ABSTRACT OF THESIS

Madelen Worley Rey

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ABSTRACT OF THESIS

THE TOXICITY OF SOME CHLORALIDES OF α-HYDROXYCARBOXYLIC ACIDS TO THE COMMON HOUSE FLY

Submitted by

Madelen Worley Rey

In partial fulfillment of the requirements

for the Degree of Master of Science

Colorado

Agricultural and Mechanical College

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ABSTRACT

A great deal of interest in the insecticidal activity of synthetic organic compounds has been displayed in the past decade. Thousands of compounds have been evaluated as to their insecticidal value in laboratories all over the country in order to eliminate from further consideration ineffective chemicals and to obtain the necessary information to make more detailed studies of promising compounds.

In accordance with this widespread testing program, a project entitled "The Systematic Chemical and Biological and Applied Investigation of Compounds Related to DDT and Synthetic Plant Hormones" was initiated at the Colorado Agricultural and Mechanical College Experiment Station at Fort Collins.

In preliminary screening tests performed during the summer of 1946, workers on this project found that several chloralides of α-hydroxy carboxylic acids seemed to show promising insecticidal value. These tests were uncontrolled, however, and the experiment was never followed through in detail due to lack of time and personnel.

The principal objective of this study has been to make a detailed investigation of the activity of some of these chloralides as insecticides.

The problem

What toxic effects do malic, tartaric, lactic, salicylic, and citric acid chloralides have on the common house fly?

Problem analysis.--1. How effective are these chloralides used as contact poisons on the house fly?

2. How effective are these chloralides used as fumigants on the house fly?

3. What are the plant growth-regulating effects of these compounds at various concentrations?

Delimitation.--This study has been limited to the chloralides of malic, tartaric, lactic, salicylic, and citric acids.

Definition of terms. A chloralide may be defined as the main product formed when chloral or chloral hydrate reacts with an acid, usually an acid containing a hydroxyl group attached to a carbon adjacent to a carboxyl group.

Materials and methods

The chloralides of malic, tartaric, lactic, salicylic and citric acids used in this study were prepared by condensing the acids with chloral in the presence of sulfuric acid. Combustion analyses and molecular weight determinations were made in order to establish their identity.

When investigating the activity of the compounds under study as contact poisons, the Kearns wind tunnel spray apparatus was used. The compounds were dissolved in a 1:2 acetone and Deobase (deodorized kerosene) medium and applied at concentrations of .04 M., .20 M., and .40 M. Each series of treatments contained the five chloralides all at the same concentration, a DDT solution, a Chlordane solution standardized to give a 50% kill with the Kearns apparatus in 24 hours, and a blank of acetone and Deobase. Four replications of 50 flies each were used and mortality readings taken 24 hours after spraying.

In the study of the action of the chloralides as fumigants, it was necessary to heat the compounds in order to vaporize them. One gram of the chemical was dissolved in 10 grams of corn oil, heated to between 85° and 100° C. and the vapors passed over the test insects. The apparatus used consisted of a side-arm flask containing the fumigant attached to the insect chamber which was a glass tube, 1 1/4 inches in diameter and 10 inches long. Also attached to the side-arm flask was a large jar calibrated in one-half liters for use in controlling the flow of the fumigant and air through the apparatus. This was accomplished by regulating the rate of the flow of water into the jar, thus forcing the mixture of air and fumigant through the

insect chamber at the same rate. By preliminary experimentation, the rate of air flow was controlled so that approximately one-tenth of a gram of chloralide passed over the insects in eight minutes. A carbon disulfide standard and a blank of corn oil were also included in this test. Mortality readings were taken after 24 hours.

Three tests were used in the study of plant growth-regulating effects of the chloralides. These were the single droplet water test, Went's pea test and an aqueous spray test.

A concentration of .001906 M. was used in the single droplet water test. Emulsions were made by dispersing the compounds in Carbowax 1500 in the ratio of 1 to 10 by weight. One drop (.05 ml.) of this test substance was then placed on the mid-rib of one of the primary leaves of bean seedlings (Pencil pod variety). Ten plants were treated with each compound. On the 15th day after treatment, the fresh weight of that portion of each plant above the second node was obtained. The sodium salt of 2,4-dichlorophenoxyacetic acid was used as a standard and untreated controls as the zero effect.

Solutions for use in Went's pea test were made on a molar basis such that the strongest of the 10 concentrations used corresponded to .001906 M. and the weakest to .000003 M. The chemicals were dissolved in Carbowax then made up to volume with water. Dilutions

were made in such a way that each concentration was half the strength of the preceding one. Ten pea stems were treated with each concentration of each compound. The sodium salt of 2,4-dichlorophenoxyacetic acid was used as a standard in this test.

Tomato plants (Marbon variety) eight inches tall were used in the aqueous spray test. Chemicals were applied at concentrations of .08 M., .06 M., .04 M., .02 M., and .01 M. Solutions were made by dissolving the compound in tri-ethanol amine in the ratio of 1 to 10 by weight and making up to volume with water. DDT was used as a comparison standard in this test. Burning of the leaves and any other visible effects were observed during a period of two weeks after spraying.

Results and discussion

None of the chloralides showed any significant effect as contact poisons on house flies when tested with the Kearns apparatus.

Lactic acid chloralide gave nearly a 100 percent average kill in the fumigation experiments as did the carbon disulfide standard. The chloralides of malic and citric acids also showed significant effects. Mortality rates of 12% to 24% were obtained with these compounds.

The chloralides of salicylic and malic acids produced significant growth-regulating action in both

the single droplet water test and in Went's pea test at the three highest concentrations used. Significant action was also exhibited by lactic acid chloralide in the water droplet test and by tartaric acid chloralide in the pea test.

As aqueous sprays, all of the chloralides produced a slight burning of the lower leaves at the three highest concentrations used. It was also observed that plants treated with these concentrations seemed to show an increase in height growth after the two week observation period when compared to the untreated control plants.

As has already been mentioned, various chloralides were shown in preliminary tests to have a toxic effect on house flies as kerosene sprays. However, no toxic effect was obtained when these compounds were applied in an acetone and Deobase (deodorized kerosene) medium with the Kearns apparatus. The question arises whether some constituent of the kerosene may have been responsible for the toxic effects produced, or whether kerosene may have affected the chloralides in some way causing them to be more toxic.

Suggestions for further study

1. Other chloralides could be investigated as to biological activity using a variety of test insects.

2. Effects of different solvents on the toxicity of various chloralides might be investigated.

3. Since lactic acid chloralide was shown to give a mortality rate of nearly 100 percent when used as a fumigant, further studies to determine the minimum lethal dosages under varied experimental conditions are suggested.

4. Significant growth-regulating action shown when the chloralides were used in Went's pea test and the single droplet water test suggests that other concentration ranges might be investigated for growth-regulating effects.

5. The clue that chloralides may cause an increase in height of tomato plants which was obtained incidently in this study should be further investigated.

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WE HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER OUR SUPERVISION BY MADELEN WORLEY REY

ENTITLED THE TOXICITY OF SOME CHLORALIDES OF - HYDROXYCARBOXYLIC ACIDS TO THE COMMON HOUSE FLY

BE ACCEPTED AS FULFILLING THIS PART OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE.

CREDITS 10

Committee on Graduate Work

W. E. Ray — Geo. M. List — X. Patton — Jas L. Faulks

Major Professor — Minor Professor

W. E. Ray — L. M. Dunell

Head of Department — Dean of Division

Committee on Final Examination

Examination Satisfactory

L. M. Dunell — W. E. Ray

X. Patton — Geo. M. List

A. E. Carlson

David H. Morgan

Dean of the Graduate School

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Chapter I

INTRODUCTION

A great deal of interest in the insecticidal activity of synthetic organic compounds has been displayed in the past decade. Thousands of compounds have been evaluated as to their insecticidal activity in laboratories all over the country in order to eliminate from further consideration ineffective chemicals and to obtain the necessary information to make more detailed studies of promising compounds.

In order to make a thorough study of some specific compound shown to possess insecticidal possibilities, several procedures must be followed. Inasmuch as a positive test concerning the activity of a certain compound as a contact or stomach poison does not preclude the possibility of fumigant action, it becomes necessary to investigate each possibility separately. Further, if evidence of contact or stomach action is shown, tests on the tolerance of plant foliage must follow before any decision can be made as to the real insecticidal value of any new compound to be used on plant insects.

To date, in spite of the great numbers of compounds tested and the precision used in these tests,

fewer than 10 materials have actually reached economic importance, DDT 1/, Chlordane 2/, Toxaphene 3/, and benzene hexachloride being among the best known. Since even these show certain limitations in practical use, the search continues for new compounds in an endeavor to find specific treatments for specific purposes.

In accordance with this widespread testing program, a project entitled "The Systematic Chemical and Biological and Applied Investigation of Compounds Related to DDT and Synthetic Plant Hormones" was initiated at the Colorado Agricultural and Mechanical College Experiment Station at Fort Collins.

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The principal objective of this study has been to make a detailed investigation of the activity of some of these chloralides as insecticides.

1/ 1-trichloro-2, 2-bis (p-chlorophenyl) ethane

2/ 1, 2, 4, 5, 6, 7, 8, 7a-octachloro-4, 7-methano-3a, 4-7-7a-tetrahydroindane

3/ Chlorinated camphene

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Delimitation.--This study has been limited to the chloralides of malic, tartaric, lactic, salicylic, and citric acids.

Definition of terms.--By definition, a chloralide is the product formed by the condensation of choral or choral hydrate with an acid, usually an acid having a hydroxyl group attached to the carbon adjacent to a carboxyl group.

Chapter II

REVIEW OF LITERATURE

Comparatively little work of any nature has been done concerning the group of compounds with which this experiment is concerned, the chloralides. However, literature dealing with the testing of compounds for insecticidal and plant growth regulating activity is indeed voluminous. Therefore, an attempt has been made to limit the review presented below to the work in these fields relating directly to this experiment.

The report of the literature pertinent to this study has been organized under the following headings:

I. The chloralides.

II. Methods of testing insecticides for contact and fumigant action.

III. Methods of determining growth-regulating properties of compounds upon plants.

I. The chloralides

In 1847, through the action of sulfuric acid upon chloral Stadeler, (29) obtained a "white crystalline body" which he represented by the formula $C_5H_2Cl_6O_3$ and gave the name of chloralide. This is the first record of such a compound being synthesized.

A few scattered studies during the next 90 years show investigators intermittently studying chloralides of various compounds.

Kekule (9), in 1859, working with the first chloralide, believed Stadeler's formula for the compound to be correct and attempted to explain the way in which the compound was formed.

Some 15 years later, in 1873, Grabowsky (6) discovered a white crystalline intermediate product of the reaction of H_2SO_4 upon chloral which had been overlooked up until this time. This product he called a "combination of chloral with H_2SO_4" and gave the formula $C_8H_6Cl_{12}O_{11}S_2$. Grabowsky (4,5) dealt with this phase of the formation of chloralides twice more in the following two years giving analytical data concerning the intermediate product. In these papers he also specified experimental conditions giving the highest yield of the chloralide.

Wallach (33), in 1878, established the constitution of Stadeler's chloralide and synthesized chloralides of several other acids including lactic acid, malic acid, salicylic acid. He introduced the method of heating the reactants in sealed tubes.

Otto (15), 1888, maintained temperature to be the main factor in obtaining high yields of Stadeler's chloralide.

Trichlorolactic acid ethyl ester, citric acid

methyl ester, and tartaric acid were shown by Edeleanu and Zaharia (3), in 1895, to form chloralides in conjunction with chloral. However, when the methyl ester of tartaric acid was combined with choral only an oily product could be obtained.

Chloralides of acetone oxalic acid, acetophenone oxalic acid, and other compounds were prepared and analyzed by Schiff (23) in ~~1989~~ 1898.

Patterson and MacMillan (17), while investigating the rotation of ethyl tartrate, choral, and water in 1912, discovered that a chloralide was formed when ethyl tartrate was added to choral and also that the chloralide exhibited isomerism.

Meldrum and Bhatt (10) pointed out, in 1934, that since the choralides are saturated ring compounds and admit to cis-trans isomerism, any melting point discrepancies encountered could be due to the presence of isomers. Chloralides of a variety of acids were prepared by these workers. They discovered that carrying the reactions out at room temperature usually gave better yields than when Wallach's sealed tube method was used.

The chloralide of benzilic acid was obtained for the first time by Shah and Alimchandani (25) in 1934. It was found necessary to use Wallach's sealed tube method in preparing chloralides of aromatic acids. Benzilic acid, tartaric acid, and citric acid chloralides

and several others were prepared and reduced with zinc dust and glacial acetic acid.

In a study performed by Shah (23), in 1939, butyl chloral hydrate was condensed with several ∝-hydroxy carboxylic acids. Yields of the butylchloralides obtained were uniformly inferior compared to the yields of the corresponding simple chloralides.

No mention of the biological activity of the chloralides could be located in the literature.

II. Methods of testing insecticides for contact and fumigant action

Contact action.--Before 1924, a simple spraying test was the most common procedure used for testing chemicals for contact insecticidal action. Insects on potted plants were sprayed directly with an atomizer or spray nozzle. Although such a method is still used for purely routine testing, most investigators prefer a more precise method.

Undoubtedly the most widely used apparatus for the application of contact insecticides is the testing chamber first described by Peet and Grady (19) in 1928. The use of this apparatus was accepted in 1932 by the National Insecticide and Disinfectant Manufacturers' Association as the official method for testing household sprays.

The principal of the Peet-Grady method consists

of atomizing the insecticide into a large chamber containing insects that may move unrestricted. These workers attempted to standardize the time, temperature, humidity, concentration of insecticide, pressure of spray, angle of spray, kind of insect used and the condition of insects in connection with the insecticidal testing.

However, through the years, the method was criticized especially because of carry-over toxicity and the wide variation of the quantity of spray received by each individual insect. Consequently, a great many methods have been proposed in an effort to overcome these objectionable factors. The most widely used of these methods were described by O'Kane et al (14) in 1930, Nelson (12) in 1934, Simanton (26) in 1937, Campbell (1) in 1938, Simanton and Miller (28) in 1938, Campbell and Sullivan (2) in 1938, Murray (11) in 1940, Hoskins as described by Richardson (21) in 1940, and Kearns (8) in 194-.

From these methods, the one described by Kearns was chosen for use in this experiment. This worker attempted to minimize any means other than direct contact in which an insecticide may produce an effect, that is, residual effect or fumigant action. This spray apparatus operates on the principal of a regulated flow of air through a specially designed tube and testing area. This apparatus is shown in Figures 1 and 2.

Fumigant action.--Shepard, et al (26), in 1937,

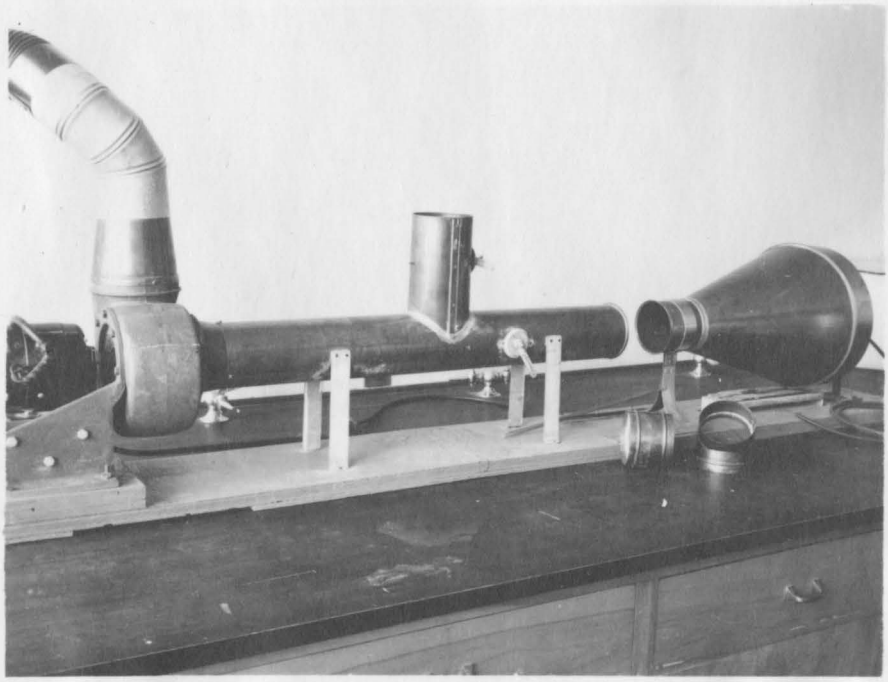

Figure 1.--The Kearns Apparatus

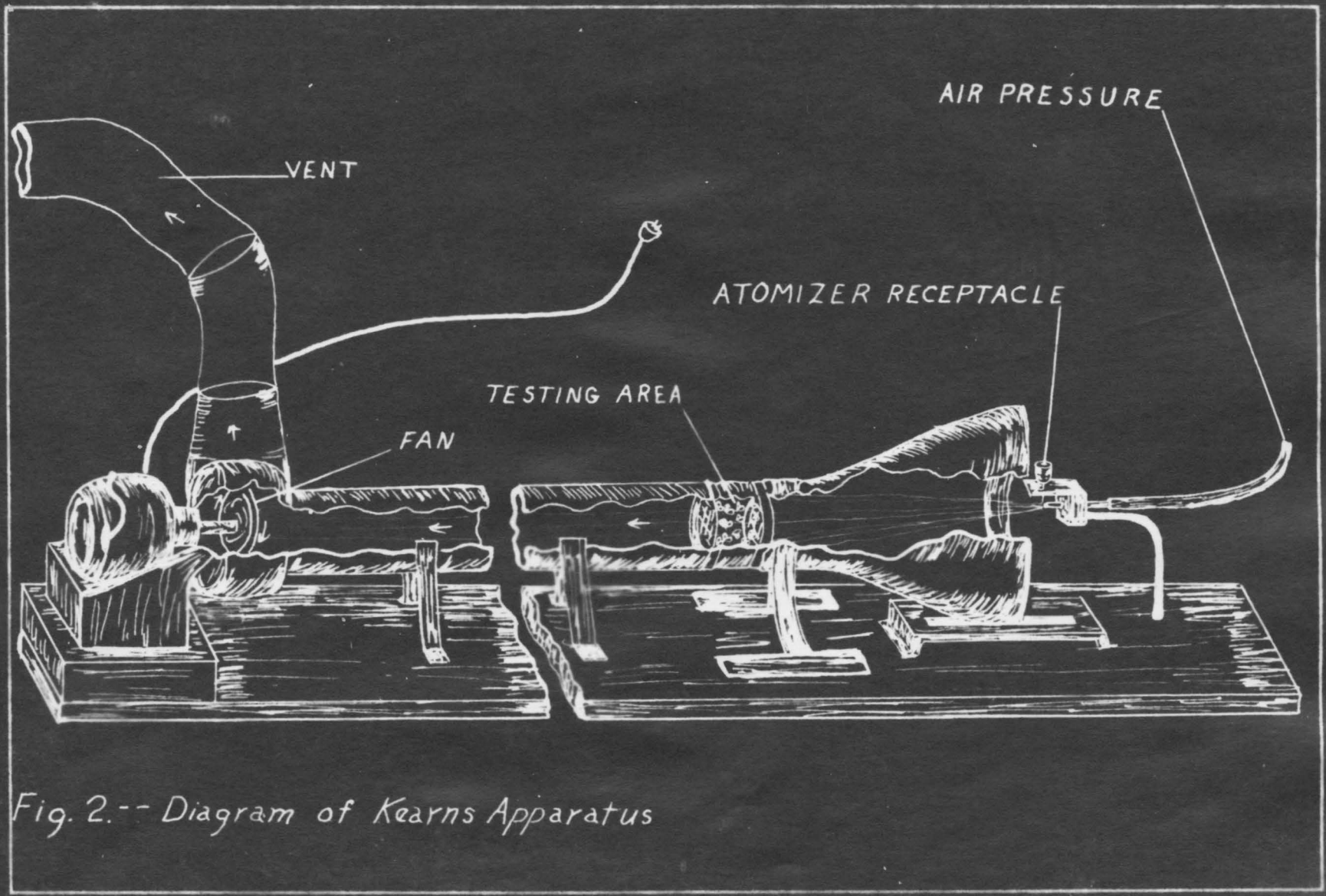

Fig. 2.-- Diagram of Kearns Apparatus

Richardson and Busbey (22) in 1937, Grayson and Swank (7) in 1941, Page and Lubatti (16) in 1940, and many others have described methods for testing fumigants. Although several of these methods have been shown to give very satisfactory results, they are applicable only to highly volatile fumigants. Since the compounds used in the present experiment are not highly volatile, it was found necessary to devise a method whereby the materials used could be volatilized by applying heat. This method is described later.

III. Methods of determining growth-regulating properties of compounds upon plants

There are many methods used today as physiological tests of growth-regulating effects of chemicals upon plants. A few of the principal methods will be discussed briefly.

As a means of determining the plant growth-regulating effect of chemicals at low concentrations, Went and Thimann (34), in 1937, developed a test using the inward curvature of etiolated split pea-stems. In this test the degree of inward curvature of the pea-stems corresponded to the growth-regulating activity of the chemical being treated.

During the last war, extensive studies of plant growth-regulating substances were performed by the Chemical Warfare Service. Most of these studies were performed

in 1944 and 1945, but due to wartime security policies were not published until 1946 or later.

Thompson et al (32), in one of these studies, developed a test using the measurement of the inhibition of root elongation of germinating corn seed by 2,4-dichlorophenoxyacetic acid. A concentration of 10 p.p.m. was used. Swanson (30) found this test to be an accurate bio-assay method for 2,4-dichlorophenoxyacetic acid at conentrations of .01 to 4.00 p.p.m. but not above.

Thompson (32) also used a single-droplet test wherein a drop of either water or oil containing a known weight of test substance was placed on the mid-rib of the primary leaf of a red kidney bean. Measurement of the inhibition of tissue production above the primary leaves was used as the basis of comparisons. Plants treated with 2,4-dichlorophenoxyacetic acid were used as standards.

Swanson (31) used an oil spray test. The material being tested was suspended or dissolved in oil and applied with an atomizer at a regulated rate and pressure. The fresh weight of all growth above the second trifoliate leaves of soy bean plants was determined 21 days after treatment and was used as a basis of comparison.

Chapter III

MATERIALS AND METHODS

Chemicals used

The chloralides used in the present study were prepared in the chemistry laboratory of the Colorado Experiment Station at Fort Collins, Colorado. The same general method was used in preparing all of the chloralides. The acids were condensed with choral hydrate in the presence of sulfuric acid at a temperature of 40-50° C. 1/ Analytical data concerning these chloralides are shown in Appendix A, Table A.

The DDT used for comparative purposes in the following tests was prepared in the Colorado Experiment Station laboratory and purified by recrystallization from an alcohol and ether mixture. No attempt was made to isolate any specific isomer. The mono-hydrated form of the sodium salt of 2,4-dichlorophenoxyacetic acid was obtained from the J. T. Baker Company of Phillipsburg, New Jersey.

When making tests with the Kearns spray

1/ Details concerning the preparation of each of the chloralides may be found in Appendix B.

apparatus, a solution of Chlordane 2/ was obtained from the Julius Hyman Company of Denver, Colorado and used as a standard. The Deobase (deodorized kerosene) used as a carrier for the other chemicals in this test was obtained from the Mine and Smelter Supply Company of Denver, Colorado.

The corn oil used as a carrier when testing the chemicals for fumigant action was the commercial Mazola oil (Corn Products Company, Argo, Illinois).

C.P. chemicals were used in all other instances.

Common house flies, Musca domestica L. were used in all insecticidal tests. The insects were reared by a method similar to that described by Richardson (20).3/ Since uniformity of vigor and development was considered an important factor in this experiment, five to seven day old flies were used in all cases. Therefore, it was arranged to have insects emerging at regular intervals of two or three days.

Testing with the Kearns spray apparatus

The simplified Kearns wind tunnel spraying

2/ Chlordane solution used contained 125 mg. per liter and was standardized for .50 ml. to give 50% kill with the Kearns apparatus.

3/ The composition of the rearing medium used in this study is given in Appendix C.

apparatus was used for all tests investigating the effect of the compounds as contact poisons.

Each series of tests with this apparatus contained the five chloralides, malic acid chloralide (MAC), tartaric acid chloralide (TAC), lactic acid chloralide (LAC), citric acid chloralide (CAC) and salicylic acid chloralide (SAC) all at the same specified concentration. 4/ A .04 M. solution of DDT, the Chlordane standard solution and controls of a 1:2 mixture of acetone and Deobase were also included in each series.

Three concentrations for the chloralides were used, .04 M., .20 M., and .40 M. Solutions were made by directly weighing the compound, dissolving in acetone and making up to volume with Deobase. Acetone and Deobase were used in a ratio of 1:2. Strengths above .40 M. were avoided because considerably more acetone would have been required to obtain complete solution of the chloralides. Because of the toxicity of acetone to flies, this increase was considered undesirable.

The test chemicals were introduced into the receptable of the atomizer while the machine was in operation. A one ml. pipette graduated to tenths was used for measuring purposes. Treatments using amounts of both .50 ml. and 1.00 ml. were performed with each

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4/ From here on the chloralides often will be referred to using capitalized abreviations.

concentration of each of the chloralides.

To facilitate counting, the flies were anaesthetized with carbon dioxide. Fifty were then placed into each of the testing cages and allowed to recover from the anaesthetic before testing. After spraying, the flies were again anaesthetized for a brief moment and placed in recovery cages along with a source of food. One quart size cylindrical ice-cream cartons with the ends replaced by screen wire disks were used for this purpose. Disposable inner linings of heavy paper were used so that these cages could be used time after time without danger of contamination from previous treatments.

Percentage mortality readings were taken at 12 and 24 hour periods.

After each series of tests, the testing cages and the screen wire disks from the ends of the recovery cages were thoroughly cleaned with chemicals.

Testing for fumigant action

As a preliminary test for fumigant action, one gram of the chloralides was placed between two sheets of filter paper and pressed into the top of a large petrie dish. Thirty flies were put into the bottom half of the dish and the top of the dish replaced. Observations were made after 24 and 48 hour periods.

After obtaining almost no kill with the chloralides in the preliminary test, a method whereby the

chloralides were volatilized by applying heat was adopted.

The apparatus used consisted of a 125 ml. side arm flask containing the fumigant connected to the insect chamber. This chamber was a heavy glass tube 1 1/4 inches in diameter and 10 inches long. Also connected to the side arm flask was a large jar, calibrated in one-half liters, used to control the flow of vapor and air through the apparatus as shown in Figure 3. This was accomplished by regulating the rate of flow of water into the jar, thus forcing the mixture of air and vapor through the insect chamber at the same rate. A rate of one-half liter per minute was used in this test.

In preparing the test substances, one gram of the chemical was weighed into the side arm flask and dissolved in 10 grams of corn oil. The total weight of the flask and test substance was recorded.

Before beginning the test, the flies were anaesthetized with carbon dioxide and 30 counted out into each of the insect chambers. The flask containing the fumigant was then placed in a paraffin oil bath, connected to the insect chamber and heated to the temperature required for vaporization of the chloralide. A range of 85° to 100° C. was used. At this point, the water jar was connected to the flask and the flow of air started through the apparatus.

After eight minutes, the insect chamber was

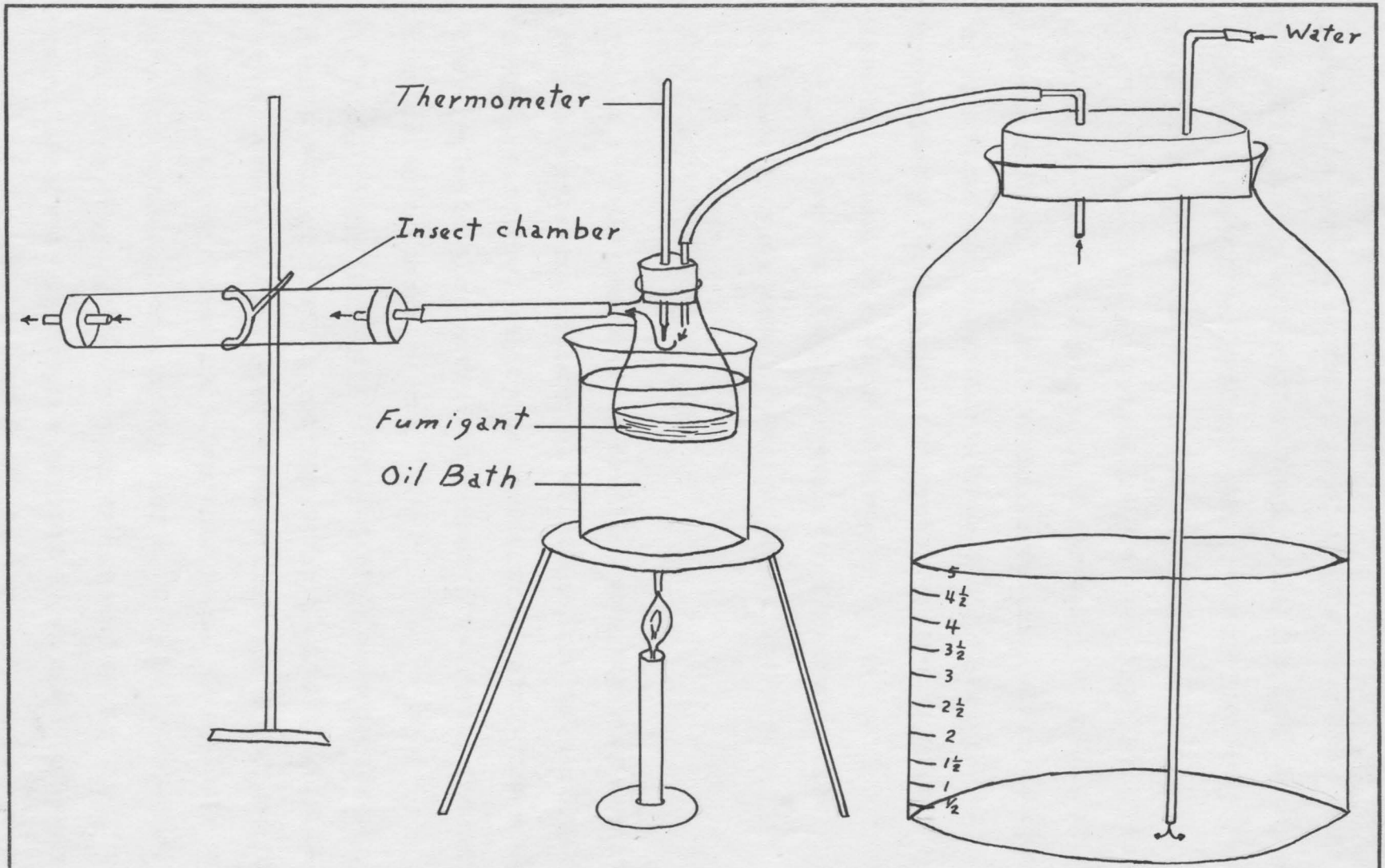


Figure 3.--Diagram of fumigation apparatus

removed, a source of food placed inside, and the ends plugged loosely with cellucotton. The tube was then set aside and mortality readings taken after 24 hours. Four replications were made with each chemical.

The loss of weight due to vaporization of the chloralides was determined by difference of weight of the side arm flask. By preliminary experimentation, the rate of air flow and temperatures were controlled so that approximately one-tenth of a gram of fumigant passed over the insects in eight minutes.

Carbon disulfide was used for comparative purposes in this test as was a blank of corn oil.

Tests for growth-regulating action

Three tests were used in investigating the growth-regulating properties of the chloralides of malic, lactic, tartaric, salicylic, and citric acid. These were the single droplet water test, Went's pea test, and an aqueous spray test.

Experiment one.--Single droplet water test. Bean seedlings (Pencil pod wax variety) five inches in height that had developed primary leaves one and one-half inches in width were used for this test. Ten plants were treated with .05 ml. portions of a .001906 M. aqueous emulsion. This concentration was chosen because of its relationship to the sodium salt of 2,4-dichlorophenoxy-

acetic acid in p.p.m., i.e., 600 p.p.m. Emulsions were made by dispersing the compounds in hot Carbowax 1500 in the ratio of one to 10 by weight, adding a drop of wetting agent (Triton 100x) and making up to volume with distilled water. A single droplet (.05 ml.) of test emulsion was applied one centimeter from the base of the upper surface of one of the primary leaves along the mid-rib. Following treatment, the plants were grown under normal greenhouse conditions of moisture, light and temperature. On the 15th day after treatment, the fresh weight of that portion of each plant above the second node was obtained. The sodium salt of 2,4-D was used as a standard and untreated controls as the zero effect.

Experiment two.--Went's pea test. This test, using the inward curvature of split stem-tips of etiolated peas was conducted as described by Went (34) except that in this study the stems were placed in the test emulsions immediately after cutting instead of first soaking them in water. For each chemical tested, 10 concentrations were used and 10 pea stems treated with each concentration.

Solutions were made on a molar basis such that the strongest concentration corresponded to .001906 M. (No. 1) and the weakest to .000003 M. (No. 10). The highest concentration was obtained by weighing the compound directly, dispersing in Carbowax 1500 and making up to volume with distilled water. Concentrations, thereafter,

were made by dilution in such a manner that each dilution was one-half the strength of the preceding one. The sodium salt of 2,4-D was used as a standard and water control as the zero effect.

Experiment three.--Aqueous spray test. In this test the tomato plants (Marbon variety) used were eight inches tall at the time of treatment. Chemicals were applied in the form of an aqueous spray with an atomizer at the rate of 7 ml. per square foot. Molar concentrations of .08, .06, .04, .02, and .01 were used. This range of concentration was selected to include the .04 M. concentration used in the insecticidal testing. Visible damage done to the plants, burning of leaves, etc., was recorded at two day intervals for two weeks after spraying.

The .08 M. emulsions were made by directly weighing the compounds and dissolving them in tri-ethanol amine in the ratio of 1 to 10 by weight, adding a drop of wetting agent (Triton 100x) and making up to volume with distilled water. Tri-ethanol amine was used in this test since it was found to be a more effective emulsifier than the Carbowax 1500 used in the two preceding tests. The remaining emulsions were obtained by dilution.

DDT was used as the standard and untreated controls as the zero effect. Blanks containing tri-ethanol amine and the wetting agent were also included in

this experiment.

Chapter IV

ANALYSIS OF DATA

This study is concerned with the toxic effects of malic acid, lactic acid, tartaric acid, salicylic acid, and citric acid chloralides on the common house fly, Musca domestica L. In undertaking such a study it seemed necessary to investigate the effect of the compounds as both contact poisons and as fumigants and also to investigate their effect as plant growth-regulators.

Results obtained in studying these three phases of the major problem are presented below.

Contact poison action as determined by the Kearns spray apparatus

Results using the chloralides of malic, lactic, tartaric, salicylic, and citric acids as contact poisons as determined by using the Kearns apparatus were consistently negative.

A variance analysis made of the five chloralides, shown in Table 1, indicated that no significant difference in mortality existed either between treatments or between concentrations within treatments, i.e., there was no significant difference in the mortality rate irregardless of which chloralide was under consideration or of which

concentration was used. It would therefore be reasonable to expect no significant toxicity to house flies using the chloralides of malic, lactic, tartaric, salicylic, and citric acids as contact poisons in acetone-Deobase solution at concentrations of .04 M., .2 M., or .4 M. if such a test as has been described in the study were to be repeated.

Table 1.--COMPLEX ANALYSIS OF VARIANCE OF MORTALITY RATES OF HOUSE FLIES TREATED WITH TAC, MAC, SAC, CAC, AND LAC USING THE KEARNS APPARATUS

Variability due to	D/F	Sum of Squares	Mean Squares	F Value	Required F .05	Required F .01
Between treatments	4	17.33334	.16667	0.550	2.54	3.68
Within treatments	55	0.66667	.30303			
Totals	59	16.66667				
Between concentrations within treatments	10	3.16667	.31667	1.055	2.055	2.74
Within concentrations within treatments	45	13.50000	.30000			

The .04 M. solution of DDT used for comparative purposes and the Chlordane standard solution used in each series of treatments repeatedly gave a 100 percent and very near to a 50 percent mortality rate respectively in 24 hours while the blank of acetone and Deobase proved to be non-toxic in all replications.

Testing for fumigant action

A variance analysis made of this portion of the study indicated that the vapors produced when all of the chloralides under study are heated, with the exception of tartaric and salicylic acid chloralides, are toxic in varying degrees to house flies under controlled conditions.

As shown in Table 2, lactic acid chloralide appeared to be highly superior to the other chloralides. It gave nearly a 100 percent average kill in 24 hours as did the carbon disulfide comparative standard. The chloralides of malic acid and citric acid also had significant toxic effects showing percentage mortality rates of 12 and 24 percent after the 24 hour observation period.

Table 2.--MORTALITY RESULTS OF FUMIGATION TESTS PERFORMED ON HOUSE FLIES USING FIVE CHLORALIDES, CARBON DISULFIDE, AND CORN OIL

Compound	MAC	LAC	TAC	SAC	CAC	Carbon Di-sulfide	Oil
Average number dead in 24 hours[1]	3.75	29.25	1.75	3.25	7.25	30.00	1.00
Average percent kill in 24 hours	12.50	97.50	5.83	10.83	24.17	100.00	3.33
Significance	*	**	xx	xx	**	**	xx

[1] Four replications of 30 flies each were used to obtain the average mortality rates.

* Significant at .05 level. Minimum difference between means required = 2.52.

**Significant at .01 level. Minimum difference between means required = 3.45.

Testing for growth-regulating action

Experiment one.--Single droplet water test. Mean differences between the green weights of treated bean plants and untreated plants showed the chloralides of malic, lactic, and salicylic acids to significantly retard tissue production at a concentration of .001906 M. Fischer's "t" test of difference of means was used to evaluate these differences.

Table 3 shows malic acid chloralide to have the

most pronounced growth regulating effect of the five chloralides tested. However, the "t" values show the effect of MAC to be much less significant than the effect of the sodium salt of 2,4-D used as a standard. The values for LAC and SAC follow the value for MAC in significance, while the chloralides of citric acid and tartaric acid show no significant effect on tissue production in this test.

Table 3.--GROWTH REGULATION EFFECTS OF FIVE CHLORALIDES, DDT AND 2,4-D ON THE GROWTH OF BEAN SEEDLINGS AS MEASURED BY THE SINGLE DROPLET WATER TEST USING .001906 MOLAR EMULSIONS

Treatments	Mean green weight of 10 bean plants above second node	Standard deviation	"t" value
	grams		
TAC	1.56	.406	1.02
MAC	1.47	.346	*2.84
SAC	1.39	.515	*2.18
CAC	1.62	.314	0.58
LAC	1.37	.533	*2.50
DDT	1.54	.493	1.77
2,4-D	1.22	.381	**4.45
Control	1.84	.180	xx

* Indicates values significant at the .05 level.

**Indicates values significant at the .01 level.

Experiment two.--Went's pea test. In order to eliminate error due to treatments and within treatments,

variance analyses were made for each compound with the water control. The criteria of significance of differences between means of each pair were graphed and interpreted as described by Payne and Fults (18). This is shown in Figures 4 and 5.

The results indicate that three of the chloralides show a slight plant growth-regulating action at the higher concentrations used. Specifically, as shown in Table 4, MAC showed significant action at the four highest concentrations and TAC and SAC showed significant action at the three highest concentrations. CAC and LAC showed no significant action at any of the concentrations used.

Table 4.--PEA TEST REACTIONS OF FIVE CHLORALIDES, DDT AND 2,4-D

Chemical	Concentrations									
	1	2	3	4	5	6	7	8	9	10
TAC	S*	S*	S**	NS	NS	NS	NS	NS	NS	NS
MAC	S**	S**	S**	S**	NS	NS	NS	NS	NS	NS
SAC	S**	S**	S*	NS	NS	NS	NS	NS	NS	NS
CAC	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
LAC	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
DDT	S**	S**	S**	S**	S**	S**	S	S	NS	NS
2,4-D	S**	S**	S**	S**	S**	S**	S**	S**	S**	S**

S* Growth reaction significant at .05 level.

S**Growth reaction significant at .01 level.

NS No significant reaction.

Emulsions were made on a molar basis such that the strongest (No. 1) corresponded to .001906 M. and the weakest (No. 10) corresponded to .000003 M. Weaker concentrations were made by dilution in such a manner that each dilution was one-half the strength of the preceding one.

Experiment three.--Aqueous spray test. All five of the choralides and DDT produced a slight burning at the highest concentrations used (.04 M., .06 M., and .08 M.) especially of the lower leaves. This effect was not evident, however, until about four days after treatment.

Although no measurements were made, the tomato plants treated with each of the chloralides at concentrations .04 M., .06 M., and .08 M. seemed to show a considerable increase in height growth in comparison to the simultaneous untreated controls.

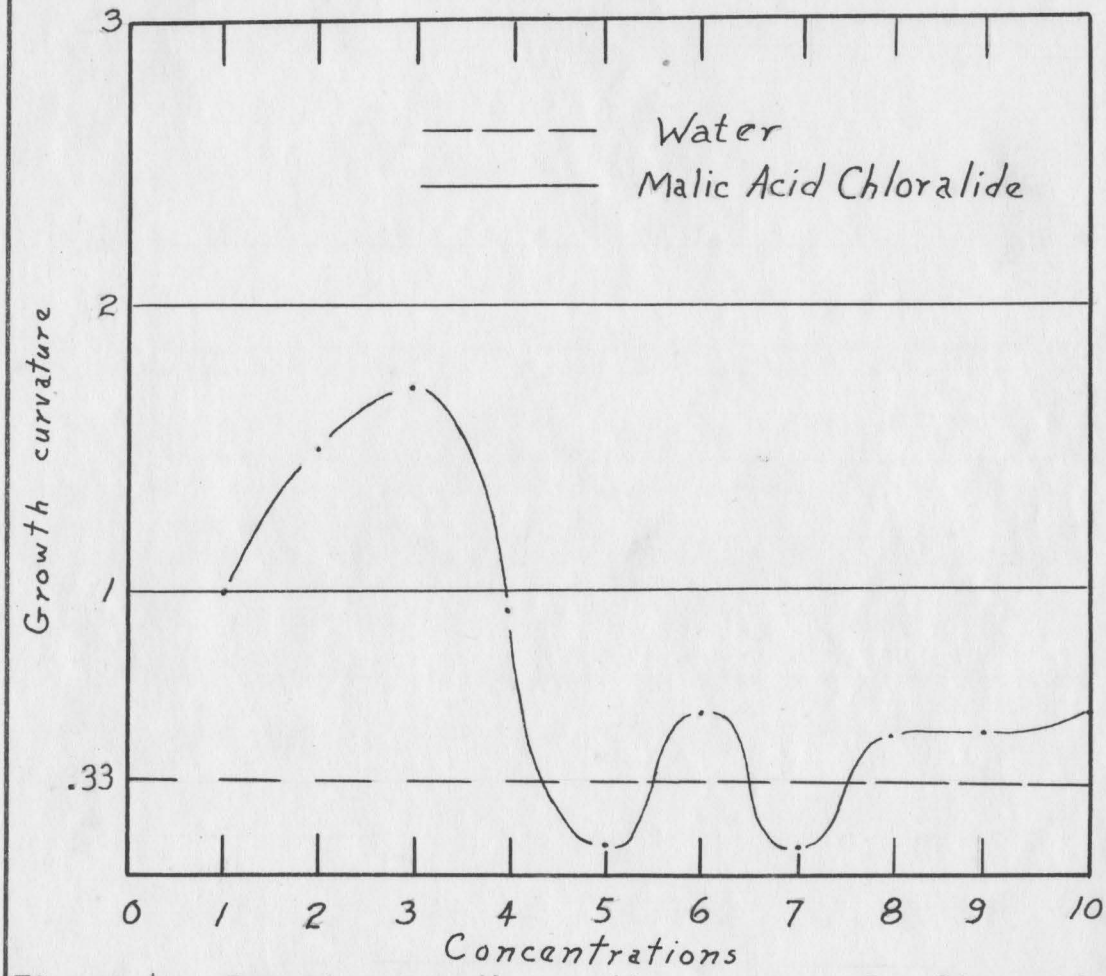


Figure 4.--Pea stem growth reaction curves of malic acid chloralide and water

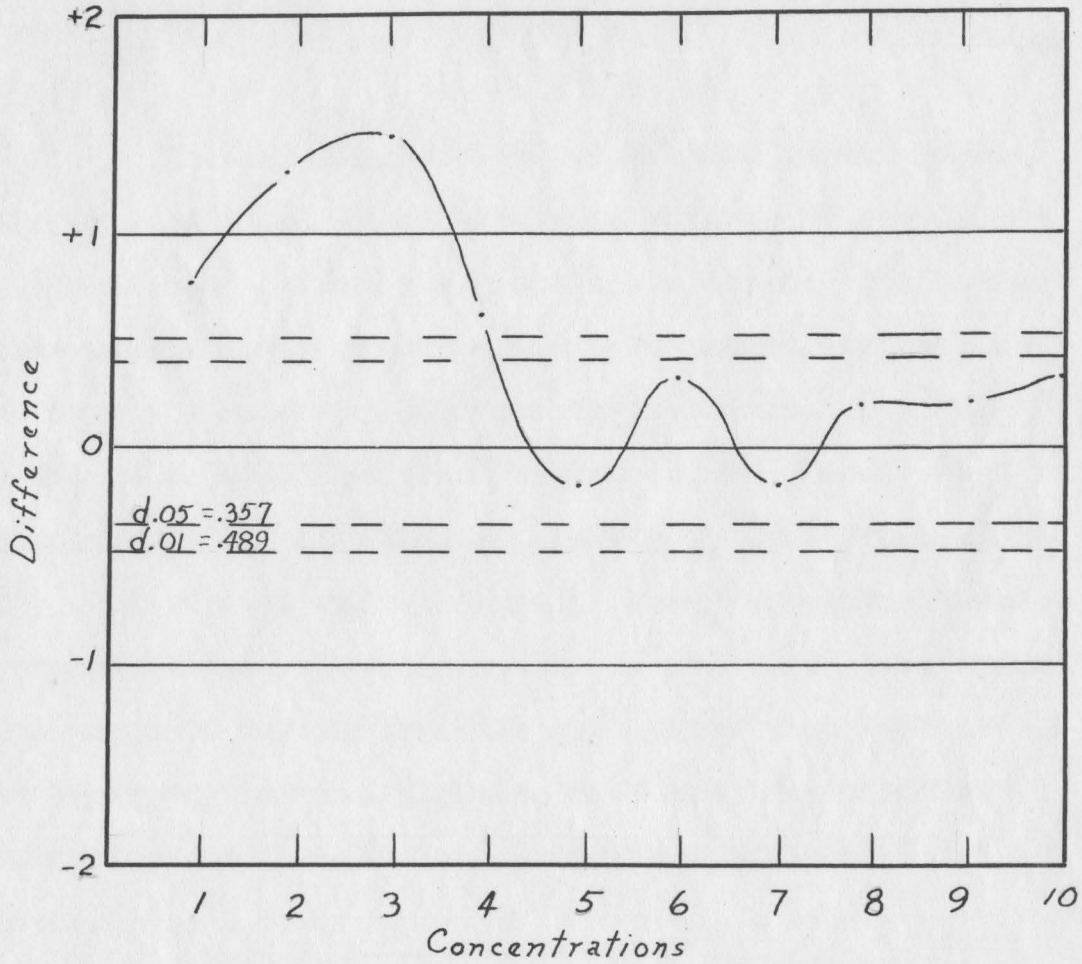


Figure 5.--Criteria of significance of data shown in Figure 4

Chapter V

DISCUSSION

The principal objective of this study was to make an investigation of the toxic effects of the chloralides of malic, tartaric, lactic, salicylic, and citric acids upon the common house fly. To accomplish this objective, the major problem was divided into three subquestions already specified in Chapter I. These subquestions will be treated separately in this discussion.

The chloralides used in these experiments were prepared in much the same manner as Shah and Alimchandani (25) prepared the chloralides with which they worked. It was observed that too high temperatures decreased the yield of product as had been suggested by Meldrum and Bhatt (10).

In order to determine how effective these chloralides are when used as contact poisons on the house fly, the Kearns apparatus was used. It was already available and its construction design decreased greatly the possibility of obtaining toxic effects due to causes other than direct contact of the spray to the insect, such as fumigant action or residual effect.

Testing with this apparatus proved to be fairly

easy as far as the actual operation of the machine was concerned. Fairly consistent results were obtained within replications and comparative values seemed to be consistent when repeated comparisons were made with the standard solution of Chlordane and with DDT solutions.

Tests were first made using the weaker .04 M. solutions. The strength of the solutions was then increased to the higher concentrations if and when the preceding concentrations failed to give significant kill. Although the .40 M. solution gave no significant mortality, no higher concentrations were used. Even this concentration was considered to be uneconomical. Also, because of the relative insolubility of the chloralides in the Deobase-acetone carrier used, solutions above this concentration would have been impractical in performing the experiment. An amount of acetone so great as to cause considerable mortality in itself would have been necessary to effect complete solution before making up to volume with Deobase.

As has been mentioned previously, workers in an uncontrolled preliminary test at the Colorado Experiment Station obtained what appeared to be promising results when the chloralides used in the present study were dispersed in kerosene and applied as a spray to house flies. However, when these chloralides were put into Deobase (deodorized kerosene) and acetone medium and

used in controlled tests with the Kearns apparatus, no significant kill was obtained. This would indicate that some constituent of the kerosene might have been responsible for the results obtained in the preliminary tests. The questions arise, however, whether this unknown constituent was in itself toxic or whether it affected the chloralides in some way as to increase their toxicity.

From a practical point of view, it would seem that the toxicity of the chloralides should be investigated using solvents other than the Deobase-acetone mixture suggested for use in the Kearns apparatus.

When making tests to determine how effective these chloralides are when used as fumigants on the common house fly, humidity was unmeasured. Since the rate of flow was regulated by the displacement of air by water, the humidity was undoubtedly relatively high in all tests.

No provision was made for sampling or analyzing the vapor passing over the test insects; therefore, an accurate determination of the concentration of vapor was impossible. The use of rubber stoppers in the equipment may also have been a source of error since some of the vapor may have been adsorbed by these connections. Although the temperature of the insect chamber was not controlled during each test, preliminary tests showed it to be only sightly above room temperature.

Despite the limitations due to simplicity in

the method used in this test, the results obtained in successive replications were fairly consistent when compared to the carbon disulfide standard. Thus it is felt that the data obtained are at least indicative of the relative toxicity of the vapors of the chloralides under study.

The single droplet water test, Went's pea test, and an aqueous spray test were used to evaluate the plant growth regulating properties of these compounds.

Only .001906 M. solutions were used in the single droplet water test. This concentration was chosen to correspond to a solution of the sodium salt of 2,4-dichlorophenoxyacetic acid in parts per million, i.e., 600 p.p.m., so that comparisons could be made to this compound.

The results show three of the chloralides, MAC, SAC, and LAC to retard tissue production significantly. This finding suggests that a series of concentrations including strengths both stronger and weaker than .001906 M. might be investigated.

Data obtained when using Went's pea test indicate that the top four concentrations (the highest being .001906 M.) show significant growth regulating action. This again suggests that a range of concentrations higher than .001906 M. might be worthy of study.

In the aqueous spray test it was desired to

determine the growth-regulating effects of the chloralides at the concentrations to be used as insecticides. At the time that these tests were made, it was thought that .04 M. would be the highest concentration used in the insecticidal testing. This accounts for the fact that the two highest concentrations finally used in the insecticidal tests, .2 M. and .4 M. were not included in this spray test.

Although plants treated with the three highest concentrations of all of the chloralides showed only a slight degree of burning of the lower leaves, these concentrations also seemed to show a considerable increase in height growth in comparison to the untreated control plants. No measurements were made since determination of differences in height growth had not been included in the objectives of the experiment.

Correlations and comparisons

MAC and SAC in both the single water droplet test and in Went's pea test exhibited growth-regulating action of significance. In neither test were they as active as 2,4-D.

LAC produced significant growth-regulation in the water droplet test but not in Went's pea test. On the other hand, TAC produced significant growth-regulation in the pea test but not in the water droplet test.

DDT produced no significant growth-regulation in the droplet test but was more active than any of the chloralides in Went's pea test.

Suggestions for further study

1. Other chloralides could be investigated as to biological activity, perhaps using a variety of test insects.

2. Effects of different solvents on the toxicity of various chloralides might be investigated.

3. Since lactic acid chloralide was shown to give a mortality rate of nearly 100 percent when used as a fumigant, further studies to determine the minimum lethal dosages under varied experimental conditions are suggested.

4. Significant growth-regulating action shown when the chloralides were used in Went's pea test and the single droplet water test suggests that other concentration ranges might be investigated for growth-regulating effects.

5. The clue that chloralides may cause increased height of tomato plants which was obtained incidentally in this study should be further investigated.

Chapter VI

SUMMARY

Although several authors over a period of nearly one hundred years have dealt with the group of compounds known as the chloralides in one way or another, no mention has been found in the literature of any worker investigating the biological activity of any of these compounds.

Preliminary tests performed with several chloralides of ∝-hydroxy carboxylic acids in kerosene, at the Colorado Agricultural Experiment Station in Fort Collins, Colorado, revealed that some of these compounds might possess insecticidal properties. It was because of this indication that this study to determine the toxicity of the chloralides of malic, lactic, salicylic, tartaric, and citric acids to the common house fly, Musca domestica L. was instigated.

The chloralides in a Deobase-acetone medium were shown to have no toxic effect as contact poisons on the house fly when used with the Kearns spray apparatus.

Fumigant action was shown by three of the five chloralides at a temperature high enough to volatilize them. Lactic acid chloralide was superior to the others

giving nearly a 100 percent kill as did the carbon disulfide used as a standard. Mortality rates of 12 and 24 percent were shown by the chloralides of malic and citric acids.

Three tests were used to determine the possible plant growth-regulating effects of the chloralides. Salicylic and malic acid chloralides were shown to exhibit significant growth-regulating action in both the single droplet water test and Went's pea test. Significant results were also obtained by lactic acid chloralide in the water droplet test and by tartaric acid chloralide in Went's pea test.

All of the chloralides used in this study showed a slight degree of burning of the leaves of Marbon tomatoes eight inches tall when applied in an aqueous spray.

A P P E N D I X

APPENDIX TABLE OF CONTENTS

Appendix A.--ANALYTICAL DATA

Table A.--RESULTS OF COMBUSTION ANALYSES AND MOLECULAR WEIGHT DETERMINATIONS PERFORMED ON FIVE CHLORALIDES

Compound	TAC	MAC	SAC	CAC	LAC
Formula	$C_6H_6O_6Cl_3$	$C_6H_5O_5Cl_3$	$C_8H_5O_3Cl_3$	$C_8H_7O_7Cl_3$ $2H_2O$	$C_5H_5O_3Cl_3$
Found					
Molecular weight	255.3 274.2	288.4 264.8	*494.9 492.8	*652.0 682.0	Unstable
% Carbon	25.68 25.55	27.33 26.98	37.35 37.28	26.87 26.67	Unstable
% Hydrogen	1.30 1.49	2.02 2.10	2.41 2.46	2.57 2.77	Unstable
Calculated					
Molecular weight	279.5	263.5	255.5	321.0	219.5
% Carbon	25.76	27.32	37.57	26.85	27.33
% Hydrogen	1.79	1.90	1.95	3.08	2.28
Difference					
Molecular weight	-24.2 - 5.3	+24.9 + 1.3	-16.1 -18.2	+10.0 +40.0	xxx xxx
% Carbon	-0.08 -0.21	+ 0.01 - 0.34	- 0.22 - 0.29	+ 0.02 - 0.18	xxx xxx
% Hydrogen	-0.49 -0.30	+ 0.12 + 0.20	+ 0.46 + 0.51	- 0.51 - 0.30	xxx xxx

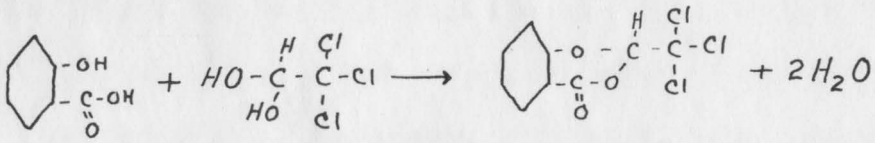
*These compounds were found to be in a dimolecular state.

% carbon and % hydrogen were determined by regular combustion analyses. (13) Molecular weight determinations were made by using the depression of the freezing point of napthalene. (The molal freezing point constant of napthalene is equal to 6.8.)

Appendix B.--PREPARATION OF CHLORALIDES

Preparation of the Chloralides

Salicylic acid chloralide

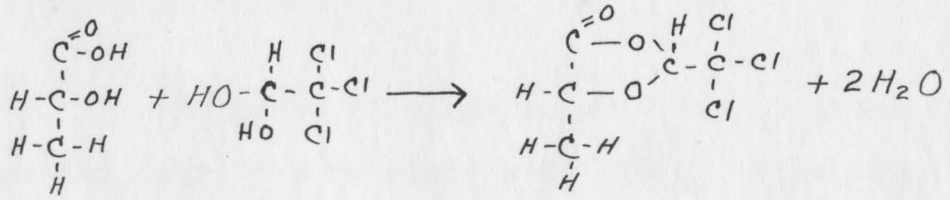


34 grams of chloral hydrate and 27.6 grams of salicylic acid were mixed with 150 ml. of concentrated sulfuric acid. The mixture was warmed slightly and allowed to stand. After 24 hours the mixture was poured into two liters of cold water, the resulting precipitate washed twice with water and filtered with suction. The white granular powder was allowed to dry in the air.

Yield; 36 grams. (68.6%)

Theoretical 52.5 grams.

Lactic acid chloralide



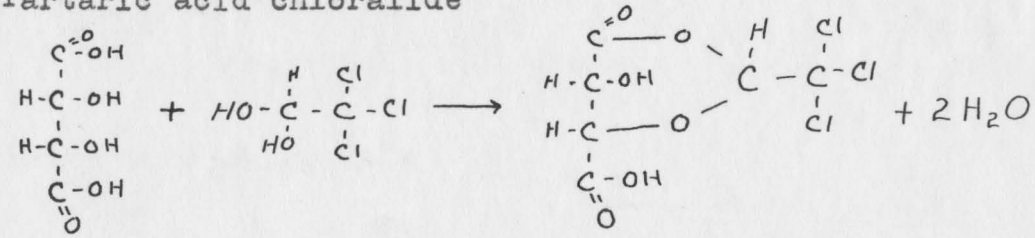
31 grams of chloral hydrate were added slowly and with cooling to 18 grams of lactic acid. When the initial reaction was over, 50 ml. of concentrated sulfuric acid were added and the mixture heated

to 90° on a water bath. After two and one-half hours the mixture, almost black from sulfuric acid carbonization was poured into 800 ml. of ice-water and extracted three times with 50 ml. portions of ether. Extracts were dried over sodium sulfate and decolorized by shaking with carbon. The ether was then evaporated en vacuo. A yellowish oil was obtained. This oil was cooled in an attempt to start crystallization and in one and one-half hours had crystallized to a semi-solid mass. This mass was washed with ice-cold petroleum ether. The resulting yield was white needle-like crystals with a terpene-like odor.

Yield; 11 grams. (26.7%)

Theoretical 41.2 grams.

Tartaric acid chloralide

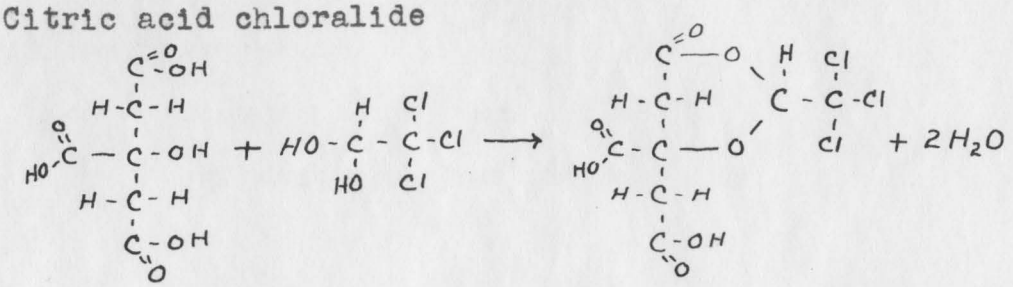


31 grams of tartaric acid and 34 grams of chloral hydrate were mixed with 100 ml. concentrated sulfuric acid and the mixture heated to between 40 and 45° to dissolve the chloral hydrate. After about 18 hours, the mixture was poured into two liters of cold water, the precipitate washed twice with water and filtered.

Yield; 34.5 grams (59.4%)

Theoretical 58 grams.

Citric acid chloralide

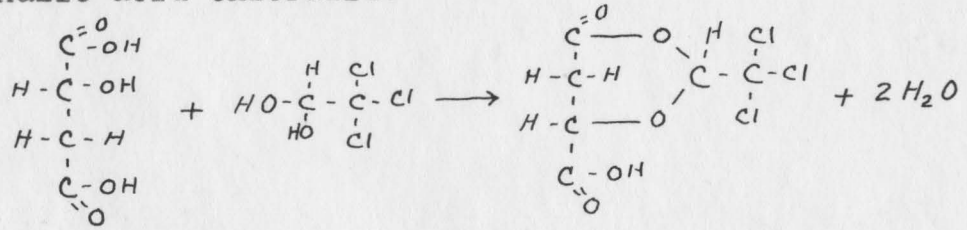


38.4 grams of citric acid and 33 grams of chloral hydrate were dissolved in 150 ml. of concentrated sulfuric acid and allowed to stand at room temperature for about 20 hours. After this length of time the presence of an appreciable quantity of solid was noted. This mixture was then poured into ice-water, washed, and filtered.

Yield; 45 grams. (70.2%)

Theoretical 64.1 grams.

Malic acid chloralide



26.8 grams of malic acid and 33 grams of chloral hydrate were dissolved in concentrated sulfuric acid and allowed to stand with frequent shakings. After about three and one-half hours the contents of the flask

crystallized to an almost solid mass of fine white crystals. The material was poured into two liters of cold water, filtered, washed twice with cold water, again filtered and dried.

Yield; 38.5 grams. (73.3%)

Theoretical 52.6 grams.

Appendix C.--REARING OF THE HOUSE FLY

Medium for Rearing the House Fly

1 pound of a 1:2 mixture of alfalfa meal and bran.
12 grams of malt extract dissolved in warm water.
8 grams of compressed or dry yeast dissolved in luke-warm water.
700 ml. cold water.

Among the incidental problems encountered in making this study was the growth of mold in the insect rearing medium after the eggs had hatched. This moldy condition nearly always resulted in a great decrease of insects reaching the pupa stage and in stunted adults unsuitable for testing purposes. It was observed during the course of the experiment, however, that mold usually did not occur in jars into which a relatively large number of eggs (between 1500 and 2000) had been placed, and, therefore, contained a large population of maggots.

LITERATURE CITED

LITERATURE CITED

1. Campbell, F. L. Cooperative tests of housefly sprays, 1935-36. Washington, Department of Agriculture, 1938. 20p.processed. (U.S. Bureau of Entomology and Plant Quarentine. E-436.)

2. Campbell, F. L. and Sullivan, W. N. Testing fly sprays; A metal turntable method for comparative tests of liquid spray contact insecticides. Soap and Sanitary Chemicals, 14:119-25, 149, June 1938.

3. Edeleanu, L. and Zaharia, Al. Einwirkung von Chloral auf die Oxalsauren in Gegenwort von Swefelsaure. Chemisches Zentralbatt, 66:212-13, 1895.

4. Grabowsky, Julijan. Ueber Chloralid und unlosliches Chloral. Deutsche Chemische Gesellshaft. Berichte, 8:1433-37, 1875.

5. Grabowsky, Julijan. Ueber die Einwirkung von Swefelsaure auf Chloral. Deutsche Chemische Gesellshaft. Berichte, 6:225-26, 1873.

6. Grabowsky, Julijan. Ueber die Verbindung von Chloral mit Swefelsaure. Deutsche Chemische Gesellshaft. Berichte, 6:1070-72, 1873.

7. Grayson, J. M. and Swank, G. R. A laboratory method for testing fumigants: Results with methyl bromide against the firebrat. Journal of Economic Entomology, 34:65-67, February 1941.

8. Kearns, C. W. A method of testing insects. Urbana, Illinois, the author, Department of Entomology, University of Illinois, 194-, 3 p. Mimeographed.

9. Kekule, Aug. Ueber die Chloralid. Justus Liebig's Annalen die Chemie, 105:293-95, 1858.

10. Meldrum, A. N. and Bhatt, D. M. Chloralides of α-hydroxycarboxylic acids. Bombay University. Journal, 3:149-52, 1934.

11. Murray, C. A. A fundamental error in the Peet-Grady method. Soap and Sanitary Chemicals, 16:111, 113, 115, 117, 119, 125, 1940.

12. Nelson, F. C. Changes and additions to the Peet-Grady method. Soap and Sanitary Chemicals, 10:81-83, 85, 97, 99, 101, 103, August 1934.

13. Niedrl, J. B. and Niedrl, V. Micromethods of quantitative organic analysis. New York, John Wiley and Sons, 1942. 347 p.

14. O'Kane, W. C. and others. Studies on contact insecticides. I. Surface tension, surface activity, and wetting ability as factors in the performance of contact insecticides. New Hampshire. Agricultural Experiment Station. Technical bulletin, 39:4-42, February 1930.

15. Otto, Robert. Zur Kenntnifs der β-Dichlor-propionsaure. Justus Liebig's Annalen die Chemie, 239:261-66, 1888.

16. Page, A. B. P. and Lubatti, O. F. Recent experiments on fumigation. Chemistry and Industry, 59:172-79, March 16, 1940.

17. Patterson, T. S. and MacMillan, Andrew. The action of chloral on ethyl tartrate and on ethyl malate. Chemical Society. Journal, 101:788-803, 1912.

18. Payne, M. G. and Fults, J. L. Effects of ultraviolet light on 2,4-dichlorophenoxyacetic acid and related compounds. Science, 106:37-39, July 11, 1947.

19. Peet, C. H. and Grady, A. G. Studies in insecticidal activity. I. Testing insecticides against flies. Journal of Economic Entomology, 21:612-17, August 1928.

20. Richardson, H. H. An efficient medium for rearing houseflies throughout the year. Science, 76:350-51, October 14, 1932.

21. Richardson, H. H. Testing contact insecticides. (In: American Association for the Advancement of Science. Laboratory procedures in studies of the chemical control of insects. Washington, D.C., The Association, 1943. p. 126-35.)

22. Richardson, H. H. and Busbey, R. L. Laboratory apparatus for fumigation with low concentrations of nicotine: With studies on aphids. Journal of Economic Entomology, 30:576-82, August 1937.

23. Schiff, Robert. Acetylbrenstraubensaure--Chloralid. Deutsche Chemische Gesellshaft. Berichte, 31:1305-06, 1898.

24. Shah, N. M. Chloralides; The condensation of butyl-chloral with α-hydroxycarboxylic acids. Indian Chemical Society. Journal, 16:285-86, 1939.

25. Shah, N. M. and Alimchandani, R. L. Chloralides from α-hydroxycarboxylic acids and their reduction products. Indian Chemical Society. Journal, 11:545-50, 1934.

26. Shepard, H. H. and others. The relative toxicity of insect fumigants. University of Minnesota. Agricultural Experiment Station. Technical bulletin, 120:3-23, April 1937.

27. Simanton, W. A. Evaluating liquid insecticides; Comments on the 1937 official method and use of the official control insecticide in grading liquid household sprays. Soap and Sanitary Chemicals, 13:103, 105, 107, 115, October 1937.

28. Simanton, W. A. and Miller, A. C. Greater speed and accuracy with modified Peet-Grady method. Soap and Sanitary Chemicals, 14:115, 117, May 1938.

29. Stadeler, A. Ueber die Bildung des Chlorals aus Starke und ein neues Zersetzungsproduct desselben. Justus Liebig's Annalen die Chemie, 61:101-14, 1847.

30. Swanson, C. P. A simple bio-assay method for the determination of low concentrations of 2,4-dichlorophenoxy acetic acid in aqueous solutions. Botanical Gazette, 107:507-09, June 1946.

31. Swanson, C. P. Two methods for the determination of the herbicidal effectiveness of plant growth-regulating substances in oil solution on broad leaf plants. Botanical Gazette, 107:560-62, June 1946.

32. Thompson, H. E. and others. New growth-regulating compounds: I. Summary of growth-inhibitory activities of some organic compounds as determined by three tests. Botanical Gazette, 107:476-507, June 1946.

33. Wallach, O. Ueber Chloralid und Chloralidartige Verbindungen. Justus Liebig's Annalen die Chemie, 193:1-61, 1878.

34. Went, F. W. and Thimann, K. V. Phytohormones. New York, MacMillan, 1937. 294 p.

Thesis

The effect of some chloralides of α-hydroxy carboxylic acids on the common house fly

Data

Madelen W. Rey

(March, 1949)

Contents

The chloralides used in the following tests were:

malic acid chloralide (MAC)
salicylic acid chloralide (SAC)
tartaric acid chloralide (TAC)
citric acid chloralide (CAC)
lactic acid chloralide (LAC)

Testing for activity of the Chlorakides as contact poisons using the Kearns wind tunnel spray apparatus.

Four replications of fifty flies each were used. ½ ml of test substance was used for each treatment.

	1st Replication concentrations			2nd Replication concentrations			3rd Replication concentrations			4th Replication concentrations		
compound	.04M	.2M	.4M	.04M	.2M	.4M	.04M	.2M	.4M	.04M	.2M	.4M
MAC	0	0	0	1	1	0	0	0	1	2	1	0
CAC	1	1	0	1	0	0	0	1	0	0	0	0
LAC	0	0	2	0	0	0	0	0	0	1	1	0
SAC	0	0	0	0	0	0	1	0	0	0	0	1
TAC	1	1	0	0	0	0	0	1	0	1	0	0
DDT	50	50	50	50	50	50	50	50	50	50	50	50
Chlordane	24	38	21	18	23	24	23	25	26	29	19	22
Blank	0	1	0	4	0	1	0	1	0	0	0	1
control	0	0	0	0	1	0	1	1	0	0	0	1

Blank = 1:2 mixture acetone and Deobase
Control = untreated flies
DDT = always at .04M strength

Contact action (cont.)

"Complex Variance - 5⁻ Chloralides

Variability due to:	D/F	Sums of squares	mean squares	F	required F at .05	required F at .01
totals	59	17.33334				
between treatments	4	.66667	.16667	.550	5.70	13.68
within treatments	55	16.66667	.30303			
Between concentrations within treatments	10	3.16667	.31667	1.055	2.05	2.75
Within concentrations within Treatments	45	13.50000	.30000			

$\Sigma X = 20$

$\Sigma X^2 = 24$

$\frac{(\Sigma X)^2}{60} = 6.66666$

$\frac{(\Sigma T)^2}{12} = \frac{88}{12} = 7.33333$

a = .04M Bill, b = .2M Bill, c = .4M Bill

replication

I. $\frac{(\Sigma a)^2 + (\Sigma b)^2 + (\Sigma c)^2}{4}$

II. $\frac{(\Sigma a)^2 + (\Sigma b)^2 + (\Sigma c)^2}{4}$

III $\frac{(\Sigma a)^2 + (\Sigma b)^2 + (\Sigma c)^2}{4}$

IV $\frac{(\Sigma a)^2 + (\Sigma b)^2 + (\Sigma c)^2}{4}$

} add $= \frac{42}{4} = 10.5$

$10.5 - 6.66666 - .66667 = 3.16667$

contact action (cont.)

Variance analysis - 5 chloralides, blank + control

Variability due to:	D/F	Sums of Squares	Mean Squares	F	Required F at .05	Required F at .01
Totals	83	38.23810				
Between Treat.	6	3.23810	.53968	1.20	2.21	3.04
Within Treat.	78	35.00000	.44872			

$\Sigma X = 34$

$\Sigma X^2 = 52$

$\frac{(\Sigma X)^2}{84} = 13.76190$

$\frac{(\Sigma T)^2}{12} = \frac{204}{12} = 17.00000$

Testing the Chloralides as fumigants by applying heat to volatilize them.

(a) .1 gram of chloralide passed over insects in 8 minutes
(b) Temperature range (85°-100° C)
(c) 30 flies per treatment
(d) Four replications

	Replications			
Compound	1	2	3	4.
MAC	*1	7	3	4
CAC	8	7	6	8
LAC	30	27	30	30
SAC	4	2	2	5
TAC	1	0	4	2
CS_2	30	30	30	30
oil check	3	0	1	0

* numbers indicate number of dead flies in 24 hrs

fumigation (cont.)

Variance analysis of 5 chlorides, CS_2 and ail check

Variability due to	D/F	Sums of Squares	mean Squares	obs. F	required F	
					at .05	at .01
totals	27	4074.6786				
Between treatments	6	4024.9286	670.8214	283.05	3.81	7.23
Within treatments	21	49.7500	2.37			

$\Sigma X = 305$

$\Sigma X^2 = 7,397$

$\frac{(\Sigma X)^2}{28} = \frac{93,025}{28} = 3,322.3214$

$\frac{(\Sigma T)^2}{4} = \frac{29,389}{4} = 7,347.2500$

Variance analysis of four chlorides and oil check

Variability due to	D/F	Sums of Squares	mean Squares	obs. F	required F at .05	required F at .01
totals	19	136.80				
between treatments	4	93.80	23.45	8.2	2.77	4.25
within treatments	15	43.00	2.86			

Lactic acid chloride was omitted because its yield of nearly 100% was considered out of the class of the remaining chlorides

$\Sigma X = 68$

$\Sigma X^2 = 368$

$\frac{(\Sigma X)^2}{20} = \frac{4624}{20} = 231.2$

$\frac{(\Sigma T)^2}{4} = \frac{1300}{4} = 325.00$

msd $\begin{cases} .05 = \text{"t"}\ 2.101 \sqrt{2.86(\frac{2}{4})} = 2.52 \\ .01 = \text{"t"}\ 2.878 \sqrt{2.86(\frac{2}{4})} = 3.45 \end{cases}$

Testing for plant growth-regulating effect of Chloralides.

Three tests were used –

1. Single droplet water test
2. Avena's pea test
3. Aqueous spray test

1. Single droplet water test
(concentration of chemicals used = .001906 M)
Beans (Pencil pod wax variety) used
treated Nov. 1, 1947 -
Harvested Nov. 15, 1947
Green wt. of leaves above second node taken as follows; ten plants per treatment.

treatment	POT	Grams wt	treatment	Pot	Grams wt.
MAC	1	1.35	LAC	1	1.26
	2	1.15		2	2.32
	3	2.07		3	1.35
	4	1.15		4	0.56
	5	1.42		5	1.02
	6	1.19		6	0.82
	7	1.19		7	1.45
	8	2.25		8	1.91
	9	1.45		9	0.90
	10	1.52		10	2.30

droplet test (cont)

treatment	pot	grams weight
TAC	1	1.90
	2	1.39
	3	1.75
	4	1.40
	5	2.72
	6	1.99
	7	1.87
	8	1.35
	9	1.35
	10	1.35

treatment	pot	grams weight
CAC	1	1.35
	2	1.15
	3	1.95
	4	1.16
	5	1.48
	6	1.72
	7	1.72
	8	1.61
	9	2.15
	10	1.91

treatment	pot	grams weight
SAC	1	1.45
	2	1.90
	3	1.68
	4	1.34
	5	1.20
	6	1.60
	7	1.20
	8	1.69
	9	1.15
	10	1.85

treatment	pot	grams weight
Na salt of 2,4-D	1	1.30
	2	0.80
	3	1.61
	4	0.80
	5	1.55
	6	0.85
	7	0.95
	8	1.96
	9	1.42
	10	1.00

droplet test (cont.)

treatment	pot	grams weight	treatment	pot	grams weight
control (no treatment)	1	2.00	DDT	1	1.20
	2	1.80		2	1.10
	3	1.85		3	1.60
	4	2.05		4	1.32
	5	1.65		5	0.75
	6	1.82		6	1.80
	7	1.40		7	1.65
	8	1.85		8	2.05
	9	1.95		9	2.57
	10	1.98		10	1.30

Analysis of data (Single droplet water test)

"t" required for significance at .05 level
= 2.101
at .01 level
= 2.878

Control

X	X^2
2.00	4.00
1.80	3.24
1.85	3.42
2.05	4.20
1.65	2.72
1.82	3.31
1.40	1.96
1.85	3.42
1.95	3.80
1.98	3.92
total 18.35	$33.99 = \Sigma X^2$

$\overline{X_1} = 1.84$ $\quad s = .18$

$$s = \frac{1}{N}\sqrt{N(\Sigma X^2) - (\Sigma X)^2}$$

$N = 10$

$$s = \frac{1}{10}\sqrt{10(33.99) - (18.35)^2}$$

$= .18$

droplet test (cont.)

TAC	
X	X^2
1.80	3.61
1.39	1.93
1.75	3.06
1.40	1.96
2.72	7.40
1.79	3.20
1.87	3.49
1.35	1.82
1.35	1.82
1.35	1.82
total = 16.87	30.11 $= \Sigma X^2$

$\bar{X}_2 = 1.69 \quad s = .406 \quad t = 1.02$

$$t = \frac{|\bar{X}_1 - \bar{X}_2 - d|}{\sqrt{\frac{N_1 S_1^2 + N_2 S_2^2}{N_1 + N_2 - 2}}} \cdot \sqrt{\frac{N_1 N_2}{N_1 + N_2}}$$

$d = 0$

$$t = \frac{1.84 - 1.69}{\sqrt{\frac{10(.48)^2 + 10(.406)^2}{18}}} \cdot \sqrt{\frac{100}{20}} = 1.02$$

diapeet test (cont.)

$\overline{X}_2 = 1.47$ $S = .346$ $t = 2.84$

MAC	
X	X^2
1.35	1.82
1.15	1.32
2.07	4.28
1.15	1.32
1.42	2.02
1.19	1.41
1.19	1.41
2.25	5.06
1.45	2.10
1.52	2.31
total 14.74	23.05 $= \Sigma X^2$

dropout test (cont.)

LAC

X	X^2
1.26	1.59
2.32	5.38
1.35	1.82
0.56	0.31
1.02	1.04
0.92	0.85
1.45	2.10
1.91	3.65
0.90	0.81
2.03	4.12
total = 13.72	21.67 = ΣX^2

$\bar{X}_2 = 1.37$, $s = .533$, $t = 2.50$

droplet test (cont.)

CAC

X	X^2
1.35	1.82
1.15	1.32
1.95	3.80
1.16	1.35
1.48	2.19
1.72	2.96
1.72	2.96
1.61	2.59
2.15	4.62
1.91	3.65
total = 16.20	27.26 = ΣX^2

$\bar{X}_2 = 1.62$, $s = .314$, $t = .576$

Dioptest test (cont.)

SAC

X	X^2
1.45	2.10
1.90	3.61
1.68	2.82
1.34	1.75
1.60	2.54
1.20	1.44
1.69	2.86
1.15	1.32
1.85	3.42
total = 13.86	21.86 = ΣX^2

$\bar{X}_2 = 1.39$, $s = .515$, $t = 2.18$

Droplet test (cont.)

Na salt of 2,4-D

X	X^2
1.30	1.69
0.80	0.64
1.61	2.59
0.80	0.64
1.55	2.40
0.85	0.72
0.95	0.90
1.96	3.84
1.42	2.02
1.00	1.00
total = 12.24	16.44 = ΣX^2

$\bar{X}_2 = 1.22$, $s = .381$, $t = 4.45$

droplet test (cont.)

DDT

X	X^2
1.20	1.44
1.10	1.21
1.60	2.56
1.32	1.74
0.75	0.56
1.80	3.24
1.65	2.72
2.05	4.20
2.57	6.60
1.30	1.69
total = 15.34	25.96 = ΣX^2

$\overline{X}_2 = 1.53$, $s = .493$, $t = 1.77$

2. Went's pea test.

Degrees of reaction

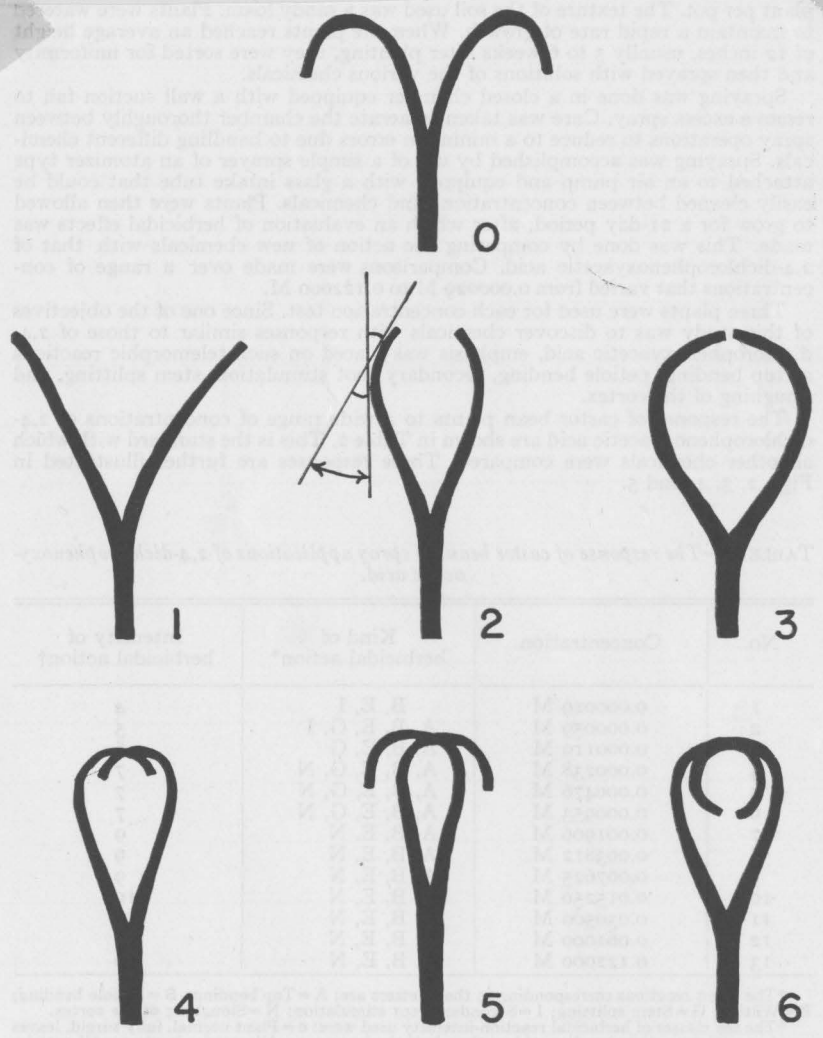


FIG. 1.—Pea stem growth-curvature classes. 0 = Zero inward curvature, typical of distilled water; 1 = Slight inward curvature; 2 = Tips parallel to an inward curvature of 45°; 3 = Average inward curvature between 45° and 90°; 4 = Average inward curvature between 90° and 135°; 5 = Average inward curvature between 135° and 180°; 6 = Average inward curvature greater than 180°.

(Payne and Fults)

Pea planted October 24, 1947
tested October 31, 1947
read November 1, 1947

Water check - Avent's pea test
3 dishes of distilled water used -
10 pea stems per dish

Dish no.	Reaction							
	0	1	2	3	4	5	6	ave.
	number of peas							
I	5	5						.5
II	9	1						.1
III	6	4						.4
							total	1.0

overall average = $\frac{1.0}{3} = .33$

DDT Hents pea test (cont.)

no. of peas per concentration per degree of reaction

conc.	reaction 0	1	2	3	4	5	6	ave.
	no. of peas							
1			6	4				2.4
2			5	5				2.5
3			5	5				2.5
4			4	6				2.6
5			5	5				2.5
6			6	4				2.4
7			10					2.0
8	2	6	2					1.0
9								0.6
10								0.8

- 10 concentrations
- 10 peas per concentration
- highest concentration = .001906 M.
- lowest concentration = .000003 M.
- every concentration = ½ strength of preceding one
- No. 1 = highest
- No. 10 = lowest

DDT with H_2O check Results per test (cont.)

X	X^2	F	FX^2
0	0	76	0
1	1	51	51
2	4	44	176
3	9	29	261
4	16	0	0
5	25	0	0
6	36	0	0
		200	488

$N = 200$

$\Sigma X = 226$

$\Sigma X^2 = 488$

$\frac{\Sigma T_T^2}{100} = 383.38$

$\frac{\Sigma T_C^2}{20} = \frac{5468.57}{20} = 284.29$

$488 = \Sigma X^2 \quad \frac{(\Sigma X)^2}{200} = 255.38$

Mean reactions

Conc	H_2O	DDT	Totals	diff.
1	.33	2.4	2.73	+2.07
2	.33	2.5	2.83	2.17
3	.33	2.5	2.83	2.17
4	.33	2.6	2.93	2.27
5	.33	2.5	2.83	2.17
6	.33	2.4	2.73	2.07
7	.33	2.0	2.33	1.67
8	.33	1.0	1.33	0.67
9	.33	0.6	093	0.27
10	.33	0.8	1.13	0.47
Totals	3.33	1.93	2.26	

Concentrations

H_2O	DDT	Totals
3.3	24	27.3
3.3	25	28.3
3.3	25	28.3
3.3	26	29.3
3.3	25	28.3
3.3.	24	27.3
3.3	20	23.3
3.3	10	13.3
3.3	6	9.3
3.3	8	11.3
33.0	193	226.0

DDT and H_2O check

Variance Analysis

Variability due to	Sums of Squares	D/F	mean square variance	obs. F	required F at.05	required F at.01
Treatments	128.00	1	128.00	319.2	3.89	6.76
Concentrations	28.91	9	3.21	8.00	1.92	2.50
Residual (Error)	75.71	189	0.401			
Totals	232.62	199				

generalized standard error =

$\sqrt{.401} = .633$

standard error of difference between two means =

$\sqrt{2/10 \times .401} = .283$

Criteria for significance:

$d_{.05} = 2.101(.28) = .588$

$d_{.01} = 2.878(.28) = .806$

MAC

		reaction						
Conc.	0	1	2	3	4	5	6	ave.
		number	of	peas				
1	1	8	1					1.0
2		5	5					1.5
3		3	7					1.7
4	2	7	1					0.9
5	9	1						0.1
6	4	6						0.6
7	9	1						0.1
8	5	5						0.5
9	5	5						0.5
10	3	7						0.7

MAC with H_2O check

X	X^2	F	FX^2
0	0	105	0
1	1	81	81
2	4	14	56
3	9	0	0
4	16	0	0
5	25	0	0
6	36	0	0
		200	$137 = \Sigma X^2$

$N = 200$

$\Sigma X = 109$

$\Sigma X^2 = 137$

$\frac{\Sigma T^2_T}{100} = 68.65$

$\frac{\Sigma T^2_C}{20} = 72.13$

$\frac{(\Sigma X)^2}{100} = 59.45$

mean reactions

conc	H_2O	MAC	diff
1	.33	1.0	+.7
2	.33	1.5	+1.2
3	.33	1.7	+1.4
4	.33	0.9	+.6
5	.33	0.1	-.2
6	.33	0.6	+.3
7	.33	0.1	-.2
8	.33	0.5	+.2
9	.33	0.5	+.2
10	.33	0.6	+.3

concentrations

H_2O	MAC	totals
3.3	10	13.3
3.3	15	18.3
3.3	17	20.3
3.3	9	12.3
3.3	1	4.3
3.3	6	9.3
3.3	1	4.3
3.3	5	8.3
3.3	5	8.3
3.3	7	10.3
33.0	76	109.0

MAC and H_2O check

Variability due to	Sums of Squares	D/F	mean square variance	obs. F	required F at.05	required F at.01
treatments	9.24	1	9.24	63.28	3.89	6.76
concentrations	12.72	9	1.41	7.66	1.92	2.50
Residual (Error)	27.63	189	.146			
totals	49.59	199				

generalized standard error = $\sqrt{.146}$ = .382

standard error of difference between two means = $\sqrt{2/10 \cdot .146}$ = .17

Criteria for significance:

$d_{.05}$ = 2.101 (.17) = .357

$d_{.01}$ = 2.878 (.17) = .489

SAC

Conc.	reaction							
	0	1	2	3	4	5	6	
	number of peas							ave.
1		5⁻	5					1.5⁻
2	3		5⁻	2				1.6
3	4	2	4					1.0
4	5⁻	5⁻						.5⁻
5	5⁻	5						.5⁻
6	7	3						.3
7	5⁻	5⁻						.5⁻
8	3	7						.7
9		10						1.0
10	2	8						.8

SAC with H_2O check

X	X^2	F	FX^2
0	0	101	0
1	1	83	83
2	4	14	56
3	9	2	18
4	16	0	0
5	25	0	0
6	36	0	0
		200	$157 = \Sigma X^2$

$N = 200$

$\Sigma X = 117$

$\Sigma X^2 = 157$

$\frac{\Sigma T_T^2}{100} = 81.45$

$\frac{\Sigma T_C^2}{20} = 77.06$

$\frac{(\Sigma X)^2}{200} = 68.45$

mean reactions

conc.	H_2O	SAC	diff
1	.33	1.5	1.17
2	.33	1.6	1.27
3	.33	1.0	.67
4	.33	.5	.17
5	.33	.5	.17
6	.33	.3	.03
7	.33	.5	.17
8	.33	.7	.37
9	.33	1.0	.67
10	.33	.8	.47

concentrations

H_2O	SAC	totals
3.3	15	18.3
3.3	16	19.3
3.3	10	13.3
3.3	5	8.3
3.3	5	8.3
3.3	3	6.3
3.3	5	8.3
3.3	7	10.3
3.3	10	13.3
3.3	8	11.3
33.0	84	117.0

SAC with H_2O checks

Variance Analysis

Variability due to	sums of squares	D/F	mean square variance	obs. F	required F at.05	required F at.01
treatments	13.00	1	13.00	36.72	3.89	6.76
concentrations	8.61	9	.957	.27	1.92	2.50
Residual (Error)	66.94	189	.354			
totals	88.55	199				

generalized standard error =

$\sqrt{.354} = .59$

standard error of difference between two means =

$\sqrt{2/10 \cdot .354} = .27$

Criteria for significance =

$d_{.05} = 2.101(.27) = .567$

$d_{.01} = 2.878(.27) = .777$

CAC

	reaction							
	0	1	2	3	4	5	6	
Conc.	number of peas							ave
1	2	8						.8
2	4	6						.6
3	3	7						.7
4	4	6						.6
5	4	6						.6
6	3	7						.7
7	6	4						.4
8	7	3						.3
9	8	2						.2
10	8	2						.2

CAC with H_2O check

X	X^2	F	FX²
0	0	116	0
1	1	84	84
2	4	0	0
3	9	0	0
4	16	0	0
5	25	0	0
6	36	0	0
		200	84

$N = 200$

$\Sigma X = 84$

$\Sigma X^2 = 84$

$\frac{\Sigma T_r^2}{100} = 36.80$

$\frac{\Sigma T_c^2}{20} = \frac{7485}{20} = 37.43$

$\frac{(\Sigma X)^2}{200} = 35.28$

mean reactions				concentrations		
conc	H_2O	CAC	diff	H_2O	CAC	totals
1	.33	.8	+.47	3.3	8	11.3
2	.33	.6	+.27	3.3	6	9.3
3	.33	.7	+.37	3.3	7	10.3
4	.33	.6	+.27	3.3	6	9.3
5	.33	.6	+.27	3.3	6	9.3
6	.33	.7	+.37	3.3	7	10.3
7	.33	.4	+.07	3.3	4	7.3
8	.33	.3	−.03	3.3	3	6.3
9	.33	.2	−.13	3.3	2	5.3
10	.33	.2	−.13	3.3	2	5.3
				33.0	51	84.0

CAC with H_2O check

Variance Analysis

Variability due to	Sums of Squares	D/F	mean square variance	obs. F	required F at .05	required F at .01
treatments	1.62	1	1.62	6.81	3.89	6.76
concentrations	2.15	9	.239	1.00	1.92	2.50
Residual (Error)	44.95	189	.238			
totals	48.72	199				

generalized standard error =

$$\sqrt{.238} = .49$$

standard error of difference between two means =

$$\sqrt{2/10} \cdot \sqrt{.238} = .22$$

Criteria for significance:

$d_{.05} = 2.101\ (.22) = .462$

$d_{.01} = 2.878\ (.22) \quad .633$

TAC

	reaction							
	0	1	2	3	4	5	6	
Conc	number of peds							ave.
1			2	8				1.8
2			6	4				2.4
3	1	2	7					1.6
4	3	5	2					.9
5	5	5						.5
6	6	4						.4
7	2	8						.8
8	3	7						.7
9	6	4						.4
10	5	5						.5

TAC with H_2O check

X	X^2	F	FX^2
0	0	68	0
1	1	75	75
2	4	23	92
3	9	4	36
4	16	0	0
5	25	0	0
6	36	0	0
		200	$203 = \Sigma X^2$

$N = 200$

$\Sigma X = 133$

$\Sigma X^2 = 203$

$\frac{\Sigma T^2_T}{100} = 110.98$

$\frac{\Sigma T^2_C}{20} = 91.42$

$\frac{(\Sigma X)^2}{200} = 88.45$

mean reactions

conc.	H_2O	TAC	diff.
1	.33	1.8	+ 1.47
2	.33	2.4	2.07
3	.33	1.6	1.27
4	.33	.9	.57
5	.33	.5	.17
6	.33	.4	.07
7	.33	.8	.47
8	.33	.7	.37
9	.33	.4	.07
10	.33	.5	.17

concentrations

H_2O	TAC	totals
3.3	18	21.3
3.3	24	27.3
33	16	19.3
3.3	9	12.3
3.3	5	8.3
3.3	4	7.3
33	8	11.3
3.3	7	10.3
3.3	4	7.3
3.3	5	8.3
330	100	133.0

TAC with H_2O check

Variance Analysis

Variability due to	Sums of Squares	D/F	mean square variance	Obs. F	Required F at .05	Required F at .01
treatments	22.44	1	22.44	47.74	3.89	6.76
Concentrations	2.97	9	.33	.702	1.92	2.50
Residual (Error)	89.14	189	.47			
totals	114.55	199				

generalized standard error =

$\sqrt{.47} = .69$

standard error of difference between two means =

$\sqrt{2/10 \cdot .47} = .31$

Criteria for significance:

$d_{.05} = 2.01 (.31) = .651$

$d_{.01} = 2.878 (.31) = .892$

LAC

		reaction						
	0	1	2	3	4	5	6	
Conc	number of peaks							ave
1	6	4						.4
2	4	6						.6
3	8	2						.2
4	7	3						.3
5	5	5						.5
6	1	9						.9
7	1	9						.9
8	1	9						.9
9	3	7						.7
10	1	9						.9

LAC with H_2O check

X	X^2	F	FX^2
0	0	114	0
1	1	96	96
2	4	0	0
3	9	0	0
4	16	0	0
5	25	0	0
6	36	0	0

$N = 200$

$\Sigma X = 96$

$\Sigma X^2 = 96$

$\frac{\Sigma T_T^2}{100} = 50.58$

$\frac{\Sigma T_C^2}{20} = 49.39$

$\frac{(\Sigma X)^2}{200} = 46.08$

mean reactions

Conc	H_2O	LAC	diff
1	.33	.4	+.07
2	.33	.6	+.27
3	.33	.2	-.13
4	.33	.3	-.03
5	.33	.5	+.17
6	.33	.9	+.57
7	.33	.9	+.57
8	.33	.9	+.57
9	.33	.7	+.37
10	.33	.9	+.57

concentrations

H_2O	LAC	totals
3.3	4	7.3
3.3	6	9.3
3.3	2	5.3
3.3	3	6.3
3.3	5	8.3
3.3	9	12.3
3.3	9	12.3
3.3	9	12.3
3.3	7	10.3
3.3	9	12.3
33.0	63	96.0

3. Aqueous spray test

- 8" Marbon variety tomatoplants used
- Concentrations of .08M, .06M, .04M .02M, .01M
- Ten plants treated with each concentration
- Chlorides dissolved in tri-ethanol amine 1:10 by weight and dispersed in water

Cmpd	Concentrations at which burning occurred			
	Degree of burning			
	0	1	2	3
MAC	.01M, .02M	.06M., .04M	.08M	
SAC	.01M, .02M .04M	.06M	.08M	
TAC		.04M, .02M .01M	.08M, .06M	
LAC			.08M. .04M, .06M.	
CAC		.04M .02M, .01M	.08M., .06M	
* tri-ethanol amine	no burning			
DDT		.02M .04M	.08M .04M. .06M	

degrees of burning:
0 = none
1 = very slight
2 = slight
3 = moderate

* blank

Combustion analyses of the Chloralide

tube A = Ascarite
tube D = Dehydrite

Calculated
25.76% - C
1.79 % - H

TAC $C_6H_6O_6Cl_3$

determined
25.55% C
1.49 % H

trial I

tube A = 17.67 mg.
tube D = 5.60 "
boat = 16.87 "
boat + TAC = 53.65 "
TAC = 36.87 "

tube A + CO_2 = 52.21 ; CO_2 = 17.67 ; C = 9.42 ; $\frac{9.42}{36.87}$ = 25.55 %C

tube D + H_2O = 10.55 ; H_2O = 4.95 ; H = .55 ; $\frac{.55}{36.87}$ = 1.49% H

trial II

---

tube A = 99.07 mg
tube D = 92.25 "
boat = 8.03
boat + TAC = 55.04
TAC = 47.01

determined
25.68 % C
1.30 % H

tube A + CO_2 = 139.68 mg
CO_2 = 40.61 "
C = 11.07
$\frac{11.07}{47.01}$ = 25.68% C

tube D + H_2O = 97.74 mg
H_2O = 4.47 "
H = .50 "
$\frac{50}{47.01}$ = 1.30 %

MAC - $C_6H_5O_5Cl_3$

Calculated
27.32 % C
1.90 % H

Trial I

tube A = 19.77 mg
tube D = 40.09 "
boat = 16.14 "
boat + MAC = 68.62 "
MAC = 52.43 "
tube A + CO_2 = 72.00 mg
CO_2 = 52.23 "
C = 14.24 "
$\frac{14.24}{52.43}$ = 27.33 %

determined
27.33 % C
2.02 % H

tube D + H_2O = 49.65 mg
H_2O = 8.56
H = .95
$\frac{.95}{52.43}$ = 2.02 %

Trial II

tube A = 136.60 mg
tube D = 130.28 "
boat = 14.06 "
boat + MAC = 60.76 "
MAC = 46.70 "
tube A + CO_2 = 182.79 "
CO_2 = 46.19 "
C = 12.60 "
$\frac{12.60}{46.70}$ = 26.98 %

determined
26.98 % C
2.10 % H

tube D + H_2O = 139.11
H_2O = 8.83
H = .98
$\frac{.98}{46.70}$ = 2.10 %

SAC - $C_8H_5O_3Cl_3$

Calculated
37.57% C
1.95% H

trial I

tube A = 42.60 mg
tube D = 54.31 "
boat = 14.16 "
boat + SAC = 60.26 "
SAC = 46.10 "
tube A + CO_2 = 108.94 "
CO_2 = 63.84 "
C = 17.31 "
$\frac{17.31}{46.10}$ = 37.35%

tube D + H_2O = 64.29
H_2O = 9.98
H = 1.11
$\frac{1.11}{46.10}$ = 2.41%

Determined
37.35% C
2.41% H

---

trial II

tube A = 105.82 mg
tube D = 64.45 "
boat = 16.02 "
boat + SAC = 57.02 "
SAC = 41.00 "
tube A + CO_2 = 161.85 "
CO_2 = 56.03 "
C = 15.28 "
$\frac{15.28}{41.00}$ = 37.28%

tube D + H_2O = 73.56 mg
H_2O = 9.09 "
H = 1.01 "
$\frac{1.01}{41.00}$ = 2.46%

Determined
37.28% C
2.41% H

CAC -

Calculated
26.85% C
3.08% H

trial I

tube A = 48.42 mg
tube D = 12.49 "
boat = 14.14 mg
boat + CAC = 61.25 "
CAC = 47.11 "
tube A + CO_2 = 94.86
CO_2 = 46.44
C = 12.66
$\frac{12.66}{47.11}$ = 26.87%

determined 26.87% C
2.57% H

tube D + H_2O = 23.37 mg
H_2O = 10.88 "
H = 1.21 "
$\frac{1.21}{47.11}$ = 2.57% H

---

trial II

tube A = 95.05 mg
tube D = 23.79 "
boat = 14.13 "
boat + CAC = 62.62 "
CAC 48.49 "
tube A + CO_2 = 142.50 "
CO_2 = 47.45 "
C = 12.93
$\frac{12.93}{48.49}$ = 26.67% C

determined
C = 26.67%
H = 2.77%

tube D + H_2O = 35.85 "
H_2O = 12.06
H = 1.34
$\frac{1.34}{48.49}$ = 2.77% H

LAC

UNSTABLE

combustion analysis could not be made

Molecular weight determinations of chloralides.

$$M = \frac{K \times \text{wt solute} \times 1000}{\text{wt solvent} \times \Delta t}$$

depression of freezing pt. of napthalene used
molal constant (K) for napthalene = 6.8

TAC Calculated M.W. = 279.5

trial I
wt napth. = 28.25 gr.
wt TAC = .56 gr
Δt = .57

$M = \underline{255.3}$

trial II
wt. napth = 24.66 gr.
wt TAC = .48
Δt = .49

$M = \underline{274.2}$

MAC calculated M.W. = 263.5

trial I

wt. naphth. = 37.20 gr
wt MAC = .42 "
Δt = .27 °
M = 288.4

trial II

wt. naphth = 29.61
wt. MAC = .52
Δt = .39°
M = 264.8

SAC - calculated M.W = 255.5

trial I

wt. naphth = 24.62 gr
wt SAC = .53 "
Δt = .30°
M = 494.9
(dimolecular)

trial II

wt. naphth = 31.34 gr
wt SAC = .47 "
Δt = .21 °
M = 492.8
(dimolecular)

CAC - calculated M.W. = 321

trial I

wt. napth = 28.20 gr.
wt CAC = .53 "
Δt = .19°
M = 682.3
(dimolecular)

trial II

wt. napth = 30.60 gr
wt. CAC = .52 "
Δt = .18°
M = 652.3
(dimolecular)

LAC - UNSTABLE
molecular weight determinations could not be made.