

Bound and Conjugated Pesticide Residues

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Gaylord D. Paulson, and Suresh K. Bandal**

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Bound and Conjugated Pesticide Residues

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FOREWORD

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PREFACE

Production of adequate supplies of food and fiber presently requires the use of pesticides. Pesticides are used deliberately to alter the ecology, that is, to eliminate or restrict undesirable species in favor of species considered necessary for man's continued existence. The ubiquitous nature of many biological and biochemical processes makes it likely that even highly specific pesticides will affect some nontarget organisms. It is therefore imperative to determine what ecological changes pesticides may produce, which changes are permanent or temporary, and to decide which are acceptable or unacceptable.

In the past, the inability to reisolate a pesticide or its degradation products enabled us to conclude glibly that it was detoxified, degraded, metabolized, or eliminated by some unknown mechanism from any need for further concern. Radiolabeled pesticides and more sophisticated analytical technology, however, have brought a halt to such practices. We now recognize that our inability to isolate a chemical does not constitute metabolism or complete detoxication to innocuous products, but rather it constitutes a complex environmental research problem requiring the most sophisticated inputs of a multitude of scientific disciplines.

This workshop was organized because of current interest and concern for bound and conjugated pesticide residues in animals, plants, and soils. The objective of the conference was to bring together scientists with biological, chemical, and physical expertise in environmental fate of pesticides so that they could examine in some detail the formation and fate, synthesis, extraction, and methods of characterization of such pesticide residues, and if possible, provide scientific insights to future considerations regarding their overall significance. In the absence of definitive conclusions it was thought that the information discussed would provide the foundation for further research toward such a goal.

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Bound and Conjugated Pesticide Residues

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Man has developed the capacity to manufacture and use on a vast scale, organic compounds which are initially foreign to our own body and to our environment. Such synthetic organic compounds are used as drugs for sickness, pesticides of various kinds for agriculture and health purposes, coloring matters, emulsifiers and stabilizing agents for food and drink, dyes for clothes, plasticizers, lubricants, coolants, and cleansing agents for all sorts of purposes, flame retardants, beauty preparations, spermicides and ovicides for population control, explosives and poison gases for military use and so on. Past research experience has clearly demonstrated that certain of these compounds, or their degradation products can enter into almost every phase of our environment and civilized existence. It is therefore essential that we know what happens to these compounds if and when they enter our bodies, our foodstuffs, and our environment so as to avoid any damaging effects or, if they cause damage, their use can be avoided in favor of less harmful compounds, or the risks associated with their continued use can be adequately evaluated.

Almost as rapidly as man has learned to generate complex new organic chemicals our environment has adjusted to cope with not all, but the majority of these chemicals. Investigations of the environmental fate, behavior, and metabolism of synthetic organic chemicals have revealed many new and fascinating environmental processes, metabolic pathways, and chemical and physical reactions which heretofore were either not recognized, or their significance not fully understood or appreciated. We all quickly recognize where man would be without the development of synthetic and natural organic chemicals in medical science. Similarly, where would environmental science and food production technology be today if it were not for the advent of agricultural chemicals. We know that agricultural chemicals are dissipated or utilized by many mechanisms and biochemical processes including oxidation, reduction, hydrolysis, dehalogenation, dehydrohalogen-

ation, ring cleavage, etc.

In the past metabolic changes of foreign organic compounds were referred to as "detoxication." In the past the inability to isolate a chemical enabled us to glibly conclude that it was degraded, metabolized, or by some unknown mechanism eliminated from any need for further concern. The advent of radio-labeled pesticides and more sophisticated analytical technology, however, gradually called a halt to what many have referred to as "bathtub chemistry." We now recognize that our inability to isolate a chemical does not constitute metabolism or complete detoxication to innocuous products, but rather, it constitutes a complex environmental research problem requiring the most sophisticated inputs of not only a multitude of scientific disciplines, but economic, resource management, and development techniques, as well.

We know now that all chemical or metabolic changes which occur with a pesticide do not necessarily lead to complete degradation or mineralization of the pesticide molecule. In some instances a parent pesticide molecule may be converted to a more toxic substance. Indeed, a few pesticides actually require molecular changes for activation. Other pesticides or their metabolites enter into synthetic reactions which frequently result in the formation of molecules far more complex than the parent pesticide molecule. It is this latter phenomenon which brings us to our present conference regarding bound and conjugated pesticide residues.

A review of radiolabeled pesticide degradation or dissipation studies reveals numerous basic similarities. Briefly, these similarities can be characterized as three experimental fractions:

- A. Volatilized or eliminated products.
- B. Extractable products.
- C. Unextractable (or residual) products.

Volatilized materials would include respired materials, i.e., parent or intermediate products, and CO_2 , as well as materials lost by the physical processes of volatilization. Other products may be eliminated in wastes or excreta. Generally speaking, the products lost by these mechanisms are readily trapped and characterized once the process and the environmental factors affecting the process are recognized.

For purposes of this presentation extractable products are considered as those products readily removed from the treated material by any one or more of a variety of solvents and extraction techniques. Although characterization of extractable products has challenged our very best minds and technology, they too represent at present a more easily workable and identifiable fraction. Included in this fraction are some of the parent compound and its many metabolic products resulting from both synthetic and degradative reactions. Generally, but not always, degradative reactions lead toward

more polar products, hence the tendency to use solvent extraction techniques designed for maximum polar product removal. Included in this polar solvent extract are products resulting from synthetic reactions occurring in the treated material. Experience has demonstrated that these synthetic products are frequently the result of various conjugative processes which occur within the treated material. Part of the purpose of this conference is to examine in some detail the synthesis, extraction, and methods of characterization of these products, i.e., conjugates, and if possible provide scientific insights to future considerations regarding their overall significance.

Isolation and removal of the extractable products from the original pesticide treated material invariably involves sacrificing the natural physical state of the treated material. While the resulting mass of unextractable materials is generally known and describable in plants as: lipids, proteins, and structural carbohydrates; in animals as: lipids, fats, proteins, and skeletal materials; and in soil as: sand, silt, clay, and organic matter; its precise chemical nature has generally defied completely meaningful and accurate description. It is frequently within this portion of the pesticide treated material that anywhere from a few percent to nearly 100% of some pesticide residues will remain in an extractable form. It is this unextractable and heretofore largely undescribable pesticide residue which has been loosely characterized by many scientists as the "bound residue." As with many such general quasi-scientific terms, however, this terminology is not fully understood or appreciated by all pesticide scientists, regulatory agencies, or their administrators. Thus, a second part of our conference is to examine what is known about the "bound residue": What is it? How can it be characterized, defined, or identified? and if not, What is its significance? How far must we go in characterizing it? If it is truly "bound", and not readily available to significant biological systems, must it be fully characterized? What does the qualification of "readily available" mean? These are but a few of the questions we hope to either answer or lay the foundation for further research to find the answer for during this conference.

Conjugates

There are very few chemicals which enter into biological systems that are not subject to chemical changes. A few "biochemically inert" compounds remain unchanged, although they may be toxicologically active. The type of change which occurs depends primarily upon the structure of the compound, but other factors such as species, route of entrance, and nutritional balance may also be important. There are several types of synthetic reactions common to pesticides. Conjugate type reactions which have been observed include (1):

A. Reactions with carbohydrates (glycoside formation):

1. Glucuronic acid conjugation.
 2. Glucoside formation.
 3. Other (riboside, gentiobioside).
- B. Reactions with aminoacids:
1. Simple amino acids (glycine, alanine, etc.)
 2. Complex amino acids (glutathione, cysteine, etc.)
- C. Reactions involving sulfur (sulfate conjugation):
and
- D. Reactions involving alkylation and acylation.

Reactions of pesticides and pesticide metabolites with carbohydrates and amino acids are common in plant and animal metabolism. Few, if any, carbohydrate-pesticide conjugates have been isolated from soil. This is somewhat surprising in view of the relative ease with which simple sugars can be reacted with aromatic amines in the laboratory. On the other hand such conjugates may be quickly metabolized by soil microorganisms and therefore seldom isolated.

Sulfate conjugation is a common reaction in animal metabolism. Reports have also indicated the occurrence of sulfate conjugation in plants and microorganisms. Alkylation and acylation reactions are common to plants, animals, and soil microorganisms.

Other types of synthetic reactions involving pesticide residues and metabolites include condensation type reactions yielding dimeric and polymeric compounds such as have been observed in soils. These will be discussed further under soil bound residues.

Whether or not a given compound will undergo any of the above syntheses depends upon its possessing particular chemical groups or reactive sites. If the compound does not initially contain such a group, it may acquire one by oxidation or reduction or some other process. Perhaps the simplest example of such a reaction is the hydroxylation of benzene to phenol which is subsequently conjugated through the hydroxyl group.

Several hypotheses have been put forward regarding the purpose or significance of conjugative reactions in biological systems. These hypotheses include:

- A. Chemical defense (2).
- B. Surface tension (3).
- C. Increased acidity (4).

Briefly, the chemical defense hypothesis is based on the assumption that metabolic products of foreign compounds are less toxic and more soluble than their precursors. Such is not always the case, however. The surface tension hypothesis observes that compounds which lower the surface tension of water tend to accumulate at cell surfaces and thereby attain toxic concentrations. Conjugated products do not lower surface tensions appreciably, and thus do not accumulate to toxic concentrations at surfaces. The hypothesis of increased acidity notes that conjugation generally changes a weak acid

which the body can not eliminate to a strong acid which it can eliminate. While all three hypotheses seem to account for certain aspects of the problem, none of them provides a general explanation of how and why such processes occur. It is not the primary objective of this conference to determine why such conjugates form but rather, how are they formed, how are they isolated and characterized, and what is their possible role in the formation of the unextractable or bound residues observed in various biological systems. Also, what is their possible significance in terms of biological availability and toxicity in subsequent food chain organisms. In general conjugates are considerably more polar and less lipophilic than the parent pesticide molecule, and as such are therefore more readily eliminated from animals. Plants, however, do not have efficient excretory mechanisms, thus while conjugation may lead to detoxication of the pesticide it does not necessarily lead to elimination. The role of such conjugates in catabolism and ultimate binding of the pesticide molecule is not clear. An alternative mechanism to binding, however, may be the direct interaction of the pesticide with functional groups on protein or complex carbohydrate molecules.

Bound Residues

Perhaps the first objective of this section should be to define the term "bound residue." Like many new terms, the definition or interpretation of what a bound residue is, has varied with every individual scientist, and is to a very large degree dependent upon the extraction techniques used. To a certain extent, it has been an elusive concept not only to the pesticide scientist, but to our administrators and regulatory agencies as well. Concern for bound residues has varied all the way from total preoccupation with characterization of that elusive few percent of unextractable radiolabeled chemical, to incredulous disbelief that an unextractable product should be of any concern whatsoever. Responsible science, however, dictates that we know at least something about that unextractable entity. If we can not describe it precisely as (e.g.) a radiolabeled aspartic acid molecule linking together other components of a protein, then we should at least be able to indicate that when passed through another living organism which is most likely to encounter that treated product, it is or is not biologically available to that organism, and if it is available it is eventually eliminated or has no significant effect on that organism. If it is an unknown soil residue incorporated into soil organic matter and is slowly released at a rate representing only a small percent of what was originally applied, can it be considered safe and acceptable? These are only a few of the many questions

which we hope to answer in the course of this conference.

I am not aware of any already existing definition of a bound residue in plants or animals. While it is evident that individual scientists have developed their own concepts of bound residues in plants or animals, there does not appear to have been any coordinated effort to provide a definition. Last Fall (1974) an American Institute of Biological Sciences-Environmental Chemistry Task Group attempted to provide such a definition for soil bound residues. As some of you are aware, this was a committee organized by AIBS for The Environmental Protection Agency to develop a series of protocols designed to provide the information necessary to meet the Pesticide Registration Guidelines.

The definition which we cast at that time was considered only an "interim definition" in recognition of the fact that a more precise or meaningful definition could evolve from this very conference. The definition: A soil bound residue is "that unextractable and chemically unidentifiable pesticide residue remaining in fulvic acid, humic acid, and humin fractions after exhaustive sequential extraction with nonpolar organic and polar solvents." In retrospect, perhaps a better definition would have included reference to plant roots, or to decomposition by soil microorganisms, or resistance to release by specific cell free enzymes. It seems entirely reasonable, however, that analogous definitions could be cast for unextractable pesticide residues in plants and animals. Such a definition based on a more nearly universal methodology has the advantage of providing a standard point of reference with which to more objectively evaluate individual chemicals and groups of chemicals. The great difficulty with all fractionation procedures, however, is that the methods employed either separate out products which are not definite chemical entities, or form artifacts which do not have the properties of the original material. Nevertheless, the various fractionation procedures can prove useful for investigation and characterization of bound pesticide residues.

There are many critical questions to be asked concerning bound residues. Perhaps the three most critical questions are:

1. What is their nature and/or identity;
2. What is their significance (toxicity, availability, accumulative nature, etc.); and
3. What is their source?

Within certain limits answering either one of the first two questions can obviate the need for answers to the other two. For example, if the radiolabeled bound residue is identified as a natural product its significance can possibly be assumed on the basis of previous knowledge. On the other hand if its identity is not determined, but its availability and/or toxicity is determined to be of no significance, then

its identity becomes academic.

Question three can be important if it is known through hydroponic or sterile rooting medium studies that absorbed radiolabeled pesticide or its plant metabolites are not translocated into edible portions of the plant. Radiolabeled products entering into edible portions of plants grown in pesticide treated nonsterile soil, therefore must come from soil degradation products of the pesticide. Knowledge of the source and the possible soil degradation products available to the plant can provide a clue as to what radiolabeled products might be present in the plant and what extraction procedures are necessary to isolate and characterize them.

Concern for the identity or nature of the bound residue centers around the question of whether or not the bound residue consists of intact pesticide or 1st or 2nd generation degradation products which are adsorbed, incorporated, or entrapped in the plant, animal, or soil matrix and may be released at some future date, or whether it is a common ordinary metabolite which has reached a metabolic state where it can be reincorporated into normal organic building materials. In soil, concern has also been expressed for the long range effects of "polychlorinated or polytrifluoromethylated" soil organic matter on vital soil processes and conditions.

Considerable information exists regarding adsorption mechanisms and sites for pesticides in plants, animals, and soil clay and organic matter. Discussion of this information and its significance will be presented by several of the speakers. There is also a growing body of information which indicates that a number of aliphatic and simple aromatic pesticides are extensively metabolized in one or more systems and subsequently reincorporated as natural products. In the past we have been optimistic in believing that all pesticides would or should be completely mineralized to CO_2 , H_2O , N_2 , etc., after their function has been fulfilled. In these times of energy crises and shortages we should take comfort in knowing that nature does not wantonly discriminate in the utilization of a simple organic acid, amino acid, or sugar molecule originally derived from a pesticide molecule over one derived from its own synthetic efforts. There are a number of pesticides for which complete metabolic pathways are known from the parent pesticide molecule to simple organic acids. For example, the herbicide dalapon is metabolized to pyruvate and alanine (5,6); TCA is metabolized to serine (7); the 2,4-dichlorophenolic portion of 2,4-D goes through a long series of reactions ultimately yielding succinic acid (8). While it is true that most of these metabolic pathways have been worked out in isolated systems free of many competitive adsorptive or metabolic reactions, it is reasonable to expect that at least a small percent of the parent pesticide molecule will be metabolized through to such naturally occurring products

in the environment.

We are always relieved in soil metabolism investigations to see large quantities of $^{14}\text{CO}_2$ evolved from pesticide treatments. This may be an unreasonable expectation, however, when following metabolism of ^{14}C -aromatic moieties in soil. The formation of humic substances in soil is a dynamic process involving the action of soil microorganisms on plant materials and other organic residues. Macromolecules are formed at the expense of carbohydrates of plant origin. These include bacterial gums, alginic and pectic acids, and other less well-defined polymeric carboxylic acids. Aromatic polyphenols formed by way of quinone oxidation can condense with amino acids to ultimately give humic-like substances. Basidiomycetes as well as other microscopic fungi have been found to degrade lignin (polyphenol) and form appreciable amounts of humic-acid like polymers. Phenolic units from ^{14}C -labeled aromatic compounds have been incorporated into fungus synthesized polymers.

It is generally believed that there is a generic relation between the various humic substances. Fulvic acid is believed to consist of polycondensation material from simpler molecules. Continuation of the polymerization process and chemical modification leads to the less soluble humic acid and eventually to insoluble humin which has the highest molecular weight and structure most resistant to degradation. When radiolabeled pesticide degradation occurs in soil we become concerned when large amounts of the radiolabel remain adsorbed or bound in the soil for long periods of time. The concern for the release of intact pesticide or pesticide metabolites and contamination of subsequent crops is real and justifiable. It is just as real and justifiable, however, to expect that those or other residues will remain in soil for long periods of time in an innocuous manner, or be released so slowly as to be insignificant in comparison to other products produced in the soil system.

Sorenson (9) studied the degradation of ^{14}C -labeled glucose and cellulose in three soils. After a rapid initial breakdown, half-lives of 5 to 9 years were reported for the remaining ^{14}C to be degraded. Other investigators have demonstrated the formation of humin from readily decomposable organic compounds (10,11). These data imply that even readily metabolized compounds are incorporated into humic substances and the extent to which degradation occurs is limited. Although Fuhr (12) has shown that ^{14}C -lignin and -humic acid are not taken up by plants, the significance of plant uptake of pesticides or pesticide products incorporated into soil organic matter is not fully understood. In our own research with chloroaniline residues we have isolated and tentatively identified large polymeric type structures from both soil and isolated microbial cultures. These materials appear analogous in structure to those products of 4-chloroaniline identified