PROGRESS REPORT

ORGANIC CHEMICALS DIVISION

TECHNOLOGY DEPARTMENT

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PROJECT TITLE

ENVIRONMENTAL ANALYTICAL PROGRAM - BIODEGRADATION STUDIES

PROJECT OBJECTIVE

Development and application of laboratory test procedures to measure the susceptibility of Organic Division products to primary biodegradation via the biological action of bacteria.

SUMMARY

A number of commonly employed biodegradation testing procedures were reviewed and three, representing different biological environments, were chosen for experimental evaluation. The biodegradation testing procedures selected are entitled, "Semi-Continuous Activated Sludge (SCAS), River Die-Away, and Soil Percolation (SP)". Each test determines the susceptibility of a compound toward degradation via bacterial action by measurement of the disappearance of the material using chromatography and/or spectroscopy techniques.

The SCAS test simulates a secondary sewage treatment facility, the RDA and SP tests the action of the natural bacteria present in rivers and soils.

A 26-unit SCAS testing facility was set up and has been operational since January of this year. The experimental variables involved in this test procedure were statistically evaluated and the optimum operating conditions defined.

The susceptibility (rate of disappearance) of 20 Organic Division products and associated materials to primary bacterial degradation were investigated using the SCAS test facility. From the data gathered, a tentative biodegradability classification scheme was set up which employes four categories as determined by the rate at which the material disappears: 90-100% disappearance - Biodegradable; 50-90% - Partially Biodegradable I; 25-50% disappearance - Partially Biodegradable II; 0-25% disappearance - Refractory.

The materials tested are classified as follows with this system:

Biodegradable	Partially Biodegradable I
o-Phthalic Acid	Monoisopropylated Biphenyl (MIPB)
p-Chloroaniline	Aroclor 1221 (21% Chlorinated Biphenyl)
Linear Alkyl Benzene Sulfonate (LAS)	Branched Alkyl Benzene Sulfonate
Partially Biodegradable II	**Refractory**
Hydrogenated Terphenyl (HB-40)	MCS 1016 (41% Chlorinated Biphenyl)
Santicizer 711M (S-711M)	Aroclor 1242 (42% Chlorinated Biphenyl)
p-Nitrochlorobenzene (PNCB)	2,2'-Dichlorodiphenyl Ether
o-Nitrochlorobenzene (ONCB)	Aroclor 1142 Distillation Residue (~51% chlorinated biphenyl)
3,4-Dichloronitrobenzene (Nitrated Orthene)	Aroclor 1254 (54% chlorinated biphenyl)
Arobromochlor 1232 (21% Cl, 11% Br-Halogenated Biphenyl)	TCC
MCS 1043 (30% Chlorinated Biphenyl)	

On the basis of the data and experience gained from the SCAS testing of the above materials a standard screening protocol was statistically designed for classification of future test materials. The protocol, which eliminates much of the manpower required by minimizing the number of data points needed to classify a test material, will be issued.

FUTURE PLANS

Work Scheduled for Completion by 10-1-71

I. SCAS testing of General Chemical Products (PNCB, ONCB, p-CA, nitrated orthene); Plasticizers (Santicizer 711M); and Specialty Products (Aroclor 1221).

II. SCAS analytical method development for: Specialty Products - tolylxylsulfone and trichlorodiphenyl ether; Plasticizers - Santicizer 160; and Food and Fine Chemicals - Santophen I.

III. Measurement of SCAS disappearance rates for: Specialty Products - Aroclor 5432, Aroclor 5460, Santomelt 990 CR and UCAR.

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DETAILS

Since the biodegradation program was initiated in May, 1970 primary emphasis has been placed on the refinement and application of the semi-continuous activated sludge test procedure. This biodegradation testing procedure is patterned after the test method recommended by the Sub-Committee on Biodegradation Test Methods of the Soap and Detergent Association for evaluation of surfactants [JAOCS 42, 986 (1965)].

In the semi-continuous activated sludge test, biodegradability is measured using activated sludge from a sewage treatment plant as the source of micro-organisms. A given level of test compound and a synthetic sewage as an energy source are fed on a daily basis to the activated sludge contained in a specially designed aeration chamber. Samples of the mixed liquor are taken shortly after feeding and near the end of the aeration period to determine the disappearance rate of the test compound during the cycle. The cycle is repeated for as long as needed to obtain consistent biodegradation rate data.

A facility for simultaneous operation of 26 SCAS units is now in operation. A more detailed description of the apparatus and procedure is given in Analytical Chemistry Method 71-32.

Twenty Organic Division products and related materials have been evaluated by means of the SCAS test procedure and a tentative classification system set up. In Table I, the materials tested are diagrammed according to the level at which they were fed and the observed mean per cent disappearance rate. The chemical structure of each material is also shown as well as the biodegradability group into which the materials have been classified. The linear alkylbenzene sulfonate, dodecene-1, is included in the diagram for reference purposes. In cases where more than one feed level was employed, the disappearance at the highest feed level is shown. The actual disappearance rates for the polyhalogenated biphenyls were obtained using cycle times greater than 24 hours, an estimate of the 24 hour disappearance rate was made for classification purposes.

Additional details and special study reports references are given in the following section arranged according to their product group.

I. Food and Fine Chemicals

A. Products Tested: TCC.
B. Discussion of Results: Analytical Chemistry Special Study 71-5.

II. General Chemicals

A. Products Tested: p-Chloroaniline, p-Nitrochlorobenzene (PNCB), o-Nitrochlorobenzene (ONCB), and 3,4-Dichloronitrobenzene (nitrated orthene).
B. Discussion of Results:

SCAS testing of these materials has been completed at two feed levels using a 24-hour cycle period. All materials were monitored by ultraviolet spectroscopy (Anal. Chem. Methods 71-11, 12, 13 and 14) after isolation from the mixed liquor by extraction (Anal. Method 71-18).

II. General Chemicals (Cont'd)

B. Discussion of Results: (Cont'd)

In the following table, the mean % disappearance rates with 95% confidence limit for the two feed levels employed are shown in Table I.

Table I

Test Material	SCAS Mean % Disappearance Rate Feed Level/24 Hour Cycle 5 Mg	10 Mg
p-Chloroaniline	97.3±1.6	99.8±0.0
ONCB	37.5±8.8	40.3±8.6
PNCB	33.9±2.9	30.7±9.4
Nitrated Orthene	40.3±3.2	32.6±4.7

Nitrated orthene appears to present a special case. During the initial part of the sampling period for the 5 mg feed level, a mean % disappearance rate of 71.3±5.5 was observed. A fairly abrupt decrease in the rate to 40.3±3.2 occurred subsequently and continued for the duration of the sampling period. The feed rate was later increased to 10 mg per 24-hour cycle for which a mean % disappearance rate of 32.6 ±4.7 was observed.

The UV absorption curve of the sludge extracts offers a possible explanation. The UV curve shows not only the characteristic absorption maximum of nitrated orthene at 265 mu but two additional maxima are evident at 300 mu and 242 mu. These wavelengths correspond to the same absorption maxima generally observed for chloroanilines. Mass spectral analysis indicates the likely presence of a chloroaniline. The origin of the chloroaniline could be either the build up of a less degradable minor component in the original test mixture or a less degradable metabolic intermediate derived from the major component. Since there is some overlap in the UV absorption, the presence of this component has an effect on the calculated disappearance rate. Further effort will be required to isolate and identify the material which is building up.

III. Plasticizers

A. Products and related materials tested: Santicizer 711M and o-Phthalic Acid.

B. Discussion of Results:

SCAS testing on S-711M was carried out at a feed rate of 10 mg per 24-hour cycle. The observed mean % disappearance rate of 48.3±7.7 classifies this material in Partially Biodegradable Group I. The S-711M was monitored by UV spectroscopy (Anal. Chem. Method 71-15) after isolation from the mixed liquor by extraction (Anal. Chem. Method 71-18). Although S-711M consists primarily of linear esters (C_7+C_{11}), some branched esters are also present. Since the linear

III. Plasticizers (Cont'd)

B. Discussion of Results: (Cont'd)

esters are likely to be more biodegradable than the branched esters, analysis of the SCAS test residues is of interest. Analysis of the sludge extracts by flame ionization gas chromatography are, therefore, planned to determine the composition of the residue. An additional factor which might be revealed by such analyses would be the contribution of hydrolysis to the observed disappearance rate. The presence of free alcohols in the extracts would indicate the extent to which hydrolysis is occurring.

Since o-phthalic acid is a hydrolysis product of S-711M and a possible metabolic intermediate, SCAS testing of this material was also carried out. Because o-phthalic acid has a significant water solubility, extraction of the mixed liquor was not necessary. The mixed liquor was, therefore, filtered and the o-phthalic acid determined directly without extraction by UV spectroscopy (Anal. Chem. Method 71-30). Testing at feed levels of 25, 50, 100, 250 and 1000 mg per 24-hour cycle was carried out. For feed levels up to and including 250 mg, complete (>99%) disappearance of o-phthalic acid was observed in 24 hours. At the 1000 mg feed level, the disappearance rate fell to essentially zero. A significant lowering of the pH indicated that the buffing capacity of the unit had been exceeded. The resultant low pH (3.8) caused the cessation of bacterial activity.

IV. Specialty Products

A. Products and related materials tested: HB-40, MIPB, Aroclor 1254, Aroclor 1242, Aroclor 1142 distillation residue, Aroclor 1221, MCS 1016, MCS 1043, Arobromochlor 1232, 2,2'-dichlorodiphenyl ether, trichlorodiphenyl ether, tolylxylyl sulfone, Aroclor 5460, and Aroclor 5432.

B. Discussion of Results

1. HB-40 and MIPB: Analytical Chemistry Special Report 71-4.

2. MCS 1016 and Aroclor 1142 Distillation Residue: Analytical Chemistry Special Study Report 71-7.

3. Polychlorinated Biphenyls (Aroclor 1254, Aroclor 1242, MCS 1016, MCS 1043, Arobromchlor 1232, and Aroclor 1221): Analytical Chemistry Special Study Report 71-6.

4. Polychlorinated Terphenyls (Aroclor 5460 and Aroclor 5432).

a. SCAS testing on Aroclor 5460 and 5432 is now in progress at a feed level of 1 mg per 48-hour cycle. Refinement of the analytical method is nearly completed and sampling will be started shortly. These materials will be monitored by electron gas chromatography after isolation from the mixed liquor by extraction. Feeding of the units was started approximately eight months ago at a feed rate of 1 mg per

IV. Specialty Products (Cont'd)

4-a. (Cont'd)

24-hour cycle. The cycle was lengthened after 4 months to 48 hours when the sludge settling characteristics, particilarly for Aroclor 5432, became decidedly poorer. After six months of feeding, possible toxic effects from the test compound were noted for the Aroclor 5460 unit in the form of a decreasing suspended solids concentration and an observable decrease in LAS degradation. Feeding of test compound was stopped for a period and the unit appeared to recover. Feeding of Aroclor 5460 has since been resumed. A similar decrease in suspended solids appears to be re-occurring since the resumption of feeding. No similar toxic effects have been noted for Aroclor 5432 yet, although its sludge settling characteristics remain poor.

5. Dielectric Fluid Substitutes (2,2'-dichlorodiphenyl ether, trichloro diphenyl ether, and tolylxylylsulfone).

SCAS testing on 2,2'-dichlorodiphenyl ether at a feed level of 5 mg per 24-hour cycle has been completed. The material was monitored by electron capture gas chromatography, after isolation from the sludge by extraction. A mean disappearance rate of 10.2 ± 3.7 was observed which would place this compound in the Refractory Group. Testing on trichlorodiphenyl ether and tolylxylylsulfone are now in progress at a feed rate of 5 mg per 48 hour cycle.

6. Santomelt 990 CR and UCAR Runway De-Icer Fluids.

SCAS testing on Santomelt 990 CR and the competitive UCAR runway de-icer fluid are also in progress at a feed rate of 1 gram per 24-hour cycle. The formamide and urea components in the Santomelt 990 CR and the ethylene glycol and urea components of the UCAR fluid are being monitored colorimetrically (Anal. Chem. Methods 71-8, 9 and 10). Because of their water solubility, the analysis is carried out directly on the filtered aqueous phase. Initial results indicate both components in each fluid show a greater than 90% disappearance rate in 24 hours.

V. New Technology

In an attempt to refine the SCAS test method, a statistical study of the operating variables was undertaken. Three model compounds: biphenyl, 2,4'-dichlorodiphenyl ether, and 2,2',4,4',6,6'-hexachlorobiphenyl were used in the 1/64 replicate of a 2^7 factorial design. The experimental design was a Plackett and Burman screening design which permits the study of seven variables in eight experimental runs The variables and levels of the variable employed in the study are shown in Table II.

V. New Technology (Cont'd)

Table II

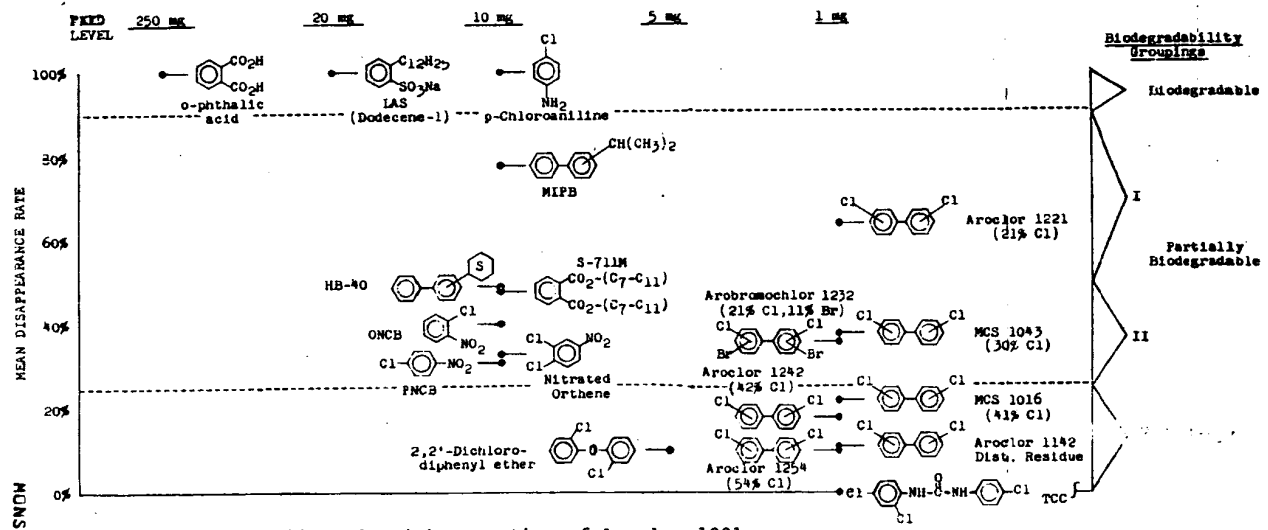
Variable	Low Level	High Level
Time Cycle	24 Hrs.	48 Hrs.
Suspended Solids	1250 mg/liter	2500 mg/liter
Amount of Test Material Fed	1 mg	5 mg
Concentration of Test Material in Solvent	1,5 mg/100 ul	1,5 mg/200 ul
Method of Feeding	Surface Injection	Deep Injection
Aeration Rate	0.5 CFH	1 CFH
Method of Sampling	Sidearm Sampling Port	Pipette

The conclusions obtained from the study were that longer time cycles, higher amount of test material fed, surface feeding and sidearm sampling resulted in higher disappearance rates for biphenyl and 2,4'-dichlorobiphenyl. For 2,2', 4,4',6,6'-hexachlorobiphenyl, the deep injection feeding and the low concentration in the solvent gave the higher disappearance rate. Aeration rate appeared to have no effect while the longer time cycle and lower suspended solids gave more reproducible results.

In retrospect the selection of model compounds for this study was poor. Two readily degradable (biphenyl and 2,4'-dichlorobiphenyl) and a refractory (2,2',4,4',6,6'-hexachlorobiphenyl) compounds were used. The effects of changes in the variables could not be as easily seen with these compounds as they would have been for compounds in the partially biodegradability I and II region.

FIGURE I

BIODEGRADABILITY OF ORGANIC DIVISION PRODUCTS



NOTE: Rates for all PCB's with exception of Aroclor 1221 were obtained at cycle times greater than 24 hours. The observed rates were mathematically extrapolated to a cycle time of 24 hours.

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TABLE III

BIODEGRADATION PROGRAM

Revision III
8-24-71

Time Frame	Method Development: Product	Man Wks	Biodegradation Testing: Product	Man Wks	Other Work: Product	Man Wks
Aug.-Sept.	Tolylxylsulfone	1	Aroclor 5432	4-6	o-Nitrochlorobenzene	2*
	Trichlorodiphenyl Ether		Aroclor 5460	4-6	p-Nitrochlorobenzene	
	Santicizer 160	1	Santomelt 990 CR	4-6	Nitrated Orthene	
	Santophen I	1	UCAR, Runway De-Icer	4-6	p-Chloroaniline	
					Santicizer 711M	1**
					Aroclor 1221	1***
Oct.-Nov.	Plasticizer Candidates to be Scheduled		Tolylxylsulfone	4-6		
			Trichlorodiphenyl Ether	4-6		
	3,4-Dichlorodiphenyl Methane		Santicizer 160	4-6		
			Santophen I	4-6		

*Re-scheduled from June-July Time Frame
1. Evaluation of volatility losses.
2. SCAS testing at higher feed levels.
3. Isolation and identification of material building up in nitrated orthene SCAS unit.
4. RDA degradation rates.

**FID/GC analysis of sludge extracts for alcohols and branched esters.

***Complete EC/GC analysis of SCAS sample extracts.

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