Pyridine-Alkali Reactions in the Analysis of Pesticides Containing Active Halogen Atoms

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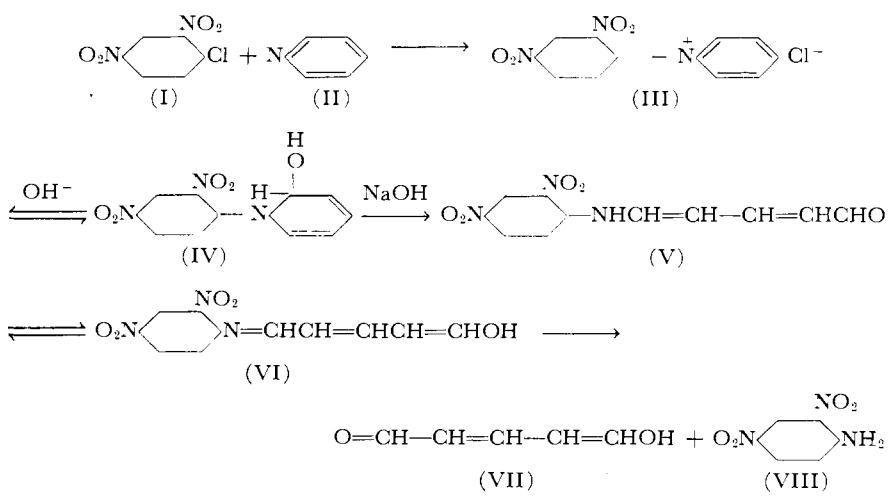
A number of pesticides such as the dichloro-*s*-triazines, 1-fluoro-2,4-dinitrobenzene, 3,4-dichlorotetrahydrothiophene-1,1-dioxide, and *N*-(trichloromethylthio)-4-cyclohexene-1,2-dicarboximide, which contain active halogen, can be determined quantitatively by colorimetric procedures, which are based on reactions with pyridine and alkali. Qualitative tests have been obtained with other fungicides, insecticides, herbicides, and fumigants. These reactions may have broad applications in the analysis of many of the pesticides used in agriculture.

THE REACTION which takes place between pyridine and 1-chloro-2,4-dinitrobenzene (CDNB) was originally described by Vongerichten (*36*), but the mechanism was first investigated in detail by Zincke and associates (*39*, *40*). The successive steps as presented by Mosher (*23*) are:



1-Chloro-2,4-dinitrobenzene (I) reacts with pyridine (II) to form a quaternary pyridinium halide (III) which undergoes addition of a hydroxyl group to form the carbinol base (IV). In the presence of alkali, the heterocyclic ring opens to yield an anil of glutaconic dialdehyde (V) which probably exists in equilibrium with the enol form (VI). This hydrolyzes on standing to form glutaconic dialdehyde (VII) and 2,4-dinitroaniline (VIII). The latter reaction can be reversed by the addition of an excess of amine. The Schiff base formed from 1-chloro-2,4-dinitrobenzene is red-violet in color, follows Beer's law, and is suitable for absorptiometric analysis in the visible region of the spectrum.

This reaction was first used analytically for the detection and determination of organic bases such as pyridine (*36*) and nicotinamide (*16*, *34*) using 1-chloro-2,4-dinitrobenzene as the reagent. Schechter and Haller (*27*) reversed this procedure by employing pyridine as a reagent for the detection of traces of 1-chloro-2,4-dinitrobenzene in the insecticide 2,4-dinitroanisole. These latter authors heated the aryl halide for 30 minutes in pyridine solution in a boiling water bath, and after cooling developed the color by diluting with ethyl alcohol and adding a 2% solution of sodium hydroxide in ethyl alcohol. Color formation took place in the absence of alkali but it was not as intense.

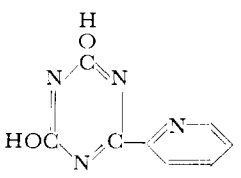
This technique was adapted by Burchfield and Storrs (*9*) to the quantitative determination of the soil fungicide 1-fluoro-2,4-dinitrobenzene (*22*) (experimental compound NP-1083). In this case, the aryl halide was dissolved in 10% aqueous pyridine and the solution made 1*N* with respect to sodium hydroxide, after standing for 20 minutes at room temperature. The red-violet color which developed had an absorption maximum at 548 mμ. It faded at a rate of about 1% per minute, but quantitative results could be obtained if readings were made precisely 2 minutes after the addition of the alkali. The molar absorptivity at the maximum was about 3.7×10^4.

A similar procedure was developed earlier (*6*) for the analysis of the foliage fungicide 2,4-dichloro-6-(*o*-chloroanilino)-*s*-triazine (Dyrene) and several related compounds (*30*, *37*, *38*). However, in these cases yellow colors were obtained with maxima at 440 mμ. The molar absorptivity obtained with Dyrene was 6.9×10^4 2 minutes after the addition of the alkali. Color intensity was dependent on the concentration of alkali; and to a lesser extent on the concentration of pyridine. The absorbance was increased by about 40% when the reagent (70% aqueous pyridine) was saturated with glycine, and decreased by about 10% in the presence of 0.03*M* phosphate buffer, so it was necessary to maintain careful control of the composition of the reaction mixture in order to obtain reproducible results. When this was not feasible, the *s*-triazines were extracted from the aqueous phase with petroleum ether or dichloromethane and redissolved in aqueous pyridine after evaporation of the solvent. This separated the *s*-triazine from the buffer and amino acids present in the original medium. As the color faded at a rate of 3% per minute at 25° C., the temperature and timing had to be controlled accurately to obtain quantitative results.

In view of this, results were always computed with reference to standards run at the same time and under the same conditions as the unknowns.

The interfering effects of pigments extracted from green plants and fungus spores were eliminated by carrying out the first step of the reaction in anhydrous pyridine and diluting with petroleum ether (boiling point 30° to 60° C.). The petroleum ether layer was then extracted with water, and the color was developed by the addition of sodium hydroxide to the aqueous phase. The plant pigments remained in the organic phase while the quaternary corresponding to III was removed quantitatively by a single extraction.

There remains some doubt whether the mechanism of this reaction is the same as that described by Zincke and associates (*39*, *40*) for 1-chloro-2,4-dinitrobenzene, as the absorption maximum is at 440 mμ rather than 548 mμ. However, the difference in color is probably caused by the absence of nitro groups in the *s*-triazine. Two moles of chloride are eliminated during the reaction, and aqueous solutions of the intermediate are highly surface-active, which would be expected if a quaternary base is formed (*6*). However, *N*-alkylation might not be the only reaction which occurs as Saure (*25*) has shown that compounds such as



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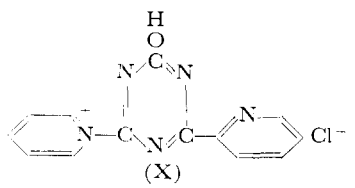
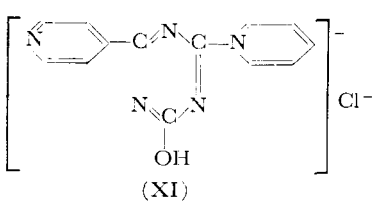


(X)

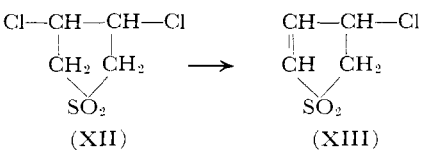
are formed on reaction of cyanuric chloride with aqueous pyridine. Menon, Nath, and Aggarwal (*21*) found the principal reaction products, in hot or cold acetone, to be X and



(XI)

so evidently *C*-alkylation as well as *N*-alkylation can occur, while one of the chlorine atoms may be replaced by a hydroxyl group. This latter step is less likely to occur with the 6-anilino-*s*-triazines, as these compounds hydrolyze extremely slowly in aqueous solution (*7*). Possibly some *C*-alkylation occurs, but the *N*-alkylation products are probably responsible for the color which is formed in alkaline solution.

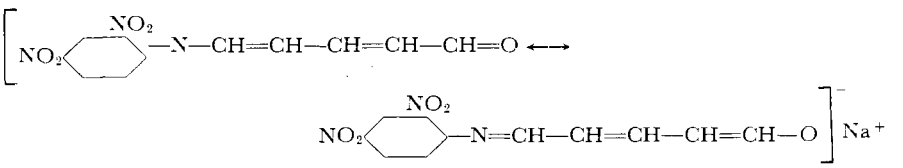
The best indirect evidence for the stepwise conversion of an active halogen compound to its corresponding amine and glutaconic dialdehyde was obtained in the case of the nematocide (*12*, *28*, *29*) 3,4-dichlorotetrahydrothiophene-1,1-dioxide (PRD). This compound (XII) probably undergoes a preparatory dehydrohalogenation step in the pyridine reagent to form 3-chloro-2,3-dihydrothiophene-1,1-dioxide (XIII), as both materials yield reaction products with identical optical characteristics (*6*).



(XII) (XIII)

These compounds were found to be much less reactive than the dichloro-*s*-triazines or 1-fluoro-2,4-dinitrobenzene (FDNB), so it was necessary to heat them for 30 minutes in 60% pyridine at 100° C. to obtain the precursors of the chromophores on which the colorimetric measurements were made. Maximum color was not developed if the solutions were made alkaline immediately after cooling. Thus the molar absorptivities increased by more than 100% when the solutions were held at 20° to 25° C. for 18 hours before developing color. During this time, they took on faint brown colors even when alkali was absent. However, when the solutions were reheated they reverted to their original conditions, indicating the possibility of an equilibrium between the analogs of the quaternary (III) and the pseudobase (IV), in which the formation of the former is favored at high temperatures, and the formation of the latter predominates at low temperatures. The fact that the intensity of the color formed after the addition of alkali was higher after incubation at room temperature could be explained on the premise that the analogs of V and VI can be formed directly from the analog of IV, while III must first be converted to IV. As compounds such as V and VI hydrolyze to form colorless products, maximum color intensity would be obtained when their rates of formation are much higher than their rates of hydrolysis, and this is more likely to be the case when the precursor is IV rather than III.

Color formation was found to take place immediately after the addition of alkali, but unlike the compounds previously described the intensity increased slowly for the first 30 minutes and thereafter remained approximately constant for 10 to 15 minutes before fading began. During this interval the absorption maximum shifted from 440 to 455 mμ, indicating the possible conversion of V or VI to the salt form which is an intensely colored resonance hybrid of the following two structures



Because of the weakly acidic character of the salt, its rate of formation from V or VI might be slow, thus accounting for the shift in the maximum. The molar absorptivity at maximum color development was found to be 2.2×10^4. On heating or prolonged standing at room temperature, the solutions became colorless. However, when *p*-aminobenzoic acid was added, a rose color developed. This was probably caused by the formation of a dianil of glutaconic dialdehyde.

When this procedure was first developed, a 50% solution of pyridine in water was arbitrarily selected as the reagent because of experience with similar compounds. Good results were obtained when the initial concentration of XII or XIII did not exceed 10 γ per ml. However, when 50 to 200 γ per ml. were used and the solutions were diluted to a suitable concentration range (2 to 8 γ per ml.) before adding alkali, recoveries were always low by about 10 to 15%—indicating that Beer's law was not obeyed. Further investigation showed that maximum color intensity was developed at 60 rather than at 50% pyridine and that under these conditions satisfactory recoveries could be obtained regardless of the initial concentration of 3,4-dichlorotetrahydrothiophene - 1,1 - dioxide. The reasons for this extreme sensitivity with regard to concentration of pyridine are unknown.

3,4-Dichlorotetrahydrothiophene - 1, 1-dioxide (XII) was readily extracted from plant tissues and soils with dichloromethane, and many interfering materials were removed by absorption on a mixture of Norite A and Florisil. Lipides were removed by replacing the dichloromethane with petroleum ether and then extracting with water in which 3,4-dichlorotetrahydrothiophene - 1,1 - dioxide is appreciably soluble. When necessary, the 3,4-dichlorotetrahydrothiophene-1,-1-dioxide was concentrated by re-extracting the aqueous phase with dichloromethane and evaporating the solvent. This procedure removed almost all interfering substances and permitted a rapid estimation of 3,4-dichlorotetrahydrothiophene-1,1-dioxide in soils, and plant

tissues in amounts as low as 0.1 γ per gram of soil or tissue.

The selective pre-emergency herbicide (*24*) 2-chloro-4,6-bis(diethylamino)-*s*-triazine (Geigy 444) also yielded colored reaction products when solutions of it were treated first with pyridine and then with alkali. This material resembled 3,4-dichlorotetrahydrothiophene - 1,1 - dioxide (XII) more closely in chemical activity than the related dichloro-*s*-triazines as the solutions had to be heated in order to obtain maximum color development. More recently a quantitative method has been developed in these laboratories for the analysis of the fungicide Captan [*N*-(trichloromethylthio)-4-cyclohexene - 1,2 - dicarboximide]. The reaction conditions are similar to those used for the determination of the dichloro-*s*-triazines, but the absorptivity at the maximum is not as high.

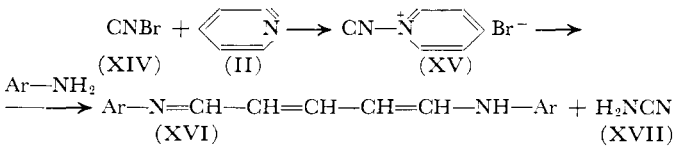
Alternate Reagents

Up to the present pyridine has been used almost exclusively for quaternary salt formation and sodium hydroxide for ring cleavage but other reagents may be employed. Burchfield and Storrs (*6*) developed satisfactory quantitative procedures for the dichloro-*s*-triazines using 2-aminopyridine or 2-aminopyrimidine as heterocyclic bases, while benzyltrimethylammonium hydroxide gave better color stability than sodium hydroxide.

The ease with which quaternary salts are formed is regulated by substituents in the heterocyclic ring. Vompe and Turitsyna (*35*) have shown that pyridine derivatives substituted in the 3 and 4 positions with methyl and alkoxyl groups react readily with 1-chloro-2,4-dinitrobenzene while those substituted with chlorine, bromine, nitro, and ester groups are inert. Similarly, the nature of the substituents in the pyridine ring as well as the nature of the alkylating group can affect the ease of ring cleavage, so it should be possible to select the reagents most suitable for any given determination by taking these properties into consideration.

Aldridge (*1*, *2*) describes a method for the determination of hydrogen cyanide which is based on converting it to cyanogen bromide, which is then made to react with a mixture of pyridine and benzidine dihydrochloride to form an orange color. The reaction of cyanogen bromide with pyridine was studied by König (*18–20*), who found that *N*-cyanopyridinium bromide is formed. This compound reacts with aniline to form the dianil of glutaconic dialdehyde. Sodium hydroxide is not required; the cyano group is sufficiently electronegative to permit cleavage of the heterocyclic ring by the organic base. The cyano group is then eliminated as cyanamide (XVII) while the glutaconic dialdehyde combines with the aromatic amine to form the colored product (XVI)

Thus the series of reactions in the Aldridge test is probably

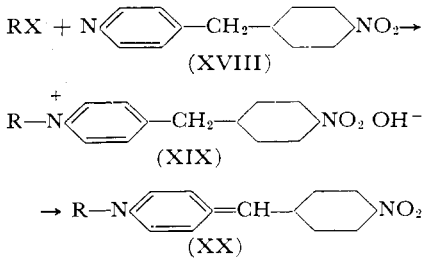


(XIV) (II) (XV) (XVI) (XVII)

In the cases of the *s*-triazines, the halonitrobenzenes, and the thiophene dioxides, it is likely that the color of the monanil formed on ring opening is measured; while for cyanogen bromide the color is caused by the formation of a dianil of glutaconic dialdehyde with the primary aromatic amine. Epstein (*10*) describes a similar method for the determination of hydrogen cyanide which depends upon converting it to cyanogen chloride and making the latter compound react with a mixture of pyridine and phenylmethylpyrazolone. A blue color is formed which is read at 630 mμ. A small quantity of bispyrazolone must be present in order to stabilize it. Saville (*26*) has described some novel applications of this method for the determination of "thiolgenic" compounds.

These modifications may have some applications to the analysis of pesticides in cases where the intermediate monanils are highly unstable or absorb light weakly, as intensely colored dianils could be regenerated from the glutaconic dialdehyde by the addition of aromatic or heterocyclic primary amines. In some cases it might be desirable to hydrolyze the monanils and regenerate dianils even when the former are stable as measurement of the blue color generated by phenylmethylpyrazolone would be possible in the presence of the yellow colors obtained on many plant and soil extracts.

Epstein, Rosenthal, and Ess (*11*) describe a method for the detection of ethyleneimines and alkylating agents which is based on the finding by Koenigs, Köhler, and Blindow (*17*) that γ-(4-nitrobenzyl) pyridine reacts with methyl iodide to form a quaternary salt which yields a blue dye in alkaline solution. In this case, color formation is believed to be caused by dehydration of the quaternary base rather than ring cleavage



(XVIII) (XIX) (XX)

While alkylation of a heterocyclic nitrogen is the primary step, color formation takes place through a different route. Examples of compounds which can be detected in this manner include diethyl sulfate, benzene sulfonyl chloride, diphenylchloroarsine, diethyl phosphorofluoridate, ethyleneimine, and a large number of alkyl and aryl halides. This procedure may be particularly valuable for the determination of pesticides which are essentially nonpolar in nature, as the heterocyclic rings of compounds, such as *N*-methylpyridinium iodide, are not readily cleaved by bases to yield colored products (*23*, p. 425). However, it is not applicable in all cases, as efforts made in these laboratories to adapt the method to the quantitative determination of 3,4-dichlorotetrahydrothiophene-1,1-dioxide were unsuccessful.

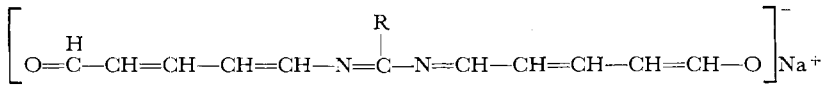
Fujiwara Reaction

The Fujiwara test (*13*) differs from the Zincke reaction in that the pyridine and alkali are added simultaneously to the sample and the mixture is heated. Colors which vary from yellow to red are obtained—depending on the nature of the halogen compound. Snell and Snell (*32*) describe the application of this method to the quantitative determination of chloroform, carbon tetrachloride, trichloroethylene, and chloral hydrate. More recently Seto and Schultze (*31*) have applied it to analysis of trichloroacetic acid, trichloroethanol, and trichloroethylene in bovine urine. They have reviewed the literature adequately with respect to its industrial applications, use in forensic medicine and pharmacology, and in studies on the migration of fumes through soil. They state that the nature of the colored compound and the stoichiometry of the reaction are unknown; hence, the test must be regarded as empirical.

However, in some cases, at least, the reactions may be similar to those obtained in the Zincke test as the same ingredients are present, although they are treated in a different manner. When the methodology used in this test was applied to the analysis of 3,4-dichlorotetrahydrothiophene - 1,1 - dioxide (XII) quantitative results were obtained but the molar absorptivity was only about 10% as high as when the reaction was carried out in two steps.

Red colors are often produced from compounds containing trichloromethyl groups in contrast to the yellow colors obtained with *s*-triazines and 3,4-dichlorotetrahydrothiophene-1,1-dioxide in the Zincke reaction. This might be interpreted to mean that the reactions take different courses, but it is also possible that the shifts in absorption maxima are caused by reaction of more than one

halogen atom attached to a single carbon to produce salts such as



This method is interesting from the standpoint of pesticide residue analysis because of the use of salts of trichloroacetic acid and related compounds as herbicides. Furthermore, its application to the determination of low molecular weight compounds containing halogen atoms, such as chloroform, suggests that it might be useful for the estimation of soil and grain fumigants.

Qualitative Tests

A number of pesticides have been found to give qualitative tests in the pyridine-alkali-aromatic amine reactions for which quantitative applications have not been developed. Johnson has shown (*14*, *15*) that chlordan and toxaphene give colored products when treated with pyridine and alcoholic potassium hydroxide, while heptachlor gives a colored product in the presence of pyridine, aniline, and alcoholic potassium hydroxide. Burchfield and Storrs (*6*) have obtained colored products by treating chlordan, heptachlor, toxaphene, and lindane with pyridine and aqueous alkali under various conditions. Most of these latter compounds probably contain allylic halogen or can be converted to compounds containing allylic halogen through a preparatory dehydrochlorination step in the pyridine reagent similar to the conversion of 3,4-dichlorotetrahydrothiophene-1,1-dioxide (XII) to XIII. For example, heptachlor contains a chlorine atom beta to a double bond, while Sternburg and Kearns (*33*) have shown that lindane can be converted to an unsaturated compound enzymatically.

Color reactions of varying intensity are also obtained when active halogen compounds in anhydrous pyridine solution are shaken with 7.*N* aqueous sodium hydroxide. Two layers are formed and the colored products appear in the pyridine phase where they are often very stable. Dichloral urea, hexachloroacetone, and sodium trichloroacetate give intense red colors in this test while Dalapon (2,2-dichloropropionic acid) gives a faint rose color only after prolonged heating. Other pesticides giving strong positive tests include the fungicides Spergon (chloranil) and Phygon (dichlone), the herbicide Oktone (octachlorocyclohexanone), and the soil fumigants DD mixture (a mixture of dichloropropane and dichloropropene), Nemagon (a mixture of 1-chloro-2,3-dibromopropane and other halogenated C_3 compounds), and Larvacide (chloropicrin). These tests are summarized in Table I. Dieldrin gives a faint yellow color after prolonged heating, but it is unlikely that this method would be useful for the detection or estimation of materials of this class unless more favorable reaction conditions or more reactive reagents can be found. 2,4-Dichlorophenoxyacetic acid does not give colored products under any of the conditions investigated. However, the reactivity of pesticides containing active halogen atoms with pyridine and alkali is quite general.

Useful qualitative tests for determining the distribution of deposits of the dichloro-*s*-triazines of leaf surfaces can be obtained by substituting β-picoline for pyridine. Detached leaves are pressed between filter papers soaked with the picoline, for 10 to 15 minutes. Leaf imprints are formed on which the location of the fungicide can be detected by the presence of brilliant crimson spots. No alkali is required. The products formed on reacting cyanuric chloride with alpha, beta, and gamma picolines have been described by Menon, Nath, and Aggarwal (*21*).

Applications to Pesticide Research

Burchfield and Storrs (*3*, *4*, *8*, *9*) have applied the colorimetric procedure for the determination of 2,4-dichloro-6-anilino-*s*-triazine to a study of the effects of halogen substitution in the benzene ring on the accumulation of these materials by conidia of *Neurospora sitophila* (Mont.) Shear and Dodge. They have shown that halogen substitution increases uptake in all cases, but that the amount and rate of uptake are also regulated by the position of the entering group. The accumulation of chemicals is accompanied by changes in the permeabilities of the spore membranes which permit the outward diffusion of some of the fungicide as well as intracellular phosphorus, but at doses up to 1000 γ of chemical per gram of spores germination is not seriously impaired. About 75% of the toxicant which is taken up reacts irreversibly with cellular constituents within a few minutes and cannot be recovered by extraction. Following this, slower reactions take place which ultimately result in loss of spore viability.

These methods have also been used to show that 2,4-dichloro-6-(*o*-chloroanilino)-*s*-triazine (*7*, *9*) as well as 1-fluoro-2,4-dinitrobenzene is able to react with many constituents of protoplasm. This may account for their toxic action. The most reactive small molecules on which data are available include cysteine, glutathione, *p*-aminobenzoic acid, hydroxyproline, proline, and tyrosine. Other molecules such as aspartic acid react very slowly, while the reactivity of compounds such as the purines, pyrimidines, and glycolic acid cannot be detected under physiological conditions. Only the ionized functional groups (NH_2 and $-S^-$) can take part in the reactions, while the $-NH_3^+$ and $-SH$ groups are inert. As the latter groups predominate at pH 7.0, only a small fraction of the potential reactivity of these compounds is utilized under physiological conditions. It is possible, however, that the functional groups of protein molecules can interact to produce regions where a higher degree of the latent reaction potential can be utilized (*3*). Thus the application of these methods may ulti-

Table I. Pesticides Giving Color Tests[a]

Material	Method[b]	Color
Fungicides		
Dyrene(triazine)[a]	A	Yellow
NP-1083(FDNB)[a]	A	Red-violet
Captan	B	Orange-yellow
Phygon(dichlone)	A	Orange-red
Spergon(chloranil)	A	Yellow-green
Herbicides		
TCA(trichloroacetate)[a]	B	Red
Dalapon	B	Pale rose
Oktone	B	Yellow
Hexachloroacetone	B	Red
Dichloral urea	B	Red
Geigy-444(triazine)	A	Yellow
Insecticides		
Lindane	A	Pale yellow
Heptachlor	A	Pale yellow
Chlordan	A	Green-brown
Toxaphene	A	Pale rose
Nematocides and fumigants		
PRD[a]	B	Yellow
DD-mixture	A	Brown
Nemagon	A	Greenish-yellow
Larvacide(chloropicrin)	A	Pale yellow

[a] Quantitative methods developed.
[b] In method A the reaction is carried stepwise; in method B the pyridine and concentrated alkali are added together.

mately serve to obtain a better understanding of the mode of action and the importance of molecular configuration of pesticides which function primarily as alkylating agents.

These procedures are also useful for studying the interactions of compounds of this class with their environments, in order to obtain information on the persistence of residues, distribution in soil, and uptake of plants. Thus Schuldt, Burchfield, and Bluestone (*29*) have shown that the nematocide 3,4-dichlorotetrahydrothiophene-1,1-dioxide (XII) has a half life of 46 days in composted garden soil at 21° C. This is increased by lower temperatures and decreased by higher temperatures. This compound moves downward in soil in a zone 3 to 4 inches wide in response to rainfall (*29*). However, the compound moves more slowly than the infiltration rate of the water, so evidently it is loosely adsorbed to soil particles in a manner analogous to a chromatographic column. When the soil dries out, the compound moves upward in the direction of the water movement so that it becomes concentrated in the top 1 inch. In response to alternating periods of rainfall the zone spreads out so that ultimately it is distributed more or less uniformly throughout the first 12 inches of soil. Studies such as these are useful in planning application schedules as they indicate for how long and at what level in the soil the materials will be effective, and how to avoid phytotoxic effects and the accumulation of undesirable residues in plants.

Conclusions

The analytical applications of the reactions of active halogen compounds with pyridine have followed several independent lines of development, and this paper stresses their underlying unity even though the mechanisms and reaction products may vary in individual cases. These tests are useful for the analysis of pesticides as the techniques are simple and applicable to a variety of compounds. Thus quantitative methods have been worked out for the fungicidal *s*-triazines (*6*), 1-fluoro-2,4-dinitrobenzene (*9*), 3,4-dichlorotetrahydrothiophene-1,1-dioxide (*5*), trichloroacetic acid (*31*), and Captan, while current investigations suggest that it will be possible to develop similar tests for the herbicidal *s*-triazines. Qualitative tests have been obtained with heptachlor, chlordan, lindane, toxaphene, dichlone, Spergon, and several other compounds, so the possibility that some of these can also be determined by variations of this method cannot be excluded. Modifications may be developed for the determination of fumigants such as chloropicrin and DD-mixture, while procedures have been described for the determination of cyanide (*1*, *2*, *11*).

The reagents and the techniques employed must be decided in each case by the properties of the compound for which the method is being developed. If the anil of glutaconic dialdehyde formed on cleavage of the pyridine ring hydrolyzes extremely readily it may be necessary to redevelop the color by the addition of an aromatic amine such as benzidine (*1*, *2*) or phenylmethylpyrazolone (*10*). On the other hand, the Schiff bases formed from the *s*-triazines and 1-fluoro-2,4-dinitrobenzene are sufficiently stable for direct measurement. The ease with which the pyridine ring is cleaved is dependent upon the electronegativity of the entering group. Cyanogen chloride gives a color with pyridine and an organic base alone, while the *s*-triazines and similar materials require the presence of an alkali metal hydroxide or a quaternary base On the other hand, the heterocyclic rings of compounds such as methylpyridinium iodide (*23*, p. 425) are not cleaved at all under normal conditions of temperature and pressure. For the analysis of compounds with nonpolar or very weak electronegative substituents, it may be necessary to make the compound react with γ-(4-nitrobenzyl)pyridine as described by Epstein, Rosenthal, and Ess (*11*) and develop the color through dehydration rather than ring cleavage. Probably, some alkylating agents which do not function through active halogen such as esters of inorganic acids, or derivatives of ethyleneimine, would have to be determined in this way. Compounds such as trichloroacetic acid (*31*) can be determined satisfactorily by merely heating them with pyridine and alkali.

The nonspecificity of the method gives it many special advantages and disadvantages. The former are self-evident, while the latter may be more apparent than real. Many of these compounds decompose within a few days to a few months when mixed with soil or plant breis, so intensive build-up of residues would not be anticipated. Furthermore, they vary considerably in their physicochemical characteristics, so that mixtures could be separated by extraction or chromatography while some compounds could be determined in the presence of one another by taking advantage of differences in their properties. 3,4-Dichlorotetrahydrothiophene-1,1-dioxide could be separated quantitatively from the *s*-triazines, by extracting a petroleum ether solution containing the two compounds with water, as the 3,4-dichlorotetrahydrothiophene-1,1-dioxide would be concentrated in the aqueous phase, while the triazine would remain in the hydrocarbon phase. Alternatively, the *s*-triazine could be determined in the presence of 3,4-dichlorotetrahydrothiophene-1,1-dioxide because it reacts with pyridine at room temperature while 3,4-dichlorotetrahydrothiophene-1,1-dioxide requires the application of heat. 1-Fluoro-2,4-dinitrobenzene could be determined in the presence of either the thiophene dioxide or the *s*-triazine by making use of the fact that its reaction product has an absorption maximum at 548 mμ compared to 440 to 455 mμ for the other compounds.

These methods have a serious shortcoming with regard to residue analysis—only material containing active halogen can be detected. Thus the total amount of pesticide in terms of the amount of unaltered material plus the amount combined chemically with the products of metabolism might be larger than indicated by the analytical results. However, this could be turned to an advantage if the reaction products were proved harmless in animal toxicity tests.

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INSECTICIDE RESIDUES

Colorimetric Estimation of Malathion Residues in Animal Products

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A colorimetric method for the estimation of spray residues of malathion on plant material has been adapted to the analysis of animal products. This has led to a more detailed study of the basic procedure, and a number of improvements and modifications have been made. Methods for the determination of residues in meat, fat, liver, milk, and eggs are presented.

In connection with a program to extend the commercial application of the organic phosphorus insecticide, malathion [*S*-(1,2-dicarbethoxyethyl)-*O,O*-dimethyldithiophosphate], to insect control on livestock and poultry, suitable methods were needed for determining residues in edible products obtained from the treated animals. The colorimetric method reported for the determination of malathion residues on plant material (*4*) has been adapted successfully, with a number of improvements and modifications, for this purpose. This method is based upon the alkaline decomposition of malathion in ethyl alcohol–carbon tetrachloride solution to sodium dimethyl dithiophosphate, sodium fumarate, and ethyl alcohol. The sodium dimethyl dithiophosphate is extracted into an aqueous solution, converted to a copper complex, which is extracted into carbon tetrachloride with the formation of a yellow color, the intensity of which is a measure of the malathion present. Amounts as low as 20 γ of malathion may be determined by the revised procedure. These methods will be used to obtain the residue data now required for federal and state insecticide licensing and registration purposes.

The methods described were developed for the determination of malathion residues in meat, fat, liver, milk, and eggs. The applicability of the methods was tested by analyzing prepared samples containing added amounts of the insecticide. Results of these analyses are shown in Tables I, II, and III. The method for analysis of pork meat was used as described; for beef and chicken meat, the sample size and volumes of all reagents were doubled, except that 30 ml. of carbon tetrachloride was used to extract the final colored copper-dithio complex. These modifications were made in order that a Klett-Summerson photoelectric colorimeter with 4-cm. cells might be used in place of a spectrophotometer without loss of sensi-

Table I. Recovery of Malathion from Meat

Type	Sample Represented by Extract Analyzed, G.	Malathion, P.P.M. Added	Malathion, P.P.M. Found	Recovery, %
Beef	200	0.24	0.16	67
	200	0.24	0.16	67
	200	0.47	0.37	79
	100	0.47	0.39	83
	200	0.47	0.35	74
	200	0.52	0.39	75
	200	0.94	0.77	82
	100	0.94	0.77	82
	200	0.94	0.67	71
	200	1.03	0.80	78
	200	1.18	0.93	79
	100	1.88	1.63	87
	100	2.06	1.75	85
	160[a]	0.47	0.33	70
	163[a]	0.94	0.72	77
	100[a]	1.88	1.41	75
Chicken (fowl)	200	0.24	0.17	71
	200	0.24	0.17	71
	200	0.47	0.40	85
	200	0.47	0.33	70
	180	0.47	0.32	68
	200	0.94	0.72	77
	200	0.94	0.64	68
	100	1.88	1.50	80
Chicken (fryer)	200	0.24	0.16	67
	200	0.47	0.37	79
	200	0.94	0.78	83
Pork	98	0.50	0.41	82
	98	0.50	0.41	82
	120	1.00	0.95	95
	120	1.00	0.87	87
	31	5.00	5.00	100

[a] Extracts stored for 2 weeks in dark, under refrigeration, before analysis.