SEMI-CONTINUOUS ACTIVATED SLUDGE DEGRADATION STUDIES

PRESENTED BY DR. E. S. TUCKER

Before entering into a detailed discussion of our recent efforts to develop biodegradation testing procedures, I feel it would be helpful to first establish a common understanding of what is meant when something is said to be "biodegradable". One definition, recently proposed by a sub-committee of the standard methods committee for the Water Pollution Control Federation is that:

Slide I

"Biodegradation consists of a change of a chemical or group of materials by life processes with an accompanying transfer of energy."

Although this is an adequate general definition of biodegradability for the purpose of our discussions this morning, "biodegradation" will be defined as:

Slide I

"The conversion of chemicals which are potential environmental pollutants to innocuous substances via the biological action of living organisms."

Within the framework of this definition, one can have an infinite number of biological transformations that can and do occur. Two such levels that can easily be identified are primary and ultimate biodegradation. These terms are generally defined in the following manner:

Slide I

Primary Biodegradation - Minimum alteration of the chemical structure of the material in question to an extent that the characteristic properties of the original material are no longer evident.

Ultimate Biodegradation - Complete conversion of the material in question to CO_2, H_2O, inorganic salts, and products normally associated with the natural metabolic process of living organisms.

With these definitions in mind, the objective of our research at the onset of this program was to -

Slide II

"Develop a laboratory test procedure which will measure the susceptibility of selected Organic Division Products to primary biodegradation via the biological action of bacteria. Furthermore, the procedure should produce results in a reasonable period of time at a reasonable cost which can be easily extrapolated to the environment.

Slide II

You will note in our preferred definition that the phrase "potential environmental pollutants" has been replaced by "selected Organic Division Products", which is of course what we are interested in as members of the Organic Division. The term "living organisms" has been replaced by "bacteria", since they are by far

the most important microorganisms in the environments receiving our wastes, that are capable of degrading them to environmentally compatible materials. We have also chosen to study the "primary biodegradation" of the selected Organic Division Products. Although monitoring the disappearance of the parent material (primary degradation) is rather subjective in that it is dependent upon the analytical procedure employed, its study is justified in that it addresses itself to our current primary concern, namely, the analytical detectability of our products in the environments receiving them. Additionally, the proof of ultimate biodegradation involves techniques considerably beyond the capabilities of the average analytical laboratory.

In addition to the above, any biodegradation test should simulate as closely as possible the actual environment and as such it should employ a suitably mixed bacterial population vs a pure bacterial culture as well as mixed foods (natural food + test material) vs the test material as the sole carbon source. This is not to say that valuable information cannot be obtained from studies employing pure bacterial cultures and the material in question as the sole carbon source. Only that our choice reflects the desire for data that can be directly extrapolated to the environment.

Additionally, we will initially restrict ourselves to the study of aerobic bacterial oxidations rather than anaerobic bacterial oxidations. There are two reasons for this; first aerobic studies are obviously easier to physically carry out and, secondly, it is generally felt (possibly from a lack of data) that the bacterial degradation of aromatic materials predominantly occurs aerobically rather than anaerobically. Most of the materials we have studied are aromatic in character.

A number of biodegradation tests are available that meet the desired criteria. After reviewing the biodegradation testing procedures listed in Slide II, in light of the data produced, their general utility, etc., and our current capabilities, knowledge, and needs, three of the eight reviewed were chosen for further investigation. Of the three chosen, most of our work has been with the semi-continuous activated sludge (SCAS) procedure and as such we will discuss the data obtained from this test. The river die-away test has been employed with moderate success, when applicable, and we do plan further work with this test as well as future work with the soil percolation technique.

Slide II

Now, before describing the SCAS test as we have used it and the data obtained, I would like to briefly review two literature examples of proposed pathways of bacterial oxidation of aromatic ring structures. The two aromatic compounds chosen as examples are benzene and phenanthrene. Incidentally, these general pathways were proposed from work with pure bacterial cultures using the compounds in question as the sole carbon source.

Here we have outlined the proposed reaction sequences for metabolism of these aromatic ring structures. It has been shown that fission of phenanthrene occurs via a dihydroxylated polynuclear aromatic compound and that subsequent reactions produce saligenin and salicylic acid and eventually catechol, a metabolic intermediate common

Slide III

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to the microbial degradation of benzene. Two proposed reaction sequences for the further degradation of catechol are also shown. In the first one, it is converted to cis, cis-muconic acid and on to β-keto adipic acid via the β-oxo-adipic acid enol-lactone and subsequent hydrolysis of the lactone ring. It has also been shown that a pseudonymous can metabolize the catechol to α-hydroxymuconic semi-aldehyde which loses formic acid to producing 2-oxo-4-hydroxy valeric acid. The valeric acid then undergoes an aldol cleavage yielding acetaldehyde and pyruvic acid. These examples illustrate that bacterial transformations are simply chemical oxidations carried out enzymatically so that the bacteria can acquire the energy and chemicals necessary for their life processes.

The SCAS test procedure we are employing is patterned after the procedure recommended by the Sub-Committee on Biodegradation Test Methods of the Soap and Detergent Association [JAOCS 42, 986 (1965)]. The SCAS unit itself, depicted graphically in the next slide, is simply a glass cylinder with provisions for an air inlet, for aeration of the mixed liquor (activated sludge + liquor) a septum for injection of the test material, a siphon for removal of the liquor, and a stirrer for agitation of the mixed liquor. The standard operating conditions are a total mixed liquor volume of 1500 ml, a suspended solids or activated sludge concentration of 2500 ppm (w/v) and an air flow rate of approximately 0.5 ft.^{3} per hour. The activated sludge used in the test SCAS units was in all cases obtained from the Sugar Creek treatment plant of the metropolitan St. Louis Sewer District. 1500 milliliters of mixed liquor (activated sludge and liquor) is charged to the aeration chamber. The aeration chamber is then connected to a suitable source of compressed air. The actual test is initiated by the addition of a compound to be tested and 10 milliliters of a synthetic sewage solution (dextrose, nutrient broth, and KH_2PO_4 in tap water) to the unit. The mechanical cycle is then as follows:

Slide IV

After addition of synthetic sewage and the test compound the mixed liquor is aerated and stirred for approximately one hour at which time a sample of the mixed liquor is taken for analysis ("0" time) "after" feeding sample. The aeration and stirring is then continued for 21 hours and a "before" feeding sample taken.

At this point, the stirring and air are interrupted and the activated sludge allowed to settle for ~0.5 - 1.5 hours. The sludge volume is estimated, two-thirds (1ℓ) of the supernatant withdrawn, and the pH of the liquor measured. Aeration and stirring are resumed, 1ℓ of tap water added, and the cycle repeated. The cycle is repeated for as long as needed to obtain consistent data.

The suspended solids content (determined gravimetrically) of the mixed liquor is maintained between 2000-3000 mg per liter by periodic removal of the mixed liquor (generally once a week) and replacement with tap water.

The compounds so far tested are generally quite insoluble in water and except for a limited amount which may be dissolved in the aqueous phase the test compound is adsorbed by the sludge. In order to disperse the test compound upon feeding, it is fed with a microliter

syringe in the form of an absolute ethanol solution (e.g. 5-10% w/v). The actual feed level is generally dictated by the sensitivity of the analytical procedure employed and by the effect the material has upon the unit's operating characteristics as witnessed by the pH and the sludge growth and settling rates. Feed rates in the range of 1-10 mg per cycle have generally been employed.

Operating in this fashion five 24-hour cycles and one 72-hour cycle can be carried out in a normal work week. On occasion, 48-hour and longer cycle periods have been employed when degradation rates were not large enough to be statistically observed above the inherent scatter. The biodegradation data are generally expressed in terms of a % disappearance rate

$$\%\ \text{disappearance rate} = \frac{(\text{mg @ "O" Time}) - (\text{mg, 23 Hr. Sample})}{(\text{mg @ "O" Time})} \times 100$$

qualified with respect to the cycle time and feed level employed.

Now assuming

1. A test compound feed level of 10 mg
2. A constant cycle time
3. A constant bacteria activity

and 4. That we incur no mechanical losses with the exception of that due to the compounds water solubility

let's examine what type data we should theoretically expect for a water soluble, and a water insoluble mixture of materials in which residual material left after each cycle is either degradable or non-degradable.

I should probably mention at this point that the SCAS test procedure is designed for the study of water soluble materials and as such water insoluble compounds present more difficult problems. This fact will become very evident as we examine graphically the three types of materials.

Slide V The first graph illustrates what should be observed for the water soluble-residue degradable-nondegradable case.

Here, we have plotted the observed level of material present in the unit as a function of the number of cycles assuming the materials % disappearance rates are 0%, 10%, and 50%. The highest value in each case represents the amount found after each feeding. The lower symbol, connected by the line is the amount found just before the unit is re-fed and a new cycle repeated. In this case, we are also assuming neglectible adsorption of the test mixture by the sludge. As can be readily seen in the case of the 0% disappearance rate, the unit reaches for all practical purposes which is termed a "steady state" in 5 cycles. The amount left, assuming that two-thirds of the amount present in the unit is discarded each cycle, is simply:

$10 + \frac{10}{3} + \frac{10}{9} + \frac{10}{27} + \frac{10}{81} + \frac{10}{243}$ . . . such that when the unit is fed at the rate of 10 mg per cycle the amount present in the unit levels at ∿15 mg. The same consideration applies to the cycle time in that a 24-hour cycle time is actually equivalent to a 36-hour retention time. The % disappearance rates of 10% and 50% are represented by the solid and open circles. The next two graphs will show the behavior of materials which are more closely related to most of those we have studied. The next graph depicts the water insoluble residue-degradable case. Again, the observed level before and after feeding is plotted vs the number of cycles. It becomes at once apparent that if the % disappearance rate is 10% or less, that it takes a significantly larger number of cycles to achieve a "steady state" and that analytical problems are to be expected since we would be measuring a small difference between two much larger growing numbers. So that as the % disappearance rate becomes larger, better analytical data can be expected in a shorter period of time.

Slide VI

The next graph illustrates the worst situation; namely, a water insoluble material in which the residue is non-degradable. The evident problem here is that we have no mechanical means by which to remove the non-degradable residue which builds up on the sludge. Under these conditions, accurate estimation of a 10% disappearance is even more difficult. This effect can be counteracted somewhat in the case where the residue is degradable by going to longer time cycles, i.e., 48 hours, or in the case where the residue is non-degradable, by feeding less often and at the lowest level analytically detectable.

Slide VII

In reality the situation is much more complex than can be shown in the simplified graphs. Bacterial populations change and mechanical as well as physical losses can and do occur. Isolating the magnitude of the effects that these losses can have upon the observed disappearance rate is difficult at best.

To date, we have tested a total of sixteen materials. These sixteen materials are shown in the table along with their general molecular structure, the feed level, and cycle time employed. The observed % disappearance rates range from 0 for TCC to greater than 97% for p-chloroaniline. Also shown in the table are the material's water solubility, vapor pressure and the methods of analysis used to monitor its disappearance.

Slide VIII

The observed data from which the disappearance rates were calculated are shown in the next set of graphs. The first graph shows the data for p-chloroaniline which had an observed (%) average disappearance rate of 97.3 ±1.6 when fed at the rate of 5 mg/24 hour cycle. It is an example of a water soluble degradable material. In the top chart we have plotted the total mg found in the unit before and after feeding. The after feeding sample is denoted by the solid circle; the before by the arrowhead; the connecting line indicates the amount lost during each cycle.

Slide IX

The lower chart is a plot of the % disappearance rate vs elapsed time. As you can see, the rate is consistent and the scatter about the average minimal.

The next graph shows the raw data for isopropylate biphenyl. The average % disappearance rate for this mixture was 78.2 ±3.2%. This is an example of a water insoluble readily degradable material. With this particular material because of its low water solubility and relatively high vapor pressure, it was checked for volatility losses. Although the level lost was measurable, it was not significant in that it amounted to less than 3% of the 10 mg fed per 24-hour cycle.

Slide X

The next graph illustrates an example of an intermediately degradable water insoluble material, HB-40. The observed % disappearance rate was 48.6 ±6.9% with no measurable volatility losses. Inspection of the earlier points in the upper graph seems to indicate that the bacteria require a longer acclimation period than in the previous example.

Slide XI

In the next set of graphs, an example of another intermediately degradable material, nitrated orthene, is shown. The initial % disappearance rate was 71.3 ±5.5% which after a short period of time plunged to 40.3 ±3.2%. It appears from changes in the UV absorption curves that an intermediate material derived from the parent material is building up and being calculated as undegraded nitrated orthene. We are currently attempting to further identify the material or materials responsible for the apparent interference.

Slide XII

Next is an example of a water insoluble material, TCC, judged to be very resistant to bacterial degradation by this test. The level found in the unit built up very rapidly at a rate about equal to the amount fed during each cycle. As expected for this class of compound the data were quite scattered.

Slide XIII

The Aroclor materials studied were for us a special case in that our objective here was to show experimentally that the second generation products, MCS 1043 and MCS 1016, were better environmentally than our original Aroclor 1242 product.

For this reason, I have chosen to discuss the data for these materials last and in a more detailed manner. Earlier work with these materials had indicated that a 24-hour cycle time was not long enough to demonstrate a statistical significant difference between the % disappearance rates for MCS 1016 and Aroclor 1242. In fact, although MCS 1016 and Aroclor 1242 showed some evidence of degradation, neither rate was significant.

A second study was therefore initiated using a longer cycle time between feedings in an attempt to resolve this problem.

The next two slides show what some of the data obtained for MCS 1043 and Aroclor 1242 looks like using a 48 hour cycle time.

Here again we have plotted in the upper graph total mg found after and before feeding vs elapsed time and in the lower, the calculated % disappearance rate vs elapsed time. As you can see, the data for MCS 1043 is reasonably consistent but scattered. Comparing this slide with the next one which shows the data for Aroclor 1242 we can see an appreciable difference. The observed average %

Slides XIV - XV

disappearance rate for Aroclor 1242 was 26.3 ±15.3% vs a 56.2 ± 15.5% for MCS 1043. The data for Aroclor 1242 was much less consistent and even included some negative % disappearance rates.

Although the data in all cases were better using the longer cycle time the observed % disappearance rates for MCS 1016 and Aroclor 1242 could not be differentiated statistically, indicating that the resolution of the test had not been improved enough by the change.

We are currently testing MCS 1016 and the residue left when Aroclor 1142 is distilled to produce MCS 1016. Preliminary data indicates that in terms of their % disappearance rates, MCS 1016 is a major improvement over the fraction removed by our distillation.

With these materials, we also carried out a more detailed analysis of the residue from the units via electron capture gas chromatography to check for isomeric alterations. The next set of slides will demonstrate what we observed chromatographically.

Slide XVI

The first slide illustrates what happens in the case of Aroclor 1221. The top trace is a chromatogram of the Aroclor 1221 that was fed to the unit, the middle trace is of the residue isolated after a 24-hour exposure period and the bottom trace is of an Aroclor 1242 standard run under equivalent GC conditions.

In interpreting these chromatograms, it is extremely important that we remember that we are using an electron capture detector (ECD). The ECD is inherently much more sensitive to chlorine containing molecules and its sensitivity can and generally does increase significantly as the degree of chlorination increases. So that minor amounts of highly chlorinated biphenyls can and do appear to be major components in the chromatograms when in actuality they are not. To illustrate this point, let's look at the standard chromatogram for Aroclor 1221. The numbers above each peak indicate the dominant homolog represented by that peak as determined by GC mass.

Casual inspection assuming a constant detector response would indicate that this material is dominantly dichlorobiphenyl with approximately equal amounts of monochlorobiphenyls and trichloro and higher biphenyl isomers. In reality, Aroclor 1221 contains ∿20% biphenyl which is not even observed, ∿55% monochlorobiphenyl, ∿20% dichlorobiphenyl (the apparent major component), and something less than 5% higher chlorinated biphenyls on a weight per cent basis. With this in mind, if we compare the standard Aroclor 1221 chromatogram to the residue after 24 hours of exposure, we can observe that the dominant components in this product are almost completely consumed during each cycle and that the minor higher chlorinated isomers appear to be more refractory toward degradation. Comparison of the residue chromatogram to the standard Aroclor 1242 chromatogram simply illustrates that the resistant residue in this product is comprised of the major components in the next higher chlorinated product.

If the supply of Aroclor 1221 were not constantly renewed every cycle appreciable disappearance of the isomers observed in the 24-hour residue would probably occur.

Slide XVII

The next slide demonstrates what is observed with MCS 1043. Again we can see that the isomers or homologs which disappear most rapidly are the lower chlorinated ones. The disappearance of the trichloro and some tetrachloro isomers becomes apparent only if we mentally ratio the areas of all components with elution times less than two minutes to those with elution times greater than two minutes. We also interestingly note that although no penta or hexa isomers are observable in the MCS 1043 fed, they are easily observed in the residue.

Slide XVIII

The next slide shows the EC chromatograms for MCS 1016 and the residue isolated after a 48-hour exposure period. The observed average % disappearance rate for this product was 32.9 ±13.8%. The chromatograms simply re-illustrate what was observed earlier in the MCS 1043 chromatograms.

Slide XIX

In the next slide are chromatograms for Aroclor 1242 and the residue. We can see again that the isomers which disappear most rapidly are the lower chlorinated ones and that the environmentally resistant penta and hexachlor isomers build up. We can also qualitatively note by the difference in the disappearance rates of the isomers for each homolog that the chlorine substitutional positions affect the rate at which they disappear.

I have not included chromatograms for Aroclor 1254 since little if any isomeric alteration was observed over the entire duration of the experiments. Its % disappearance rate, as you noted earlier in the table was 15.0 ±37.7%, which is for all practical purposes, zero. This is, of course, what we would have predicted on the basis of our earlier environmental work.

Slide XX

In the last slide, for comparative purposes, I have plotted all the average % disappearance rates vs a product index number assigned in the earlier table. The solid symbols indicate a 24-hour cycle time and the open, a 48-hour cycle time. The type of symbol simply indicates the feed level per cycle: hexagon - 20 mg, triangle - 10 mg, square - 5 mg, and circle - 1 mg, and the lines drawn through the symbols indicate the range of the % disappearance rates.

From this graph, it can be seen that we can rank the biodegradability of each material according to feed level, cycle time, and the variability of the data.

Further extrapolation of these results to the actual environment will require the study of more model materials with known ecological histories.

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SLIDE I

"BIODEGRADATION CONSISTS OF A CHANGE OF A CHEMICAL OR GROUP OF MATERIALS BY LIFE PROCESS AS WITH AN ACCOMPANYING TRANSFER OF ENERGY."

Definition proposed by a sub-committee of the standard methods committee for the W.P.C.F.

"The conversion of chemicals which are potential environmental pollutants to innocuous substances via the biological action of living organisms."

Primary Biodegradation - Minimum alteration of the chemical structure of the material in question to an extent that the characteristic properties of the original material are no longer evident.

Ultimate Biodegradation - Complete conversion of the material in question to CO_2, H_2O, inorganic salts, and products normally associated with the natural metabolic processes of living organisms.

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SLIDE II

"Develop a laboratory test procedure which will measure the susceptibility of selected Organic Division Products to primary biodegradation via the biological action of bacteria. Furthermore, the procedure should produce results in a reasonable period of time at a reasonable cost which can be easily extrapolated to the environment."

Potential Environmental Pollutants		Selected Organic Division Products
Living Organisms	-	Bacteria
Conversion	-	Primary Biodegradation
Mixed Bacterial Population	or	Pure Bacterial Culture
Mixed Foods	or	Sole Carbon Source
Aerobic	or	Anaerobic (Slower (Difficult

✓ River Die-Away Test (~$1X10^5$ cells/ml)
Fortified and Inoculated Water Test
Shake Culture Test
Laboratory Trickling Filters
Continuous Flow Activated Sludge
Batch Sludge Die Away
✓ Semi-Continuous Activated Sludge ($2X10^8$ cells/ml)
✓ Soil Percolation

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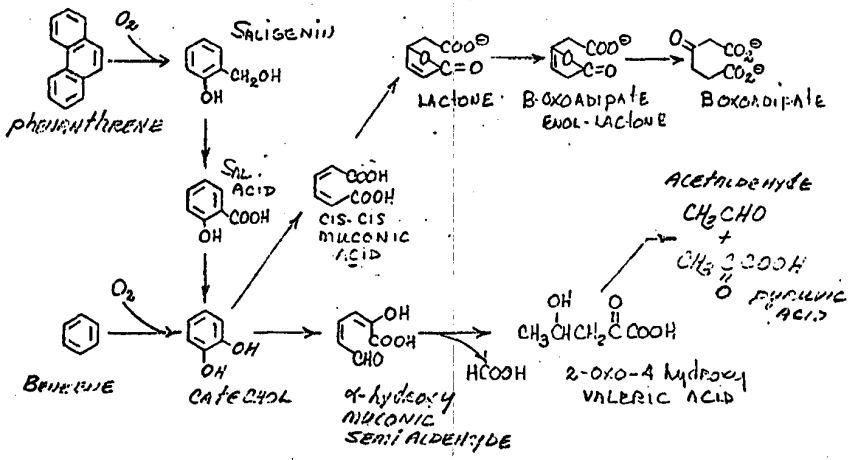
SLIDE III



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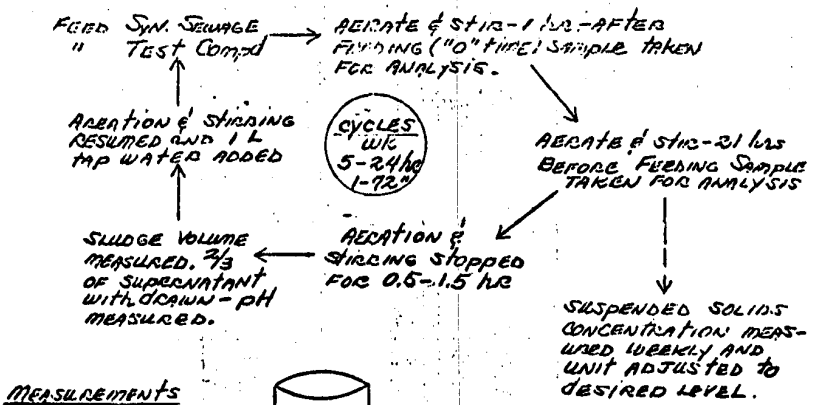
SLIDE IV

Standard Operating Conditions

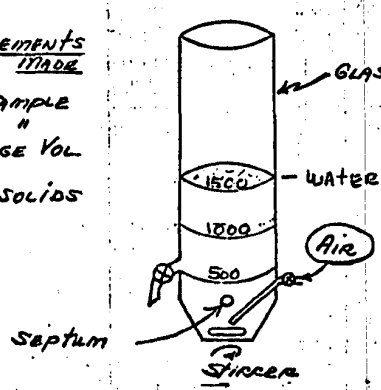
Vol.	1500 mL.
SS	3750 mg (2500 ppm) Activitated Sludge
Air	½ ft^3/hr
Feed	Synthetic Sewage { Dextrose, Nutrient broth, KH_2PO_4
	Test compd (EtOH)

Mechanical Cycle



Measurements made

1. AF Sample
2. BF "
3. Sludge Vol
4. pH
5. Sus. Solids



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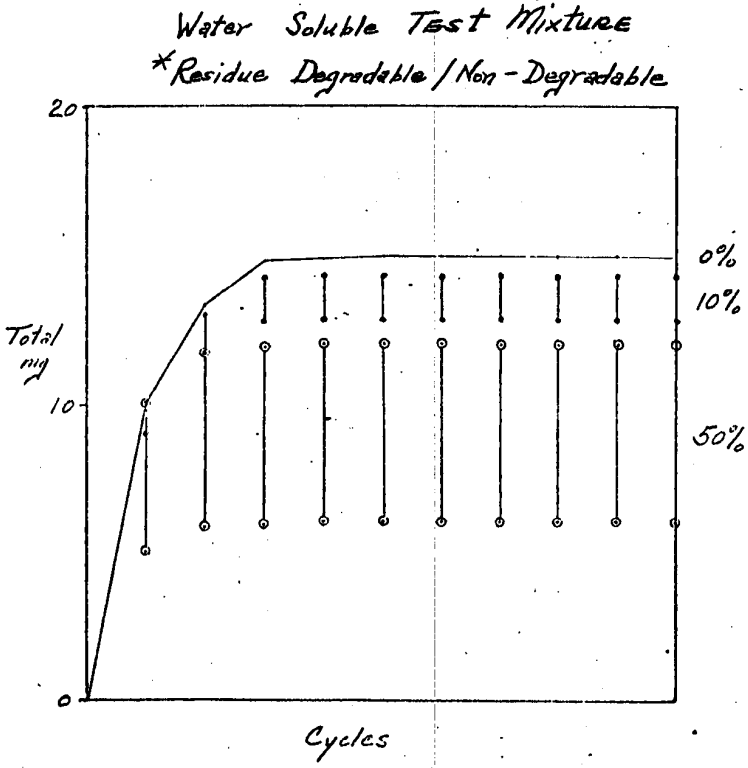
SLIDE V



*PORTION OF TEST MIXTURE NOT CONSUMED DURING EACH CYCLE.

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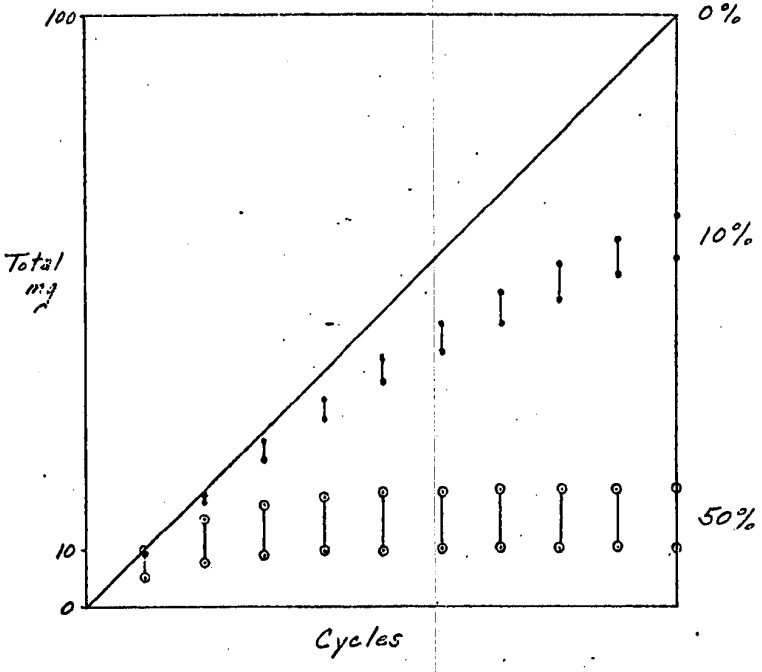
SLIDE VI

Water Insoluble Test Mixture Residue Degradable*



* portion of test mixture not consumed during each cycle.

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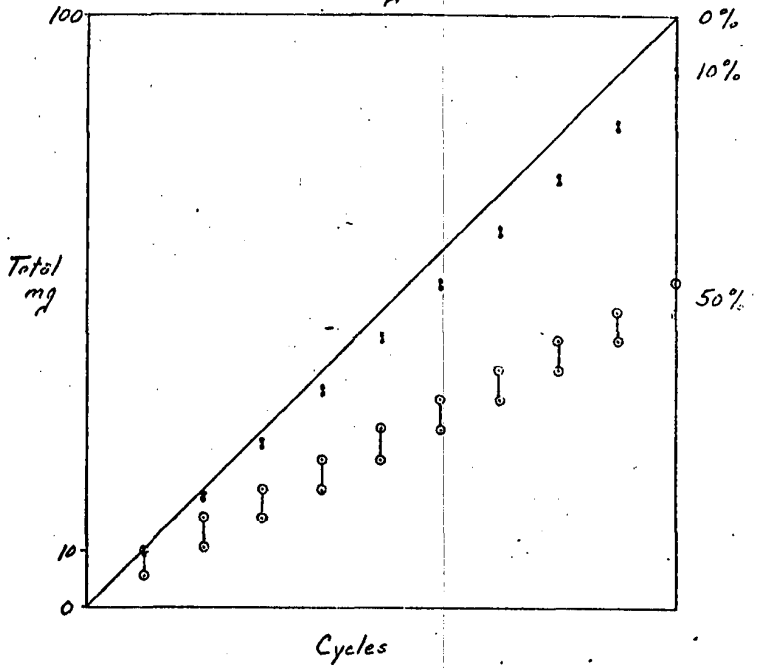
SLIDE VII

Water Insoluble Test Mixture
Residue Non-Degradable*



* portion of test mixture not consumed during each cycle.

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SLIDE VIII

Product Index	Test Compound	Molecular Structure	Feed Level (mg)	Cycle Time (hr)	Disappearance Rate (%)	Water Solubility %	Vapor Pressure mmHg (25°)	Method of Analysis
1	LAS (Dodecene-1)		20	24	>99.5	Soluble		Colorimetric
2	p-Chloroaniline		5	24	97.3±1.6	0.5	0.1	UV
3	MIPB		10	24	78.2±3.2		0.005	UV, FID-GC
4	HB-40		10	24	48.6±6.9		N.S.	UV, FID-GC
5	S-711 M		10	24	48.3±7.7			UV
6	o-NCB		5	24	37.5±8.8	0.050		UV
7	Nitrated Orthene		5	24	*40.3±3.2			UV
8	p-NCB		5	24	33.9±2.9	0.005		UV
9	Aroclor 1221	 21% Cl	1	48	~70.0		0.005	UV
10	2,2' Dichloro Diphenyl Ether		5	24	12.0±4.8			EC-GC
11	MCS 1043	 ~30% Cl	1	48	56.2±15.5			UV, EC-GC
12	Arobromoclor 1232	 21% Cl 11% Br	1	48	54.1±19.3			UV, EC-GC
13	MCS 1016	 41% Cl	1	48	32.9±13.8			UV, EC-GC
14	Aroclor 1242	 42% Cl	1	48	26.3±15.3	2×10^{-5}	1.4×10^{-3}	UV, EC-GC
15	Aroclor 1254	 54% Cl	1	48	15.0±3.7	5×10^{-6}	3.4×10^{-5}	EC-GC
16	TCC		1	24	0	<0.001		UV

* Initial Disappearance Rate 71.3 ± 5.5 %

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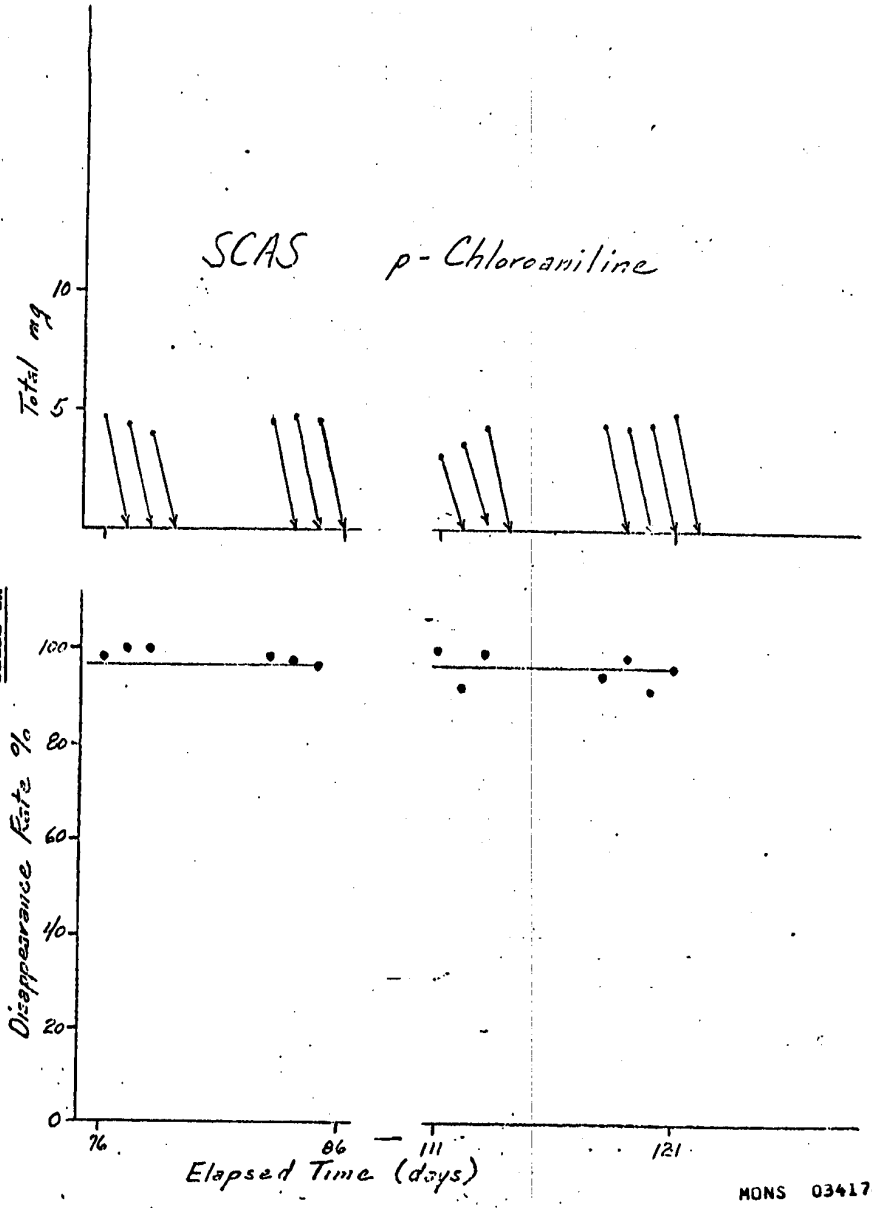
SLIDE IX



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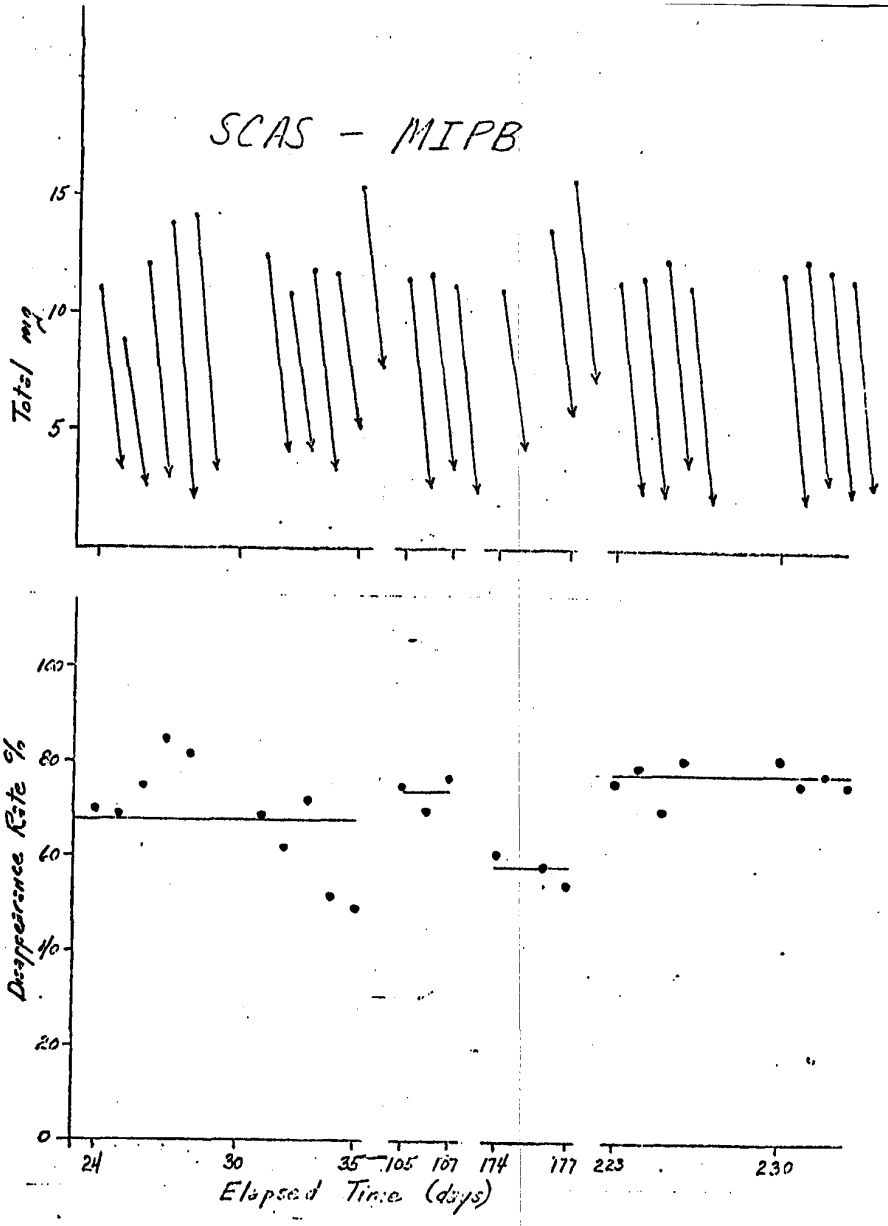
SLIDE X



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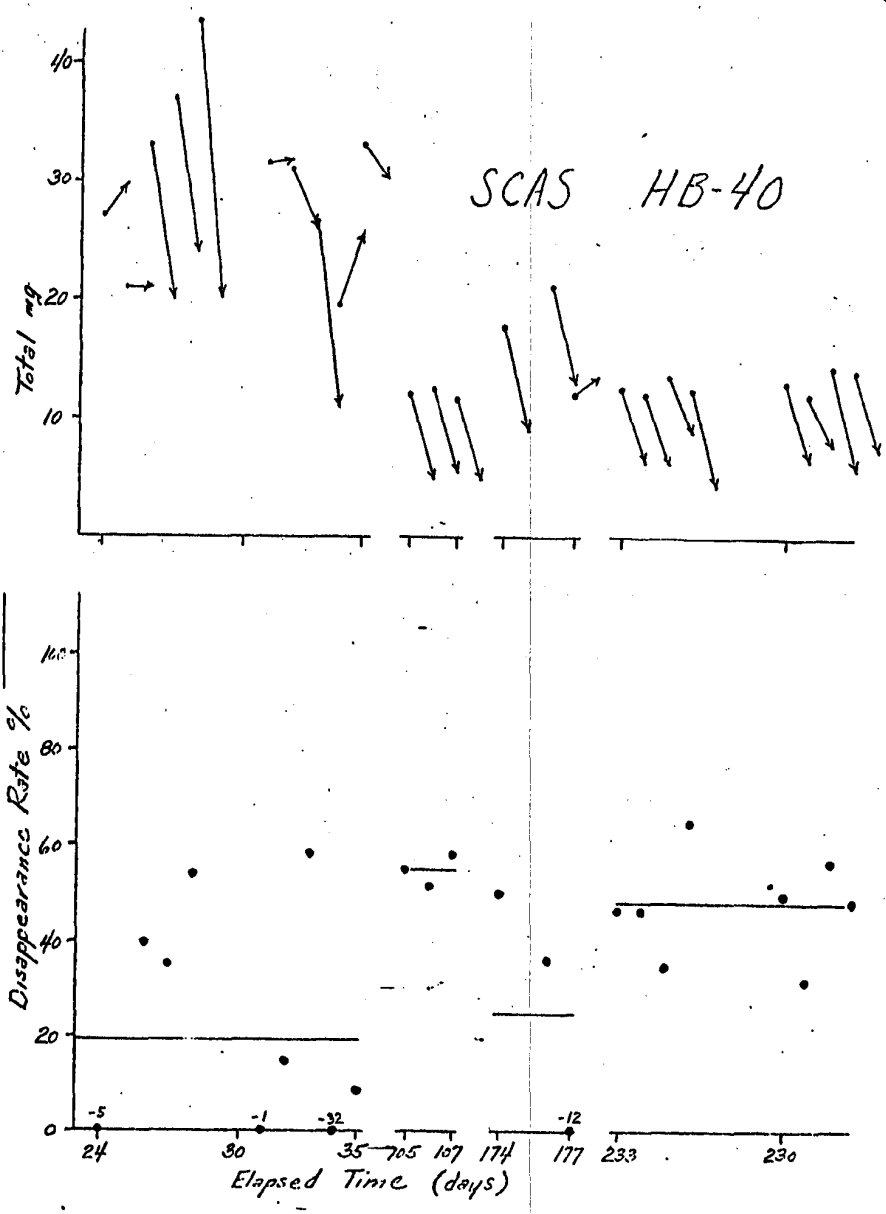
SLIDE XI



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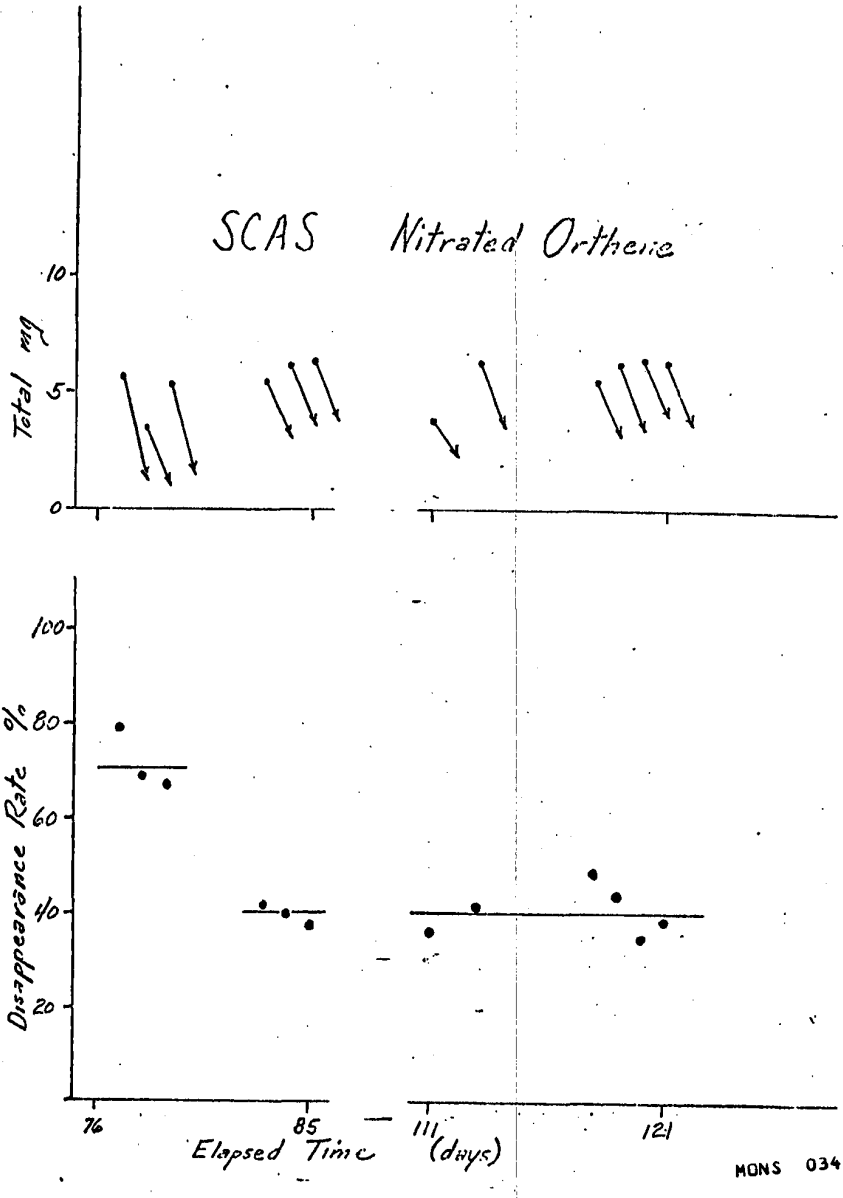
SLIDE XII



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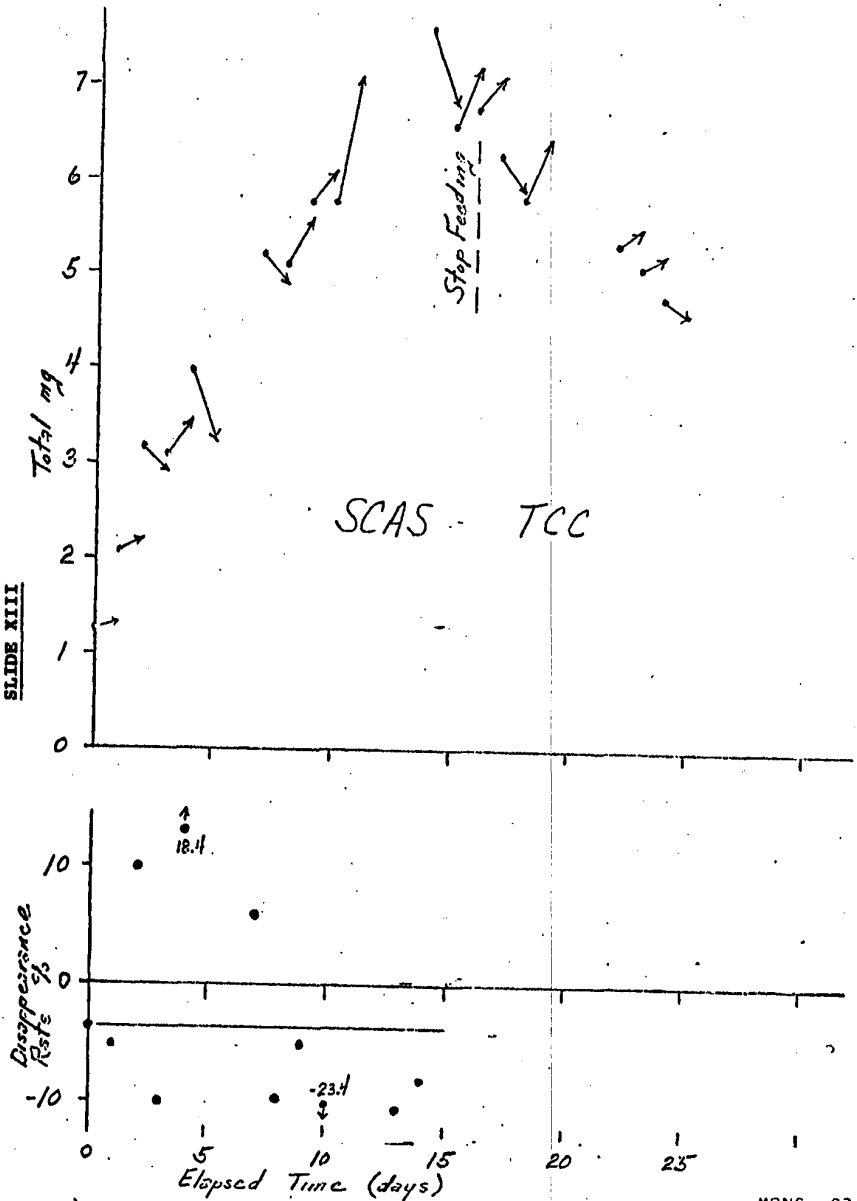
SLIDE XIII



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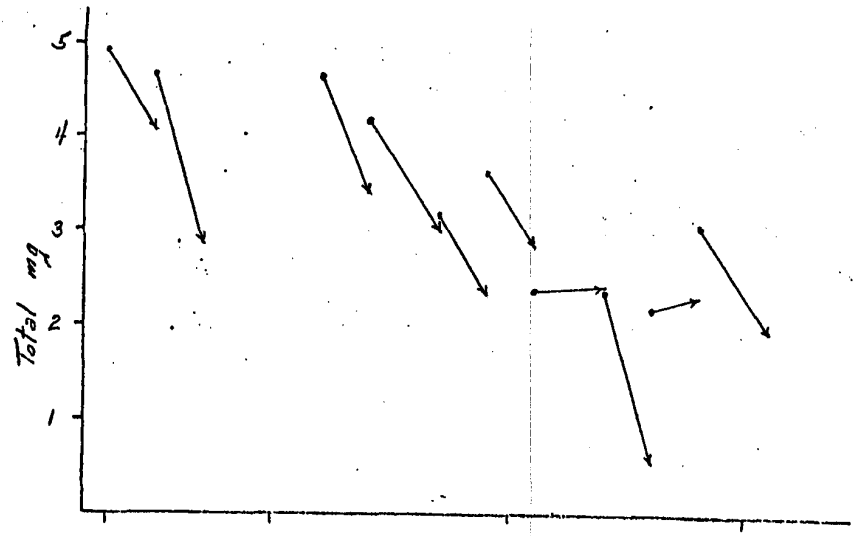
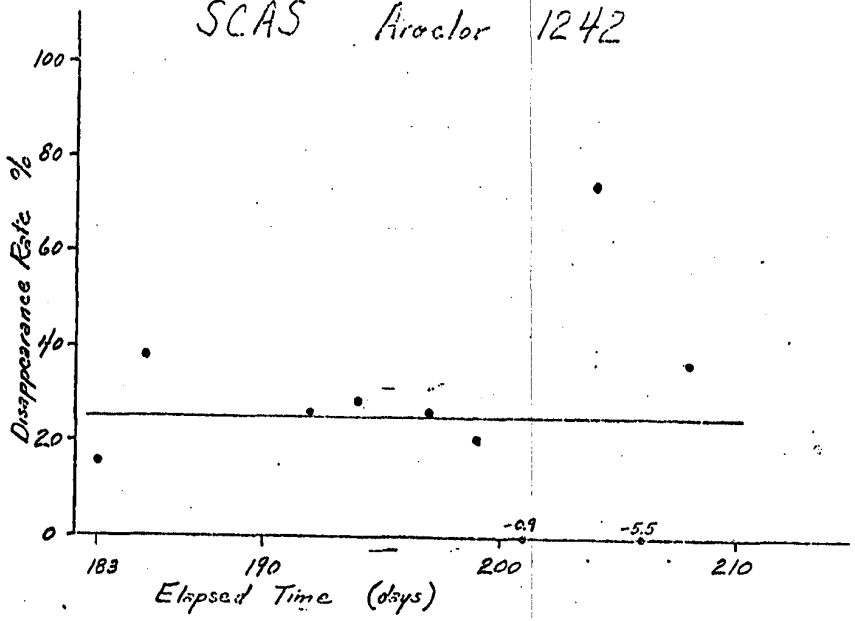
SLIDE XIV



SCAS Aroclor 1242



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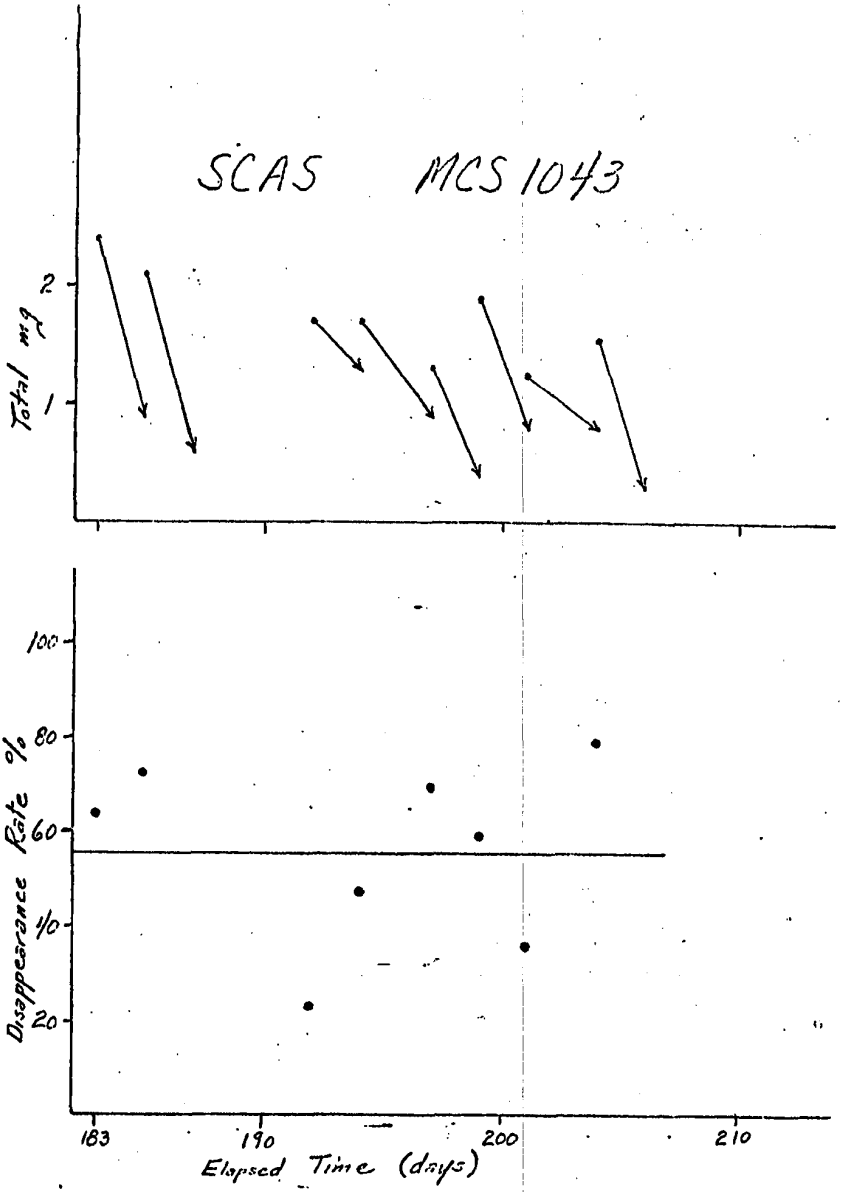
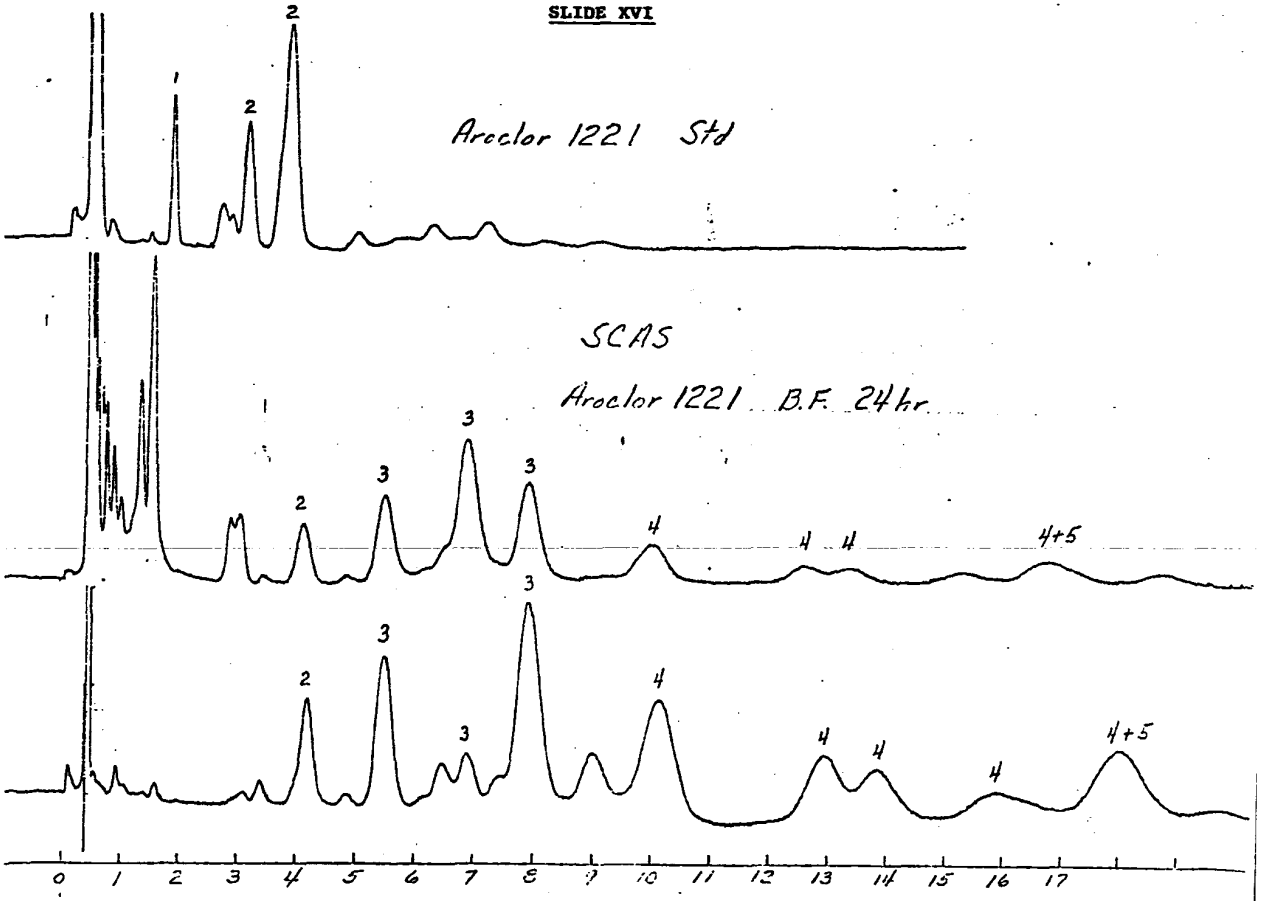
SLIDE XV



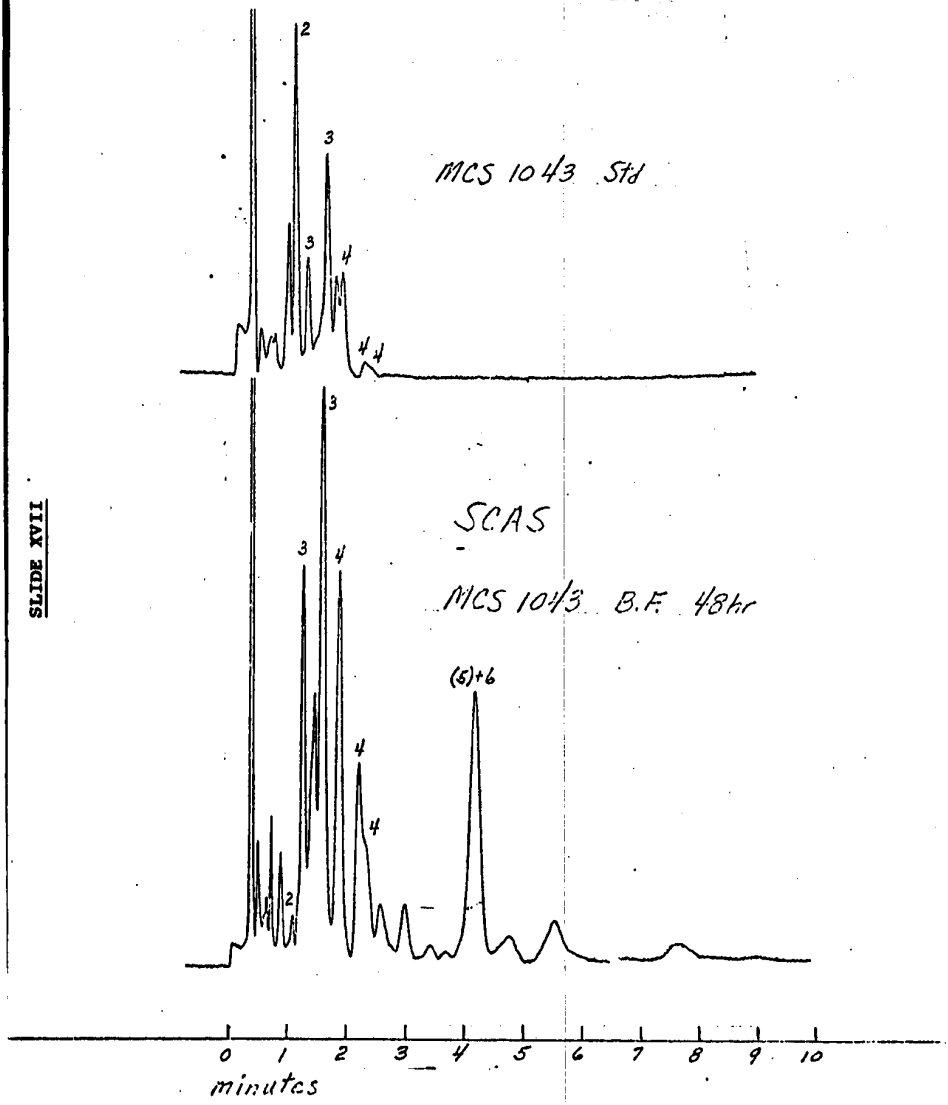
MONS 034184

SLIDE XVI



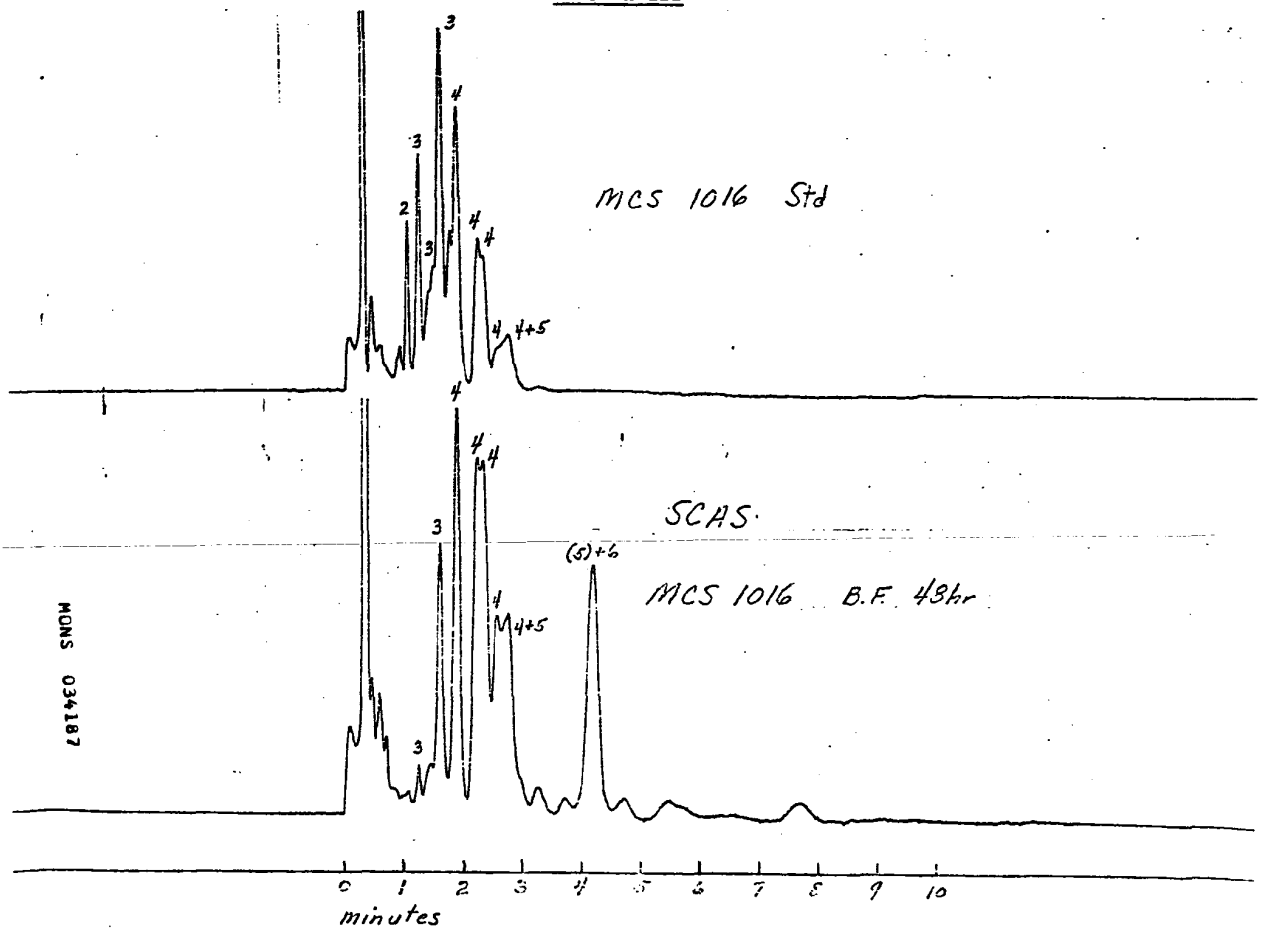
MONS 034185

SLIDE XVII



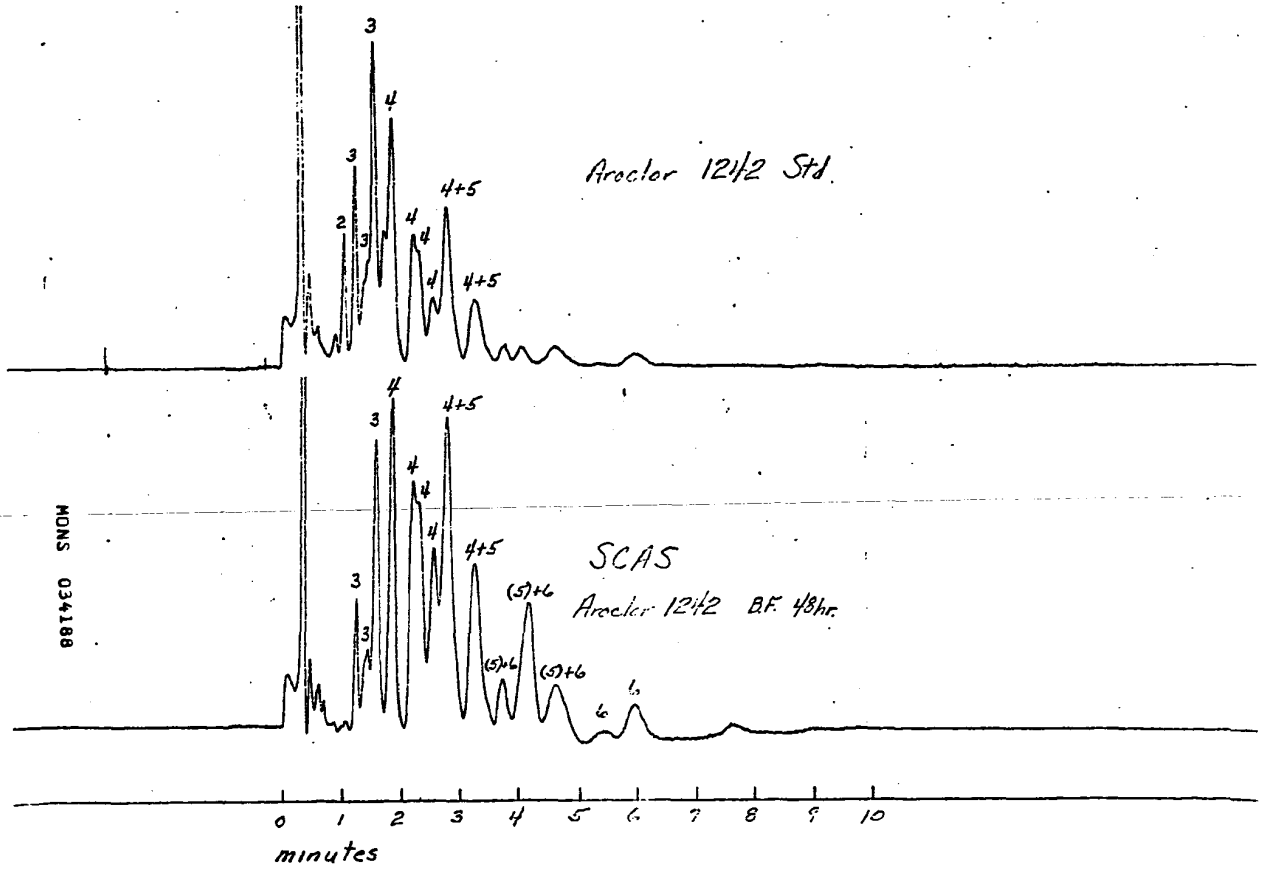
MONS 034186

SLIDE XVIII

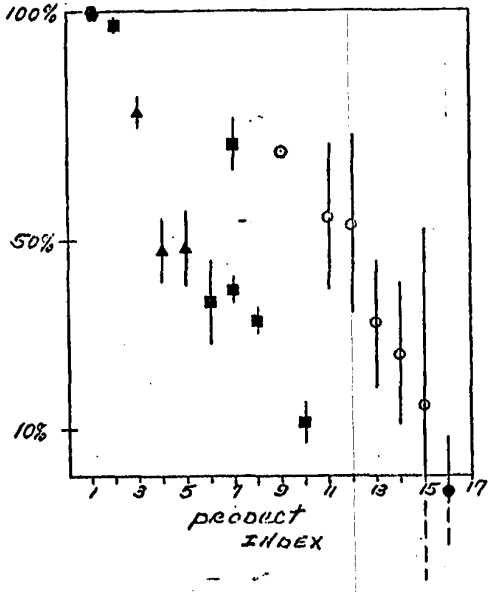


MONS 0341187

SLIDE XIX


Aroclor 1242 Std.
SCAS
Aroclor 1242 B.F. 48hr.
MONS 034188
0 1 2 3 4 5 6 7 8 9 10
minutes

SLIDE XX



MONS 034189