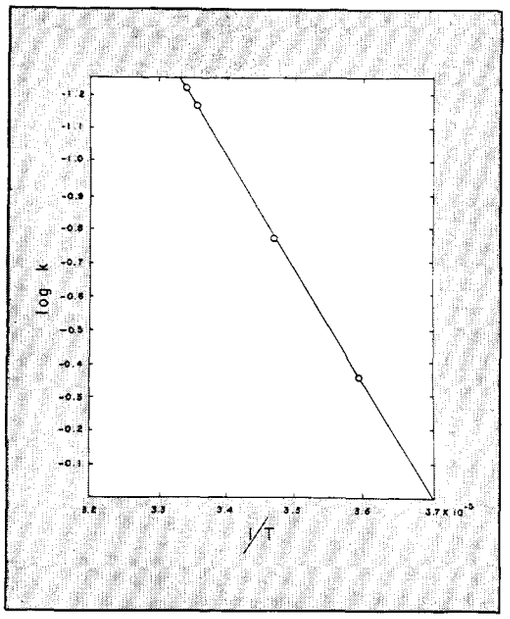

Figure 6. Activation energy curve for monochlorinated schradan

to 4.7. This would indicate an increase in phosphorylating ability of even a phosphate ester on introduction of an electrophilic group. A similar enhancement was observed on the chlorination of octaethylpyrophosphoramide, and acetaldehyde was identified after adding the product to water. This substantiates the proposed mechanism for chlorination of schradan when formaldehyde was found.

The optimum pI_{50} value of 5.8 as shown in Figure 4 is below the actual value for the monochlorinated derivative, since the di- and trichlorinated schradan have been lost by hydrolysis, and the product is diluted with unchanged schradan. If one corrects for the amount of monochlorinated derivative hydrolyzed before assaying (at the end of 30 minutes) together with the value for the actual amount of monochlorinated derivative in the mixture (21%), then the pI_{50} value is increased to 6.7, or almost a 100,000-fold increase in anticholinesterase activity.

Summary

Chlorination of schradan in an anhydrous medium proceeds with the release of hydrogen chloride in a heterogeneous manner and not stepwise. The amount of chlorine added determines the ratio of schradan and mono-, di-, tri-, and tetrachlorinated products. Formaldehyde is produced in amounts directly proportional to the amount of chlorine introduced.

Increasing substitution renders the compound increasingly reactive to water so that schradan with a half life of ten years is reduced in the dichloro derivative to a half life of 4 minutes at room temperature. The increase in activity is indicated by a net increase in the anticholinesterase activity of almost 100,000 times. However, an optimum is reached, due to the instability of the more highly chlorinated material which is hydrolyzed before being able to react with and inactivate the enzyme.

Conclusions

Evidence is offered to support the concept of activation of schradan. The introduction of an electrophilic group, in this case chlorine, renders the anhydride less stable and therefore a better anticholinesterase in vitro. Increased chlorination renders the compound so unstable that its anticholinesterase activity is greatly reduced due to rapid hydrolysis.

The chlorination of schradan in an anhydrous medium has been shown by a kinetic study of rates of hydrolysis to be heterogeneous and not stepwise.

Acknowledgment

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EMULSION TESTING

A Basis for Tests for Emulsifiable Concentrates of Agricultural Chemicals

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ESTABLISHMENT OF RECOGNIZED methods of evaluating emulsifiers by test emulsions is of critical interest to several industries, but data that would provide a basis for devising suitable test procedures are not available. This is due in part to the complexity of the problem, confusion resulting from the many tests that have been devised for divergent applications, and lack of reproducibility of results even with a given test. The differences observed are often due to a lack of

A multitude of different emulsion tests are presently employed by manufacturers of emulsifiable concentrates. Many of these tests lack reproducibility, resulting in inadequate quality control or inconclusive research experiments. One of the major difficulties encountered is the preparation of the emulsion, which is known to be a critical operation. There have been no data published on the effects to be expected from the many variations that can be encountered in this operation, such as mode of addition of ingredients, agitation, and other preparation conditions. Data are presented indicating the effects of these and other variables on the laboratory preparation of emulsions from emulsifiable concentrates. A test method using equipment which is commercially available has been devised and described.

respect for the effects that occur with slight variations in emulsification technique.

At the present time an industry that markets an emulsifiable concentrate usually sets up its own simple laboratory emulsion test. Though the procedure for preparing the emulsion may have been thoughtfully devised, it may defeat the purpose of the test. Likewise, improperly styled observations lead to erroneous conclusions. It is the purpose of this paper to outline and discuss several of the factors in emulsion preparation and observation and their importance in developing a laboratory procedure. A testing procedure based on data presented in this paper has been presented previously by Griffin and Behrens (*1*).

The subject of emulsions, emulsifiable concentrates, dispersions, and their ramifications presents unusual difficulties to anyone who endeavors to establish a suitable general evaluation procedure (*4*). In fact, there is little question but that a single procedure will be inadequate, though by careful selection it should be possible to establish a small number of procedures that will provide for the evaluation of almost any type of product.

For the major portion of the study, a single insecticide concentrate has been used. This permits correlation of the data from one series of tests to another with suitable precautions. The formula employed was a 2-pound-per-gallon DDT formulation employing xylene as the solvent. Insofar as is known the properties shown apply to all emulsifiable concentrates, although the quantitative effect may be different for each formula that might be studied.

Emulsification Procedure

The preparation of an emulsion for analytical evaluation requires control over many factors that may be ignored or subordinated if the emulsion is for practical use. The emulsion must be prepared by a method that is reproducible and does not overshadow emulsification characteristics by excessive agitation or faulty preparation otherwise. It is readily apparent that if a concentrate is formulated to be emulsified with a minimum amount of effort, a test using vigorous agitation would be misleading. The chief concern is the repeated preparation of the emulsion with minimum agitation to give the same result on critical observation.

Minor deviations in the agitation, a different order of addition of ingredients, and the use of a vessel of different shape are some of the variables involved that may influence the results to a greater extent than small changes in the ingredients or surface active agents.

	Shaking	Stirring
Order of addition of ingredients	Of major importance	Of major importance
Speed of addition of last ingredient (emulsifiable concentrate)	Of little importance within wide limits	Of considerable importance if done while stirring is in progress, which is usual
Size and shape of container	Of little importance as long as volume emulsion is about the same	Of extreme importance since vigor of action of propeller or stirring rod depends upon the shape and depth of the liquid
Air incorporation	A nominal amount at a low energy point in the system	A variable amount at the periphery of the propeller, a high energy point, which promotes dispersion
Vigor defined by	Speed and length of stroke with a given bottle and the specified volume of emulsion	Size, speed, and shape of stirring rod or propeller, location in vessel by height and relationship to sidewalk, shape and size of vessel, shape, size, and location of baffles for a given volume of liquid

Agitation Two general methods of preparation are possible—shaking and stirring (or propeller agitation). The characteristics of both methods at their optimum conditions (which includes fixed speed motors, etc.) have been tabulated.

It would appear that the common practice of preparing emulsions in stoppered graduated cylinders is really a

Figure 1. Effect of order of addition of ingredients

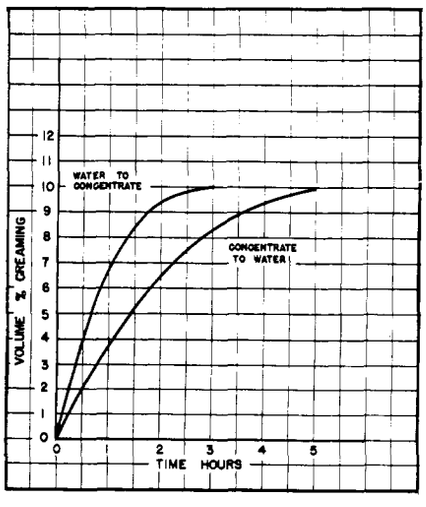
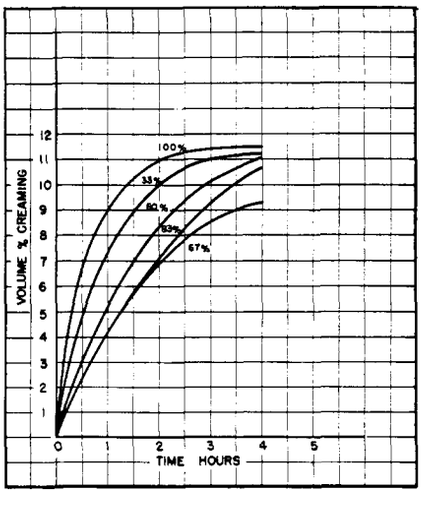


Figure 2. Effect of ratio of volume of emulsion to volume of bottle

Volume of emulsion as percentage of bottle capacity



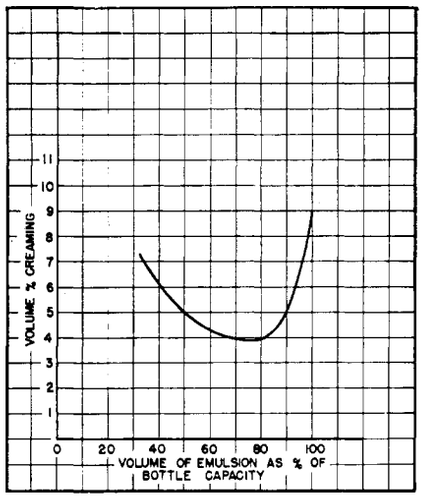


Figure 3. Effect of volume of emulsion on stability after 1 hour

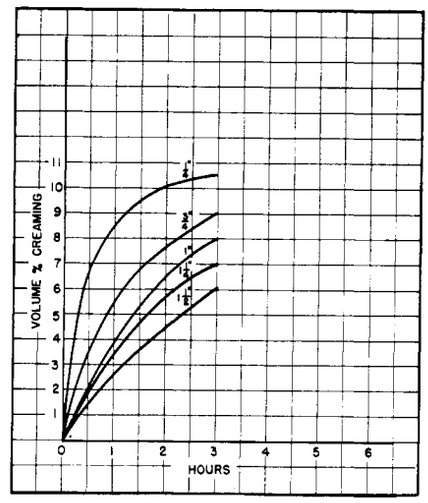


Figure 4. Effect of stroke length

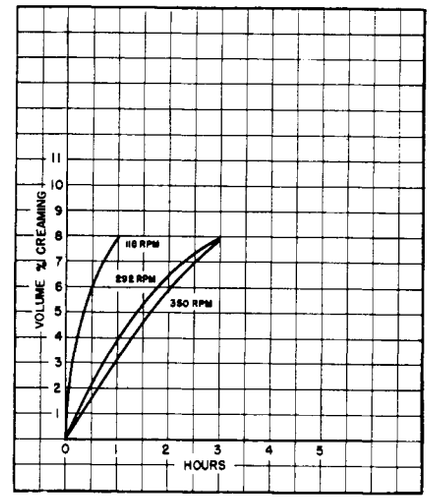


Figure 5. Effect of rate of shaking

shaking technique although it probably is not often considered such.

The variables present in stirring or propeller mixing are far more difficult to establish between laboratories than those for the shaking method. Conditions such as the pitch of the propeller blades (including individual variations in pitch of the blades of a single propeller), proper baffling to exclude air, and similar conditions are often all important in degree of emulsification. Based on these considerations shaking was considered the most suitable method of emulsion preparation for evaluation procedures, particularly where preparation of large numbers of emulsions is contemplated.

These conclusions are, of course, particularly apt for emulsifiable concentrates. They also apply to emulsions that are to be prepared with a minimum of plant equipment and mechanical effort. For those emulsions that are to be milled or homogenized, or to receive some other fixed form of emulsification procedure in plant preparation, it may be necessary to incorporate such steps in their evaluation preparation procedure.

Emulsification preparation by shaking may be broken down into two phases—combination of the ingredients, and shaking or vigor of shaking.

The order of addition of ingredients must influence the final results as little as possible, and must be easily followed in laboratory practice. For an emulsifiable concentrate there are two ingredients—the concentrate and the water.

Experimental

Addition of Ingredients In order to obtain experimental data showing the effect of variables, series of tests were carried out, including a series in which the concentrate was added to the water, and also one in which the water was added to the concentrate (the combination being effected in the bottle in which shaking was to occur). The results of these tests using a 1 to 11 ratio of concentrate to water are shown in Figure 1. It is interesting that the addition of the concentrate to the water gives a more stable emulsion. Compositions are known in which the reverse is true.

Containers The shape and size of the bottle influence the results to some extent. Most of our tests were carried out with the bottle filled to $^2/_3$ of its total volume, justification for which will be presented shortly. In the interest of uniformity of results and ease of repetition, regular laboratory (A. H. Thomas Co., Catalog No. 6284-B) 4-ounce, tall-form screw cap jars were employed for our studies.

Volume of Emulsion The quantity of fluid in a shaker bottle influences greatly the effectiveness of a given amount of shaking. Observed stabilities of emulsions prepared under similar conditions but varying the volume of emulsion in the jar are shown in Figures 2 and 3. The amount of agitation or stability of the emulsion goes through a maximum when the volume of the emulsion in the jar is approximately $^2/_3$. This was chosen as the most favorable volume ratio for further work.

Vigor of Agitation The vigor of agitation is of utmost importance. Since vigor must remain constant throughout a series of tests, a mechanical shaker is necessary. Three factors that may be controlled in such a device are length of stroke, speed or cycles per minute, and time of shaking or number

Figure 6. Effect of time of shaking

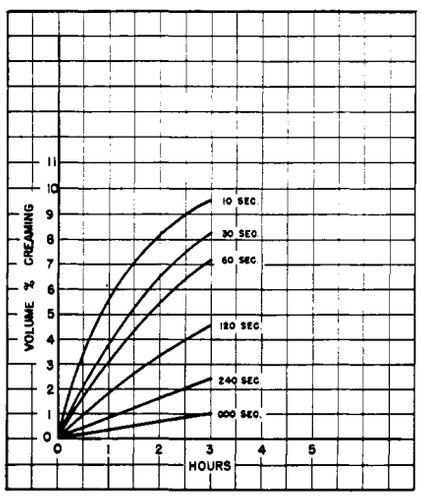


Figure 7. Effect of time of shaking after 1 hour

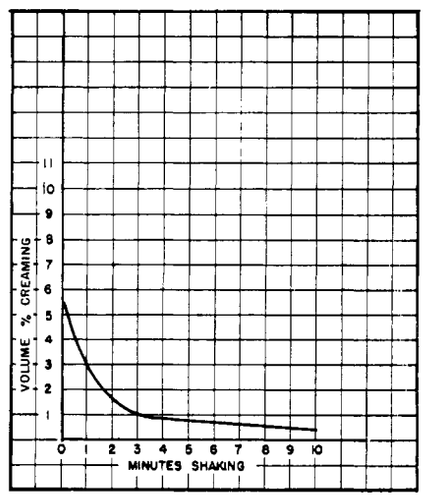
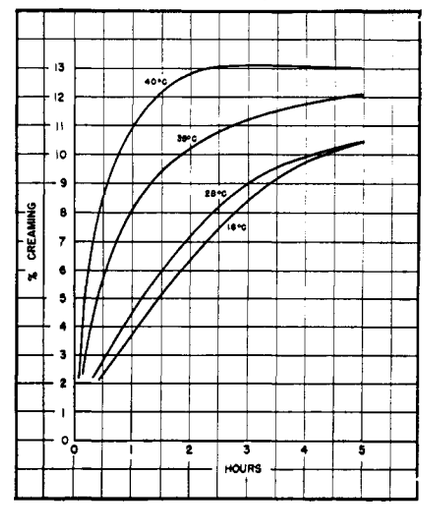


Figure 8. Effect of temperature of test



of strokes. A laboratory shaker was modified to permit a stroke length from $^1/_4$ inch to $1^1/_2$ inches and a speed of from 118 to 350 strokes per minute.

In Figure 4 the effect of the length of the stroke is depicted. The curves are similar until the stroke is reduced to less than $^3/_4$ inch. Since reproducibility of test data is desired, a stroke of 1 inch was chosen as optimum. With creaming rates as shown for $^1/_4$-inch strokes, laboratory manipulation is difficult unless each emulsion is prepared separately to avoid any time lag between end of preparation and first observations.

The number of strokes per minute followed a somewhat similar pattern (Figure 5), in that with 118 strokes per minute rapid breakdown of the emulsion occurred. Again, to avoid excessive agitation, a medium rate of 250 to 300 was chosen as optimum.

When using a mechanical shaker to agitate emulsions the vigor of agitation is defined by the length of the stroke and the strokes per minute. However, to regulate the total amount of agitation, the interval of shaking must be specified. Accordingly a series of experiments were made in which emulsions were shaken for 10-, 30-, 60-, 120-, 240-, and 600- second intervals using a 1-inch stroke at a rate of 292 r.p.m. The data are presented in Figures 6 and 7.

In Figure 7 the volume per cent creaming exhibited by each emulsion after standing 1 hour was plotted against the interval the emulsion was shaken during preparation. The amount of creaming decreased rapidly as the shaking period was increased from 10 seconds to 3 minutes. Further agitation provided only slight additional improvement in emulsion stability.

Since agricultural emulsions are normally prepared with a minimum of agitation, a shaking interval of 30 seconds was chosen to avoid subjecting the emulsion to excessive agitation.

Temperature One variable frequently overlooked in liquid-liquid emulsions is that of temperature. Observations were made on emulsions prepared and stored at various temperatures.

Fortunately, major changes are not noted until the temperatures are beyond normal room temperature fluctuations of 25 to 30° C. At higher temperatures (35 to 40° C.) definite differences are noted (Figure 8).

These, then, appear to be the major variables inherent to preparation technique when shaking is employed. When the procedures are followed as outlined, results have shown a high degree of reproducibility.

Observation of Emulsions

Emulsions are rated by many methods, including room temperature shelf storage, high and low temperature storage, freezing, centrifuging, dilution, microscopic examination (particle size growth), creaming and oil separation observation, viscosity changes on aging, behavior under circulating pumping (pressure), behavior in an electric current, behavior on evaporation of water, behavior with vibration (shipping), and others. All have their place in the industrial evaluation of emulsions; however, the selection of the method of observation employed should be made with due consideration.

Centrifuging is frequently used, and the writers believe that this is often done erroneously. Centrifuging accelerates the effect of gravity and thus some consider it as an accelerated aging test. However, on aging, the interfacial film between the phases of an emulsion may change and result in the destruction of the emulsion. A centrifuged emulsion exhibits only the separation that occurs with the interfacial film in the condition prevailing at the time of centrifuging, which is usually only a short time after preparation.

Dilution is a frequently used means of emulsion testing. Particularly if the dilution is accompanied by a decrease in viscosity, it is probable that the dilute emulsion will break down more rapidly than the concentrated emulsion.

Microscopic examination to determine the rate of growth of the size of the particles is usually too time-consuming for general use.

Creaming observation, particularly for fluid emulsions, is one of the methods most often used. An emulsion does not truly break until the particles coalesce and the two phases are present in an undispersed form. However, industry has expanded this definition to include emulsions that cream, even though coalescence does not occur. For this reason, the rate of creaming and of oil separation is of prime importance.

Viscosity changes on aging, behavior on recirculation in a pumping (pressure) system, behavior with vibration, and behavior on water evaporation are usually related to a particular end use and should be employed when indicated.

Creaming and Separation For the type of emulsifiable concentrate under study the rate of creaming and possible oil separation are the criteria. Considering this field alone, there is considerable variation in setting up tests and test equipment. Observations are all too frequently made in containers that are not ideal for this purpose, either because of heavy glass, graduations, lighting, improper shape of container, etc.

Observation Equipment. From an over-all consideration it was deemed advisable to view emulsions in an unetched vessel. The lines on a graduated cylinder can easily obscure a cream-over-cream separation. An ungraduated cylinder was thought to be the most suitable vessel, and to establish its size a series of tests were run.

Figure 9. Effect of depth of emulsion

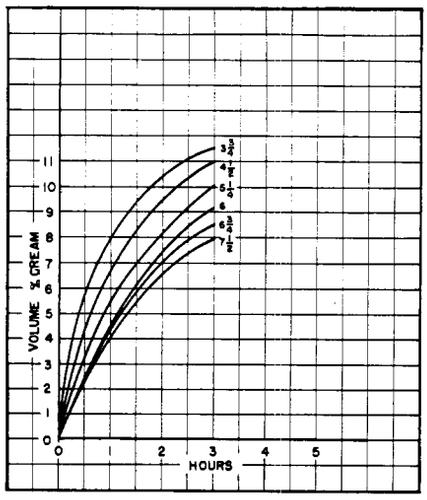


Figure 10. Volume percent cream after 1 hour at various emulsion depths

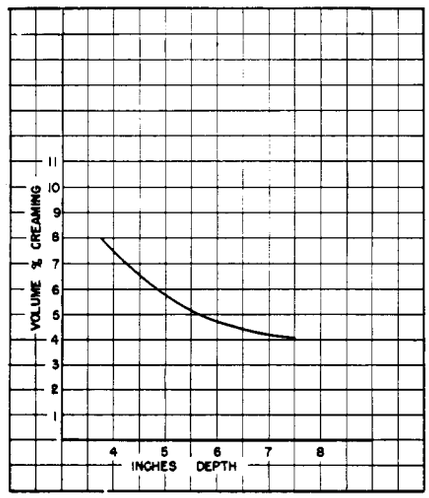
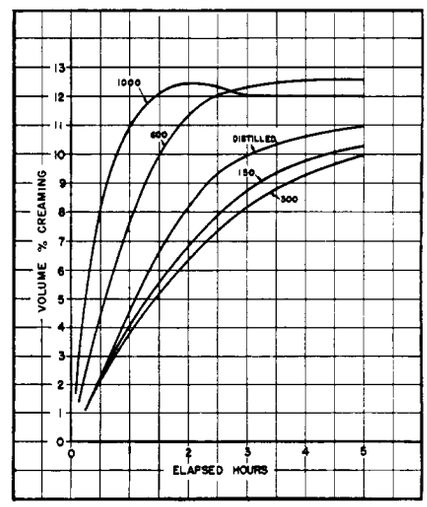


Figure 11. Effect of water hardness

Water hardness as p.p.m. of $CaCO_3$



Early in the study, it was realized that in many instances, a cream-over-cream separation had occurred that was difficult to detect. By viewing the emulsion with transmitted light, the cream line was easily defined. For this type observation, a diameter of 1 inch appeared to be maximum with about $^3/_4$ inch as optimum. Thin walls for the container reduced reflection along with a suitably designed light source and rack for the tubes. The optimum tube was found in present laboratory equipment as a 50-ml. Nessler tube (tall form), (*2, 5*).

Emulsion Depth. The depth of emulsion influences results as shown in Figures 9 and 10, illustrating the necessity of standardization and a minimum depth of 7 to 8 inches.

A method of evaluating emulsifiable concentrates has been devised using the above data and has been published (*1*). It has been in use in our laboratory for about 4 years with gratifying results.

Ingredients

The ingredients of the emulsion influence the results; one of the principal ingredients, the water, is sufficiently common to be disregarded often, and yet it is of paramount importance. Particularly is this true with emulsifiable concentrates that will be emulsified by the consumer, with water of unknown hardness (*3*). A brief study of the effect of a change in water hardness has been made, and the results are presented in Figure 11.

For a given formulation, the emulsification efficiency usually goes through a maximum at one hardness. It is possible to formulate concentrates that are most efficient at low hardness, or at medium, or high hardness. With a truly satisfactory product, efficiency is not markedly affected by varying considerably the hardness, but a measurable difference is obtained. This allows formulation for the hardness that is most likely to be encountered, and evaluation under these conditions.

For special conditions of high or low pH, or unusual salt concentrations, an evaluation test should be carefully scrutinized to make sure that the conditions imposed yield the proper degree of critical conditions.

Summary and Conclusions

Experimental results have been presented on the effect of a number of variables that influence the design of an emulsion test procedure.

The preparation of the test emulsion has been found to be critical, with the amount and vigor of agitation, test temperature, and mode of addition and ratio of ingredients influencing results. Regulation of agitation was accomplished by shaking 100 ml. of emulsion in 4-ounce, wide-mouth, screw cap jars with a commercial shaker operated at 292 r.p.m. with a 1-inch stroke for an interval of 30 seconds.

The observation of emulsions was studied with the finding that the depth of the emulsion and the shape and size of the container produced effects on the creaming rate. Standardization was obtained using a commercial emulsion viewer employing 50-ml. Nessler tubes filled to a depth of $7^3/_4$ inches, and equipped with a shaded light to permit examination of the test emulsion by uniform transmitted light.

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TRICHLOROACETIC ACID

Colorimetric Method for Quantitative Determination in Plant Tissue

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A method is described for the separation of trichloroacetic acid from plant tissue and the colorimetric estimation of the amount of the chemical recovered. The plant tissue is homogenized with 0.1 *N* acetic acid and the fat-soluble pigments (chlorophylls and carotenoids) and the insoluble cellular components are effectively separated from the chemical by filtration through asbestos. An aliquot of this filtrate is added to a tube containing pyridine and sodium hydroxide. The solution is heated to develop a red-violet color which is measured colorimetrically. The procedure has been used effectively to determine the quantity of chemical in the roots and above ground portions of several different plants.

ACCURATE DETERMINATION of an herbicidal chemical within plant tissue may often facilitate an understanding of the differential response to the chemical by plants of various species. Further, a quantitative estimate of the amount of herbicide accumulated in various plant parts may give information concerning the center of activity of that chemical and thus provide a clue to the mechanism by which it brings about its particular plant response. With the advent of each promising new herbicidal compound a diligent search is made for a suitable method of determination. Biological assays have been described for use with some herbicides but are often limited in scope because of variability