ml. aliquots to 100 ml. with absolute methanol. The resulting solutions will contain, respectively, 10, 15, and 20 γ per ml. of Co-Ral.

Procedure. Weigh accurately approximately 0.2 gram of technical Co-Ral and place in a 250-ml. volumetric flask. Add 100 ml. of 1,4-dioxane and 5 ml. of 0.1N hydrochloric acid, stopper, and shake until the Co-Ral is dissolved. Dilute to volume with absolute methanol (Solution 1). Pipet a 2-ml. aliquot of the solution into a 100-ml. volumetric flask, and again dilute to volume with absolute methanol (Solution 2). Measure the absorbance in a 1-cm. silica cell at 290 mμ in a suitable spectrophotometer. Use a 0.8% v./v. solution of 1,4-dioxane in absolute methanol as a blank.

For the estimation of chloromethylumbelliferone, pipet 50 ml. of Solution 1 into a 100-ml. volumetric flask. Pipet in 50 ml. of 1% aqueous sodium carbonate solution. Cool rapidly to room temperature and dilute to volume with absolute methanol. Within 15 minutes of the sodium carbonate addition, read the absorbance in a 1-cm. cell at 380 mμ against a blank solution containing the same proportions of methanol, 1,4-dioxane, and aqueous sodium carbonate as are present in the sample solution. Calculate the chloromethylumbelliferone content by reference to the appropriate calibration curve. Multiply the chloromethylumbelliferone concentration by 0.67 and subtract from the Co-Ral value obtained at wave length 290 mμ.

Discussion

Reproducibility. The reproducibility of both methods is shown in Table II. The results show that the hydrolysis procedure is capable of somewhat greater precision than the spectrophotometric method. This is to be expected. However, the reproducibility of the spectrophotometric procedure is satisfactory for analytical control purposes. The precision of the spectrophometric method is reduced by running duplicate determinations. In general, the spectrophotometric method is preferred, because it permits a ready correction for chloromethylumbelliferone.

Table II. Reproducibility of Methods for Co-Ral Determination

Method	Material	Mean Value[a]	Standard Deviation
Hydrolysis	Recrystallized	100.73 (9)	0.15
Spectrophotometric	Technical	96.3 (4)	0.6
		94.9 (4)	0.5
		93.8 (4)	0.5
		92.3 (4)	0.6

[a] Number of values used to calculate each mean shown in parentheses.

The accuracy of the method for determination of chloromethylumbelliferone in Co-Ral was tested by preparing synthetic mixtures of the two compounds. The chloromethylumbelliferone results are shown in Table III. It is apparent that the method described will give very accurate values for chloromethylumbelliferone in the presence of Co-Ral.

Table III. Determination of Chloromethylumbelliferone Content of Co-Ral

Chloromethylumbelliferone, %	
Present	Found
0.99	0.99
0.99	0.99
2.00	1.98
2.01	2.00
3.02	3.01
3.01	2.99

The presence of more than small amounts of chloromethylumbelliferone can be detected by the hydrolysis method, although the actual concentration of interfering material cannot be determined accurately by this method. The presence of chloromethylumbelliferone will result in a change in the relative number of moles of acid obtained on titration to pH 7.5 and 3.0. In the case of pure Co-Ral, this ratio is 3 to 2 (Figure 1), while for chloromethylumbelliferone it is 1 to 1.

Acknowledgment

The authors thank Fred N. Larsen for technical assistance.

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Received for review April 22, 1959. Accepted August 10, 1959. Presented, in part, before the Division of Agricultural and Food Chemistry, 134th Meeting, ACS, Chicago, Ill., September 1958.

FUNGICIDE ASSAY

Determination of Dyrene and Cyanuric Chloride in Technical Materials

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Dyrene (2,4-dichloro-6-*o*-chloroanilino-*s*-triazine), previously known as Kemate or B-622, is a new organic fungicide which has proved outstandingly effective for the control of fungus diseases on a number of crops. It is prepared by the reaction of cyanuric chloride with *o*-chloroaniline in the presence of sodium carbonate. This study was undertaken to develop a suitable assay method for this product in the presence of possible contaminants.

A residue method for Dyrene (*5*) is based on acid hydrolysis to form *o*-chloroaniline. The *o*-chloroaniline is then diazotized and coupled with *N*-1-naphthylethylenediamine to produce a colored complex. This method is time-consuming, as the hydrolysis requires 2 hours of reflux with hydrochloric acid. A second colorimetric procedure involving the Zincke reaction (*2*) has also been described. In general, volumetric methods are more accurate than colorimetric procedures in cases where the former can be employed. Therefore, it was decided to investigate the hydrolysis of the chlorine atoms of Dyrene, because chloride ion can be readily determined volumetrically. A second volumetric

The hydrolysis of Dyrene and cyanuric chloride has been investigated in acid, neutral, and alkaline aqueous media. On treatment of Dyrene under reflux with 1*N* sodium hydroxide for 10 minutes, both of the chlorine atoms on the cyanuric chloride ring are released. Under the same conditions the three chlorine atoms in cyanuric chloride are liberated. On the other hand, refluxing with water completely liberates the chlorine from cyanuric chloride in 20 minutes, while under the same conditions no chlorine is liberated from Dyrene. These facts are utilized in an analytical method for Dyrene which contains cyanuric chloride. Inorganic chloride in the material is determined by solution in ice water and direct titration with silver nitrate.

possibility would have been to titrate the *o*-chloroaniline, formed on acid hydrolysis, with standard nitrite. However, the chloride determination has some advantages over the nitrite titration and therefore the former approach was investigated.

In the development of the chloride method for Dyrene, the principal interferences to be considered are inorganic chloride and cyanuric chloride, both of which are commonly present in the technical material as impurities. Considering the wide use of cyanuric chloride as a raw material, information on its reactions with aqueous reagents is surprisingly meager and, occasionally, contradictory. Fierz-David and Matter (*4*) state that cyanuric chloride is almost unaltered after heating for a short time with potassium hydroxide solution and with further heating is saponified completely to tripotassium cyanurate. In the same paper, however, they report that at 100° C., 10% sodium hydroxide gives only the dihydroxy compound. Comp and Heckle (*3*), on the other hand, found that these conditions yield cyanurate in 2 hours.

Experimental

Hydrolysis of Dyrene. The hydrolysis of Dyrene was investigated in aqueous media by refluxing 0.5 gram of recrystallized material for varying periods of time with 1*N* sodium hydroxide, water, and 1*N* hydrochloric acid. The curves obtained are shown in Figure 1. The figures for Dyrene hydrolyzed are based on the release of two equivalents of chloride per mole. In the case of the alkali hydrolyzates, the solution was acidified with nitric acid before titration with 0.1*N* silver nitrate, using a glass-silver electrode system to detect the end point. The aqueous and acid hydrolyzates were titrated without further treatment, allowance being made in the case of the hydrochloric acid hydrolyzate for the chloride added.

As can be seen, the alkaline hydrolysis is virtually complete within 10 minutes. With acid the reaction is much slower, although still appreciable. With water, there was no appreciable hydrolysis at least up to 30 minutes.

Hydrolysis of Cyanuric Chloride. The cyanuric chloride (Matheson Coleman & Bell, Cincinnati, Ohio), melting point 144 to 146 ° C., was hydrolyzed in 1*N* sodium hydroxide, 1*N* hydrochloric acid, and water by determining the chloride. In each case, a 0.3-gram sample of material was reacted with 50 ml. of reagent. The extent of hydrolysis in each medium was determined at 15 °, 25°, and 50° C., and reflux for varying periods of time. The results are shown in Figures 2, 3, and 4. The cyanuric chloride figures are based on 3 equivalents of chloride per mole.

The results show that cyanuric chloride is much more rapidly hydrolyzed in alkali than in water or acid. In the former medium, hydrolysis is virtually complete in 20 minutes even at 25° C. On the other hand, in acid or water, reflux temperature is required to obtain complete reaction in a reasonable length of time.

Comparison of Figures 1 and 4 shows that on refluxing with water, the hydrolysis of cyanuric chloride is essentially complete in 20 minutes. Under the same conditions, Dyrene is virtually unaffected. Thus, a possible method of determining cyanuric chloride in the presence of Dyrene presented itself.

As small amounts of inorganic chloride are liable to be present in technical Dyrene, a method for determination of cyanuric chloride in the presence of the former contaminant was sought. From Figure 4, it is shown that the rate of hydrolysis of cyanuric chloride decreases rapidly as the temperature is lowered.

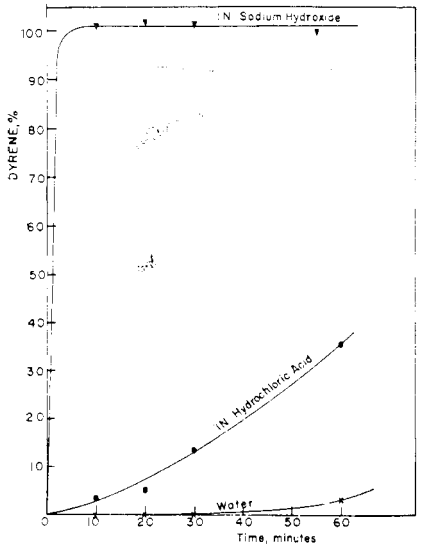

Figure 1. Hydrolysis of Dyrene at reflux

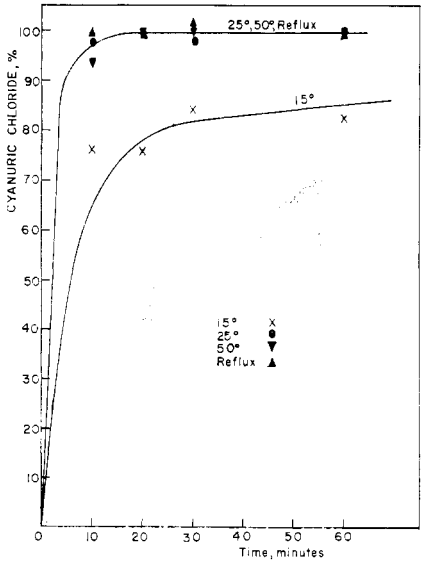

Figure 2. Hydrolysis of cyanuric chloride in 1*N* sodium hydroxide

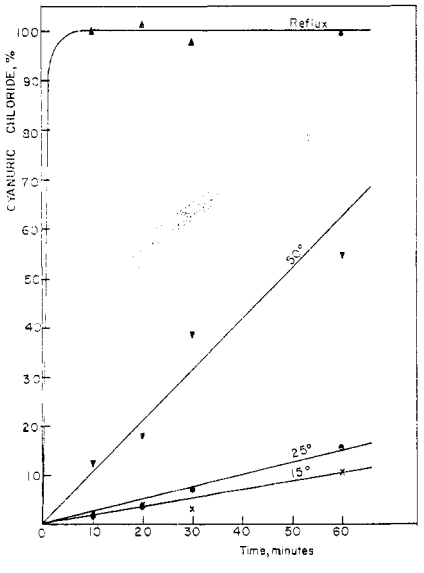

Figure 3. Hydrolysis of cyanuric chloride in 1*N* hydrochloric acid

The possibility that at 0° C. the hydrolysis of cyanuric chloride might be sufficiently slow to allow the direct titration of inorganic chloride was investigated. The results showed that a small amount of hydrolysis of cyanuric chloride occurs even at 0° C. Under these conditions, cyanuric chloride was 0.24% hydrolyzed in 10 minutes. However, a rapid titration of inorganic chloride in a solution held at 0° C. would give an almost correct result, if the cyanuric chloride content of the sample is not too great. Fierz-David and Matter (*4*) have reported that no hydrolysis of cyanuric chloride occurs in 12 hours at 0° C. The authors have been unable to confirm their results.

Application to Analysis of Technical Dyrene. Consideration of the various hydrolysis curves (Figures 1 to 4) indicated the following approach to the problem of analyzing technical Dyrene.

1. Determination of total hydrolyzable chloride by refluxing for 30 minutes with sodium hydroxide. This will determine chloride from Dyrene, cyanuric chloride, and sodium chloride.
2. Determination of water-hydrolyzable chlorine by refluxing with water. This will determine chloride from cyanuric chloride and sodium chloride.
3. Determination of sodium chloride alone by dissolving in ice cold water.

Determination of Total Hydrolyzable Chlorine. The method was first applied to recrystallized Dyrene by refluxing 0.5 gram for 30 minutes with 1*N* sodium hydroxide, acidifying with nitric acid, and titrating the liberated chloride potentiometrically with 0.1*N* silver nitrate. Dyrene is initially insoluble in the reagent, but rapidly passes into solution when hot. Eleven determinations on a recrystallized material gave a mean of 100.68% with a standard deviation of ±0.34%, a mean significantly higher than 100. This may possibly have been due to the formation of small amounts of cyanate. A further series of six determinations was carried out. The hydrolyzate was acidified under reflux for 30 minutes before titration. A mean of 99.19% was obtained with a standard deviation ±0.14%. Apparently, some volatile or acid-hydrolyzable material was removed as a result of the refluxing.

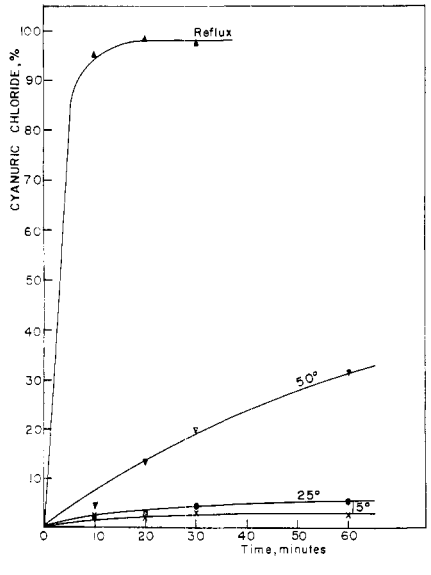

Figure 4. Hydrolysis of cyanuric chloride in water

Table I. Reproducibility of Various Chlorine Determinations on Mixtures of Dyrene, Cyanuric Chloride, and Sodium Chloride

Determination	Calcd., %	Found, % $\bar{x}$	s	n
Total hydrolyzable chlorine, as Dyrene	101.81	101.65	±0.11	6
Water hydrolyzable chlorine, as cyanuric chloride	3.42	3.45	±0.05	6
	3.46	3.48	±0.04	5
Ionizable chlorine, as sodium chloride	0.98	0.96	±0.07	5
	0.90	0.96	±0.04	5
	0.98	0.99	±0.01	5

Determination of Water-Hydrolyzable Chlorine. From the hydrolysis curves of Dyrene and cyanuric chloride it would appear that 20 minutes would be the optimum reflux time for the estimation of the latter compound in the presence of the former. However, under these conditions Dyrene is hydrolyzed to the extent of about 0.2%. Under the condition where a small amount of cyanuric chloride (less than 3%) is present in a large amount of Dyrene, the error due to Dyrene hydrolysis becomes appreciable. In an effort to compensate for this error, the hydrolysis time was reduced to 10 minutes. Under this condition the cyanuric chloride is only about 95% hydrolyzed, but the value will be compensated by a very slight hydrolysis of Dyrene.

Determination of Ionizable Chloride. The method proposed for this constituent was simply to stir the sample for 5 minutes with ice-cold water containing chipped ice and titrate potentiometrically with 0.1*N* silver nitrate at the same temperature. Because of the comparatively small cyanuric chloride content of technical Dyrene, interference from the hydrolysis of this compound would be expected to be negligible.

Analytical Procedure

Method. Weigh a 0.5-gram sample into a 300-ml. Erlenmeyer flask. Pipet 50 ml. of 1.0*N* sodium hydroxide into the flask, swirl, and attach a reflux condenser. Reflux for 30 minutes. Cool and rinse down the condenser with water. Add one drop of 1% phenolphthalein followed by sufficient dilute nitric acid—1 to 2—to discharge the color, then use 10 ml. in excess. Reflux for 30 minutes more. Cool, rinse down the condenser, and transfer the solution to a 400-ml. beaker. Insert a bright silver cathode and glass anode connected to a suitable potentiometer and titrate to the end point with 0.1*N* silver nitrate. One milliliter of 0.1*N* silver nitrate is equivalent to 3.546 mg. of chloride. Let the total hydrolyzable chlorine be A%.

Table II. Analysis of a Mixture of Dyrene, Cyanuric Chloride, and Sodium Chloride

Component	Present, %	Found, %
Dyrene	92.90	92.80
Cyanuric chloride	2.61	2.64
Sodium chloride	1.13	1.16

Weigh a 5-gram sample into a 300-ml. Erlenmeyer flask. Add 100 ml. of water, swirl, and reflux under a condenser for 10 minutes. Titrate with silver nitrate by the same method as above. Let the water-hydrolyzable chlorine be B%.

Weigh a 5-gram sample into a 400-ml. beaker. Add 100 ml. of ice-cold water and sufficient chipped ice to maintain the temperature during titration. Stir for 10 minutes and titrate with silver nitrate as before. Let the ionizable chloride be C%.

Calculation:

$$\text{Dyrene content} = (A - B) \times 3.886\%$$

$$\text{Cyanuric chloride content} = (B - C) \times 1.733\%$$

$$\text{Sodium chloride content} = C \times 1.648\%$$

Reproducibility and Accuracy. In order to test the reproducibility and accuracy of the analytical method developed, several synthetic mixtures of Dyrene, cyanuric chloride, and sodium chloride were prepared. These were analyzed for total hydrolyzable chlorine, water-hydrolyzable chlorine, and inorganic chloride. The results, as percentages, are shown in Table I. This experiment was designed to show the reproducibility of the different steps. These results cannot be used to calculate the actual Dyrene and cyanuric chloride contents of the samples, as the same mixtures were not used throughout. Table I shows that both accuracy and reproducibility are adequate for each of the three determinations. The results for inorganic chloride do not appear to be increased significantly due to slight hydrolysis of cyanuric chloride.

In reporting an analysis, the Dyrene content is calculated from the difference of two determinations, as is the cyanuric

Table III. Total Hydrolyzable Chlorine in Cyanuric Chloride

Conditions	$\bar{x}$	s	n
Direct titration	99.78	±0.32	5
30-minute reflux after acidification	100.08	±0.20	5

chloride content. As a final check on the procedures, a mixture of this Dyrene with cyanuric chloride and sodium chloride was analyzed for all components. The results are shown in Table II. Each of the determinations was carried out five or six times. It can be shown that the differences between the calculated and found figures for the mixture are not significant as based on the standard deviations of the previous table. If each determination were carried out in duplicate, a standard error for the Dyrene content would be ±0.13%, for cyanuric chloride ±0.05%, and for sodium chloride ±0.04%. Both accuracy and reproducibility are satisfactory for control purposes.

The method is subject to interference by pesticides such as DDT or BHC which are capable of undergoing alkaline dehydrohalogenation.

Application to the Analysis of Technical Cyanuric Chloride. Following on from the above, there seemed no reason why the relevant methods should not be applied to the assay of technical cyanuric chloride. The published method (*1*) for this determination involves the reaction of excess sodium methylate with cyanuric chloride, the excess reagent being hydrolyzed and determined acidimetrically in the usual way. The results with aqueous sodium hydroxide suggested a somewhat simpler procedure.

From the hydrolysis curves (Figure 2), one would expect hydrolysis at room temperature to be erratic. This was confirmed by hydrolyzing a series of 0.25-gram samples for 30 minutes in 1.N sodium hydroxide at about 22° C. The liberated chloride was determined by direct titration in acid solution. Five determinations gave a mean of 93.32% with a standard deviation of ±2.46. As hydrolysis at room temperature was obviously unsatisfactory, the reaction was reinvestigated by heating for 30 minutes at reflux. In this study the effect of boiling after acidifying the hydrolyzate was investigated for cyanuric chloride as it had been for Dyrene. The results obtained by the refluxing procedure are shown in Table III. Theoretical values are obtained whether the posthydrolysis boiling step is included or not and this step was omitted in applying the procedure to a mixture of cyanuric chloride and sodium chloride (2.47%). In this case a mean value of 100.0% (standard deviation ±0.19 for seven determinations) was obtained. The calculated value for the sample was 99.92%.

The method for free chloride as described for Dyrene was unsatisfactory for cyanuric chloride because of a small amount of hydrolysis of the latter compound in ice water. Accordingly, a procedure (*1*) involving solution of the sample in chloroform and shaking for 2 minutes with ice-cold water was employed. In this method the inorganic chloride is supposed to be extracted into the water without causing any hydrolysis of cyanuric chloride. When this method was applied to pure cyanuric chloride, five determinations gave a mean value of 0.09% as sodium chloride. This indicated that a slight hydrolysis of cyanuric chloride was occurring even under these conditions. That the value is real and not due to a slight contamination of the cyanuric chloride with inorganic chloride was shown by the fact that a plot for the hydrolysis of cyanuric chloride in water at 0° C. went through the origin. If there had been as much as 0.1% of inorganic chloride present, this would not have been true.

When the method was applied to a sample of cyanuric chloride containing 2% of added sodium chloride, the mean of six determinations gave a value 0.1% higher than the theoretical. However, for control purposes this was not considered serious.

The total hydrolyzable chlorine procedure is suggested as an alternative to the methylate procedure for the analysis of cyanuric chloride. It may be corrected satisfactorily for ionizable chloride by the method described above.

Acknowledgment

The authors acknowledge the assistance of Daniel MacDougall in the preparation of the manuscript.

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Received for review May 18, 1959. Accepted August 27, 1959. Division of Agricultural and Food Chemistry, 135th Meeting, ACS, Boston, Mass., April 1959.

ACARICIDE BIOASSAY

Two Organisms Suitable for Bioassaying Specific Acaricides

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BIOASSAY with susceptible organisms is a well established method for determining residues of pesticides. However, the specific acaricides—pesticides specifically toxic to mites but virtually nontoxic to insects—cannot be successfully determined by bioassay with insects commonly used for this purpose.

Phytophagous mites, notably the two-spotted mite *Tetranychus telarius* (L.), have been used to screen candidate pesticides for acaricidal activity. Reports on this subject, found in the unpublished files of pesticide manufacturers, indicate a great deal of variability in response to any given level of toxicant. Several reasons may exist for such variability, but undoubtedly difference in host plants is a prominent factor. Thus variation in age and nutrition of plants probably affects the mites, and their almost continual dependence on plant food necessitates careful dipping or spraying practices, which are in turn modified by interactions between pesticide and plant surface. The instability of response to a given level of toxicant accounts for the present lack of bioassay methods for specific acaricides.

A current investigation on the useful-

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