RESIDUE REVIEWS

VOLUME 51

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Residues of Pesticides and Other Contaminants in the Total Environment

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VOLUME 51

SPRINGER-VERLAG
NEW YORK · HEIDELBERG · BERLIN
1974

Library of Congress Catalog Card Number 69-18595.
Printed in the United States of America.

New York: 175 Fifth Avenue, New York, N.Y. 10010
Heidelberg: 6900 Heidelberg 1, Postfach 1780, West Germany

ISBN 0-387-90079-9 Springer-Verlag New York Heidelberg Berlin
ISBN 3-540-90079-9 Springer-Verlag Berlin Heidelberg New York

Foreword

Worldwide concern in scientific, industrial, and governmental communities over traces of toxic chemicals in foodstuffs and in both abiotic and biotic environments has justified the present triumvirate of specialized publications in this field: comprehensive reviews, rapidly published progress reports, and archival documentations. These three publications are integrated and scheduled to provide in international communication the coherency essential for nonduplicative and current progress in a field as dynamic and complex as environmental contamination and toxicology. Until now there has been no journal or other publication series reserved exclusively for the diversified literature on "toxic" chemicals in our foods, our feeds, our geographical surroundings, our domestic animals, our wildlife, and ourselves. Around the world immense efforts and many talents have been mobilized to technical and other evaluations of natures, locales, magnitudes, fates, and toxicology of the persisting residues of these chemicals loosed upon the world. Among the sequelae of this broad new emphasis has been an inescapable need for an articulated set of authoritative publications where one could expect to find the latest important world literature produced by this emerging area of science together with documentation of pertinent ancillary legislation.

The research director and the legislative or administrative advisor do not have the time even to scan the large number of technical publications that might contain articles important to current responsibility; these individuals need the background provided by detailed reviews plus an assured awareness of newly developing information, all with minimum time for literature searching. Similarly, the scientist assigned or attracted to a new problem has the requirements of gleaning all literature pertinent to his task, publishing quickly new developments or important new experimental details to inform others of findings that might alter their own efforts, and eventually publishing all his supporting data and conclusions for archival purposes.

The end result of this concern over these chores and responsibilities and with uniform, encompassing, and timely publication outlets in the field of environmental contamination and toxicology is the Springer-Verlag (Heidelberg and New York) triumvirate:

Residue Reviews (vol. 1 in 1962) for basically detailed review articles concerned with any aspects of residues of pesticides and other chemical contaminants in the total environment, including toxicological considerations and consequences.

Bulletin of Environmental Contamination and Toxicology (vol. 1 in 1966) for rapid publication of short reports of significant advances and discoveries in the fields of air, soil, water, and food contamination and pollution as well as methodology and other disciplines concerned with the introduction, presence, and effects of toxicants in the total environment.

Archives of Environmental Contamination and Toxicology (vol. 1 in 1973) for important complete articles emphasizing and describing original experimental or theoretical research work pertaining to the scientific aspects of chemical contaminants in the environment.

Manuscripts for *Residue Reviews* and the *Archives* are in identical formats and are subject to review, by workers in the field, for adequacy and value; manuscripts for the *Bulletin* are not reviewed and are published by photo-offset to provide the latest results without delay. The individual editors of these three publications comprise the Joint Coordinating Board of Editors with referral within the Board of manuscripts submitted to one publication but deemed by major emphasis or length more suitable for one of the others.

November 21, 1973 Joint Coordinating Board of Editors

Preface

That residues of pesticide and other contaminants in the total environment are of concern to everyone everywhere is attested by the reception accorded previous volumes of "Residue Reviews" and by the gratifying enthusiasm, sincerity, and efforts shown by all the individuals from whom manuscripts have been solicited. Despite much propaganda to the contrary, there can never be any serious question that pest-control chemicals and food-additive chemicals are essential to adequate food production, manufacture, marketing, and storage, yet without continuing surveillance and intelligent control some of those that persist in our foodstuffs could at times conceivably endanger the public health. Ensuring safety-in-use of these many chemicals is a dynamic challenge, for established ones are continually being displaced by newly developed ones more acceptable to food technologists, pharmacologists, toxicologists, and changing pest-control requirements in progressive food-producing economies.

These matters are of genuine concern to increasing numbers of governmental agencies and legislative bodies around the world, for some of these chemicals have resulted in a few mishaps from improper use. Adequate safety-in-use evaluations of any of these chemicals persisting into our foodstuffs are not simple matters, and they incorporate the considered judgments of many individuals highly trained in a variety of complex biological, chemical, food technological, medical, pharmacological, and toxicological disciplines.

It is hoped that "Residue Reviews" will continue to serve as an integrating factor both in focusing attention upon those many residue matters requiring further attention and in collating for variously trained readers present knowledge in specific important areas of residue and related endeavors involved with other chemical contaminants in the total environment. The contents of this and previous volumes of "Residue Reviews" illustrate these objectives. Since manuscripts are published in the order in which they are received in final form, it may seem that some important aspects of residue analytical chemistry, biochemistry, human and animal medicine, legislation, pharmacology, physiology, regulation, and toxicology are being neglected; to the contrary, these apparent omissions are recognized, and some pertinent manuscripts are in preparation. However, the field is so large and the interests in it are so varied that the editors and the Advisory Board earnestly solicit suggestions of topics and authors to help make this international book-series even more useful and informative.

"Residue Reviews" attempts to provide concise, critical reviews of timely advances, philosophy, and significant areas of accomplished or needed endeavor in the total field of residues of these and other foreign chemicals in any segment of the environment. These reviews are either general or specific, but properly they may lie in the domains of analytical chemistry and its methodology, biochemistry, human and animal medicine, legislation, pharmacology, physiology, regulation, and toxicology; certain affairs in the realm of food technology concerned specifically with pesticide and other food-additive problems are also appropriate subject matter. The justification for the preparation of any review for this book-series is that it deals with some aspect of the many real problems arising from the presence of any "foreign" chemicals in our surroundings. Thus, manuscripts may encompass those matters, in any country, which are involved in allowing pesticide and other plant-protecting chemicals to be used safely in producing, storing, and shipping crops. Added plant or animal pest-control chemicals or their metabolites that may persist into meat and other edible animal products (milk and milk products, eggs, etc.) are also residues and are within this scope. The so-called food additives (substances deliberately added to foods for flavor, odor, appearance, etc., as well as those inadvertently added during manufacture, packaging, distribution, storage, etc.) are also considered suitable review material. In addition, contaminant chemicals added in any manner to air, water, soil or plant or animal life are within this purview and these objectives.

Manuscripts are normally contributed by invitation but suggested topics are welcome. Preliminary communication with the editors is necessary before volunteered reviews are submitted in manuscript form.

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January 18, 1974

F.A.G.
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By

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I. Introduction

Diazinon is a broad-spectrum insecticide of the following chemical structure:

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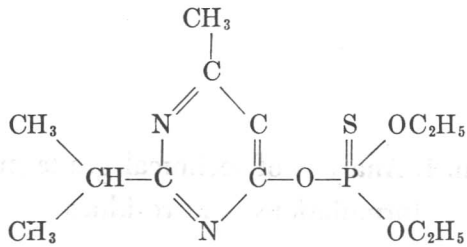

O,O-Diethyl-*O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate

It is effective against a variety of orchard, vegetable, and soil pests, ectoparasites, and vectors (flies, lice, fleas). The acute oral toxicity of diazinon is LD_{50} for mouse, 85 to 135 mg./kg. and LD_{50} for rat, 150 to 220 mg./kg. It is a colorless liquid of boiling point 83° to 84°C/0.0002 mm., refractive index n_D^{20} 1,4978, solubility in water at 20°C 40 p.p.m., and vapor pressure at 20°C 1.4×10^{-4} mm. of Hg.

II. Identification

The first identification of diazinon as well as other pesticides by means of the infrared spectrum was published by FISCHER and UHLICH (1960). Infrared spectra have also been used for the characterisation of the possible by-products TEPP, monothiono-TEPP, and dithiono-TEPP by MARGOT and GYSIN (1957). A new infrared technique for the identification and for the detection of trace amounts of diazinon was developed by HERMANN (1965) with Frustrated Multiple Internal Reflectance. The phosphorescence characteristics have also been successfully used for the characterisation of diazinon as well as other organophosphorus compounds (MOYE and WINEFORDNER 1965). The mass spectrum of diazinon, investigated by DAMICO (1966), and the NMR spectrum, published by KEITH *et al.* (1968), represent excellent analytical tools for confirmation.

III. Analysis of technical grade product

A method for the exact determination of the diazinon content in various formulated products (SUTER *et al.* 1955) uses the titration with perchloric acid in glacial acetic acid and 1-naphtholbenzene as an indicator. This method is appropriate if perchloric acid consuming by-products are absent. In technical grade diazinon this is not the case. Therefore, MARGOT and STAMMBACH (1964) described an improved method where all perchloric acid reacting by-products are eliminated by shaking the technical material, dissolved in petroleum ether, with 3*N*-sulfuric acid without any loss of the active ingredient. The isolated pure diazinon is then titrated with perchloric acid. In

order to distinguish between the two procedures, the first is called determination of "total diazinon," the latter determination of "pure diazinon." The 3*N*-sulfuric acid step is adaptable to most analyses of diazinon formulation products. The procedure has disadvantages for the routine determination of large sample series because of the time and equipment required. Therefore, a new method was developed by SCHNELLER (1963) where the extraction with 3*N*-sulfuric acid was replaced by a column chromatographic separation of basic by-products from the pure compound.

Recently, a gas chromatographic (GLC) method was published by MURPHY *et al.* (1971). This procedure, using a flame ionization detector and aldrin as an internal standard, is well suited to determine both the quality of diazinon technical materials and the diazinon content of various formulations. The diazinon content obtained by the GLC procedure corresponds to the "pure diazinon" content in the titration method.

a) Determination of "total diazinon" by titration

Accurately weigh 0.4 to 0.6 g. of the sample in a 150-ml. beaker and dissolve in approximately 80 ml. of glacial acetic acid. Titrate with glass/silver electrodes.

Let w = weight of sample in g.
a = standard 0.1 *N*-perchloric acid used in ml.

Then:

$$\text{Percent "total diazinon"} = \frac{a \times 304.4 \times 100}{w \times 10{,}000}$$

Note: If the diazinon contains epoxides as stabilizer, it is necessary to deactivate the epoxides before titration. In this case pour the sample into a 200-ml. ground-joint Erlenmeyer flask, dissolve in 50 ml. of glacial acetic acid, reflux for 15 minutes, cool, rinse into the titration beaker, and titrate.

b) Determination of "pure diazinon" by titration

1. Apparatus. —

Chromatography columns, 25 mm. i.d. × 400 mm., equipped with Teflon stop-cocks
Tamping rod, 55 cm. long with flat surface of diameter to fit loosely inside of chromatography column
Electric kitchen mixer
Powder funnel
Erlenmeyer flasks, 250 and 500 ml. with 24/40 joints
Beakers, 50-ml. and 150-ml. capacity

Reflux condensers, all glass, 24/40 glass joints
Hot plates
Magnetic stirrers
Soxhlet extraction apparatus
Separatory funnel, 250 ml. with 24/40 glass joint

2. Reagents. —
Petroleum ether, 30 to 60°C boiling range
Ethyl ether, anhydrous, reagent grade.
Hyflo Super-Cel (Johns Manville Co.)
Sulfuric acid, 3*N*, aqueous
Perchloric acid, 0.1*N*, in acetic acid
Silica gel, Merck, 0.05- to 0.2-mm. particle size
Glacial acetic acid, reagent grade

3. Preparation of the 3N-sulfuric acid-column. — Two-hundred and fifty g. of Hyflo Super-Cel (this amount is enough for ten columns) is transferred to the mixing bowl. The bowl is put into position under the beaters with the bowl in the off-center position; 150 ml. of the 3*N*-sulfuric acid is added slowly while mixing. After all of the acid has been added, the mixing is continued until a homogeneous mixture is obtained. As the mixing proceeds, the Hyflo Super-Cel must be scraped from the sides of the bowl. After a uniform mixture has been obtained, the bowl is removed from the electric mixer. (*Caution:* The electric mixer must not be used for the subsequent steps in the procedure since petroleum ether is used and an explosion could readily occur from sparks generated by the electric mixer).

Enough petroleum ether is added to the sulfuric acid-Hyflo Super-Cel mixture, while stirring by hand using a porcelain spatula, so that the Hyflo Super-Cel is covered with petroleum ether; this slurry is now ready for packing into the columns. A glass wool plug is tamped into position at the bottom of the column. Portions of the slurry are added to the column through a powder funnel. Each portion is tamped gently with the tamping rod. As the excess petroleum ether is pressed out, it is allowed to drain off by opening the stop-cock. Packing is continued with portions of the slurry until each column is packed to a height of five inches. Enough petroleum ether is retained in the column so that the Hyflo Super-Cel is just covered with solvent at all times. Properly packed columns allow the petroleum ether to pass through dropwise. After the column is packed, a small glass wool plug (¼ to ½ in. high) is tamped into position on the top.

4. Column chromatography and titration. — Accurately weigh 0.4 to 0.6 g. of diazinon into a 25-ml. Erlenmeyer flask and transfer with about five to ten ml. of petroleum ether into the column. Open the stop-cock and allow the solution to penetrate into the column to the level of the petroleum ether phase. Wash the Erlenmeyer flask quanti-

tatively twice each with five ml. and ten ml. of petroleum ether, add the petroleum ether fractions to the column, and allow them to penetrate into the column. Now add 30 ml. of petroleum ether, set up the dropping funnel which contains 200 ml. of petroleum ether, and elute while keeping the stop-cock open until all petroleum ether has dropped through the column. Collect the eluate in a 500-ml. Erlenmeyer flask. Evaporate the petroleum ether and rinse the distillation residue with about 80 ml. of glacial acetic acid into a 150-ml. beaker. Finally titrate potentiometrically with 0.1*N*-perchloric acid in glacial acetic acid as described in section **III** *a*).

c) Determination of "pure diazinon" by gas chromatography

1. Standard solutions. — (α) Aldrin internal standard solution. Weigh 4.0 ± 0.1 g. of technical grade aldrin into a 600-ml. beaker. Slurry with 400 ml. of acetone to dissolve and filter through paper into a one-l. volumetric flask; wash with several 100-ml. portions of acetone. Dilute to volume and mix well. Standards should be ≧90 percent pure and must contain no impurities that elute at diazinon retention time.

β) Diazinon standard solution. Accurately weigh approximately 125 mg. of diazinon of known purity (CIBA-GEIGY Chemical Corp., Ardsley N.Y. 10502) into a four-oz. round bottle with an aluminum foil-lined screw cap. Pipet in 50 ml. of aldrin internal standard solution and shake well.

2. Preparation of sample. — Accurately weigh sample into a four-oz. aluminum foil-lined screw-cap bottle to provide approximately 110 mg. of diazinon. Pipet in 50 ml. of aldrin internal standard solution. Cap and shake.

3. Gas chromatography. — Use an instrument equipped with a flame ionization detector and a 1.8 m. × 4 mm. i.d. glass column packed with ten percent silicone DC-200 (Applied Science Laboratories, Inc.) on 80/100 mesh Gas-Chrom Q. Condition 24 hours at 240°C with nitrogen or helium flow at approximately 40 ml./min; this column should develop ≧2,000 theoretical plates. Operate at 240°C, carrier gas 80 to 100 ml./min., air and hydrogen flows as recommended for detector, attenuation varied so that peak heights of diazinon and aldrin are 60 to 80 percent full scale; retention times are diazinon five to six min. and aldrin ten to 12 min.

4. Determination. — Inject three μl. aliquots of standard solution until the peak-height ratio diazinon:aldrin varies ≦ one percent for successive injections. Make duplicate injections of sample followed by duplicate injections of standard. Peak-height ratios of standards must be within ± one percent of first accepted standard values or repeat series of injections. Repeat for additional samples.

5. Calculations. — Calculate peak-height ratios for duplicate

standard injections both preceeding and following sample. Average the four values (R_s), calculate and average peak-height ratios of the two samples (R_x), then percent diazinon = $(R_x/W_x) \times (W_s \times P/R_s)$, where W_s = mg. of standard, W_x = mg. of sample, and P = percent purity of diazinon standard.

IV. Analysis of formulations

a) Procedures for wettable powders, dusts, and granules

If wettable powders, dust formulations, or granules are analysed by titration the active ingredient is extracted by ethyl ether. Emulsifiers, if present, are adsorbed by column chromatography using silica gel as adsorbent. After removal of the solvent, the residue is dissolved in petroleum ether and the basic by-products of diazinon are separated on a column filled with Hyflo Super-Cel impregnated with 3*N*-sulfuric acid. The "pure diazinon" content is determined in the eluate by potentiometric titration with perchloric acid in glacial acetic acid using glass/silver electrodes. Titration in glacial acetic acid without chromatography on the 3*N*-sulfuric acid/Hyflo column yields the "total diazinon" content. The weight of sample should correspond to 0.4 to 0.6 g. of diazinon.

The GLC-analysis of powders, dusts, or granules is preferably achieved by directly adding 50 ml. of the aldrin internal standard solution to an appropriate quantity of formulation containing 100 to 150 mg. of diazinon. After shaking, the analysis is performed according to section **III** *c*).

b) Procedures for emulsifiable solutions

For the "total diazinon" content by titration the appropriate amount of sample is dissolved in glacial acetic acid and titrated according to section **III** *a*). If the "pure diazinon" is determined, the emulsifier has to be removed by a silica gel column prior to the separation of by-products by the Hyflo column. The detailed procedure is described below.

Fill a chromatographic tube with ethyl ether to about two-thirds of the tube height. Pour 30 ml. of silica gel into a dry graduated cylinder and transfer the adsorbent to the column, while agitating with a glass rod. Drain off the solvent above the silica gel level. Then accurately weigh the appropriate amount of sample (w.g.) into a 25-ml. Erlenmeyer flask, dissolve in ten ml. of ethyl ether, and put the solution into the column. Open the stop-cock and allow the solution to penetrate into the column just above the level of the adsorbent. Wash the Erlenmeyer flask five times with five-ml. portions of ethyl ether, add the fractions to the column, and allow them to

penetrate into the column nearly to the adsorbent level. Now add 50 ml. of ether, attach the dropping funnel which contains 200 ml. of ether, and elute. Keep the stop-cock open until the ether has passed through the column. Collect the eluate in a 500-ml. Erlenmeyer flask and distil off the ether. Now determine the "pure diazinon" as described in section **III** *b*). For the gas chromatographic determination of "pure diazinon," apply directly the method in section **III** *c*).

c) *Procedure for aerosol sprays*

Connect the accurately-weighed aerosol bomb to a polyethylene tube by means of a suitable adapter. Insert the tube into a 300-ml. Erlenmeyer flask containing about ten ml. of petroleum ether. Open the valve so that a gentle stream of aerosol enters the Erlenmeyer flask and is collected below the surface of the petroleum ether. The propellant evaporates and the diazinon remains dissolved in the solvent. Close the valve, disconnect the tube, and weigh the aerosol bomb again. Evaporate the petroleum ether and proceed according to section **III** *a*), **III** *b*), or **III** *c*).

d) *Comparison of titration and gas chromatographic methods*

The analytical titration method described in sections **III** *a*) and **III** *b*) was applied successfully in many laboratories for almost two decades to the routine determination of diazinon in thousands of samples. In 1965 it was adopted as the Official CIPAC method. The reproducibility of the procedure was tested as follows (Suter and Nowak 1969):

A sample of technical grade diazinon was analysed at weekly intervals by different analysts of CIBA-GEIGY Basle using different equipment and reagents. The statistical evaluation of the results is summarized below:

N (no. of analyses)	54
$\bar{x}$ (mean value of percent "pure diazinon")	96.75
S^2 (variance)	0.034
S_{abs} (absolute standard deviation for single value)	0.184
CL_{95} (confidence limits for single value)	0.369

The gas chromatographic procedure described in section **III** *c*) was collaboratively studied in 1970 by 13 collaborators within Europe and the United States and yielded an over-all coefficient of variation of 1.0 percent. This method was adopted as AOAC Official in 1971.

Analytical results obtained by both procedures agree satisfactorily as demonstrated in Table I (Suter 1971).

Recently a collaborative test was performed between eight laboratories in Europe and the United States. The analytical results demonstrate a very good agreement of both procedures (Eberle *et al.* 1974).

Table I. *Comparison of titration and gas chromatographic methods* (SUTER 1971).

Diazinon product analysed	"Pure diazinon-content" (%)	
	By titration (CIPAC-method)	By GLC (AOAC-method)
Technical grade diazinon	96.5, 96.5	97.2, 97.1
Technical grade diazinon	92.8, 92.9	92.5, 92.5
Diazinon formulation (25 EC)	25.4, 25.4	25.6, 25.6
Diazinon formulation (2 D)	2.00, 2.02	2.01, 2.00

V. Residue analysis

a) Extraction

Many procedures are reported in the literature for extracting diazinon and other organophosphorus insecticides from various products. SUTER *et al.* (1955) used a mixture of ethyl ether-petroleum ether and ammonia for the extraction of diazinon from milk and BLINN and GUNTHER (1955) extracted diazinon from milk by shaking with petroleum ether after freeze-drying the milk. Petroleum ether was also used by GUNTHER *et al.* (1958) for removal of diazinon from oranges and by BRODERICK *et al.* (1967) for the extraction of grapes. LEONI (1971) applied the same solvent to continuous liquid extraction of diazinon from natural waters. MENDOZA *et al.* (1970) reported a hexane extraction procedure for the simultaneous determination of azinphosmethyl, carbophenothion, diazinon, and four other organophosphorus insecticides in whole-wheat flour. Benzene was recommended as the extraction solvent for diazinon from water by KONRAD *et al.* (1967) who followed the degradation and leaching of ^{14}C-labelled diazinon in soil.

Mixtures of petroleum ether with more polar solvents have been described by TEASLEY and COX (1963) who used repetitive extractions with an ethyl ether-petroleum ether mixture to remove diazinon from water. In a study of the persistence of various insecticides including diazinon, STATHOPOULOS *et al.* (1971) used petroleum ether-acetone (9:1) for the extraction of soils and carrots. A hexane-acetone mixture was recommended for removing diazinon residues from various soil types by LICHTENSTEIN *et al.* (1967), by GETZIN (1968), and by SUETT (1971). Vegetable crops such as carrots, Brussels sprouts, beans, broccoli, and spinach were macerated in acetone:hexane (1:4) in a residue study with 31 pesticides including diazinon (SISSONS *et al.* 1970). Excellent recoveries were obtained by BRO-RASMUSSEN *et al.* (1968)

with a petroleum ether-ethanol (1:2) mixture to remove diazinon residues from soils by rigorous shaking of the mixture for 60 minutes.

More polar solvents are usually applied when residues of parent orgonophosphorus compounds and metabolites are determined in crops or animal tissues. The rapid determination of diazinon and diazoxon in small samples of blood and animal tissues was performed by grinding the samples with sodium sulfate and sand and eluting the mixture from a column with ethyl ether or methanol (MACHIN *et al.* 1969). Methanol is also used as extraction solvent in the routine residue methods of CIBA-GEIGY Basle for determining diazinon and metabolites in soil and plant and animal tissues (EBERLE and NOVAK 1969, Voss 1971). MCRAE and MCKINLEY (1963) recommended blending of crop material with acetone for the extraction of diazinon from apples, lettuce, cabbage, and oranges. GETZIN and ROSEFIELD (1966) and GETZIN (1967) used acetone in a radioactive study on the fate of diazinon in soils. The authors found that the maximum amount of radioactive substance in treated soil was removed by a mixture of water and acetone. The addition of calcium chloride facilitated the precipitation of interfering colloidal clay. Acetone was also used as extraction solvent by BOWMAN *et al.* (1971) and by BOWMAN and BEROZA (1971) in pesticide residue studies including diazinon in a wide range of fruits, vegetables, meats, dairy products, and fats.

ROBBINS (1957) followed the pattern of metabolism of ^{32}P-labelled diazinon when fed to cows and recommended ethanol-hexane for the extraction of milk and hexane for the extraction of blood. An extraction method developed by MODDES and COOK (1959) and improved by GETZ (1962) and by MILLS *et al.* (1963) was widely used for removal of various organophosphorus esters including diazinon from leafy crops and fruits. This procedure consists of chopping the sample and subsequent blending a 100-g. subsample with 200 ml. of acetonitrile and ten g. of Celite. This extraction procedure was recommended in the official FDA-method and many authors applied it with minor changes for the development of residue methods for detecting diazinon and metabolites besides other organophosphorus insecticides in various crop materials, soil, food, and animal feed products (STORHERR *et al.* 1964 and 1971; COFFIN and MCKINLEY 1964; MCCAULEY 1965; GUNNER *et al.* 1966; NELSON 1964, 1966, and 1967; WELLS 1967; WESSEL 1967; MENDOZA *et al.* 1968 b; WALES *et al.* 1968; SIEWERSKI and HELRICH 1970; MENDOZA and SHIELDS 1971).

In the search for rapid extraction procedures especially suitable for routine determination, WATTS and STORHERR (1965), STORHERR and WATTS (1965 and 1968), and WATTS *et al.* (1969) recommended ethyl acetate instead of acetonitrile as an extraction solvent after comparison of the extraction efficiencies of both solvents with field-treated crops. The authors blended 25 g. of chopped crop material with 80