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Submitting Organization
PROCTER & GAMBLE CO

Contractor

Document Title
ULTIMATE BIODEGRADABILITY VIA CO2 PRODUCTION, WITH COVER LETTER DATED 5/23/96

Chemical Category
POLYETHYLENE GLYCOL 4-(TERT-OCTYL)PHENOL ETHER (9002-93-1)

OFFICE OF TOXIC SUBSTANCES REV. 7/27/82

CODING FORM FOR GLOBAL INDEXING

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May 23, 1996

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Attn: 8(d) Health and Safety Reporting Rule (Notification/Reporting)

RE: SUBMISSION IN RESPONSE TO TSCA: 8(D) FINAL RULE-TECHNICAL AMENDMENTS HEALTH AND SAFETY REPORTING

(Federal Register 2/28/96; 61FR7421, OPPTS-82048; FRL-4996-9)

Dear Sir/Madam:

This submission is being made by The Procter & Gamble Company to satisfy 8(d) Health and Safety Reporting requirements as specified above.

We are submitting a variety of different types of tests, including sensitization, mutagenicity irritation, biodegradation, toxicity and cytogenetics. An index of test substances for these studies is attached along with the name of one study currently in progress.

In addition, we are providing a list of studies conducted by CMA (Chemical Manufacturer's Association). We understand that the CMA APE Panel plans to provide EPA and ITC with copies of these studies under a Section 8(d) extension. In keeping with the Agency's interest in reducing paperwork and knowing that the Agency will be receiving copies separately, we have only listed these studies in our submission.

If you wish further information regarding this submission, please contact me at 513-627-6145.

8696000524
To
8696000534

Sincerely Yours,
The Procter & Gamble Company

William E. Bishop, Ph.D.
Manager
Risk, Policy, & Regulatory Sciences

Attachments

HEALTH AND SAFETY STUDIES
THE PROCTER & GAMBLE COMPANY

INDEX OF SUBMISSION

	8(d) Name & CAS Number		Study Title/Description
524	Paratertiary butyl phenol	(98-54-4)	Depigmentation Studies Using Phenolic Components
525	Polyethylene glycol octylphenyl ether	(9036-19-5)	Toxicity Testing of Triton X-15 to Chinese Hamster Ovary Cells
526	Polyethylene glycol octylphenyl ether	(9036-19-5)	A Method for Evaluating the Photoallergic Property of Chemicals Using G. Pigs
527	Polyethylene glycol octylphenyl ether	(9036-19-5)	Activity of R0110 (T1772) (Triton X-15) in the In Vivo Cytogenetic Assay in Rodents
528	Polyethylene glycol octylphenyl ether	(9036-19-5)	An Evaluation of an In Vitro Cytogenetic Assay with Chinese Hamster Ovary (CHO) Cells
529	Polyethylene glycol octylphenyl ether	(9036-19-5)	Evaluation of Various Surfactants for Compatibility with the Salmonella/Microsome Test
530	Polyethylene glycol 4-(tert-octyl) phenol ether	(9002-93-1	Ultimate Biodegradability via CO_2 Production
531	Polyethylene glycol 4-(tert-octyl) phenol ether	(9002-93-1)	Acute Oral Toxicity of Wyandotte Experimental Nonionic X-100
532	Polyethylene glycol 4-(tert-octyl) phenol ether	(9002-93-1)	Eye Irritation of Triton X-100
533	Polyethylene glycol 4-(tert-octyl) phenol ether	(9002-93-1)	Acute Oral Toxicity of Triton X-100
534	Polyethylene glycol 4-(tert-octyl) phenol ether	(9002-93-1)	Comparison of SDA and OECD Biodegradation Test Methods

STUDY IN PROGRESS

Development of Cytotoxicity Screening Assays. University of Connecticut, Storrs.
8(d) Chemical Name and CAS No. - Polyethylene glycol 4-(tert-octyl) phenol ether (9002-93-1)
Estimated Completion - August 15, 1996.

CHEMICAL MANUFACTURERS ASSOCIATION
INDEX OF SUBMISSION
HEALTH AND SAFETY STUDIES

8(d) Name & CAS Number		Study Title/Description
Nonylphenol	(84852-15-13)	Toxicity of Nonylphenol to the Amphipod *Hyalella azteca* (Saussure)
Nonylphenol	(84852-15-13)	Toxicity of Nonylphenol to the Midge *Chironomus tentans*
Nonylphenol	(84852-15-13)	Chronic Toxicity of Nonylphenol to *Ceriodaphnia dubia*
Nonylphenol	(84852-15-13)	Early Life Stage Toxicity of Nonylphenol to the Fathead Minnow, *Pimephales promelas*

Contains No CBI

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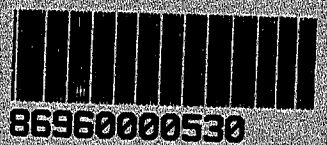


86960000530

BIODEGRADABILITY TEST SUMMARY SHEET

Laboratory: EWQRL

BTR-74023

EWQRD Code #	Material Identification	Calculations TOD, g/g active	Calculations TCO_2, g/g active	Stock Soln. Conc., mg/l active	Stock Soln. pH Before Adj.	Stock Soln. pH After Adj.	Oxygen Demand @ 2ppm COD, g/g active	Oxygen Demand @ 2ppm % TCO_2 Aerobic	CO_2 Production % TCO_2 Standard	CO_2 Production Conc., mg/l	CO_2 Production Days in Test
1. Blank	Blank	--	--	--	--	--	--	--	--	--	40
2. Dextrose	Dextrose	1.07	1.47	1074	7.1	7.1	0.97	85.0	77.5	20	40
3. APS-71.031	C_{12}LAS	2.32	2.28	1119	10.6	7.0	2.23	73.1	69.9	20	40
4. APS-71.033	C_{13}LAS	2.36	2.31	993	6.2	6.2	2.40	28.5	32.8	20	40
5. NPR-74.002	Polyglycol E-600	1.07	1.47	1336	6.3	6.3	1.31	91.9	53.1	20	40
6. NPR-74.012	Triton X-100	not sent	TOC used 1.96	1002	6.0	6.0	2.01	44.7	41.0	20	40
7. NPR-74.013	NPE 10	1.93	1.93	1160	6.2	6.2	1.73	35.6	32.7	20	40
8. NPR-74.014	Dobanol 25-9	2.27	2.30	902	5.6	7.2	2.33	38.3	25.5	20	40
9. NTG-74.005	BRIJ-35	2.31	2.13	979	9.4	7.7	1.97	57.1	30.1	20	40
10. NTG-74.006	Lauric Diethanolamide	2.54	2.45	929	7.1	7.1	2.39	83.8	79.7	20	40

Notebook-page #	Outside Lab	EWQRD
COD test:		M-3523 pg. 39
BOD test:		
CO_2 test:	--	X
Other		

Seed source: Colerain Heights
Date acclimatization started: 12-14-74
Date test started: 12-28-74
Date test completed: 2-5-75

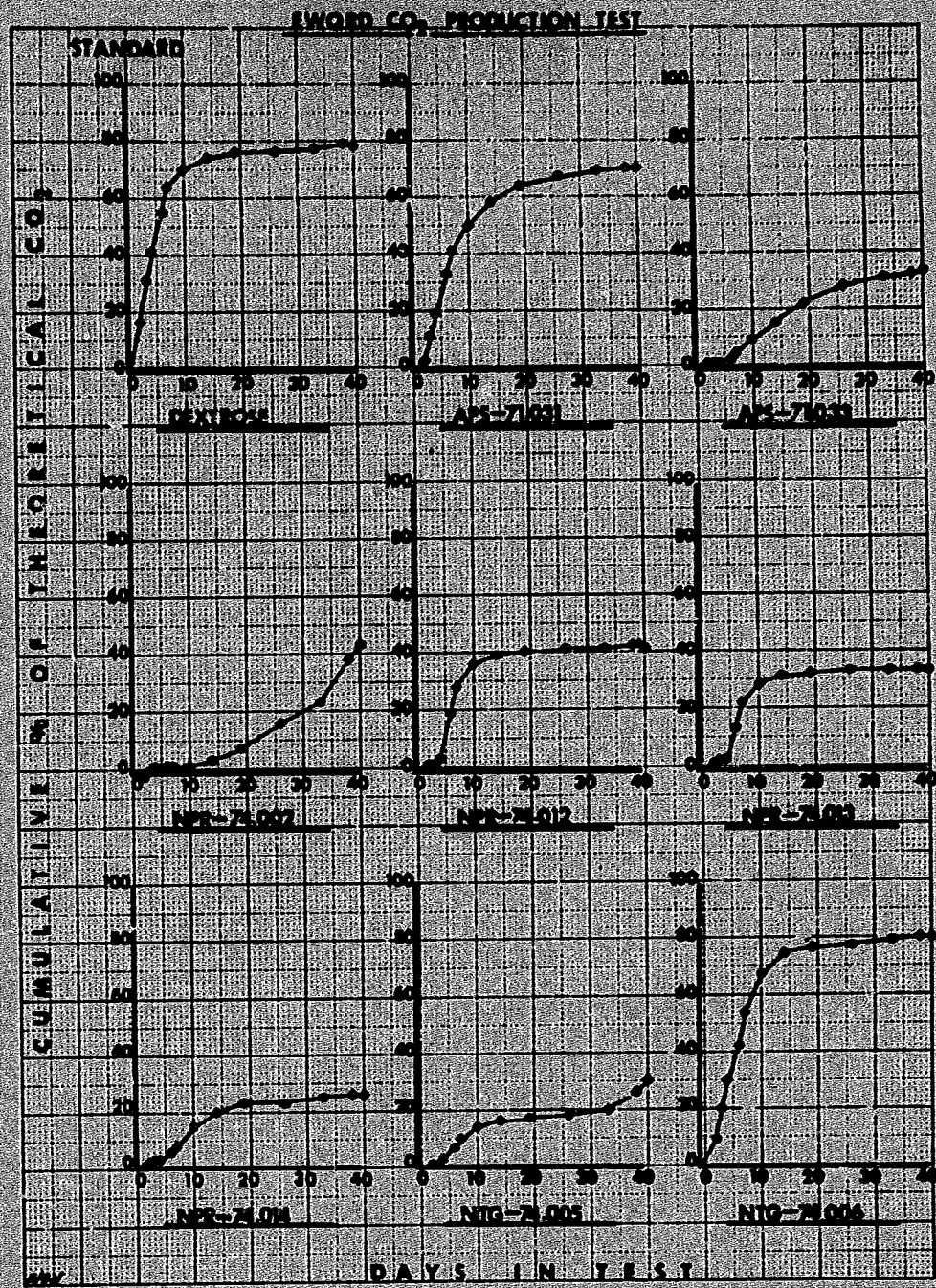
Comments:

Note: (1) This was a special test to determine if aerating the Mineral Media seed had any effect on CO_2 degradation. Results indicate a slight difference; however, air was introduced in standard test. Immediate rerun is scheduled to confirm results of this test.

(2) Three liter O_2 test

(3) BOD's in 5% composite seed.

Signed: Lee McCuistion
(experimenter)



NO. 328 MILLIMETERS 180 BY 220 DIVISIONS.
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SWORD CO_2 PRODUCTION TEST
AEROBIC
CUMULATIVE % OF THEORETICAL CO_2
DEXTROSE
ABS-71.001
ABS-71.002
NCR-74.002
NCR-74.012
NCR-74.013
NCR-74.014
NTG-74.005
NTG-74.006
DAYS IN TEST

PROTOCOL FOR THE DETERMINATION OF

ULTIMATE BIODEGRADABILITY VIA CO_2 PRODUCTION

Sept. 9, 1974

Theory and Scope

This screening test is based on the proposition that, as a carbonaceous material is acted upon by aerobic bacteria capable of utilizing it as a carbon and energy source, the molecule will be converted to cellular material, carbon dioxide and water. The ultimate biodegradability of a given material may then be estimated by measuring the amount of CO_2 produced or the amount of O_2 consumed by acclimated bacteria during a given time, and relating these figures to calculated theoretical yield based on the structure and molecular weight of the compound under investigation. The CO_2 gas produced by a suitably acclimatized bacterial population is trapped in barium hydroxide, thus precipitating out barium carbonate. The barium hydroxide remaining is titrated with HCl, and the amount of evolved CO_2 is then calculated, relative to a suitable blank, of course. If a known quantity of test material containing a known amount of carbon is added to the system, then the evolved CO_2 can be related directly to the carbon input, and biodegradation can be expressed as a "percent of theoretical CO_2."

Test System

The CO_2 production test is usually set up in a battery of 10 carboys consisting of a blank carboy, eight test carboys, and one carboy containing dextrose as the test material to serve as a positive control that confirms that the test system is running correctly.

As shown in Fig. 1, the carboys are preceded by a scrubbing system that introduces CO_2-free air into the carboys, consisting of a rotameter, a silica gel/Drierite drying bottle, an Ascarite CO_2 absorbing bottle, and a $Ba(OH)_2$ check bottle. The carboy is followed by three 100 ml. $Ba(OH)_2$ bottles in series to trap all the evolved CO_2 from the test system.

Drying Train Apparatus

The train is in series, in the order described (use heavy walled glassware):

1 air rotameter
1 1-liter Erlenmeyer filled 2/3 full with activated carbon and with a wad of cotton on the carbon surface, to remove trace organics.
1 2-liter Erlenmeyer filled 2/3 full with indicating silica gel, to remove moisture.
1 2-liter Erlenmeyer filled 2/3 full with a 50:50 mixture of regular and indicating Drierite, to remove moisture.
1 2-liter Erlenmeyer filled 2/3 full with a 50:50 mixture of indicating Drierite and Ascarite, to remove CO_2.

1 2-liter Erlenmeyer filled 2/3 full with Ascarite, to remove CO_2.
1 2-liter Erlenmeyer, filled 2/3 full with 0.05 N $Ba(OH)_2$, to remove CO_2, to indicate if the absorber train is working, and to remoisturize the air.
1 1-liter bottle filled with glass wool, for pressure equalization and to prevent liquid carryover.

Acclimation Apparatus

9 2-liter Erlenmeyer flasks
9 500 ml volumetric flasks

CO_2 Production Apparatus

10 1 gallon amber bottles, soft glass
10 15¼" long, ¼" O.D. pieces of glass tubing
10 4" long, ¼" O.D. pieces of glass tubing
30 4½" long, ¼" O.D. pieces of glass tubing with one end tempered to a 1/8" O.D. opening
10 #6 three hole stoppers
30 #4 two hole stoppers
1 box ¼" I.D. rubber tubing
~150 "French squares" (i.e. 100 ml barium hydroxide absorber bottles)
1 10 ml burette
0.025N $Ba(OH)_2$
0.05 N HCl, standardized

Reagents

a. BOD water stock solutions

Ferric chloride solution - dissolve 0.25 g $FeCL_3 \cdot 6H_2O$ in distilled water and dilute to 1 liter.

Magnesium sulfate solution - dissolve 22.5 g $MgSO_4 \cdot 7H_2O$ in distilled water and dilute to 1 liter.

Calcium chloride solution - dissolve 27.5 g anhydrous $CaCl_2$ in distilled water and dilute to 1 liter.

Phosphate buffer solution - dissolve:

8.5 g potassium dihydrogen phosphate, KH_2PO_4;
21.75 g dipotassium hydrogen phosphate, K_2HPO_4;
33.4 g disodium hydrogen phosphate heptahydrate, $Na_2HPO_4 \cdot 7H_2O$;
1.7 g ammonium chloride, NH_4Cl;

in 500 ml of distilled water and dilute to 1 liter. The pH of this buffer should be 7.2 without further adjustment.

The BOD water reagents should therefore contain the following:

$FeCl_3$ stock: 250 mg/l $FeCl_3 \cdot 6H_2O$; 150.2 mg/l $FeCl_3$; 31.8 mg/l Fe
$MgSO_4$ stock: 22500 mg/l $MgSO_4 \cdot 7H_2O$; 10976 mg/l $MgSO_4$; 2195 mg/l Mg; 8780 mg/l SO_4

$CaCl_2$ stock: 27500 mg/l $CaCl_2$; 9910 mg/l Ca; 17590 mg/l Cl
Phosphate buffer: 12187 mg/l K; 2866 mg/l Na; 29652 mg/l PO_4, 572 mg/l NH_4; 1128 mg/l Cl.

b. BOD water

For each 1 liter of BOD water, add 1 ml each of the ferric chloride, magnesium sulfate, calcium chloride, and phosphate buffer stock solutions and make up to 1 liter with distilled water. The BOD water should now contain:

0.052 mg/l Fe^{+++}	8.780 mg/l $SO_4^=$
2.195 mg/l Mg^{++}	18.718 mg/l Cl^-
9.910 mg/l Ca^{++}	29.652 mg/l $PO_4^=$
12.187 mg/l K^+	(equivalent to 9.675 mg/l P)
2.866 mg/l Na^+	
0.572 mg/l NH_4^+	

c. Barium hydroxide, 0.025 N - dissolve 7.89 g $Ba(OH)_2 \cdot 8H_2O$ distilled water and dilute to 2 liters. Filter through filter paper and cap off the clear solution to prevent absorption of CO_2 from the air.

d. HCl, 0.05 N - dissolve contents of one HCl ampule of Chemtam (or equivalent) in one liter of distilled water to make 1 N HCl. Dilute 50 ml of this solution to 1 liter in a volumetric flask. Store in the dark.

e. Yeast extract - dissolve 5 g in distilled water and make up to 500 ml.

Sample Preparation

This portion of the protocol is extremely important, since the % biodegradation depends directly on having the correct amount of carbon added to the test system. For this reason, the following sample preparation routine should be adhered to:

a. Initial stock solution - weigh out a homogeneous aliquot of the sample (e.g. shake well, but avoid foaming that tends to concentrate the active disproportionately; for solid samples, melt and mix the entire contents of the sample bottle before taking the aliquot), and make up a 1000 mg/l stock solution of the active material in distilled water (i.e. stocks are usually made up on an "active" basis, not on a "total" or "as-is" basis). Usually, a 500 ml or 1 liter stock solution is made up.

b. Problem stocks - It is of the utmost importance that the stock be a true solution, which by definition here means "clear to the eye." If the stock solution is turbid, contains insoluble particles, contains floating material, or contains material clearly observable as being adsorbed to the walls, heat the stock gently (<90°C) while stirring overnight. After cooling, it may be possible to obtain a clear solution. If this is not the case, dilute a small portion of the stock 1:50 in distilled water to see if a 20 mg/l solution of active (the final test concentration) would dissolve. In many cases, simple dilution will be sufficient to obtain a clear solution. If this also

fails, filter the entire contents of the stock through a prewashed 0.45 u Millipore membrane filter. Obviously, such a procedure done on heterogeneous materials will change the nature or distribution of the material in the filtrate relative to what was in the original sample. The submitter should be aware of this. Another alternative is to suspend the insoluble material with a hydrotrope or a non-biodegradable surfactant, such as ABS. This works especially well for insoluble, floating materials, such as mineral oil. Obviously, two test carboys would have to be set up in this case - an "ABS + test material" carboy and an "ABS alone" control carboy - and the CO_2 produced by the latter must be subtracted from the CO_2 produced by the former, in order to measure the CO_2 produced by the test material alone.

c. pH adjustment - check the pH of each (clear) stock solution. If the pH is between 6.0 and 8.5, nothing need be done except recording the pH. However, if the pH is outside these limits, the pH should be brought inside these limits, and preferably close to pH 7, with a small amount of NaOH or HCl. The pH both before and after adjustment should be recorded.

d. Organic carbon analysis - In order to ascertain exactly how much active is in the stock solution, the stock solution should be analyzed for Total Organic Carbon. This is usually best accomplished by running a TOC analysis of a 1:10 dilution of the stock on a Beckmann Model 915 Total Carbon Analyzer. This will give the mg/l carbon in the stock.

e. TCO_2 and TOC calculations - The theoretical amount of CO_2 that can be generated by a test material, or TCO_2, may be calculated as follows:

$$TCO_2, \text{ mg } CO_2/\text{mg active} = \frac{\#\text{ of carbons in active} \times \text{MW of } CO_2}{\text{MW of active}}$$

For example, dextrose ($C_6H_{12}O_6$) contains 6 carbons. Therefore, TCO_2 of dextrose $= \frac{6 \times 44}{180} = 1.467$ mg CO_2/mg dextrose

For mixtures, the TCO_2 of the total active is obviously a weighted average of the TCO_2's of the individual components. Once the TCO_2 has been calculated, and the TOC has been measured on the stock solution, the active concentration in the stock solution may be calculated as follows:

$$\text{Active concentration in stock, mg/l} = \left(\frac{\text{mg carbon}}{\text{l stock}}\right) \times \left(\frac{44 \text{ mg } CO_2}{12 \text{ mg carbon}}\right) \times \left(\frac{\text{mg active}}{\text{mg } CO_2}\right)$$

$$\text{or: } \frac{\text{mg active}}{\text{l stock}} = \frac{\text{TOC} \times 44}{TCO_2 \times 12} = \frac{\text{TOC} \times 3.67}{TCO_2}$$

For example, if the TOC analysis on a dextrose stock solution that had been made up to a nominal concentration of 1000 mg/l was 425 mg/l carbon, then:

$$\frac{\text{mg dextrose}}{\text{l stock}} = \frac{425 \times 3.67}{1.467} = 1063 \text{ mg/l } \underline{\text{actual}} \text{ dextrose concentration.}$$

It is this actual stock concentration as measured by TOC that is to be used in spiking the carboys with test material.

If, however, the sample that has been submitted is of unknown structure and molecular weight, meaning that the TCO_2 is not available, a reverse calculation should be performed, i.e. assume that the stock solution is exactly correct (i.e. 1000 mg/l), and using the TOC, calculate what the TCO_2 should be, as follows:

$$TCO_2 = \frac{TOC \times 3.67}{mg/l \text{ active}} = \frac{TOC \times 3.67}{1000}$$

For example, suppose we didn't know the TCO_2 or MW of dextrose, but had weighed out a 1000 mg/l stock (nominal) and had obtained a TOC of 425 mg/l. The calculated TCO_2 for dextrose would then be:

$$TCO_2 = \frac{425 \times 3.67}{1000} = 1.560$$

This "analyzed" TCO_2 of 1.560 is clearly different than the previously calculated value of 1.467, but for the purposes of obtaining the correct % degradation in the CO_2 test, this is irrelevant, since in both cases, an identical amount of carbon is placed into the test, based on 20 mg/l active added. This will be demonstrated below.

f. Discrepancies - If the calculated active concentration and the nominal concentration (the value it "should be") differ by a wide amount - more than 25% either high or low - the submitter should be contacted to resolve the discrepancy.

Acclimatization

The biodegradability screening test is preceded by a fourteen day acclimatization period during which microorganisms contained in raw sewage are selected and adapted for biodegradation of a specific test material. The acclimatization cultures which consist of a 10% dilution of sewage in BOD water, are set up as follows:

Into a 2 l Erlenmeyer flask add:

a. 1320 ml of BOD water
b. 75 mg of yeast extract (freshly made)
c. 30 ml of a 1000 mg/l stock solution of test sample (since accuracy is not critical in the acclimatization stage, TOC's on the stock are not necessary yet, and a 1000 mg/l concentration may be assumed)
d. 150 ml of settled influent sewage from a non-industrial local municipal sewage treatment plant. The sewage should be added last.

The final volume should be 1500 ml. The flask is shaken and then placed loosely capped with aluminum foil in the dark at room temperature (24°C) for 14 days. The flask should be shaken or swirled periodically during this period.

A standard acclimatization set-up consists of one dextrose flask and eight test flasks, each containing 20 mg/l active in 10% sewage. Each flask should be set up separately, according to the above directions.

CO_2 Test Set-Up

At the end of the 14 day acclimatization period, a "composite seed" is made up that contains 350 ml of each of the 9 acclimatization cultures mixed together and then filtered through glass wool into a 6 liter carboy to remove the large biological floc and slime particles, yielding a total volume of 3150 ml of composite seed.

Note: These 3150 ml are sufficient seed only for the CO_2 test.

Since the BOD test will require the identical composite seed, it may be desirable to make up a larger volume of composite, e.g. 500 ml of each of the 9 acclimatization cultures, filtered to yield a total volume of 4500 ml of composite seed.

Since the CO_2 test employs a 10% seed (i.e. 10% dilution of the composite seed, or a 1% dilution of the original sewage seed used to make up the acclimatization cultures), it is necessary to make dilutions in BOD water. This is most easily achieved as follows:

a. To each of the 10 1 gal. test carboys, add 2200 ml of distilled water.

b. To each of the 10 1 gal. test carboys, add 3 ml each of the ferric chloride, magnesium sulfate, and calcium chloride stock solutions used for making the BOD water; add 6 ml of the phosphate buffer stock solution ("double" buffer is used here for improved pH control).

c. To each of the 10 1 gal. test carboys, add 300 ml of "composite seed."

d. These additions should now total 2515 ml in each carboy.

This mixture is now aerated with CO_2-free air for 24 hours, in order to remove residual CO_2 from the system. At the end of this aeration period, the test materials are added to the carboys to begin the test period, as follows:

a. The amount of test material stock solution required in the carboy is calculated:

$$\frac{\text{ml stock required}}{\text{carboy}} = \left(\frac{\text{target carboy concentration, mg/l}}{\text{mg/l active in stock}}\right) \times \left(\frac{3 \text{ liters}}{\text{carboy}}\right) \times \left(\frac{1000 \text{ ml}}{\text{liter}}\right)$$

For the usual target concentration of 20 mg/l, this works out to:

$$\frac{\text{ml stock required}}{\text{carboy}} = \frac{60000}{\text{mg/l active in stock}}$$

b. Sufficient stock solution to reach the desired test concentration, as calculated above, plus enough distilled water to make 485 ml of (stock solution + distilled water) are added to the appropriate carboys. For the blank control carboy, which contains no test materials, 485 ml of distilled water are added.

c. The final volume of each carboy is now 3000 ml.

The pH of the carboy contents is measured, and the test is started by bubbling CO_2-free air through the solution at a rate of 50-100 cc/min per carboy (~1-2 "bubbles/second"). The three CO_2 absorber bottles are now filled with 100 ml 0.025 N $Ba(OH)_2$ and connected in series to the exit air line of each test carboy. The CO_2 produced in each carboy is trapped in the barium hydroxide and is precipitated out as barium carbonate; the amount of CO_2 produced is quantitated by titrating the remaining $Ba(OH)_2$ with 0.05 N HCl (see below). Periodically, the CO_2 absorber most proximal to the carboy is removed for titration. The remaining two absorbers are each moved one place closer to the carboy, and a new absorber filled with 100 ml of fresh 0.025 N $Ba(OH)_2$ is placed at the distal end of the series. Titrations are usually made daily for the first 12 days, and then every other day thereafter until the 25th day.

On the 25th day, the pH of the carboy contents are measured again, and then 1 ml of concentrated HCl is added to each of the test carboys, in order to drive off inorganic carbonate plus dissolved CO_2. The carboys are aerated overnight, and a final titration is made on day 26. Once again, the pH is measured on the acidified contents of the carboy. If desired, the test period may be extended beyond 26 days, especially if the curve of % TCO_2 vs. time is still rising.

Titrations

Titrations are carried out as follows:

a. 10 ml of the $Ba(OH)_2$ CO_2 absorber solution from the bottle most proximal to the carboy are pipetted into a 50 ml beaker.

b. The $Ba(OH)_2$ is then titrated to a phenolphthalein endpoint with standardized 0.05 N HCl from a burette.

c. The control carboy serves as a "seed blank" to correct for CO_2 which may be produced through endogenous respiration of the bacteria as well as through biodegradation of organic material remaining in the "seed" at the end of the 14 day acclimatization culture. Since the measurement of CO_2 production consists of back-titrating the residual $Ba(OH)_2$ in the absorbers, the amount of CO_2 produced by a test material is determined by the difference (in ml of titrant) between the experimental and blank carboy.

For example, Blank = 4.80 ml HCl titrated
Experimental = 4.50 ml HCl titrated
Test material = 0.30 ml HCl titrated
= 3.30 mg CO_2 evolved (see Table I)

Calculation of CO_2 Production

Equations

a. Consumption of CO_2:

$$\underset{171}{xs\ Ba(OH)_2} + \underset{44}{CO_2} \rightleftharpoons \underset{197}{BaCO_3}\downarrow + \underset{18}{H_2O}$$

molecular weights

b. Back-titration with HCl:

$$\underset{171}{Ba(OH)_2} + 2\ \underset{73}{HCl} \rightleftharpoons \underset{208}{BaCl_2} + \underset{36}{2H_2O}$$

molecular weights

0.025 N $Ba(OH)_2$ = 2.138 g/l = 2.138 mg/ml $Ba(OH)_2$
0.050 N HCl = 1.825 g/l = 1.825 mg/ml HCl

$\frac{171}{73} = 2.342$ and $\frac{2.138}{1.825} = 1.171$ ($Ba(OH)_2$:HCl ratios)

Therefore, 1 ml of 0.05 N HCl will neutralize 2 ml of 0.025 N $Ba(OH)_2$.

If the $Ba(OH)_2$ absorber contains 100 ml, and a 10 ml aliquot is taken for titration, then the maximum amount of $Ba(OH)_2$ present in the aliquot is:

$$(10\text{ ml } Ba(OH)_2)\left(\frac{2.138\text{ mg } Ba(OH)_2}{\text{ml } Ba(OH)_2}\right) = \frac{21.38\text{ mg } Ba(OH)_2}{10\text{ ml aliquot}}$$

These 21.38 mg $Ba(OH)_2$ are capable of consuming:

$$\left(\frac{21.38\text{ mg } Ba(OH)_2}{10\text{ ml aliquot}}\right) x \left(\frac{44\text{ mg } CO_2}{171\text{ mg } Ba(OH)_2}\right) = \frac{5.50\text{ mg } CO_2}{10\text{ ml aliquot}} \text{ or } \frac{0.55\text{ mg } CO_2}{\text{ml } Ba(OH)_2}$$

Since 1 ml of $Ba(OH)_2$ can consume 0.55 mg CO_2, and since each 2 ml of 0.025 N $Ba(OH)_2$ can be neutralized by 1 ml of 0.05 N HCl, it is clear that 1 ml of HCl titrated in a 10 ml aliquot represents 1.10 mg CO_2, or, for the complete 100 ml sample in the absorber, 11.00 mg CO_2.

Thus, Table I shows the conversion from ml 0.05 N HCl titrated in a 10 ml aliquot to mg of CO_2 consumed in a 100 ml absorber of 0.025 N $Ba(OH)_2$.

Once the amount of CO_2 produced is calculated, it is a simple matter to calculate the % of theoretical CO_2 that was biodegraded.

$$\%\ TCO_2 = \frac{\text{mg } CO_2 \text{ produced}}{(\text{mg active added in test})(\text{mg } CO_2/\text{mg active})} \times 100$$

$$= \frac{\text{mg } CO_2 \text{ produced}}{(\text{mg active added in test})(TCO_2)} \times 100$$

Example: Blank = 4.80 ml 0.05 N HCl titrated
Dextrose = 4.65 ml 0.05 N HCl titrated
Difference = 0.15 ml 0.05 N HCl titrated

From Table I, 0.15 ml .05 N HCl is equivalent to 1.65 mg CO_2 consumed in the absorber.

Since dextrose has a TCO_2 of 1.47 mg CO_2/mg dextrose, and since 60 mg of dextrose were placed in the test (i.e. 3 liters x 20 mg/l), the %

of theoretical CO_2 that was degraded on that particular day was:

$$\% TCO_2 = \frac{1.65 \text{ mg } CO_2 \text{ produced}}{(60 \text{ mg dextrose})(1.47 \text{ mg } CO_2/\text{mg dextrose})} \times 100 = 1.87\%$$

Presentation of Results

Calculate the % TCO_2 for each titration, and plot the cumulative % TCO_2 vs. time, as shown in the attached graph. The final 26-day result should also be recorded along with other pertinent data, as shown on the attached summary sheet.

Radioactive $^{14}CO_2$ Production

If desired, this test may be modified for biodegradation studies on radio-tagged compounds, in which evolved $^{14}CO_2$ is measured. In this case, the only change in the protocol is the use of 0.05 N NaOH instead of 0.025 N $Ba(OH)_2$ in the CO_2 absorber bottles.

References

1. Sturm, R. N., "Biodegradability of Nonionic Surfactants: Screening Test for Predicting Rate and Ultimate Biodegradation," J. Am. Oil Chemists Soc., 50, No. 5, 159-167 (1973).

2. "Standard Methods for the Examination of Water and Wastewater," 13th Edition, American Public Health Association, New York (1971).

E. A. Schon

L. McElrath

S. L. Schaffer

kb
Attachments

TABLE I

CONVERSION VALUES FOR ml OF 0.05 N HCl USED VS. mg CO_2 PRODUCED

FROM 0.025 N $Ba(OH)_2$ WHEN TITRATING A 10 ml ALIQUOT FROM A 100 ml TOTAL VOLUME

ml* HCl	mg CO_2 Prod.	ml* HCl	mg CO_2 Prod.
0.05	0.55	1.05	11.55
0.10	1.10	1.10	12.10
0.15	1.65	1.15	12.65
0.20	2.20	1.20	13.20
0.25	2.75	1.25	13.75
0.30	3.30	1.30	14.30
0.35	3.85	1.35	14.85
0.40	4.40	1.40	15.40
0.45	4.95	1.45	15.95
0.50	5.50	1.50	16.50
0.55	6.05	1.55	17.05
0.60	6.60	1.60	17.60
0.65	7.15	1.65	18.15
0.70	7.70	1.70	18.70
0.75	8.25	1.75	19.25
0.80	8.80	1.80	19.80
0.85	9.35	1.85	20.35
0.90	9.90	1.90	20.90
0.95	10.45	1.95	21.45
1.00	11.00	2.00	22.00

*ml HCl difference between blank and experimental sample

Formula for calculation of mg CO_2 Produced:

$$\text{mg } CO_2 \text{ produced} = \text{ml } 0.05 \text{ N HCl titrated} \times \left[\frac{2 \text{ ml } 0.025 \text{ N } Ba(OH)_2 \text{ consumed}}{\text{ml } 0.05 \text{ N HCl titrated}} \times \frac{2.138 \text{ mg } Ba(OH)_2}{\text{ml } 0.025 \text{ N } Ba(OH)_2} \times \frac{44 \text{ mg } CO_2}{171 \text{ mg } Ba(OH)_2} \times \frac{100 \text{ ml sample}}{10 \text{ ml aliquot}} \right]$$

$$= \text{ml } 0.05 \text{ N HCl titrated} \times 11.0$$

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TABLE II

CODE LISTING FOR REAGENTS

For CO_2 Determination

	Fisher Scientific Code (order #)
Ferric Chloride Solution, 0.25% W/V	So-F-97
Magnesium Sulfate Solution, 2.25%	So-M-109
Calcium Chloride Solution, 2.75%, W/V	So-C-10
Phosphate Buffer Solution, pH 7.2	So-P-341
Hydrochloric Acid Solution, 0.1 N	So-A-54
Drierite (Calcium sulfate)	7-577-3B
Drierite, indicating	7-578-3B
Silica Gel, indicating, 14-20 mesh, grade 43	S-707
Ascarite, indicating, 8-20 mesh	7-572A or B
Activated charcoal, 6-14 mesh	5-685A or B
Barium Hydroxide, crystal, $Ba(OH)_2 \cdot 8H_2O$	B-46
Yeast extract	Difco DBL-11929

All reagents used in the screening test can be ordered locally from Fisher Scientific Company.

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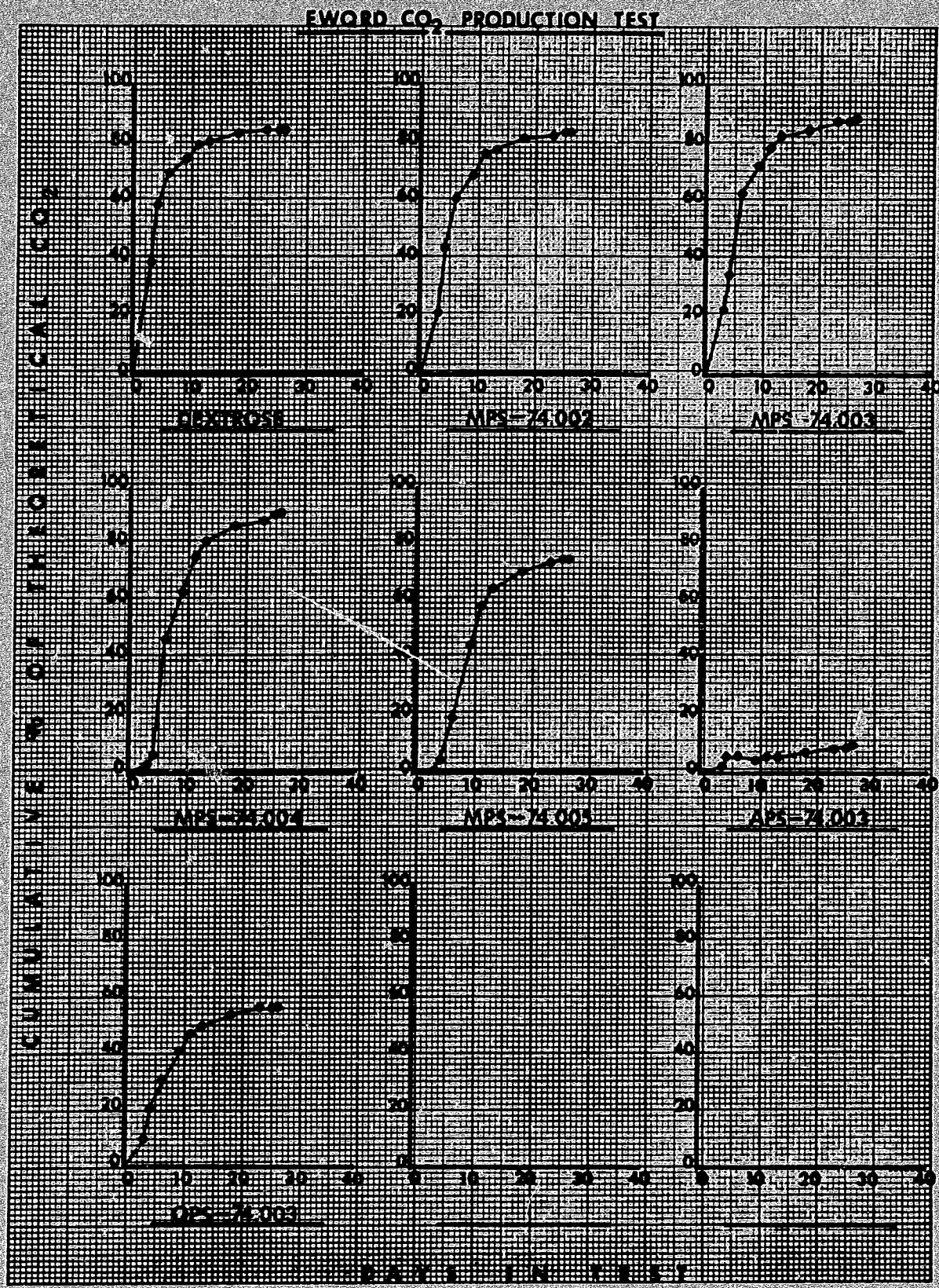
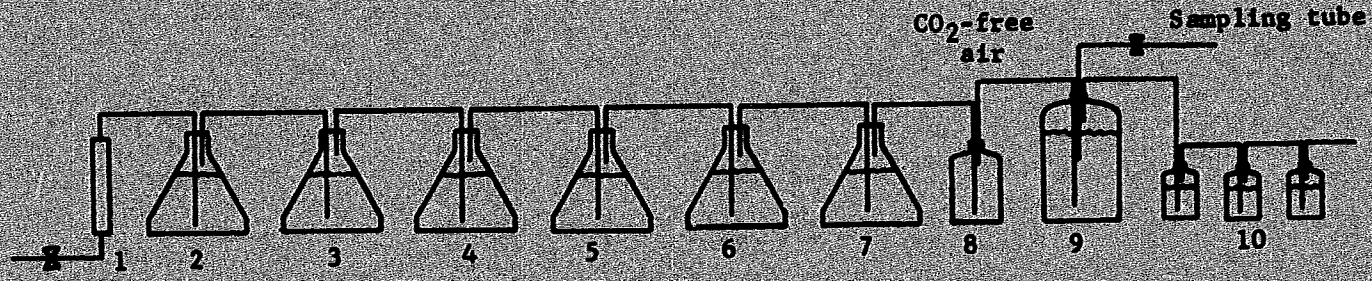
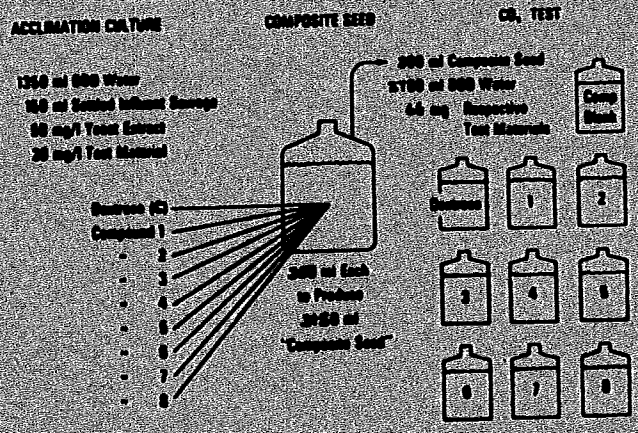


NO. 329 MILLIMETERS 160 X 250 DIVISIONS
COCEX GRAPH PAPER IN STOCK DIRECT FROM CODEX BOOK CO., NORWOOD, MASS. 02062
PRINTED IN U.S.A.

Diagram of Individual CO_2 production test unit.
Air supply and drying train for entire 10 unit battery
rather than for each individual unit.



1. Rotameter
2. Organic removal - activated carbon
3. Moisture removal - Silica gel
4. Moisture removal - Drierite
5. CO_2 removal - Drierite + Ascarite
6. CO_2 removal - Ascarite
7. CO_2 removal and remoisturization - $Ba(OH)_2$
8. Train protection
9. Test carboy
10. CO_2 absorber bottles



General protocol for a 10 unit CO_2 production test.

BIODEGRADABILITY TEST SUMMARY SHEET

Laboratory: EWQRD

EWQRD Code #	Material Identification	Calculations TOD, g/g active	Calculations TCO_2, g/g active	Stock Soln. Conc., mg/l active	Stock Soln. pH Before Adj.	Stock Soln. pH After Adj.	Oxygen Demand @ 20°C COD, g/g active	Oxygen Demand @ 20°C BOD_5* % TOD	Oxygen Demand @ 20°C BOD_{20}* % TOD	CO_2 Production CO_2, % TCO_2	CO_2 Production Conc., mg/l	CO_2 Production Days in Test
1. OPS-74.001			1.573	1100	2.7	7.8	1.15	9.6	0.0	13.2	20	26
2. OPS-74.002			1.485	1069	2.7	7.8	0.79	0.0	79	17.3	"	"
3. OPS-74.003			1.060	1221	6.3	7.8	0.66	-14	-4.5	8.3	"	"
4. OPS-74.004			1.404	1183	2.7	7.8	0.75	12	55	11.0	"	"
5. OPS-74.005			1.940	1045	2.7	7.8	1.32	-2.0	11	9.5	"	"
6. OPS-74.006			1.874	904	2.6	7.3	1.04	0.0	2.4	8.3	"	"
7. OPS-74.007			1.203	1143	7.2	7.2	0.81	10	-3.6	13.1	"	"
8. OPS-74.008			1.980	1013	2.7	7.8	1.21	-2.1	-4.1	9.6	"	"
9. --	Dextrose	1.07	1.46	1056	7.0	7.0	--	65	--	74.4	"	"
10.												

Notebook-page#	Outside Lab	EWQRD
COD test:		M-5304-36
BOD test:		M-5304-36
CO_2 test:		M-5413-38
Other		

Comments:

*BOD's based on % COD rather than % TOD

Seed source: Colerain Township STP Influent
Date acclimatization started: 7/3/74
Date test started: 7/18/74
Date test completed: 9/13/74

Signed: Kirk E. Biehge
(experimenter)

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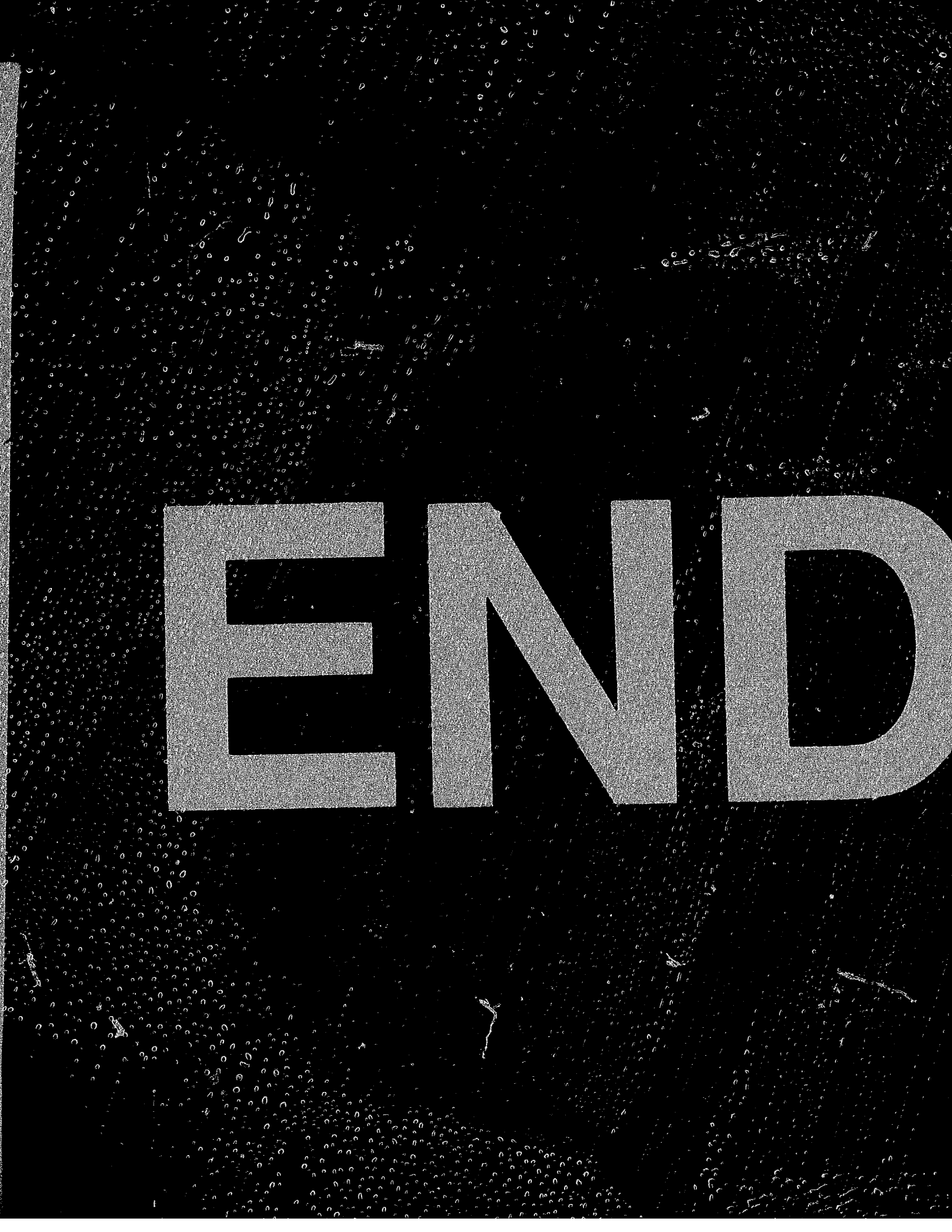
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