A POLYSTYRENE GEL PERMEATION METHOD OF SAMPLE PREPARATION IN THE ANALYSIS OF MALATHION IN WHEAT

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ABSTRACT

A POLYSTYRENE GEL PERMEATION METHOD OF SAMPLE PREPARATION IN THE ANALYSIS OF MALATHION IN WHEAT

by

Norman L. Aker

A most important step in insecticide analysis is the quantitative separation of the insecticide from interfering substances, mainly lipids and pigments. This procedure, commonly known as cleanup, consists predominantly of methods utilizing adsorption materials.

After preliminary evaluation of a number of adsorbents appropriate for column chromatography including silicic acid, Florisil®, DeCalso®, two different types of clay, porous glass, and several gas chromatographic supports, the work on malathion (S-[1,2-bis(ethyoxycarbonyl) ethyl]O,O-dimethyl phosphorodithioate) separation was confined to the use of a Bio-bead® column. Iso-octane as the eluting solvent gave unsatisfactory results, but satisfactory elution was obtained with 10 percent iso-octane in benzene. In the final procedure, benzene is used for swelling the Bio-beads in column preparation. Impurities in the Bio-beads necessitated the washing of new columns with approximately 200 to 300 ml of benzene to remove materials interfering with gas liquid chromatography (GLC).

Elution patterns of fortified wheat extracts applied to the columns gave two major fractions. The first materials to move through

the column were the exclusion substances, or molecules too large for the pore size of the Bio-beads. Malathion followed in a different, colorless fraction. The elution patterns were established by collecting two-milliliter fractions which were analyzed by GLC. Samples containing up to 1 milligram of malathion could be handled on a column bed 20 mm in diameter by 360 mm high. Flow rates of 34 - 40 ml per hour gave optimum separation. Flow rates up to 125 ml per hour, using pressure, still gave adequate separation.

Recoveries of malathion applied to the Bio-bead columns ranged from 94 to 112 percent for 10.0 to 1000 µg quantities. Mixtures of other insecticides, including DDT, lindane, heptachlor and heptachlor epoxide, aldrin, and parathion, were added to determine their interference in this analysis. Aldrin and parathion were not separated from malathion by GLC, however, aldrin was separated from malathion by the Bio-bead column.

The Bio-bead column can be used repeatedly for sample cleanup, with no apparent change.

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Introduction

During the past 25 years, a large number of organic compounds have been introduced to control insects, weeds and plant diseases. Some are used on crops, pastures, forest lands and animals as well as for household purposes. The population of much of the world is constantly exposed to small amounts, and occasionally to large doses of these compounds by accident. Because of this chronic exposure, residues of some insecticides have been found in the tissues of many people. Residues of new pesticides, or of those for which new uses are discovered, may also be found in human tissue.

The effectiveness of modern pesticides in controlling agricultural pests helps keep food costs down and the quality high. It has been estimated that if pesticides were to be completely withdrawn from farm use, crop and livestock production in the United States would drop by 25 to 30 percent (89). The U.S.D.A. urges that all users of pesticides exercise constant vigilance to assure the protection of human health by avoiding unnecessary exposure of crops, livestock, fish and wildlife. Chemicals used in combating pests are designated economic poisons, and are subject to the provisions of the Federal Insecticide, Fungicide, and Rodenticide Act of 1947. In addition, those used in such a manner as to be associated with foodstuffs designed to be consumed by humans are also subject to the Federal Food and Drug, and Cosmetic Act of 1938, and provisions of the Pesticide Residue Amendment of 1954, and the Food Additives Amendment of 1958 (1,10).

The organophosphorus insecticides are extremely effective against a wide range of insects. Many are toxic to warm-blooded animals because of their cholinesterase inhibiting action. There has been relatively little development of insect resistance to these compounds compared to that for the organochlorine insecticides (84). Even though more toxic to mammals than the organochlorine insecticides, they usually do not leave highly persistent residues. Because of this, the organophosphorus residues are less likely to accumulate in animal tissues, or in the soil, from which the residues could be absorbed by new crops and subsequently endanger the consumer (1). The F.D.A. has established a tolerance of 8 ppm of malathion in small grains (1). Therefore, a suitable method to determine malathion residues in wheat is needed.

LITERATURE REVIEW

I. History

A. Development

The unusual behavior of organophosphorus compounds was studied during World War II by a German chemist, Gerhard Schrader, who was looking for chemical warfare agents while employed by I. G. Farbenindustrie. His work was the first to demonstrate the insecticidal possibilities of organophosphorus compounds. Parathion was originally designated by Schrader as E-605. Amoung his compounds were other effective pesticides including schradan and TEPP (38). An American team of scientists which visited Germany at the end of the war to obtain information on scientific advances brought back much information on these organophosphorus compounds. Research has since been active and many potent insecticides have been discovered; some which have been put into use are malathion, methyl parathion, demeton, EPN, Dipterex®, DDVP, Phosdrin®, Co-Ral®, Guthion® and ronnel* (84). Patents were issued for certain organic phosphorus compounds useful as insecticides, rodenticides, and fungicides, such as phosphate esters (52), carbamylalkyl phosphates (21), and cyanoalkyl phosphates (22).

*See Appendix.

In 1951, a patent was issued covering adducts of diesters of dithiophosphoric acid and maleic and fumaric esters (20). This covered compounds having the general formula $(RO)_2$ PSS CH (CO_2R') CH_2CO_2R' and having possible use as pesticides, especially in view of their low toxicity in warm-blooded animals. They are prepared by the addition of diesters of dithiophosphoric acid, $(RO)_2$ PSSH, to esters of maleic and fumaric acid. Malathion synthesis is accomplished by the addition of O,O-dimethyl phosphorodithioic acid to diethyl maleate (94).

B. Malathion Uses

Malathion is used on plants and livestock, and in the home. It may be used for direct contact or as a residue, and is applied in either dust or spray form (10).

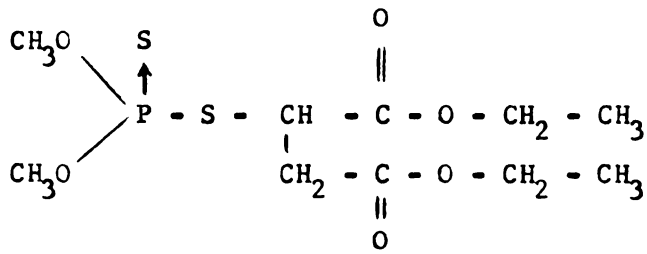
In 1952, Reynolds et al. (77) described work in controlling mites in California with malathion. Many uses for malathion are listed in The Handbook of Pest Control (57), Pests of Stored Grain and Grain Products (32), and the Agriculture Handbook No. 313 (1). An important use for malathion is in the control of the cereal leaf beetle (Oulema melanopus) in small grains (1), and especially in Michigan wheat.

II. Malathion

S-[1,2-bis(ethoxycarbonyl)ethyl]O,O-dimethyl phosphorodithioate

or S-(1,2-dicarbethoxyethyl)-O,O-dimethyl dithiophosphate*

or O,O-dimethyl dithiophosphate of diethyl mercaptosuccinate*



A. Physical and Chemical Properties

1. Physical

Molecular weight	330.4
Melting point	2.9°C
Boiling point	156 - 157°C at 0.7 mm Hg
Vapor pressure	0.0004 mm Hg at 30°C
Refractive index	n_D^{25} 1.4985
Specific gravity	1.2315 at 25°C
Viscosity--at 25°C	36.78 centipoise
--at 40°C	17.57 centipoise

Solubility in H_2O approximately 145 ppm at 25°C. Completely soluble in most alcohols, esters, ketones and aromatic solvents; limited solubility in petroleum hydrocarbons.

*The Merck Index, 7th Ed.

2. Chemical

Rapid hydrolysis occurs at pH values above 7.0 or below 5.0; but it is stable in aqueous solution buffered at pH 5.0 (83). Alkaline hydrolysis under controlled conditions results in quantitative yields of 0,0-dimethyl phosphorodithioate salts (94).

B. Residue Characteristics in the Field

Residues on field crops are considered non-persistent. Coffin (23) found a malathion residue decrease from 11.4 ppm at 4 hrs. to less than 0.1 ppm at 10 days. Malaoxon and 3 unidentified metabolites were detected, at times, up to 2 days after application. When malathion is exposed to air, an atom of sulfur is often replaced with an oxygen atom. Many derivatives and degradation products cannot be detected by the analytical methods employed for the parent compound (13).

C. Toxicity

1. Mechanism

Following World War II, studies were made on these new organophosphorus compounds. Schwartz (80) in 1951 reported that his insecticide studies with frogs indicated that these compounds may block the action of oxidative enzymes. DuBois and Coon (37) in 1952 reported that a class of substances, the alkyl

pyrophosphates and the alkyl thiosphosphates which includes malathion, were active inhibitors of cholinesterase activity.

Many organophosphorus compounds inhibit the action of cholinesterase (ChE). The ChE enzymes catalyze the hydrolysis of acetylcholine to choline and acetic acid. The organophosphorus insecticides are capable of phosphorylating a serine residue at the active center of the enzyme, thus inhibiting its action. Since de-acetylation of acetycholine is essential to nerve function, these compounds act as neurotoxins.

Gardiner (41) reported that since schradan in vitro has low anticholinesterase activity, but still inhibits acetylcholinesterase, it must be converted into a much more potent enzyme inhibitor. He found that incubation with liver does convert the compound into an active poison and he stated that the same is apparently true of other organophosphorus compounds. Gardiner (41) also found that parathion was converted to a compound 1000-times more effective as a ChE inhibitor. Tsuyuki et al. (87) in 1955 published extensive findings on the mechanisms involved with the oxidation of schradan and its increased effectiveness as a ChE inhibitor. Malaoxon is the active principle of

malathion poisoning and paraoxon is the active principle of parathion poisoning (13, Vol. II). Cook et al. (29) in 1957 reported that malathion, in the absence of other organophosphorus insecticides, was believed to be acted upon by an esterase enzyme in liver homogenate which destroyed its anticholinesterase activity and could no longer be tested for by the colorimetric method. Continued work reported in 1958 (30) revealed an enzyme identified as malathionase (MA), which converted malathion into a compound which was a poor anticholinesterase (anti-ChE) agent. From these results, it appears that: 1) malathion is not as highly toxic to mammals as many other organophosphorus insecticides, because it is detoxified rapidly by an enzyme system; 2) this enzyme system seems to be very sensitive to small amounts of a number of other organophosphorus compounds; 3) these compounds were not effective inhibitors unless converted from P double-bond S to the oxygen analog, similar to those of high anti-ChE activity. Additional work by Cook and Yip (31) reported that malathionase (MA) acting on malathion formed the mono-acid by removing one of the ethyl groups.

2. Symptoms of poisoning

The more severe symptoms of organophosphorus poisoning are blurred vision, abdominal cramps and tightness of the chest. Other symptoms are weakness, headache, non-reactive pinpoint pupils, salivation, sweating, nausea, vomiting, and diarrhea.

Repeated exposure to ChE inhibitors may without warning cause prolonged susceptibility to very small doses of any ChE inhibitor.

Since studies involving humans are limited, the toxicities of insecticides are expressed in terms of the results of studies on animals. These results can then be extrapolated to estimate the effects on humans.

Oral LD_{50} white rats, males -- 1375 mg/kg

females -- 1000 mg/kg

Dermal LD_{50} white rats, both sexes -- 4444 mg/kg

III. Residue Analysis

A. Extraction Methods

1. Stripping consists of adding an appropriate solvent to the sample and tumbling or mixing without appreciably grinding or reducing the particle size of the original sample. Mills (63) used this method on soft fruits and vegetables to extract organochlorine insecticides. Klein et al. (55), used this method, along with soxhlet

extraction and the Waring Blendor method in his study on extraction procedures for organochlorine insecticides. He preferred the Waring Blendor method. Ford and Ottes (40) recommended a tumbling procedure as a result of their studies on a method of extracting parathion from leafy vegetables. Hardin and Sarten (51) also used tumbling in their study on extraction procedures for recovery of DDT from collards treated in the field, but found that the Waring Blendor method gave better recoveries than tumbling.

2. Soxhlet extraction consists of discontinuous reflux extracting, using a soxhlet extraction apparatus, for an extended period of time. This method is sometimes termed, exhaustive extraction. Klein et al. (55), Burke and Porter (19) used it in extraction studies as a reference method. Dawson et al. (34), used a reflux method of extraction to determine parathion and related insecticides in cocoa beans. Saha (78) used a 6 to 8 hour soxhlet extraction to determine organochlorine insecticides in wheat.

3. Blendor extraction consists of macerating or mixing a sample, at high speed in a Waring Blendor® or Omini-mixer®, with a solvent or mixture of solvents. Klein et al. (55), concluded that a Waring Blendor extraction

using isopropyl alcohol first, then adding benzene and blending again, was better than drying followed by soxhlet extraction or than tumbling (stripping) for organochlorine insecticides in soft foods. The method is fast and is efficient, according to Burke and Porter (19) who extracted 99% of that obtained by an exhaustive extraction method (soxhlet) for pp′ TDE, (98% of the diazinon, and 94% of the parathion) in vegetables. Mills (63) used this method for small fruits and dried materials. In the widely used Mills-Onley-Gaither method (65), 100 g of a representative sample are blended at high speed in a Waring Blendor for 1-2 minutes with 200 ml of acetonitrile and about 10 g of Celite 545 (Johns-Manville Company).

Watts and Storherr (90) blended various crops to obtain extracts for their sweep co-distillation cleanup method for organophosphorus residues (85). Samuel (79) described a Waring Blendor extraction for organochlorine and organophosphorus insecticides in foods and feeds.

B. Cleanup - Separation

One of the first applications of column chromatography to separate compounds was the use of a silicic acid column by Ramsey and Patterson (76) to separate mixtures of fatty acids. Barrette and Payfer (6) used a silicic acid column

to separate insecticides in formulations and found that it allowed them to observe the separation without using dyes or special equipment. Boone (11) used silicic acid to clean up the extracts from some fruits and vegetables containing Dibrom® and DDVP*.

Tsuyuki et al. (87) used a silica gel column to purify oxidation products in a study of schradan. Walker and Beroza (88) used silica gel TLC cleanup in their work with 62 insecticides. Pesic (71) obtained separations of malathion from potato extract using Kieselguhr TLC. Bates et al. (8) used columns of acid-washed alumnia to clean up barley extracts prior to malathion determinations, and used columns of fuller's earth for extracts of rice bran.

Florisil®(Floridin Co., Pittsburgh, Pa., 15222) was used by Mills (63, 64) in organochlorine insecticide cleanup. Moddes (67) discovered that unless Florisil was properly activated, insecticides required more solvent for elution. McKinley and Mahon (62) reported that aldrin, dieldrin and endrin* were eluted only partially from activated Florisil. Wood (91) found that unless Florisil was partially deactivated, dieldrin, endrin and heptachlor epoxide were not eluted at all or only partially recovered. McCaulley and Cook (60) discussed the use of Florisil prior to determining the infrared

*See Appendix.

spectra of organophosphorus compounds and Double (36) used it to separate malathion from other insecticides in formulations for infrared spectrophotometry. Nelson (70) investigated the Florisil cleanup of organophosphorus insecticides in crop extracts and noted that better recoveries would be desirable. However, Burke and Porter (19) in a study involving pp′ TDE, diazinon and parathion,* concluded that using Florisil and the Mills, Onley and Gaither method (65) gave satisfactory results. Samuel (79) recognized some of the disadvantages of Florisil and developed a several-step alternative method for use with organochlorine and organophosphorus insecticides. This consisted of column chromatography with a mixture of 8 parts anhydrous sodium sulfate, 8 parts celite, 2 parts Attapulgus clay, 3 parts activated carbon and 6 parts aluminum oxide. A hexane solution of the residue, concentrated to about 15 ml, was extracted with four 15 ml portions of acetonitrile. These fractions were added to about 350 ml of water and 100 ml of petroleum ether was added. The mixture was shaken to partition the insecticides into the petroleum ether phase, and the aqueous phase was discarded.

*See Appendix.

The petroleum ether fraction containing the insecticides was next chromatographed on a silicic acid column. The organochlorine insecticides were eluted with hexane followed by 0.75% nitromethane in hexane (by volume). Alternatively, elution by hexane saturated with nitromethane recovered the organothiophosphates with the remaining organochlorine insecticides. Coffin and McKinley (24, 25) used polyethylene coated alumina in their studies of several organophosphorus insecticides and their metabolites in vegetable crops. McKinley et al. (61) also used this material in a cleanup method for malathion.

Vacuum distillation, or sublimation, was utilized by McCaulley (58) and Farrow et al. (39) for the cleanup of organophosphorus residues; however, recoveries were not good for some of the insecticides, and additional study would be necessary to achieve precision and accuracy. Storherr and Watts (85) developed a sweep co-distillation method for the cleanup of organophosphorus insecticides in crop extracts, similar in principle to gas chromatography. Gunther et al. (49) followed the same principle, but constructed an apparatus which was better adapted to the cleanup of butterfat.

Beroza and Bowman (9, 12) used a system of binary solvents

for sample extract cleanup and established a series of Nernst coefficients which they designated as p-values (fraction of total partitioning into the upper phase) for a number of organophosphorus and organochlorine insecticides.

An ion exchange resin (Dowex®1-X8) was used by Plapp and Casida (73, 74) in studies of the hydrolysis and metabolism of organophosphorus insecticides. Gehrt (43) used Dowex 2-X8 for the separation of the systemic insecticide ronnel* from its decomposition products extracted from feeds.

Porous glass has not been used for insecticide residue cleanup, however, it was used as a molecular sieve by Zhdanov et al. (93) to separate methane, ethane and ethylene by GLC. Dobychin et al. (35) used porous glass to separate toluene from methyl cyclohexane, and benzene from cyclohexane. Its application to insecticide residue cleanup should be investigated.

Moore (68, 69) described the use of a cross-linked polystyrene gel and its application to separating low molecular weight polymers. This work showed that structurally modified polystyrene gels, when compatible solvents were used, had utility in making molecular size fractionations of hydrophobic molecules. The structure of these

*See Appendix

gels has been described as anologous to that of ion exchange resins but without ionic sites. They are composed of rigid cross-linked beads prepared by suspension copolymerization of styrene with a difunctional monomer (cross-linking agent) such as divinylbenzene. The separation of molecular species according to size is believed to occur as a result of differences in the extent to which different species permeate the gel particles. Molecules whose size is too great to allow them to enter the gel pores, pass through the column solely by way of the interstitial volume. Smaller molecules permeate to a greater or lesser degree, depending upon their size. The largest molecules emerge first, followed by smaller molecules.

C. Analysis - Detection

There are a number of methods for the analysis and detection of organochlorine and organophosphorus insecticides which are applicable to pure compounds or formulation mixtures, but most residues extracted from environmental materials require purification before satisfactory analytical results can be obtained.

The colorimetric method for malathion determination (53) consists of its decomposition, in carbon tetrachloride solution by the addition of ethanol and sodium hydroxide, to 0,0-dimethyl dithiophosphate. This is then converted to

the yellow colored cupric salt, the absorption of which is measured at 418 nm. The amount of malathion present is determined from a standard calibration curve which has a range of 0.1 mg to 1.0 mg of malathion. Compounds other than malathion which are converted to 0,0-dimethyl dithiophosphoric acid by alkali treatment will interfere (94). Known interfering compounds are 0,0,0,0-tetramethyl trithiopyrophosphate and the disulfide of dimethyl dithiophosphoric acid, namely 0,0,0,0-tetramethyl 2,3-dithiotetraphosphane-1,1,4,4-dithiotetroate. Also both half-esters of malathion, namely S-[(1 carboxy-2-carbethoxy)-ethyl]0,0-dimethyl dithiophosphate and S-[(2 carboxy-1-carbethoxy)-ethyl]0,0-dimethyl dithiophosphate are converted to 0,0-dimethyl dithiophosphoric acid (94). Conroy (26) in 1959 found that Delnav®, Niagara 1137, Nialate®, Thimet® and Guthion®* interfered to varying degrees; while Trithion®, parathion, methyl parathion and EPN* did not. In 1957 Cook et al. (29) used the colorimetric method of analysis in an enzyme study involving malathion in liver homogenate.

In 1954, Cook (27) separated and identified organophosphorus insecticides by paper chromatography. Mitchell (66) in 1960 described a method of separating pure organophosphorus insecticides. In 1962 Getz (44) described a paper

*See Appendix.

chromatographic method for determining malathion and other organophosphorus insecticide residues in leafy vegetables. Coffin (23) used paper chromatography in metabolism and residue persistence studies of parathion and malathion with lettuce.

In 1963 Walker and Beroza (88) established R_f values on thin layer chromatograms with various solvent combinations for 62 organochlorine and organophosphorus insecticides. Stanley (82) described a method for organophosphorus insecticide determination using microchromatoplates. Pesic (71) used TLC to determine malathion residues in potatoes.

The use of housefly bioassays for determining organophosphorus insecticide residues was discussed by McCaulley and Cook (59) in 1959. Tighe (86) described the method and equipment needed for a housefly bioassay. The method is not specific, but detects the presence of toxic materials.

The property of inhibiting serum cholinesterase enzyme activity in vitro is one measure of the potential toxicity of an organophosphorus insecticide residue. A method reported by Cook (28) made use of this property as a spot test for the detection of enzyme inhibitors separated by paper chromatography. Burchfield and Johnson (13, Vol. II) describe a colorimetric method of determining anticholinesterase activity. In this test the residue is allowed to

react with known amounts of ChE. The degree of inhibition is determined by adding a known amount of acetylcholine (ACh), allowing it to react for a pre-determined time, and determining the remaining ACh colorimetrically after color development with hydroxylamine in the presence of ferric iron. Yip and Cook (92) used Systox® to compare four methods of analysis for organophosphorus insecticides based on their anti-ChE activity. They concluded that each method had advantages and disadvantages, but that any of the methods could be used. Getz and Friedman (45) in 1963 developed a spot test for detecting ChE inhibitors. Boone (11) in 1965 stated that the disadvantage of an anti-ChE method was its lack of specificity.

In 1964 Double (36) used infrared spectrophotometry to estimate the malathion content of pesticide formulations after its separation on Florisil. In 1960 McCaulley and Cook (60) discussed the application of infrared spectra of organophosphorus pesticides to some problems of analysis and McCaulley (58) used I.R. spectroscopy in 1965 for verification after the gas chromatographic analysis of organophosphorus insecticides.

In 1952 James and Martin (54) started anew on Martin's earlier discovery of gas chromatography. Coulson et al. (33) proposed the use of micro-coulometric gas chromatography in

1960. Burke and Johnson (18) further investigated the micro-coulometric detector in 1962. Burke and Holswade (16), in 1964, determined GLC retention times for many insecticides using this detector and noted that GLC detection was improved if the residues were cleaned up. Nelson (70) used this detector in a study involving 16 organophosphorus insecticides and 25 crops. He also noted improved chromatograms after residue cleanup.

In 1964 Giuffrida (46) discussed the use of a sodium thermionic detector. It was a modified flame ionization detector sensitive to organophosphorus compounds, but relatively insensitive to other compounds. Farrow et al. (39) used a sodium thermionic detector in vacuum sublimation cleanup studies, and noted that it was considerably less sensitive than the electron capture detector. Storherr and Watts (85) also used this detector in sweep co-distillation cleanup studies in 1965. Giuffrida et al. (47) in 1966, described an improved thermionic detector using potassium instead of sodium salts in its construction.

The suitability and application of the electron capture (electron affinity) detector to organochlorine and organophosphorus insecticides has been discussed by Gaston (48) and also by Burchfield and Johnson (13). Burke and Giuffrida (15) tried electron capture with parathion and

were successful, however, they noted decreased response following analysis of uncleaned extracts. Dawson et al. (34) used electron capture GLC to determine fenitrothion and parathion* residues in cocoa. They found that the minimum detectable amounts were 0.2 ng (nanogram, 10^{-9}g) for parathion, fenitrothion and chlorothion.* However, paraoxon could not be detected below 5.0 ng. Oxygen analogues of the organophosphorus insecticides appear to have poor response with electron capture detectors. In 1965 Beroza and Bowman (9, 12) used an electron capture detector in a study of liquid partitioning methods for 131 pesticides and related compounds.

A general purpose GLC column packing, consisting of 10% DC 200 (12,500 cst) on Gas Chrom Q®, has evolved as a standard from many reported for specific separations. Giuffrida et al. (47) used this in a study on special ionization detector systems. This column was recommended by Shurman and Collie (81) and used by Burke and Holswade (16, 17) in a study involving 87 organochlorine and 26 organophosphorus pesticides, and also in a study on confirmatory methods by GLC. F.D.A. has standardized on this column for the district laboratories (58), (footnote on p. 660).

*See Appendix.

Methods of measuring GLC detector response and the calculations associated with analyses are discussed by Burchfield and Johnson (13). Gaul (42) discussed some of the problems associated with calculations involving GLC peaks and preferred to use peak height or what he referred to as area (product of elution time and peak height, however, it must be noted that GLC carrier gas flow must be constant). Burke (14) discussed GLC methods for pesticides in general, and suggested that extracts should be cleaned up to maintain consistent instrument response. Samples containing oil or chlorophyll are the most troublesome in contaminating detectors (48).

The procedures known as extraction and cleanup are basic to pesticide analysis and must be accomplished quantitatively for results to be meaningful. It has been estimated that the precision obtainable in residue analysis is usually no better than $\pm$ 10%. Reproducibility of results between laboratories is almost always more difficult than within a single laboratory.

METHODS AND MATERIALS

I. Analysis

The method used for the detection and quantitation of malathion (and other insecticides used) was gas-liquid chromatography (13, 72). The GLC method was selected over the official AOAC colorimetric method (53) because of increased sensitivity and convenience. An electron capture detector was used since it had the necessary sensitivity (56). However, other adequate detectors are available such as the sodium thermionic detector (46) and the micro-coulometric detector (33).

The gas chromatograph used was a Jarrell-Ash Model 28-710 with an electron capture detector having a 100 millicurie tritium source for ionization. The recorder used was a Minneapolis-Honeywell "Electronik 18." The GLC column was pyrex glass 1/4 inch o.d. and 6 feet long, packed with 10% D.C. 200 (12,500 cst.) on 100/120 mesh Gas Chrom Q (17). (Jarrell Ash Company, Waltham, Massachusetts 02154). The instrument parameters were: column temperature, 200°C; injection temperature, 290°C; detector temperature, 203°C; and nitrogen carrier gas flow, 220 ml per minute. A ten microliter Hamilton syringe was used to make the GLC injections.

Peak height was used in the calculations for GLC analysis in both the preliminary work and the proposed method. Injections of standards and samples were made near each other, as suggested by Burke and Johnson (18), rather than making a complete calibration

prior to or following a series of analyses.

II. Establishment of a Suitable Method

The wheat extracts were prepared by blending 200 or 250 g unground samples with the selected solvent, using a volume of the solvent in ml equal to the weight of the sample in grams. The samples were blended 4 minutes at low speed and 1 minute at high speed, using an explosion-proof Waring Blendor, and filtered carefully to minimize evaporation. (The 1/5 h.p., two-speed explosion-proof Waring Blendor with no load is rated at 12,200 r.p.m. at low speed and 16,100 r.p.m. at high speed.) The following investigations were used to determine a suitable procedure.

1. Seven chromatographic columns 20 mm o.d. by 200 mm were packed with a 5 to 1 mixture (w/w) of Florisil-Celite 545 to form a bed 60 mm high. Layers of anhydrous sodium sulfate were placed at the bottom and top of the columns (each layer 15 mm high). After saturating with hexane, two milliliters of hexane containing 111.4 µg malathion per milliliter were added to columns numbered 1, 2, 3, 4, and 5. To columns numbered 6 and 7 were added 75 ml of hexane extracts equivalent to 40 g samples of wheat, after first saturating with hexane. Column No. 1 was eluted with 180 ml of hexane and 12.5 ml fractions were collected. Column No. 2 was eluted first with 600 ml of hexane, then with 200 ml of acetone, and 25 ml fractions were collected. Columns No. 3 and 6 were eluted with 4 to 1 hexane-acetone (v/v), and 25 ml

fractions were collected. Columns No. 4 and 7 were eluted with ethyl ether and 25 ml fractions were collected. Column No. 5 was eluted with 9 to 1 petroleum ether-ethyl ether (v/v), and 25 ml fractions were collected.

2. A chromatographic column 20 mm o.d. by 200 mm high was packed with (in order from bottom to top) anhydrous sodium sulfate 15 mm high, 10 g of 5 to 1 (w/w) Florisil-Celite 545, 10 g Silica Gel H, and anhydrous sodium sulfate 15 mm. The column was saturated with hexane, then 2 ml of hexane containing 111.4 µg malathion per milliliter was added. The column was eluted with 9 to 1 petroleum ether-ethyl ether (v/v) and 25 ml fractions were collected.
3. Four chromatographic columns 20 mm o.d. by 200 mm high were packed with Florisil (commercial preparation) with a layer of anhydrous sodium sulfate 15 mm high placed at the top and bottom. Columns No. 1 and 2 contained 10 g of Florisil, and columns No. 3 and 4 contained 15 g of Florisil. To column No. 1 was added 150 ml of hexane extract equivalent to 100 g of wheat. To column No. 3 was added the mixture of 2 ml of hexane containing 111.4 µg malathion per milliliter, plus 10 ml of a concentrate made from the hexane extract of 100 g of wheat. To columns No. 2 and 4, 2 ml of hexane containing 111.4 µg per milliliter malathion were added. Columns No. 1 and 2 were eluted with 9 to 1 hexane-acetone (v/v), and No. 3 and 4 were eluted with 5% acetone

in ethyl ether (v/v). Twenty-five ml fractions were collected from all columns.

4. Two chromatographic columns 20 mm o.d. by 200 mm were packed with Florisil. Column No. 1 was packed with 15 g of Florisil plus 10% H_2O, and column No. 2 was packed with 15 g of Florisil plus 20% H_2O. Each column had 15 mm layers of anhydrous sodium sulfate at the top and bottom. After saturating these columns with hexane, a mixture, consisting of 2 ml of hexane containing 111.4 µg of malathion per milliliter plus a 10 ml concentrate made from the hexane extract from 100 g of wheat, was added to each column. A 5% solution of acetone in ethyl ether (v/v) was used for elution and 25 ml fractions were collected.
5. Two chromatographic columns 20 mm o.d. by 200 mm were packed with 10 g of 4 to 1 mixture (w/w) of silicic acid-Celite 545. Layers of anhydrous sodium sulfate 15 mm thick were used at the bottom and on the top of the columns. To column No. 1 was added 150 ml of hexane extract from 150 g of wheat, and to column No. 2 was added 2 ml of hexane containing 111.4 µg of malathion per milliliter. The columns were eluted with 9 to 1 hexane-acetone (v/v) and 25 ml fractions were collected.
6. Two micro columns were made from disposable pipets (approximately 5 mm i.d. by 150 mm). Column No. 1 was filled with

Anakrom® ABS 80/90 coated with 10% Carbowax 20 M. Column No. 2 was filled with confectioners sugar. To each column was added 2 ml of iso-octane extract of wheat containing 2.5 μg of malathion per milliliter. Each column was eluted 3 times with 2 ml portions of iso-octane and once with 2 ml of 25% ethanol in iso-octane (v/v). A 2 ml fraction was collected for each portion of solvent added.

7. Chromatographic columns were made from 10 ml serological pipets by breaking off the top and flaring the tubes slightly. One of these columns was packed with 30/60 mesh Chromosorb® P, non-acid washed. To the column was added 2 ml of iso-octane extract of wheat containing 2.5 μg malathion per milliliter. The column was first eluted with iso-octane, and eight 2 ml fractions were collected. Then the column was eluted with 10% ethyl ether in iso-octane (v/v), and six 2 ml fractions were collected.

8. A chromatographic column made from a 10 ml serological pipet was packed with Decalso®, (a Zeolite ion exchange material prepared for thiamine analysis). Five ml of an iso-octane extract of wheat containing 2.5 μg malathion per milliliter was added. The column was first eluted with iso-octane and sixteen 5 ml fractions were collected; next, it was eluted with 10% ethyl ether in iso-octane (v/v), and seven 5 ml fractions were collected. Finally, the column was eluted with ethyl ether, and six 5 ml fractions were collected.

9. Three chromatographic columns were made from 10 ml serological pipets. Column No. 1 was packed with ground unrefined clay. Column No. 2 was packed with ground unrefined clay with the fine material removed, and column No. 3 was packed with HCl washed Attagel®. A solution of iso-octane extract of wheat containing 2.5 µg malathion per milliliter was prepared, and 2 ml was added to column No. 1, 5 ml was added to No. 2 and 5 ml was added to No. 3. The columns were eluted first with iso-octane; seven 2 ml fractions were collected from Column No. 1, eight 5 ml fractions were collected from Column No. 2 and six 5 ml fractions were collected from Column No. 3. Next, the columns were eluted with 10% ethyl ether in iso-octane (v/v); eight 2 ml fractions were collected from column No. 1, twelve 5 ml fractions were collected from column No. 2, and none were collected from column No. 3. (No. 3 column was abandoned at this point because solvents eluted very slowly even when vacuum was applied). Finally, the columns were eluted with ethyl ether; five 2 ml fractions were collected from column No. 1, and four 5 ml fractions were collected from column No. 2.
10. Four serological pipets were prepared as chromatographic columns. No. 1 was packed with 60/80 mesh porous glass (50, 93), (Applied Science Labs., State College, Pa.).

No. 2 and 3 were packed with Sephadex LH-20 (3, 4, 5), (Pharmacia Fine Chemicals, Piscataway, New Jersey) and No. 4 was packed with 200/400 mesh Bio-beads S-X8. (2). (Bio-Rad Laboratories, Richmond, Cal.) A solution of iso-octane extract of wheat containing 10 µg of malathion per milliliter was prepared; 5 ml was added to columns No. 1 and 2, and 2 ml was added to columns No. 3 and 4. The columns were eluted with iso-octane; twenty 5 ml fractions were collected from column No. 1 and ten 5 ml fractions were collected from No. 2. Twelve 2 ml fractions were collected from column No. 3, and twelve 2 ml fractions were collected from No. 4. In addition, column No. 1 was eluted with 2% ether in iso-octane (v/v) and nine 5 ml fractions were collected, then eluted with ethyl ether and five 5 ml fractions were collected.

11. Two serological pipets were packed with Bio-beads S-X8, previously swelled in 90% benzene in iso-octane (v/v). A solution of wheat extract in 90% benzene containing 10 µg of malathion per milliliter was prepared; 2 ml was added to Column No. 1 and 0.5 ml was added to Column No. 2. The columns were eluted with 90% benzene; fifteen 2 ml fractions were collected from Column No. 1 and twelve 0.5 ml fractions were collected from Column No. 2.

12. A 20 mm i.d. by 500 mm chromatographic column, with teflon stopcock, was assembled without the fritted glass disc

(Chromaflex No. 1, Kontex of Illinois, Franklin Park, Illinois, 60131). The lower end at the constriction above the stopcock was plugged loosely with a small amount of pyrex glass to prevent the passage of the column packing into the stopcock. A 21 mm glass filter-paper disc (No. 934-AH, distributed by H. Reeve Angel and Co., Clifton, New Jersey) was placed upon the glass wool plug and 10-20 ml of 90% benzene was added to remove entrapped air. Next, a slurry of Bio-beads S-X8, previously swelled in 90% benzene iso-octane (v/v), was carefully poured into the column to give a settled bed height of 360 mm. Another glass filter-paper disc was carefully added to the leveled packing and gently pressed into place. The column was washed by allowing about 50 to 60 ml of 90% benzene to flow through it. As the last of the solvent passed into the column, the stopcock was closed and 2 ml of wheat extract fortified with malathion (10 μg/ml) was added. Fraction collection was begun immediately. The stopcock was opened and as soon as the last of the wheat extract had passed into the column, a small amount of 90% benzene was used to rinse the top of the column. When the last of the rinse had passed into the column, the column was filled with 90% benzene. Three 10 ml fractions, one 5 ml, five 2 ml and finally twenty-seven 1 ml fractions were collected. The solvent flow rate was

determined, the movement of the colored band observed, and also the color of the fractions noted.

13. The same column and packing used for trial 12 was used again, however, 1 ml of a concentrated wheat extract in 90% benzene (5 ml concentrated to 1 ml), containing 50 μg of malathion, was added to the column in the same manner as described in trial 12. The column was eluted with 90% benzene; four 10 ml fractions, one 5 ml fraction, and finally thirty-one 2 ml fractions were collected. The solvent flow rate was determined, the movement of the colored band was observed, and also the color of the fractions was noted.

14. The same packing in the same column (from trials 12 and 13) was washed with about 100 ml of 100% benzene and allowed to equilibrate. A new set of malathion standards was prepared in 100% benzene (redistilled in glass); and a new benzene extract of wheat was prepared and fortified with malathion to contain 1 μg per milliliter. Twenty milliliters of this extract were concentrated under a flowing stream of nitrogen to less than 3 ml by heating in a waterbath. The concentrate was made to exactly 4 ml with benzene, mixed well, and 2 ml added to the column in the manner described in trial 12. The column was eluted with benzene; and four 10 ml fractions, one 5 ml fraction, and twenty-seven 2 ml fractions were collected. The solvent

flow rate was determined, the movement of the colored band was observed, and the color of the fractions was noted.

III. Proposed Method

A. Supplies and Equipment

1. Gas Chromatograph equipped with a detector suitable for organophosphorus pesticides (comparable to the Jarrell Ash Gas Chromatograph Model 28-710 with a 100 mc tritium electron capture detector and Minneapolis-Honeywell "Electronik 18" stripchart recorder. Jarrell Ash Company, Waltham, Massachusetts, 02154).
2. Glass column 1/4 inch o.d. by 6 feet, coiled, and packed with 10% D.C. 200 (12,500 cst) on 100/120 mesh Gas Chrom Q. (Jarrell Ash Company, Waltham, Massachusetts, 02154).
3. Compressed nitrogen, high purity, suitable as gas chromatographic carrier.
4. Two-stage inert gas regulator.
5. Four foot, 1/4 inch o.d. copper tubing packed with 80/90 mesh Linde Molecular Sieve 5A.
6. Ten microliter syringe. (Hamilton Company, Whittier, California, 90608).
7. Chromatographic column, 20 mm i.d. by 500 mm with teflon stopcock. (Chromaflex No. 1, Kontes of Illinois, Franklin Park, Illinois, 60131).

8. Bio-beads S-X8, 200/400 mesh (Bio-Rad Laboratories, Richmond, California, 94804).
9. Pyrex glass wool.
10. Glass filter-paper discs, 21 mm diameter. (No. 934-AH, Hurlbut Paper Company, Clifton, New Jersey).
11. Malathion standard; solutions of such concentration to calibrate gas chromatographic response. (American Cyanamid Company, Agricultural Division, Princeton, New Jersey).
12. Benzene, Nanograde (Mallinckrodt Chemical Works, St. Louis, Missouri, 63160).
13. Waterbath at 60 to 70°C.
14. Source of purified nitrogen for sample concentration.
15. High speed blender (comparable to two-speed explosion-proof Waring Blendor).

B. Procedure

1. Assemble the chromatographic column, loosely plug the restriction at the lower end of the tube above the stopcock with a small amount of glass wool, and place a 21 mm glass filter-paper disc on top of the glass wool.
2. Add 10 to 20 ml of benzene to the column to remove entrapped air. Pour a slurry of Bio-beads, pre-swelled in benzene, gently into the column to obtain a settled

bed height of 380 to 400 mm. (A long glass rod or tube may be pressed lightly against the filter disc while filling the column to prevent its displacement.) Then carefully add another glass filter-paper disc to the top of the bed. Always keep column bed covered with benzene to prevent drying and subsequent "channeling."

3. Wash the Bio-bead column with 200-300 ml of benzene. Check the effluent by GLC to verify freedom from interfering substances.
4. Ascertain separating characteristics of column by using a wheat sample extract containing a known amount of malathion, and "chromatographing" as directed for samples.
5. Concentrate a suitable aliquot of the benzene extract of a wheat sample to 3 or 4 ml under a stream of nitrogen, using a water bath at 60 to 70°C.
6. Make the concentrated extract to exactly 10 ml volume with benzene and mix well.
7. Open the stopcock and allow the remaining benzene to pass into the top of the bed; then close the stopcock. Add 2 ml of the concentrated sample and begin collecting the effluent by opening the stopcock and allowing the extract to pass completely into the bed. Immediately

rinse the top of the column with a small amount of benzene and allow the rinsings to pass into the bed. Fill the top of the column (reservoir) with benzene and add more as necessary.

8. Continue collecting effluent until colored materials begin to elute. (About 50 ml effluent from the time of sample application.) Collect individual 2 ml fractions until the first colorless 2 ml fraction (8 to 9 fractions for a total volume of 16-18 ml). Immediately collect the next 10 ml of effluent and save (or other suitable volume pre-determined to contain all the malathion expected). Collect at least one additional fraction to check for the presence of malathion. Also check the first 2 ml colorless fraction collected.
9. Shake or swirl to mix the collected fraction(s) and determine the malathion by suitable gas chromatographic procedure.

IV. Experimental

Samples of 1966 Michigan soft winter wheat (Triticum vulgare; mixture of varieties Genessee and Avon) were obtained from two sources, and 1966 Michigan light bran from one source. 1966 California soft wheat (Triticum vulgare, variety Onus) was obtained from one source, and 1966 Montana-North Dakota durum

wheat (Triticum durum; mixture of varieties LaKota and Wells) from one source. In addition, a composite of random samples of 1965 Michigan soft winter wheat was obtained.

Extracts were prepared from these six different samples by blending 200 g of wheat with 200 ml of benzene in an explosion-proof Waring Blendor for 4 minutes at low speed, and 1 minute at high speed. Known amounts of malathion were added to the filtered extracts and the proposed method was used to evaluate column performance. The flow rates ranged from 32.7 to 41.7 ml/hr and were governed only by the mesh size of the Bio-beads and the amount of solvent above the bed. (The results of these tests appear in the appendix.)

The aforementioned extracts and another 1966 Michigan soft winter wheat extract were used to conduct additional tests using the Bio-bead column. Similar concentrates were prepared and lindane, heptachlor, heptachlor epoxide, DDT, aldrin and parathion were also added to determine their effect on the column performance. Further studies of performance were conducted by applying air pressure of approximately 1 psig to the column to obtain a flow rate of 125 ml/hr.

Column 1 was packed with Bio-beads S-X8 (Control No. 3874) to a height of 360 mm. Column 2 was packed with the same Bio-beads emptied from Column 1, with enough additional material added to give a height of 390 mm.

RESULTS AND DISCUSSION

Since Florisil has been used extensively for the cleanup of extracts containing organochlorine insecticides (65), it was tried first. No malathion was recovered from column No. 1; 93% was recovered from column No. 2; 86% from column No. 3; 88% from column No. 4; and 104% from column No. 5. However, the corresponding fractions from columns No. 6 and 7 indicated that pigments and other interfering materials would be eluted at the same time; therefore the separation was considered unsatisfactory.

No malathion was recovered from the column of Florisil and Silica Gel H, even though successful uses have been reported. Air pressure was required to obtain the desired flow rates.

The commercially prepared Florisil gave recoveries of 95%, 94% and 101%, however, the interfering materials had not been removed. The Florisil with added H_2O gave recoveries of 75% and 73%. The deactivation of the Florisil decreased malathion recovery and still did not accomplish satisfactory cleanup.

The column packed with Celite 545-silicic acid gave a malathion recovery of 97%. The malathion appeared in 10 of the fractions, and was not satisfactorily separated from the interfering materials.

No quantitative tests were used on the fractions from the Carbowax-Anakrom ABS and confectioners sugar columns; however, qualitative tests indicated that there had been inadequate separation.

Only qualitative tests were performed on the fractions collected from the Chromosorb P column and these indicated that no separation or

cleanup had occurred. The presence of many additional unidentified compounds was indicated by the chromatograms.

The malathion appeared in only the more polar ethyl ether fractions from the Decalso column, as did the pigments. GLC tests indicated that there had been no cleanup and additional unidentified materials were present.

The malathion was found only in the ethyl ether fractions from the unrefined clays. The fate of the malathion on the acid washed Attagel was not determined. None had been recovered before the column was abandoned. The unrefined clays removed some of the pigments, however, GLC analysis indicated the presence of additional unidentified materials.

The porous glass accomplished no apparent separation or cleanup when solvents of sufficient polarity were used to recover the malathion. Observations and GLC analysis of the fractions collected from the Sephadex LH-20 columns indicated a slight separation of the malathion from the pigments and interfering materials. However, separation was not much improved when smaller amounts of sample were applied to the column. Observations and GLC analysis of the fractions collected from the Bio-bead column indicated a partial separation, with the colored and interfering materials starting to elute before malathion.

Since gel filtration is based upon the property which these materials have of swelling in certain solvents (2, 3, 5) and it had not been established that iso-octane accomplished this (2); benzene was tried with Bio-beads (9/1 benzene-iso-octane, v/v). The first

three fractions from column No. 1 were colorless, however, GLC analysis revealed the presence of many interfering materials. (These were not found in the corresponding fractions of subsequent trials using the same packing.) Fractions 4, 5, and 6 were yellow, but GLC analysis revealed that malathion started eluting with fraction 6, and was also present in fractions 7 and 8, with only a trace in 9, and none present in the rest. The first three fractions from column No. 2 were colorless and almost free from interfering materials. Fractions 4, 5 and 6 were yellow. GLC analysis revealed that malathion started eluting with fraction 6, was present in 7, 8 and 9, with only a trace found in 10. The highest malathion concentration was in fraction 8, while the most intense yellow color was in fraction 5. During the preparation of these columns, it was observed that the bed volumes increased about 50% upon soaking in 90% benzene. These columns after packing had a bed size of about 7.5 mm diameter by 185 mm.

Complete separation of malathion had not occurred, even when only 0.5 ml of wheat extract was used. Therefore, to obtain more separating capacity, a bed volume of 20 mm diameter by 360 mm was prepared, using a column 25 mm o.d. by 500 mm. The first sixteen fractions were colorless, however, GLC analysis revealed that the same unidentified compounds were present as found in the first trials using the Bio-beads packed in serological pipets. (Again, these were not found in subsequent trials using this column.) Fractions 17 to 21 were yellow; this color began to elute when about 52 ml had been

collected. Fractions 22 to 36 were colorless. GLC analysis revealed the presence of malathion first in fraction 25; and it was found in the rest of the fractions collected, with the highest concentration in fraction 33. No interfering materials were found in any fractions after 22. The colored band moved down the column about 8 mm per ml of collected fraction. The column flow rate was 26.7 ml/hr.

Good gel filtration technique requires the application of a small volume of concentrated sample solution for optimum separation (3, 5). The first five fractions (total volume of 45 ml) were colorless, and GLC analysis revealed no interfering or unusual substances. Fractions 6 to 13 were yellow, and contained no malathion. Fractions 14 to 36 were colorless, and GLC analysis revealed that malathion was present in fractions 15 to 22 with a trace found in fraction 23. No unusual or interfering substances were found in any of the colorless fractions. GLC behavior during the analysis of the colorless fractions was good, indicating that the colored and interfering materials were eluted in fractions before those containing malathion and that the extract had been cleaned. The colored band moved down the column about 7.8 mm per ml of collected fraction. The column flow rate was 34 ml/hr.

Since the only reason for having used 90% benzene-iso-octane was that the original malathion standards used were in iso-octane, a change to 100% benzene was made to simplify the procedure. The first five fractions collected from this column (45 ml total volume) were colorless

and contained no malathion, and no unusual substances were found. Fractions 6 to 12 were yellow, while fractions 13 to 32 were colorless. GLC analysis revealed that malathion was present in fractions 14 to 18, and a trace in fraction 19; with no malathion and no unusual substances in the remaining fractions. These analyses indicated that the colored and interfering materials were eluted prior to the elution of malathion. The colored band moved down the column about 7.8 mm per ml of collected fraction. The column flow rate was 27.6 ml/hr. The estimated recovery of malathion was 143%, based on the analyses of four 2 ml fractions. Obviously this recovery figure is in error, and most probably is due to the measurement of fraction volumes. However, some of the problems involved in GLC analysis such as: calculations of peaks, are discussed by Gaul (42), detector response to successive injections was noted by Barrette and Payfer (7), and precision and accuracy was noted in The Guide to The Analysis of Pesticide Residues (13).

Results, Discussion and Limitations of Procedure

Solvent flow rates initially determined were in the range of 34 to 40 ml per hour; however, preliminary work indicated that flow rates as high as 125 ml per hour did not appreciably affect the separation and cleanup. The limiting malathion capacity of the column was not established, however, sample concentrates containing as much as 1 milligram were successfully separated and cleaned up. However, these required collecting two 10 ml fractions, rather than just one. Initial work also indicated that only those substances which have molecular

sizes close to that of malathion and which have similar gas chromatographic retention times would interfere.

From the results of the trials conducted using the proposed method, it was found that the malathion recovery from the Bio-bead Column 1 ranged from 94 to 112%. The malathion recovery from the Bio-bead Column 2 ranged from 97 to 103%. The overall average malathion recovery for both columns was 100% and the overall range was 94 to 112%. However, no difference in column performance was observed. (See table of results in appendix.)

The pressurized column gave about the same performance, however, a slight compacting of the bed was noted while pressurized. This was only temporary, and the bed rapidly returned to its normal height upon removal of the pressure.

It was noted that when flow rates began to decrease, they could be restored by back-flushing with 25-30 ml of benzene. Occasionally there was an accumulation of fine solids, either from inefficient filtration of the wheat extracts or some displacement of the Bio-beads upon the top glass filter-paper disc which retarded the flow rate. When this disc was removed carefully and another pressed into position, the flow returned to normal.

Trials using mixtures of pesticides were not pursued extensively; however, initial work indicated there would be no interference except that due to aldrin and parathion, because they were not well separated from malathion by the GLC procedure used. No percent recovery studies

were attempted for the insecticides used in the mixtures, however, GLC analysis of collected fractions indicated that cleanup of the insecticides in the extract had been accomplished. The elution volume for each constituent in the mixture appeared to be related to individual molecular weights, but only malathion and lindane were completely separated from each other. Complete separation of each insecticide from all of the others in the mixture was not accomplished.

Including the preliminary work, more than 30 wheat extracts were separated on the same column packing and no differences in column performance were apparent. All the work with Bio-beads was done using Bio-beads from control No. 3874 of April 21, 1966.

The wheat samples were chosen to represent different supply and marketing areas, and if possible, different growing areas. The California wheat resembles the soft winter wheat grown in Michigan; however, the durum is distinctly different from these. The composite of 1965 Michigan soft winter wheat was made from individual samples representing over twenty box car shipments. This composite was stored at room temperature in a closed plastic container. The lipid composition of aged wheat was found by Pomeranz (75) to differ from that of newly harvested wheat.

A typical chromatogram produced by an injection of 5 µl of 10 µg/ml of malathion dissolved in benzene may be seen in Fig. 1.

A 5 µl injection of a concentrated crude wheat extract produced the chromatogram shown in Fig. 2. The chromatogram produced by a 5 µl

injection of the corresponding wheat extract cleaned up by using the Bio-bead column (proposed method) may be seen in Fig. 3. The extract had been fortified with malathion to a concentration of 10 µg/ml.

The instrument used was a Jarrell-Ash Model 28-710 Gas Chromatograph with a 100 mc. electron capture detector. The instrument parameters were:

4 ft. by 1/4 in. pyrex glass column packed with 10% DC 200 (12,500 cst.) on 100/120 mesh Gas Chrom Q.

column temperature, 200°C

injection temperature, 290°C

detector temperature, 203°C

nitrogen carrier flow 220 ml/min.

The baseline was about 2% full scale deflection when the uncleaned sample was injected, however, even after more than 30 minutes, it had returned to only 47%. This condition was observed for more than 24 hours. The change (loss) in response of the detector due to injecting uncleaned extracts cannot be shown here, however, it has been reported by Gunther (48), Burchfield and Johnson (13), and Burke and Guiffrida (15).

SUMMARY AND CONCLUSIONS

Eight different adsorbents commonly used for column chromatography were tested for column chromatographic cleanup of malathion in wheat extracts. These were Florisil, silica gel, silicic acid, confectioners sugar, Decalso, clay, Chromosorb P, and Anakrom ABS coated with carbowax 20M. Three porous materials were tried as molecular sieves. These were porous glass, Sephadex LH-20 and Bio-beads S-X8. The Bio-beads appeared most promising, especially when the solvent system was changed to benzene. It was discovered that the colored pigments, lipids and other interfering materials were eluted prior to malathion. These elution patterns were established by collecting 30 to 40 small fractions (1 to 2 ml) which were analyzed by GLC. It was found that flow rates of 34 to 40 ml/hr gave acceptable separation. Wheat extracts containing such other insecticides as DDT, lindane, heptachlor, heptachlor epoxide, aldrin, and parathion were successfully cleaned up. However, recovery data for these insecticides were not obtained. The elution of each constituent in the mixture appeared to be related to molecular weight, even though complete separation of each insecticide from the others in the mixture was not accomplished.

Proper preparation of a column for sample extract cleanup requires that the Bio-beads first must be swelled in benzene and washed with several 100 ml portions of benzene to remove interfering substances. To ascertain the readiness of the column, the last of the benzene wash is analyzed by GLC for interfering substances, and this readiness

is maintained by keeping the benzene level above the packing when not in use.

Recovery studies using malathion fortified extracts from new and old crop soft winter wheat, and durum wheat, yielded recoveries from the Bio-bead column ranging from 94 to 112%, with an overall average recovery of 100%.

The usable life of a column packing was not determined, however, there was no apparent change in performance after thirty extracts had been eluted. This factor might be considered for cost and convenience.

APPENDIX

Recovery of Malathion from Bio-bead Cleanup Columns Using Fortified Raw Wheat Extracts

Sample	Column	Flow ml/hr.	Performance: Malathion (µg) Added	Performance: Found	% Recovery
1966 Michigan soft winter wheat					
Source A	1	34.1	0	0	---
	1	34.3	10.0	11.2	112
	1	33.9	10.0	10.3	103
	2	40.2	10.0	10.2	102
Source B	1	32.8	0	0	---
	1	32.7	10.0	9.6	96
	1	32.8	10.0	9.6	96
	1	35.7	1000.0	950.0	95
	2	41.7	10.0	10.3	103
1965 Michigan soft winter wheat	1	34.4	0	0	---
	1	33.8	20.0	20.1	100
	1	34.0	20.0	19.6	98
	2	40.2	20.0	19.3	97
	2	38.1	20.0	20.2	101
	2	39.3	20.0	19.2	100
1966 Michigan light bran	1	33.7	0	9.2*	---
	1	33.7	10.0	19.4	102
1966 California Wheat (Onus)	1	34.4	0	65.6*	---
	1	34.5	20.0	85.9	102
1966 Durum Wheat	1	35.4	0	0	---
	1	34.3	20.0	18.9	94
	1	35.4	20.0	20.6	103
	2	39.7	20.0	20.2	101
	2	39.3	20.0	19.6	98
	2	38.7	20.0	19.5	98

Col. 1 range = 94 to 112%
Col. 2 range = 97 to 103%
Overall range = 94 to 112%

*Malathion present in the extract prior to fortification.

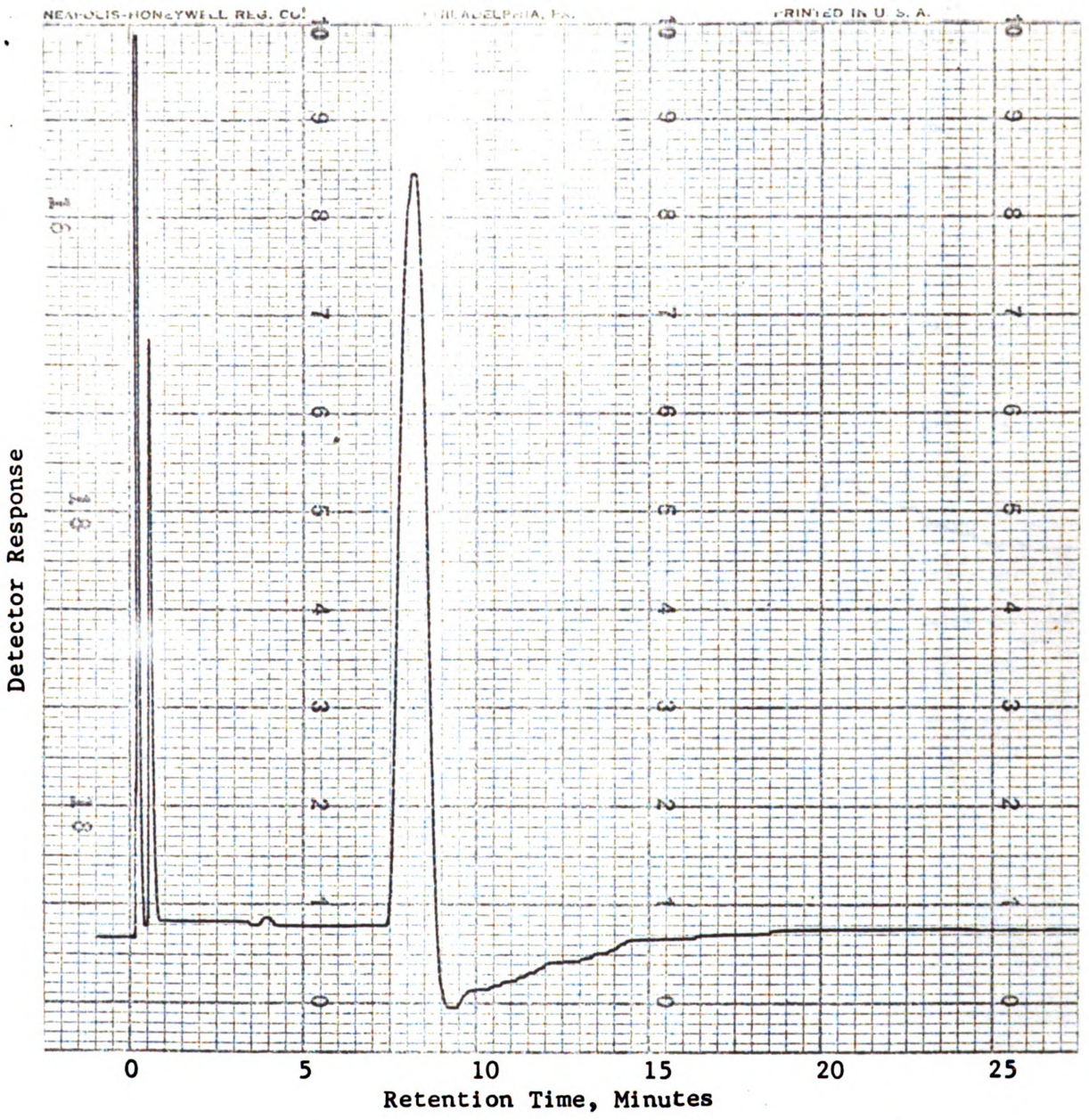


Figure 1. Typical chromatogram of a 5 µl injection of 10 µg/ml of malathion. The instrument parameters were the same as those listed previously: Column 4 ft. by 1/4 in. pyrex glass packed with 10% DC 200 on 100/120 mesh Gas Chrom Q; column temperature, 200°C; injection temperature, 290°C; detector temperature, 203°C; nitrogen carrier flow, 220 ml/min.

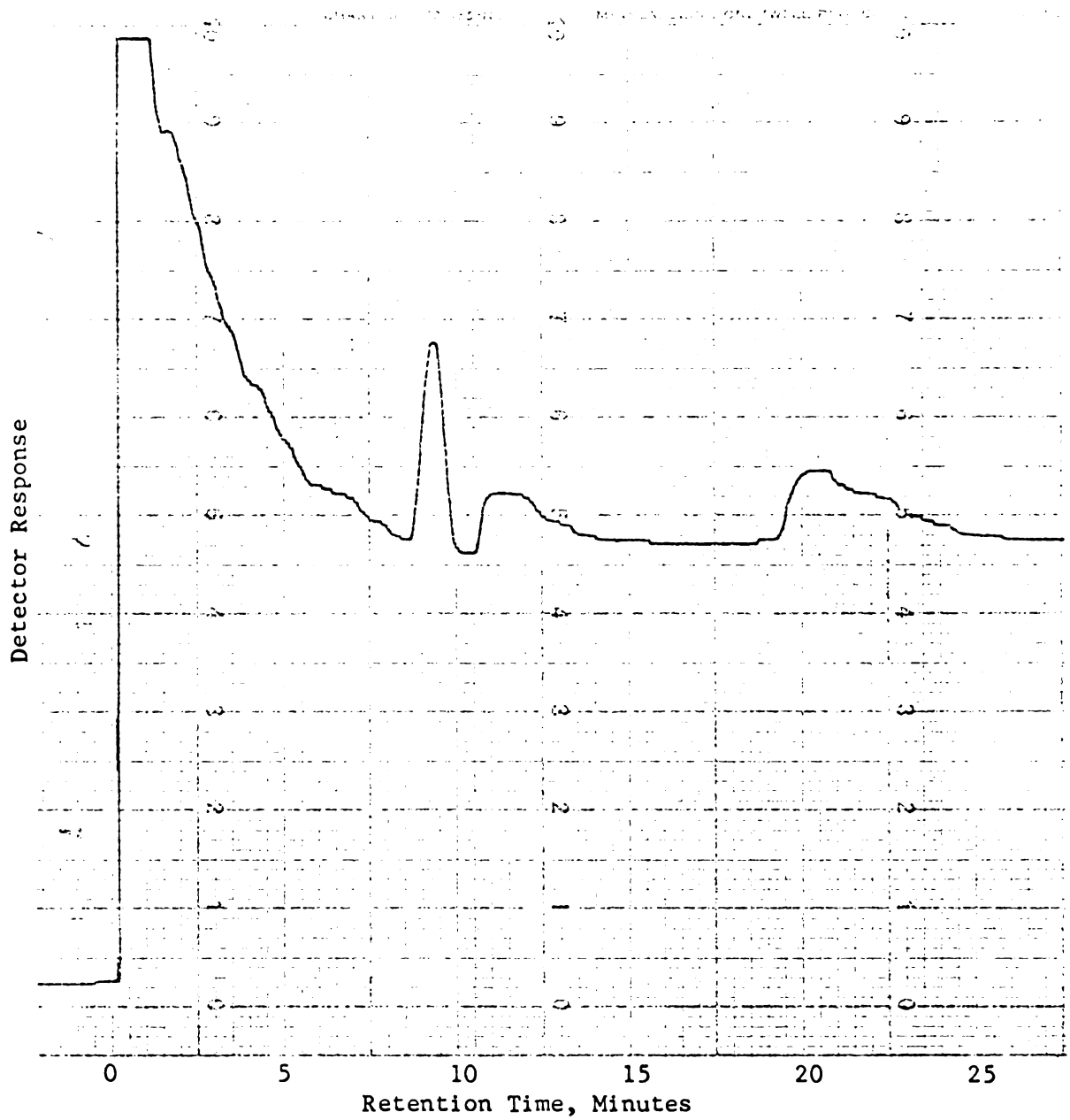


Figure 2. Typical chromatogram of a 5 µl injection of concentrated crude wheat extract from sample 65-1. The instrument parameters were the same as those listed previously.

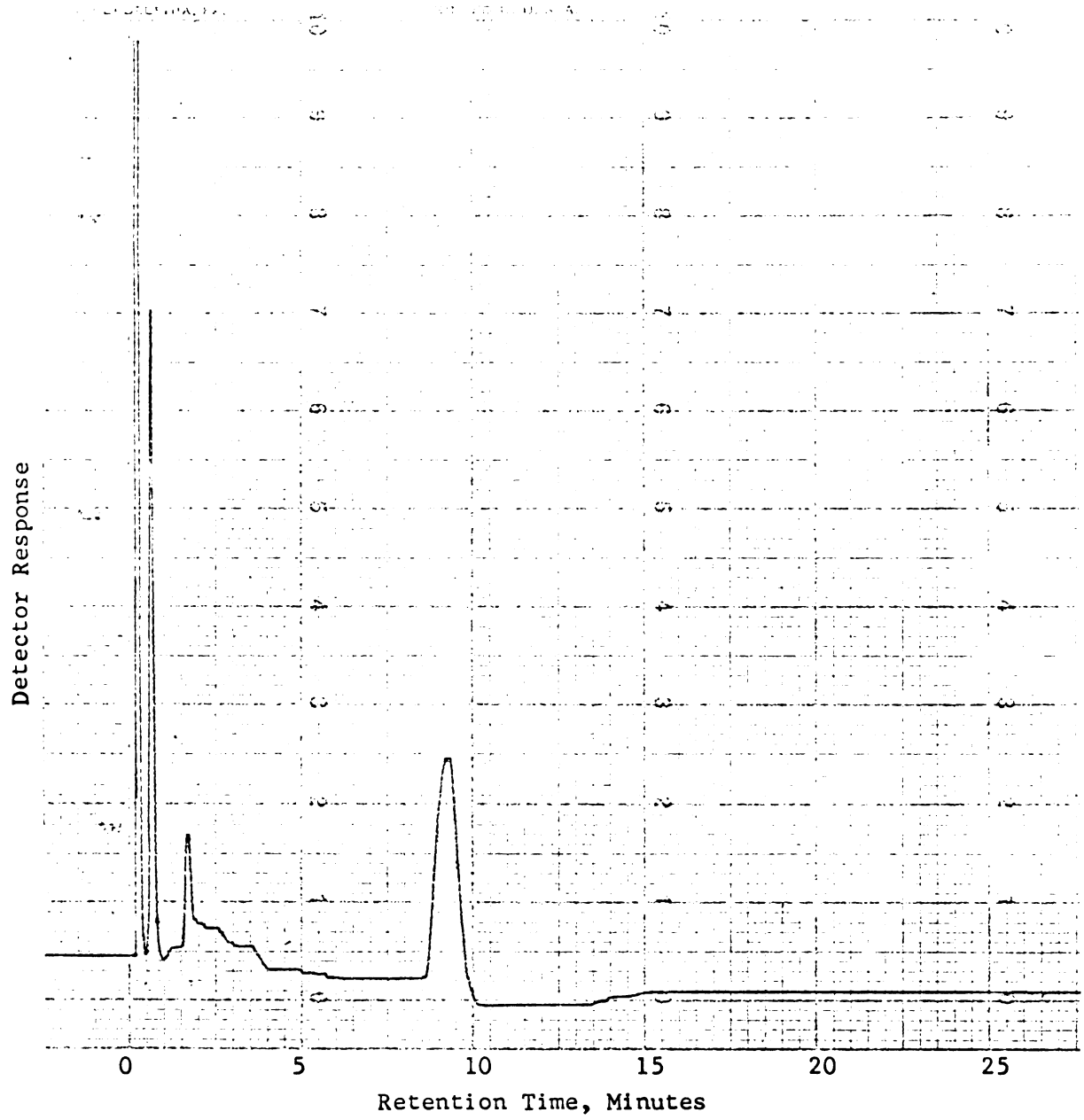


Figure 3. Typical chromatogram of a 5µl injection of Bio-bead cleaned wheat extract from sample 65-1. The instrument parameters were the same as those listed previously.

Chemical names of insecticides appearing in this paper.

aldrin

1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-1,4-endo-exo-5,8-dimenthanonaphthalene

chlorothion

O-(3-chloro-4-nitrophenyl),O,O dimethyl phosphorothioate

Co-Ral®

O,O-diethyl O-(3-chloro-4-methyl-2-oxo-2H-1-benzopyran-7-yl) phosphorothioate

DDT

1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane

DDVP

2,2-dichlorovinyl dimethyl phosphate

Delnav®

p-dioxane-2,3-diyl ethyl phosphorodithioate

demeton (Systox®)

mixture of O,O-diethyl S(and O)-2-(ethylthio)ethyl phosphorothioates

diazinon

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

Dibrom®

1,2-dibromo-2,2-dichloroethyl dimethyl phosphate

dieldrin

1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-endo-exo-5,8-dimethanonaphthalene

Dipterex®

0,0-dimethyl (2,2,2-trichloro-1-hydroxyethyl) phosphonate

endrin

1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-endo-exo-5,8-dimethanonaphthalene

EPN

0-ethyl 0-p-nitrophenyl phenylphosphorothioate

fenitrothion

fenthion

0,0-dimethyl 0-[4-(methylthio)-m-tolyl]phosphorothioate

Guthion®

0,0-dimethyl S-[4-oxo-1,2,3-benzotriazin-3(4H)-ylmethyl] phosphorodithioate

heptachlor

1,4,5,6,7,8,8-heptachloro -3a,4,7,7a-tetrahydro-4,7-methanoindene

heptachlor epoxide

1,4,5,6,7,8,8-heptachloro-2,3-epoxy-4,7-methano-3,4,7,7a-tetrahydroindane

lindane

gamma-1,2,3,4,5,6-hexachlorocyclohexane

malathion

S-[1,2-bis(ethoxycarbonyl)ethyl]0,0-dimethyl phosphorodithioate

methyl parathion

0,0-dimethyl 0-p-nitrophenyl phosphorothioate

Niagara 1137

mixture of (with alkyl ratio 75% ethyl, 25% isopropyl)

bis(dialkoxyphosphinothioyl) disulfides

Nialate®

0,0,0',0'-tetraethyl S,S'-methylenebisphosphorodithioate

parathion

0,0-diethyl 0-p-nitrophenyl phosphorothioate

Phosdrin®

0,0-dimethyl 0-(2-carbomethoxy-1-methylvinyl) phosphate

ronnel

0,0-dimethyl 0-(2,4,5-trichlorophenyl) phosphorothioate

schradan

octamethyl pyrophosphoramide

Systox®

(see demeton)

pp'TDE

1,1-dichloro-2,2-bis(p-chlorophenyl)ethane

tepp

bis-0,0-diethylphosphoric anhydride

Thimet®

0,0-diethyl S-[(ethylthio) methyl]phosphorodithioate

Trithion®

S-[(p-chlorophenylthio)methyl]-0,0-diethyl phosphorodithioate

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