

RESIDUE REVIEWS

VOLUME 56

RESIDUE REVIEWS

Residues of Pesticides and Other
Contaminants in the Total Environment

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Foreword

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

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

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

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

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

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

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

August 7, 1975

Joint Coordinating Board of Editors

The end result of this so far over three decades and responsibilities with national, encompassing and timely publication outlet in the field of environmental contamination and toxicology is the Springer-Verlag (Heldelberg and New York) monographs. As the *Bulletin* (vol. 1 in 1966) for rapidly detailed review articles connected with the current in research of pesticides and other chemical contaminants, the *Archives* and *Residue Reviews* logical considerations and consequences.

Preface

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

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

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

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

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

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Gas chromatographic determination of residues of insecticidal carbamates

By

EDWIN D. MACALLONA*

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I. Introduction

With the increasing concern over the effect of the persistent organochlorine insecticides on the living environment, alternative pesticides

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have been increasingly used, notably the organophosphates and the carbamates. There are two main groups of insecticidal carbamates, the *N*-methyl- and the *N,N*-dimethylcarbamates. The latter are used only to a limited extent, especially in the United States, because of the greater insecticidal spectrum and potency exhibited by the *N*-methylcarbamates (O'BRIEN 1967). The *N*-methylcarbamates can be further subdivided, according to the nature of the functional group attached to the carbamyl moiety, into the aryl and the oxime *N*-methylcarbamates; the aryl *N*-methylcarbamates are the most important from the standpoint of number and tonnage used in pest-control work.

Since gas chromatography (glc) has become the most used single detection technique in pesticide residue analysis, its use in the detection of insecticidal carbamates was a matter of course. Review papers which included the glc of insecticidal carbamates as part of a broader subject were those of FISHBEN and ZIELINSKI (1967 and 1969) and the biennial *Analytical Chemistry* review series on pesticide residues (WILLIAMS and COOK 1967, THORNBURG and BECKMAN 1969, THORNBURG 1971 and 1973). In these papers, emphasis was on the detection itself and no unification with the equally important pre-chromatography steps was attempted. The annual "General Referee Report on Carbamate Pesticides" by the Association of Official Analytical Chemists (AOAC) also provides insights on recent developments on the subject (BENSON 1969 and 1970, STORHERR 1971 and 1972) but its coverage is necessarily restricted.

An attempt at unifying the subject of detection with pre-chromatographic operations was made by WILLIAMS (1971) but the coverage was restricted to plants and extraction and cleanup steps were discussed only cursorily.

It is the intention here, therefore, to provide an updated and comprehensive review of the glc of carbamates. Identity confirmation is also included in response to the growing awareness that glc responses from other industrial pollutants or related pesticides could be erroneously interpreted as those coming from the pesticide analyzed, especially if the electron-capture detector is used.

II. Pre-chromatography operations

Pre-chromatography operations, singly and collectively, influence the final determinative step in terms of how closely the actual insecticide content of the substrate is estimated and the degree of interference from substrate extractives and other sources; therefore, the transfer of the insecticide from the substrate to the organic solvent(s) should be maximized while only minimal and/or reproducible losses are allowable during cleanup or other preparative steps.

Very few studies have been made to evaluate the efficacy of different extraction and cleanup techniques for the analysis of carbamates, especially with field-incurred residues, so that very few generalizations can

be made. In most cases, the reported procedures were adopted because they were found suitable and recoveries with fortified samples were considered adequate. To provide some general guidelines on these operations it is necessary, therefore, to consider results obtained with the organochlorines and the organophosphates, at the same time keeping in mind some properties unique to carbamates which may necessitate a different set of procedures.

For carbamates, their thermal and hydrolytic instability should be given consideration in the overall detection scheme. Evaporation of solvents to dryness should be avoided because insecticide loss with this operation is high and variable. Mineral oil or natural oils and waxes as keeper may minimize losses in concentration steps. Where possible, lower boiling solvents should be used in the earlier stages of the analysis so that preferential evaporation can be taken advantage of if transfer to another solvent is necessary. For evaporations to 0.1 to 0.3 ml, a micro-Snyder column attached to a Kuderna-Danish collector tube has been suggested by BURKE *et al.* (1966). Operations at pH higher than neutral should be avoided with most carbamates because of hydrolytic cleavage (ALY and EL-DIB 1970, FAUST and GOMAA 1972). Another property of carbamates which is important in the overall residue picture and has recently received increasing attention is the formation of hydroxylated metabolites; this possibility is discussed in Section II c.

a) Extraction

For routine analysis, blending or other homogenization is commonly used because of the facility of the operation and the high recoveries obtained using the proper solvent or solvent systems. Because Soxhlet extraction, despite its tediousness, results in almost complete removal of the pesticide from the substrate, it may be used to evaluate other extraction procedures. However, the extents of thermal decomposition and volatilization of carbamates should be evaluated in using Soxhlet extraction.

Extraction efficiency of field-incurred residues is also conveniently evaluated using radiolabeled compounds. In the absence of radiolabeled compounds and the inability to check on extraction efficiencies by Soxhlet extraction because of thermal degradation or volatilization, the procedure suggested by THORNBURG (1965) may be used. In this procedure, using field-treated samples, three samples are extracted as follows: (1) extraction according to the proposed procedure, (2) extraction according to the proposed procedure but conducted three times and the extracts combined for analysis, and (3) extraction according to the proposed procedure but using three times the solvent volume. Effective removal of the pesticide is indicated by good agreement among the recoveries obtained.

The choice of solvent or solvent systems in the extraction operation

may be critical as far as efficiency is concerned. This choice is dictated to a large extent by the nature of the substrate for analysis.

1. Plant tissues.—Although for organochlorines the order of efficiencies of the extraction solvents appears to be water-miscible > water-miscible-water-immiscible combination > water-immiscible (BURKE and PORTER 1966, KLEIN 1958, KLEIN *et al.* 1959), the same simplification does not appear to be operative with carbamates. Thus, WATTS (1971) found that blending with acetonitrile and ethyl acetate and Soxhlet extraction for 12 hours removed about 100% of ^{14}C -labeled carbaryl¹ from bean leaves and kale. VAN MIDDELEM and PELOW (1973) also showed that ethyl acetate resulted in more efficient extraction of ^{14}C -labeled carbofuran from weathered cabbage leaves compared to acetonitrile or methanol blending, Soxhlet extraction, and acid digestion although it was the least efficient of the five procedures for total ^{14}C which was primarily 3-hydroxy carbofuran. On the other hand, PORTER *et al.* (1969) found acetonitrile superior to methylene chloride so that acetonitrile is also recommended by the associate referee for adoption in the AOAC "Official Methods of Analysis" (STORHERR 1971).

The MILLS *et al.* (1963) procedure, which has become the most widely used extraction and cleanup system for organochlorines and organophosphates and which also formed the basis for the "Multiple Detection Scheme" of the U.S. Food and Drug Administration for these insecticides, was modified by PORTER *et al.* (1969) for the analysis of carbaryl. The basic Mills procedure involves extraction of the sample with acetonitrile, dilution of the extract or an aliquot with water, and then partitioning of the insecticides into petroleum ether. The petroleum ether is then passed directly through a Florisil column or first concentrated to five to ten ml before Florisil column chromatography. The PORTER *et al.* (1969) modification was necessary because carbaryl cannot be removed from aqueous acetonitrile with petroleum ether nor could it be eluted from the Florisil column with the eluting solvents used for organochlorines and organophosphates. The modification called for a preliminary cleanup of the acetonitrile extract by ammonium chloride-phosphoric acid solution followed by Florisil column chromatography. The insecticide was then transferred to methylene chloride by partitioning after dilution of acetonitrile with water. A final Florisil column cleanup was then made on the organic phase with methylene chloride serving to elute carbaryl.

HOLDEN (1973) also attempted to apply the multi-residue method to the analysis of carbamates by way of the 2,4-dinitrophenyl ether derivative. With several crops, petroleum ether partitioning followed by treatment of the acetonitrile phase with coagulating solution, and then removal of phenolic impurities by partitioning of a methylene chloride extract

¹Chemical characterizations of insecticides mentioned in text are given in Table XII.

with dilute alkali was adequate cleanup for detection by electron capture with good recoveries.

In the case of aldicarb and metabolites, ANDRAWES *et al.* (1971 b) found that a 1:1 mixture of ethanol and water was the most efficient extraction solvent for plant tissues. With dehydrated substrates, the use of 35% water in acetonitrile could result in higher extraction efficiencies. JOHNSON and STANSBURY (1965 a) also increased carbaryl extraction from dehydrated fibrous products by soaking the sample in water for six to 24 hours prior to extraction. Carbaryl decomposition was insignificant. For organochlorines and organophosphates, the aqueous acetonitrile solvent was also found efficient for frozen samples (BURKE and PORTER 1967) and for those substrates having a high sugar content (STORHERR *et al.* 1971).

An examination of Table I shows that methylene chloride or chloroform is the extraction solvent of choice in most cases, their use being premised on their high solubility for carbamates; however, the use of these solvents may have to be discouraged because of their inferiority to acetonitrile, ethyl acetate, or the water-miscible-immiscible solvent system recommended by GUNTHER and BLINN (1955).

2. Animal tissues.—Special problems are associated with the extraction of pesticides from this substrate; different tissues of an animal have widely different physical properties and the connective tissues are harder to break or separate compared to plant tissues. With animal tissues, therefore, obtaining a thoroughly blended sample may be difficult. Extraction problems associated with tissue water, mainly emulsion formation, can be avoided by using sodium sulfate while the sample is being ground in a mortar and pestle or while it is being blended.

An extraction procedure which eliminates desiccants was proposed by BENVILLE and TINDLE (1970) for fish samples and other resilient tissues. The sample was frozen, cut into small pieces, and ground with Dry Ice in a blender. The pulverized mixture was poured into plastic bags and stored in a freezer overnight to allowed complete elimination of the Dry Ice. A fine homogenate was obtained with this procedure.

It can be seen in Table II that methylene chloride was also the solvent most often used for extraction of animal tissues. Again, its use was premised primarily on the high solubility of carbamates in this solvent and no comparative study has been made to illustrate its superiority over other solvents or solvent systems. With carbaryl in bees, JOHNSON and STANSBURY (1965 b) favored benzene over methylene chloride because it resulted in higher recoveries; this could be due to the greater solubility of waxes in benzene.

3. Milk.—MOFFITT (1963) has indicated that since most organophosphates, carbamates, and sulfur-containing compounds are polar and are thus not fat-soluble, small or no residues from these pesticides are excreted through the milk. With Baygon, carbaryl, dimetilan, and Mobam,

Table I. Extraction and cleanup procedures for the gas chromatographic analysis of carbamate insecticides and metabolites in plants and plant materials.

Carbamate(s)	Substrate	Extraction	Cleanup	Reference
Aldicarb, metabolites	Apples, oranges, potatoes, sugarbeets	Blend with CHCl_3 , anh. Na_2SO_4 , Nuchar C-190N, Al_2O_3 , Florisil, MgO , and Hyflo Supercel	Column chromatography with Florisil and Nuchar C-190N; elute with acetone:petroleum ether mixtures	MAITLEN <i>et al.</i> (1968)
	Alfalfa, apples, cucumbers, potatoes, cottonseed	Blend with CH_2Cl_2 and anh. Na_2SO_4	Florisil column; elute the sulfone with 3:7 acetone:Skellysolve B	MAITLEN <i>et al.</i> (1969)
	Sugarbeets	Blend with CHCl_3	Hydrolysis and solvent partitioning; further cleanup (optional) by silica gel column; elute with benzene:methanol mixtures	BECKMAN <i>et al.</i> (1969)
	Carrots, corn, green beans, oranges, potatoes, silage	Blend with 1:1 acetone: CH_2Cl_2 and anh. Na_2SO_4	Florisil column; pre-elute plant pigments with CH_2Cl_2 followed by acetone:hexanel mixtures to elute aldicarb and metabolites	CAREY & HELRICH (1970)
	Cottonseed, lint	Blend with 1:1 acetone: H_2O ; soak overnight with 1:1 acetone: H_2O and rotate 4 hr in concentric rotator	Florisil column; elute with 1:1 acetone:ether after pre-eluting with 4% acetone in ether	WOODHAM <i>et al.</i> (1973)
Aldicarb, Banol, Baygon, carbaryl, carbofuran, dimet, Matacil, Mobam, Pyrolan, Zectran	Apples, broccoli, cucumbers, lettuce, spinach, string beans, tomatoes	Blend with CH_2Cl_2 and anh. Na_2SO_4	Coagulation	HOLDEN <i>et al.</i> (1969)

Banol	Alfalfa	Blend with acetone	Coagulation	BACHE & LISK (1968)
Banol, carbaryl	Apples, Bermuda grass, cucumbers, tomatoes	Blend with CH_2Cl_2	Florisisl column (deactivated, 20% H_2O); elute carbamate with CH_2Cl_2 after dilution with water followed by coagulation	COHEN <i>et al.</i> (1970)
Carbaryl	Green beans	Blend with CH_2Cl_2 and anh. Na_2SO_4	Florisisl column; elute carbamate with CH_2Cl_2	RALLS & CORTES (1965)
	Apples, broccoli, green beans, corn	Blend with acetone	Coagulation	GUTENMANN & LISK (1965)
	Alfalfa, pollen	Blend with CH_2Cl_2 and anh. Na_2SO_4	Florisisl column (deactivated, 20% H_2O); elute carbamate with CH_2Cl_2	ARGAUER <i>et al.</i> (1970)
	Pollen	Blend with CHCl_3 and add anh. Na_2SO_4	Acetonitrile:hexane partitioning followed by alumina column; elute with CHCl_3	BUTLER & McDONOUGH (1970)
	Spinach, chicory	Blend with CH_2Cl_2	Hydrolysis and solvent partitioning	TILDEN & VAN MIDDELEM (1970)
Carbaryl, Mesurol, UC 10854	Snap beans	Blend with CH_2Cl_2	Coagulation	VAN MIDDELEM <i>et al.</i> (1965)
Carbaryl, carbofuran, Mobam	Apples, potatoes, sugarbeets	Blend with CHCl_3 and anh. Na_2SO_4	Coagulation followed by Florisisl column or column only consisting of alumina, Florisisl, and Norit A; elute carbamate with CHCl_3	BUTLER & McDONOUGH (1968)

Table I. (Continued)

Carbamate(s)	Substrate	Extraction	Cleanup	Reference
Carbofuran, Mobam	Range grass	Steep overnight with CHCl_3 , tumble, and blend	Coagulation followed by Florisil column or column chromatography only with alumina, Florisil, and Norit A; elute carbamate with CHCl_3	BUTLER & McDONOUGH (1969)
Carbofuran, phenolic metabolite	Corn silage	Blend with CHCl_3 and anhydrous NaSO_4	Alumina column followed by steam distillation of phenol after hydrolysis; elute carbamate with CHCl_3 and phenol with CH_3OH	BOWMAN & BEROZA (1967)
Carbofuran, carbamate metabolites	Alfalfa	Digest chopped sample with 0.25N HCl, extract with CH_2Cl_2	Silica gel and Nuchar-Attaclay column; elute with CH_2Cl_2 and 70% petroleum ether in ethyl acetate	CASSIL <i>et al.</i> (1969)
	Corn	Digest blended sample with 0.25N HCl and extract with CH_2Cl_2	Acetonitrile:hexane partitioning followed by Nuchar-Attaclay column; elute with CH_2Cl_2 and 30% hexane in ethyl acetate	COOK <i>et al.</i> (1969)
	Lettuce	Digest with 0.25N HCl and extract with CH_2Cl_2	Nuchar-Attaclay column; elute with 30% ethyl acetate in hexane	VAN MIDDELEM <i>et al.</i> (1971)
	Cucumbers, lettuce, potatoes, tomatoes, tomato foliage	Reflux with 0.25N HCl and extract with CH_2Cl_2	Alumina-Florisil column eluted with CH_2Cl_2 followed by 3:7 petroleum ether:ethyl acetate; second cleanup with alumina column; elute carbofuran and 3-ketocarbofuran with 1:4 ether: CH_2Cl_2 and 3-OH carbofuran with 3:7 petroleum ether:ethyl acetate	BUTLER & McDONOUGH (1971)

Small fruits	Digestion as above	Minor modification from BUTLER and McDONOUGH procedure; Florisil column as additional cleanup for waxy substrates	WILLIAMS & BROWN (1973)
Apples	Blend with acetone	Coagulation	BACHE & LISK (1968)
Corn	Blend with CH_3CN and anhyd. Na_2SO_4	Alumina (deactivated, 10% H_2O), Solka floc D and Darco 660 column; elute with 3:1 hexane: ether	LAU & MARKMILLER (1970)
Rice grains	Soak overnight powdered rice in water + acetone followed by extraction with CH_2Cl_2	Silica gel-Hyflo Supercel column; eluate with 3:2 hexane: CHCl_3 ; follow with TLC in kieselguhr HF using 1:2 hexane:ethyl acetate	SUMIDA <i>et al.</i> (1970)
Apples, corn, pears	Chop in presence of buffer, blend with acetone, extract with CH_2Cl_2 ; treat aq. phase and filter cake with 2N HCl ; extract as before	Precipitation of polar extractive from benzene followed by silica gel column; elute with acetone: benzene mixtures	BOWMAN & BEROZA (1969)
Variety	Blend with ethyl acetate	Wash acidified aq. phase with hexane	PEASE & KIRKLAND (1968)
Bermuda grass	Blend with CHCl_3 and anhyd. Na_2SO_4	Alumina column; elute carbamate with CHCl_3 and phenol with CH_3OH	BOWMAN & BEROZA (1967 a)
Forest foliage	Blend with 0.5N H_2SO_4	Wash acid extract with ether and CHCl_3 , then partition insecticide into benzene after neutralization	PIEPER & MISKUS (1967)
Mesuroi, 5 metabolites			
Methomyl			
Mobam, phenol			
Zectran			