that the inextractable phosphorus was in casein; this protein is vigorously synthesized during lactation, and contains 0.8 to 0.9% of phosphorus (*11*, *12*). A sample of milk taken at 4 days was centrifuged to remove fat, and hydrochloric acid was added to pH 4.7, in order to precipitate the casein. The filtrate contained 76% of the radioactivity. The major fraction of the P^{32} in milk therefore is not casein; its identity is unknown.

Discussion

Because this study was carried out with one animal, the quantitative aspects of the results should only be tentatively accepted. However, it is clear that metabolism in the cow is substantially similar to that in the mouse, rat, and dog in that hydrolysis at the carboxyester bond accounts for most degradation. However, the fecal metabolites were mainly the product of phosphate ester hydrolysis, suggesting (but not proving) that rumen microorganisms degraded malathion by phosphatase action. If so, the reason that so little phosphatase products were found in the urine might be the fairly rapid uptake from the rumen that was observed, so that metabolism in the rumen was not very important.

Acknowledgment

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INSECTICIDE RESIDUES

Fluorometric Method for Estimation of Residues of Bayer 22,408

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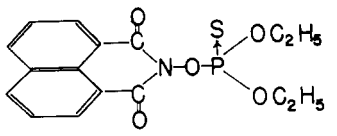
A rapid and sensitive fluorometric method for the estimation of microgram amounts of O,O-diethyl O-naphthalimido phosphorothioate (Bayer 22,408) is based on the principle that, when mixed with methanolic sodium hydroxide and dioxane in the presence of hydrogen peroxide, solutions of this compound fluoresce in proportion to concentration. The sensitivity of the method is 5 γ, and recoveries of the insecticide added to milk and to butterfat were over 90%. The method was also applied to the determination of Bayer 22,408 in plants.

ENTOMOLOGICAL interest in (*O,O*-diethyl *O*-naphthalimido phosphorothioate (Bayer 22,408) has created a need for a sensitive analytical method of detecting and estimating minute amounts of residues of this compound in animal tissues or on treated plants. Interest has centered especially on its toxic action on pests that are injurious and destructive to farm animals.

A technical grade sample of Bayer 22,408 was obtained as a brown powder from the Vero Beach Laboratories, Inc., Vero Beach, Fla. This was crystallized several times from *n*-hexane containing a little chloroform to give fine light yellow crystalline flakes melting at 160° to 161° C. The compound has the following structural formula:



It is not soluble in water but very soluble in most organic solvents, although only slightly soluble in petroleum ether.

Hornstein (*3*) reported that fluorometric methods may be worked out for a number of insecticides containing multiple-conjugated double bonds that possess a high degree of resonance stabilization. Anderson, Adams, and MacDougall (*1*) published a photofluorometric method for the determination of *O* - (3 - chloro - 4 - methylumbelliferone) *O,O* - diethyl phosphorothioate (Co-Ral, also known as Bayer 21/199), using 1*N* potassium hydroxide and heat.

In the development of a fluorometric method for the estimation of Bayer 22,-408 residues, it was found that this compound fluoresces weakly in methanolic sodium hydroxide solution. The fluorescence has a calamine blue color, and its intensity is greatly increased when dioxane containing a small amount of hydrogen peroxide is added to the reaction mixture. Work on the identification of the fluorescent compound is in progress.

Apparatus

Fisher Nefluoro-Photometer or equivalent.

Filter arrangement. Left sample filter, No. 440; light-source filter, No. 365; and right reference filter, No. 430.

Photometric cells, 15 × 90 mm. Scratch a mark on the glass at 10-ml. volume.

Reagents

Mixture of 2 parts of redistilled diethyl ether and 1 part of redistilled *n*-hexane.

Acetonitrile, reagent grade, redistilled.

Chloroform, technical grade, redistilled.

Florex, XXX grade. Wash with redistilled chloroform, filter, and wash until the filtered chloroform becomes colorless. Dry in a hood and finally in an oven at 120° C. overnight.

Dioxane, pure grade, 100 ml., plus 1 ml. of 30% hydrogen peroxide.

Sodium hydroxide, 0.1*N*, in reagent grade methanol.

Quinine Sulfate Solution. Dissolve 80 mg. of quinine alkaloid in 0.1*N* sulfuric acid and dilute to 1 liter; dilute 5 ml. of this solution to 100 ml. with 0.1*N* sulfuric acid, and dilute 1 part of the second solution with 2 parts of 0.1*N* sulfuric acid.

Sodium sulfate, anhydrous, C.P. grade.

Cotton. Extract cotton in a Soxhlet extractor with acetone, dry in air, and then dry overnight in a 100° oven.

Procedure

Preparation of Standard Curve. Transfer 50 mg. of Bayer 22,408 to a 500-ml. volumetric flask and make to the mark with chloroform. Pipet 10 ml. of the solution into a 100-ml. volumetric flask, make up to volume with chloroform, and mix well. Pipet 0-, 1-, 2-, 4-, 6-, 8-, and 10-ml. aliquots of the second solution into small Erlenmeyer flasks. Evaporate the solvent carefully on the steam bath, and remove the last traces at room temperature with a glass tube connected to a vacuum line. The standard solutions may not be stable and should be prepared freshly as needed.

To develop fluorescence, pipet 2 ml. of the 0.1*N* methanolic sodium hydroxide solution into each flask, carefully wetting the sides. Cover, and allow the flasks to stand for 5 minutes. Add 10 ml. of the dioxane reagent to each flask and mix. Filter each solution through about 1 gram of anhydrous sodium sulfate in a 7.0-cm. Whatman No. 2 filter paper. Collect the filtrate directly in a photometric cell (filtered solution should be clear). Measure the amount of fluorescence in the fluorophotometer, which is adjusted to 100% transmittance with the second and third quinine sulfate solutions in the reference and sample beams, respectively. Prepare the standard curve as shown in Figure 1.

Analysis of Milk Samples. Extract 200 ml. of the milk in a large separatory funnel with three 250-ml. portions of the diethyl ether–*n*-hexane mixture, vigorously shaking for at least 1 minute each time. Filter the solvent layer through a plug of cotton. With the aid of a three-bulb Snyder column, concentrate the combined extracts on the steam bath to approximately 25 ml.

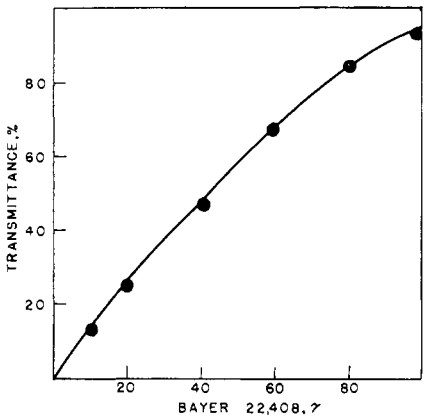


Figure 1. Standard fluorometric curve

Transfer the concentrated extract to a 125-ml. separatory funnel, using 75 ml. of *n*-hexane. Rinse the flask with 25 ml. of acetonitrile and add the rinse to the funnel. Shake the contents of the funnel vigorously for at least 1 minute. When the layers separate, transfer the acetonitrile layer into a second separatory funnel. Extract the acetonitrile in the second funnel with 50 ml. of fresh *n*-hexane by vigorous shaking. Finally, transfer the acetonitrile layer into a small Erlenmeyer flask. Extract the contents of the first and second funnels successively with two additional 15-ml. portions of acetonitrile, and finally drain the acetonitrile extracts into the same Erlenmeyer flask. Evaporate the combined acetonitrile extracts on the steam bath with the aid of a three-bulb Snyder column. Remove the last traces of solvent carefully with a little vacuum at room temperature. Add 50 ml. of chloroform to the residue.

For chromatography, place 15 grams of the activated Florex in the chromatographic tube (20 × 400 mm.), pack the adsorbent down by tapping the sides of the tube, and prewash with 50 ml. of chloroform. When the solvent level reaches the top of the Florex column, introduce the chloroform solution of the residue. From this point on, collect all the chloroform percolated through the column in a clean flask. Measure 150 ml. of fresh chloroform in a graduated cylinder, and use small portions to rinse the Erlenmeyer flask. Add each rinse to the column until all the chloroform has been used. Evaporate the combined eluate with a three-bulb Snyder column on the steam bath. Remove the last traces of chloroform at room temperature with a little vacuum. After complete removal of the solvent, proceed with the analysis as described under preparation of the standard curve, starting with the addition of 2 ml. of methanolic sodium hydroxide.

Table I. Recovery of Bayer 22,408

Added, γ	Recovered γ	Recovered %
From 200 Ml. of Whole Milk		
18.4	17.0	92.4
36.8	34.8	94.6
73.6	68.6	93.2
110.4	102.6	92.9
From 20 Grams of Butterfat		
16.4	15.2	92.7
32.8	29.6	90.2
65.6	62.8	95.7
98.4	92.8	94.3
From 200 Grams of Plant Material		
Red Cabbage		
22.6	21.8	96.5
45.2	42.8	94.7
90.4	87.0	96.2
Spinach		
18.2	17.4	95.6
36.4	33.0	90.7
91.0	85.6	94.1
Apple		
16.8	15.2	90.5
33.6	31.7	94.3
84.0	77.8	92.6

Analysis of Plant Samples. Extract a weighed amount of the chopped sample with a measured volume of the redistilled chloroform in a wide-mouthed glass jar by tumbling for at least 1 hour. Use at least 100 ml. of solvent for each 100 grams of plant material to ensure complete extraction. Filter the extract through a layer of anhydrous sodium sulfate packed on a small plug of cotton in a Gooch crucible holder. Measure the volume collected and evaporate the solvent to dryness. Dissolve the residue in 25 ml. of *n*-hexane and finish the determination as described for the milk sample, starting from the extraction with acetonitrile.

Tests Made with Method

Recovery from Whole Milk. Aliquots of a standard chloroform solution of Bayer 22,408 were added to a number of 200-ml. whole-milk samples. Each milk sample was then extracted with the *n*-hexane and diethyl ether mixture and analyzed by the procedure described. The results are shown in Table I.

Recovery from Butterfat. The per cent recovery of Bayer 22,408 added to several butterfat samples (20 grams each) was determined by the same procedure (Table I).

Recovery from Plant Material. Recovery studies were run on Bayer 22,408 added to cabbage, spinach, and apple samples. Each plant sample was chopped fine, extracted with chloroform, and analyzed by the method described (Table I).

Discussion

The fluorometric method is suitable

for the estimation of Bayer 22,408 residues in milk, butterfat, or plant samples, especially when all the material that may cause interference can be removed by the repeated solvent extractions, evaporation, and chromatography. However, to avoid excessively high reagent blanks, certain general precautions should be followed. First, all glassware should be cleaned with cleaning solution, thoroughly rinsed with water, and dried in an oven at 100° C. Second, contact of the analytical solution with stopcock grease, rubber, cork, soap powder, or soap film must be avoided, as these materials can introduce additional fluorescence and cause high readings.

With the use of a Fisher Nefluoro-Photometer and the filters specified, the range of the method is limited to 5 to 100 γ of Bayer 22,408 in the samples analyzed. However, with an Aminco-Bowman spectrophotofluorometer, the activation and fluorescence maxima under the conditions of the method were 372 and 480 mμ, respectively. As little as 0.01 γ in 1 ml. can be detected with this instrument. Therefore, the sensitivity and range of the method can be greatly increased if a spectrophotofluorometer is used or if more accurate filters of proper wave lengths are used.

Under the experimental conditions, Bayer 22,408 fluoresces strongly in the alcoholic sodium hydroxide solution. The intensity of the fluorescence is diminished when a small amount of acid is added to the reaction solution, and is completely quenched when the solution is made strongly acidic, but it does not change if an excess of alcoholic sodium hydroxide is added. Acetone and water also have a quenching effect on the fluorescence intensity.

Increasing the temperature up to 50° C. increases the intensity of fluorescence, but if the reaction mixture is kept between 20° and 30° C., the intensity will not change.

Experience shows that it is important to include hydrogen peroxide in the dioxane. Chemically purified dioxane was first tried, and the intensity of the fluorescence was very low; however, when 0.1 ml. of 30% hydrogen peroxide was added to the same solution, the intensity became extremely high. The intensity could not be further increased by the addition of larger amounts of hydrogen peroxide.

Although practically no interference has thus far been encountered in runs on milk, butterfat, and plant materials not treated with Bayer 22,408, it is always important to run such control analyses. According to reports (*2*), extracts of many plants fluoresce when exposed to ultraviolet light; therefore, in the analysis of plant samples the inclusion of untreated control samples is especially important.

Most insecticides do not interfere; however, some, such as Co-Ral and Potasan [*O*-(4-methylumbelliferone) *O,O*-diethyl phosphorothioate], give some fluorescence in this method, but will interfere only if present in amounts exceeding 10 mg.

Acknowledgment

The author is grateful to M. S. Schechter of this division for helpful suggestions and criticisms.

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HERBICIDE STRUCTURE AND STABILITY

Effect of Chemical Structure on Microbial Decomposition of Aromatic Herbicides

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The effect of molecular structure on the persistence and microbial decomposition of phenoxyalkyl carboxylic acid herbicides, chlorophenols, and some related compounds is presented. The resistance of some aromatic herbicides or their derivatives to microbial degradation is governed by the position of the halogen on the aromatic nucleus and by the linkage and type of aliphatic side chain.

THE USE of herbicides to control and eradicate weeds has presented several problems in addition to the initial plant response to the applied chemical. An aspect of these studies which becomes more important with the greater utilization of herbicides in agriculture is that dealing with the persistence and decomposition of the added material. The duration of activity of the herbicide has a distinct bearing upon agronomic practice, because phytotoxicity may be observed not only in the season of application but in succeeding years as well.

In many instances where herbicide breakdown occurs, evidence is available to show that the active agents in the process are members of the soil microflora. With the exception of a small group of compounds, however, little is known of the conditions favoring the decomposition or of the effects of molecular structure upon microbial decomposition. A study has been made to ascertain the rate of breakdown of a number of halogenated phenols and phenoxyalkyl carboxylic acids by certain soil populations and to determine which of these substances are transformed microbiologically.

Methods

The ultraviolet absorption characteristics of each of the chemicals investigated was determined with the Beckman spectrophotometer using aqueous solutions containing 10 to 100 p.p.m. of the compound. From each absorption spectrum, an absorbance was chosen for the analytical determination either at the point of maximal absorption or at a wave length close to it, with the latter choice adopted to minimize the number of individual spectrophotometric manipulations. The wave lengths used are included in Tables I and II. Of the materials tested, the absorbances were proportional to their concentration up to levels of more than 50 p.p.m. This is in agreement with the results of Dorschner and Buchholtz (*2*), who investigated the optical properties of several of these herbicides.