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DEVELOPMENT AND EVALUATION OF SIMPLIFIED APPROACHES TO RESIDUE ANALYSIS

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APPLIED CHEMISTRY DIVISION

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DEVELOPMENT AND EVALUATION OF SIMPLIFIED

APPROACHES TO RESIDUE ANALYSIS**

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ABSTRACT - Simplified analytical methods are reviewed for pesticide residues and their metabolites. One criteria used in their selection was their suitability for screening food and environmental samples. Currently, thin layer chromatography is the most practical technique. It can detect organochlorine, organophosphorus and carbamate insecticide residues together with those of phenoxy, triazine and urea herbicides. Comparative data on the accuracy of thin layer chromatographic and gas chromatographic methods is also given. The former is shown to be a very useful multiresidue procedure for identification as well as quantitation of most important classes of pesticides.

1. INTRODUCTION

This project was initiated to identify unsophisticated methods for determining pesticide residues. These methods should be capable of accommodating the needs of developing countries for food inspection purposes and an aid in establishing new residue laboratories. The objectives of the project are therefore, to select or develop adequate residue analytical methods, especially multiresidue procedures, which employ relatively simple methodology and are suitable for the enforcement of Codex maximum residue limits.

2. CRITERIA

The basic criteria considered in selecting suitable methods are, (a) Methods should be reasonably comparable in sensitivity, precision and accuracy to gas chromatographic (GC) or liquid chromatographic (LC) methods. (b) Methods should give reliable information in screening for the parent pesticides and important transformation and degradation products. (c) Methods should be capable of quantitating residue levels by different techniques with different degrees of sophistication. (d) Methods should be useful for important commodities in international trade and domestic food supplies with an unknown pesticide history. (e) Methods should not require compressed gases or large volumes or high purity organic solvents. (f) Equipment should be relatively inexpensive as compared with GC or LC.

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These criteria are fulfilled by only a few methods such as thin-layer chromatography (TLC) or spectrometry in the visible range. Other simple methods, however, may be more convenient depending on specific laboratory conditions.

3. COMPARISON OF METHODS

All pesticide residue analytical methods involve extraction, clean-up, and a subsequent determination step. In each case, the latter step dictates the extent of clean-up required. Published methods need not necessarily be used in their entirety, it is often advantageous to combine individual working steps from different references. In addition, some analytical methods can analyse a much broader range of residues than described in the literature.

TLC appears to be the most convenient procedure for screening and determination of groups of pesticides in multiresidue analysis. It is simple, fast, sensitive, and usually quite specific, being equal or surpassing other determinative steps with regard to speed, and cost (1, 2).

TLC is especially valuable for the detection and identification of residues, although the quantitative aspect is limited. Recent development in the TLC of pesticides (3) and automatization of pesticide residue determination (4) has extended the efficacy of TLC for quantitation. The technique is already in widespread use for conformation of the identity of residues found by other procedures, such as GC methods (5).

In contrast to TLC, spectrophotometry yields only quantitative results. Apart from the specificity inherent in the colour reaction, it often lacks the required selectivity and hence is more open to possible interferences. Spectrophotometric methods, however, can be useful in conjunction with TLC as a confirmatory tool.

4. THIN-LAYER CHROMATOGRAPHIC MULTIRESIDUE PROCEDURES

Detailed TLC procedures are included in the FDA "Pesticide Analytical Manual" (PAM), volume I (6), and are also outlined as "official first action" or "official final action" in the "Official Methods of Analysis" of the AOAC (7). Equivalent procedures have been published by Canada Department of National Health and Welfare (8), the Deutsche Forschungsgemeinschaft (DFG) (9), the State Commission for Pest, Plant Diseases and Weed Control Chemicals of Ministry of Agriculture of the USSR "Methods for Determination of Pesticide Residues" (SCC) (10), and in the Council of Mutual Economic Assistance countries (CMEA) Manual (11).

The current PAM methods for organochlorine pesticides are based on procedures reported by Kovacs (12, 13). Aluminum oxide coated plates spotted with aliquots of the cleaned-up extracts and reference standards are developed with either n-heptane or n-heptane/acetone (98:2). For detection, the chromogenic reagent silver nitrate/2-phenoxyethanol and exposure to unfiltered UV light are used.

The use of acid washed aluminum oxide incorporating silver nitrate (6) or pre-coated plates dipped in a silver nitrate solution (14) eliminates the need to spray the developed plates. Some commercially available precoated plates containing silver nitrate are less effective for some pesticides than when the silver nitrate solution is applied at the time of developing (15).

The Canadian multiresidue procedure (8) for semi-quantitation of eleven organochlorine pesticides employs MN-silica gel HR and four mobile solvent systems: hexane, 1% acetone in hexane, 10-50% benzene in hexane, and 1% ethanol in hexane. Methods S1-S4, in the DFG Manual (9) utilize silica gel G-coated plates developed with n-heptane containing 1-3% acetone, whereas the more recent method S9, recommends aluminum oxide-precoated plates and petroleum ether/acetone (99:1) for 29 organochlorine pesticides and metabolites. Similar procedures are found in SCC methods (10), using silica gel (Silufol) or aluminum oxide precoated plates and a mobile solvent system of hexane/acetone (6:1) for determination of organochlorine pesticides. Apart from the advantage of the easier handling, precoated plates give more reproducible but generally somewhat lower R_f values. Pesticides are visualized by the silver nitrate/ammonia reagent containing hydrogen peroxide or by o-tolidine incorporated before coating the plate, following UV-irradiation. Florisil column chromatography is commonly used for the clean-up step to remove the lipids or other interferences. Organochlorine residues elute when petroleum ether, or petroleum ether/dichloromethane (8:2), are used. Silica gel deactivated with 30% water and petroleum ether as the eluant (16), yields a highly purified extract free from fatty materials, which is suitable for TLC. Good results have also been obtained using a silica gel ASK column with benzene/hexane (3:8) as the developing solvent (17).

Caution is recommended with the use of florisol, since the quality may vary in different countries. If the developing solvent used is petroleum ether, it can be readily recovered by distillation in order to reduce solvent costs.

In the PAM method for detection and identification of organophosphorus pesticides (6), silica AR plates are developed with a mixture of 2,2,4-trimethyl-pentane, acetone, and chloroform. The pesticides are visualized with 4-(p-nitrobenzyl)-pyridine (NBP) (18).

In the SCC method (10), determination and identification of organophosphorus pesticides is performed on silica gel or "Silufol" plates developed with chloroform, hexane/acetone (4:1, 7:3) or benzene/acetone (2:1). Pesticides are visualized using chromogenic reagents such as bromophenol blue/silver nitrate/acetic acid, 2,6-dibromo-N-chloro-p-quinoneimine (DCQ), palladium chloride and NBP. Trichlorfon could also be detected with resorcinol. The lower limits of detection by TLC are 0.005 mg/l (water), 0.1 - 0.3 mg/kg (soil), 0.2 - 0.4 mg/kg (plant and animal tissues).

Detection and semiquantitative analysis by TLC can be performed for both organophosphorus and carbamate insecticides using the enzyme inhibition technique. Because of the specificity of this technique, vigorous clean-up is usually not required. The most common enzyme inhibition procedure used is based on the work of Mendoza et al. (19-22) and Wales et al. (23). MN-silica gel G-HR plates are developed in hexane/acetone (4:1) and the pesticides visualized by steer or pig liver esterase in conjunction with 5-bromo-indoxyl acetate as substrate (8).

The use of 2-naphthyl acetate as substrate for beef liver cholinesterase and Fast Blue B salt as described by Ackermann (24, 25) and Ackermann et al. (26) is preferable for analytical purposes. The detailed procedure has been accepted by the CMEA countries as a recommended method; R_f -values and detection limits are reported for 38 compounds (27). Ernst et al. (28) have compared *Drosophila* and rat liver esterases with bee-head esterase as alternative enzyme sources. The results with 65 pesticides indicated that bee-head esterase is generally more sensitive in detecting organophosphorus and carbamate pesticides at the nanogram level in vegetables and fruits, without the need for elaborate clean-up. The other two esterases are also useful in screening for these pesticides.

In addition, two review articles, Burchfield and Storrs (29) and Dorrough and Thorstenson (30), on the analysis for organophosphorus and carbamate insecticides and their metabolites include TLC quantitative techniques.

TLC methods for analyzing different groups of herbicides, e.g. chlorinated phenoxy acids, s-triazines, and phenylureas, have been used and reviewed by Yip (31). Some of the procedures cited could be adapted for routine analytical work.

In the SCC methods (10), phenylureas and some carbamates are determined in plants, water and soil matrices using aluminum oxide plates and a mobile solvent system of diethyl ether or diethyl ether and carbon tetrachloride (3:1 and 1:17) mixtures. The pesticides are visualized by sodium nitrite and 1-naphthol after thermal destruction.

TLC methods could be also used for the determination of ethyl mercury chloride and inorganic mercury compounds in water following extraction with dithizone (17). It is also possible to use TLC for the determination of bioactive pesticides, such as trichotecine and beta-exotoxine (10).

5. IDENTIFICATION BY THIN-LAYER CHROMATOGRAPHY

A high degree of clean-up often is required for an unambiguous TLC identification as compared to a GC method. Oils and waxes interfere, causing streaked zones and distorted R_f -values. In practice, the more polar fractions from column clean-up may frequently require further purification if lipids are present in the extract.

For TLC multiresidue analysis, it is important to use small spots in order to obtain highly reproducible R_f -values. Usmenceva et al. (32) concluded that R_f values for organochlorine pesticides strongly depend on the particle size of adsorbent and the extent of development by the mobile phase. Temperature and moisture were reputed to have less influence. However, Ebing (33) emphasized that a constant particle size of the sorbent layer and temperature and humidity are necessary and also possible with simple laboratory equipment. Observing these parameters, reproducible R_f -values were determined in several TLC systems for organochlorine insecticides (34) as well as for triazine, carbamate, urea, and uracil herbicides (35).

The brand of an adsorbent often cannot be substituted, since the polarity or hardness may differ, which results in altered R_f -values and may even require a modified solvent system. For this reason, standards should always be run on adjacent areas of the same plate. Confirmation of the identity of a compound can only be achieved by the use of different solvent systems, in order to obtain R_f -values, which can be correlated to provide independent evidence. The use of multiple R_f -values for identification purposes was studied by Connors (36), who recommended the pairing of solvent systems with opposite properties. A TLC procedure was described for identification of organochlorine insecticides using three types of adsorbent, seven solvent systems and two visualization techniques (37). The classical technique of UV-irradiation could also be useful to confirm the identity of organochlorine compounds (38).

6. QUANTITATIVE ANALYSIS BY THIN-LAYER CHROMATOGRAPHY

Further development of TLC methods has resulted in the ability to quantitate residues. For this purpose, precise and accurate spotting, uniform layers, uniform application of detection reagents are essential, coupled with optimum use of measuring devices.

Manual spotting is best performed using small volume disposable pipettes, where delivery errors below 1% are possible (39). For larger volumes, repeated applications are necessary. In some cases, dipping plates into chromogenic reagents often yields more uniform results than spraying.

Precoated plates are of a higher purity and more uniform than those coated by hand, and are recommended for quantitative TLC. Visual comparison of the location, size and intensity of spots from sample extracts with reference standard spots is extensively used. Thus, quantitation of cholinesterase inhibiting compounds can be achieved by comparing the area of the inhibition spot and the lowest detectable amount of the unknown chemical with those of the standard (19). However, planimetric measurement of spot size was found to be less accurate than a visual comparison of the spots (19, 26).

Klisenko (40) compared TLC analyses with results obtained by other techniques. Quantitative evaluation was made on the basis of comparison of spot sizes and standards measured with a planimeter or squared paper (tables 1 and 2).

TABLE 1. Recovery of various pesticides by thin layer chromatography

Pesticides	Substrate	Recovery, %			
		\bar{X}	S	S_r	$\Delta \bar{X}$
thiophanate	water	90	8.2	9.0	8.0
malathion	soil	85	8.2	9.6	8.4
chlorthiamid	water	80	2.9	3.6	7.4
	soil	74.5	7.8	10.5	8.2
	rice	66.1	4.9	7.5	5.2
	grass	69.6	7.1	10.3	7.5
dichlobenil	water	85.6	3.8	4.4	4.0
	soil	80.2	3.8	4.7	4.8
	rice	67.0	4.6	6.9	4.8
	grass	71.8	5.3	7.4	5.6
diphenamid	tomato	90.0	9.0	10	10
kelevan	potato	87.4	6.9		
	soil	79.3	4.2		
	grass	74.3	6.1		
dimethoate	soil	80.0	5.2		
menazon	vegetable	93.0	6.5	7.0	6.8

\bar{X} - average recovery

S - standard deviation

S_r - relative standard deviation

$\Delta \bar{X}$ - confidence level, $p = 0.95$, $n = 6$

TABLE 2. Recovery of various pesticides by the methods TLC, GC and spectrophotometry¹

Pesticide	Substrate	Method		
		TLC	GC	Spectrophotometry
terbutylazine	water	86.7 ± 5.64	-	76.0 ± 3.65
	soil	82.6 ± 6.96	-	74.2 ± 3.38
dalapon	water	72.4 ± 5.78 ²	92.6 ± 3.26 ³	
	soil	67.4 ± 5.45 ²	83.6 ± 2.86 ³	
benthiocarb	water	76.9 ± 6.54	93.5 ± 3.0	
	soil	70.3 ± 7.46	78.3 ± 4.5	
	rice (corn)	70.9 ± 5.75	81.3 ± 4.67	
dichlobenil	water	85.6 ± 4.02	88.0 ± 4.67	
	soil	80.2 ± 4.01	83.4 ± 3.62	
	rice	67.0 ± 4.85	72.3 ± 5.08	
	grass	71.8 ± 5.6	75.0 ± 4.62	

¹ expressed as $(\bar{X} \pm \Delta \bar{X} \%)$ ² as α - bischloropropionic acid³ as - methyl ether α - bischloropropionic acid

Table 3 shows the analytical results obtained by GC and TLC following the DFG method S9 for a number of commercial milk and fat samples. Corresponding GC and TLC data (41) are also given for processed plant material analyzed by a modified

TABLE 3. Comparative results for fatty substrates

Sample	Pesticides found	Concentration ¹	
		GC	TLC
Fresh Milk	alpha-BHC	0.71	0.8
	beta-BHC	0.40	0.4
	gamma-BHC	0.12	-
	p,p'-DDE	0.13	-
	p,p'-TDE	0.06	-
	p,p'-DDT	0.25	0.3
"	alpha-BHC	0.48	0.3
	gamma-BHC	0.10	-
	beta-BHC	0.35	0.3
	p,p'-TDE	0.65	0.5
	p,p'-DDT	1.40	1.0
	p,p'-DDE	0.76	0.8
"	p,p'-DDE	0.10	-
	p,p'-TDE	0.63	0.4
	p,p'-DDT	0.28	0.3
Anhydrous milk fat	toxaphene	5.2	4
"	"	8.7	6.5
Anhydrous milk fat	α -BHC	3.09	3
	γ -BHC	0.36	0.3
	β -BHC	1.84	1.5
	δ -BHC	0.12	0.1
	p,p'-DDE	0.71	0.9
	p,p'-TDE	1.54	1.5
	p,p'-DDT	2.80	2.4
Anhydrous milk fat	toxaphene	10.3	9
	"	12.3	11
	"	9.9	9
	"	4.6	4
Butter oil	α -BHC	2.33	2.6
	γ -BHC	2.34	1.2
	β -BHC	2.50	2.2
	δ -BHC	0.64	0.5
Butter oil	α -BHC	1.18	0.9
	γ -BHC	0.11	-
	β -BHC	0.50	0.4

TABLE 3 - (con't.)

	δ -BHC	0.06	-
Butter oil	dieldrin	0.45	0.3
	p,p'-DDE	0.24	0.2
	p,p'-TDE	0.15	-
	p,p'-DDT	0.60	0.5
Butter oil	α -BHC	0.93	0.6
	γ -BHC	0.05	-
	β -BHC	0.67	0.8
	p,p'-DDE	0.25	0.3
	p,p'-TDE	0.67	0.6
	p,p'-DDT	0.16	0.2
Butter oil	α -BHC	2.07	1.8
	γ -BHC	0.50	0.6
	β -BHC	0.53	0.6
	δ -BHC	0.22	0.1
	p,p'-DDE	0.61	0.6
	p,p'-TDE	2.18	2.4
	p,p'-DDT	3.10	2.8
	Toxaphene	5.1	4
	"	7.8	7
	"	10.6	8
"	8.7	6	
Chicken fat	HCB	0.10	-
	α -BHC	0.06	-
	γ -BHC	0.05	-
	β -BHC	0.27	0.3
	p,p'-DDE	0.50	0.4
	p,p'-DDT	0.15	0.1
Human milk	HCB	0.66	0.5
	β -BHC	0.48	0.4
	p,p'-DDE	3.29	3
	p,p'-DDT	0.87	0.7
	Dieldrin	0.25	0.2
Cocoa butter	Lindane	0.57	0.5
	p,p'-DDT	0.25	0.2
Cocoa butter	Lindane	0.70	0.6
	alpha-BHC	0.10	-
	p,p'-DDT	0.45	0.3
	p,p'-DDE	0.20	0.1

¹All values in mg/kg on fat basis.

AOAC procedure (table 4). TLC quantitation was based in both cases, on visual comparison of spot sizes with detection limits of 0.1 mg/kg (table 3) and 0.01 mg/kg (table 4), respectively. The results of a TLC and GC comparative study of lard, fortified with a mixture of BHC isomers, DDT, DDD, and DDE are summarized in table 5 (42).

Similar results (table 6) have been obtained for non-fatty substrates fortified with methylparathion (10). Comparison of bio-assay and TLC data (43) shows that both methods give similar results (table 7). However, bio-assay being more sensitive but not specific, can give higher residues as shown for phosalone (table 8).

Data obtained using the bio-assay method with *Drosophila melanogaster* Meig, TLC (enzyme detection) and GC (⁶³Ni-ECD) for trichloronate, fonofos and parathion in different crops and soil, after treatment with granular formulations are compared in Table 9 (44). In most cases the results obtained by TLC are reliable and indicative of its potential for use in routine residue analysis. Thin-layer densitometry has been reported to be capable of precision of 1 - 2% on a routine basis in the hands of an experienced operator. Alternative quantitative procedures involve elution of spots from the adsorbent and measurement of the pesticide residue by a variety of standard microchemical methods.

Touchstone et al. (45) have described operating parameters for quantitative spectrodensitometry of TLC plates. The use of double beam instrumentation corrected

TABLE 4. Comparative results for non-fatty substrates

Sample	Pesticides found	Concentration ¹	
		GC	TLC
Tobacco	p,p'-TDE	8.5	9
	p,p'-DDT	1.2	0.8
	p,p'-DDE	0.15	0.1
Apple purée	Parathion	0.05	0.05
	DDT	0.10	0.08
Wheat flour	Malathion	0.48	0.5
	Lindane	0.12	0.1
Apricot purée	Dimethoate	0.23	0.2
Tomato flakes	Lindane	2.13	2
	Endrin	1.30	1.2
	Parathion	0.10	0.10
Oat flakes	α-BHC	0.01	0.01
	γ-BHC	0.04	0.03
Rice flour	Malathion	0.80	0.6
	Lindane	0.05	0.04
	p,p'-DDT	0.02	0.02

¹ All values in mg/kg

TABLE 5. Results of comparative analysis of lard fortified with a mixture of BHC isomers, DDT, DDD, and DDE at the levels of 1.0 mg/kg

Compound	Concentration (mg/kg)	
	TLC	GC
α-BHC	1.0	1.24
β-BHC	1.2	1.20
γ-BHC	1.0	1.16
ζ-BHC	1.2	1.24
DDT	1.0	1.05
DDD	1.0	1.09
DDE	1.0	1.07

TABLE 6. Comparative analysis of non-fatty substrates fortified with methyl parathion at the levels of 0.16 mg/kg

Sample	Concentration (mg/kg)	
	TLC	GC
Apples	0.13 ± 0.02	0.16 ± 0.04
Pears	0.12 ± 0.01	0.16 ± 0.02
Plums	0.11 ± 0.02	0.16 ± 0.04
Potatoes	0.13 ± 0.02	0.16 ± 0.08
Carrots	0.11 ± 0.02	0.15 ± 0.03

some of the faults due to nonuniformity of layer thickness, background scatter, and sample application. Linear response was found to be dependent on a proper choice of scanning wavelengths.

Sherma and Bloomer (46) reported the determination of organochlorine pesticide residues using aluminum oxide and silica gel precoated plates impregnated with silver nitrate. Quantitation in this case was achieved by scanning with a densitometer in the double beam mode after UV-irradiation. A similar method has been described for chlorophenoxyalkanoic acid herbicides (47).

An excellent approach to quantitative measurement of organophosphorus pesticides by direct scanning has been described by Getz (48) where the reproducibility is limited only by the uniformity of the adsorbent layer. Another development involving scanning with a fiber optics densitometer has been reported using dichloro-indophenol/enzymatic procedure for the detection and quantitation of cholinesterase inhibiting

TABLE 7. Sensitivity of TLC and bioassay methods for some organophosphorus pesticides

Pesticide	Lower limit of detection (mg/l)	
	TLC ¹	Bioassay ²
dichlorvos	10	6.6 x 10 ⁻²
dimethoate	5	1.6
trichlorfon	5	2.2 x 10 ⁻²
malathion	10	1.4 x 10 ⁻²
parathion methyl	7.20 x 10 ⁻⁴	2.5

¹Adsorbent silica gel; detection with bromophenol blue-AgNO₃

²Test object: *Culex pipiens molestus*

TABLE 8. Comparative analysis of apples for pesticide residues by TLC and bioassay

Pesticide	Concentration (mg/kg)	
	TLC	Bioassay
carbaryl	24.0 ± 3.2	26.5 ± 3.4
malathion	26.1 ± 2.9	30.7 ± 3.3
phosalone	24.0 ± 1.5	49.5 ± 3.9
trichlorfon	12.2 ± 1.5	14.2 ± 1.2

TABLE 9. Comparative residue analysis for some organophosphorus insecticides after practical application to soil (mg/kg)

Sample	trichloronate			fonofos			parathion	
	bioassay	TLC	GC	bioassay	TLC	GC	TLC	GC
potatoes	0.05	0.02-0.05	-	0.05	0.05	-	-	-
"	-	0.02	0.02	-	0.05	0.05	-	-
carrots	0.1	0.05	-	0.4	0.2-0.5	-	0.05	0.05
"	0.1	0.2	-	-	-	-	-	-
"	-	0.02-0.05	-	-	-	-	-	-
beets	0.25	0.2	-	0.18	0.05-0.1	-	0.05	0.05
"	0.05	0.02	-	-	-	0.05	-	-
"	-	0.02	-	-	-	-	-	-
soil	0.25	0.2	-	0.5	0.05	-	0.1	0.09
"	0.1	0.05	-	-	0.1	0.2	-	-
"	-	0.1	0.4	-	-	-	-	-

compounds (49).

Mallet et al. (50) and McNeil and Frei (3) have published reviews on the recent application of TLC and *in situ* fluorescence spectroscopy for the determination of pesticides and other organic contaminants. These methods offer a reasonable alternative to GC. An excellent review has been prepared also by Hill (1) concerned with the merits of TLC for qualitative and quantitative multiresidue analysis.

7. TLC ANALYSIS OF PESTICIDE METABOLITES

Organochlorine pesticide metabolites (e.g. dieldrin, heptachlor epoxide) are recovered with the commonly used multiresidue procedures. However, the wide range of polarities of organophosphorus and carbamate pesticide metabolites poses several difficulties. Although several oxon analogs of phosphorothioates insecticides can be detected by TLC, most of these compounds are more polar than their parent compounds. As a result they are preferentially soluble in water and are removed during the washing step in the clean-up procedure. In the AOAC Official Methods of Analysis (7), a spectrophotometric enzyme inhibition procedure is described for determination of known water soluble anticholinesterase organophosphorus compounds. A rapid total phosphorus method has been reported for polar organophosphorus pesticides in cleaned-up water extracts from vegetables (51). Macroporous silica gel (Merck EM-gel SI 200 A) and gradient elution with acetone in hexane was used by

Getz (52) for the separation of insecticides and their metabolites from co-extractants. Considerable separation of different classes of insecticides and metabolites was observed. The quantitative recovery of DDT, DDE, methoxychlor, the two demeton isomers and their sulfone and sulfoxide analogues, carbofuran, 3-keto and 3-hydroxycarbofuran and their phenolic degradation products was achieved. Using the quantitative dichloro-indophenol method mentioned above (49), S-methyl phorate sulfone and sulfoxide can be determined at low nanogram levels, and phorate sulfone and sulfoxide after oxidation with bromine vapors. Carbofuran and its metabolites, aldicarb and its metabolites, and carbaryl were determined at similar levels.

The reviews by Burchfield and Storrs (29) and by Dorough and Thorstenson (30) are recommended for TLC procedures for organophosphorus and carbamate insecticides including several metabolites.

8. MISCELLANEOUS SIMPLE METHODS

Where adequate analytical methods or equipment are lacking, biological assay can be used as an alternative. Although these methods are non-specific, they do offer the advantage of fairly rapid analysis. The most commonly used methods are based on exposure of insects to macerated and homogenized plant tissue or to evaporated extracts (53). They have been extensively reviewed by Nagasawa (54) and Hoskins and Craig (55). Wegman et al. (56) reported on the development and application of a TLC-fungispore inhibition method for screening pesticide residues. A sensitive detection method for herbicide residues following separation by TLC has been described by Kovac and Henselova (57) and by Sackmauerova and Kovac (58). Detection is based on inhibition of the Hill reaction and it has been applied to triazine, urea and uracil herbicides.

The Weisz ring oven technique in combination with TLC has been successfully used in analyzing plant samples for organophosphorus pesticide residues (59). The same procedure was applicable to bipyridylum herbicides (60).

In spectrometry, an outstanding, simple procedure for quantitative analysis of dithiocarbamate fungicides is acid hydrolysis and colorimetric evaluation of carbon disulfide isolated by distillation. The best results were obtained using the hydrolytic conditions described by Keppel (61) and the diethanolamine/copper acetate colour reaction (9).

9. CONCLUSION

A continuing need exists for unsophisticated methods of screening and quantitating residues of pesticides and their metabolites in both food and environmental samples. Currently, TLC is the most suitable technique available. Future research efforts should be directed towards improving this technique, the development of simple, comprehensive multiresidue procedures and the search for alternative methods.

A central reference point for the coordination of such work should be established, such as that proposed in the document, "Action program for the development of practical (simple) methods of analysis of contaminants in foods." This document is included in the report of the second session of the Joint FAO/WHO Expert Consultation on Methods of Sampling and Analysis of Contaminants in Food, Rome, 1978.

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