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Comparative Study of Analytical Methods for Bioresmethrin, Fenothrin, *d*-Fenothrin, Pyrethrum I, Carbaryl, Fenitrothion, Methacrifos, Pirimiphos-Methyl and Dichlorvos on Various Grains

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Methods of extraction and analysis suited for analysis of aged, unknown residues on wheat, barley, oats, sorghum, rice in husk, milled rice, polished rice and cooked rice are reported for carbaryl, for the pyrethroids bioresmethrin, fenothrin, *d*-fenothrin and pyrethrum I, and for the organophosphorus insecticides dichlorvos, fenithrothion, methacrifos and pirimiphos-methyl. Fortification studies were performed for each insecticide on each commodity; six regimes were used to extract insecticides that had been applied to grains 3–6 months previously in order to obtain adequate methods of extraction. Amounts of aged residues of dichlorvos, fenitrothion, methacrifos and pirimiphos-methyl, after standing whole grain in methanol or ethanol for 12 hours, were over 90% of the amounts obtained after extracting for 26 or 36 hours and above the levels extracted by hexane. Recoveries of fortified samples extracted by ethanol or methanol from all commodities ranged between 89 and 100%. The organophosphorus insecticides were determined by gas-liquid chromatography (*glc*) using a phosphorus-specific detector. Residue levels of fenitrothion were also determined colorimetrically, after hydrolysis to 4–nitro–3–methylphenoxide and clean-up by the addition of barium chloride. This method was suitable for determination of residue levels greater than 2 mg/kg. At least 80% of aged residues of carbaryl was extracted by standing grain in acetone or ethanol for 12 hours. Carbaryl was determined by *glc*, using electron capture, after derivatization to 2–chloroacetyl–1–naphthol and also semi quantitatively by thin-layer chromatography with 4–nitrobenzenediazonium fluoroborate as the chromogenic reagent. A two phase procedure using dilute alkali, 2–chloroacetic-anhydride and diethyl ether resulted in >95% acetylation of 1–naphthol, 4–cyanophenol and of the acidic phenols 2,4,5–trichlorophenol, 4–nitrophenol and 4–nitro–3–methylphenol provided that the pH of the aqueous phase was close to the pK_a of the phenol. Over 90% of the amounts of the pyrethroids bioremethrin, fenothrin, *d*–fenothrin and pyrethrum I extracted from all commodities after 26–36 hours in ethanol, light petroleum or acetone was extracted after 12 hours in light petroleum, with the exception that light petroleum was inadequate for the extraction of these pyrethroids from cooked rice. The pyrethroids, after alkaline hydrolysis, were determined colorimetrically from the reaction of chrysanthemic acid with acidified mercury (II) sulphate. Recoveries of fortified samples were between 89 and 95%.

INTRODUCTION

In order to assess the environmental safety of grain protectants applied to different commodities it is necessary to have analytical procedures suitable for each commodity. The commodities investigated in this work were wheat and "other grains," namely barley, oats, sorghum, rice in husk, and raw and cooked milled and polished rice. However, previous studies which were principally on wheat, were not repeated.

Methods are available[1,2] for the determination on what of residues of the organophosphorus insecticides dichlorvos, fenitrothion, pirimphosmethyl (2–diethylamino–4–methylpyrimidin–6–yl–dimethylphosphorothionate) and methacrifos (2–methoxycarbonyl–2–methylvinyl dimethylphosphorothionate). These methods involve extraction into a polar solvent, methanol or ethanol, and determination from the response of a detector specific for phosphorus, after separation by gas-liquid chromatography (*glc*). The suitability of this method for other grains was investigated, and extraction studies were performed with methanol, ethanol and hexane.

Fenithrothion in formulations has also been determined colorimetrically,[3] after alkaline hydrolysis to 4–nitro–3–methylphenol. The suitability of this method for determination of residues of fenitrothion on wheat and other grains was investigated. A quantitative procedure and a semiquantitative procedure using thin-layer chromatography (*tlc*) are described.

Two of the procedures used for determination of carbaryl on crops or vegetables were investigated for their suitability on wheat and other grains. One was an official[4] semiquantitative method, based on alkaline hydrolysis after purification by *tlc*, and visualization by spraying with 4–nitrobenzenediazonium fluoroborate. The derivatization procedure of Arguer[5] was also investigation; this involves hydrolysis and formation of 2–chloroacetyl–1–naphthol, which is determined by an electron capture detector, after purification by *glc*. The causes of the unreliability[5] of this procedure were investigated, and a more reliable method of esterification is reported for 1–naphthol and also for 4–nitrophenol, 4–nitro–3–methylphenol, 2,4,5–trichlorophenol and 4–cyanophenol. For each method of determination of carbaryl, extraction studies are reported for acetone, ethanol and dichloromethane.

A colorimetric procedure[6,7] for determination on wheat of pyrethrum I and bioremethrin (5–benzyl–3–furylmethyl (+) *trans*–chrysanthemate) is based on the colour of the reaction between acidified mercury (II) sulphate and chrysanthemic acid. The suitability of this procedure was investigated for determination of these pyrethroids on other grains and of fenothrin (3–phenonybenzyl (+)–*trans*–chrysanthemate) and *d*–fenothrin on grains. Intraction studies were performed with ethanol, light petroleum and 20% acetone, 80% light petroleum.

The nine insecticides were investigated because they are of low mammalian toxicity and because they were effective[8–10] in controlling insects on stored wheat in supervised trials (cf. also Bengston *et al.*, unpublished results). Although it is likely that methods of determination suitable for one commodity would be suitable for other commodities, this assumption was checked and the necessary modifications described. Fortification studies were performed on all commodities. Different extraction regimes were also applied to aged unknown[11,12] residue levels on different commodities, because aged residues may be more difficult to extract than those that have been freshly applied.[2,11] Extraction is complete if it is not increased by altering the regime (e.g. by changing solvent or extending the duration of extraction) and if similar results are obtained from different regimes.[11]

Particular attention was given to extraction of pesticides from cooked rice, as the partitioning effects of absorbed water in this medium could well prevent complete extraction by non-polar solvents. For example, hexane has been shown to be ineffective at extracting malathion from high moisture wheat.[13]

MATERIALS AND METHODS

1. *Fortification Studies and Extraction of Aged Residues*

Although fortification procedures varied slightly with particular determinations, common features in all studies were the addition of 100 μl of acetone solutions containing known amounts of pesticides to weighed quantities of commodity in extraction flasks. The solvent for extraction was added one hour after the addition of the pesticide. Unless stated otherwise in the text, each unprocessed commodity was fortified at the levels of 0, 1, 2, 4 and 8 mg/kg and each processed commodity was fortified at the levels of 0, 0.25, 0.5, 1 and 2 mg/kg. For example lots of 50 g of barley were treated with 0, 50, 100, 200 and 400 μg

of fenitrothion respectively, 100 ml of ethanol was added, and, after extraction, aliquots of the supernatant were injected into the *glc*, alternating fortified samples with standards. Standards were prepared by adding 100 ml of acetone solutions containing known amounts of pesticides to volumetric flasks and making up to the mark with commodity extract just prior to determination. The commodity extract used was the supernatant from pesticide-free grain, extracted by the same procedures as used for the fortified samples. Each standard and fortified sample was determined three times, and the whole process of preparing fortified samples and standards was replicated three times. To minimize the effect of the variation in response given by *glc* procedures, the ratio of response of fortified sample to standard was calculated for subsequent injections only. The % recovery was obtained by multiplying this average ratio by 100.

In preparing standards, it was assumed that the concentration of insecticide in solvent inside the grain was the same as that in the supernatant, as has shown to be the case with fenitrothion.[2] For example, there is only approximately 70 ml of supernatant after extracting 50 g of grain in 100 ml of solvent and, in preparing standards, this supernatant was assumed to contain only 70% of the pesticide, with the other 30% being contained within the grain. If this assumption were wrong, recoveries of fortified samples would be high. Thus the fortification studies serve not only to confirm that the procedure does not lead to loss of protectant, but also tests the assumption that pesticide concentrations are homogenous in absorbed and supernatant solvent.

Adequate recovery of fortified samples is only one criterion of complete extraction. Completeness of extraction was also assessed from a comparison of results from different extraction regimes.[11,12] All grains were extracted by at least three solvents for different times. Typical quantities were 50 g of grain, or 20 g of cooked rice, in 100 ml of solvent. All grains had been treated with the protectants applied as aqueous emulsions 3–6 months before extraction studies commenced, and in all cases residues recovered by an extraction procedure were corrected for recovery of pesticides added to various grains. These fortified samples were used as one of the standards for analysis. For the organophosphorus insecticides methacrifos, pirimiphos-methyl and fenitrothion recovery studies were performed on all grains except wheat, which had been investigated previously.[2] Recoveries of dichlorvos were investigated on rice and rice products only.

Solvents used for extraction studies were generally methanol, ethanol and hexane. For carbaryl, acetone was substituted for the less volatile ethanol because the procedure required concentration of extracted solvent, and, for similar reasons, light petroleum (30–40°C) was substituted for hexane in extraction of pyrethroids. Dichloromethane was generally used in place of methanol for extraction of carbaryl, as dichloromethane is used in an official procedure for analysis of carbaryl on vegetables.[4]

2. *Gas-Liquid Chromatography (glc) Procedures for Analysis of Organophosphorus Protectants*

The methods of analysis were standard,[1,2] based on clean-up by *glc* and analysis by flame photometric detector, specific for phosphorus. The column used was chromosorb W, 100–120 mesh, coated with 4% SE 30/6% SP 2401, with column temperatures ranging from 150°C, for dichlorvos, to 210°C for fenitrothion. For analysis of residues less than 0.1 mg/kg, the column temperature was lowered 15°C, then raised at 8°C/minute following injection.

3. *Carbaryl Analysis, Semiquantitative (Thin-Layer Chromatography, tlc)*

Basis of method: The method was modified from an official procedure for vegetables,[4] based on hydrolysis of carbaryl to 1–naphthol, and coupling with 4–nitrobenzenediazonium fluoroborate on a *tlc* plate to give naphthol fast blue.

Reagents: 1 M sodium hydroxide in 90% ethanol, 10% water, V/V; 4–nitrobenzenediazonium fluoroborate saturated at 0°C in 90% ethanol, 10% digol, V/V.

Extraction: For uncooked grains, 40 g of

grain were steeped for 24 hr in 60 ml of dichloromethane, or for 8 hr in 60 ml of acetone. For cooked rice, the acetone extraction was required. The extraction flasks were shaken occasionally.

Standards: Standards were prepared by adding appropriate quantities of carbaryl to 40 g of untreated grain; for example, 0, 40, 80, 160 and 320 μg correspond to residues of 0, 1, 2, 4 and 8 mg/kg. Standards were then treated similarly to the unknown. A simplified procedure for standards was used after the technique had been tested on a particular grain. Thus solvent aliquots were taken from untreated grain in solvent, as discussed below, and appropriate quantities of carbaryl added before concentration. For aliquots of 0.5 ml, each μg of carbaryl corresponded to residues of 3 mg/kg.

Tlc procedures: Two *tlc* procedures were used for most grains, but the polyamide plate was used for cooked rice, as it absorbed the water in the extraction solvent better than the alumina plate. The systems were:

i) Alumina plates, e.g. Merck's Aluminiumoxid F 254, Type e neutral, developed with 15% ethyl acetate, 85% hexane.
ii) Polyamide plates, e. g. Merck's Polyamide F 254, developed with 60% methanol, 40% water.

Plates were spotted with 0.5 ml of extraction solvent from unknowns and standards for analysis in the range 0.2–4 mg/kg or with 0.25 ml for analysis in the range 4–8 mg/kg. Two methods were used for spotting. The first spotting procedure, which was the easiest, was to reduce the aliquot in a 1 ml vial to 0.1 ml in a stream of air, to apply the concentrate to the plate with a thin paintbrush, type 00, to add 0.01 ml of acetone to the vial, and to respot. The second spotting procedure was to reduce 10 ml of extraction solvent to 0.1 ml, and to spot 5 μl on the plates. Plates were spotted with alternate unknowns and standards.

Method of analysis: Plates, after development, were sprayed with the ethanolic base reagent, and stored in the dark at 50°C for 5 minutes. The plates were then resprayed, left to partly dry for about 1 minute, and sprayed while still wet with the chromogenic reagent. Estimation was by visual comparison of intensity of the blue spots with standards. The total spraying procedure was then repeated, as a test for completeness of reaction, and repeated until there was no change in the order of colour intensities.

The analysis was then repeated on a different *tlc* system, with unknowns, based on results of the first analysis, placed adjacent to close standards. For example, an unknown identified as having 2–4 mg/kg carbaryl was placed adjacent to standards equivalent to residues of 2.2 and 3.3 mg/kg.

Qualitative confirmatory tests: 1–naphthol showed as a blue colour when illuminated at 254 nm before the plates were sprayed with chromogenic reagent. This colour was unstable and this test was used as a separate confirmatory test. After estimation of the blue colour caused by the chromogenic spray, spraying with acetic acid produced an orange colour, detectable to 0.15 μg.

4. Carbaryl Analysis, glc, Quantitative

Basis of method: The method was based on esterification of phenols by reaction with anhydrides in dilute aqueous alkali. As such the method was based on the classical methods of Pschorr[14] and Lumiére[15] for acetylating amines, and extended by Chattaway[16] to phenols. Improvements to the method of Arguaer[5] were used to maximize yields of phenolic esters.

Extraction: Acetone extraction was used, as in the semiquantitative method.

Derivatization: 1 ml of acetone extract was added to 1 ml of 1 M sodium hydroxide in 90% ethanol, 10% water. This was left for 1 hr at 50°C with extra solvent being added as required. The mixture was then poured onto 10 ml water and extracted with 2×10 ml diethyl ether, which was discarded. The pH of the aqueous phase was adjusted to 10, cooled to 0°C, and stirred with 2×20 ml 1% chloracetic anhydride in diethyl ether. The combined organic extracts, made up to 50 ml, were dried over sodium sulphate and an aliquot of 5 μl was injected onto a *glc* column attached to an electron capture detector. 2–chloroacetyl–1–naphthol was satisfactorily re-

solved on Chromosorb W, 60–80 mesh, coated with 5% SE30, at a column temperature of 175°C and a flow rate (N_2) of 70 ml/min.

Alternatively, esterification with propionic or acetic anhydride could be used, followed by identification by flame ionization detector.

5. *Analysis of Free Phenols, or Phenols from Hydrolysis of Organophosphorus Protectants*

The derivatization procedure was identical to that for 1–naphthol, but the pH had to be adjusted not to 10 but to the pK_a of the phenol.

6. *Colorimetric Procedure for Derivatives of Chrysanthemic Acid (d–Fenothrin, Pyrethrum I, Bioresmethrin)*

Basis of procedure: The procedure was based on published colorimetric methods,[6,7] modified for multiple analysis using simple equipment, and conforming more closely to official procedure[17] for analysis of formulations.

Reagents:

Ethanol, 95%, V/V.

Light petroleum, 40–60°C.

Barium chloride solution, a 10% solution of barium chloride dihydrate in water.

Sodium hydroxide, 1 M.

Hydrochloric acid, diluted 40 parts with water 60 parts, V/V.

Phenolphthalein indicator solution, a 1 % solution in ethanol.

Sodium hydroxide, dilute, 0.2 M.

Mercury (II) sulphate solution (Deniges reagent, modified).

5 ml of concentrated sulphuric acid was added with cooling and stirring, to 1 g of yellow mercury (II) oxide in 60 ml of water. The mixture was stirred till the oxide was dissolved and then a further 55 ml of sulphuric acid was added cautiously. The reagent was kept out of light, and used on day of preparation.

Filtercel. A natural diatomaceous earth available from Johns-Manville Co. Ltd.

Extraction: 100 g of grains, or 50 g of milled or polished rice, were extracted in 100 ml of light petroleum, and left, with shaking, for 20 ± 12 hours. For cooked rice, and for simultaneous analysis of organophosphorus protectants, 50% ethanol/50% light petroleum, V/V, was substituted for light petroleum.

Workup: 75 ml of extract was decanted through a strainer (to remove light fragments) into a 350–500 ml graduated beaker containing 5 ml of sodium hydroxide (1 N) and 20 ml of ethanol. The extract was concentrated on a water bath until the solution was homogeneous, and then the beaker was covered with a watch glass and gently refluxed for 45 minutes, adding ethanol as required. The solution was then reduced to 20 ml, made up to 200 ml with water, and boiled down to 140 ml. After cooling to room temperature, 10 ml of barium chloride solution were added (reserving 10 ml of total aqueous solution for fenitrothion analysis, method A, if required). The aqueous solution was made up to 250 ml with water and either 200 ml was decanted, after leaving overnight, or 1 g of Filtercel was added, the solution was shaken vigorously and 200 ml was filtered through a fully fluted (32 folds) 150 mm Whatmans No. 1 filter paper.

200 ml of the aqueous solution was neutralized (phenolphthalein) by the dropwise addition of diluted hydrochloric acid, and 1 ml of the acid in excess was added. After mixing, the solution was transferred to a 500 ml separating funnel, rinsed with 2×10 ml of water and 50 ml of light petroleum was added to the aqueous solution plus rinsings. The funnel was shaken vigorously for at least one minute, releasing the pressure, if necessary, at intervals, and the layers were allowed to separate for at least 5 minutes, or until the lower aqueous layer was clear, before the aqueous layer was run off into a flask. The light petroleum extract was filtered through a plug of cotton wool into a 100 ml volumetric flask. The aqueous liquid was returned to the 500 ml separating funnel, and the extraction repeated with a further 50 ml of light petroleum. The separating funnel and cotton wool were rinsed with light petroleum ($2 \times$ 10 ml), and the rinsings added to the flask. It was most important that none of the aqueous phase was added to the flask. The organic phase was dried with sodium sulphate, and

decanted and rinsed into another volumetric flask. The aqueous phase was reserved for analysis of fenitrothion, method B.

2 ml of 0.2 M sodium hydroxide was added to the organic phase in the flask, which was swirled vigorously for 1 minute. After leaving for at least 5 minutes, 0.6 ml of the aqueous extract was placed into a stoppered glass cell of width 10 mm and capacity 3–5 ml. Two ml of the modified Deniges reagent was added, starting a stop watch at commencement of addition. The cell was shaken three times, and placed in a split-beam spectrometer; the absorbance at 578 and 680 nm was measured at 2 and $2\frac{1}{2}$ minutes on the stop watch, using as blank 0.6 ml of 0.2 M sodium hydroxide plus 2 ml of the modified Deniges reagent. Readings at 680 nm were constant, and close to zero. High reading denoted turbidity and unacceptable analyses.

Standards: Internal standards were prepared by adding appropriate quantities of pyrethroid to untreated wheat, adding light petroleum and treating as unknown. External standards were prepared by adding chrysanthemic acid of isomeric composition similar to that of the pyrethroid to a glass cell, making up to 0.6 ml with 0.2 M sodium hydroxide, adding Deniges reagent and monitoring the colour. For derivatives of *d–trans* chrysanthemic acid, solutions of hydrolyzed pyrethroids were standardized by official procedures.[17] The appropriate quantity of chrysanthemic acid, for a standard of χ mg/kg, was $18\chi \times 168/M.W.$, where $M.W.$ was the molecular weight of the pyrethroid. For other pyrethroids, standards were prepared by hydrolysis of known amounts.

7. *Colorimetric Analysis of Fenitrothion*

Basis of method: The method was based on measurement of the yellow colour of the phenol from fenitrothion; this colour was used also in standardization of concentrates.[3] The hydrolysis procedure was that described in Section 6 with the exception that phenolphthalein was used as an external, not internal, indicator.

Method A: The method, which was suitable for residues in excess of 3 mg/kg, was to measure the absorbance at 398 and 480 nm of the aqueous extract whose preparation was described in section 6 for fenitrothion analysis, Method A. The blank cell contained 0.2 M sodium hydroxide, and high absorbances at 480 nm indicated turbidity and unacceptable analyses. However, such absorbances did not occur provided the solution was left overnight in a stoppered vial prior to analysis. The method was suitable for all grains tested except sorghum, where a natural yellow colour survived the work up procedure.

Method B: Method B was suitable for residues <3 mg/kg, or residues on sorghum. It involved adding 5 ml of concentrated hydrochloric acid to the aqueous extract of the type indicated in Section 6 for analysis of fenitrothion, Method B, and then extracting with 2×50 ml diethyl ether, back extracting the combined ether extracts into 15 ml of 0.2 M sodium hydroxide for residues in the range 0.1 to 3 mg/kg, or into larger volumes of alkali for larger concentrations.

8. *Semiquantitative tlc Procedure for Fenitrothion*

Basis of the method: The method was based on basic hydrolysis of fenitrothion on a *tlc* plate and identification by *Rf* and the yellow colour formed. The acetone extraction and *tlc* procedures described in section 3 were suitable for purposes of identification, but for semiquantitative tests silica plates, such as Merck's Silicar F254, were preferable to Merck's polyamid plates. They also enabled suitable resolution of fenitrothion from 4–nitro–3–methylphenol.

Spray procedures: After development of the *tlc* systems, the plates were sprayed with 1 N sodium hydroxide in 90% ethanol, 10% water and left 1 hr at 60°C with respraying at intervals of approximately 20 minutes. The yellow colour was stable in indoor light, and the chromogenic reagent used to detect carbaryl did not interfere.

Preparation of standards: Internal standards, as described for carbaryl, were used. The limit of detection was 0.2 μg of fenitrothion, corresponding to a residue in grain of 0.67 mg/kg, using the extraction procedures described for carbaryl and an 0.5 ml aliquot.

RESULTS

1. *Solvents for Extraction of Residues on Cooked Rice and Other Commodities*

Extraction is considered complete when residue levels are not increased by changing the solvent or extending the duration of extraction.[11,12] Determinations of aged residues on cooked rice by extraction with alcohols or acetone were consistent and independent of time of extraction.[2,11] With less polar solvents, extraction was incomplete (Table 1).

Fortified samples were prepared by adding insecticides to cooked rice before extraction, in order to confirm that the method of extraction was suitable. Recoveries (Table 1) were satisfactory, and for all compounds the simple method of stirring overnight in a polar solvent gave reproducible results, not affected by time of extraction or choice of polar solvent.

It was found that results (±10%) obtained from 24 hr extraction in acetone, methanol or ethanol of methacrifos, fenitrothion and pirimiphosmethyl on barley, oats, sorghum, rice in husk, polished rice, milled rice and malt were similar to each other. On the same commodities results (±10%) obtained from 24 hr extraction in light petroleum of fenothrin, *d*-fenothrin and bioresmethrin were similar to those from extraction in ethanol. For carbaryl, comparable results, within the precision of the *tlc* procedure, were obtained with all commodities from 8 and 24 hr extraction in acetone, 24 hr extraction

Table 1 Aged Residues of protectants in cooked rice, as determined by different extraction regimes.

Protectant	Duration of extraction (hr)	Solvent	Residue (mg/kg)	% recovery of fortified sample
Dichlorvos	12	Ethanol	0.32	95
	36	Ethanol	0.34	93
	12	Hexane	0.12	—
	36	Hexane	0.16	—
	12	Methanol	0.33	—
Carbaryl	6	Acetone	0.4 ±0.1	100±20%
(semiquantiative	12	〃	0.4 ±0.1	100±20%
analysis)	24	〃	0.4 ±0.1	100±20%
	12	Ethanol	0.4 ±0.1	100±20%
	12	Dichloromethane	0.25±0.12	75±37%
	24	Dichloromethane	0.25±0.12	75±37%
Bioresmethrin	12	Light petroleum	0.7	96
	12	Acetone	0.8	93
	12	20% acetone, 80% light petroleum	0.8	93
d-Fenothrin	12	Ethanol	3.1	91
	24	Ethanol	2.9	—
	24	Light petroleum	1.9	87
Fenitrothion	12	Methanol	2.8	97
	24	Methanol	2.8	96
	24	Ethanol	2.9	99
	24	Hexane	0.7	—
Pilmiphos-methyl	12	Methanol	4.9	91
	24	Methanol	4.9	94
	24	Hexane	1.9	93
Methacrifos	12	Methanol	0.2	91
	24	Methanol	0.2	89
	24	Ethanol	0.2	94

in dichloromethane and 4 hr extraction of ground commodity in dichloromethane.

2. Acetylation of Phenols in Dilute Base

Acetyl–4–nitro–phenol (50 mg), prepared by the procedure of Chattaway,[16] in diethyl ether (20 ml) was stirred over water (10 ml) at pH 7.5 at 0°C for 1 hour, without any detectable hydrolysis (<1%), whereas 4–nitrophenol (25 mg) was quantitatively acetylated (>98%) in less than one minute in the presence of excess acetic anhydride. However, acetyl–4–nitro–phenol in diethyl ether, stirred over water at pH 10, was rapidly hydrolyzed, and the formation of a yellow colour (1–2% decomposition) was immediately apparent. The variable yields reported by Arguer[5] for the two phase acetylation of phenols, at room temperature and at pH 14, were doubtless due to excessively basic conditions which hydrolyzed the esters as they were formed. At pH 7.6 quantitative yields (±5%) of acetyl–4–nitro–phenol, acetyl–4–nitro–3–methyl–phenol and acetyl–2,4,5–trichlorophenol were obtained from the two phase acetylation procedure, whereas at pH 10, 83% yield was the maximum obtained for acetylation of 4–nitro–phenol. However, quantitative yields of acetyl–1–naphthol, 1–chloroacetyl–1–naphthol and acetyl–4–cyanophenol were obtained from the 2 phase reaction at 0°C, pH 10.

3. Quantitative Procedure for Carbaryl

The recoveries of carbaryl added to wheat at different concentrations are outlined in Table 2. No interference was experienced on the *glc*, principally because the procedure was specific for compounds that can be readily hydrolyzed to phenols, or amines, with other materials being removed by the various partitionings.

Table 2 Recoveries of carbaryl added to wheat.

Wt. of wheat (g)	Carbaryl (μg)	
	Added	Recovered
10, 40	0	<0.01, <0.01
10	1	0.93, 0.96
10	10	9.7 , 9.9
40	25	24, 24, 25
10	50	48, 50

4. Carbaryl, Semiquantitative tlc Procedures

The measured levels of aged residues of carbaryl were similar, within the precision of the method, after extraction of whole or ground grain in dichloromethane for 24 or 36 hr, or after extraction of ground grain in acetone for 4 or 24 hr, or after extraction of whole grain in acetone for 24 hr. Any of these methods was suitable.

The advantage of the method was that it was quick and required no expensive equipment. It was, however, only a semiquantitative method. The accuracy of the method was tested by asking chemists to grade standards in order of intensity. The results of these tests (Table 3) were consistent with a precision of about 25% at suitable concentrations. That is, an analysis can be given as 4±1 mg/kg, but not as 4±0.5 mg/kg.

The limit of detection, 0.05 μg of carbaryl, corresponded to a residue of 0.17 mg/kg, under the conditions outlined.

Residues of carbaryl determined by the *tlc* procedure were, on wheat and barley, 5±1.25 and 4±1 mg/kg, as compared with results by the *glc* procedure of 4.6±0.2, and 4.1±0.2 mg/kg.

5. Colorimetric Procedure for Pyrethroids

Results using different extraction regimes for analysis of pyrethrum I, *d*–fenothrin and fenothrin, are outlined in Table 4, and are similar to results previously obtained for

Table 3 Ability of scientists to rank carbaryl standards in correct order, using a semiquantitative *tlc* procedure.

Amount of carbaryl (μg)	No. of participants	No. of completely correct rankings
0, 0.2, 0.4, 0.8, 1.6, 3.2	5	5
0, 0.15, 0.3, 0.6, 1.2, 2.4	5	5
0.1, 0.2, 0.4, 0.6	3	3
1.2, 1.8, 2.4	2	1
0.8, 1.2, 0.2, 0.3, 0.4	3	3

Table 4 Extraction of residues of *d*–fenothrin, fenothrin, and pyrethrum I on wheat and barley.

Code No.	Protectant	Time of extraction (hr)	Solvent	Type of grain	Residues (mg/kg)
A	*d*–Fenothrin	4	Light petroleum	Wheat	0.73
A		12	〃		0.71
A		4	20% acetone, 80%		0.70
A		12	Light petroleum		0.71
A		4	Methanol		0.72
A		12	〃		0.69
A		36	〃		0.73
B		12	Light petroleum	Barley	1.1
B		12	Ethanol		1.1
C	Fenothrin	12	Light petroleum	Wheat	1.9
C		12	Ethanol		1.9
D		12	Light petroleum	Barley	1.4
D		12	Ethanol		1.4
E	Pyrethrum I	12	Light petroleum	Wheat	0.55
E		12	Ethanol		0.58
E		24	Ethanol		0.56
F		12	Light petroleum	Barley	0.41
F		24	Ethanol		0.39

bioresmethrin, in that pyrethroids were readily extracted from whole grain by a variety of non-polar or polar solvents. Polar solvents were used when organophosphorus protectants were also analyzed.

As with the *tlc* procedure for carbaryl, losses during workup were compensated by the method of preparing standards. However, losses were estimated by comparison of internal standards with external standards. For example, for all analyses performed on pyrethroids by the author in 1977, on 24 separate occasions an external standard theoretically equivalent to 1.30 mg/kg of bioresmethrin, was analyzed by the internal standards as 1.37 mg/kg, standard deviation 0.05 mg/kg. These results were consistent with a slight to negligible loss during workup, and the small standard deviation, 0.05 mg/kg, between internal and external standards was consistent with a precise, reproducible method of analysis.

With regard to absorbance, the external standard was also constant, with a mean and standard deviation of 0.242 and 0.017 respectively. Accordingly, the constant instrument response could have been used as one of the two standards necessary for quantitative analysis.

6. Colorimetric Procedure for Fenitrothion

Method A: The hydrolysis used in the analysis of pyrethroids resulted in yellow colours for grain also treated with fenitrothion, and attempts were made to quantify this colour. However, basic hydrolysis of untreated wheat also resulted in a slight yellow colour, plus some turbidity. Attempts were made to reduce these background interferences by varying solvent and workup procedures, and substitution of light petroleum for methanol was partly effective. Despite these background effects, plots of optical density versus residue of fenitrothion, using internal standards of 0, 3, 6, 9 and 12 mg/kg were linear and passed through the origin provided the 0 standard was used as reagent blank (Table 5).

The absorbance of the 0 standard, relative to 0.2 N sodium hydroxide, was greatly reduced by the addition of barium chloride to the hydrolyzed aqueous extract and became negligible when the aqueous extract was left overnight prior to analysis. In eleven experiments, the absorbance of zero internal blank, measured against 0.02 M sodium hydroxide,

Table 5 Absorbance[a] of internal standard (fenitrothion) and external standard (4–nitro–3–methylphenol), using solution derived from untreated wheat as control.

Weight of wheat[b] in decanted extract	Volume of aqueous extract	No. of occurrence	Absorbance[a] for a residue of 10 mg/kg external standard		internal standard	
			Mean[c]	S.D.	Mean[c]	S.D.
60	250	8	0.141	0.001	0.144	0.004
60	150	10	0.211	0.001	0.215	0.007
40	150[d]	7	0.141	0.001	0.142	0.003

a) Absorbance is absorbance at 398 nm less absorbance at 480 nm. The subtraction of the latter absorbance, which is zero for 4–nitro–3–methyl–phenol, reduces erros associated with turbidily, dirty cells, etc. (The absorbance is quoted for a residue of 10 mg/kg, from the line of best fit through internal standards of 3, 6, 9 and 12 mg/kg.

b) Weight of wheat in decanted extract equals weight of wheat $\times V_d/V_e$, where V_d and V_e are volumes of decanted and extraction solvent respectively.

c) The mean and standard deviation of external standards were calculated from the mean absorbance of totally duplicated standards recorded on 17 separate occasions over a six month period. Thus the small standard deviation is indicative of the stability of the standards, which were kept at room temperatures away from the light, as well as of the constancy of machine response.

d) The mean and standard deviations were calculated from results obtained over a 6 month period.

Table 6 Residues of fenitrothion as ascertained by different extraction regimes and two methods of analysis.[a]

Time of extraction (hr)	Solvent	Residues (mg/kg) by	
		Method A	*glc*
4	Light petroleum	1.6	
12	〃 〃	4.0	
36	〃 〃	5.2	
4	80% light petroleum	2.9	
12	20% acetone, V/V	5.1	
36	〃	5.5	
4	Methanol	5.6	5.3
12	〃	5.4	5.4
36	〃	5.4	5.4

a) The methods were the colorimetric procedure Method A, and a *glc* procedure.

averaged 0.005, standard deviation 0.006 and this blank reading was of little importance for residues in the range 2–10 mg/kg (absorbance 0.042 to 0.211, Table 5). However, for residues less than 2 mg/kg the error associated with the blank became unacceptably high, and it was to reduce such errors that Method B was devised.

A suitable solvent for extraction of fenitrothion was made from the data in Table 6; complete extraction required a polar solvent. Results for analysis of fenitrothion by Method A were similar to results obtained by an established *glc* procedure[2] (Table 6). While the *glc* procedure, using a specific phosphorus detector, was far quicker than Method A, the latter method had the advantages of constant machine response (Table 5) and a crystalline primary standard in 4–nitro–3–methyl–phenol.

Method B: The slope of the plot of absorbance versus concentration in Method B was ten times greater than the slope of a similar plot in Method A, because of the 10 fold concentration achieved by acidification, extrac-

tion into ether, and back extraction into 15 ml of 0.2 M base. Thus the absorbance in Method B at 1 mg/kg averaged 0.207, compared with the mean absorbance in Method A of 0.211 (Table 5) for residues of 10 mg/kg.

7. Semiquantitative Test for Fenitrothion, Useful for Determination with Carbaryl

The *tlc* procedures used for analysis of carbaryl were used as a qualitative or semiquantitative test for fenitrothion, sensitive to 0.2 μg, or 0.7 mg/kg using the same size aliquots as used for carbaryl. The basis of the method was hydrolysis by base to the phenol, though the hydrolysis required 3 separate spraying and 1 hr at 60°C before it was complete. The precision of the method was better than 50%. The procedure did not require the chromogenic spray used to detect carbaryl, but was not affected by it.

Each of the *tlc* procedures used for analysis of carbaryl was suitable for detection of fenitrothion, though the Merck's polyamid plates buckled under the procedure. An alternative *tlc* procedure was elution on silica plates (e.g. Merck's F254 Silicar) with dichloromethane, and this also gave excellent separation of fenitrothion (*Rf* 0.95) and 4–nitro–3–methyl–phenol (*Rf* 0.21). The phenol, of course, gave an immediate yellow colour when sprayed with base.

DISCUSSION

Most of the methods require little discussion, because they were chosen by standard criteria for selection of procedures suitable for aged, unknown residue levels.[11,12] There criteria are adequate recovery of insecticides added to commodities, and completeness of extraction of aged residues. Extraction is said to be complete if levels are not increased by extending the duration of extraction, and if no other extraction regime gives higher levels, and if the regime gives levels comparable to those from other regimes.

The colorimetric procedures for fenitrothion and pyrethroids had the major advantage of optical procedures over chromatographic procedures in constant machine response. That is, absorbance did not vary from day to day in the manner that peak heights varied. In addition, primary standards were directly available in 4–nitro–3–methyl–phenol[3] and chrysanthemic acid,[17] whereas primary standards were not directly available for chromatography of fenitrothion or the pyrethroids. However, the colorimetric procedures were not specific and did not distinguish between fenitrothion and fenitro-oxon or between bioresmethrin and pyrethrum I.

The semiquantitative *tlc* procedures for carbaryl and fenitrothion were rapid, required little equipment and were sufficiently precise for many purposes. In situations where variations between samples are large, many relatively imprecise analyses may be of more use than a few more precise analyses.

The use of alcohols for extraction of organophosphorus insecticides on grain has been described as undesirable,[18] because of possible hydrolysis. However, it has been shown[2] that several organophosphorus pesticides are stable in alcoholic grain extract and that grain extract, when diluted in water, has a pH of about 5. At this pH most organophosphorus insecticides are stable, and even for dichlorvos only a small percentage of dichlorvos would be hydrolyzed after 24 hr at about 20°C.[19] This conclusion is consistent with the observed 93% recovery of added dichlorvos to rice after 36 hr extraction in ethanol.

Polar solvents were required for the extraction of residues from cooked rice, and even the non-polar pyrethroids were not completely extracted by light petroleum. It should be noted that the high residues in cooked rice in Table 1 were due to treatment with insecticides of polished rice, rather than rice in husk, and that such treatments with stable insecticides were not commercial practice.

Observed losses of the protectants studied in this work during storage and processing of grains are reported in a companion paper.

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要　　約

貯蔵穀物中のビオレスメトリン，フェノトリン，*d*–フェノトリン，ピレトリンI，カルバリル，フェニトロチオン，ピリミホスメチル，ジクロルボスの分析法の比較検討

J. M. デスマーチェリーア

7種の穀物中の貯穀害虫防除剤9種の抽出および定量法を検討した．ジクロルボス，フェニトロチオン，メタクリホス，ピリミホスメチルはメタノールまたはエタノールに12時間浸漬により36時間浸漬の90％以上が抽出され，ヘキサン抽出より効率がよい．これら有機リン剤はFPDガスクロマトグラフにより定量する．添加回収率はすべての穀物で84～100％であった．フェニトロチオンは4-ニトロ-*o*-クレゾールに加水分解し，塩化バリウム添加により精製したのち比色定量することもでき，この方法は2 mg/kg以上の場合に適する．80％以上のカルバリルがエタノールに12時間浸漬により抽出される．カルバリルはナフトールとしクロルアセチル化してECDで定量するか，4-ニトロベンゼンジアゾニウム塩を発色試薬とした*tlc*で半定量する．アルカリ溶液と無水クロロ酢酸のエーテル溶液によるアセチル化は95％以上進むが，4-ニトロフェノール等の酸性フェノールでは水溶液のpHが当該フェノールのpK_aに近いことが必要である．エタノール，石油エーテル，アセトンで36時間浸漬した場合の90%以上のビオレスメトリン，フェノトリン，*d*-フェノトリン，ピレトリンIIが石油エーテル12時間浸漬により抽出される．これらピレスロイドはアルカリ分解し，菊酸と硫酸水銀の反応を用いて比色定量する．添加回収率は89～95％であった．