790 Main Street
Wareham, Massachusetts 02571**

SPONSOR: The Procter & Gamble Company

**TEST PROTOCOL: Static Acute Marine Fish Toxicity Study of
B0793.02; Principal Investigator,
21 July 1986.**

REPORT NUMBER AND DATE: #BW-86-12-2189, December 1986

STUDY NUMBER: #1011.0986.6184.500

MATERIAL: B0793.02 **DATE RECEIVED: 6 August 1986**

**DESCRIPTION: a clear, colorless liquid tested as 100% active
ingredient**

TEST DATE: 30 September - 4 October 1986

SPECIES: Cyprinodon variegatus

Total length: Mean = 30 mm; range = 23 - 36 mm; N = 30

Wet weight: Mean = 0.47 g; range = 0.22 - 0.64 g; N = 30

Source: a commercial supplier in Massachusetts

**DILUTION WATER: Natural filtered seawater from Cape Cod Canal,
Bourne, Massachusetts**

Salinity: 32 ‰

pH: 8.0

TEST TEMPERATURE: 22°C

NOMINAL TEST CONCENTRATIONS: 8.0, 13, 22, 36, 60 and 100 mg/L

RESULTS: The 96-hour LC50 was 8.8 (4.4 - 11) mg/L.

INTRODUCTION

The purpose of this study was to estimate the acute toxicity (LC50) of B0793.02 to sheepshead minnow (Cyprinodon variegatus) under static test conditions. The LC50 is defined as the concentration of the test material in dilution water which causes mortality of 50% in the exposed test population after a fixed period of time. This value is often used as a relative indicator of potential acute hazards resulting from release of the test material into aquatic environments. A 96-hour definitive test was conducted from 30 September - 4 October 1986 at the Springborn Bionomics, Inc., laboratories in Wareham, Massachusetts. All raw data produced during the study are stored at the above location.

MATERIALS AND METHODS

Test Material

The B0793.02, a clear, colorless liquid tested as 100% active ingredient, was received from The Procter & Gamble Company, Cincinnati, Ohio on 6 August 1986. Test concentrations are reported as milligrams of B0793.02 per liter of solution (mg/L).

Protocol

Procedures used in this acute toxicity study followed those described in the protocol entitled "Static Acute Marine Fish Toxicity Study of B0793.02" Principal Investigator, 21 July 1986) issued to Springborn Bionomics, Inc., by The Procter & Gamble Company, Cincinnati, Ohio. This protocol closely follows those described in "Standard Practice for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates and Amphibians" (ASTM 1980).

Test Organisms

The sheepshead minnow (Bionomics lot #86A45) were obtained from a commercial supplier in Massachusetts and held in a 500-L fiberglass tank under a photoperiod of 16 hours light and 8 hours darkness. A closed loop recirculating filtration system provided natural seawater with a salinity of 32 ‰, a pH range of 7.1 - 7.2, and a dissolved oxygen concentration range of 91 - 96% of saturation (Weekly Record of Fish Holding Water Characteristics). Test fish were maintained under these conditions for a minimum of 14 days. The temperature range in the holding tank was 21 - 22°C during this period. The fish were fed a dry commercial pelleted food, ad libitum, daily except during the 48 hours prior to testing. There was no mortality of the test fish population during this 48-hour period (Daily Record of Fish Holding

Conditions). The mean wet weight of the test fish population was 0.47 g (range 0.22 - 0.64 g, N=30) and the mean total length was 30 mm (range 23 - 36 mm, N=30) (Fish Weight and Length Log).

Reference Test

A sodium lauryl sulfate reference test was conducted with the test fish population from 30 September - 4 October 1986. The resulting 96-hour LC50 and 95% confidence interval was 1.2 (0.67 - 1.8) mg/L (Reference Test Log).

Dilution Water

The dilution water used was natural seawater collected from the Cape Cod Canal, Bourne, Massachusetts. The seawater was filtered through a 5- μ m porosity polypropylene core filter and an activated carbon canister before use. The dilution water was characterized at test initiation as having a salinity of 32‰, and a pH of 8.0.

Test Procedures

The toxicity test was conducted in 18.9-L glass aquaria which contained 15 L of test solution. The test solution depth was 18 cm with a surface area of 985 cm². A clear, colorless stock solution of 200 mg/mL was prepared by diluting 10 grams of

B0793.02 with isopropyl alcohol (IPA) to volume in a 50 mL volumetric flask. The appropriate volume of stock solution was then added to 15 L of dilution water in each test aquaria and mixed by stirring with a glass rod. Two control aquaria were established containing the same dilution water and maintained under the same conditions as the test aquaria but containing no B0793.02; one control aquarium contained the maximum quantity of solvent present in any test vessel (0.5 mL/L) and was designated the solvent control.

All test solution temperatures were controlled by a system designed to maintain temperatures at $22 \pm 1^\circ\text{C}$. Test solutions were not aerated. The photoperiod during testing was the same as that provided in the fish culture area.

Ten sheepshead minnow selected impartially from the holding tank were placed in each test aquaria within 10 minutes after the test solutions had been prepared. The resulting test organism loading concentration was 0.31 grams of biomass per liter of test solution. Fish were not fed during exposure.

Test Monitoring

All aquaria were examined after 0, 24, 48, 72 and 96 hours of exposure as follows: mortalities were recorded, dead fish were removed, and observations of the fish and the physical

characteristics of the test solutions were recorded. Dissolved oxygen concentrations and pH were measured in the controls and all test concentrations, and temperature was measured in the control aquaria. If 100% mortality of the test organism population was observed in any test concentration, water quality determinations were made at that time, but further determinations for that concentration were discontinued.

Water Quality Measurements

Salinity concentrations presented in this report were measured with a refractometer distributed by Argent Laboratories. The pH was measured with an Instrumentation Laboratory Model #175 pH meter and combination electrode. Dissolved oxygen concentrations were measured with a YSI Model #57 dissolved oxygen meter and probe. Temperatures were measured with a Brooklyn alcohol thermometer.

Statistics

The concentrations tested and the corresponding mortality data derived from the toxicity test were used to estimate the median lethal concentrations (LC50) and 95% confidence intervals for each 24-hour interval of the exposure period. The LC50 is defined as the concentration of the test material in dilution water lethal to 50% of the test organism population at the stated time interval. LC50 values were empirically estimated as being greater than the highest concentration tested when no test concentrations caused 50% or more mortalities. If at least one test concentration caused mortality of greater than or equal to 50% of the test population, then a computer program (Stephan, 1977, 1982) was used to calculate the LC50 values and 95% confidence intervals.

Three statistical methods were available in the computer program: moving average angle analysis, probit analysis, and nonlinear interpolation with 95% confidence intervals calculated by binomial probability. Moving average angle and probit analyses yield statistically sound results only if at least two concentrations produce a mortality of between 0 and 100% of the test population. The selection of reported LC50 values and 95% confidence intervals were based upon an examination of the data base and the results of the computer analysis. Selection criteria included the establishment of a concentration-effect

relationship (mortality), the number of concentrations causing partial mortalities, and the span of responses bracketing the LC50 value. If two or more statistical methods produced acceptable results, then the method which yielded the smallest 95% confidence interval was selected.

RESULTS

The nominal test concentrations, the corresponding cumulative mortalities and the observations made during the test are presented in Table 1. Table 2 summarizes the 24-, 48-, 72- and 96-hour LC50's and corresponding 95% confidence intervals. The 96-hour LC50 and 95% confidence interval for sheepshead minnow exposed to B0793.02 were calculated by probit analysis to be 8.8 (4.4 - 11) mg/L. The pH, dissolved oxygen concentrations and temperatures measured during the toxicity test are presented in Table 3.

Protocol Deviation

Protocol specifies that wide-mouth glass jars will be used as test containers. For this test 18.9 liter glass aquaria were used as test containers.

It is our opinion that this deviation did not affect the results of this study.

Richard B. Nicholson

Richard B. Nicholson
Study Director

1/6/87
date

LITERATURE CITED

APHA, AWWA, WPCF. 1985. Standard Methods for the Examination of Water and Wastewater. 16th Edition, Washington, D.C., 1268 PP.

ASTM Standard E729-80. 1980. Standard Practice for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates, and Amphibians. American Society for Testing and Materials, 1916 Race Street, Philadelphia, PA 19103.

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Stephan, C. E. 1982. U.S. EPA, Environmental Research Laboratory, Duluth, Minnesota. Personal communication to Dr. Lowell Bahner, Chairman ASTM Task Group on Calculating LC50's.

Table 1. Concentrations tested, corresponding cumulative percent mortalities and observations made during the 96-hour static exposure of sheepshead minnow (*Cyprinodon variegatus*) to B0793.02.

Nominal Concentration (mg/L)	Cumulative Mortality (%)			
	24-hour	48-hour	72-hour	96-hour
Control	0	0	0	0
Solvent	0	0	0	0
Control	0	0	0	0
8.0 ^a	0	30	30 ^b	30 ^b
13 ^a	10	80	100	100
22 ^a	60	90	90	90
36 ^a	60 ^c	100	100	100
60 ^a	70 ^b	100	100	100
100 ^a	100	100	100	100

- ^a Foam was on the surface of the test solution.
^b One of the surviving fish was at the surface of the test solution.
^c Two of the surviving fish were at the surface of the test solution.

Table 2. The LC50 values and 95% confidence intervals for sheepshead minnow (*Cyprinodon variegatus*) exposed to 80793.02 for 96 hours.

	LC50 (mg/L)	Confidence Limits	
		Lower (mg/L)	Upper (mg/L)
24-hour ^a	29	22	38
48-hour ^b	9.8	5.8	13
72-hour ^b	8.8	4.4	11
96-hour ^b	8.8	4.4	11

^a LC50 value and 95% confidence interval calculated by moving average analysis

^b LC50 value and 95% confidence interval calculated by probit analysis

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Table 3. pH, dissolved oxygen concentration, and temperature measured during the 96-hour static exposure of sheephead minnow (*Cyprinodon variegatus*) to B0793.02.

Nominal Concentration (mg/L)	0-hour	24-hour	48-hour	72-hour	96-hour
			pH		
Control	8.0	7.7	7.6	7.4	7.4
Solvent Control	8.0	7.7	7.5	7.4	7.4
8.0	8.0	7.7	7.6	7.4	7.3
13	8.0	7.7	7.6	7.5	---
22	8.0	7.8	7.7	7.7	7.7
36	8.0	7.8	7.7	---	---
60	8.0	7.8	7.6	---	---
100	8.0	7.8	--- ^a	---	---
		<u>Dissolved Oxygen, mg/L</u> (% Saturation)			
Control	6.9 (96)	4.1 (57)	3.9 (54)	2.4 (33)	2.2 (31)
Solvent Control	6.9 (96)	3.4 (47)	2.1 (29)	2.0 (28)	2.9 (40)
8.0	6.9 (96)	3.7 (51)	3.1 (43)	1.7 (24)	1.4 (19)
13	6.9 (96)	4.0 (56)	3.0 (42)	2.2 (31)	---
22	6.9 (96)	4.4 (61)	4.1 (57)	4.0 (56)	4.5 (62)
36	6.9 (96)	4.3 (60)	2.9 (40)	---	---
60	6.9 (96)	4.1 (57)	2.0 (28)	---	---
100	5.8 (94)	4.5 (62)	--- ^a	---	---
		<u>Temperature (°C)</u>			
Control	22	22	22	22	22

^a Measurements not required due to 100% mortality of test organisms at previous 24-hour interval.

0136

The data and report prepared for this study were produced and compiled in accordance with all pertinent EPA Good Laboratory Practice regulations except in the case of characterization and verification of the test substance identity. Maintenance of these records is the responsibility of the test sponsor.

Richard B. Nicholson

12/17/82

Richard B. Nicholson
Study Director

date

The data contained in this report were audited by the Quality Assurance Unit to assure compliance with the protocols, standard operating procedures, and the pertinent EPA Good Laboratory Practice Regulations on the following date: 22 December 1986. If discrepancies were found, reports were made immediately to the Study Director and management. It is the opinion of this unit that these data accurately reflect the raw data generated during this study.

Jane P. Mayo
Jane P. Mayo
Quality Assurance Unit

1/2/87
date

SUBMITTED BY:

Springborn Bionomics, Inc.
790 Main Street
Wareham, Massachusetts 02571
December 1986

STUDY DIRECTOR:

Richard B. Nicholson

Richard B. Nicholson 1/6/87
Aquatic Toxicologist

APPROVED BY:

Donald C. Surprenant

DC Surprenant 1/1/87
Director, Aquatic Toxicology

DATA AUDITED BY:

Jane P. Mayo

Jane P. Mayo 1/2/87
Quality Assurance Unit

SI²



THE PROCTER & GAMBLE COMPANY

MIAMI VALLEY LABORATORIES

August 4, 1986

P. O. BOX 39175
CINCINNATI, OHIO 45247

Mr. Robert Bentley
Springborn Bionomics, Inc.
790 Main St.
Wareham, MA 02571

Dear Mr. Bentley:

This is to authorize you to carry out the following study according to the attached protocol, and in conformance with the stipulations of our current Laboratory Services Agreement.

Protocol: Static Acute Marine Fish Toxicity Study of B0793.02

Date: 7/21/86

Sponsor's Principal Investigator:

Notice: The stipulations of this protocol are to be implemented in conformance with EPA Good Laboratory Practice Regulations (40 CFR, Part 792).

Please have the Study Director approve and complete both copies of the attached protocol by adding your study or project number, estimated starting and reporting dates, and date the test material was received. Retain one copy for your files and return the other to me.

Please return all unused portions of the test material to the Sponsor's Principal Investigator at the following address:

We understand the estimated cost for this study is \$1,125. Invoices are also to be sent to the Principal Investigator.

Three copies of the final report are to be sent to my attention at the letterhead address.

Matters involving the scientific aspects of the work can be handled directly with the Sponsor's Principal Investigator. Feel free to contact me at if you have any other questions or concerns.

Sincerely,

THE PROCTER & GAMBLE COMPANY

Approved:

Attachments
cc: Study File

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PROTOCOL

SPONSOR: The Procter & Gamble Company; Cincinnati, Ohio
Springborn Bionomics, Inc.

LABORATORY: 790 Main St.
Wareham, MA 02571

TITLE: Static Acute Marine Fish Toxicity Study of B0793.02

OBJECTIVE: To determine the 96-hr LC50 of the test material to a marine fish species.

JUSTIFICATION OF TEST SYSTEM: The Sheepshead minnow (Cyprinodon variegatus) is a readily available marine fish species, on which a large amount of toxicity data exists, and is recommended in ASTM E729-80¹, as a standard test organism.

TEST MATERIAL:

Sample Code B0793.02 Color CLEAR Form LIQUID
X Active 100 Density — Solubility LOW IN WATER
Expiration Date 11-86 Other —

Storage Conditions - ROOM TEMPERATURE

Safe Handling Precautions - AVOID SKIN OR EYE CONTACT. IF CONTACT OCCURS, FLUSH WITH WATER

The Sponsor accepts full responsibility for appropriate characterization and stability verification of this test material.

TEST MATERIAL ADDITION/PREPARATION: All calculations and measurements are to be based on the active ingredient. The maximum concentration to be tested is 1000 mg/L active ingredient. Dissolve in water (~40°C) IFA.

TEST ORGANISM:

Species - Sheepshead minnow (Cyprinodon variegatus)

Age - All fish will be from the same year class and greater than 7 days old.

Length and Weight - The longest fish will be no more than twice the standard length of the shortest fish. Average weight will not exceed 1.2 g (confirmation of lengths and weights is not required for fish less than 3 weeks old).

Acclimation - Fish will be acclimated to the test temperature for a minimum of 48 hrs in the "dilution water" or water of similar chemical composition to the "dilution water". A batch of fish will not be used for testing if cumulative mortality exceeds 3% during this acclimation period.

Feeding - Fish will be fed during holding and the acclimation period following laboratory's standard operating procedures. Fish will not be fed during the test period.

¹ASTM Standard E729-80, Standard Practice for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates, and Amphibians. American Society for Testing and Materials, 1916 Race Street, Philadelphia, PA 19103.

TEST CONTAINERS: Wide-mouth glass jars (approximately 4- or 20-L capacity) containing 3 or 15 L of test solution, respectively.

DILUTION WATER: Natural, filtered (5-micrometers) seawater, salinity 15-35 parts per thousand (‰) and pH 8.0 ± 0.5.

TEST CONDITIONS:

- 1) Temperature - 22 ± 1°C.
- 2) The dissolved oxygen concentration in seawater in each test container will be >90% of saturation on Day 0, before addition of the test material.
- 3) The fish biomass-to-water ratio will not exceed 0.8 g/L.
- 4) A control and at least five concentrations of the test material are to be used. Except for the control, the concentration of test material in each treatment must be >60% of the next higher one.

OPERATION: A minimum of 10 fish for each test concentration and the control will be randomly added to the test containers following laboratory's standard operating procedures. Fish will be added within 1 hour after the addition of the test material. One treatment should kill or affect more than 65% of the fish and one treatment (not the control) should kill or affect less than 35% of the exposed fish. The test will be conducted for 96 hours, commencing when the fish are first exposed to the test material.

If any additive (solvent or carrier other than seawater or deionized water) is used to solubilize the test material, another control containing the greatest volume of additive present in any container will also be maintained.

The test will not be valid if mortality in either control is >10%.

WATER CHEMISTRY: Dissolved oxygen (DO) concentrations and pH will be determined at 0, 48, 72, and 96 hours for all test concentrations and the control(s). When 100% mortality is observed in a test concentration, DO and pH determinations are to be made then, but further determinations for that concentration are discontinued.

OBSERVATIONS: Observations of mortality, behavior, and morbidity are to be made and recorded at each 24 hour period for all test concentrations and control(s). Of specific interest are (a) erratic swimming, (b) partial loss of equilibrium, (c) complete loss of equilibrium, (d) any lack of appendage (gill bailer) movement, and (e) no response to touching with a glass rod. Dead fish will be removed during these observations.

RANGE-FINDING TEST: Will be conducted at the discretion of the testing laboratory unless otherwise specified.

CALCULATIONS: Test results are used to calculate the 96-hr LC50 following laboratory's standard operating procedure. The 24, 48, and 72 hour LC50's are calculated when possible. The LC50 is defined as the calculated concentration of the test material which causes 50% mortality in populations of test fish at the specified time of exposure. Results are to be calculated and reported on the basis of added (nominal) test concentrations or from concentrations confirmed by actual analysis, if requested.

RECORDS TO BE MAINTAINED: All records necessary to reconstruct the study and demonstrate adherence to the Protocol.

PROTOCOL CHANGES: If a change in the approved protocol becomes necessary, verbal agreement should be made between the Study Director and Sponsor's Principal Investigator. As soon as practical thereafter, this change and the reasons for it should be put in writing, approved by both persons, and attached to the protocol as an addendum.

REPORTING: The report is to be a typed document in triplicate, describing the results of the study and is to be signed and dated by the Study Director, Quality Assurance Officer and Laboratory Manager. It is to include, but is not limited to, the following:

- 1) Identification of test material by sample code, percent active, color, form, and date received.
- 2) Procedures followed for test material preparation and addition.
- 3) Reference to laboratory notebook or other file containing raw data.
- 4) Date definitive test was conducted.
- 5) Species tested, source, age, and mean and range of the length and weight if fish are greater than 3 weeks old.
- 6) Percentage mortality in all test containers, including the control.
- 7) Calculated LC50 values, 95% confidence intervals, and reference to the method used to calculate these values.
- 8) Description of dilution water used, including the source, measured pH, and salinity.
- 9) Description of holding conditions and acclimation procedures.
- 10) All temperature, pH, and DO determinations and all visual observations.
- 11) Laboratory Study Number.
- 12) Reference to Protocol (title, author, and date) and addenda if made, and any analytical procedures used.
- 13) Any Protocol deviations and their implications.
- 14) Description of the quality assurance methods used to insure the quality of the data.

ALTERNATE PRINCIPAL INVESTIGATOR

PHONE: _____

NOTED: _____

PHONE: _____

APPROVE

PHONE: _____

TO BE COMPLETED BY STUDY DIRECTOR:

Study No. 104-0920-6184-50

Estimated Starting Date 6 Oct. 1986

Estimated Reporting Date 2 January 1987

Date Test Material Received 6 August 1986

Approved

Richard B. Anderson
Study Director

9/2/86
Date

PHONE: 617 295-2550

bg:PRSAMP

1143

A. MATERIAL

Formula or nature of product: (Exact Structure/Nominal Composition; Analytical Information)

Octadecyl-dimethylamine

Active Molecular Weight 297

SPECIAL INFORMATION: (additional information of value not included in above, reference to testing done on similar formulations or chemicals, etc.).

Originator _____ Dept. Code _____ Date: 2/3/86

B. ENVIRONMENTAL TESTS:

Algae Toxicity (Microcystis and Selenastrum) ^{Lab} *Melinda Proulx*
- Marine Fish Toxicity (Sheepshead minnow) *Brown*
Marine Invertebrate Toxicity (mysid shrimp) *"*

SAMPLE IDENTIFICATION

B0793-02

Est. Cost \$8,500

Est. Reporting Date 9/10/86

Date: 7/18/86

RHE392/dh
7/18/86

11 X 4 4

TEST SUBSTANCE CHARACTERIZATION REPORT (TSCR)

Test Substance Identification Number (TSIN): D0793-02
 Safety Test Request Number: _____
 Principal Investigator: _____

Product or Ingredient: _____ Brand Notebook Ref: _____
 Physical Description: Clear Liquid Solubility: NK pH: NK
 Recommended Storage Conditions: Room Temperature Expiration Date: 11-86
 Hazards (i.e. flammability, toxic gases): Alkaline Corrosive
 Dept. of Transportation Hazard Classification: Cor. HAZ713 CAS No. (a): 124-28-7

Formulated Composition (b)

Component (c)	Nom. Wt.	Nominal Level (X by Wt.)	Acceptable Range	Stock Code No.	Supplier	Lot Number (NB-Ref.)
(Alkyl Dimethylamine)	297	100% -NK-	7/2/85	NK	Ethyl Corp	ADMA 8-0

- (a) Include CAS number(s) for the three most major components of a formulation or for single chemical products. Footnote to the material with which the respective number is associated.
- (b) If information requested is not known, then the symbol NK will be entered.
- (c) Chemical names which are inconveniently long may be abbreviated in tables but should be listed in full in referenced footnotes. Non-chemical names, such as Tergitol 15-8-0 or Yellow Dye #10, may not be acceptable but should be previewed with the responsible toxicologist. Nondescriptive identification (e.g. Aquad, BC-base) is not acceptable.

The above information provided by: _____ (Name) _____ (Signature) 0/23/85 (Date)

The above information reviewed and accepted by: _____
 Principal Investigator: _____ (Signature) 7/2/85 (Date)

Test Substance Identification Number (TSIN): 20793-02

Analyzed Composition

<u>Date Submitted</u>	<u>Submitter Code</u>	<u>Analysis Code/Analysis</u>	<u>Estimated Value</u>	<u>Measured Value</u>	<u>Testing Laboratory</u>
-----------------------	-----------------------	-------------------------------	------------------------	-----------------------	---------------------------

Analytical Information Verified By:

(Signature)

Date:

to: 2/4/82

This test substance is suitable for human (clinical) safety testing.

Principal Investigator:

(Signature)

Date:

11 1 4 5

ENVIRONMENTAL SAFETY TEST SUMMARY REVIEW

Contains No. 100

Test Material Name: CAS RN = 124-28-7
Test Substance Identification Number: 80793.01 project # suffix): (02)
Type of Study: Daphnia static acute Report or Project #: 85-4-1758
Name of Originator: _____
Laboratory Involved: Springer Biomatrix; Waverham, MA
Date Report Written: 5/16/85 Date Received by Operations Section: 5/21/85

This report has been received and found in agreement with the Protocol and there appear to be no inaccuracies in the numerical data or written portions with the following exceptions:
None

Logistics Review _____ Date 6/26/85

This report has been reviewed for scientific quality and is summarized with the following comments:

RECEIVED
OPPT CBIC
94 JUL 14 AM 9:13

Principal Investigator _____ Date 6/28/85

This report is recommended for entry into _____
Date 7/17/85

File approved for entry: _____
Date 7-25-85

Entered into _____ Date _____ By _____

Microfilming Completed: Date _____ By _____

Copy returned to: _____ Date _____

TEST SUBSTANCE CHARACTERIZATION REPORT (TSR)

Test Substance Identification Number (TSIN): ED793.D1

Principle Investigator: _____

Product or Ingredient: _____
Physical Description: White Solid/Semi solid Solubility: NK pH: NK
Recommended Storage Conditions: Room Temperature Expiration Date: > 1 year
Hazards (i.e. flammability, toxic gases): Non-Hazardous
Dept. of Transportation Hazard Classification: Non-Hazardous CAS No. (a): 124-28-7

Formulated Composition (b)

<u>Component (c)</u>	<u>Nom. Wt.</u>	<u>Nominal Level (X by Wt.)</u>	<u>Acceptable Range</u>	<u>Stock Code No.</u>	<u>Supplier</u>	<u>Lot Number (NB-Ref.)</u>
<u>Di-Methyl Stearyl Amine (</u>	<u>297</u>	<u>100.00</u>		<u>NK</u>		<u>4069-S-666334</u>

- (a) Include CAS number(s) for the three most major components of a formulation or for single chemical products. Footnote to the material with which the respective number is associated.
- (b) If information requested is not known, then the symbol NK will be entered.
- (c) Chemical names which are inconveniently long may be abbreviated in tables but should be listed in full in referenced footnotes. Non-chemical names, such as Tergitol 15-S-0 or Yellow Dye #10, may not be acceptable but should be previewed with the responsible toxicologist. Nondefinitive identification (e.g. Arquad, DC-base) is not acceptable.

The above information provided by:

12/18/84
(Date)

The above information reviewed and accepted by:

Principle Investigator:

12/18/84
(Date)

Test Substance Identification Number (TSIN):

Analyzed Composition

<u>Date Submitted</u>	<u>Submitter Code</u>	<u>Analysis Code/Analysis</u>	<u>Estimated Value</u>	<u>Measured Value</u>	<u>Testing Laboratory</u>
-----------------------	-----------------------	-------------------------------	------------------------	-----------------------	---------------------------

See page 1

Analytical Information Verified By:

(Signature)

Date:

This test substance is suitable for animal (non-clinical) safety testing.

Principle Investigator:

(Signature)

Date:

This test substance is suitable for human (clinical) safety testing.

Principle Investigator:

(Signature)

Date:



THE PROCTER & GAMBLE COMPANY

MIAMI VALLEY LABORATORIES

P. O. BOX 39175
CINCINNATI, OHIO 45247

January 24, 1985

Mr. Robert Bentley
Springborn Bionomics, Inc.
790 Main St.
Wareham, MA 02571

Dear Mr. Bentley:

This is to authorize you to carry out the following study according to the attached protocol, and in conformance with the stipulations of our current Laboratory Services Agreement.

Protocol: Static Acute Freshwater Invertebrate Toxicity of B0793.01

Date: 12/21/84

Sponsor's Principal Investigator

Notice: The stipulations of this protocol are to be implemented in conformance with EPA Good Laboratory Practice Regulations (40 CFR, Part 792).

Please have the Study Director approve and complete both copies of the attached protocol by adding your study or project number, estimated starting and reporting dates, and date the test material was received. Retain one copy for your files and return the other to me.

Please return all unused portions of the test material to the Sponsor's Principal Investigator at the following address:

The Procter & Gamble Company
Sharon Woods Technical Center
11520 Reed Hartman Hwy.
Cincinnati, OH 45241

We understand the estimated cost for this study is \$750. Invoices are also to be sent to the Principal Investigator.

Matters involving the scientific aspects of the work can be handled directly with the Sponsor's Principal Investigator. Feel free to contact me at (513) 245-2120, if you have any other questions or concerns.

Sincerely,

THE PROCTER & GAMBLE COMPANY

0 1 5 0

PROTOCOL

SPONSOR: The Procter & Gamble Company; Cincinnati, Ohio
Springborn Economics, Inc.

LABORATORY: 790 Main St.
Wareham, MA 02571

TITLE: Static Acute Freshwater Invertebrate Toxicity Study of D0793.01

OBJECTIVE: To determine the 48 hr LC₅₀ of the test material to a freshwater invertebrate species.

JUSTIFICATION FOR TEST SYSTEM: Daphnia magna is a readily available freshwater invertebrate species, on which a large amount of toxicity data exists, and is recommended by the USEPA¹ as a standard test organism.

TEST MATERIAL:

Sample Code D0793.01 Color Milky Form liquid or gel
% Active 100 Density -- Solubility 5-10% in IPA
Expiration Date in progress Other Material will melt and clarify at 150°F

Storage Conditions - Ambient

Safe Handling Precautions - USE CAUTION. Avoid contact with eyes and skin, if exposure occurs, rinse thoroughly with water. Strong fishy odor. *Prepare stock solution under hood, as per OSHA instructions if applicable*
The Sponsor accepts full responsibility for appropriate characterization and stability verification of this test material.

TEST MATERIAL ADDITION/PREPARATION: All calculations and measurements are to be based on the active ingredient. The maximum concentration to be tested is 1000 mg/L active ingredient. Stock solution to be prepared in IPA. Maximum solvent in test as per laboratory SOI.

TEST ORGANISM:

Species - Daphnia magna

Handling and Preparation - On the day preceding test initiation, reproductively mature adults are to be isolated from any young to assure only adult sizes are present in the stock cultures. The young produced by these isolated adults are to be removed from the stock cultures and used for testing. The maximum time to initiate a test following isolation is 36 hours.

TEST CONTAINERS: 250 ml glass beakers containing 200 ml of test solution. Beakers are to be covered with glass or clear plastic during the test period to minimize evaporation.

¹USEPA, Committee on Methods for Toxicity Tests with Aquatic Organisms (1975) Methods for Acute Toxicity Tests with Fish, Macroinvertebrates, and Amphibians. EPA-3-75-009.

DILUTION WATER: Water of the same characteristics as the water normally used for culturing Daphnia in the laboratory.

TEST CONDITIONS:

- (1) Maintain temperature at $21 \pm 1^{\circ}\text{C}$.
- (2) The dissolved oxygen concentration in each test container must be at least 90% of saturation on Day 0.
- (3) A control and at least five concentrations of the test material are to be used. Except for the control, the concentration of test material in each treatment must be at least 36% of the next higher one.

OPERATION:

Five Daphnia are randomly assigned to each test container, following the laboratory's standard operating procedure, within 30 minutes after the addition of the test material. A minimum of three test containers are to be used for each test concentration and the control. One treatment must have killed or affected more than 65% of the Daphnia, and one treatment (not the control) must have killed or affected less than 35% of the Daphnia. The test is conducted for 48 hours, commencing when the test Daphnia are first exposed to the test material. Daphnia are not to be fed during the test period.

If any additive (e.g. solvent) is used to solubilize the test material, another control containing the greatest amount of solvent present in any other container is also required. A test is not valid if there is a greater than 10% mortality in either control.

WATER CHEMISTRY: Dissolved oxygen (DO) and pH are measured at 0 and 48 hours in at least one replicate for all test concentrations, including the controls. The 0 hour measurements are taken prior to the addition of the test organisms. If 100% mortality is observed in a test concentration at 24 hours, determinations are to be made at that time, but further determinations for that concentration are not required.

SPECIAL INSTRUCTIONS (If any, for operation, sampling, analyses, or monitoring):

RANGE-FINDING TEST: To be conducted at the discretion of the laboratory unless otherwise specified.

OBSERVATIONS: Mortality observations are to be made and recorded at 24 and 48 hours for all test containers, including the control. The criteria for death is the lack of reaction to gentle prodding.

CALCULATIONS: Test results are used to calculate the 48 hour LC_{50} . The 24 hour LC_{50} is calculated when possible. The LC_{50} is defined as the calculated concentration of the test material which causes 50% mortality in populations of test organisms at the specified time of exposure. Results are to be calculated and reported on the basis of added (nominal) test concentrations or from concentrations confirmed by actual analysis, if requested.

RECORDS TO BE MAINTAINED: All records necessary to reconstruct the study and demonstrate adherence to the Protocol.

PROTOCOL CHANGES: If a change in the approved protocol becomes necessary, verbal agreement should be made between the Study Director and Sponsor's Principal Investigator. As soon as practical thereafter, this change and the reasons for it should be put in writing, approved by both persons, and attached to the protocol as an addendum.

REPORTING: The report is to be a typed document in triplicate, describing the results of the study and is to be signed and dated by the Study Director, Quality Assurance Officer and Laboratory Manager. It is to include, but is not limited to, the following:

- 1) Identification of test material by sample code, percent active, color, form, and date received.
- 2) Procedures followed for test material preparation and addition.
- 3) Reference to laboratory notebook or other file containing raw data.
- 4) Date definitive test was conducted.
- 5) Species tested, including the source and age.
- 6) Percentage mortality in all test treatments, including the control.
- 7) The calculated LC₅₀ values, 95% confidence intervals, and a reference to the method used to calculate these values.
- 8) Description of dilution water used, including a range of the measured pH, hardness, alkalinity, and conductivity.
- 9) All temperature, pH, and DO determinations and all visual observations.
- 10) Laboratory Study Number.
- 11) Reference to the Protocol (title, author, and date) and addenda if made, and any analytical procedures used.
- 12) Any Protocol deviations and their implications.
- 13) Results of reference toxicant and date conducted.
- 14) Description of the quality assurance methods used to insure the quality of the data.

ALTERNATE PRINCIPAL INVESTIGATOR

NOTED: _____ Date

APPROVED: _____ 12/21/84
Date

TO BE COMPLETED BY STUDY DIRECTOR:

Study No. 1011-0185-6126-110

Estimated Starting Date 1-25-85

Estimated Reporting Date 3-22-85 (Nebel 2-1-85)

Date Test Material Received 1-25-85

Approved Richard B. Thibault 1/5/85
Study Director Date

(617)
PHONE: 295-2550

PROTO/eja

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ACUTE TOXICITY OF B0793.01

TO Daphnia magna

TOXICITY TEST REPORT

SUBMITTED TO

THE PROCTER & GAMBLE COMPANY

CINCINNATI, OHIO

BIONOMICS REPORT #BW-85-4-1758

BIONOMICS STUDY #1011-0185-6126-110

Springborn Bionomics, Inc.
Aquatic Toxicology Laboratory
790 Main Street
Wareham, Massachusetts
April, 1985

DATE: 5/21/85

0154

SUMMARY

48-Hour Static Toxicity Test: Freshwater Invertebrate

**Springborn Bionomics, Inc.
Aquatic Toxicology Laboratory
790 Main Street
Wareham, Massachusetts**

CLIENT: The Procter & Gamble Company

PROTOCOL CITED: Static Acute Freshwater Invertebrate

Toxicity Study of B0793.01; Principal

Investigator

21 December 1984.

REPORT NUMBER AND DATE: BW-85-4-1758, April, 1985

STUDY NUMBER: 1011-0185-6126-110

MATERIAL: B0793.01

**DESCRIPTION: a cloudy white-colored gel tested as 100%
active ingredient**

DATE MATERIAL RECEIVED: 25 January 1985

TEST DATE: 30 January - 1 February 1985

SPECIES: Daphnia magna

Age: <24 hours old

Source: Bionomics culture facility

DILUTION WATER: Fortified well water

Total hardness as CaCO₃: 160 mg/L

Total alkalinity as CaCO₃: 110 mg/L

pH: 7.9

Specific conductivity: 500 umhos/cm

TEST TEMPERATURE: 20°C

METHOD OF TEST MATERIAL ADDITION: stock solution; 0.60 mg of
B0793.01/mL isopropyl
alcohol

TEST CONCENTRATIONS: 0.30, 0.18, 0.11, 0.066, 0.039, and
0.023 mg/L of B0793.01

RESULTS: Results are reported as nominal concentrations of
B0793.01 based on 100% active ingredient. The 48-
hour LC50 was calculated by moving average angle
analysis to be 0.042 mg/L with a 95% confidence
interval of 0.034-0.050 mg/L.

INTRODUCTION

The purpose of this study was to estimate the acute toxicity of B0793.01 to daphnids (Daphnia magna) under static conditions. A 48-hour definitive test was conducted from 30 January - 1 February 1985, at the Aquatic Toxicology Laboratory of Springborn Bionomics, Inc., Wareham, Massachusetts. All raw data generated are stored at the above location.

MATERIALS AND METHODS

The B0793.01, a cloudy white-colored gel tested as 100% active ingredient, was received from The Procter & Gamble Company, Cincinnati, Ohio, on 25 January 1985. Nominal test concentrations are reported as milligrams of B0793.01 active ingredient per liter of solution (mg/L).

Procedures used in this acute toxicity study followed those described in "Methods for acute toxicity tests with fish, macroinvertebrates, and amphibians" (U.S. EPA, 1975) and the protocol entitled "Static Acute Freshwater Invertebrate Toxicity Study of B0793.01" (R. H. Hall, Principal Investigator, 21 December 1984).

The daphnids used in this toxicity test were obtained from laboratory stocks cultured at Springborn Bionomics, Inc., Wareham, Massachusetts. The culture water was prepared

0157

by fortifying well water based on the formula for hard water (U.S. EPA, 1975) and filtering it through a carbon filter and an Amberlite XAD-7 resin column to remove any potential organic contaminants. This water had total hardness and alkalinity ranges as calcium carbonate (CaCO₃) of 160-180 mg/L and 110-130 mg/L, respectively, a pH range of 7.9-8.3, a temperature of 20 ± 1°C, a dissolved oxygen concentration of greater than 60% of saturation and a specific conductance range of 400-600 micromhos per centimeter (umhos/cm).

The daphnid culture area received a regulated photoperiod of 16 hours of light and eight hours darkness. Light at an intensity of 5-10 hectolux at the culture solutions' surface was provided by a combination of Sylvania Growlux^R and Cool White^R fluorescent bulbs. Daphnids were fed a solution of green algae (Ankistrodesmus sp.) and yeast suspension once daily. The ambient air temperature in the culture area was controlled in order to maintain the culture solution temperatures at 20 ± 1°C.

A sodium lauryl sulfate reference test was conducted with the daphnid test population from 29-31 January 1985. The 48-hour LC50 and 95% confidence interval was 6.3 (5.2-7.7) mg/L (Reference Test Log).

The toxicity test was conducted in 250-milliliter (mL) glass beakers. The dilution water used (IRC #474, IWQ-6 Log #

0 1 5 8

Book) was from the same source as the culture water previously mentioned and was characterized as having a total hardness and alkalinity of 160 and 110 mg/L as CaCO₃, respectively, a pH of 7.9 and a specific conductivity of 500 umhos/cm.

A clear, colorless primary stock solution of 2.6 mg/mL was formulated by diluting 0.26 grams of B0793.01 with isopropyl alcohol (IPA) to volume in a 100-mL volumetric flask. A subsequent working stock solution of 0.60 mg/mL was prepared by diluting 23.08 mL of the primary stock solution with isopropyl alcohol to volume in a 100-mL volumetric flask. Test solutions were formulated by adding appropriate volumes of working stock solution (0.60 mg/mL) to dilution water to total 1000 mL. Each solution was mixed on a magnetic stirrer for one minute and then divided into three beakers to provide replicate exposure treatments, each containing 200 mL. The excess 400 mL of test solution was discarded. The test solution depth was 6.2 centimeters (cm) with a surface area of 33 cm². Two sets of three control beakers containing the same dilution water and maintained under the same conditions as the exposure concentrations, but containing no B0793.01, were established. One set contained the maximum quantity of IPA in any test solution (0.5 mL). The air temperature in the laboratory was controlled in order to maintain test solution temperatures at 20 ± 1°C. Test solutions were not aerated. The test area was illuminated

with Sylvania Growlux^R and Cool White^R fluorescent lights at an intensity of 7 hectolux at the solutions' surface. The photoperiod during the test was the same as in the culture area.

Fifteen daphnids, ≤ 24 hours old, were impartially distributed to each concentration (five daphnids per replicate) within twenty minutes after the test solutions had been prepared. Daphnids were not fed during the exposure. Mortalities in replicate test solutions were recorded at 24 and 48 hours of exposure. Biological observations and observations of the physical characteristics of each replicate test solution were also made and recorded at 0, 24, and 48 hours. The pH's and dissolved oxygen concentrations were measured at 0 and 48 hours in one replicate vessel of each test concentration and the controls. Temperature was measured in one replicate vessel of the control at 0, 24 and 48 hours of exposure.

Total hardness concentrations presented in this report were measured by the EDTA titrimetric method and total alkalinity concentrations were determined by potentiometric titration to an endpoint of pH 4.5 (APHA et al., 1980). Specific conductivities were measured with a Yellow Springs Instrument Company (YSI) Model #33 salinity-conductivity-temperature meter and probe; the pH's were measured with an Instrumentation Laboratory Model #175 pH meter and

combination electrode; the dissolved oxygen concentrations were measured with a YSI Model #57 dissolved oxygen meter and probe and the temperatures were measured with a Brooklyn alcohol thermometer. Light intensity was measured with a General Electric type 213 light meter.

Statistics

The concentrations tested and the corresponding mortality data derived from the toxicity test were used to estimate 24- and 48-hour median lethal concentrations (LC50) and 95% confidence intervals. The LC50 is defined as the concentration of the test material in dilution water which caused mortality of 50% of the test animal population at the stated exposure interval. If sufficient toxicant-related mortality occurred during the test (e.g., presence of at least one test concentration causing mortality of $\geq 50\%$ of the animals in the test population), then a computer program (Stephan, 1982, personal communication) was used to calculate the LC50 values. The computer program scanned the data base, identified the most appropriate statistical methods and performed the analyses. Three statistical methods, in the following order of preference, were available in the computer program: moving average angle analysis, probit analysis, binomial probability (Stephan, 1977). The binomial probability method calculates only the 95% confidence interval. In such a case, a point estimate of the LC50 is

obtained by nonlinear interpolation (i.e., logarithm transformation of the concentration and the angle transformation of the percent dead) (Stephan, 1982). The method selected was determined by the above order of preference and by the characteristics of the data base (e.g., presence or absence of several test concentrations causing mortality of a partial number of animals in the respective test population). LC50 values were empirically estimated when insufficient toxicant-related mortality occurred.

RESULTS

The concentrations tested, corresponding mortalities and observations made during the toxicity test are presented in Table 1. The 48-hour LC50 value and 95% confidence interval for D.magna exposed to B0793.01 were calculated by moving average angle analysis to be 0.042(0.034-0.050) mg/L. Table 2 summarizes the LC50 values and 95% confidence intervals. The results of the water quality analyses, presented in Table 3, illustrate that the pH and dissolved oxygen concentrations were unaffected by the concentrations of B0793.01 tested.

0 1 5 2

LITERATURE CITED

APHA, ANWA, WPCF. 1980. Standard methods for the examination of water and wastewater. 15th Edition, Washington, D.C., 1134 pp.

Stephan, C. E. 1977. Methods for calculating an LC50, Aquatic Toxicology and Hazard Evaluation, ASTM STP 634, F. L. Mayer and J. L. Hamelink, Eds., American Society for Testing and Materials, pp. 65-84.

Stephan, C. E. 1982. U.S. EPA, Environmental Research Laboratory, Duluth, Minnesota. Personal communication to Dr. Lowell Bahner, Chairman ASTM Task Group on Calculating LC50's.

U.S. EPA. 1975. Methods for acute toxicity tests with fish, macro-invertebrates, and amphibians. Ecological Research Series (EPA-660/3-75-009), 61 pp.

0-163

Table 1. Concentrations tested, corresponding mortalities and observations made during the 48-hour exposure of daphnids (Daphnia magna) to B0793.01

Nominal concentration (mg/L)	Cumulative Mortality (%)							
	24-hour				48-hour			
	A	B	C	X	A	B	C	X
0.30	100	100	100	100	100	100	100	100
0.18	0	60	40	33 ^{ab}	100	100	100	100
0.11	0	100	0	33	100	100	100	100
0.066	20	0	0	7	80	80	80	80 ^d
0.039	0	0	0	0	60	40	60	53 ^b
0.023	0	0	0	0	20	0	0	7 ^c
solvent control	0	0	0	0	0	0	0	0
control	0	0	0	0	0	0	0	0

^aOne of the surviving daphnids was at the surface of the test solution.

^bSeveral surviving daphnids were lethargic.

^cOne of the surviving daphnids was lethargic and on the bottom of the test vessel.

^dAll of the surviving daphnids were lethargic and on the bottom of the test vessel.

Table 2. The estimated 24- and 48-hour LC50 values for daphnids (Daphnia magna) exposed to B0793.01.

LC50 ^a (mg/L)	
24-hour	48-hour
0.16 (0.13-0.19)	0.042 (0.034-0.050)

^aLC50 values and 95% confidence intervals were calculated by moving average angle analysis.

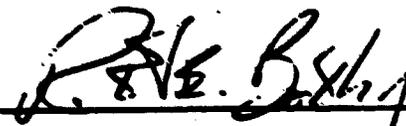
Table 3. The pH's, dissolved oxygen concentrations and temperatures measured during the 48-hour exposure of Daphnia magna to B0793.01.

	Nominal concentration (mg/L)	0-hour	24-hour	48-hour
pH	0.30	8.2	— ^b	7.8
	0.18	8.2	—	7.8
	0.11	8.2	—	7.8
	0.066	8.2	—	7.8
	0.039	8.2	—	7.8
	0.023	8.2	—	7.8
	solvent control	8.1	—	7.8
	control	8.2	—	7.8
Dissolved oxygen (mg/L)	0.30	8.6(93) ^a	—	7.8(85)
	0.18	8.8(96)	—	7.8(85)
	0.11	8.8(96)	—	8.2(89)
	0.065	8.8(96)	—	8.2(89)
	0.039	8.8(96)	—	8.2(89)
	0.023	8.8(96)	—	8.3(90)
	solvent control	8.7(94)	—	8.0(87)
	control	8.5(92)	—	8.0(87)
Temperature (°C)	control	20	20	20

^aPercent of saturation.

^bMeasurement not required at stated time interval.

The data contained in this report were audited by the Quality Assurance Unit to assure compliance with the protocols, standard operating procedures and the pertinent EPA Good Laboratory Practice Regulations on the following dates: 22 March and 10 May 1985. If discrepancies were found, reports were made immediately to the Study Director and management. It is the opinion of this Unit that these data accurately reflect the raw data generated during this study.



Robert E. Bentley
Director, Quality Assurance and
Special Projects

SUBMITTED BY:

Springborn Bionomics, Inc.
Aquatic Toxicology Laboratory
790 Main Street
Wareham, Massachusetts
April, 1985

STUDY DIRECTOR:

Richard B. Nicholson

Richard B. Nicholson 5/16/85

Aquatic Toxicologist

STUDY DIRECTOR:

Donald C. Surprenant

Donald C. Surprenant 4/17/85

Director, Aquatic Toxicology

DATA AUDITED BY:

Robert E. Bentley

R. E. Bentley

Director, Quality Assurance Unit

18

ENVIRONMENTAL SAFETY TEST SUMMARY REVIEW

Test Material Name: CAS RN 124-28-7

Contains No CBI

Test Substance Identification Number: B0793.01

Suffix #: .08

Type of Study: Anaerobic Digestion Inhibition Test (ADIT) Report #: 85-017 1

Name of Originator:

Division:

ORD #:

Laboratory Involved: Weston

Date Report Written: 11-08-85

Date Received by Operations Section: 12-04-85

This report has been received and found in agreement with the Protocol and there appear to be no inaccuracies in the numerical data or written portions with the following exceptions:

None, except as noted on pg 405; and corrections as per Jan letter of 1/3/86.

Logistics Reviewer

Date 6/26/86

This report has been reviewed for scientific quality and is summarized with the following comments:

Principal Investigator

Date 8/6/86

RECEIVED
OPPT/CBC
94 JUL 14 AM 9:12

This report is recommended for entry into

Safety Data System:

Corp Liaison

Date 8/11/86

File approved for entry into the SDRS:

Section Head

Date 3-12-86

(Return

Entered into Safety Data System: Date 9-16-86 By

Microfilming Completed: Date 2-12-87 By

11 169

TEST SUPPORT SHEET

A.

B. Text: (Note - tabulations must be limited to 71 characters in width)

Test Type: Lab Digester

Test Protocol: Anaerobic Digester Study on B0793.01;

3/5/85

Sludge Source: Ocean County, NJ, South Treatment Plant

Initial Conditions (control unit - day 0); mg/l

<u>TSS</u>	<u>VSS</u>	<u>T Alk</u>	<u>Bi Alk</u>	<u>V Acids</u>	<u>COD</u>	<u>pH</u>
21,352	13,328	2,900	2,817	100	29,000	7.3

Results:

<u>Material</u>	<u>Conc. (mg/l)</u>	<u>OEC/NOEC</u>	<u>P₂ Cum gas (ml)</u>	<u>P₂ Rate Day⁻¹</u>	<u>Lag Days</u>
Phenol	250	NOEC	459 ± 27	0.34 ± 0.06	0.0
Phenol	1500	OEC	230 ± 15	0.29 ± 0.04	0.0
<u>B0793</u>	<u>0.1</u>	<u>NOEC</u>	<u>420 ± 107</u>	<u>0.49 ± 0.10</u>	<u>0.0</u>
<u>B0793</u>	<u>1.0</u>	<u>NOEC</u>	<u>410 ± 68</u>	<u>0.46 ± 0.18</u>	<u>0.0</u>
<u>B0793</u>	<u>10</u>	<u>NOEC</u>	<u>323 ± 31</u>	<u>0.46 ± 0.00</u>	<u>0.0</u>
<u>B0793</u>	<u>100</u>	<u>NOEC</u>	<u>325 ± 4</u>	<u>0.48 ± 0.03</u>	<u>0.0</u>
<u>B0793</u>	<u>1,000</u>	<u>NOEC</u>	<u>573 ± 29</u>	<u>0.44 ± 0.01</u>	<u>0.0</u>
<u>Control</u>	<u>----</u>	<u>----</u>	<u>447 ± 2</u>	<u>0.46 ± 0.00</u>	<u>0.0</u>

OEC = observed effect conc.
NOEC = no observed effect conc.

Test Period: 17 days

Test Temperature: 32 ± 1 degrees C

Material tested as 100% active

Test Material Addition: [] added via stock solution in [] H₂O [] _____
[x] added via direct weighing

Comments:

C. Project Status Sheet Code No. B0793.01.08

D. Test Type: LDIG Lab Digester
KEYWORD

E. Test Material: CAS RN = 124-28-7

F. Title/Data: File No. Anaerobic Digestion Inhibition Test (ADIT) on

G. Test Location: Wester H. Lab Project No.: PS-017

I. Date: 85/12/21
YY MM DD

K. Prepared by:

M. TSIN: B0793.01

N. Date Work Done: 6/22/85 - 7/9/85

bg:TSSANA

TEST SUBSTANCE CHARACTERIZATION REPORT (TSCR)

Test Substance Identification Number (TSIN): 92191.D1
 Safety Test Request Number: _____
 Principle Investigator: _____

Product or Ingredient: _____
Physical Description: White Solid/Semi solid Solubility: HK pH: HK
Recommended Storage Conditions: Room Temperature Expiration Date: > 1 year
Hazards (i.e. flammability, toxic gases): Non-Hazardous
Dept. of Transportation Hazard Classification: Non-Hazardous CAS No. (a): 124-28-7

Formulated Composition (b)

<u>Component (c)</u>	<u>Nom. Wt.</u>	<u>Nominal Level (Σ by Wt.)</u>	<u>Acceptable Range</u>	<u>Stock Code No.</u>	<u>Supplier</u>	<u>Lot Number (NB-Ref.)</u>
<u>Ni-Methyl Stearyl Amine</u>	<u>197</u>	<u>100.00</u>		<u>HK</u>	<u>Shorex</u>	<u>4069-S-666334</u>

- (a) Include CAS number(s) for the three most major components of a formulation or for single chemical products. Footnote to the material with which the respective number is associated.
- (b) If information requested is not known, then the symbol HK will be entered.
- (c) Chemical names which are inconveniently long may be abbreviated in tables but should be listed in full in reference/footnotes. Non-chemical names, such as Tergitol 15-S-9 or Yellow Dye #10, may not be acceptable but should be provided with the responsible toxicologist. Nondefinitive identification (e.g. Arquad, DC-base) is not acceptable.

12/13/91
(Date)

The above information reviewed and accepted by:

12/13/91
(Date)

TEST SUBSTANCE CHARACTERIZATION REPORT (TSCR)

Side 2 of 2

Test Substance Identification Number (TSIN):

Analyzed Composition

<u>Date Submitted</u>	<u>Submitter Code</u>	<u>Analysis Code/Analysis</u>	<u>Estimated Value</u>	<u>Measured Value</u>	<u>Testing Laboratory</u>
-----------------------	-----------------------	-------------------------------	------------------------	-----------------------	---------------------------

None

Analytical Information Verified By:

(Signature)

Date:

This test substance is suitable for *animal (rodent) acute subcut.*

Principle Investigator:

Date: *8/5/85*

This test substance is suitable for human (clinical)

Principle Investigator:

(Signature)

Date:



THE PROCTER & GAMBLE COMPANY

MIAMI VALLEY LABORATORIES

January 3, 1986

P. O. BOX 39175
CINCINNATI, OHIO 45247

Mr. Peter J. Marks
Roy F. Weston, Inc.
Weston Way
West Chester, PA 19380

Dear Pete:

We have reviewed the following test report and ask that the items listed below be addressed.

Anaerobic Digestion Inhibition Test (ADIT) on B0793.01; Weston Proj. No. 85-017

1. Summary page - M. Stapleton's and D. Therry's signatures are barely legible. If available, please send a better copy.
2. Page 1 - the protocol title in section 2.0 is incorrect. Also, material is misspelled in the 4/16/85 addendum. Would you provide us a copy of this addendum?
3. Page 2 - the 1.0 mg/l is missing from section 3.b.2.
4. Page 3 - please include the temperature range with the test conditions. This is not a protocol requirement but something you've provided in the past.
5. Page 4 - for future reports, changes in the protocol addressed in amendments should not be reported as deviations (first two items in section 5).
6. Table F-2 - test material concentrations are not included.

Any corrections or additions should be made via an amendment to the report, and be signed and dated by the study director. Three copies of each are to be sent to my attention.

If you have any questions, give me a call.

Sincerely,

THE PROCTER & GAMBLE COMPANY
Research & Development Department

0 1 7 3

WESTON

ANAEROBIC DIGESTION INHIBITION TEST (ADIT)

MATERIAL B0793.01

Prepared for:

THE PROCTER AND GAMBLE COMPANY

CINCINNATI, OHIO

Michael G. Stapleton
Michael G. Stapleton 10/25/85
Study Director

WESTON Project No. 85-017

Dianne S. Therry
Dianne S. Therry 11/1/85
Quality Assurance
Coordinator

Peter J. Marks
Peter J. Marks 11/8/85
Project Director

RECEIVED BY

0174

ANAEROBIC DIGESTION INHIBITION TEST (ADIT)

MATERIAL B0793.01

Prepared for:

THE PROCTER AND GAMBLE COMPANY

CINCINNATI, OHIO

[Handwritten signature]

**Michael G. Stapleton
Study Director**

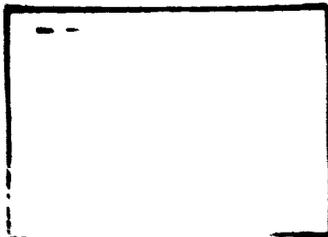
WESTON Project No. 85-017

[Handwritten signature]

**Dianne S. Therry
Quality Assurance
Coordinator**

[Handwritten signature]

**Peter J. Marks 11/8/85
Project Director**



ANAEROBIC DIGESTION INHIBITION TEST (ADIT)

TEST MATERIAL B0793.01

1.0 OBJECTIVE

To determine a no observed effect concentration (NOEC) and an observed effect concentration (OEC) of the test material on a laboratory anaerobic digestion test system.

2.0 EXPERIMENTAL PROTOCOL

R. H. Hall's Protocol of 13 March 1985 entitled -

"Batch Anaerobic Digestion Inhibition Test on B0793.01" Procter & Gamble Environmental Standard Test Method, VIII C-4, Issue 3 (July 7, 1982).

Protocol Addendum, M. Stapleton to R.H. Hall 30 May 1985: Change in test material addition from 2-propanol solution to direct weight addition.

Protocol Addendum, P. Marks to J.W. Williams 15 May 1985: Change of study director from D. Russell to M. Stapleton.

Sample analyses were performed using the following analytical methods:

Parameter	Analytical Methods
pH	SM ¹ 423
Total Solids (TS)	SM 209A
Total Volatile Solids (TVS)	SM 209E
Chemical Oxygen Demand (COD)	EPA 410.4
Total Alkalinity (TA)	O'Brien & Donolan ³
Bicarbonate Alkalinity (BA)	O'Brien & Donolan
Volatile Acids (VA)	O'Brien & Donolan

¹ SM - Standard Methods for the Examination of Water and Wastewater, 15th Edition, Washington, DC. American Public Health Association and American Water Works Assoc. 1980.

*m. Stapleton
2/5/86*

ANAEROBIC DIGESTION INHIBITION TEST (ADIT)

TEST MATERIAL B0793.01

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To determine a no observed effect concentration (NOEC) and an observed effect concentration (OEC) of the test material on a laboratory anaerobic digestion test system.

2.0 EXPERIMENTAL PROTOCOL

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Sample analyses were performed using the following analytical methods:

Parameter	Analytical Methods
mg l^{-1}	mg l^{-1} 423
Total Solids (TS)	SM 209A
Total Volatile Solids (TVS)	SM 209E
Chemical Oxygen Demand (COD)	EPA 410.4
Total Alkalinity (TA)	O'Brien & Donolan ³
Bicarbonate Alkalinity (BA)	O'Brien & Donolan
Volatile Acids (VA)	O'Brien & Donolan

¹ SM - Standard Methods for the Examination of Water and Wastewater, 15th Edition, Washington, DC. American Public Health Association and American Water Works Assoc. 1980.

2 EPA-600/4-79-020, Methods for Chemical Analysis of Water and Wastes, Revised March 1983.

3 O'Brien & Donolan (1977), "A Direct Method for Differentiating Bicarbonate and Acetate in Digester Sludge."

3.0 TEST MATERIAL, INTERNAL STANDARD, AND APPARATUS

a. Test Material Description

Sample Code:	B0793.01
Percent Active:	100
Expiration Date:	In Progress
Physical Form:	gel/liquid
Color:	Opaque
Solubility in Water:	Low
Date Received:	19 March 1985

b. Addition of Test Material

1. The test material was added by direct weight addition to the reaction vessel. This was due to the low solubility of the test material in water.
2. Test material active concentrations to be studied were given in the "Batch Anaerobic Digestion Inhibition Test on B0793.01, R.H. Hall, 13 March 1985". Test solutions were prepared as follows:

Test Material Nominal Concentration (mg/L)	Weight of Test Material Added Per 477 Total mL of Test Solution (mg)
0.1	0.05
1.0	0.5
10.0	4.8
100.0	47.7
1000.0	477.0

c. Internal Standard Description

Compound:	Phenol
Physical Form:	Crystal
Active Ingredient:	99%
Color:	White
Solubility:	Completely water soluble at test concentrations
Source:	Mallinckrodt Lot DHD

M. H. H. H.
2/5/86

2 EPA-600/4-79-020, Methods for Chemical Analysis of Water and Wastes, Revised March 1983.

3 O'Brien & Donolan (1977), "A Direct Method for Differentiating Bicarbonate and Acetate in Digester Sludge."

3.0 TEST MATERIAL, INTERNAL STANDARD, AND APPARATUS

a. Test Material Description

Sample Code:	B0793.01
Percent Active:	100
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Physical Form:	gel/liquid
Color:	Opaque
Solubility in Water:	Low
Date Received:	19 March 1985

b. Addition of Test Material

1. The test material was added by direct weight addition to the reaction vessel. This was due to the low solubility of the test material in water.
2. Test material active concentrations to be studied were given in the "Batch Anaerobic Digestion Inhibition Test on B0793.01, R.H. Hall, 13 March 1985". Test solutions were prepared as follows:

Test Material Nominal Concentration (mg/L)	Weight of Test Material Added Per 477 Total mL of Test Solution (mg)
0	0
0.1	0.05
10.0	4.8
100.0	47.7
1000.0	477.0

c. Internal Standard Description

Compound:	Phenol
Physical Form:	Crystal
Active Ingredient:	990
Color:	White
Solubility:	Completely water soluble at test concentrations
Source:	Mallinckrodt Lot DED

0 1 7 9

d. Preparation of Internal Standard Solution

1. A stock solution of phenol was prepared at a concentration of 14,455 mg/L based on the total weight of the phenol.
2. Test solutions were prepared as follows:

Active Ingredient Nominal Concentra- tion (mg/L)	Volume of Stock Solution Used per 477 total mL of Test Solution (mL)
250	8.3
1500	50.

4.0 TEST CONDITIONS

- a. Testing was performed at WESTON's Laboratory in West Chester, Pennsylvania. Michael G. Stapleton was the Study Director and responsible for initial set-up of the apparatus, pre-test analyses, maintaining and adjusting all apparatus, and conducting post test analyses with the exception of COD. Ms. Emily Carfioli conducted the COD analyses.
- b. Raw data, calculations, and analytical results are recorded in bound WESTON Laboratory Notebook # 625. pages 1-32.
- c. The test temperature was $32 \pm 1^{\circ}\text{C}$ which was maintained during the test.

d.

Item	Conditions
Test Dates	22 June 1985 (day 0) - 17 July 1985 (day 17)
Anaerobic Digester Sludge Source	Ocean County South Treatment Plant, Ocean County, NJ Secondary anaerobic digester sludge was collected by Tom Baylis from the locations shown in Figure D. Initial sludge characteriza- tions were:

M. Stapleton
2/5/86

d. Preparation of Internal Standard Solution

1. A stock solution of phenol was prepared at a concentration of 14,455 mg/L based on a total weight of the phenol.
2. Test solutions were prepared as follows:

Active Ingredient Nominal Concentra- tion (mg/L)	Volume of Stock Solution Used per 477 total mL of Test Solution (mL)
--	--

250	8.3
1500	50.

4.0 TEST CONDITIONS

- a. Testing was performed at WESTON's Laboratory in West Chester, Pennsylvania. Michael G. Stapleton was the Study Director and responsible for initial set-up of the apparatus, pre-test analyses, maintaining and adjusting all apparatus, and conducting post test analyses with the exception of COD. Ms. Emily Carfioli conducted the COD analyses.
- b. Raw data, calculations, and analytical results are recorded in bound WESTON Laboratory Notebook # 625, pages 1-32.

c. Item Conditions

Test Dates	22 June 1985 (day 0) - 17 July 1985 (day 17)
------------	---

Anaerobic Digester Sludge Source	Ocean County South Treatment Plant, Ocean County, NJ Secondary anaerobic digester sludge was collected by Tom Baylis from the locations shown in Figure D. Initial sludge characteriza- tions were:
-------------------------------------	---

Total Solids = 22,400 mg/L
Total Alkalinity = 3,330 mg/L
Volatile Acids = 90 mg/L
pH = 7.2

Inquiry of the plant operator indicated that the anaerobic digester was operating normally and within acceptable limits.

Primary Sludge
Source:

Ocean County South Treatment
Plant, Ocean County, NJ

Primary sludge was collected by Tom Baylis from the location shown in Figure D.

Initial sludge characteristics were:

Total Solids = 35,840 mg/L
Volatile Acids = 1,200 mg/L
pH = 5.0

5.0 DEVIATIONS FROM THE TEST METHOD

Michael Stapleton assumed the role of Study Director replacing David Russell (letter from Pete J. Marks to 15 May 1985).

The method of test material addition was changed from a 2-propanol/test material solution to direct weight addition of the test material to reaction vessels. Water was used to dilute the digester/primary sludge in the reaction vessels to 477 mls.

Normal operating temperatures were in the range of $32 \pm 1^\circ\text{C}$ rather than the $33 \pm 1^\circ\text{C}$ ranged specified in the test method. The overlap between the specified and actual temperature ranges was considered to make the deviation insignificant.

Primary sludge used in conducting the test had a total solids value less than the $50,000 \pm 10,000$ mg/L limit. The average value of the post test and pretest primary sludge samples was 36,832 mg/L. This deviation did not appear to have any significant effect on the test.

Gas production in the control reaction vessels (RV) at T-14 is specified in the protocol to be at least 500 mL. Both

reactor vessels (RV) produced less than 500 mLs: RV 4 at 465 mLs and RV 14 at 460 mLs. (Frocter and Gamble Company) was contacted on 8 July 1985, informing him of the low gas production. He felt this would not cause a problem with the results and there was no need to invalidate the test.

6.0 RESULTS/SUMMARY

Table 1 gives preliminary results for observed effect concentrations (OEC) and no observed effect concentrations (NOEC) for:

- cumulative measured gas production
- changes in chemical characteristics
- computed ultimate cumulative gas production (P_1)
- computed rate constant (P_2)

Table 2 references Tables A-F which present the analytical data for T=0, T=16, and chemical changes that occurred during the test period for all relevant reaction vessels, and figures A-C which graphically represent gas products, and P_1 and P_2 rates related to the test material.

7.0 OBSERVATIONS AND DISCUSSION OF RESULTS

Table 1 will be used in this discussion to differentiate between OEC and NOEC for the materials under test. All test material concentration (0.1 - 1,000 mg/L) did not show any inhibitory effect on the test organisms, NOEC were shown for all four (4) areas.

The 250 mg/L phenol internal standard demonstrated NOEC's in all areas, while the 1,500 mg/L phenol internal standard showed OEC's for cumulative measured gas production, changes in chemical characteristics, and computed ultimate cumulative gas production (P_1). This clearly demonstrated that the 1,500 mg/L internal standard had an inhibitory effect on the test organisms.

A description of the quality assurance methods used to ensure the quality of the data may be found in Appendix A.

8.0 CONCLUSION

NOEC characteristics were observed for the 250 mg/L phenol internal standard and the 1,500 mg/L internal showed OEC characteristics.

NOEC characteristics was observed for all concentrations (0.1 - 1,000 mg/L) of the test material.

Table 1. Preliminary Results for OGCs (a) and MOGCs (b) Deduced from Cumulative Measured Gas Production/Changes in Chemical Characteristics of the Test System, Computed Ultimate Cumulative Gas Production, and Computed Rate Constants.

Material Under Test	Residual Concentration (mg/L)	Cumulative Measured Gas Production	Changes in Chemical Characteristics	Computed Ultimate Cumulative Production, P_1	Computed Rate Constant, P_2
Phenol	250	MOGC	MOGC	MOGC	MOGC
Phenol	1500	OGC	OGC	OGC	MOGC
B0793.01	0.1	MOGC	MOGC	MOGC	MOGC
B0793.01	1.0	MOGC	MOGC	MOGC	MOGC
B0793.01	10.0	MOGC	MOGC	MOGC	MOGC
B0793.01	100.0	MOGC	MOGC	MOGC	MOGC
B0793.01	1000.0	MOGC	MOGC	MOGC	MOGC

(a) OGC = Observed Effect Concentration.

(b) MOGC = Me Observed Effect Concentration.

(c) P_1 = Statistically computed ultimate cumulative gas production volume (asymptote to a first order rate model of the gas production process), mL.

(d) P_2 = Statistically computed rate constant for cumulative gas production based upon a first order rate model of the gas production process, day⁻¹.

Table 2
Cross Reference of Data

Table	Figure	Data Description
A		Initial Conditions of Primary and Anaerobic Digester Sludge ($t \leq 0$)
B		Initial Conditions of Each Reaction Vessel ($t = 0$)
C		Cumulative Gas Readings Recorded During the Test Period ($0 \leq t \leq 17$ days)
D		Data Summary of Post Test Chemical Analyses ($t = 17$ days)
E		Data Summary of Sludge Conditions over the Test Period ($0 \leq t \leq 17$ days)
F-1		Statistical Results of Control and Internal Standard
F-2		Statistical Results of Control and Test Material
	A	Cumulative Gas Production Over Time ($0 \leq t \leq 17$ days)
	B	Computed Coefficient P_1 Related to Test Material Concentration
	C	Computed Gas Production Rate Coefficient P_2 Related to Test Material Concentration

Table A

Data Summary of Initial Conditions
for Primary and Digester Sludge
Test Material B0793.01
(T = - days)

	PH (PHU)	TS (MG/L)	VS (MG/L)	TA (MG/L)	SA (MG/L)	VA (MG/L)	COD (MG/L)	VS/VS (MG/L)	SA/TA (MG/L)	VA/TA (MG/L)
PRIMARY PNE SFT-UP	5.2	36372	20236	1400	246	1390	52000	0.76	0.10	0.99
PRIMARY POST SFT-UP	5.2	36692	20144	1430	326	1330	49000	0.77	0.23	0.93
ANAEROBIC DIGESTER PNE SFT-UP	7.3	22404	13024	3320	3270	70	22000	0.62	0.98	0.02
ANAEROBIC DIGESTER POST SFT-UP	7.3	23472	14224	3300	3242	70	22000	0.61	0.98	0.02

REFERENCE NYW LABORATORY NOTEBOOK 6625, PAGES 41 AND 43

Table B

Data Summary of Pre Test Analysis
Test Material B0793.01

	PH (PHU)	TS (MG/L)	VS (MG/L)	TA (MG/L)	BA (MG/L)	VA (MG/L)	COO (MG/L)	VS/TS (MG/L)	BA/TA (MG/L)	VA/TA (MG/L)
CONTROL	7.3	21352	13328	2900	2817	100	20000	0.62	0.97	0.03
250 MG/L INTERNAL STANDARD	7.3	22408	14144	2900	2817	100	21000	0.63	0.97	0.03
1500 MG/L INTERNAL STANDARD	7.3	21996	13932	2910	2794	100	24000	0.63	0.95	0.03
0.1 MG/L	7.2	21876	13832	2850	2792	70	21000	0.63	0.98	0.02
1.0 MG/L	7.3	21748	13664	2850	2792	70	28000	0.63	0.98	0.02
10 MG/L	7.3	21572	13412	2800	2825	90	20000	0.62	0.97	0.03
100 MG/L	7.3	21892	13892	2800	2859	50	23000	0.63	0.99	0.02
1000 MG/L	7.3	21372	13464	2820	2712	130	24000	0.63	0.96	0.05

REFERENCE NFW LABORATORY NOTEBOOK 8625 , PAGES 23-26, AND 31

THE PROCTER AND GAMBLE CO.
 PERIODIC DAILY AND CUMULATIVE GAS PRODUCTION
 TEST MATERIAL B0793.01
 TABLE C

REACTION VESSEL			2	8			
CONCENTRATION			0.1 MG/L	0.1 MG/L			
DAY	DATE	TIME	BAROMETRIC PRESSURE	DAILY PRODUCED	TOTAL PRODUCED	DAILY PRODUCED	TOTAL PRODUCED
0	06/22/85	1930	764	0	0	0	0
0	06/22/85	1215	765	*	110	*	145
1	06/23/85	1845	763	145	145	185	185
1	06/23/85	1015	763	*	205	*	260
2	06/24/85	1655	761	90	235	110	295
3	06/25/85	1650	761	45	280	55	350
4	06/26/85	1430	762	20	300	35	385
5	06/27/85	1715	762	15	315	25	410
6	06/28/85	1200	761	10	325	20	430
7	06/29/85	1800	761	25	350	20	450
8	06/30/85	1500	767	0	350	0	450
9	07/01/85	1800	767	-5	345	20	470
10	07/02/85	1130	765	0	345	10	480
11	07/03/85	1614	759	5	350	20	500
12	07/04/85	0930	762	0	350	-5	495
13	07/05/85	1635	765	0	350	5	500
14	07/06/85	1320	766	0	350	15	515
15	07/07/85	1430	760	-10	340	5	520
16	07/08/85	1600	760	0	340	0	520
17	07/09/85	1500	759	5	345	0	520

REFERENCE: RFW LABORATORY NOTEBOOK ADIT B0793.01, pages 17-22.

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THE PROCTER AND GAMBLE CO.
 PERIODIC DAILY AND CUMULATIVE GAS PRODUCTION
 TEST MATERIAL B0793.01
 TABLE C (CONT'D)

REACTION VESSEL			1		3		
CONCENTRATION			1 MG/L		1 MG/L		
DAY	DATE	TIME	BAROMETRIC PRESSURE	DAILY PRODUCED	TOTAL PRODUCED	DAILY PRODUCED	TOTAL PRODUCED
0	06/22/85	1930	764	0	0	0	0
0	06/22/85	1215	765	*	110	*	120
1	06/23/85	1845	763	145	145	155	155
1	06/23/85	1015	763	*	210	*	220
2	06/24/85	1655	761	100	245	95	250
3	06/25/85	1650	761	50	295	45	295
4	06/26/85	1430	762	30	325	25	320
5	06/27/85	1715	762	30	355	15	335
6	06/28/85	1200	761	15	370	10	345
7	06/29/85	1800	761	20	390	10	355
8	06/30/85	1500	767	5	395	0	355
9	07/01/85	1800	767	20	415	0	355
10	07/02/85	1130	765	15	430	5	360
11	07/03/85	1614	759	10	440	10	370
12	07/04/85	0930	762	10	450	-10	360
13	07/05/85	1635	765	15	465	5	365
14	07/06/85	1320	766	5	470	-5	360
15	07/07/85	1430	760	0	470	0	360
16	07/08/85	1600	760	5	475	0	360
17	07/09/85	1500	759	0	475	5	365

REFERENCE: RFW LABORATORY NOTEBOOK ADIT B0793.01, pages 17-22.

THE PROCTER AND GAMBLE CO.
 PERIODIC DAILY AND CUMULATIVE GAS PRODUCTION
 TEST MATERIAL B0793.01
 TABLE C (CONT'D)

REACTION VESSEL			13	15			
CONCENTRATION			10 MG/L	10 MG/L			
DAY	DATE	TIME	BAROMETRIC PRESSURE	DAILY PRODUCED	TOTAL PRODUCED	DAILY PRODUCED	TOTAL PRODUCED
0	06/22/85	1930	764	0	0	0	0
0	06/22/85	1215	765	*	130	*	150
1	06/23/85	1845	763	160	160	180	180
1	06/23/85	1015	763	*	225	*	250
2	06/24/85	1655	761	95	255	100	280
3	06/25/85	1650	761	45	300	55	335
4	06/26/85	1430	762	30	330	25	360
5	06/27/85	1715	762	15	345	40	400
6	06/28/85	1200	761	20	365	0	400
7	06/29/85	1800	761	20	385	15	415
8	06/30/85	1500	767	0	385	5	420
9	07/01/85	1800	767	10	395	10	430
10	07/02/85	1130	765	5	400	20	450
11	07/03/85	1614	759	10	410	10	460
12	07/04/85	0930	762	5	415	-5	455
13	07/05/85	1635	765	5	420	10	465
14	07/06/85	1320	766	0	420	5	470
15	07/07/85	1430	760	5	425	0	470
16	07/08/85	1600	760	-5	420	0	470
17	07/09/85	1500	759	0	420	0	470

REFERENCE: RFW LABORATORY NOTEBOOK ADIT B0793.01, pages 17-22.

THE PROCTER AND GAMBLE CO.
 PERIODIC DAILY AND CUMULATIVE GAS PRODUCTION
 TEST MATERIAL B0793.01
 TABLE C (CONT'D)

REACTION VESSEL			9	10			
CONCENTRATION			100 MG/L	100 MG/L			
DAY	DATE	TIME	BAROMETRIC PRESSURE	DAILY PRODUCED	TOTAL PRODUCED	DAILY PRODUCED	TOTAL PRODUCED
0	06/22/85	1930	764	0	0	0	0
0	06/22/85	1215	765	*	120	*	125
1	06/23/85	1845	763	155	155	160	160
1	06/23/85	1015	763	*	215	*	230
2	06/24/85	1655	761	90	245	100	260
3	06/25/85	1650	761	40	285	40	300
4	06/26/85	1430	762	30	315	20	320
5	06/27/85	1715	762	15	330	20	340
6	06/28/85	1200	761	15	345	20	360
7	06/29/85	1800	761	10	355	10	370
8	06/30/85	1500	767	5	360	0	370
9	07/01/85	1800	767	10	370	20	390
10	07/02/85	1130	765	15	385	0	390
11	07/03/85	1614	759	10	395	10	400
12	07/04/85	0930	762	0	395	0	400
13	07/05/85	1635	765	10	405	0	400
14	07/06/85	1320	766	5	410	10	410
15	07/07/85	1430	760	-10	400	0	410
16	07/08/85	1600	760	5	405	0	410
17	07/09/85	1500	759	0	405	0	410

REFERENCE: RFW LABORATORY NOTEBOOK ADIT B0793.01, pages 17-22.

THE PROCTER AND GAMBLE CO.
 PERIODIC DAILY AND CUMULATIVE GAS PRODUCTION
 TEST MATERIAL B0793.01
 TABLE C (CONT'D)

REACTION VESSEL			7	16			
CONCENTRATION			1000 MG/L		1000 MG/L		
DAY	DATE	TIME	BAROMETRIC PRESSURE	DAILY PRODUCED	TOTAL PRODUCED	DAILY PRODUCED	TOTAL PRODUCED
0	06/22/85	1930	764	0	0	0	0
0	06/22/85	1215	765	*	135	*	150
1	06/23/85	1845	763	175	175	200	200
1	06/23/85	1015	763	*	250	*	270
2	06/24/85	1635	761	105	280	100	300
3	06/25/85	1650	761	55	335	75	375
4	06/26/85	1430	762	45	380	40	415
5	06/27/85	1715	762	25	405	35	450
6	06/28/85	1200	761	20	425	20	470
7	06/29/85	1800	761	20	445	10	480
8	06/30/85	1500	767	5	450	10	490
9	07/01/85	1800	767	15	465	10	500
10	07/02/85	1130	765	10	475	15	515
11	07/03/85	1614	759	20	495	15	530
12	07/04/85	0930	762	-5	490	0	530
13	07/05/85	1635	765	10	500	10	540
14	07/06/85	1320	766	5	505	5	545
15	07/07/85	1430	760	-5	500	5	550
16	07/08/85	1600	760	5	505	0	550
17	07/09/85	1500	759	0	505	0	550

REFERENCE: RFW LABORATORY NOTEBOOK ADIT B0793.01, pages 17-22.

THE FACTOR AND GAMBLE CO.
 PERIODIC DAILY AND CUMULATIVE GAS PRODUCTION
 TEST MATERIAL B0793.01
 TABLE C (CONT'D)

REACTION VESSEL			4				14	
CONCENTRATION			CONTROL		CONTROL			
DAY	DATE	TIME	BAROMETRIC PRESSURE	DAILY PRODUCED	TOTAL PRODUCED	DAILY PRODUCED	TOTAL PRODUCED	
0	06/22/85	1930	764	0	0	0	0	
0	06/22/85	1215	765	*	140	*	135	
1	06/23/85	1845	763	175	175	175	175	
1	06/23/85	1015	763	*	250	*	245	
2	06/24/85	1655	761	95	270	100	275	
3	06/25/85	1650	761	60	330	55	330	
4	06/26/85	1430	762	30	360	25	355	
5	06/27/85	1715	762	20	380	25	380	
5	06/28/85	1200	761	15	395	15	395	
7	06/29/85	1800	761	15	410	10	405	
8	06/30/85	1500	767	5	415	5	410	
9	07/01/85	1800	767	10	425	15	425	
10	07/02/85	1130	765	5	430	10	435	
11	07/03/85	1614	759	20	450	10	445	
12	07/04/85	0930	762	0	450	5	450	
13	07/05/85	1635	765	10	460	5	455	
14	07/06/85	1320	766	5	465	5	460	
15	07/07/85	1430	760	0	465	0	460	
16	07/08/85	1600	760	0	465	0	460	
17	07/09/85	1500	759	0	465	0	460	

REFERENCE: RFW LABORATORY NOTEBOOK ADIT B0793.01, pages 17-22.

THE PROCTER AND GAMBLE CO.
 PERIODIC DAILY AND CUMULATIVE GAS PRODUCTION
 TEST MATERIAL B0793.01
 TABLE C (CONT'D)

REACTION VESSEL			5				11	
CONCENTRATION			INTERNAL STANDARD 250 MG/L		INTERNAL STANDARD 250 MG/L			
DAY	DATE	TIME	BAROMETRIC PRESSURE	DAILY PRODUCED	TOTAL PRODUCED	DAILY PRODUCED	TOTAL PRODUCED	
0	06/22/85	1930	764	0	0	0	0	
0	06/22/85	1215	765	*	115	*		
1	06/23/85	1845	763	145	145	160	125	
1	06/23/85	1015	763	*	200	*	160	
2	06/24/85	1655	761	90	235	90	215	
3	06/25/85	1650	761	60	295	55	250	
4	06/26/85	1430	762	40	335	30	305	
5	06/27/85	1715	762	20	355	20	335	
6	06/28/85	1200	761	20	375	15	355	
7	06/29/85	1800	761	20	395	15	370	
8	06/30/85	1500	767	0	395	5	385	
9	07/01/85	1800	767	20	415	10	390	
10	07/02/85	1130	765	10	425	10	400	
11	07/03/85	1614	759	30	455	20	410	
12	07/04/85	0930	762	0	455	0	430	
13	07/05/85	1635	765	20	475	10	430	
14	07/06/85	1320	766	10	485	15	440	
15	07/07/85	1430	760	10	495	5	455	
16	07/08/85	1600	760	10	505	10	460	
17	07/09/85	1500	759	5	510	5	470	
							475	

REFERENCE: RFW LABORATORY NOTEBOOK ADIT B0793.01, pages 17-22.

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THE PROCTER AND GAMBLE CO.
 PERIODIC DAILY AND CUMULATIVE GAS PRODUCTION
 TEST MATERIAL B0793.01
 TABLE C (CONT'D)

REACTION VESSEL			6		12		
CONCENTRATION			INTERNAL STANDARD 1500 MG/L		INTERNAL STANDARD 1500 MG/L		
DAY	DATE	TIME	BAROMETRIC PRESSURE	DAILY PRODUCED	TOTAL PRODUCED	DAILY PRODUCED	TOTAL PRODUCED
0	06/22/85	1930	764	0	0	0	0
0	06/22/85	1215	765	*	45	*	45
1	06/23/85	1945	763	60	60	60	60
1	06/23/85	1015	763	*	85	*	85
2	06/24/85	1655	761	35	95	35	95
3	06/25/85	1650	761	20	115	20	115
4	06/26/85	1430	762	15	130	20	135
5	06/27/85	1715	762	20	150	20	155
6	06/28/85	1200	761	10	160	10	165
7	06/29/85	1800	761	15	175	15	180
8	06/30/85	1500	767	10	185	5	185
9	07/01/85	1800	767	10	195	10	195
10	07/02/85	1130	765	10	205	10	205
11	07/03/85	1614	759	10	215	10	215
12	07/04/85	0930	762	5	220	0	215
13	07/05/85	1635	765	10	230	0	215
14	07/06/85	1320	766	5	235	5	220
15	07/07/85	1430	760	0	235	-5	215
16	07/08/85	1600	760	0	235	0	215
17	07/09/85	1500	759	5	240	0	215

REFERENCE: RFW LABORATORY NOTEBOOK ADIT B0793.01, pages 17-22.

Table D

Data Summary of Post Test Analysis
Test Material B0793.01

REACTION VESSEL	CONTROL		INTERNAL STANDARD		0.1 MG/L		1.0 MG/L		TEST MATERIAL B0793.01		1000 MG/L					
	0 MG/L	MG/L	250 MG/L	1500 MG/L	0.1 MG/L	1.0 MG/L	10 MG/L	100 MG/L	10 MG/L	100 MG/L	1000 MG/L	10000 MG/L				
PH (PHU)	4	14	5	11	6	12	2	8	1	3	13	15	9	10	7	16
TOTAL SOLIDS (TS) (MG/L)	7.6	7.6	7.5	7.6	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.6	7.5	7.5	7.5	7.6
VOLATILE SOLIDS (VS) (MG/L)	19140	19396	19260	19140	20372	20036	20176	20304	20280	19060	10316	19404	20212	20096	20172	19012
TOTAL ALKALINITY (TA) (MG/L)	11520	11220	11392	10944	12304	11932	11996	12264	12164	11804	10392	11272	12144	12124	12152	10052
BICARBONATE ALKALINITY (BA) (MG/L)	3190	3070	3230	3150	3120	3230	3130	3180	3260	3120	3100	3150	2550	3170	3200	3130
VOLATILE ACID (VA) (MG/L)	3149	3012	3180	3092	2907	3114	3080	3122	3193	3005	3050	3042	2492	3100	3239	3122
CHEMICAL OXYGEN DEMAND (COD) (MG/L)	50	70	60	70	160	140	60	70	80	90	60	130	70	80	50	70
VS/TS	10000	15000	21000	19000	24000	24000	22000	22000	21000	20000	19000	35000	21000	19000	23000	30000
BA/TA	0.60	0.50	0.59	0.57	0.63	0.60	0.59	0.60	0.60	0.59	0.59	0.57	0.60	0.55	0.6	0.57
VA/TA	0.99	0.98	0.98	0.98	0.96	0.96	0.98	0.98	0.98	0.98	0.98	0.98	0.98	0.98	0.98	0.98
	0.02	0.02	0.02	0.02	0.05	0.04	0.02	0.02	0.02	0.03	0.03	0.02	0.03	0.03	0.03	0.02

REFERENCE: NFM NOTEBOOK 6625, PAGES 27-30

Table E
Data Summary of Sludge Conditions
Over Test Period
Test Material B0793.01

	CONTROL		INTERNAL STANDARD						TEST MATERIAL B0793.01							
	0	MG/L	250	MG/L	1500	MG/L	0.1	MG/L	1.0	MG/L	10	MG/L	100	MG/L	1000	MG/L
REACTION VESSEL	4	14	5	11	6	12	2	8	1	3	13	15	9	10	7	16
PH (PMU)	0.3	0.3	0.2	0.3	0.2	0.2	0.3	0.3	0.2	0.2	0.2	0.3	0.2	0.2	0.2	0.3
TOTAL SOLIDS (TS) (MG/L)	-2204	-1956	-3140	-3268	-1824	-1960	-1700	-1492	-1460	-1880	-3256	-2168	-1680	-1396	-1200	-2360
VOLATILE SOLIDS (VS) (MG/L)	-1808	-2108	-2752	-3280	-1628	-2000	-1836	-1568	-1500	-1868	-3020	-2140	-1748	-1768	-1312	-2612
TOTAL ALKALINITY (TA) (MG/L)	298	170	330	250	210	320	280	330	410	270	200	250	350	270	460	360
BICARBONATE ALKALINITY (BA) (MG/L)	332	195	362	275	193	320	288	330	401	253	235	237	367	245	527	410
VOLATILE ACID (VA) (MG/L)	-50	-30	-40	-30	60	40	-10	0	10	20	-30	40	20	30	-80	-60
CHEMICAL OXYGEN DEMAND (COD) (MG/L)	-2000	-1000	0	-2000	0	0	1000	1000	1000	-8000	-1000	15000	-2000	-4000	-1000	6000
VS/TS	-0.02	-0.05	-0.04	-0.06	-0.02	-0.04	-0.03	-0.03	-0.03	-0.04	-0.03	-0.05	-0.03	-0.04	-0.03	-0.06
BA/TA	0.02	0.01	0.01	0.01	.00	.00	.03	.00	.00	.00	.00	0.01	-0.01	-0.01	0.03	0.02
VA/TA	-0.02	-0.01	-0.02	-0.01	0.02	0.01	-0.01	.00	.00	.00	.00	-0.01	0.01	0.01	-0.03	-0.02



TABLE F-1: STATISTICAL RESULTS FOR THE INTERNAL STANDARD AND CONTROL

80793.01

Test Material Concentration, mg/L	Control		Internal		Standard	
	N/A		250		1500	
Reaction Vessel	4	14	5	11	6	12
P ₁ Cumulative gas produced	448	445	478	440	241	220
mL of Gas						
Average	446.8		459.1		230.2	
Standard Deviation	2.24		27.23		14.82	
Student t-Test	N/A		0.25		-4.45*	
Probability	N/A		0.806		0.002	
P ₂ Rate Constant	0.4587	0.4621	0.3019	0.3880	0.2119	0.2659
Day ⁻¹						
Average, Day ⁻¹	0.4604		0.3450		0.2589	
Standard Deviation	0.0024		0.0609		0.0382	
Student t-Test	N/A		-2.68		-8.19	
Probability	N/A		0.227		0.076	
C = Lag period, days	0.0	0.0	0.0	0.0	0.0	0.0
Average, days	0.0		0.0		0.0	

*Denotes significant difference at 5% risk level.
N/A Non Applicable

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TABLE F-2: STATISTICAL RESULTS FOR THE TEST MATERIAL AND CONTROL

Test Material	Control	Test Material 80793.01						
		0.1 mg/L	1.0 mg/L	10 mg/L	100 mg/L	1,000 mg/L		
Concentration	N/A							
Reaction Vessel	4	2	3	13	15	9	10	7
P ₁ = cumulative gas produced	448	347	458	361	411	455	392	492
mL of Gas								
Average	446.8	422.9	409.5	432.8	394.6	512.79		
Standard Deviation	2.24	107.34	68.30	31.39	3.67	28.81		
Student t-test	--	-0.49	-0.77	-0.29	-1.07	1.32		
Probability	--	0.637	0.466	0.780	0.315	0.212		
P ₂ = rate constant	0.4587	0.5593	0.4174	0.377	0.5863	0.4645	0.4673	0.4588
Day ⁻¹								
Average Day ⁻¹	0.4604	0.4884	0.4620	0.4659	0.4772	0.4601		
Standard Deviation	0.0024	0.1003	0.1758	0.0070	0.0261	0.0093		
Student t-Test	--	0.39	0.01	2.50	0.91	2.51		
Probability	--	0.761	0.992	0.134	0.528	0.004		
C = lag period, days	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Average, days	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

*Denotes significant difference at 5% risk level.

M. H. ...

TABLE F-2: STATISTICAL RESULTS FOR THE TEST MATERIAL AND CONTROL

Test Material	Control	Test Material 80793.01												
		4	14	2	8	1	3	13	15	9	10	7	16	
Concentration	N/A													
Reaction Vessel	4	14	2	8	1	3	13	15	9	10	7	16		
F ₁ - cumulative gas produced	448	445	347	499	458	361	411	455	392	397	492	533		
ml. of Gas			422.9		409.5		432.8		394.6			512.79		
Average	446.8		107.34		68.30		31.39		3.67			28.81		
Standard Deviation	2.24		-0.49		-0.77		-0.29		-1.07			1.35		
Student t-Test	--		0.637		0.466		0.780		0.315			0.212		
Probability	--													
P ₂ - rate constant Day ⁻¹	0.4587	0.4621	0.5593	0.4174	0.377	0.5863	0.4645	0.4673	0.4588	0.4957	0.4025	0.4159		
Average Day ⁻¹	0.4604		0.4884		0.4620		0.4659		0.4772			0.4092		
Standard Deviation	0.0024		0.1003		0.1758		0.0020		0.0261			0.0095		
Student t-Test	--		0.39		0.01		2.50		0.91			-7.41		
Probability	--		0.761		0.992		0.134		0.528			0.068		
C - lag period, days	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		
Average, days	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		

*Denotes significant difference at 5% risk level.

Figure A Test Material B0793.01
Gas Production (ml) vs Time (days)

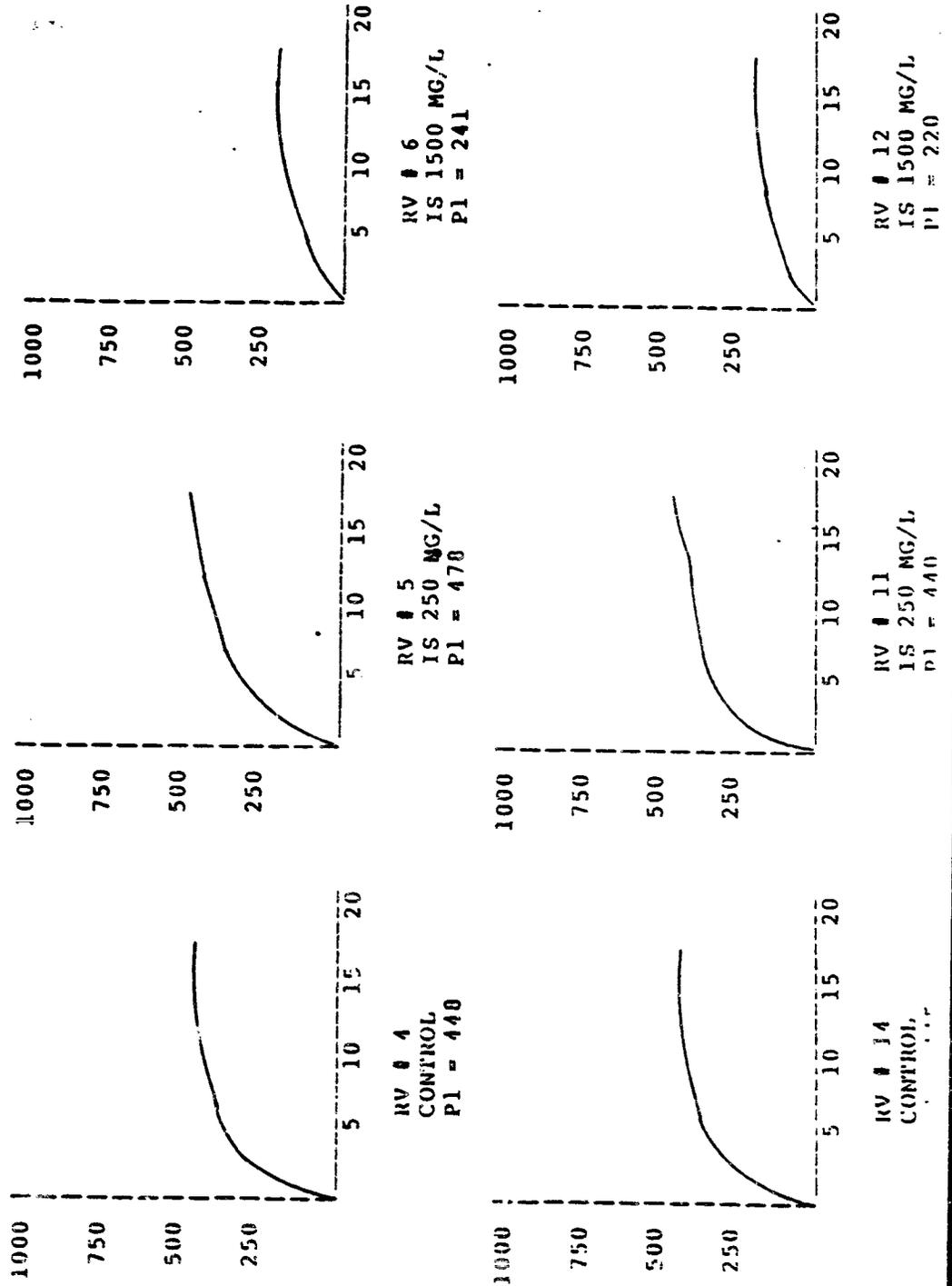


Figure A Test Material B0793.01
Gas Production (ml) vs Time (days)

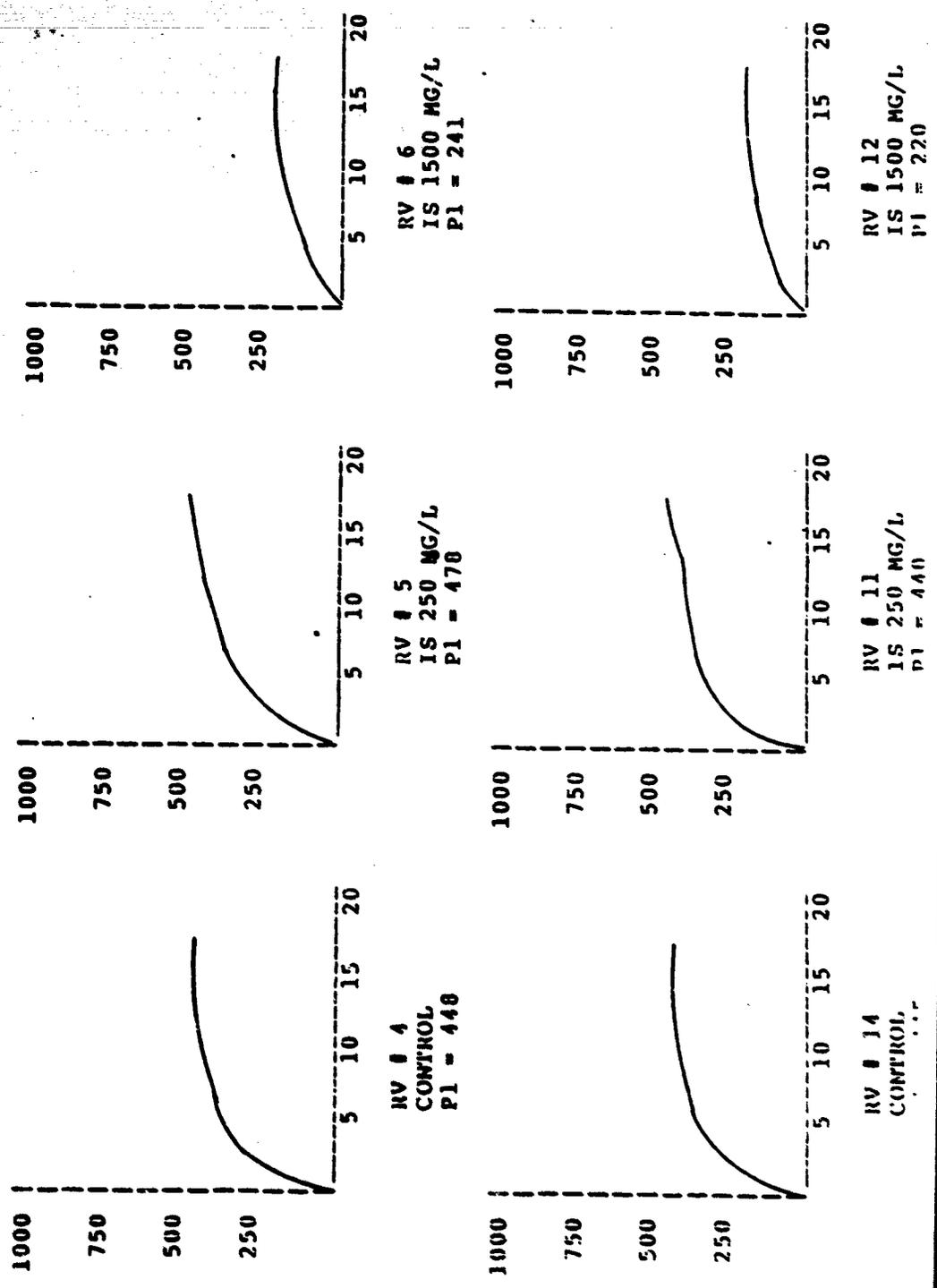


Figure A (continued) Test Material B0793.01
 Gas Production (ml) vs Time (days)

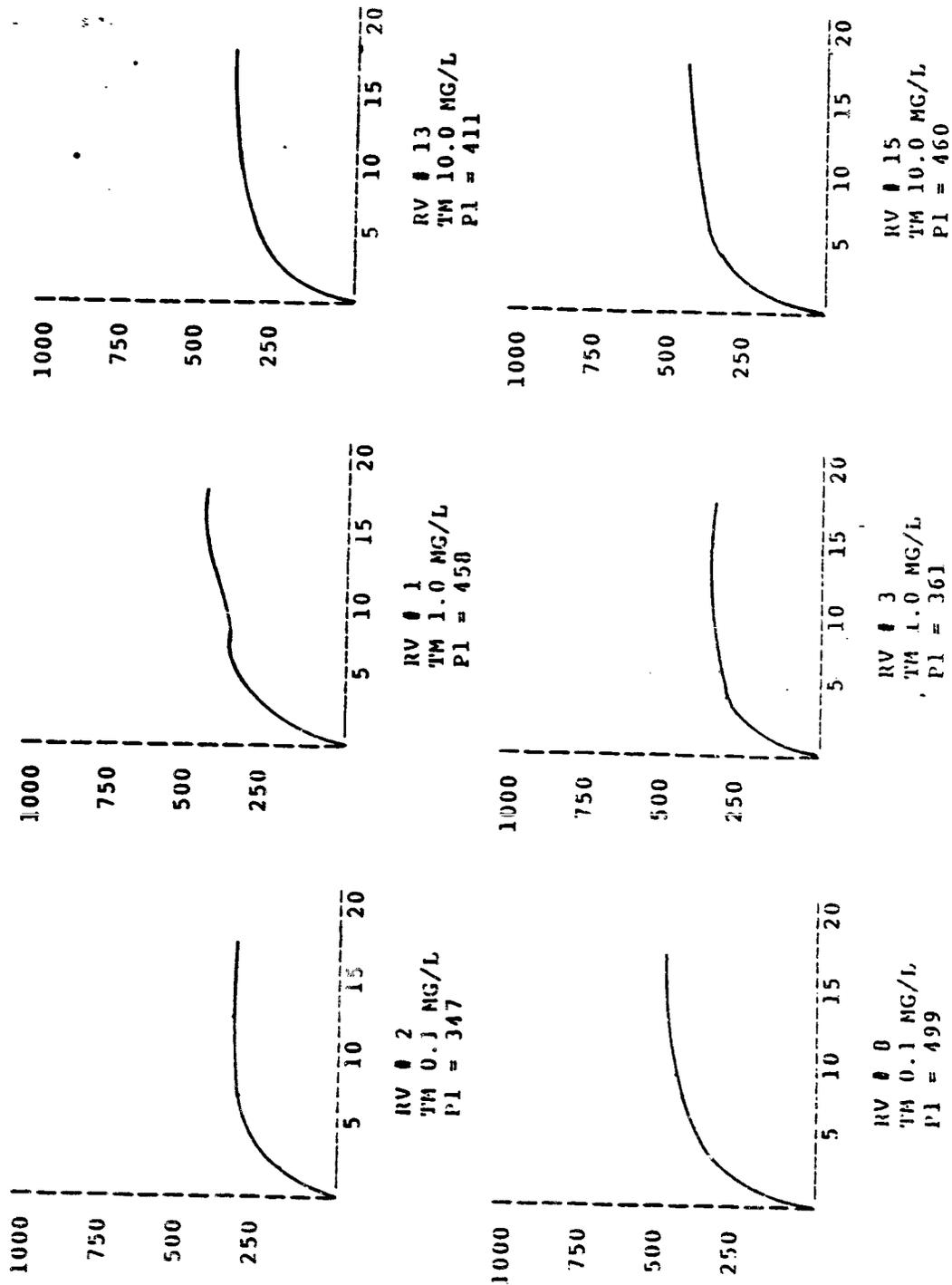
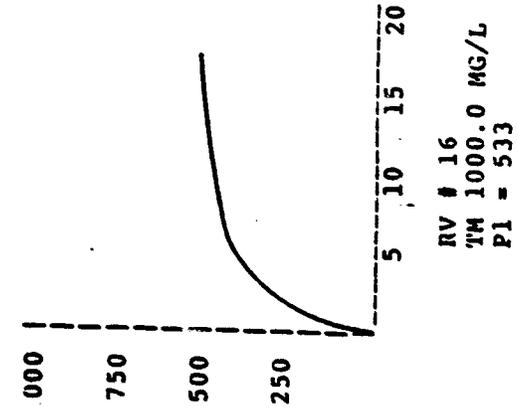
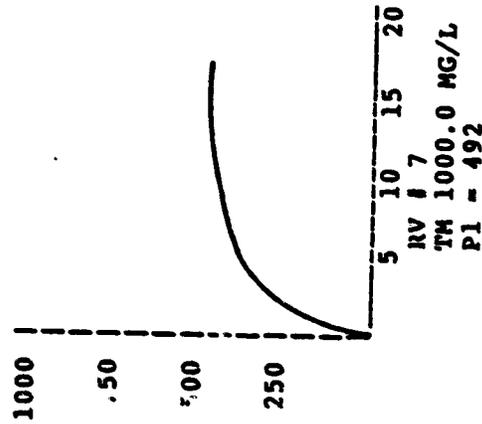
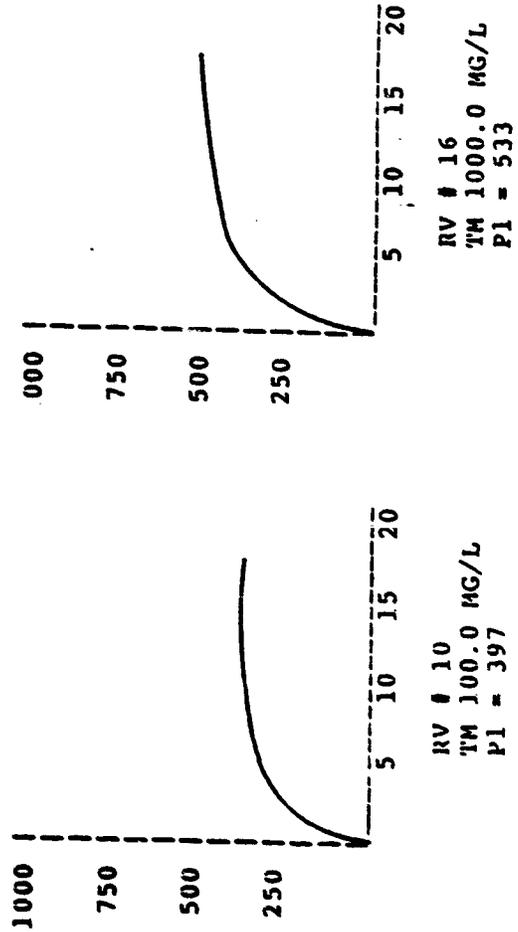
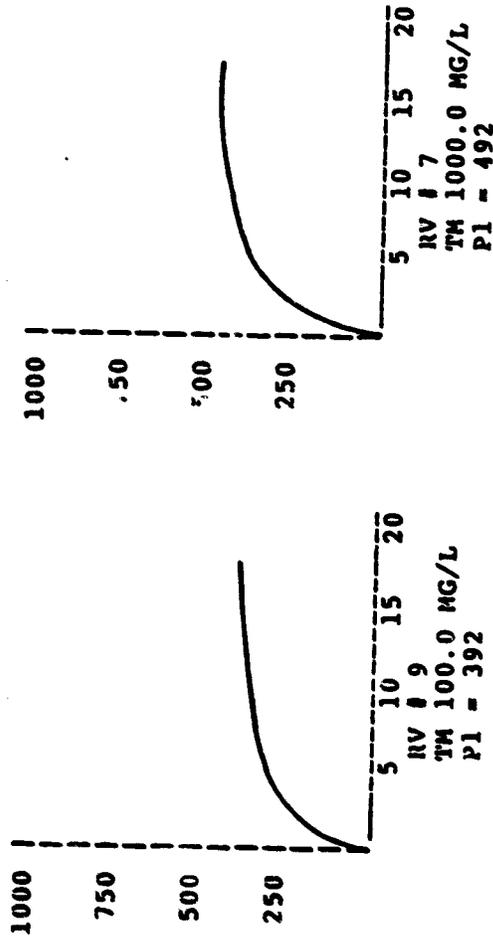


Figure A (continued) Test Material B0793.01
 Gas Production (ml) vs Time (days)



46 / 520

Test Material Concentration, mg/L

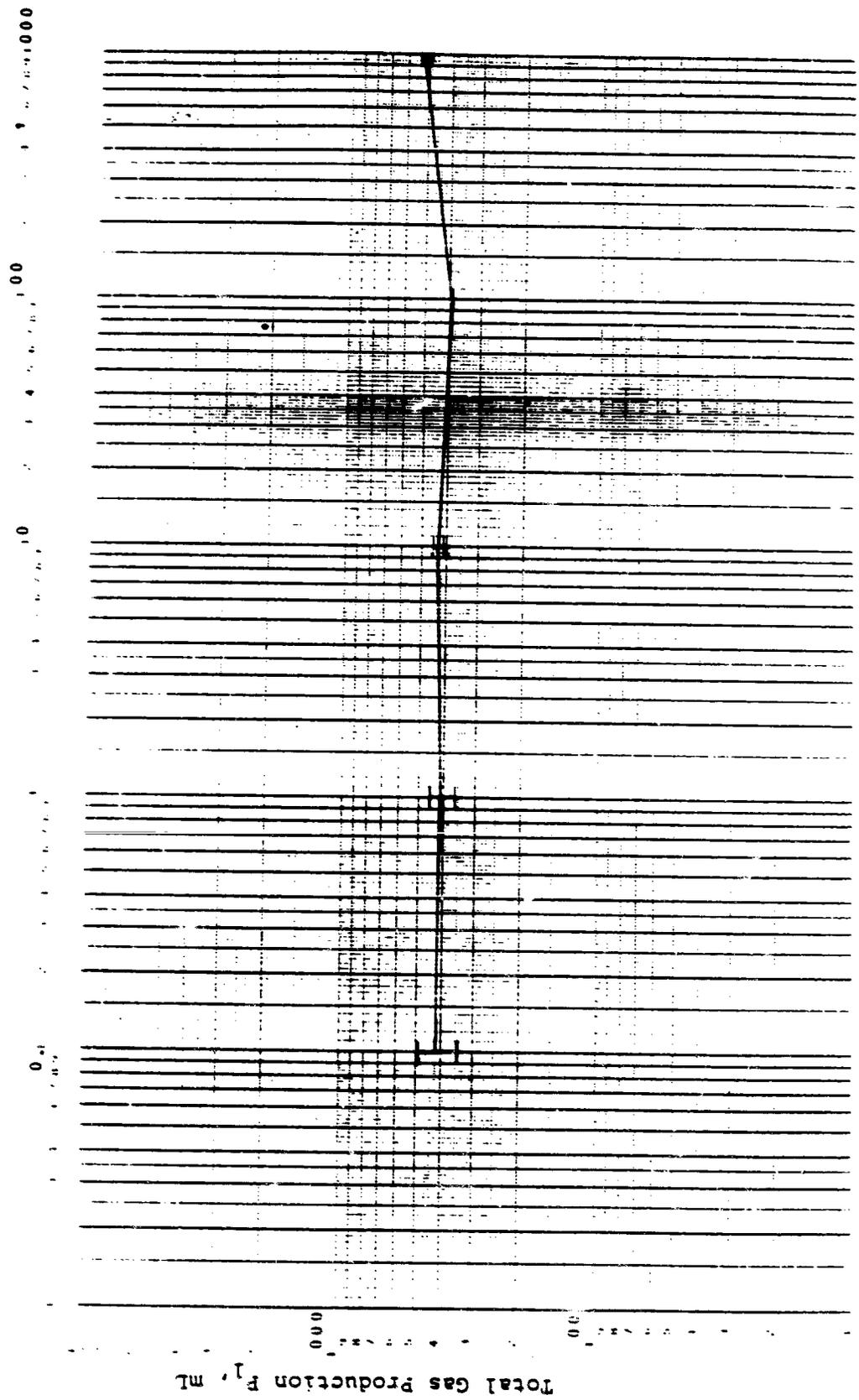
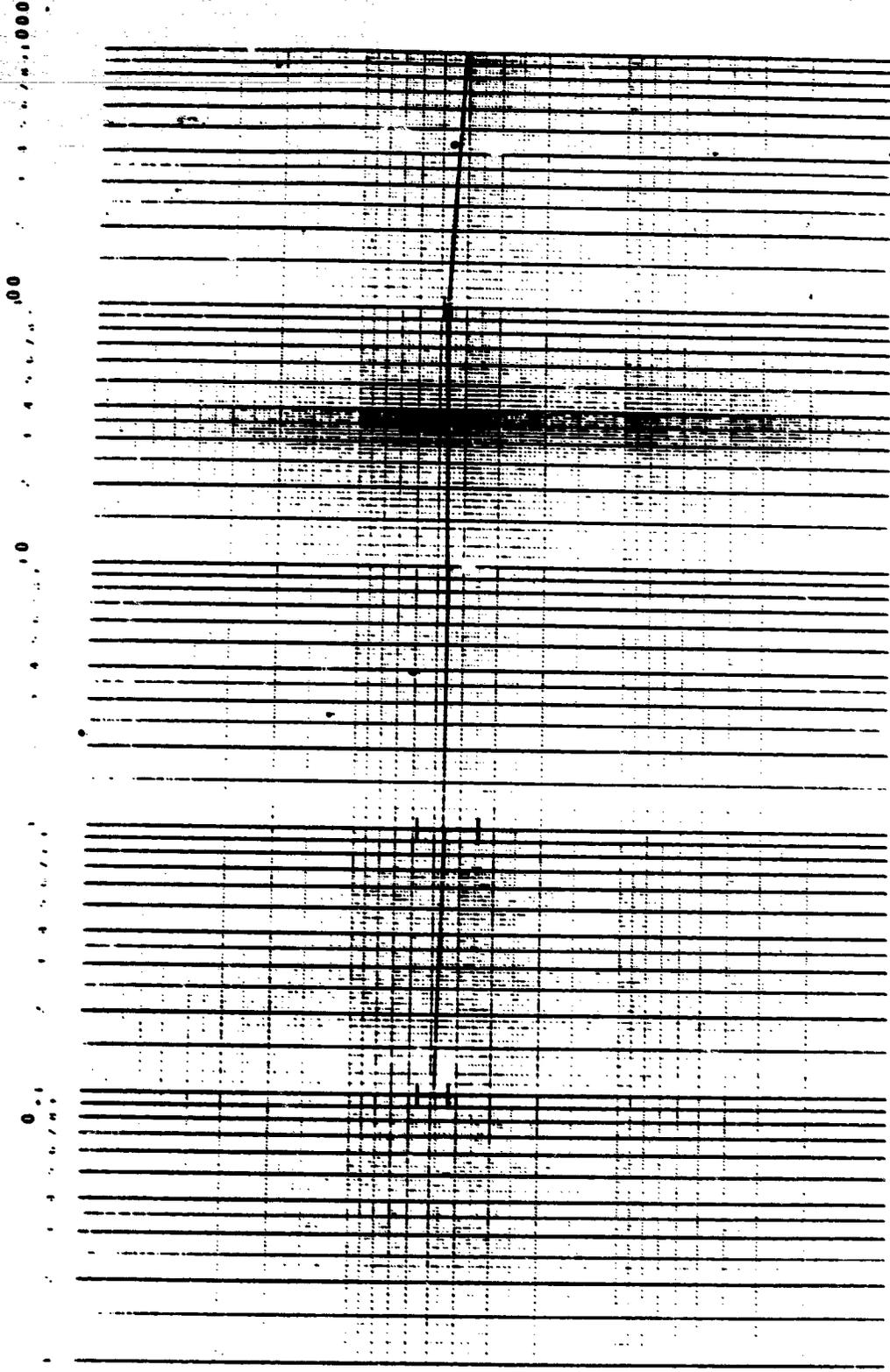


Figure B - Test Material No. B0793.01

1. 2. 3. 4. 5. 6. 7. 8. 9. 10. 11. 12. 13. 14. 15. 16. 17. 18. 19. 20. 21. 22. 23. 24. 25. 26. 27. 28. 29. 30. 31. 32. 33. 34. 35. 36. 37. 38. 39. 40. 41. 42. 43. 44. 45. 46. 47. 48. 49. 50. 51. 52. 53. 54. 55. 56. 57. 58. 59. 60. 61. 62. 63. 64. 65. 66. 67. 68. 69. 70. 71. 72. 73. 74. 75. 76. 77. 78. 79. 80. 81. 82. 83. 84. 85. 86. 87. 88. 89. 90. 91. 92. 93. 94. 95. 96. 97. 98. 99. 100.

10/15/70

Test Material Concentration, Mg/L



Gas Rate Constant, P_2 (Day⁻¹)

Figure C - Test Material No. B0793.01

WESTERN

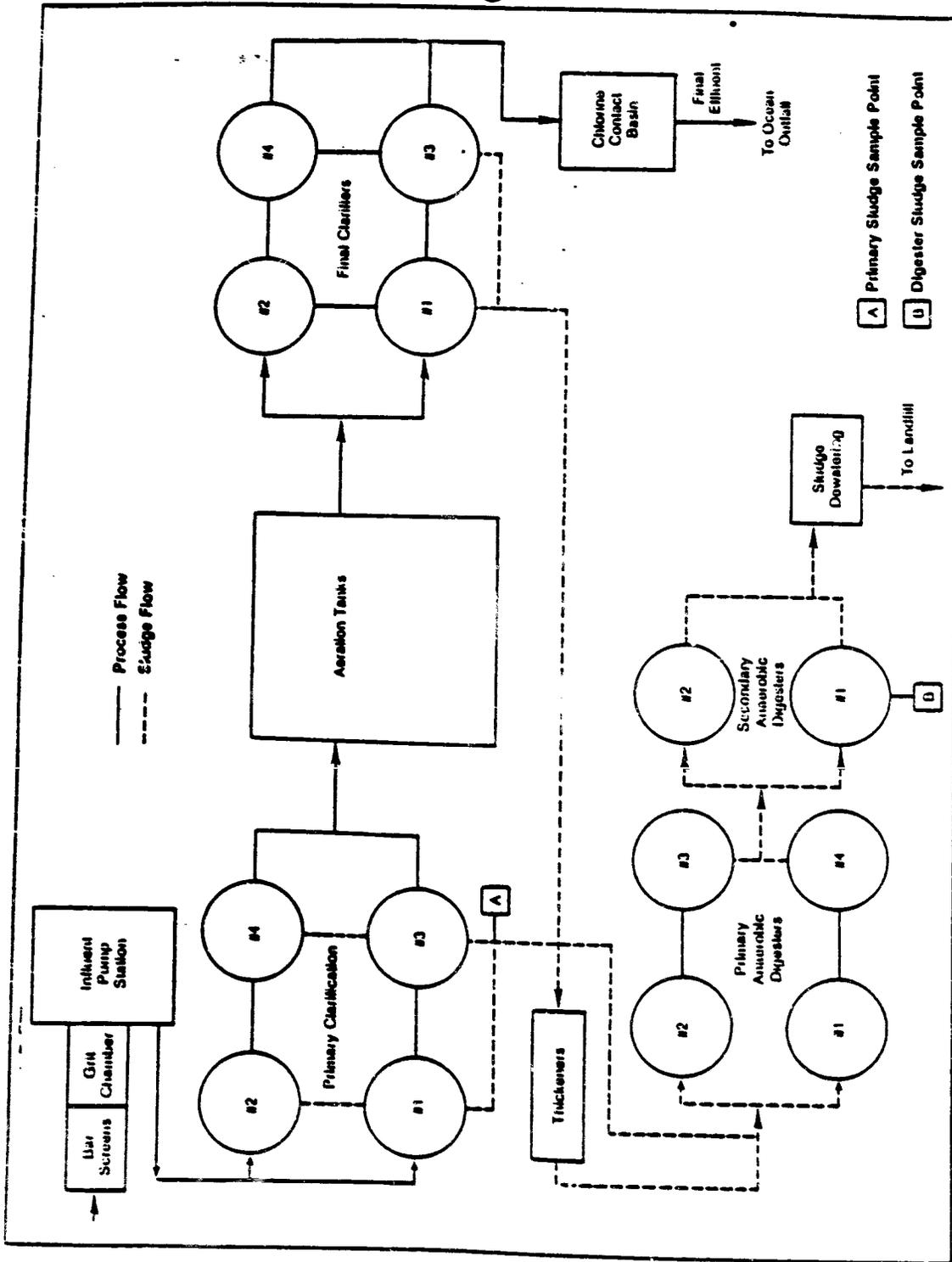


FIGURE D OCEAN COUNTY SOUTH TREATMENT PLANT
OCEAN COUNTY, NEW JERSEY

Appendix A

Quality Assurance Methods



QUALITY ASSURANCE METHODS

This report and related records have been audited by the Quality Assurance Coordinator for adherence to protocol, laboratory standard operating procedures, and pertinent EPA Good Laboratory Practices. If non-compliance items were identified, management and the Study Director were notified immediately for corrective action.

Audits for the study were conducted on the following dates:

24 June 1985

10 July 1985

29 October 1985

Dianne S. Therry

Quality Assurance Coordinator



THE PROCTER & GAMBLE COMPANY

MIAMI VALLEY LABORATORIES

March 13, 1985

P. O. BOX 39175
CINCINNATI, OHIO 45247

Mr. Peter J. Marks
Roy F. Weston, Inc.
Weston Way
West Chester, PA 19380

Dear Mr. Marks:

This is to authorize you to carry out the following study according to the attached protocol, and in conformance with the stipulations of our current Laboratory Services Agreement.

Protocol: Batch Anaerobic Digestion Inhibition Test on B0793.01
Date: 3/5/85

Sponsor's Principal Investigator:

Notice: The stipulations of this protocol are to be implemented in conformance with EPA Good Laboratory Practice Regulations (40 CFR, Part 792).

Please have the Study Director approve and complete both copies of the attached protocol by adding your study or project number, estimated starting and reporting dates, and date the test material was received. Retain one copy for your files and return the other to me.

Please return all unused portions of the test material to the Sponsor's Principal Investigator at the following address:

The Procter & Gamble Company
Sharon Woods Technical Center
11520 Reed Hartman Hwy.
Cincinnati, OH 45241

We understand the estimated cost for this study is \$5,000. Invoices are also to be sent to the Principal Investigator.

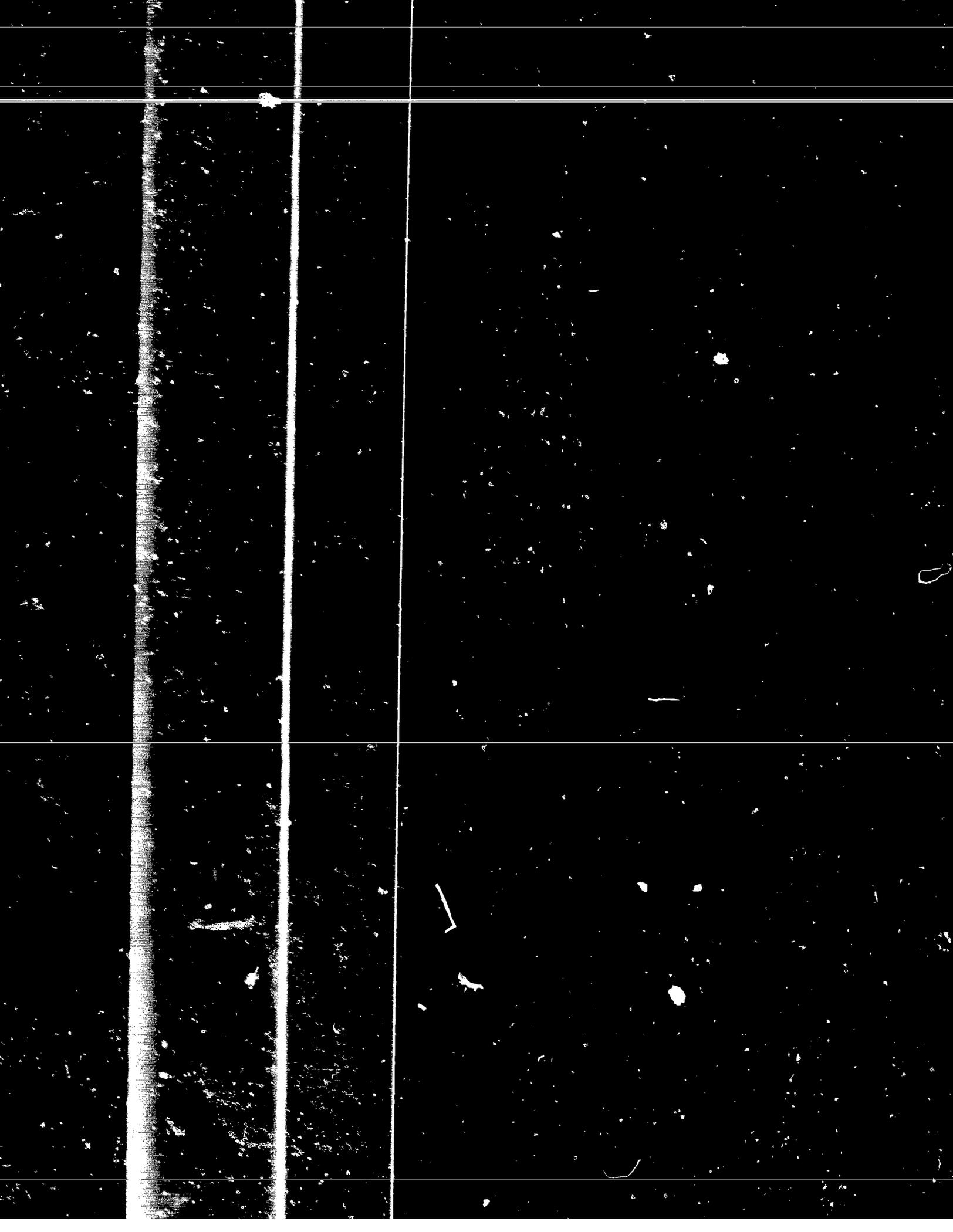
Matters involving the scientific aspects of the work can be handled directly with the Sponsor's Principal Investigator. Feel free to contact me at if you have any other questions or concerns.

Sincerely,

THE PROCTER & GAMBLE COMPANY

Approved:

Attachments
cc:





THE PROCTER & GAMBLE COMPANY

MIAMI VALLEY LABORATORIES

P.O. BOX 39175
CINCINNATI, OHIO 45247

June 13, 1985

Mr. Peter J. Marks
Roy F. Weston, Inc.
Weston Way
West Chester, PA 19380

Dear Pete:

This is to correct an error Dianne Therry identified in our standard protocol for the Batch Anaerobic Digestion Inhibition Test. On page 1 in special instructions 1.b., the less than (<) symbols should be greater than (>) for both the 200 ml and 500 ml TGP.

This correction should be made on the protocols for the studies listed below. Please have Dave Russell sign this letter and include a copy in each of the study files, and return a signed copy to me.

Sincerely,

THE PROCTER & GAMBLE COMPANY
Research & Development Department

bg

| <u>TSIN</u> | <u>Weston Project No.</u> |
|-------------|---------------------------|
| P1138.01 | 84-034 |
| F1195.01 | 84-035 |
| W0924.01 | 85-008 |
| B0792.01 | 85-016 |
| B0793.01 | 85-017 |
| B0807.01 | 85-015 |

PROTOCOL ADDENDUMS

B0793.01

Batch Anaerobic Digestion Inhibition Test

A. Test Material Additions

The protocol calls for test material additions to be made in a stock solution using isopropanol as the solvent. Due to the toxic effects of isopropanol on the anaerobic system, and the non-soluble properties of the test material, test material additions will be made by direct weight.

Principal Investigator (Date) Study Director Michael D. DePinto 3/14/86 (Date)

M. DePinto
2/5/86

1

WESTON

PROTOCOL ADDENDUM

Batch Anaerobic Digestion Inhibition Test on B0793.01

**Michael G. Stapleton has been designated as Study Director for
Batch Anaerobic Digestion Inhibition Test on B0793.01.**

 5/16/85

**Peter J. Marks
Project Director
WESTON (A Business Trust)**

(Date) -

(Date)

The Procter & Gamble Company

0213

R-2

Laboratory Project No. 85-017

PROTOCOL

SPONSOR: The Procter & Gamble Company, Cincinnati, Ohio.
Roy F. Weston, Inc.

LABORATORY: Weston Way
West Chester, PA 19380

TITLE: Batch Anaerobic Digestion Inhibition Test on B0793.01.

OBJECTIVE: To determine a no observed effect concentration (NOEC) and an observed effect concentration (OEC) of the test material on a laboratory anaerobic digestion test system.

JUSTIFICATION FOR TEST SYSTEM: Many of P&G's products are sewerred by the consumer and disposed through municipal wastewater treatment systems. Some quantity of this material will reach the anaerobic digester either sorbed to the sludge or as a soluble component in the liquid fraction of the sludge.

TEST MATERIAL:

| | | | |
|------------------|--------------------|---------------------|-----------------------|
| Sample Code | <u>B0793.01</u> | Physical Form | <u>gal/liquid</u> |
| Percent Active | <u>100</u> | Color | <u>Opaque</u> |
| Molecular Weight | <u>297</u> | Solvent | <u>—</u> |
| Expiration Date | <u>in progress</u> | Solubility in Water | <u>low</u> |
| Density | <u>--</u> | Other | <u>Soluble in IPA</u> |

Storage Conditions - room temperature

Safe Handling Precautions - Caution: May cause eye and skin irritation. If exposed rinse with plenty of water. Strong fishy odor - use hood.

The Sponsor accepts full responsibility for the appropriate characterization and stability verification of this test material.

REFERENCE TEST METHOD: Batch Anaerobic Digestion Inhibition, Procter & Gamble Environmental Standard Test Method

SPECIAL INSTRUCTIONS AND/OR MODIFICATIONS:

1. The Principal Investigator is to be contacted if any of the following criteria in the Reference Test Method are not met:
 - a. Test Procedures; C-3-b (EQ.1) by t=21 days
 - b. Expt. Sampling & Analyses; B-1 (TGP<200 ml by t=48 hr and/or TGP<500 ml by t=14 days)
 - c. Data Analyses; B (EQ.4).

TEST MATERIAL ADDITION/PREPARATION:

All calculations and measurements are to be based on the active ingredient of the test material. The test material is to be introduced to the reactors as an IPA solution. All test flasks and controls must contain equal solvent levels (i.e. level in highest test concentration).

The following five test material concentrations are to be studied:

| <u>Treatment</u> | <u>Concentration (mg/L)</u> |
|------------------|-----------------------------|
| 1 | 0.1 |
| 2 | 1.0 |
| 3 | 10.0 |
| 4 | 100.0 |
| 5 | 1000.0 |

RECORDS TO BE MAINTAINED: All records necessary to reconstruct the study and demonstrate adherence to the Protocol. Example formats for the data are available in the appendix of the reference test method.

PROTOCOL CHANGES: If a change in the approved protocol becomes necessary, verbal agreement should be made between the Study Director and Sponsor's Principal Investigator. As soon as practical thereafter, this change and the reasons for it should be put in writing, approved by both persons, and attached to the protocol as an addendum.

REPORTING: The report is to be a typed document describing the results of the study. It is to be signed and dated by the Study Director, Quality Assurance Officer, and Laboratory Manager. It is to include, but is not limited to, a description of the quality assurance methods used to insure the quality of the data, and the items in the Reporting of Results section in the Reference Test Method.

ALTERNATE PRINCIPAL INVESTIGATOR _____

NOTE

Lu/ps _____ PHONE: _____
Date

APPX

Lu/ps _____ PHONE: 5: _____

Principal Investigator

Date

TO BE COMPLETED BY STUDY DIRECTOR:

Laboratory Project No. 85-017

Estimated Starting Date 4/30/85

Estimated Reporting Date 6/30/85

Date Test Material Received 3/19/85

Approved

David R. Kimmel
Study Director

3/20/85
Date

PHONE: 215-642-3030

0 2 1 5

Procter & Gamble Amine No. 73 is identified as Amines, C14-18-
Alkyldimethyl (CAS # 68037-93-4) under current TSCA nomenclature.

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WV 380

RESEARCH AND DEVELOPMENT DEPARTMENT MEMORANDUM
Research Division

E. V. Bumbler

October 1, 1954

Guinea Pig Closed Patch Test (# V1724-74)
(BTS #359)

Material(s) Tested Procter & Gamble A-ine No. 73 (hydrogenated tallow dimethyl amine).

Reasons for Testing Determination of sensitization potential.

Patching Schedule Once weekly for 5 weeks. Challenge with 2% in dimethyl phthalate.

| <u>Test Material</u> | <u>Cons.</u> | <u>Vehicle</u> | <u>Results*</u> |
|--------------------------------------|--------------|--------------------|-----------------|
| F&G A-ine No. 73 (XND-833, UDK-1779) | 2% | Dimethyl phthalate | 0/31 |
| Dimethyl phthalate | - | - | 0/6 |

* No. of Positive Reactors/Total No. Tested

Remarks None of the test animals were sensitized when compared to controls challenged with dimethyl phthalate.

Copies To: L. J. Beck; NVL Library File
J. P. Griffith

E. V. Bumbler

Subjects

Guinea Pig Closed Patch Test
F&G A-ine No. 73 (hydrogenated tallow dimethyl amine)
Dimethyl phthalate

Procter & Gamble Amine No. 73 is identified as Amines, C14-18-
Alkyldimethyl (CAS # 68037-93-4) under current TSCA nomenclature.

Contains No CBI

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CONFIDENTIAL

RESEARCH AND DEVELOPMENT
Research Division

E. A. Bowman

September 2, 1960

Rabbit Skin Irritation (SV1988-27)

Material(s) Tested Procter & Gamble Amine No. 73.

Reasons for Testing Safety evaluation requested prior to test sales as an industrial chemical (EPC 7339).

| <u>Test Material</u> | <u>Mean Score (3)</u> | | <u>Rabbits/Sample</u> | | <u>Primary Irritation Index**</u> |
|---|-----------------------------------|--------------|----------------------------------|--------------|-----------------------------------|
| | <u>3 Abraded Sites*
Ryth.</u> | <u>Edema</u> | <u>3 Intact Sites*
Ryth.</u> | <u>Edema</u> | |
| P&G Amine No. 73
(EPC-833, UDE-1779) | 4.0 | 3.0 | 4.0 | 3.0 | 7.0 |

* Average of 24 and 72 hour scores.

** 0-2 = Mild; 2-5 = Moderate; 6 or above = Severe

Remarks P&G Amine No. 73 was rather irritating to the skin of rabbits producing necrosis on all test sites, and is classified as a severe irritant.

Copies To: L. W. Best; NYL Library File
J. P. Griffith

E. A. Bowman

Subjects

Rabbit Skin Irritation
P&G Amine No. 73

Product Identification

Procter & Gamble Amine No. 73
(EPC-833) (hydrogenated tallow
dimethyl amine) made at Kansas
City from 20265 stock and di-
methyl amine.

0 2 0 6

Procter & Gamble Amine No. 73 is identified as Amines, C14-18-
Alkyldimethyl (CAS # 68037-93-4) under current TSCA nomenclature.

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EXPERIMENTAL TEST RESULTS & CONCLUSIONS

BTS No. 159

1. Acute Oral Toxicity (200-300 gm rats)

V184-153

| Material | Concentration | LD ₅₀ | 95% Confidence Limits |
|-------------------------------|---------------|--------------------------|-----------------------|
| P40 Amino No. 73
(XNO-833) | 100% | 1.3 ml/kg
(1.0 gm/kg) | 1.1 - 1.6 |

P40 Amino No. 42 has an LD₅₀ of 1.4 ml/kg (1.1 gm/kg).

2. Eye Irritation (rabbits)

V2000-7

| Material | Concentration | Average Max. Score | Corneal Involvement | Eyes Normal in Indicated No. of Days |
|-------------------------------|---|--------------------|---------------------|--------------------------------------|
| P40 Amino No. 73
(XNO-833) | 100% _{HR}
100% _R | 45.0
9.3 | 3/3
0/3 | 3 in 14
2 in 4; 1 in 7 |

Non-rinsed P40 Amino No. 73 produced mild to moderate corneal clouding, bleeding of the mucosa of the eye lids, and necrosis of the exterior of the lids. Rinsing reduced ocular involvement to conjunctivitis and slight necrosis of the lids. P40 Amino No. 73 was essentially equivalent to P40 Amino No. 42 in eye irritant potential.

3. Skin Irritation (rabbit)

V1925-27

| Material | Concentration | Irritation Index | Degree of Irritation |
|-------------------------------|---------------|------------------|----------------------|
| P40 Amino No. 73
(XNO-833) | 100% | 7.0 | Severe |

(6 or above = severe)

P40 amino No. 73 produced necrosis of all test sites. Amino No. 73 was essentially equivalent to P40 Amino No. 42 in skin irritancy.

4. Skin Sensitisation (guinea pigs)

V1724-34

| Material | Concentration | Reactions Indicative of Sensitization |
|-------------------------------|-------------------------|---------------------------------------|
| P40 Amino No. 73
(XNO-833) | 2% in dimethylphthalate | 0 of 31 |
| Dimethyl Phthalate | undiluted | 0 of 6 |

A closed patch, repeated insult (once weekly for 6 weeks), patch test and subsequent challenge did not produce any evidence of sensitization.

Conclusions:

The above results indicate P40 Amino No. 73 would not be expected to present ingestion or skin sensitization hazards when used for industrial applications, and when handled according to good industrial hygiene practices. The results indicate the material is a primary skin irritant. In addition, it was shown that the amino may produce ocular injury if not removed from the eye immediately. P40 Amino No. 73 is considered safe for marketing as an industrial chemical, provided appropriate cautionary labeling, relative to eye and skin irritation, is used.

Signature J. E. Carter Date 12/2

CONFIDENTIAL

RESEARCH AND DEVELOPMENT DEPARTMENT MEMORANDUM
Research Division

E. V. Bushler

August 31, 1944

Acute Oral Toxicity (S V1877-158)
(MS #357)

Material (s) Tested P80 Amine No. 73.

Species: Charles River SD Rat (200-300 gm).

| <u>Test Material</u> | <u>Conc.</u> | <u>Dose Level</u> | <u>Dead/Total</u> | <u>LD₅₀</u> | <u>Conf. Limits</u> |
|---------------------------------------|--------------|-------------------|-------------------|------------------------|---------------------|
| P80 Amine No. 73
(MS-833, MS-1779) | 100% | 0.678 ml/kg | 0/10 | 1.39 ml/kg | 1.09-1.62 |
| | | 0.95 ml/kg | 1/10 | | |
| | | 1.33 ml/kg | 6/10 | | |
| | | 1.86 ml/kg | 8/10 | | |

Remarks P80 Amine No. 42, recently tested, has an LD₅₀ value of 1.39 ml/kg.

Copies To: L. W. Bush; NVL Library File
J. P. Griffith

E. V. Bushler

Subjects
Acute Oral Toxicity
P80 Amine No. 73

Product Identification

P80 Amine No. 73 (MS-64)

Hydrogenated tallow dimethyl amine.
Made at Kansas City from 20265 stent
and dimethyl amine.

NV #210

Procter & Gamble Amine No. 73 is identified as Amines, C14-18-
Alkyldimethyl (CAS # 68037-93-4) under current TSCA nomenclature.

Contains No CBI

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RESEARCH AND DEVELOPMENT PERMITS PROGRAM Research Division

E. A. Newman

September 2, 1960

Eye Irritation (4 V2000-9)

Material(s) Tested Procter & Gamble Amino No. 73.

Reasons for Testing Safety evaluation requested prior to test sales as an industrial chemical (ETS 7559).

| <u>Test Material</u> | <u>Conc. (%) & Treatment*</u> | <u>MAG**</u> | <u>Corneas Involved</u> | <u>Eyes Normal in Indicated No. of Days</u> |
|---|-----------------------------------|--------------|-------------------------|---|
| 140 Amino No. 73
(IND-833, UDL-1779) | 100% (NR) | 45.0 | 3/3 | See remarks. |
| | 100% (R) | 9.3 | 0/3 | 2 in 4; 1 in 7 |

* (NR) No Rinse (R) Rinse (MAG) Max. Avg. Score ** Not Related to Treatment

Remarks 140 Amino No. 73 produced diffuse areas of corneal translucency which cleared within fourteen days. This product, however, produced bleeding of the palpebral conjunctiva and necrosis of the exterior part of the eye lids. Except for loss of hair on the lids, all eyes appeared grossly normal after fourteen days. Rinsing greatly reduced the ocular involvement so that only conjunctivitis and slight necrosis of the lids were noted.

Copies For: E. A. Beck; NVL Library File
J. F. Griffith

E. A. Newman

Subjects

Eye Irritation
40 Amino No. 73

10769

Eye Irritation Scores of Individual Rabbits

| F&O Amino No. 73 | Test Material | Treatment | No. 1 Hr. | Days | | | | | |
|------------------|---------------|-----------|-----------|-------|--------|--------|--------|--------|--------------|
| | | | | 1 Day | 2 Days | 3 Days | 4 Days | 7 Days | 14 Days |
| 688 | 100% (IR) | 1/5cat | 6 | 4 | 3 | 3 | 3 | 3 | See remarks. |
| | | | 10 | 20 | 30 | 30 | 30 | 30 | See remarks. |
| | | | 6 | 4001 | 3 | 3 | 3 | 3 | See remarks. |
| 692 | 100% (R) | 1/5cat | 2 | 4 | 4 | 4 | 4 | 4 | 0 |
| | | | 2 | 4 | 4 | 4 | 4 | 0 | |
| | | | 2 | 12 | 2 | 2 | 2 | 0 | |

1 = Iritis
 0 = Corneal Involvement

Product Identification

Procter A. Double Amino No. 73 (K14-64)
 (hydrogenated tallow dimethyl amine)
 made at Kansas City from 20265 stock
 and dimethyl amine.



CERTIFICATE OF AUTHENTICITY

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