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REPORT FILE

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Monsanto Industrial Chemicals Co.
St. Louis, Missouri

MS-77-538-551

Date: March 23, 1976.

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Reported By: O. Hicks, V. W. Saeger, R. G. Kaley, J. P. Mieure

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BIODEGRADABILITY OF PLASTICIZERS

OBJECTIVE

To determine the biodegradability of current, potential and competitive products to aid in the assessment of their environmental compatibility.

SUMMARY

[redacted] metabolites. The plasticizers studied included esters of o-phthalic acid, [redacted] phthalate, mono (2-ethylhexyl) phthalate, o-phthalic acid and 2-ethylhexanol. These studies demonstrated that as a group the phthalic acid and adipic acid ester plasticizers should not persist for long times in the environment. The biodegradability assessment for individual plasticizers is as follows (no ranking within groups):

Readily degraded	Butylbenzyl phthalate [Santicizer 160]
	Butylglycolylbutyl phthalate [Santicizer 416]
	Di(2-ethylhexyl) phthalate [DEHP, DOP]
	Di(heptyl, nonyl) adipate [Santicizer 97A]
	Di(2-ethylhexyl) adipate [DOA]
	Di-n-hexyl adipate
	1,3-Butylene glycol adipic acid polyester [Santicizer 334F]
Intermediate degradation rate	Di(heptyl, nonyl, undecyl) phthalate [Santicizer 711]
	Diundecyl phthalate [DUP]
Slowly degraded	Tri(heptyl, nonyl) trimellitate [Santicizer 79TM]

The degradation pathway for the phthalic acid esters was shown to involve conversion to the half ester and/or phthalic acid followed by ring cleavage to smaller non-aromatic molecules. Primary biodegradation of three expected metabolites (monobutyl phthalate, o-phthalic acid and 2-ethylhexanol) was very rapid. Ultimate biodegradation carbon dioxide determinations for Santicizer 160, Santicizer 711, di(2-ethylhexyl) phthalate and mono(2-ethylhexyl) phthalate showed essentially complete conversion to CO_2 and H_2O.

The adipic acid esters similarly were shown to undergo rapid primary and eseentially complete ultimate biodegradation.

- Mono (2-ethyl hexyl phthalate)
- Monobutyl phthalate (Butyl acid phthalate)
- o-phthalic acid
- 2-ethyl hexanol

TEST METHODS

I. Ultimate Biodegardation

We define ultimate biodegradation as complete conversion of an organic material to carbon dioxide, water, inorganic salts and normal cellular products of bacteria. The rate and extent of CO_2 production by bacterial action is dependent on many variables. However, the extent of CO_2 production in a given time under constant conditions can be used as a measure of ultimate biodegradability of a material. Several test procedures have been developed for this purpose. Readily degradable materials such as dextrose and linear alkylbenzene sulfonate (LAS) typically yield 80-90% and 60-80% of theory, respectively, by these procedures. Since the bacterial systems used in these procedures are relatively week, a material showing a high degree of conversion to CO_2 during the test interval is not likely to persist in the environment.

A. Thompson-Duthie-Sturm Procedure

In this procedure [J. Amer. Oil Chem. Soc. 50, 159 (1973); J. Water Poll. Control Fed. 40, 306 (1968)] a nine-liter bottle containing 500 ml of acclimated bacterial seed and 5,500 ml of standard BOD water is prepared for each test material and a control. Except for the control bottle, each bottle receives a weighed quantity (approximately 120 mg) of the appropriate test material. The bottles are connected to a source of CO_2-free air and the effluent air passed through a set of aqueous barium hydroxide scrubbers. The evolved CO_2 is trapped as barium carbonate and quantitated by titration of the remaining barium hydroxide with 0.1 N HCl. CO_2 values obtained from the control are subtracted from those obtained for the test material. The CO_2 produced during an experiment (28-35 days) is compared to the theoretical yield based on the carbon composition and weight of the material.

B. Monsanto Shake Flask Procedure

The shake flask system is similar to that described by Gledhill [Appl. Microbiol. 30, 922 (1975)]. In the shake flask procedure, 100 ml of acclimated bacterial seed is mixed with 400 ml of standard BOD water in fluted 2-liter Erlenmeyer flasks. A weighed quantity (approximately 15 mg) of the appropriate test material is added to each flask except for the control. After aerating the medium with 70% oxygen in nitrogen, an open reservoir containing 10 ml of 0.1 N barium hydroxide is suspended via a glass tube inserted in a rubber stopper. Provisions for removal and addition of the barium hydroxide solution, aeration and sampling are provided. After sealing, the flasks are agitated on a rotary shaker at 80 rpm in the dark at ambient temperature. Periodic removal (3, 7, 14, 21, 28 and 35 days) and titration of the barium hydroxide solution are used to determine the CO_2 evolved Fresh barium hydroxide solution is added back at each sampling point and the flasks sparged with 70% oxygen. CO_2 evolution values obtained with the control are subtracted from values for test material.

II. Primary Biodegradation

Primary biodegradation is defined as the disappearance of the original material due to bacterial action as evidenced by a specific analytical technique. Primary biodegradation measurements alone may not suffice to establish biodegradability because some materials which undergo complete primary biodegradation may be converted to more persistent intermediates. The use of specific analytical methods allows one to work with a wide variety of bacterial systems and conditions. Two procedures which have been widely used in primary biodegradation measurements are the semi-continuous activated sludge (SCAS) test - simulating a secondary-sewage treatment process - and the river die-away (RDA) test - simulating the natural environment of a river.

A. Semi-Continuous Activated Sludge (SCAS) Procedure

In our SCAS procedure (Analytical Chemistry Method 71-32), mixed liquor (activated sludge and supernatant) from a local domestic sewage treatment plant is charged to a magnetically-stirred vessel of 1.5 liter capacity. Means for aeration and sampling are provided. The SCAS unit is generally operated using a retention or aeration cycle of 23 to 167 hours. At the beginning of each cycle, a given level of test material (generally in ethanol solution) and synthetic (300 mg glucose, 200 mg nutrient broth and 130 mg K_2HPO_4) and/or raw sewage are added to the mixed liquor (2,500 mg/liter suspended solids concentration). Aeration is maintained until the end of the cycle, at which time the sludge is settled and one liter of supernatant drained. The cycle is then re-initiated by the addition of tap water, sewage and test material. Primary biodegradation is determined during one cycle each week by analyzing 50 ml mixed liquor samples withdrawn after feeding (C_o) and at the end of the aeration cycle (C_n). The percent biodegradation is calculated from the following equation:

$$\% \text{ Primary Biodegradation} = (C_o - C_n)/C_o \times 100$$

B. River Die-Away (RDA) Procedure

The RDA test consists of exposing a low level (usually 1 ppm) of test material to the natural microorganisms in replicate river water samples. Changes in the level of test material are monitored as a function of time by analyzing samples at selected intervals. The river water supply (approximately six gallons) is obtained from either the Meramec or Mississippi Rivers. After settling for two days, the supernatant is transferred to a five-gallon carboy and 200 ml portions withdrawn and added to 16 ounce screw-cap bottles. Four µl portions of an ethanol solution of the test material (50 µg/µl) are injected into each bottle. each bottle is sealed with a foil-lined cap, mixed by swirling and stored in the dark at ambient temperature. Sterile water controls are included to verify that a decrease in the initial level is due to biodegradation and not some physical or chemical phenomena. A set of river water and sterile water samples are also prepared with 4 ppm LAS and monitored for LAS concentration to provide a control measure of biological activity. At each sampling point, the total contents of a river water and the corresponding sterile river water bottle are analyzed for test material. The duration of the test is generally six weeks. The result may be expressed as a half-life - days required for the initial level to decrease by 50 percent.

ANALYTICAL METHODS FOR MONITORING PRIMARY BIODEGRADATION

Both SCAS and RDA samples for the phthalic acid diesters, adipic acid diesters, Santicizer 79TM and 2-ethylhexanol were extracted with hexane and prepared for analysis using the procedures outlined in Analytical Chemistry Method No. 71-18. Analyses were carried out by either flame ionization gas chromatography or ultraviolet spectrophotometry. SCAS and RDA data for monobutyl phthalate and o-phthalic acid were obtained by direct analysis of the aqueous phase using ultraviolet spectrophotometry. Recoveries obtained from spiking and analysis of blank SCAS mixed liquor samples are tabulated with the appropriate analytical method reference in Table I.

BIODEGRADATION TEST RESULTS

Ultimate and primary biodegradation test results for the plasticizers and metabolites are listed in Table I. In Figures 1 and 2, CO_2 evolution curves are plotted as a function of time. Additional details on the phthalic acid ester and metabolite biodegradation may be found in the attached reprint, Appl Environ. Microbiol. 31, 29(1976).

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Attachments(3)

Monsanto Industrial Chemicals Co.
Applied Sciences
St. Louis, Mo.

2/76 - O. Hicks, R. G. Kaley, V. W. Saeger, J. P. Mieure

FIGURE 1

PHTHALIC ACID ESTER CO_2 EVOLUTION

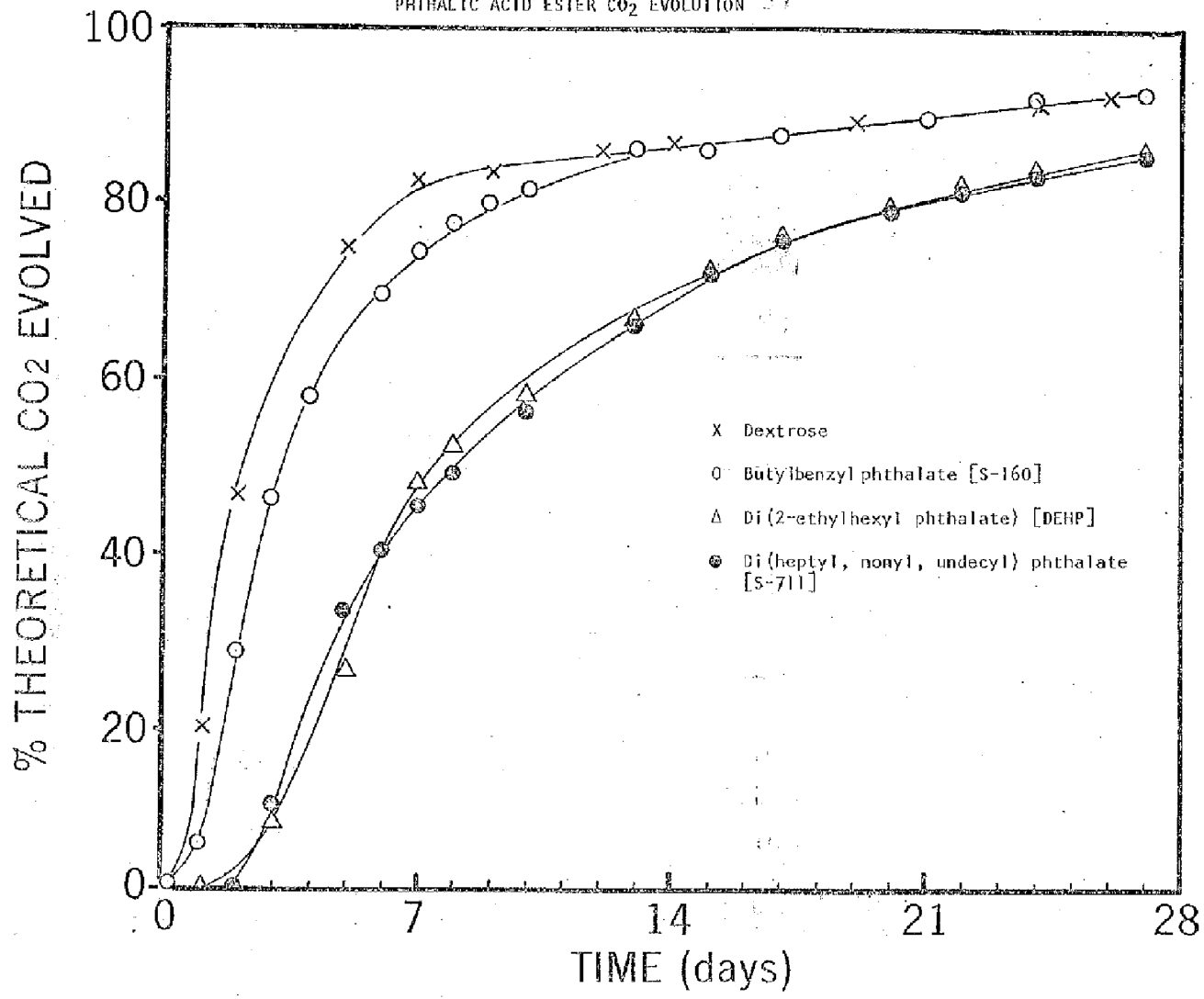


FIGURE 2

ADIPIC ACID ESTER CO_2 EVOLUTION

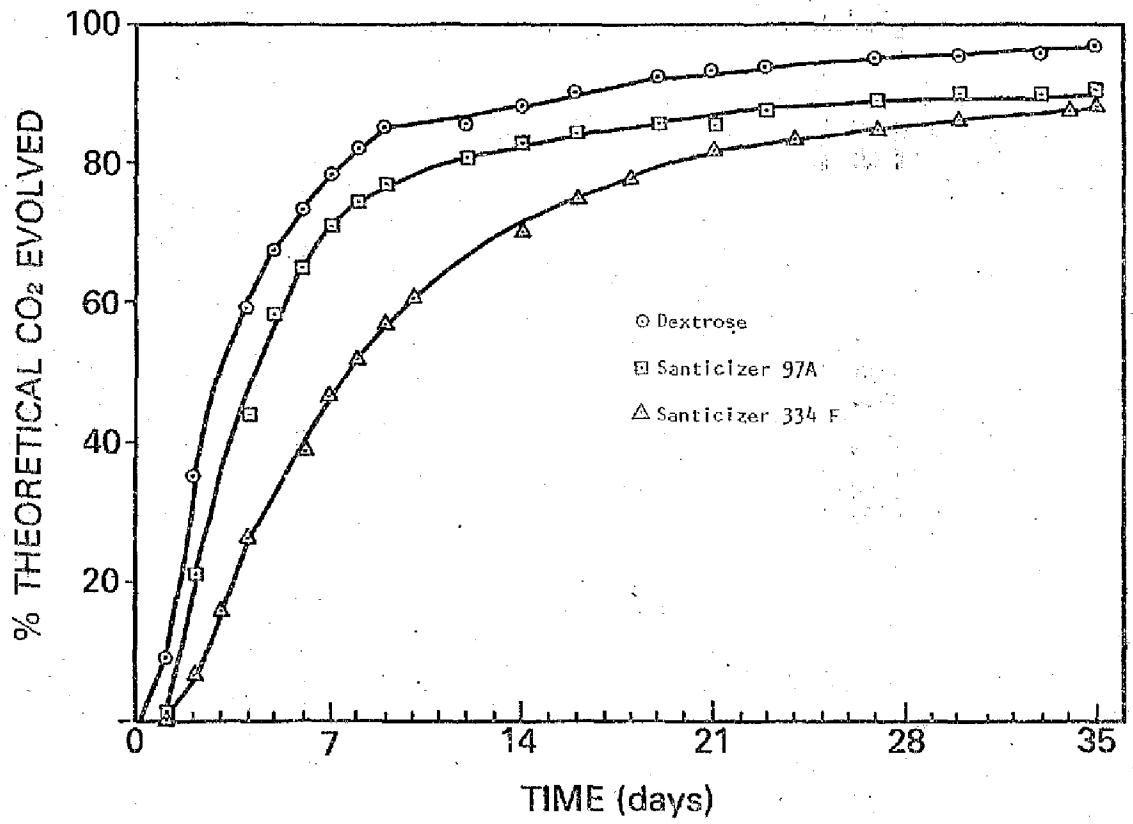


TABLE I

PRIMARY AND ULTIMATE BIODEGRADABILITY OF PLASTICIZERS AND RELATED METABOLITES

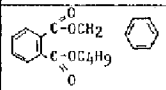
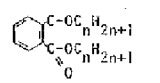
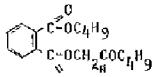
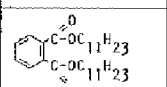
Test Material	Structure/ Composition	Ultimate Biodegradability (% of Theoretical CO_2 Evolution)		Primary Biodegradability						
				Semi-Continuous Activated Sludge (SCAS)			River Die-Away (RDA)		SCAS and RDA Analytical Method	
		Thompson -Duthie -Sturm procedure	Monsanto Shake Flask procedure	Addition Rate (ppm added per cycle)	Primary % Degradation Rate ±95% C.L.	Comments	Initial Conc(ppm)	Days Req'd for 50% Primary Degradation	% Recovery (Mixed Liquor Extraction)	Method No.
						No significant volatility loss for any ester. +, ++, and +/- indicate moderate, strong, and variable inhibition of sludge growth rate respectively.				
MO-77-538 Santicizer 160 (Butylbenzyl phthalate)		96 (27-day)		3 134	93 ± 6 > 99	For more detail see AC-73-SS-20	1	2	105.7 ± 3.8	AC-71-M-31 AC-72-M-4
MO-77-539 Santicizer 711 [Di(heptyl, nonyl, undecyl) phthalate]	 n = 7, 9, 11	86 (27-day)		3 7 13	52 ± 10 48 ± 8 54 ± 7	For more detail see AC-73-SS-20	1	> 35	100.7 ± 3.3	AC-71-M-15 AC-72-M-4
MO-77-540 Santicizer 816 (Butylglycolyl butyl phthalate)				3 13 67 134	> 99 > 99 > 99 > 99	For more detail see AC-73-SS-20	1	1	79.0 ± 2.4	AC-72-M-4
MO-77-541 Diundecyl phthalate (DUP)				3 13	45 ± 11 29 ± 7	For more detail see AC-73-SS-20	1	24	89.9 ± 6.4	AC-72-M-4

TABLE I

(2)

PRIMARY AND ULTIMATE BIODEGRADABILITY OF PLASTICIZERS AND RELATED METABOLITES

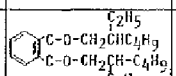
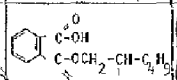
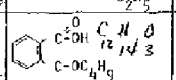
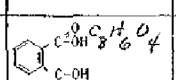
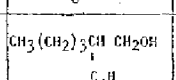
Test Material	Structure/ Composition	Ultimate Biodegradability (% of Theoretical CO_2 Evolution)		Primary Biodegradability					SCAS and RDA Analytical Method	
				Semi-Continuous Activated Sludge (SCAS)			River Die-Away (RDA)			
		Thompson -Duthie -Sturm procedure	Monsanto Shake Flask procedure	Addition Rate (ppm added per cycle)	Primary % Degradation Rate ±95% C.L.	Comments	Initial Conc (ppm)	Days Req'd for 50% Primary Degradation	% Recovery (Mixed Liquor Extraction)	Method No.
						No significant volatility loss for any ester. +, ++, and +/- indicate moderate, strong, and variable inhibition of sludge growth rate respectively.				
MO-77-542 Di(2-ethylhexyl) phthalate [DEHP, DOP]				3 3	70 ± M 78 ± 3	 For more detail see AC-73-SS-20	1	24	89.9 ± 6.4	AC-72-M-4
MO-77-543 Mono(2-ethylhexyl) phthalate [2-Ethylhexyl acid phthalate]		87 (27-day) 89 (35-day)								
MO-77-544 Monobutyl phthalate [Butyl acid phthalate]				67	> 99				100.0 ± 1.4	AC-73-M-29
MO-77-545 o-Phthalic Acid CP42547				33 67 167	> 99 > 99 > 99	 For more detail see AC-73-SS-20	12.5 25 50	11 15 19	93.0 ± 4.0	AC-71-M-30
MO-77-546 2-Ethylhexanol (34) CP9598				3 13	> 92 > 98	 For more detail see AC-73-SS-13			71.9 ± 6.8	AC-73-M-16

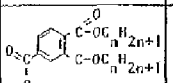
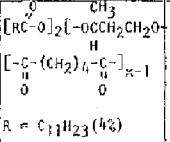
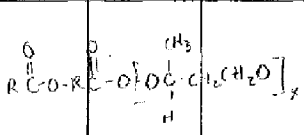
TABLE I

PRIMARY AND ULTIMATE BIODEGRADABILITY OF PLASTICIZERS AND RELATED METABOLITES

Test Material	Structure/ Composition	Ultimate Biodegradability (% of Theoretical CO_2 Evolution)		Primary Biodegradability						
				Semi-Continuous Activated Sludge (SCAS)			River Die-Away (RDA)		SCAS and RDA Analytical Method	
		Thompson -Duthie -Sturm procedure	Monsanto Shake Flask procedure	Addition Rate (ppm added per cycle)	Primary % Degradation Rate ±95% C.L.	Comments	Initial Conc (ppm)	Days Req'd for 50% Primary Degradation	% Recovery (Mixed Liquor Extraction)	Method No.
						No significant volatility loss for any ester. +, ++, and +/- indicate moderate, strong, and variable inhibition of sludge growth rate respectively.				
110-77-547 Santicizer 97A	$O = C(CH_2)_4C = O$ $O \quad O$ $C_nH_{2n+1} \quad C_nH_{2n+1}$ n = 7 (45%) n = 9 (55%)	90(35-day)	82(35-day) 79(35-day)	3 13	67 ± 14 88 ± 5	For more detail see AC-72-SS-11			88.6 ± 4.3	AC-72-M-15
110-77-548 Di(2-ethylhexyl) adipate [DOA] CP 3180	$O = C(CH_2)_4C = O$ $O \quad O$ $CH_2 \quad CH_2$ $C_2H_5{-}CH \quad CH{-}C_2H_5$ $C_4H_9 \quad C_4H_9$	94(35-day)	82(35-day)	3 13	92 ± 4 73 ± 8	+ For more detail see AC-72-SS-11			91.0 ± 4.5	AC-72-M-15
110-77-549 Di-n-hexyl adipate [DHA] $C_{18}H_{34}O_4$ CP 1163	$O = C(CH_2)_4C = O$ $O \quad O$ $n\text{-}C_6H_{13} \quad n\text{-}C_6H_{13}$	90(35-day)	81(35-day)	3 13	> 99 95 ± 3	For more detail see AC-72-SS-11			89.6 ± 3.7	AC-72-M-15

TABLE I

PRIMARY AND ULTIMATE BIODEGRADABILITY OF PLASTICIZERS AND RELATED METABOLITES

Test Material	Structure/ Composition	Ultimate Biodegradability (% of Theoretical CO_2 Evolution)		Primary Biodegradability						
				Semi-Continuous Activated Sludge (SCAS)			River Die-Away (RDA)		SCAS and RDA Analytical Method	
		Thompson -Duthie -Sturm procedure	Monsanto Shake Flask procedure	Addition Rate (ppm added per cycle)	Primary % Degradation Rate ±95% C.L.	Comments	Initial Conc(ppm)	Days Req'd for 50% Primary Degradation	% Recovery (Mixed Liquor Extraction)	Method No.
						No significant volatility loss for any ester. +, ++, and +/- indicate moderate, strong, and variable inhibition of sludge growth rate respectively.				
MO-72-SS6 Santicizer 79 TM Tri(heptyl,nonyl) trimellitate [Hepty], nonyl ester of 1,2,4-benzene tricarboxylic acid]	 C_nH_{2n+1} n = 7 (45%) n = 9 (55%)			3 13	26 ± 13 25 ± 10	For more detail see AC-72-SS-10			73.5 ± 11	AC-72-M-14
MO-72-SS7 Santicizer 334F (1,3-Butylene glycol adipic acid polyester - terminated by fatty acids)	 R = $C_{11}H_{23}$ (4%) $C_{13}H_{27}$ (45-55%) $C_{15}H_{31}$ (35-45%) $C_{17}H_{35}$ (10%) MW = 1700-2200	38 (35-day)	78 (35-day)			For more detail see AC-72-SS-8				

Monsanto | INDUSTRIAL CHEMICALS CO.

FROM (NAME & LOCATION): V. W. Saeger - T2B

DATE : March 23, 1976

CC:

SUBJECT : REPORT AC-75-SS-8

REFERENCE :

TO : Report Distribution

PLW
FRJ
JGL
File — as with earlier rept. — Can we put on computer?

The enclosed report summarizes our biodegradation testing to date for the product group or chemical type indicated. A comprehensive report of this type will be issued on an annual basis. As you are aware, many of our biodegradation tests require several months to complete. To keep each group leader informed on future interim test results, an updated copy of the Biodegradation Testing Work Request form (sample enclosed) will be returned to him monthly. To facilitate this reporting format, a request form must be completed when testing is initiated on a product. Utilization of this form will not only provide Applied Sciences with the necessary information for proper test selection, but will also be a rapid vehicle for communicating results.

V. W. Saeger

V. W. Saeger

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Enclosures (2)

IN - 10 REV. 1/72