pCl = 6.5. This corresponded to a chloride concentration of $10^{-2}M$ in our system.

Under these conditions standards containing various amounts of soil gave reliable readings.

cis- and *trans*-1,3-dichloropropene and *cis*- and *trans*-3-chloroallyl alcohol were qualitatively monitored during a run by direct, flame ionization gas chromatography on a 5-foot $^1/_8$-inch glass column of 5% diethylene glycol succinate on HMDS-treated Chromosorb W at 110°. Each dichloride isomer was examined separately. The respective emergence times were: *cis*-1,3-dichloropropene 0.5 minute, *trans*-1,3-dichloropropene 0.6 minute, *cis*-3-chloroallyl alcohol 1.3 minutes, and *trans*-3-chloroallyl alcohol 1.7 minutes. No peaks other than water and these materials were discernible.

Product Analyses. Ordinary rate runs for chloride release were carried out in bottles or in stationary beakers covered with aluminum foil. Typically, for product analysis, 300 cc. of soil were added to 1 liter of an aqueous solution of $1 \times 10^{-2}M$ *trans*-1,3-dichloropropene. The flask was swirled occasionally. After 66 days the chloride ion concentration was $8.9 \times 10^{-3}M$. A 500-ml. sample was withdrawn from the flask after 1 hour of mechanical shaking. The mixture was saturated with ammonium sulfate and extracted five times with ether. The ether extracts were dried over sodium sulfate. The ether was removed through a small Vigreux column and the residue was dissolved in 1 ml. of *n*-hexane.

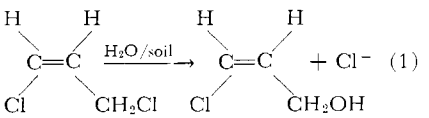
Gas chromatography of the hexane solution upon a 6-foot Dow Corning −710 column at 90° showed only one peak, which corresponded exactly with that of authentic *trans*-3-chloroallyl alcohol. The product substance was repeatedly trapped from 50-μl. injections. Its infrared spectrum was identical with that of authentic *trans*-3-chloroallyl alcohol. The amount of chloroallyl alcohol in the hexane solution was quantitated by the addition of authentic material. The over-all yield based upon chloride produced was 40%.

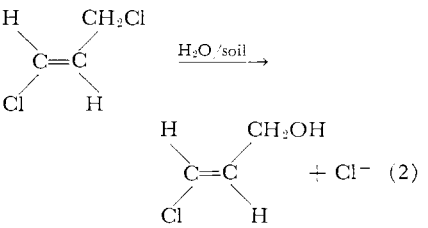
Under identical conditions *cis*-1,3-dichloropropene had hydrolyzed to the extent of 50% in 20 days. The product *cis*-3-chloroallyl alcohol was identified by gas chromatography and its infrared spectrum, both of which were identical with an authentic sample. The yield of alcohol was 40%.

Results and Discussion

The rate of chloride release from a $10^{-2}M$ solution of *cis*-1,3-dichloropropene buffered at either 6.9 or 7.5 pH is depicted in the lower curve of Figure 1. The other points represent chloride release from $10^{-2}M$ solution of this halide when mixed with soil in the ratios: soil-solution of 0.5, 1, 2, and 3. Points represent an average of at least two independent runs. The maximum pH change during a run was from 7.5 to 7.7. Reproducibility in the soil water runs was within 10%. The points are also corrected for small blank readings of chloride in soil water mixtures of the same composition but without added dihalopropene. These amounted to, at most 0, 0.1×10^{-3}, and 0.2×10^{-3} after 1, 2, and 3 days, respectively. The rate of halide release is relatively insensitive to the amount of soil present. Thus from initial slopes the rate of hydrolysis of *cis*-1,3-dichloropropene at a soil-solution ratio of 0.5 is only 1.4 times that in the absence of soil. Moreover, a change from a ratio of 1 to 3 elicits no significant change in the kinetics. The mixtures of higher soil content enhance the rate to about the same extent and at best only threefold.

In accord with the kinetics, the only detectable organic products from either





cis- or *trans*-1,3-dichloropropene are the corresponding allylic alcohols (Equations 1 and 2). Neither the starting halides nor the alcohols were isomerized by these conditions. The yields of the alcohols determined were approximately 40% and we attribute the lack of a complete material balance to the difficulty of extracting the alcohols from soil-water mixtures.

Soil does not inhibit the normal solvolysis (*1*) of *cis*- or *trans*-1,3-dichloropropene and the biocidal properties of the chloroallyl alcohols must be considered when the dihalide fumigants are employed. It is significant that the chloroallyl alcohols are rather wide-range biocides (*5*). The fate of these alcohols in soil is under investigation.

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HERBICIDE COLORIMETRIC PROCEDURES

Evaluation of the Pyridine-Alkali Colorimetric Method for Determination of Atrazine

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THE pyridine-alkali method for the determination of chloro-*s*-triazines and the reactions involved in color development have been reviewed by Burchfield and Schuldt (*2*), Gysin and Knüsli (*3*), Knüsli *et al.* (*5*), and Ragab (*7*). The mechanism of the reaction between pyridine and the chloro-*s*-triazines is probably similar to the mechanism described by Zincke (*10*) for the reaction between pyridine and 1-chloro-2,4-dinitrobenzene.

The first application of this reaction to the analysis of a 2-chloro-*s*-triazine herbicide (simazine) was by Ragab (*7*), who investigated several modifications of the pyridine-alkali reaction leading to conversion of the unstable yellow color to other more stable colors. Reaction of the yellow complex with ethyl cyanoacetate gave a red color showing maxi-

An evaluation of the pyridine-alkali and pyridine-alkali–ethyl cyanoacetate techniques for the colorimetric determination of atrazine (2-chloro-4-ethylamino-6-isopropylamino-*s*-triazine) showed that color intensity was influenced by the pH of the system and temperature of reaction of the atrazine-pyridine complex with alkali. Color intensity increased with increased acidity and a suitable temperature for color development is 20° ± 2° C. The sensitivity of the pyridine-alkali–ethyl cyanoacetate method was 0.02 p.p.m. with a precision of ±1.5%. Either technique is adaptable to other 2-chloro-*s*-triazine herbicides.

mum absorbance at 550 mμ, whereas other modifications were more involved and time-consuming. The color formed in the pyridine-alkali reaction was found to be subject to daily fluctuations which would also cause similar variations in the color formed by the pyridine-alkali–ethyl cyanoacetate reaction. Daily comparison with standards was recommended to compensate for daily variations (*5*). Information concerning the cause of such fluctuations was largely unavailable. However, the use of glycine to increase the color intensity indicated that pH could be important (*7*).

The pyridine-alkali reaction is compared to the pyridine-alkali–ethyl cyanoacetate reaction on the basis of varying pH and temperature to determine the most accurate and reproducible method for the determination of atrazine.

Reagents

Stock solution, purified atrazine prepared in 95% ethanol (250 p.p.m.).

Standard solutions, prepared from stock solution by dilution with water or buffer solution.

Buffer solutions, 0.01*M* with respect to citric acid, monobasic sodium phosphate, and boric acid; the desired pH obtained by titration with NaOH or HCl; 0.25*M* citric acid buffer titrated to pH 4.0 with NaOH.

Ethyl cyanoacetate, Matheson Coleman & Bell Division, b.p. 97–99° C. at 15 mm.

Pyridine, reagent grade, diluted to 70% (v./v.) with water.

Procedure

The two methods employed for the colorimetric determination of atrazine were essentially those of Ragab (*7*) with the following modifications: buffering of atrazine solutions, control of the solution temperature on addition of NaOH, and control of the time intervals between addition of NaOH and measurement of the yellow color intensity or addition of ethyl cyanoacetate.

Pyridine-Alkali (P-A) Technique. Atrazine solutions (5 ml.) of 0 to 3 p.p.m. were pipetted into test tubes and 70% pyridine (1 ml.) was added. When it was inconvenient to prepare the atrazine directly in buffer, 0.25*M* citric acid buffer (1 ml.) was added to the atrazine solutions (4 ml.). The tubes were placed in a boiling water bath for 30 minutes, and cooled to 0°, 20°, or 30° C. in water baths. Large glass marbles, placed on the tubes, served as condensers to prevent excessive evaporation. After allowing time for temperature equilibration, 9*N* NaOH (1 ml.) was added, the contents of the tubes were mixed, and the absorbances of the resulting yellow solutions were measured at 436.5 mμ. Each absorbance reading was made 1 minute after addition of NaOH—i.e., the time of maximum color intensity. The color faded rapidly after 1 minute.

Pyridine-Alkali–Ethyl Cyanoacetate (P-A-E) Technique. The technique was basically a modification of the P-A technique as follows: One minute following the development of the yellow color as described above, ethyl cyanoacetate (1 ml.) was added. The contents of the tubes were mixed, 95% ethanol (1 ml.) was added, and the mixtures were agitated. By this means the unstable yellow color was converted to a stable red color showing an absorption maximum at 550 mμ.

Color Stability. Samples were analyzed by the P-A technique, with development of the yellow color at 0°, 20°, and 30° C. Color stability was determined by making absorbance measurements over a period of 20 minutes while maintaining each sample at 0°, 20°, or 30° C.

Light sensitivity of the color in both the P-A and P-A-E techniques was investigated by a comparison of color stability in light and dark over a 4-hour period.

Determination of Atrazine in Water Extracts of Soils. Soil extracts were obtained by continuous recycling of a mineral salts–sucrose medium through columns of three soils for several days, utilizing perfusion apparatus similar to those described by Lees (*6*). The perfusion medium consisted of: K_2HPO_4 (0.2 gram), $CaSO_4$ (0.2 gram), $MgSO_4$ (0.2 gram), $FeSO_4$ (1 mg.), NH_4NO_3 (0.3 gram), and sucrose (0.1 gram) per liter of solution. Samples of soil extracts were withdrawn from the perfusion units and known amounts of atrazine added to the sampled solution. The solutions were buffered by the addition of pH 4.0 citrate (1 ml.) to each sample (4 ml.); temperature prior to addition of NaOH was maintained at 20° C. The accuracy of the P-A-E method in determining atrazine in the presence of substances appearing in soil extracts was ascertained by estimating the recovery of added atrazine.

Soil Analysis. Organic matter determinations were made by chromic acid oxidation (*9*) and clay determinations by the method described by Jackson (*4*). Soil pH was measured on a thin soil paste, equilibrated for 30 minutes.

Statistical Methods. For regression through the origin

$$b = \frac{\Sigma XY}{\Sigma X^2}$$

where b = absorbance per p.p.m. and Y = absorbance of a solution of atrazine concentration × p.p.m.

The coefficient of variability is defined as the standard error of estimate of Y expressed as a percentage of the mean absorbance, $\bar{Y}$ (*8*).

Each coefficient of variability and corresponding absorbance per part per million was calculated on the basis of 27 samples, which consisted of three separate standard curves of nine samples each run over a period of 2 days.

Results and Discussion

The rate of formation and fading of the color developed in the pyridine-alkali (P-A) method was dependent on the temperature of the solution at the time of color development (Figure 1). Rapid fading rates at 20° and 30° C. necessitated use of an equal time interval between color development and measurement of absorbance. Measurement of absorbance 1 minute after color development at 20° C. provided highly reproducible results (Table I). The sensitivity of the determination decreased linearly with time at a rate of approximately 12% in 20 minutes at 20° C. It is essential, therefore, that the time interval between color development and absorbance reading be carefully determined and held as short as possible. Replication at 0° C. was unsatisfactory even at time intervals corresponding to maximum color intensity. Fluctuations in absorbance were attributed to difficulty in maintaining temperatures at 0° C. Maintenance of a constant temperature was more critical than at 20° or 30° C. because color intensity exhibited greater temperature dependence near 0° C.

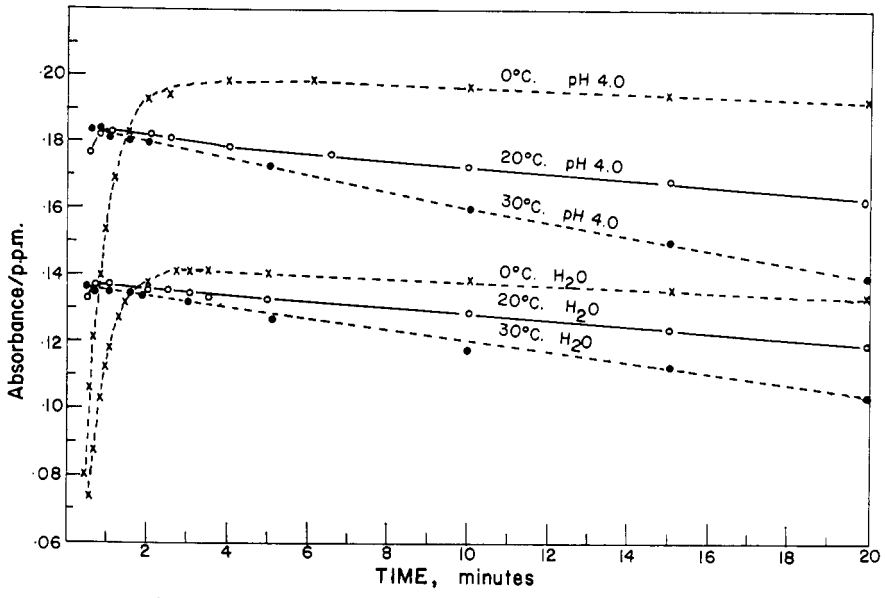

Figure 1. Effect of temperature on intensity and stability of color in P-A method

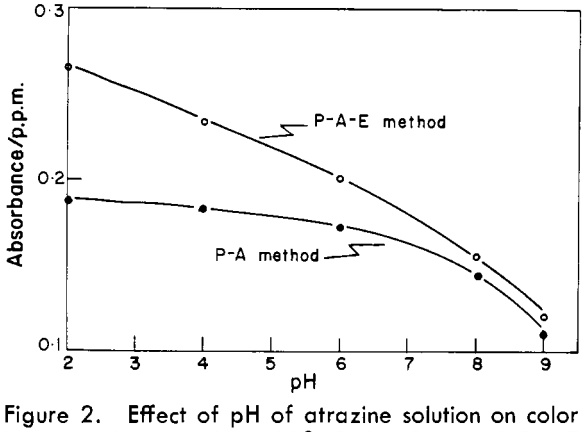

Figure 2. Effect of pH of atrazine solution on color intensity developed at 20° C. by P-A and P-A-E methods

Table I. Effect of pH on Determination of Atrazine by P-A and P-A-E Methods at 20° C.

Method	Buffer	Absorbance/ P.P.M.	Coefficient of Variability, %
P-A	None	0.137	2.2
P-A	pH 4	0.183	1.9
P-A-E	None	0.170	4.2
P-A-E	pH 4	0.237	1.5

Table II. Absorbance as Affected by Temperature Using P-A-E Method

Temp., ° C.	Absorbance/P.P.M. In H_2O	Absorbance/P.P.M. In pH 4.0 buffer
0	0.127	0.155
20	0.170	0.237
30	0.164	0.237

The intensity of the color obtained by the P-A method increased with decreasing buffer pH and elimination of pH fluctuations through use of a buffered system led to improved reproducibility and sensitivity (Table I and Figure 2).

The red color developed in the pyridine-alkali–ethyl cyanoacetate technique was formed directly from the yellow color obtained by the P-A technique and again necessitated temperature control (Table II). The temperature trend of these absorbances at 1 minute after color development was the same as that for the yellow color (Figure 1). When longer periods of time elapsed between development of the red and yellow colors, the absorbances followed the sequence expected from Figure 1.

The pH dependence of the P-A-E method is shown in Figure 2 and Table II. Although the absorbance of the red color increased with decreasing buffer pH, the addition of a strong acid (2 to 3 ml. of 6.V HCl) to the system after color formation completely destroyed the color. As in the P-A method, elimination of pH variances by use of buffer solutions greatly increased the precision of the P-A-E method (Table I).

Inconsistencies in the color stability of the P-A-E method were attributed to light sensitivity of the red color. Samples placed in darkness immediately after development of the red color were completely stable for at least 4 hours, whereas samples placed in light faded from 10 to 25% per hour, depending on the light intensity. Shadowing samples diminished light intensity sufficiently so the red color was stable for about 30 minutes and then faded approximately 5% in the next 30 minutes. However, fading of samples in light was erratic and poor replication was obtained unless absorbance was measured immediately after color development. The yellow color developed by the P-A method was totally unaffected by light.

The absorbance of both the red and yellow colors obeyed Beer's law, with the P-A-E method being more sensitive than the P-A method. The use of buffered systems increased the precision of both determinations, although fluctuations in color intensity in nonbuffered systems were most pronounced with the P-A-E method.

Highly satisfactory results were obtained when the temperature was controlled at 20° ± 2° C. Although minor variations in temperature were not critical, cooling was done in a controlled temperature water bath because cooling quenches the reaction between pyridine and atrazine. The pyridine-atrazine reaction was not complete at 30 minutes and the reaction continues slowly if longer heating times are employed. Failure to cool the solution rapidly resulted in fluctuations in color intensity due to further reaction.

The P-A-E method offered the advantage of color stability in darkness, so that timed measurement was not required. For soil and plant extracts which often appear yellow, the red color of the P-A-E method is subject to less interference than the yellow color of the P-A method.

In related studies, it has been necessary to determine atrazine present in the water extracts of soils obtained in soil perfusion studies. In the perfusion system, a solution containing atrazine is continually recycled through a soil column and the rate of atrazine degradation in the system is followed by periodically sampling the perfusate and determining the concentration of atrazine remaining. Atrazine added to soil perfusates was successfully determined using the P-A-E technique (Table III). Although the recovery values were somewhat greater than 100%, the results were within acceptable error limits in view of the appreciable amounts of contaminating soil components present in the perfusate. The method shows no interferences when applied to water extracts of soils and is less time-consuming than ultraviolet spectrophotometric methods, which require a prior hydrolysis

Table III. Recovery by P-A-E Method of Atrazine Added to Water Extracts of Soils

Soil Type	Soil pH	Organic Matter, %	Clay, %	Atrazine, μg. Added	Atrazine, μg. Found	Recovery, %
Poygon sil	6.9	13	26	6.16	6.36	103
Ella ls	4.6	4	5	6.16	6.40	104
Kewaunee c	7.3	2	40	6.16	6.36	103

of atrazine to hydroxyatrazine. Interferences in the ultraviolet method are numerous when applied to determination of atrazine in a microbiological medium. Furthermore, in investigations requiring determination of atrazine and its degradation product, hydroxyatrazine, the preferred method is colorimetric determination of atrazine and ultraviolet determination of hydroxyatrazine after removal of atrazine by chloroform extraction (*1*). No interference in the colorimetric determination of atrazine is encountered from the presence of hydroxyatrazine.

Both the P-A and P-A-E techniques were investigated for the determination of simazine and propazine. The factors affecting the determination of atrazine were similarly important in the determination of the other two *s*-triazine herbicides. The absolute absorbance for each compound was different, and the techniques are probably readily adaptable to the analysis of other 2-chloro-*s*-triazine herbicides.

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STRUCTURE AND SYNERGISM

Some Structural Requirements of Methylenedioxyphenyl Derivatives as Synergists of Carbamate Insecticides

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Sixty-two compounds, many of them new, were evaluated as synergists for 1-naphthyl *N*-methylcarbamate (carbaryl), 3,4-dimethoxyphenyl *N*-methylcarbamate, and 4-dimethylamino-3,5-xylenyl *N*-methylcarbamate against the common housefly. Maximum synergistic activity is associated with the planar methylenedioxyphenyl ring system. However, replacement of oxygen by sulfur causes only a slight decrease in activity. Synergistic activity in esters of piperonyl alcohol is considerably modified by the nature and position of the side chain on the alpha-carbon.

The essentiality of the 3,4-methylenedioxyphenyl moiety (1,3-benzodioxole) in synergism of the pyrethrins with sesamin was established by Haller *et al.* (*26, 27*). This discovery led to the evaluation of thousands of compounds as potential pyrethrin synergists (*8, 28, 32*) and several, such as piperonyl butoxide, piperonyl cyclonene, *n*-propyl isome, sulfoxide, sesoxane, and sesamex, have become commercially important. However, few structure-activity relationships have been developed. Moore and Hewlett (*37*) demonstrated the inactivity of pyrethrin synergists of compounds containing isopropylidenedioxy (2,2-dimethyl-1,3-benzodioxole), carbonyldioxyphenyl (1,3-benzodioxole-2-one), and ethylenedioxyphenyl (1,4-benzodioxane) groups (*21, 45*).

The discovery of Moorefield (*38, 39*) that the established methylenedioxyphenyl synergists could greatly enhance the activity of the carbamate insecticides stimulated renewed interest in the field, chiefly because of their possible potential use in combating carbamate-resistant strains of insects. A considerable amount of work has since been undertaken on a variety of aspects of carbamate synergism (*22, 24, 25, 33, 34*). However, the only study relating synergist structure to carbamate synergism is that of Moorefield and Weiden (*40*), who evaluated various substituted benzyl acetals and related compounds as synergists for carbaryl (Sevin) and concluded that the 1,2-methylenedioxyphenyl structure was important for maximum synergism.

In contrast to the situation with the pyrethrins, where little is known regarding the site or mode of action or the detoxication process, considerably more data are available on these aspects of carbamate toxicology (*13, 14, 19, 20*). Therefore, it appeared of value to study the structure-activity relations for carbamate synergists in order to further the knowledge of the mode of action of these compounds and to clarify the detoxication pathways of carbamates in susceptible and resistant strains of insects. Three major aspects were studied: the effect of the nature of the side chain on activity of the 1,3-benzodioxole nucleus, the effect of nuclear substitution, and the effect of alterations in the 1,3-benzodioxole ring.

Materials and Methods

The physical constants of the compounds synthesized and evaluated as carbamate synergists in this investigation