of allethrin were added to the milk samples taken prior to the start of the spray program in amounts ranging from 0.1 to 0.5 p.p.m. Samples of 200 grams were then analyzed as described under the heading of procedure. The zero point of the scale was set with the fresh reagent (Table I).

Known amounts of allethrin in a volatile solvent were added to 100-gram samples of meat. The solvent was allowed to vaporize and the meat extracted and tested as described under procedure (Table II).

Qualitatively, the method indicated that no allethrin or its component, chrysanthemummonocarboxylic acid, appeared in the milk or meat during the spray program.

Quantitatively, the method indicated that allethrin, if present, was less than 0.1 p.p.m.

Literature Cited

(1) Assoc. Offic. Agr. Chemists, Washington, D. C., "Methods of Analysis," 7th ed., p. 72, 1950.
(2) Fischer, W. Z., *Anal. Chem.* **113,** 1 (1938).
(3) Schreiber, A. A., McClellan, D. B., *Ibid.*, **26,** 604 (1954).

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INSECTICIDE RESIDUES

Colorimetric Determination of Residual Perchloroethylene in Fumigated Wheat

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The use of tetrachloroethylene in fumigants to control insect infestation in grains makes it necessary to determine the residual amount of this compound present after fumigation. A simple, sensitive colorimetric method for determining small amounts of perchloroethylene in wheat—utilizing a new-found color reaction of perchloroethylene with pyridine, aniline, and sodium methylate—is presented.

PUBLISHED ANALYTICAL METHODS for small amounts of perchloroethylene fall into three general classes. First, a direct spectrophotometric measurement in either the infrared or ultraviolet regions as reported by Bernstein, Semeluk, and Arends (*1*), Berton (*2*), and Hanson (*6*). Even assuming that sufficient sensitivity could be obtained by this method, the problem of "cleanup" of other absorbing materials present in the wheat prohibits its use. A second general method involves decomposition of the perchloroethylene by one of several methods with subsequent determination of the liberated chloride, generally on a micro scale. Such procedures are reported by Mapes and Shrader (*9*), Buscarons and Mir (*3*), Winteringham (*12*), Johnson (*7*), and Elliot (*4*). Although results are good the procedures are generally lengthy, always exacting, and highly susceptible to extraneous chloride pickup. The third method is an application of the Fujiwara (*5*) color reaction of halogenated compounds with pyridine and aqueous caustic as reported by Lugg and Wright (*8*) and Webb, Kay, and Nichol (*10*). While the Fujiwara reaction provides a simple and sensitive colorimetric method for most chlorinated methanes and ethanes, perchloroethylene does not enter into the reaction nearly so readily. The sensitivity is poor and the color fades rapidly.

When perchloroethylene is refluxed with a pyridine-aniline mixture for about 15 minutes, the sodium methylate solution is added, and the refluxing is continued for about 45 minutes, a sensitive and reproducible color reaction is obtained. This is the basis for determining residual perchloroethylene in wheat.

The sample of grain is digested in 0.15*N* sulfuric acid solution under reflux. The condenser is held at a temperature of approximately 65° C., which holds back most of the water but permits the released perchloroethylene to be swept over into pyridine absorbers when aided by a continuous sweep of aspirated air through the system. The perchloroethylene is then determined colorimetrically.

Procedure

The digestion-aeration train is shown in Figure 1. Absorption tube, *A*, is packed with activated carbon aimed at removing halogenated compounds that might be present in the air. The digestion flask, *B*, is heated by a heating mantle with Variac control. The temperature of the 8-inch, straight-tube condenser, *C*, is conveniently maintained at 65° C. by means of a flame-heated, 15-foot coil of $^1/_4$-inch copper tubing connected directly to the cold water tap. Tube *D* serves as a condensate trap; bulbs *E* and *F*, sulfuric acid scrubbers, remove some of the unwanted material which distills over from the wheat, and water vapor, which has the undesirable effect of decreasing the color intensity of the perchloroethylene reaction. Bulb *G* serves as a mist trap and *H* and *J* are pyridine absorbers for the perchloroethylene.

Ten milliliters of Karl Fischer grade pyridine, measured with a pipet, are divided—approximately 7 ml. and 3 ml.—between the first and second pyridine absorbers, 5 ml. of concentrated sulfuric acid are pipetted into each of the sulfuric acid scrubbers, and the absorbers are placed in the train. With the train completely assembled and 65° C. water flowing through the condenser, 200 ml. of 0.15*N* sulfuric acid are added to the digestion flask. Eighty grams of wheat sample are then quickly weighed and added to the flask, and the flask is stoppered. By means of a bleed control on the line going to the aspirator, a rate of sweep of about 150 ml. of air per minute is maintained through the system. The current applied to the heating mantle is adjusted so that the wheat suspension will boil in 10 to 15 minutes. Heating and sweeping are continued for an additional 45 minutes, the air sweep is then stopped by venting the bleed line, and the plug in the neck of the flask is removed. The contents of the first pyridine absorber are transferred to a 125-ml. flat-bottomed, standard-taper Soxhlet extraction flask. A rubber squeeze bulb is helpful in forcing the liquid through the frit.

The contents of the second pyridine absorber are used to rinse the first absorber before transfer to the flask. Both absorbers are finally rinsed with 5 ml. of pure pyridine, measured with a

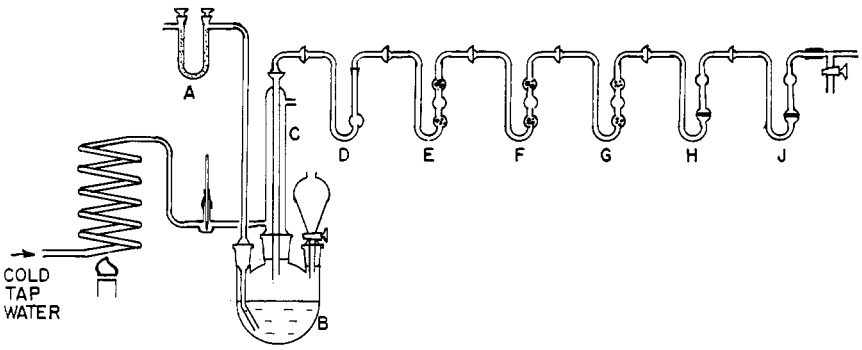

Figure 1. Digestion aeration train

A. Activated carbon packed U-tube
B. 500-ml. three-necked, round-bottomed flask, standard taper joints
C. Hot water condenser
D. Condensate trap, 15-mm. tube, 25-mm. outside diameter tube
E,F. Sulfuric acid scrubbers, 25-mm. outside diameter bulbs packed with beads
G. Mist trap
H,J. Pyridine absorbers for perchloroethylene, 25-mm. outside diameter bulb with a medium porosity frit.

pipet, first rinsing the second absorber and then the first. Five milliliters of colorless aniline are pipetted into the flask containing the perchloroethylene-pyridine mixture, a 12-inch straight-tube water condenser is fitted to the flask without lubrication and the mixture is refluxed for 15 minutes. Approximately 0.2 ml. of a 5% w./v. solution of sodium hydroxide in methanol is then added through the condenser by means of a pipet, without interrupting the heating. After a total reflux time of 1 hour, the flask is removed from the hot plate, cooled under the tap, and the orange solution is transferred to a 50-ml. Nessler tube. The flask is rinsed once with methanol, the rinsings are added to the Nessler tube, and the tube is diluted to the mark with methanol and mixed by inverting. Eight minutes after removing from the hot plate the absorbance is read on a Fischer filter photometer against a distilled water blank using a blue filter and 23-ml. cells. The amount of perchloroethylene in the wheat is then read from a prepared analytical working curve.

Experimental

Construction of Working Curve. A working curve was constructed by pipetting 1-ml. aliquots of standard solutions of perchloroethylene in methanol directly to 80 grams of untreated wheat in the digestion flask and then following the procedure just described to obtain the points on the curve. Three determinations were made and averaged for each point on the curve, resulting in a straight line intercepting the y axis, indicating a small blank value. From the scattering of the points on the curve an accuracy for the method of about ± 1 p.p.m. is indicated. When blank determinations were performed on 80-gram samples of untreated wheat and the values were plotted as additional points on the curve, the average value fell on the y axis at the point of intersection. A yellow coloration was observed in the pyridine-aniline–sodium methylate solution from the blank wheat determinations, but fortunately this color disappears when the solution is diluted with methanol in the Nessler tubes.

Blank values were also obtained for the pyridine-aniline–sodium methylate solution itself and were only slightly lower than the untreated wheat blanks, showing that interfering components from the wheat contribute very little indeed to the color.

Recovery Data. Recovery information of perchloroethylene from the wheat was obtained in two ways. First, another curve was prepared from standard solutions of perchloroethylene in pyridine, without being carried through the train. The two curves agree exactly showing that when perchloroethylene is added to wheat and immediately distilled out, 100% recovery is obtained. While this proves that the technique of distillation-aeration is sound and that the design of the train is also sound, it does not prove that 100% recovery can be obtained for perchloroethylene that has remained in contact with wheat for a period of time. To check this, known amounts of perchloroethylene were added to 80-gram wheat samples in 500-ml., 3-necked, round-bottomed flasks and the flasks were sealed. After standing for various periods of time, the flasks were placed on the train and analyzed. Recovery data from these experiments are shown in Table I. Within the accuracy of the method, 100% recovery of perchloroethylene from fumigated wheat is obtained.

Table I. Perchloroethylene Recovery from Treated and Sealed Wheat Samples

(Eighty grams of wheat treated; 37.0 p.p.m. perchloroethylene added)

Samples Exposed, Days	Perchlor. Found, P.P.M.	Perchlor. Recovery %
3	36.5	98.6
7	37.0	100.0
10	37.7	101.9

Sodium Methylate Concentration. The effect of the sodium methylate concentration on color intensity was studied by using 0.1, 0.2, 0.4, and 0.6 ml. of sodium methylate which gave 0.443, 0.438, 0.440, and 0.390 absorbance values, respectively. Within a relatively large sodium methylate concentration range, there is no difference in color intensity. The sodium methylate concentration, though important, is not critical. Two-tenths milliliter of a 5% w./v. solution of sodium hydroxide in methanol was the amount chosen for the procedure.

Preliminary Heating Time. The perchloroethylene, pyridine, aniline, and methanolic caustic cannot be brought together at the same time as a rather intense blank color develops. If the perchloroethylene, pyridine, and aniline are first heated for a short time, then the methanolic caustic solution can be added and the heating continued, without blank color development, and the color intensity is proportional to the perchloroethylene concentration.

A study of final color intensity against preliminary heating times of 15, 30, 45, and 60 minutes gave absorbance values, respectively, of 0.435, 0.430, 0.430, and 0.428. A preliminary heating time of 15 minutes is sufficient for full color development and this is the time chosen for the procedure. No color appears in the blank after a preliminary heating period of 15 minutes.

Secondary Heating Time. The minimum heating period required for full color development after adding the sodium methylate solution was also studied. Figure 2 shows that a 45-minute secondary heating period is sufficient. The color reaction procedure is: 15 ml. of the pyridine-perchloroethylene solution plus 5 ml. of aniline is refluxed for 15 minutes, 0.2 ml. of the sodium methylate solution is added through the condenser, and the refluxing is continued for an additional 45 minutes.

Color Stability. The decrease in absorbance of the reaction solution with

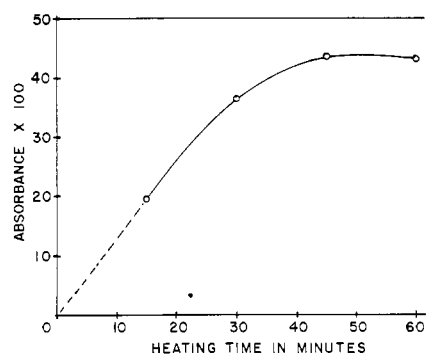

Figure 2. Final color intensity *vs.* secondary heating time

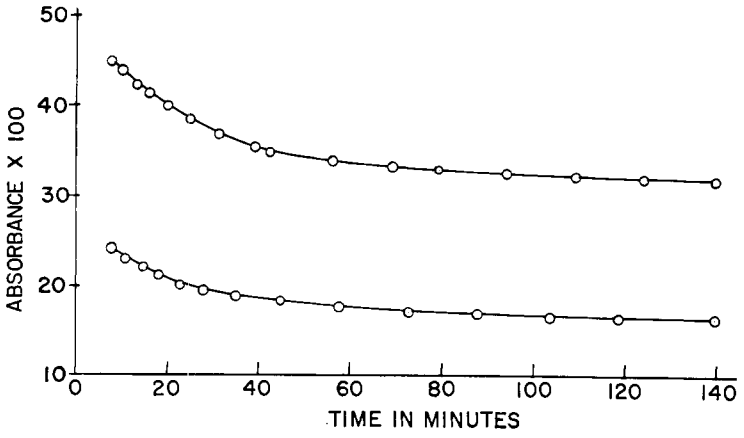

Figure 3. Color stability at two concentration levels

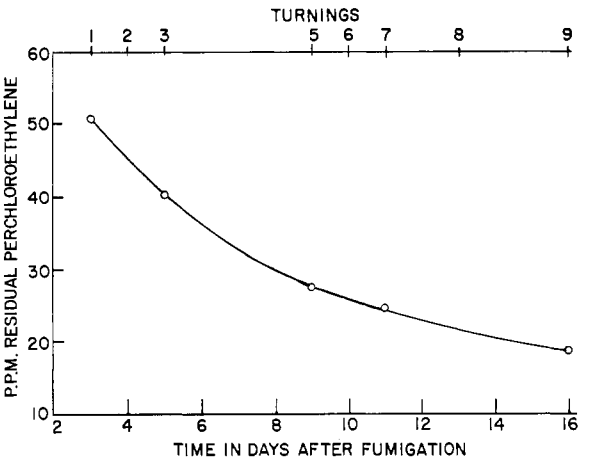

Figure 4. Decrease with time of residual perchloroethylene in wheat fumigated at single dosage level of 400 p.p.m.

time at two concentration levels is shown in Figure 3. As the solutions came off of the hot plate, the color was observed to vary from yellow-orange for low concentrations of perchloroethylene to orange-red for high concentrations. When diluted to 50 ml. with methanol in the Nessler tubes the color shade differences are not nearly so noticeable—all solutions appear yellow. There is a comparatively rapid decrease in absorbance at first with a leveling off of the color intensity, and a stable color is reached after about 90 minutes. Solutions left standing in the Nessler tubes for days showed no visible change in color.

Obviously, if one can afford the time, best reproducibility will be obtained by waiting about 90 minutes before taking the absorbance readings. For the work of this study, very good results were obtained by taking all absorption measurements 8 minutes after removing the solutions from the hot plate.

Fumigation and Sampling Procedure. The fumigated wheat was prepared by adding known amounts of a commercial fumigant to untreated wheat. The major component of the fumigant is perchloroethylene and the minor components, by actual test, do not interfere with the method. The fumigation and weathering was performed indoors at about 25° C.

Two 20-pound samples of untreated wheat were placed in separate 2.5-gallon open-top pails. The first was fumigated at the single dosage level which is equivalent to about 400 p.p.m. of perchloroethylene and the second was fumigated at the triple dosage level which is equivalent to about 1200 p.p.m. of perchloroethylene. The fumigation consisted of sprinkling the required amount of fumigant over the top surface of the grain after which a loose fitting lid was placed on top. After standing for 3 days, the wheat was "turned" by pouring from the pails into second containers and then back into the pails. Immediately following this first turning, three separate samples were taken for analysis from both the single dosage and triple dosage treated wheat. The sample consisted of enough small portions of wheat from different points in the pail to make a composite sample of approximately 100 grams. These composite samples were then sealed in screw-cap jars until the analyses were performed—generally on the same day as the sampling. Ideally, analyses should be performed immediately after sampling. Upon standing, perchloroethylene vapors are lost to the air space in the sampling container.

Results

The perchloroethylene remaining with the single dosage, 400 p.p.m., fumigated wheat is shown in Figure 4 in which the residual parts per million of perchloroethylene is plotted against the time in days after fumigation. Each point is an average value of three separate determinations. A very rapid initial decrease in the perchloroethylene content is apparent. Also, the amount of perchloroethylene that stays on or in the wheat is small. Similar data were obtained for a dosage level of 1200 p.p.m. After 16 days, the perchloroethylene remaining with the wheat had been reduced to about 65 p.p.m.

Discussion

The method is not specific for perchloroethylene; other halogenated compounds will react. Further study of reaction conditions for the other halogenated methanes, ethanes, and ethylenes should prove a sufficient difference in their reactivity to establish a quantitative method for certain ones of these compounds in the presence of others. For example, trichloroethylene yields an intensely colored solution immediately upon addition of the sodium methylate solution, whereas perchloroethylene requires 45 minutes of refluxing for full color development.

The use of amines other than aniline, the effect of water, and the caustic concentration are three possible influencing factors, the control of which might permit an analysis for one compound in the presence of others.

By use of the method as hereby reported, increased sensitivity and color stability for perchloroethylene as compared to the Fujiwara reaction is obtained. Whether or not this is true for other halogenated methanes, ethanes, and ethylenes was not determined, nor was the order of reactivity of these compounds as compared to the Fujiwara reaction determined.

The mechanism of the reaction is not fully established. Probably, the reaction proceeds through a pyridinium salt with subsequent ring opening to yield an anil of glutaconic dialdehyde which then reacts with aniline to form a di- or trianil. Some light is thrown on this reaction by the work of Zincke (*13*), and Wechsler (*11*).

Assuming the reaction proceeds to the trianil of glutaconic dialdehyde, the initial color fading observed with subsequent formation of a stable color may be due to reversion to the dianil. If this were true, then use of a secondary amine instead of aniline should produce a stable color immediately, as only the dianil could be formed. This point was not investigated.

Literature Cited

(1) Bernstein, R. B., Semeluk, G. P., Arends, C. B., *Anal. Chem.* **25,** 139–42 (1953).
(2) Berton, A., *Bull. soc. chim. France* **1949,** p. 858–66.
(3) Buscarons, F., Mir, P., *Anal. Chim. Acta* **7,** 185–90 (1952).
(4) Elliot, F. L., *J. Assoc. Offic. Agr. Chem.* **16,** 383 (1933).
(5) Fujiwara, K., *Sitz. Nat. Ges. Rostock* **6,** 33 (1916).
(6) Hanson, V. F., *Ind. Eng. Chem., Anal. Ed.* **13,** 119–23 (1941).

(7) Johnson, G. M., *J. Assoc. Offic. Agr. Chemists* **17,** 451–3 (1934).
(8) Lugg, G. A., Wright, A. S., *Australia, Dept. Supply, Defence Research Labs., Circ.* **14,** 1–48 (1953).
(9) Mapes, D. A., Shrader, S. A., J. AGR. FOOD CHEM. **2,** 202–3 (1954).
(10) Webb, F. J., Kay, K. K., Nichol, W. E., *J. Ind. Hyg. Toxicol.* **27,** 249–55 (1954).
(11) Wechsler, H., *J. Polymer Sci.* **11,** 233 (1953).
(12) Winteringham, F. P. W., *J. Soc. Chem. Ind. (London)* **61,** 186–7 (1942).
(13) Zincke, Th., *Ann. Chem. Liebigs* **330,** 367 (1904).

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MEAT COLOR RETENTION

Effects of Package Type, Irradiation, and Treatment with Aureomycin on Redness of Vacuum-Packaged Beef Cuts

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Redness of fresh beef samples, vacuum-packaged in cans and in film packages, was measured. Samples in cans with small head space had better red color than those in any film-type package. Of the film materials, a coated polyethylene gave the best red-color preservation. Aureomycin treatment of samples prior to packaging was detrimental to red-color retention. Irradiation at a 500,000-rep. level was detrimental to the red color of samples in cans or film packages.

PREPACKAGED SELF-SERVICE MEAT has largely replaced individual butcher service in the store. There is a problem in maintaining good color in meat in self-service distribution.

Oxygenated myoglobin gives fresh raw meat its bright red color; hence prepackagers maintained conditions that they believed would produce and retain oxymyoglobin.

According to researchers, in certain chemical systems, metmyoglobin, having grayish brown color, is produced from reduced myoglobin at a rapid rate when the partial pressure of oxygen in the surrounding atmosphere is about 1 mm. of mercury. The rate of metmyoglobin formation decreases with increasing partial pressure of oxygen until the value of 30 mm. of partial pressure of oxygen is reached. At this point, the rate becomes constant up to 80 mm. of partial pressure of oxygen. At and above this oxygen tension, reduced myoglobin is converted to oxymyoglobin, which is stable as long as the surrounding atmosphere contains free oxygen at a pressure in excess of 80 mm. of mercury.

Prepackagers concluded that the only way to obtain a satisfactory color in prepackaged meat is to maintain free oxygen at a pressure of at least 80 mm. in contact with the meat. They sought a packaging film which had a high transmission rate of oxygen, to allow oxygen of the air to have access to the meat, and a low transmission rate for moisture, to prevent dehydration. Cellophane, coated on one side with a moisture-resistant material, answered the purpose best when packaged under normal atmospheric conditions. According to Landrock and Wallace (*8*) and others, the cellophane, used in this manner, is saturated with water from the meat, permitting it to transmit oxygen, but the coating on the outside prevents the water from getting out—to a degree.

Results reported herein point strongly to the desirability of following a course diametrically opposed to that which is now in use.

In the currently used package, oxygen can reach the meat from the atmosphere, resulting in the retention of a pleasing bright red color or "bloom" in the meat after packaging. Within 48 hours, however, this bloom is lost and a discoloration, due to the change of the red pigments (myoglobin and oxymyoglobin) to the brown pigment (metmyoglobin), as described by Brooks (*3*) and George and Stratmann (*5*), has occurred. This brown discoloration is unacceptable to the consumer and necessitates the packaging of meat in the individual store as loss of redness would occur during transportation from a central point to retail outlets. Meat, packaged after slaughter and hanging, distributed to retail stores from a central point would result in greater economy and efficiency in distribution to the consumer.

In 1949, an investigation (*1, 4, 10–12*) was started by the Departments of Animal Husbandry and Food Technology, N. J. Agricultural Experiment Station, to find a technique of prepackaging which would permit centralized prepackaging of fresh meat. The project was concentrated on vacuum packaging throughout, with the basic control package being tin-plate cans hermetically sealed under high vacuum.

Fresh meat packed under vacuum lost its red color very quickly and the higher the vacuum and the storage temperature, the higher the rate of color change. However, as vacuum-packed meat was held, red color returned at a variable rate, which was generally low. Under the conditions established in the experiments, 2 weeks or longer were needed for a good color to return. Accelerating the return of red color to meat in cans and finding a transparent film in which the action could be duplicated were the next steps.

In the earliest work, cans were used in which only 20% or less of the volume of the can was occupied by the sample. As a perfect vacuum in the cans could not be obtained, reducing the size of the can in comparison to the volume of the sample (40% of can volume) brought about an accelerated return of red color. Later a can was adopted in which the sample occupied at least 90% of the can volume. In this can, with high vacuum, the initial loss of color is greatly reduced and good red color returns in from 2 to 4 days after packaging.

In the above project and in studies by others, meat has been packaged experimentally in materials differing widely in permeability to moisture and to oxygen (*1, 4, 9*) and in atmospheres of inert gases and atmospheres containing oxygen at various pressures (*12*). Antioxidants (*11*) and enzyme inhibitors (*6*) have been employed. Carbon monoxide will stabilize fresh meat color (*2*). Vacuum packaging has been favored by some (*1, 4*) and is said by others to be undesirable (*2*). The relation-