Spectrophotofluorometry for Pesticide Determinations

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A number of organic pesticides have been shown to fluoresce in the ultraviolet region. Maximum activation and fluorescence wave lengths are reported. These measurements, which offer a greater degree of specificity to fluorometric analysis, are possible because of recent improvements in instrumentation. The lower limit of sensitivity for the compounds exhibiting fluorescence is about 0.01 γ per ml.

THE DETERMINATION of pesticide residues in concentrations of less than 0.1 p.p.m. necessitates the handling of large samples, and demands that the analyst work at the limit of accuracy of the methods available.

Spectrophotometric measurements in the visible and ultraviolet regions of the spectrum provide the most specific and sensitive procedures for such determinations. However, few of the spectrophotometric methods developed for the determination of pesticides can detect less than 1 γ per ml. of a compound. Fluorometric methods, on the other hand, can be used often to determine as little as 0.01 γ per ml. The extreme sensitivity of fluorometric methods, when applicable, should permit the reliable determination of hundredths of a part per million of a pesticide in small samples. A brief survey has therefore been made of the potential applicability of spectrofluorometry to the determination of trace amounts of pesticides. Cleanup procedures for the separation of pesticides from plant materials and animal tissues prior to application of this technique have not been investigated.

Recent advances in instrumentation have enlarged the field of application of fluorometric methods. Bowman, Caulfield, and Udenfriend (*1*), Udenfriend and associates (*4*, *5*), and Duggan and coworkers (*2*) have described the design and analytical applications of a spectrophotofluorometer, now commercially available, in which a xenon arc lamp produces a continuous source of activating radiation in the region of 220 to 800 mμ. Two monochromators are used, one to supply monochromatic radiation from this xenon arc and the other, at right angles to the incident beam, to isolate the fluorescent light emitted by the sample. By this arrangement, the wave length—in the visible or ultraviolet—of the activation maximum as well as the fluorescence peak can be determined. Specificity of such measurements is thus greatly increased.

In general, fluorescence may be expected in molecules containing multiple-conjugated double bonds that possess a high degree of resonance stabilization. Molecules exhibiting fluorescence therefore include those containing aromatic nuclei; unsaturated heterocyclic nuclei, such as quinoline and indole; and long-chain conjugated polyenes, such as vitamin A and its precursors. Substituents may greatly alter the degree of fluorescence of the parent compound.

Electron-donating groups, such as $-NH_2$ and OH, may increase fluorescence, whereas electron-withdrawing groups, such as $-NO_2$ and halogens, may diminish and even destroy fluorescence. The magnitude of these effects is also dependent upon the position of such groups in the molecule. The general nature of fluorescence has been discussed by West (*6*, *7*) and by Oda and Yoshida (*3*). Practical considerations in spectrophotofluorometry are discussed by Duggan and coworkers (*2*).

Experimental

Apparatus. Aminco-Bowman spectrophotofluorometer or its equivalent.

Silica cells, 1-cm. cross section; 4 sides transparent.

Procedure. Stock solutions were prepared containing 10 γ per ml. of the pesticide in methanol. Measurements were made at a dilution of 1 γ per ml. in methanol and, as pH often affects fluorescence intensity, in aqueous solutions at four pH values—pH 1 (0.1*N* sulfuric acid), pH 7 (phosphate buffer), pH 11 (1*N* ammonium hydroxide), and pH 14 (1*N* sodium hydroxide).

For each sample the fluoresence spectrum was scanned while the activation wave length was changed 10 mμ per scan until the entire activating range was covered. When a fluorescence band was observed, the wave lengths of maximum activation, as well as the fluorescence peak, were determined. In those samples where pH was important, the pH of maximum fluorescence was used in subsequent studies. Sensitivities were determined by continued dilution of the stock solutions. The final volume of solution for all measurements was 5 ml.

Practical sensitivity has been defined (*2*) as "that concentration which gives a fluorescent intensity reading equal to 10% of full-scale deflection on the meter of the Aminco-Bowman instrument, at highest sensitivity using $^1/_{16}$-inch defining slit (band pass = 6 mμ) and a 1P28 photomultiplier." The sensitivity data in Table I are based on this definition, except that a $^1/_8$-inch defining slit (band pass = 12 mμ) and a 1P21 photomultiplier tube were used. The wave lengths of maximum activation and fluorescence and the solvent media are also tabulated. The observed wave lengths are dependent to a degree on the particular instrument used and are therefore not absolute values. For analytical purposes these values are adequate.

Discussion

The results of tests with compounds that were found to fluoresce are given in Table I. Several of these compounds show two activation maxima for one fluorescence peak. The higher activation wave length gives the greater fluorescence intensity. For example, the sensitivity value for naphthaleneacetic acid at pH 7 and activation wave length 282 is 0.02, while, at the same pH and at activation maximum 230, the sensitivity drops to 0.1 This increase in sensitivity may be in a large part attributable to the difference of intensity of the xenon arc at the two activation wave lengths. The xenon arc has a low intensity at 225 mμ, rises sharply from 225 to 350 mμ, and is relatively constant from 350 to 450 mμ—peaking at 400 mμ. The arc output then drops to about 50% of the peak value at around 600 mμ, then rises again at about 800 mμ.

Guthion, one of the new organophosphorus insecticides, contains a benzotriazine group. At pH 11, Guthion hydrolyzes to form a fluorescent compound. Anthranilic acid, a possible

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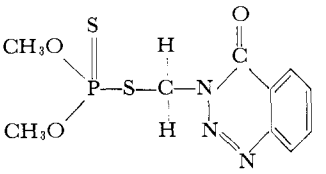
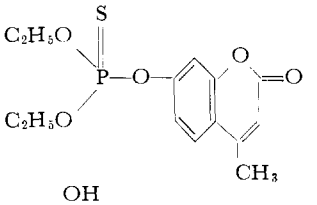
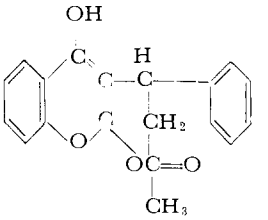
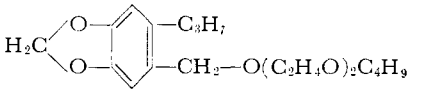
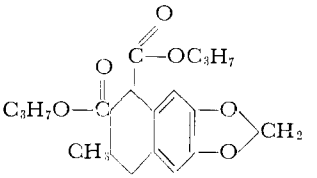
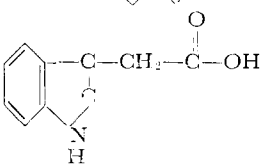
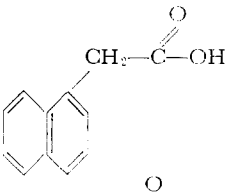
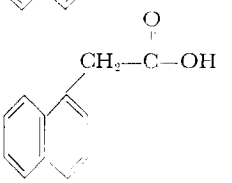
Table I. Sensitivity and Activation and Fluorescence Wave Lengths for Several Pesticides

Compound	Structure	Wave Length, Mμ: Activation λ_{max}	Activation λ_{50} [1]	Fluorescence, λ_{max}	Solvent	Sensitivity, γ/Ml.
Guthion[2]		312 250	298, 327	380	Water (pH 11)	0.007 0.04
Potasan		320	292, 345	385	Methanol	0.04
Warfarin		320	290, 342	385	Methanol	0.04
Piperonyl butoxide		292 248	282, 302	318	Methanol	0.01 0.05
n-Propyl isome		292 248	280, 305	326	Methanol	0.01 0.05
Indoleacetic acid		285	265, 295	345	Water (pH 7)	0.008
Naphthaleneacetic acid		282 230	270, 305	327	Water (pH 11)	0.02 0.10
Naphthaleneacetamide		286 239	270, 305	327	Water (pH 11)	0.02 0.14

[1] Activation wave lengths at which fluorescence intensity decreases to 50% of peak value while fluorescence wave length is kept constant at its maximum value.

[2] This solution must stand for 0.5 hour before readings are taken. Fluorescence is caused by a hydrolysis product.

hydrolysis product, is markedly fluorescent and may be the compound actually measured; Guthion in methanol or in strong acid exhibits little fluorescence.

The fluorescence exhibited by the other compounds in Table I may be attributed to the following structures: Potasan, a phosphorus insecticide, and warfarin, a rodenticide, both contain a hydroxycoumarin nucleus. The pyrethrin synergists piperonyl butoxide and *n*-propyl isomer have in common the methylenedioxyphenyl group. Indoleacetic acid, and presumably similar plant-growth regulators, fluoresce because of the indole nucleus. The naphthalene group is responsible for the fluoresence exhibited in plant-growth regulators of the naphthalene derivative type.

Other compounds that were studied at a concentration of 1 γ per ml. but failed to show fluoresence included DDT, TDE, methoxychlor, (2,4-dichlorophenoxyacetic acid), (2,4,5-trichlorophenoxyacetic acid), *p*-chlorophenoxyacetic acid, Diazinon, rotenone, and Aramite.

Chlorinated organic insecticides such as the isomers of hexachlorocyclohexane, toxaphene, aldrin, dieldrin, chlordan,

and heptachlor are not expected to fluoresce, as no conjugated double bonds are present in their structures. DDT-type insecticides do not fluoresce because of their halogen-substituted ring structure, and the same is apparently true for the chlorinated phenoxyacetic acid materials. Rotenone contains a dimethoxyphenyl group, but this apparently does not contribute to fluorescence in the solvent systems used. Methoxychlor, in which two methoxyphenyl groups are present, is also inactive. Aramite, in which electron donation through hyperconjugation in the *p-tert*-butylphenoxy nucleus is not possible, also fails to fluoresce, as does Diazinon, which contains a pyrimidine nucleus.

In the low concentration range (below 1 γ per ml.), fluorescence is directly proportional to concentration; at higher concentrations self-quenching becomes significant and may lead to low results.

For comparative purposes the absorbance of piperonyl butoxide at λ_{max} 288 using a Beckman Model DU and 1-cm. silica cells was determined. At a concentration of 1 γ per ml., this absorbance was 0.027 unit. The sensitivity using the spectrofluorometer is 0.01 γ per ml.

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ISOTOPE-LABELED FERTILIZERS

Preparation of Radioactive Polynutrient Fertilizers Having Specified Phosphate Solubilities

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Several series of radioactive mixed fertilizers, having different degrees of water solubility, were prepared in a small-scale production plant for use in greenhouse and field investigations. The mixtures were classified as mixed-salt types, prepared by the blending of suitably chosen inorganic salts, and commercial types, derived from the treatment of phosphate rock with sulfuric or nitric acid. High phosphate availability and close control of the phosphate water solubility were obtained in the mixed-salt types. More difficulty was encountered in regulation of solubility and availability in the commercial types.

THE RADIOISOTOPE of phosphorus, phosphorus-32, is commonly employed as a tracer in agronomic investigations of soil, plant, and fertilizer reactions. Since 1947, the Fertilizer Investigations Research Branch, as a result of an agreement with the Atomic Energy Commission, has been engaged in the small-scale production of fertilizers and fertilizer materials containing this tracer element (*3*). The labeled products are shipped to a number of universities and state or federal cooperating agencies.

The type of material produced has been determined by the needs and desires of the using groups. Recently, attention has been focused on polynutrient combinations in which the water solubility of the contained phosphate falls within defined limits. Field and greenhouse investigations are being conducted at several locations on the influence of the degree of this solubility and on the effects of granulation to various particle sizes. A summary of the information gained by experience in the preparation of these materials, including a description of known factors which influence variability in products, is presented.

Manufacturing Methods

The labeled polynutrient fertilizer mixtures were classified as: mixed-salt types, prepared by the blending of suitably chosen inorganic salts, and commercial types, derived from superphosphate or from nitric acid treatment of phosphate rock (nitric phosphate). All of these materials were prepared by a slurry process. Certain of the manufacturing operations, such as labeling, blending and drying of mixtures, and granulation of pulverulent products, are common to the preparation of both types and will be described separately.

Labeling. Radioactive phosphorus is obtained from the Oak Ridge National Laboratory as an aqueous solution of potassium dihydrogen phosphate of negligible nonorthophosphate content. In the fertilizer manufacture, portions of this solution are added to each phosphate source to supply a specific activity in the product of approximately 0.15 mc. per gram of phosphoric oxide.

Radiation intensities normally encountered in the manufacture of radioactive fertilizers are such that precautionary safeguards against exposure and particulate contamination must be strictly observed. Protective clothing, special equipment and tools, and adequate shielding and ventilation are necessary (*3*). All of the processing equipment, such as grainers, grinders, granulators, and screeners, are mounted in hooded enclosures; chemical reactions, such as acidification, ammoniation, precipitation, and filtration, are carried out in a hood equipped with facilities for the collection of waste materials.

Blending and Drying. These operations are carried out in a grainer, a specially designed mixer-evaporator,