para-oxon in either system was small. This is in agreement with in vivo studies with wheat plants administered parathion, where only small quantities of para-oxon were recovered based on cholinesterase inhibition studies (*5*). Schradan is converted to a more potent anticholinesterase agent by the EDTA-Fe^{+2}-ascorbic acid system (*8*), presumably through an initial oxidative attack at a nitrogen. The oxidation of thioether groups to their sulfinyl and sulfonyl derivatives is rapid in plants (*14*), while phosphorothionates and *N,N*-dimethyl phosphoramidates usually oxidize more slowly. It might then be anticipated that the peroxidase–hydrogen donor system would also oxidize the thioether grouping.

In addition to oxidation, peroxidase and the EDTA-Fe^{+2} complex catalyzed the hydrolysis of parathion and para-oxon. The large amount of hydrolysis products from parathion may explain the low para-oxon yields. The ratio of hydrolysis products to para-oxon formed has been found to be fairly constant for the peroxidase-catalyzed reactions. On the basis of this observation and the greater stability of para-oxon to hydrolysis by those oxidative systems, the thiono sulfur must play an important role in the hydrolysis reaction. In contrast, parathion is considerably more stable to hydrolysis by alkali than para-oxon. This has been explained on the basis of the lower electronegativity of sulfur than of oxygen. The approach of the hydroxyl ion for nonenzymatic hydrolysis depends on the relative electronegativities of the central phosphorus atom and of the attached substituents, on the degree to which the transition state is stabilized, and on steric hindrance by groups surrounding the phosphorus atom (*9*).

The results presented are consistent with the assumption of Buhler and Mason (*2*) that oxygen is activated as a free perhydroxyl radical by the peroxidase–hydrogen donor system. Free perhydroxyl radicals are formed by the reaction of molecular oxygen and the hydrogen donor (*2*) as distinguished from the EDTA-Fe^{+2}–ascorbic acid system in which free hydroxyl radicals are produced by the reaction of ascorbic acid with hydrogen peroxide generated in the reaction (*2, 18*). Free perhydroxyl or hydroxyl radicals may bring about the formation of an activated intermediate of parathion which rapidly decomposes by displacement of sulfur or of an ethoxy or *p*-nitrophenyl radical. Characterization of the hydrolysis products from parathion would establish the site of hydrolysis and aid in explaining how a free radical causes both oxidation and hydrolysis.

The results obtained with peroxidases in vitro suggest that peroxidases in plants may play a role in the metabolism of phosphorothionates in vivo.

Literature Cited

(1) Bowman, J. S., Casida, J. E., J. Agr. Food Chem. **5,** 192 (1957).
(2) Buhler, D. R., Mason, H. S., *Arch. Biochem. Biophys.* **92,** 424 (1961).
(3) Casida, J. E., Chapman, R. K., Stahmann, M. A., Allen, T. C., *J. Econ. Entomol.* **47,** 64 (1954).
(4) Dautermann, W. C., Viado, G. B., Casida, J. E., O'Brien, R. D., J. Agr. Food Chem. **8,** 115 (1960).
(5) David, W. A. L., Aldridge, W. N., *Ann. Appl. Biol.* **45,** 332 (1957).
(6) Devlin, T. M., "Methods in Enzymology," Vol. **II,** p. 770, Academic Press, New York, 1955.
(7) Fallscheer, H. O., Cook, J. W., *J. Assoc. Offic. Agr. Chemists* **39,** 691 (1956).
(8) Fenwick, M. L., *Biochem. J.* **70,** 373 (1958).
(9) Heath, D. F., *J. Chem. Soc.* **1956,** 3796.
(10) Heath, D. F., Lane, D. W. J., Park, P. O., *Phil. Trans. Roy. Soc. London* **B 239,** 191 (1955).
(11) Larsson, L., Hansen, B., *Svensk Kem. Tidskr.* **68,** 521 (1956).
(12) Mandeles, S., *J. Chromatog.* **3,** 256 (1960).
(13) Mason, H. S., "Proceedings of International Symposium on Enzyme Chemistry, Tokyo and Kyoto," p. 220, Maruzen Co., Maruzen, Tokyo, 1958.
(14) Metcalf, R. L., Reynolds, H. T., Winton, M., Fukuto, T. R., *J. Econ. Entomol.* **52,** 435 (1959).
(15) O'Brien, R. D., "Toxic Phosphorus Esters," p. 346, Academic Press, New York, 1960.
(16) Seume, F. W., O'Brien, R. D., J. Agr. Food Chem. **8,** 36 (1960).
(17) Sober, H. A., Gutter, F. J., Wyckoff, M. M., Peterson, E. A., *J. Am. Chem. Soc.* **78,** 756 (1956).
(18) Udenfriend, S., Clarke, C. T., Axelrod, J., Brodie, B. B., *J. Biol. Chem.* **208,** 731 (1954).
(19) Umbreit, W. W., Burris, R. H., Stauffer, J. F., "Manometric Techniques," p. 238, Burgess Publishing Co., Minneapolis, Minn., 1957.

Received for review March 31, 1961. Accepted July 12, 1961. Approved for publication by the Director of the Wisconsin Agricultural Experiment Station. Study supported in part by the Research Committee of the Graduate School from funds supplied by the Wisconsin Alumni Research Foundation and by grants from the U. S. Atomic Energy Commission [Contract No. AT(11-1)-64, Project No. 14], U. S. Public Health Service, and National Institutes of Health (Contract RG-5304 and Regional Research Project NC-33).

GRAIN FUMIGANT DETERMINATION

Polarographic Determination of Methyl Bromide, Ethylene Dibromide, Acrylonitrile, Chloropicrin, and Carbon Tetrachloride in Air

BEN BERCK

Canada Department of Agriculture Research Station, Winnipeg, Man., Canada

To expedite a factorial investigation (*7*) of the concept that wheat may behave as a chromatographic column toward fumigant gases (*3, 5*), polarographic methods were developed for the measurement of methyl bromide, ethylene dibromide, acrylonitrile, chloropicrin, and carbon tetrachloride in concentrations as low as 10^{-5} *M* with a precision within ±5.1% in air samples taken in duplicate, and within ±0.3% in aliquots of a given sample in solution. These fumigants were applied singly and in combination with carbon tetrachloride to the surface of $7^1/_2$-foot columns of wheat, following which the composition of the gas-air mixture that emerged from the bottom was determined as a function of time. This report presents details of the sampling and analytical methods that were used.

Aspects of Research Methods

In the initial stages of development of the methods, carbon tetrachloride was determined spectrophotometrically by the Ramsey method (*18*), employing the Fujiwara pyridine-alkali reaction. However, since the cumulative time required to fit several thousand samples of unknown concentrations to the relatively narrow range of optimum measurement (0.1 to 1.0 mg. of carbon tetrachloride) was considerable, the Ramsey method was replaced by the direct polarographic method of Kolthoff *et al.* (*13*), modified as described herein.

Methods for acrylonitrile (*8*) and chloropicrin (*11*) determination were

Polarographic methods were developed for rapid measurement of methyl bromide, ethylene dibromide, acrylonitrile, chloropicrin, and carbon tetrachloride in amounts as low as $10^{-5}M$ with a precision within ±5.1% in air samples taken in duplicate. Methods are described for quantitative separation of binary mixtures containing carbon tetrachloride, and of methyl bromide–ethylene dibromide mixtures. Sampling of fumigant gases in absorption vessels was improved by use of a "bubble-fractionating" gas-dispersion tube.

similarly time-consuming and, in addition, the presence of carbon tetrachloride in high concentrations affected the accuracy of measurement. The titrimetric method (*1*) for acrylonitrile was accordingly replaced in this work by adapting the polarographic method of Fuller and Norris (*12*) in which the cathodic wave of acrylonitrile ($E_{1/2}$ = approximately −2.1 volts *vs.* S.C.E. in aqueous solution with 0.05*M* tetramethylammonium hydroxide as supporting electrolyte) afforded direct measurement in amounts as low as $1.08 \times 10^{-5}M$ acrylonitrile with no interference from carbon tetrachloride.

Chloropicrin was rapidly measured by preliminary hydrolysis in capped sample tubes for 4 hours at 60° C. with 3.5% monoethanolamine in 60% 1-propanol to yield inorganic Cl^-. Up to 72 samples at a time were thus hydrolyzed; under these conditions carbon tetrachloride did not interfere. The anodic wave of hydrolyzed chloropicrin at +0.22 volt *vs.* S.C.E. was measured more rapidly and reproducibly with the D.M.E. than with the rotating platinum electrode. Development of an equally new, but direct, method by which chloropicrin could be measured by its polarographic reduction at approximately −1.8 volts in 95% ethanol was not attained because of generally erratic and uncertain wave plateaus. The direct method has since been substantially modified (*6*) to give highly reproducible results down to $9.13 \times 10^{-6}M$ chloropicrin without interference from carbon tetrachloride, methyl bromide, ethylene dibromide, ethylene dichloride, or chloroform.

Quantitative separation of methyl bromide–carbon tetrachloride, methyl bromide–ethylene dibromide, and ethylene dibromide–carbon tetrachloride mixtures was initially accomplished by differential alkaline hydrolysis, followed by amperometric titration of the Br^- and Cl^- (*2*). When Br^- was present together with Cl^-, the Br^- was selectively oxidized to BrO_3^- (*15*), the starch-iodide end point being determined amperometrically (*2*). In attempts to measure methyl bromide directly, reconnaissance tests after von Stackelberg and Stracke (*19*) were undertaken. Methyl bromide was reduced at −1.6 volts in 80% methanol with various quaternary ammonium compounds as supporting electrolytes, but losses due to its volatilization during oxygen removal with nitrogen were appreciable even at 0° C., and therefore the hydrolysis method (*2*) was retained, with modifications as described herein.

Ethylene dibromide yielded a well-defined, stable, and highly reproducible wave ($E_{1/2}$ = −1.55 volts) in 85% methanol with 0.05*M* tetramethylammonium hydroxide as supporting electrolyte, and this served as the basis for a new, simple, and direct method. The wave for ethylene dibromide coincided with the second wave of carbon tetrachloride. In a mixture of ethylene dibromide and carbon tetrachloride, it was possible to measure both compounds in a single sample by first determining the carbon tetrachloride by the height of its first wave at −1.2 volts. The second wave at −2.0 volts represents the sum of ethylene dibromide and carbon tetrachloride, and the concentration of ethylene dibromide as such was determined by subtracting from the wave height the amount corresponding to the carbon tetrachloride, calculated by multiplying the height of the first wave by an empirical factor.

Various methods of sampling gas-air mixtures were tested. Reproducibility of measurement was obtained by taking air samples of 25 to 100 ml. with precalibrated glass syringes fitted with a mechanical stop, as described by Chaney (*10*). The samples were discharged at a rate of 30 to 33 ml. per minute, at first manually through 20-gage, 6-inch stainless steel hypodermic needles, and later semiautomatically by controlled vacuum (*7*) into chilled gas-trapping solvent, as indicated in Figure 1.

Apparatus and Reagents

Apparatus. Recording Polarograph. A Radiometer Model PO_3 (Radiometer Co., Copenhagen, Denmark) with a galvanometer sensitivity of 0.00035 μa. per mm. full scale was used to record polarographic waves. The $m^{2/3}t^{1/6}$ values of the D.M.E. capillary were 2.404, 2.375, 2.334, 2.234, and 2.126 $mg.^{2/3}sec.^{-1/2}$ at −0.4, −0.75, −1.2, −1.75, and −2.0 volts at 25° C. All D.M.E. potentials were measured against an external saturated calomel reference electrode. Three salt-agar bridges, modified as shown in Figure 2, were used, respectively, to simplify changing the cell assembly from one potential range to another in measuring the various fumigants.

Polarographic Cells. One dozen cells were made by cutting off the lower ends of 25-mm. borosilicate glass test tubes $2^1/_2$ inches from the bottom (Figure 2). Such cells are easily filled, emptied, and washed and were preferable to the standard H-cell for work with thousands of samples. They permitted measurement in from 2 to 20 ml. of solution with unimpaired accuracy. Deaeration time was lowered when smaller samples were used.

Sample Tubes. Two types were used: 125 × 16 mm. outside diameter, borosilicate glass, without rim (Kimble No. 45048), when hydrolysis was not required, and 125 × 16 mm. outside diameter, borosilicate glass, fitted with Bakelite screw-cap and Teflon liner (Kimble No. 45066-A) when hydrolysis of methyl bromide or chloropicrin was required.

Constant Temperature Bath. This was maintained at 60° ± 0.5° C. to hold racks of sample tubes immersed to a depth of 4 inches.

Glass Syringes, hypodermic, Luer-lok. These ranged in capacity from 25 to 100 ml. and were fitted with mechanical stops (*10*) and calibrated by weighing designated volumes of water. Stainless steel needles, 18- and 20-gage and in 2-, 4-, and 6-inch lengths, were used according to requirements.

Gas Absorption Assembly (Figure 1). This consisted of a machined brass sampling head, *A*, to which was fitted a gas inlet tube, *B*, of 14-gage stainless steel (s.s.) tubing, to accommodate a Luer-lok syringe with 18-gage needle placed on top. The s.s. tube projects upward by $^3/_{16}$ inch and is fitted with a perforated serum rubber stopper, small size (Scientific Glass Apparatus Co., Bloomfield, N. J.), to ensure a snug fit to the shoulders of the syringe needle. The bottom of the s.s. tube is closed, except for two pairs of holes for the gas-air outlets at *E*. The rubber stopper, *C*, is machined to fit a test tube, *D* (lipless, 16 × 125 mm.). The two pairs of holes of the "bubble-fractionating" tip, *E* (shown also in the inset of Figure 1), were drilled with a 0.022-inch watchmaker's pivot drill, with one pair at 90° to and slightly above the other. Two pieces of 24-gage s.s. wire were placed through the holes, and the wire ends crimped, as shown in the inset. The crossed wires fractionated the gas-air

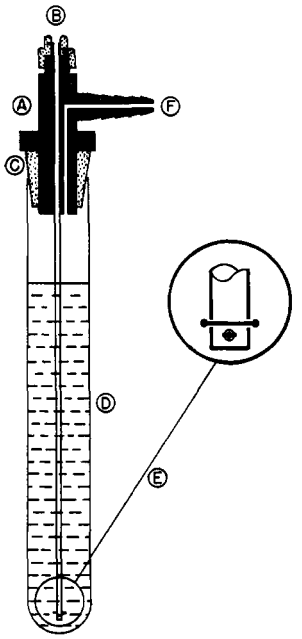

Figure 1. Gas absorption assembly

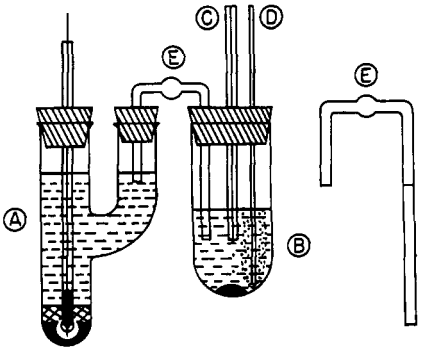

Figure 2. Cell assembly and bridge tube

A. Saturated calomel reference electrode
B. Polarographic cell
C. D.M.E.
D. Capillary tube for nitrogen, placed above surface of solution after oxygen removal. Hole in stopper is slightly oversize to permit venting of nitrogen
E. Salt-agar bridge tube, filled in one of three ways described in text, according to voltage span required

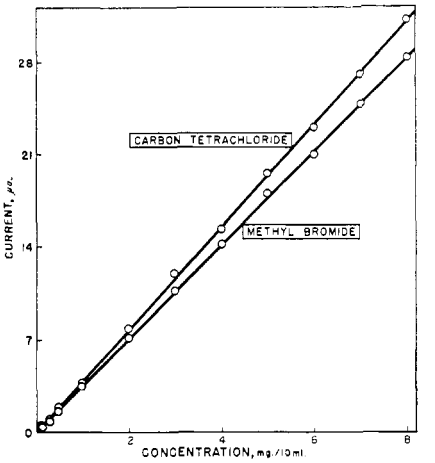

Figure 3. Calibration curves for carbon tetrachloride (first wave) and methyl bromide

bubbles and yielded a steady stream of fine bubbles when slight vacuum was applied through the air outlet, *F*. The air outlet led to a flowmeter (Scientific Glass Apparatus Co.) that was operated by needle valve–controlled vacuum to permit discharge of the sample in the syringe at a rate of 30 to 33 ml. per minute.

Reagents. FUMIGANT STANDARDS. The methyl bromide and chloropicrin standards, ranging in concentration from 5.0 to 0.01 mg. per ml. in 60% 1-propanol, were made from stock solutions of 50 to 100 mg. per ml. that were checked polarographically after hydrolysis against standard Br^- and Cl^- solutions. Primary methyl bromide standards were prepared from weighed ampoules as described by Busbey and Drake (*9*). Secondary standards were made by dispensing through gas dispersion tubes into chilled 1-propanol predetermined volumes of methyl bromide, 99% pure, from a 1-pound lecture bottle of compressed gas (Matheson Co., East Rutherford, N. J.). Acrylonitrile standards, ranging from 5.0 to 0.01 mg. per ml. of water, were made from freshly distilled acrylonitrile (American Cyanamid Research Laboratories, Stamford, Conn.). Ethylene dibromide and carbon tetrachloride standards, 10.0 to 0.05 mg. per ml. of 85% methanol, were prepared from stock solutions made from the redistilled fumigants. All standards were kept in the refrigerator in Teflon-lined screw-cap reagent bottles until required.

Tetramethylammonium hydroxide, polarographic grade (Southwestern Analytical Chemicals, Austin, Tex.).

Tetrabutylammonium bromide, polarographic grade (Southwestern Analytical Chemicals, Austin, Tex.).

Methanol, 85% (v./v.), used in sampling ethylene dibromide and carbon tetrachloride in air.

Monoethanolamine, 3.5% (v./v.) in 60% 1-propanol, used in sampling and subsequent hydrolysis of methyl bromide and chloropicrin in air.

Nitric acid, approximately 8*N*.

Gelatin solution, 1% (w./v.), prepared in a boiling water bath, and stored between use in a refrigerator. A fresh solution was made after 5 days.

NITROGEN FOR OXYGEN REMOVAL. Tank nitrogen, "high purity, dry," 99.99% pure (Linde Gases Division, Union Carbide Canada, Ltd., Toronto, Ont.), presaturated with a portion of the blank solution, was used in deaeration of some of the fumigant solutions in the measurement cell. Oxygen removal was necessary only in measurement of carbon tetrachloride and ethylene dibromide, and occasionally measurement of acrylonitrile was improved when oxygen was absent. It was found empirically that 5 minutes of deaeration with nitrogen, regulated by a flowmeter to emerge in small bubbles through a capillary tube at a rate of 30 ml. per minute, gave satisfactory oxygen removal in 8 to 10 ml. of sample solution, and accordingly 10 ml. was designated as the standard volume of solution prior to measurement. While concentrations of ethylene dibromide and acrylonitrile were unchanged by deaeration for 10 to 20 minutes, periods longer than 5 minutes at 30 ml. of nitrogen per minute at 23° C. contributed to significant losses of carbon tetrachloride, and therefore 30 ml. of nitrogen per minute for 5 minutes were used throughout the experimental procedures.

AGAR BRIDGES. Three types were used to encompass the polarographic working range for the various fumigants. The following agar mixtures were prepared in a boiling water bath in 50-ml. boiling flasks:

Agar A. 3% agar + 23% KCl solution, w./v.

Agar B. 3% agar + 30% KNO_3 solution, w./v.

Agar C. 3% agar + 23% tetrabutylammonium bromide solution, w./v.

For use in the range 0 to −2.0 volts (carbon tetrachloride and ethylene dibromide) a bridge tube (Figure 2) was filled with agar A only.

For use in the range 0 to +0.45 volt (methyl bromide and chloropicrin) the bridge tube was filled to the parallel mark with agar A, and then, after setting of A, to the end of the sample-side arm with agar B.

For use in the range −1.7 to −2.4 volts (acrylonitrile) the bridge tube was similarly filled to the parallel mark with agar A, and then with agar C.

Use of a 5-ml. syringe fitted with a 4-inch, 18-gage stainless steel needle simplified the filling of the 4-mm. bore tubing with hot agar solution, and eliminated the entrapment of air bubbles. Diffusion in the combination bridges of K^+ or Cl^- ions to the bridge–sample side interface was slow and could readily be detected in the blank. For continuous use, combination bridges were replaced every week or sooner, depending on the amount of use. The sample-side end of the various bridges became discolored in due course from repeated immersion in alcoholic alkaline solutions, and such bridges were replaced whenever browning was observed.

Procedures

Carbon Tetrachloride. Using standard solutions of carbon tetrachloride as indicated, prepare a calibration curve for the range 0.05 to 10.0 mg. by pipetting

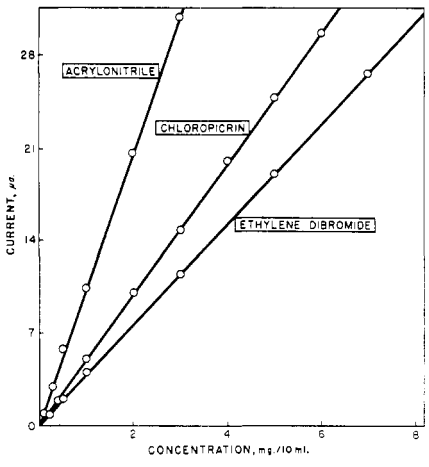

Figure 4. Calibration curves for acrylonitrile, chloropicrin, and ethylene dibromide

the equivalent of 0.05, 0.1, 0.3, 0.5, 1.0, 2.0, 4.0, 6.0, 8.0, and 10.0 mg. of carbon tetrachloride in duplicate into 85% methanol in 16 × 125 mm. test tubes precalibrated to a 10-ml. mark. Add 0.5 ml. of 1*M* tetramethylammonium hydroxide and adjust to 10 ml. with 85% methanol. Transfer the solution to the electrolysis cell, deaerate for 5 minutes at a nitrogen flow of 30 ml. per minute, and record the waves through the range −0.4 to −2.0 volts. Two waves, with $E_{1/2}$ of about −0.85 and −1.75 volts, respectively, will be obtained. Measure their respective diffusion currents at −1.2 and −2.0 volts, deducting the residual current of the blank solution. Plot the diffusion current values of the first wave of carbon tetrachloride. The relationship between the diffusion current and concentration should be linear (Figure 3).

To measure the contribution of carbon tetrachloride to the total diffusion current shown by a mixture of carbon tetrachloride and ethylene dibromide, multiply the data of the first carbon tetrachloride wave by 1.69, and deduct the resultant values from the total diffusion current at −2.0 volts. The factor 1.69 represents 0.925 × 1.83, where 0.925 comprises the change of $m^{2/3}t^{1/6}$ characteristics between −1.2 and −2.0 volts, and 1.83 represents the ratio of the total diffusion current constant to that of the first carbon tetrachloride wave [cf. Kolthoff *et al.* (*13*)].

Ethylene Dibromide. Prepare a standard curve in the range 0.05 to 10.0 mg. of ethylene dibromide, as indicated above for carbon tetrachloride, using 85% methanol and tetramethylammonium hydroxide as supporting electrolyte. Deaerate for 5 minutes (longer periods may be used) and record the wave ($E_{1/2}$ = approximately −1.55 volts) in the range −1.2 to −2.0 volts. Plot the diffusion current values, correcting for residual current (Figure 4).

Ethlyene dibromide-carbon tetrachloride admixtures in air are quantitatively differentiated in a single sample by absorbing the sample in 9.5 ml. of 85% methanol (use an ice bath). Add 0.5 ml. of 1*M* tetramethylammonium hydroxide, deaerate for 5 minutes, and record the waves in the range −0.4 to −2.0 volts. Determine the proportion of carbon tetrachloride in the second wave by the procedure indicated in the previous section.

Acrylonitrile. Prepare a calibration curve from standard solutions for the range 0.025 to 5.0 mg. by pipetting the equivalent of 0.025, 0.05, 0.1, 0.5, 1.0, 2.0, 3.0, 4.0, and 5.0 mg. of acrylonitrile in duplicate into cool water in 16 × 125 mm. tubes. Add 0.5 ml. of tetramethylammonium hydroxide and adjust the volume to 10 ml. with water. Record the acrylonitrile wave ($E_{1/2}$ = approximately −2.1 volts) through the range −1.7 to −2.5 volts. It is generally not necessary to deaerate the test solution, although sometimes waves with improved definition are obtained after 3 or 4 minutes of oxygen removal. Plot the diffusion current values *vs.* concentration, correcting for the blank (Figure 4).

To separate acrylonitrile from carbon tetrachloride in air, two separate samples are necessary. The first is absorbed in 9.5 ml. of water and the second in 9.5 ml. of 85% methanol contained in 16 × 125 mm. tubes in an ice bath. After absorption, add 0.5 ml. of tetramethylammonium hydroxide to both tubes and process them separately for acrylonitrile and carbon tetrachloride, as already indicated. The presence of the one will not interfere with measurement of the other.

Chloropicrin. Prepare a calibration curve from standard solutions for the range 0.05 to 5.0 mg. by pipetting into screw-cap Teflon-lined 16 × 125 mm. tubes the equivalent of 0.05, 0.1, 0.3, 0.5, 1.0, 2.0, 3.0, 4.0, and 5.0 mg. of chloropicrin in duplicate into 60% 1-propanol containing 3.5% of monoethanolamine (the total volume should be approximately 8.5 ml.). Place the capped tubes in a constant temperature water bath at 60° C. for 4 hours, agitating the tube rack several times for a few seconds during the reaction period. After cooling, add 1 ml. of 8*N* nitric acid and 0.5 ml. of 1% gelatin solution, and adjust to 10 ml. Transfer to the polarographic cell and record the Cl^- waves ($E_{1/2}$ = +0.22 volt) in the range 0 to +0.45 volt. Deaeration is not necessary. A double wave will occur at the higher concentrations, but the plateau of the main wave is very reproducible. Graph the net diffusion current *vs.* concentration (Figure 4).

To separate chloropicrin from carbon tetrachloride in air samples, two separate samples are needed. The first is absorbed in 8.5 ml. of propanol-monoethanolamine reagent and the second in 9.5 ml. of 85% methanol. Process as indicated for carbon tetrachloride and chloropicrin, respectively, using the appropriate agar bridges. Record the carbon tetrachloride and chloropicrin waves in the ranges −0.4 to −1.2 volts and 0 to +0.45 volt, respectively. The presence of the one will not interfere with measurement of the other.

Methyl Bromide. Prepare a calibration curve from standard solutions of methyl bromide in 60% 1-propanol for the range 0.1 to 10.0 mg. in a manner similar to that prescribed for chloropicrin. Place the capped tubes in a constant temperature water bath at 40° C. for 4 hours, agitating the tube rack several times during the reaction period. After cooling, add 1 ml. of 8*N* nitric acid and 0.5 ml. of 1% gelatin solution, and adjust to 10 ml. Transfer to the polarographic cell and record the Br^- wave ($E_{1/2}$ = +0.15 volt) in the range 0 to +0.45 volt. As with chloropicrin, deaeration is not necessary. A double wave will occur at the higher concentrations. Plot net diffusion current *vs.* concentration (Figure 3). Check against standard Br^- solutions.

To separate methyl bromide from carbon tetrachloride in air samples, take two separate samples. The first is absorbed in 8.5 ml. of monoethanolamine-propanol reagent, and the second in 9.5 ml. of 85% methanol placed in tubes in an ice bath. Process as indicated for methyl bromide and carbon tetrachloride, respectively, using the appropriate agar bridges. Record the carbon tetrachloride and methyl bromide waves in the ranges −0.4 to −1.2 volts and 0 to +0.45 volt, respectively.

To separate methyl bromide from ethylene dibromide in air, take two samples and process by either of these methods:

A. Hydrolyze the first sample in 8.5 ml. of monoethanolamine-propanol reagent at 3° to 5° C. (refrigerator) for 24 hours with occasional agitation of the tube rack. [At this temperature and time, ethylene dibromide will not react (*2*).] Hydrolyze the second sample similarly at 98° C. for 3 hours in a constant temperature air oven (*2*) with occasional agitation. (Use pliers to tighten the tube caps.) Cool, stop the reaction in both cases with 1 ml. of 8*N* nitric acid, add 0.5 ml. of 1% gelatin, adjust to 10 ml. with 60% 1-propanol if necessary, and determine the Br^- content in each sample. By deducting the Br^- value of the first sample (methyl bromide) from that of the second (methyl bromide + ethylene dibromide) one obtains the Br^- due to ethylene dibromide. Precede such determinations by methods calibration data, using aliquots of solutions of admixtures of known composition.

B. Hydrolyze the first sample at 3° to 5° C. for 24 hours with monoethanol-

amine reagent as in the previous method and determine the methyl bromide content as Br^-. Absorb the second sample in 9.5 ml. of 85% methanol, add 0.5 ml. of tetramethylammonium hydroxide, and volatilize-hydrolyze the methyl bromide by bubbling nitrogen through the sample at a rate of 30 ml. per minute for about 20 minutes. (The optimum time for elimination of methyl bromide can be determined empirically by pretesting with known solutions of methyl bromide as such and in combination with ethylene dibromide.) Record the wave for ethylene dibromide in the range −1.2 to −2.0 volts as previously described.

Results and Discussion

Linear relationships between diffusion current and concentration were obtained for all five gases in the ranges specified (Figures 3 and 4). Plot points below 0.1 mg. and above 8.0 mg. per 10 ml. were necessarily omitted for purposes of the illustrations. As indicated by the different slopes, the current-concentration ratios varied with the nature of the gas. Thus, methyl bromide, ethylene dibromide, acrylonitrile, chloropicrin, and carbon tetrachloride had i_d/C values of 3.50, 3.85, 10.02, 4.96, and 4.00 μa. per mg. per 10 ml. of sample solution at 25° C., or of 3.32, 7.24, 5.44, 8.17, and 6.16 μa. per millimole.

The precision between replicates was satisfactory, and was within ±1.2% at $10^{-3}M$ levels and within ±5.1% at $10^{-5}M$ levels. It was generally not necessary to measure in the $10^{-5}M$ region because the concentration could be raised by absorbing a larger sample. However, where experimental conditions impose limits on the size of air sample—e.g., taking "point" samples of 5 to 10 ml. of the interstitial air of sacked grain, flour, soil, packaged goods, etc., that have been fumigated—one can use the method of standard addition (*14*), in which case fortification of the solution with 1.0 mg. of fumigant standard is suggested. Sample solutions that are too high in gas concentration can be diluted readily, although sometimes it is as easy to prepare a fresh sample by using a smaller volume of air.

The gas absorption assembly (Figure 1) worked well, and was modified for sampling of grain columns (*7*) and glass fumigation flasks (*8*) to give results reproducible to within ±2.3% in the range 0.25×10^{-4} to $10^{-4}M$. The efficiency of gas absorption of a single absorption tube was 98 to 100%, as determined by placing a second tube in series with the first. Major factors in efficient absorption were the use of chilled "trapping" solvent (ice bath temperature), a slow rate of discharge of air sample (30 to 33 ml. per minute), at least a 4-inch path for bubble travel, and the design of the bubble-fractionating tip which imparted a spin to the fine bubbles that emerged from the outlets, and thereby increased the path of travel. Since the final volume of solution in each tube was only 10 ml., lower limits of sensitivity of measurement were thereby achieved, and this made it possible to work with small gas-air samples (20 to 100 ml.). To absorb gas-air samples larger than 300 ml., faster rates of sample discharge would be required to maintain the total sampling time within reasonable limits, in which case an assembly with two tubes in series should be used for each sample.

The volume of sample solution was standardized at 10 ml. for convenience in use with the simple polarographic cell shown in Figure 2. Once the sample is in solution in the calibrated tube, brought to volume, and mixed, placing a definite or exact volume of solution in the polarographic cell is unimportant to the final result; the proportion of electro-oxidizable or -reducible substance would be the same in a 0.1-ml. aliquot as in the original 10 ml. of solution, and the same D.M.E. may be used in either case with identical results. Although 2 ml. could have been readily used in a narrower polarographic cell with appreciable reduction of time required for deaeration, this was not done for the grain column investigation (*7*), but has been adopted for current work.

The constantly renewable surface of the D.M.E. gave results for Br^- and Cl^- (methyl bromide and chloropicrin) that were more consistently reproducible at higher concentrations than amperometric titration with Ag^+ and a rotating platinum wire electrode. The platinum electrode had to be cleaned often, and a fresh sample was needed if the original sample was too high in gas concentration. In the latter regard, concentrations could be more readily adjusted to the optimum range when the D.M.E. was used, and did not require repeat samples that were sometimes unobtainable in a kinetic study.

Differentiation between carbon tetrachloride and ethylene dibromide in a single sample is relatively simple, since carbon tetrachloride, if present, would exhibit two well-defined waves ($E_{1/2}$ = −0.85 and −1.75 volts) and ethylene dibromide would yield only one wave at approximately −1.55 volts. Interestingly, no wave is shown for ethylene dichloride in 80% methanol and $0.05M$ tetramethylammonium hydroxide. This can be applied in measurement of its presence in a tertiary mixture with the other two gases by first determining the total organic halogen content of the sample (*2, 16, 17, 20*) and then deducting the values of carbon tetrachloride and ethylene dibromide determined by the methods described here.

The methods can be used in determining residues in fumigated foods, textiles, tobacco, soils, etc. The method for acrylonitrile was adapted for such a purpose (*4*). Measurement of fumigant residues of grain in columns (*7*) was not within the terms of reference of the investigation and residues were therefore not determined. In addition to applications for residue problems of concern to agricultural research and Food and Drug Administration agencies, the methods may be applied to research in air pollution, plant and animal toxicology, industrial processing, reaction kinetics, etc.

Acknowledgment

The author is particularly grateful to his assistant, John Solomon, for helping to execute many developmental tests and for designing a crossed-wire bubble-fractionating tip to improve gas recovery. Thanks are also due to Ray Cunningham, Head, Instrument Shop, at this station, for constructing the various sampling assemblies that were developed.

Literature Cited

(1) Beesing, D. W., Tyler, W. P., Kurtz, D. M., Harrison, S. A., *Anal. Chem.* **21,** 1073 (1949).
(2) Berck, B., Can. Dept. Agr., Publ. **1101** (1961).
(3) *Ibid.*, **1104** (1961).
(4) Berck, B., J. Agr. Food Chem. **8,** 128 (1960).
(5) Berck, B., *Proc. Xth Intern. Congr. Entomology* **4,** 99 (1956).
(6) Berck, B., Solomon, J., *Anal. Chem.* **34,** 514 (1962).
(7) Berck, B., Solomon, J., J. Agr. Food Chem. **10,** 163 (1962).
(8) Berck, B., Solomon, J., Liscombe, E. A., unpublished manuscript.
(9) Busbey, R. L., Drake, N. L., *Ind. Eng. Chem., Anal. Ed.* **10,** 390 (1938).
(10) Chaney, A. L., *Ibid.*, **10,** 326 (1938).
(11) Feinsilver, L., Oberst, F. W., *Anal. Chem.* **25,** 820 (1953).
(12) Fuller, L. T., Norris, M. B., Research Div., American Cyanamid Co., Stamford, Conn., private communication.
(13) Kolthoff, I. M., Lee, T. S., Stocesova, D., Parry, E. P., *Anal. Chem.* **22,** 521 (1950).
(14) Kolthoff, I. M., Lingane, J. J., "Polarography," 2nd ed., Chap. XVIII, Interscience, New York, 1952.
(15) Kolthoff, I. M., Yutzy, C. H., *Ind. Eng. Chem., Anal. Ed.* **9,** 75 (1937).
(16) Mapes, D. A., Shrader, S. A., *J. Assoc. Offic. Agr. Chemists* **40,** 180 (1957).
(17) Munsey, V. E., Mills, P. A., Klein, A. K., *Ibid.*, **40,** 201 (1957).
(18) Ramsey, L. L., *Ibid.*, **40,** 175 (1957).
(19) Stackelberg, M. v., Stracke, W., *Z. Elektrochem.* **53,** 118 (1949).
(20) Stenger, V. A., Mapes, D. A., *J. Assoc. Offic. Agr. Chemists* **40,** 196 (1957).

Received for review May 8, 1961. Accepted October 16, 1961. Division of Agricultural and Food Chemistry, 138th Meeting, ACS, New York, September 1960. Contribution No. 86, Canada Department of Agriculture, Research Station, Winnipeg, Man., Canada