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NON-EXTRACTABLE PESTICIDE RESIDUES IN SOILS AND PLANTS

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NON-EXTRACTABLE PESTICIDE RESIDUES IN SOILS AND PLANTS

The information available on the nature, identity and properties of non-extractable (bound) pesticide residues formed in plants and soils is reviewed. In particular, definitions of non-extractable residues are given and progress in our understanding of the nature and significance of these materials is critically appraised.

Most work has been carried out on non-extractable residues in soils since in a number of cases such material may constitute a large proportion of the residual pesticide (as detected using radiochemical techniques). Apart from studies of the factors affecting formation of non-extractable residues, model experiments involving incubation of humic acid monomers with chlorinated anilines have been conducted to provide insight into the nature of covalent binding that occurs. Examples of the bioavailability of soil non-extractable residues to plants are given.

In the case of plants it is particularly important to distinguish between the binding or incorporation of parent pesticide, pesticide metabolites or (radio-labelled) CO₂ with plant macromolecules. Plant growth is one of the important factors which can have an effect on formation of non-extractable residues. Sequential extraction schemes have been used on a case-by-case basis to try to establish the localisation of binding (e.g. with lignin, polysaccharides etc).

It is concluded that a sequential approach to studying non-extractable residues is recommended. Information on bioavailability of soil residues to plants, and of plant residues to animals is likely to be of greater importance than a knowledge of chemical identity of the residues in the majority of cases.

1. INTRODUCTION

Bound and conjugated pesticide residues in plants and soils were the focal point of an ACS Conference in Vail/Colorado (ref. 1). The progress in our understanding of non-extractable residues since then is critically reviewed. Criteria used in evaluation of their significance are considered in light of the IUPAC definitions. Published methods to establish the nature of bound residues are discussed, referenced, and may be of assistance to an investigator of such residues.

The major question still under discussion is the significance of non-extractable residues even when the magnitude formed is known. A survey is given of the results obtained in recent years on the correlation of binding with the chemical structure of the parent compounds, time course of binding, environmental factors influencing binding rates, binding sites and mechanisms, laboratory techniques for the liberation of non-extractable residues, and their chemical identity as well as their persistence and bioavailability.

2. DEFINITIONS

Non-extractable residues (sometimes referred to as "bound" or "non-extracted" residues) in plants and soils are defined as chemical species originating from pesticides, used according to good agricultural practice, that are unextracted by methods which do not significantly change the chemical nature of these residues. These non-extractable residues are considered to exclude fragments recycled through metabolic pathways leading to natural products.
Chemical species in this context refers either to the parent material or to derivatives or fragments of it.

Methods in this context refer to any procedures, such as solvent extraction and distillation, used to exhaustively remove chemical species from a soil or plant matrix. In each reference to a non-extractable residue, the extraction procedure must be given.

2.1 Properties and relevance of non-extractable pesticide residues

When significant concentrations of the non-extractable pesticide residues (structurally related to the parent pesticide) occur in soil or plants, the properties of these residues should be investigated, the most relevant being:

In soil
- The bio-availability to plants and to soil organisms
- The persistence in soil
- The mobility in soil

In plants
- The bio-availability to man and animals
- The distribution within the plant

Following these investigations, if a non-extractable pesticide residue in soil is:

(a) not bio-available to plants
(b) not persistent or
(c) not mobile

or a non-extractable residue in plants is:

(a) not bio-available to man or animals or
(b) not located within the edible parts of the plant, then such residues can be considered insignificant.

If, however, based on the above criteria, a non-extractable pesticide residue is considered relevant, further work on a case-by-case basis, depending on the chemical nature of the residue may be required by registration authorities.

3. PESTICIDE RESIDUES BOUND IN SOIL

Soils have received major attention as an environmental component where non-extractable residues may constitute a significant fraction of a residual pesticide.

Table 1 contains a partial review of published information on non-extractable residues found in soil. These examples demonstrate the chemical classes investigated which resulted in non-extractable residues (refs. 2-25).

The chlorinated hydrocarbons, such as the dodecachloropenta-cyclododecane insecticides (e.g. kepone and kelevan) or cyclodiene insecticides (e.g. aldrin and dieldrin), do not form significant concentrations of non-extractable residues in soil, whereas phenols, anilines, and their derivatives have a high binding potential. Carbamates, s-triazines, and organophosphates also form considerable amounts of non-extractable residues. Some organophosphates evaluated in this list (parathion, paraaxon-methyl, fonofos, fenitrothion, phorate and phosalone) contain amino groups or form amino groups by metabolic reactions, which might contribute to their high soil binding rates.

Tables 2 and 3 give further examples of the relationship between soil-binding tendency of pesticides and their chemical structure. The data have been obtained by Klein and co-workers in a series of long-term experiments with plant-soil systems under outdoor conditions (refs. 2,42 and refs. cited therein).

3.1 Effect of chlorination on binding

Table 2 shows the percentages of unextractable residues, based on total radioactivity recovered from soil, for two free anilines and four pesticides which form anilines by metabolic reactions. In the case of free anilines, the portion of non-extractable residues is lower for the representatives with higher chlorine content. For the pesticides, also, the percentage of non-extractable residues decreases with increasing number of chlorines present in the metabolic aniline molecules.
Table 1. Non-Extractable Pesticide Residues in Soil
(in % of applied amount)

<table>
<thead>
<tr>
<th>Chemical class</th>
<th>No. of representatives evaluated</th>
<th>Time of exposure</th>
<th>% Residue bound (range)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Free phenols</td>
<td>2</td>
<td>1 vegetation period</td>
<td>50-58</td>
<td>3, 4</td>
</tr>
<tr>
<td>Anilines without N-substitution</td>
<td>2</td>
<td>1 vegetation period</td>
<td>31-58</td>
<td>5, 6</td>
</tr>
<tr>
<td>Triazines</td>
<td>4</td>
<td>6 weeks</td>
<td>56-65</td>
<td>7</td>
</tr>
<tr>
<td>Urea herbicides</td>
<td>2</td>
<td>4-12 months</td>
<td>49-57</td>
<td>8, 9</td>
</tr>
<tr>
<td>Carbamates</td>
<td>2</td>
<td>30-32 days</td>
<td>17-57</td>
<td>12, 13</td>
</tr>
<tr>
<td>Organophosphates</td>
<td>6</td>
<td>7-84 days</td>
<td>18-80</td>
<td>14, 15, 16, 17, 18, 19</td>
</tr>
<tr>
<td>Anilines with N-substitution</td>
<td>5</td>
<td>7 months</td>
<td>7-21</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>12 months</td>
<td>20-56</td>
<td>21</td>
</tr>
<tr>
<td>Dodecachloropentacyclodecane</td>
<td>2</td>
<td>1 vegetation period</td>
<td>1-9</td>
<td>22, 23</td>
</tr>
<tr>
<td>insecticides</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cyclodiene insecticides</td>
<td>2</td>
<td>1 vegetation period</td>
<td>1-8</td>
<td>24, 25</td>
</tr>
</tbody>
</table>

Table 2. Influence of Aniline Formation of $^{14}$C-Labelled Chemicals on the Formation of Bound Residues in Soil under Outdoor Conditions

<table>
<thead>
<tr>
<th>Chemical applied</th>
<th>Aniline metabolites identified</th>
<th>%* unextractable in soil</th>
</tr>
</thead>
<tbody>
<tr>
<td>p-Chloroaniline</td>
<td></td>
<td>95</td>
</tr>
<tr>
<td>3,4-Dichloroaniline</td>
<td></td>
<td>84</td>
</tr>
<tr>
<td>Monolinuron</td>
<td>p-Chloroaniline derivatives</td>
<td>74</td>
</tr>
<tr>
<td>Buturon</td>
<td>p-Chloroaniline</td>
<td>53</td>
</tr>
<tr>
<td>Chloraniformethan</td>
<td>3,4-Dichloroaniline</td>
<td>32</td>
</tr>
<tr>
<td>Pentachloronitrobenzene</td>
<td>Pentachloroaniline</td>
<td>11</td>
</tr>
</tbody>
</table>

* % of total radioactivity recovered in soil at harvest.
Table 3. Influence of Phenol Formation Tendency of $^{14}$C-Labelled Chemicals on the Formation of Bound Residues in Soil under Outdoor Conditions

<table>
<thead>
<tr>
<th>Chemical applied</th>
<th>Phenolic metabolites identified</th>
<th>% (a) unextractable in soil</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,4,6-Trichlorophenol</td>
<td></td>
<td>91</td>
</tr>
<tr>
<td>Pentachlorophenol</td>
<td></td>
<td>87</td>
</tr>
<tr>
<td>2,2'-Dichlorobiphenyl</td>
<td>Dichlorobiphenyols</td>
<td>42</td>
</tr>
<tr>
<td>Chloroalkylene-9</td>
<td>Dichlorobiphenyols</td>
<td>40</td>
</tr>
<tr>
<td>2,5,4'-Trichlorobiphenyl</td>
<td>Trichlorobiphenyols</td>
<td>19</td>
</tr>
<tr>
<td>Lindane</td>
<td>Trichlorobiphenyols</td>
<td>-</td>
</tr>
<tr>
<td>Pentachloronitrobenzene</td>
<td>Pentachlorophenol</td>
<td>11</td>
</tr>
<tr>
<td>2,4,6,2',4'-Pentachlorobiphenyl</td>
<td>Not identified</td>
<td>7</td>
</tr>
<tr>
<td>Hexachlorobenzene</td>
<td>Not identified</td>
<td>1</td>
</tr>
</tbody>
</table>

(a) % of total radioactivity recovered in soil at harvest.

The results in Table 3 show a similar relationship for two phenols and seven chemicals which form phenols in soil. Here also, the amounts of non-extractable residues decrease with the increasing number of chlorine atoms in the phenolic metabolites. Although polychlorinated biphenyls are not used as pesticides, they were included in this Table to demonstrate the relationship between the degree of chlorination of the metabolites and the formation of non-extractable residues. Results from 2,4,6,2',4'-pentachlorobiphenyl and hexachlorobenzene, showed the formation of very low levels of soluble metabolites, which are suspected to be phenolic. This has not been unequivocally confirmed. The very low conversion to phenols is considered responsible for the low level of non-extractable residues.

3.2 Microbial degradation of non-extractable residues

Laboratory experiments conducted by several research groups showed an increase of non-extractable residues in soil with time for two organophosphates, parathion-methyl and dyfonate, and two chlorinated hydrocarbon insecticides, dieldrin and DDT (ref. 15).

Within 28 days all the test substances showed a decrease in total residues, a decrease in extractable residues, and an increase in non-extractable residues. However, as discussed below, non-extractable residues are susceptible to microbiological degradation. Thus, if the time of exposure is extended to several years, the formation of non-extractable residues from the parent compound or its soluble metabolites may appear to be constant. This is because the total residues decrease with time and move through the non-extractable residue pool which stays nearly constant. The degradation of aldrin in soil is an example of the importance of the dynamics of the non-extractable residue pool in soil, in relation to the total degradation of compound.

The contribution of biotic reactions to the overall formation rate of non-extractable residues in soil for a metabolite of the herbicide monolinuron under laboratory conditions has been reported (ref. 26). The unextractable portion is considerably lower after soil sterilization; however, binding is not fully suppressed by sterilization. Indeed, it has been demonstrated that in the case of anilines, abiotic chemical reactions contribute significantly to the formation of insoluble complexes with inorganic as well as organic soil constituents.

3.3 Adsorption sites

The formation of non-extractable residues from carbaryl has been described for five different soils (ref. 13). The binding rate is roughly correlated to the organic matter content; no correlation seems to exist with the particle size distribution (content of clay...
or silt). In most cases the formation of unextractable residues in soil cannot be attributed to a unique soil fraction. Therefore, the question of localizing the binding sites in soil has been of research interest. The classical fractionation scheme for separating soil organic matter into humin, humic acids and fulvic acids by alkaline treatment followed by acid precipitation has been used by several authors (refs. 9, 20, 27, 28). Non-extractable pesticide residues were detected in all these fractions.

A more sophisticated fractionation scheme has been described (refs. 20, 29) which permits the complete separation of soil into a number of organic and inorganic fractions. Non-extractable residues of the herbicide butralin were detected in all of these fractions, although humic acid and silt were the major binding sites. It is concluded that both organic and mineral soil constituents must be considered for binding mechanisms of pesticides and their metabolites to soil.

The adsorption sites between the tetrahedral and octahedral silicate sheets are normally discussed (ref. 30) in the context of adsorption-desorption studies, i.e. the adsorption to these sites is assumed to be reversible. However, the occurrence of non-extractable residues in mineral soil fractions suggests that at these sites, a non-extractable binding of a pesticide molecule is possible. Hysteresis (= non-coincidence of adsorption and desorption isotherms), a phenomenon which has been observed in many pesticide adsorption studies including those with sterilized soils (ref. 30), is a further indication supporting this assumption. Chemical structures suitable for such binding would be ionic for basic substances such as phenols or anilines.

Normally, the organic soil materials are regarded as the primary site of irreversible binding of chemical residues. As an example, Figure 1 shows a portion of a humic acid macromolecule, which could be responsible for some soil organic matter binding although it does not explain the presence of bound parent molecules. It is evident that, in the genesis of humic acids, aniline units (marked by the spotted squares) could be replaced by chlorinated anilines derived from other sources. Similarly, chlorinated phenols may be incorporated into soil organic matter.

Figure 1  Type structure for humic acid

Hsu and Bartha (ref. 31) have carried out model experiments involving incubation of humic acid monomers with chlorinated anilines at room temperature and obtained polymeric materials with benzaldehyde, p-benzoquinone, 4-methylcatechol and indamine. In later experiments You et al. (ref. 32) and Saxena and Bartha (ref. 33) prepared well defined, monomeric compounds by reaction of 3,4-dichloroaniline with 4-methylcatechol and 2-methylbenzo-1,4-quinone, respectively. Upon hydrolysis the latter compound behaved similarly to dichloroaniline-humic acid complex, whereas the former did not. Therefore these compounds are models for hydrolysable and non-hydrolysable bonds in non-extractable chloroaniline residues. Since neither microbial inoculum nor any other biotic reagent was added, these reactions may be regarded as mainly abiotic. You and Bartha (ref. 34) found that the ratio...
of hydrolysable and non-hydrolysable, non-extractable residues of $[^{14}\text{C}]3,4$-dichloroaniline decreases with time. However, the hydrolysable part does not decrease below 50%.

Methods to elucidate the chemical identity of non-extractable pesticide residues should cleave the bonds fixing the xenobiotic compound to the natural macromolecule as completely as possible. However, the reagents should not cause chemical changes of the xenobiotic molecule itself. Hydrolytic (refs. 5,31,35,36) and pyrolytic (refs. 9,20,37) methods have been proposed. For anilines, both methods are applicable and release the non-extractable xenobiotic molecule unchanged ready for identification. However, for chemicals sensitive to hydrolytic or thermal attack the problem of identifying soil non-extractable residues remains as yet unsolved.

Those xenobiotics which are non-extractable from soil constituents may represent the parent compound, which was applied to the soil, or conversion products formed biotically or abiotically in the soil.

Anilines, when placed in soil, are bound as the parent compounds (refs. 5,31,35,37). The herbicide prometryn is bound both as the parent compound and in the form of its mono- and didealkylated and hydroxylated derivatives (ref. 9). The insecticide aldrin, however, is non-extractable only in the form of its polar ring cleavage product. This product was identified after alkaline hydrolysis of non-extractable residues in soil treated either with the insecticide itself (ref. 38) or with the aldrin metabolite trans-4, 5-dihydroxy-4, 5-dihyroaldrin (ref. 36).

All non-extractable residues discussed so far may be regarded as xenobiotic residues in soil. However, when soil biota degrade the xenobiotic to small fragments or $^{14}\text{C}$CO$_2$, the soil microorganisms may assimilate the radiocarbon to yield bound radioactivity although by definition this is not a non-extractable residue. These residues consist only of derivatives originating from the radiocarbon which was contained in the test pesticide, in most cases they are found in the form of natural cell components. This is supported by the results from studies with 12 chemicals in a laboratory soil-plant system which show a linear correlation between the mineralization rate and the formation of non-extractable residues in soil (ref. 39), as shown in Figure 2. It is noteworthy that two substances, namely aniline and phenol, do not fit into this correlation. This is in line with the previously discussed mechanisms of incorporation of anilines and phenols into humic acids, which occur without any preceding biotic degradation.
An analytical separation and quantitation of non-extractable residues into the parent xenobiotic, metabolic fragments and natural compounds which have incorporated small fragments or CO₂ from the xenobiotic would then imply the degradation of all the macromolecules.

The persistence of non-extractable residues, i.e. their susceptibility to mineralization mechanisms is, as mentioned in the introduction, a central point of interest for the evaluation of their significance. As an example, Hsu and Bartha described the ¹⁴CO₂ evolution from cultures of Aspergillus versicolor utilizing 3,4-dichloroaniline–humic acid complexes (ref. 31). The results reveal that Aspergillus versicolor makes little, if any, distinction between hydrolysable and non-hydrolysable complexes and oxidizes both at comparable rates. Similar biodegradation studies with non-extractable residues of other pesticides show, depending on the chemical nature of pesticides, large variations. However, the fact that microorganisms are able to break heterocyclic bonds that resist acid as well as alkaline hydrolysis, indicates that xenobiotic residues non-extractable in any form may become bioavailable to plants and thereby be a source for natural product synthesis.

Table 4. Bioavailability of Soil-Bound Pesticide Residues to Plants

<table>
<thead>
<tr>
<th>Pesticide</th>
<th>Plant species</th>
<th>Time</th>
<th>% of Soil-bound residue taken up</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Butralin</td>
<td>Soybean</td>
<td>4 weeks</td>
<td>0.27</td>
<td>( )</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10 weeks</td>
<td>0.74</td>
<td>( )</td>
</tr>
<tr>
<td>Chlornidine</td>
<td>Soybean</td>
<td>4 weeks</td>
<td>0.16</td>
<td>( )</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10 weeks</td>
<td>0.46</td>
<td>( )</td>
</tr>
<tr>
<td>Dinitramine</td>
<td>Soybean</td>
<td>4 weeks</td>
<td>1.07</td>
<td>( )</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10 weeks</td>
<td>1.07</td>
<td>( )</td>
</tr>
<tr>
<td>Fluchloralin</td>
<td>Soybean</td>
<td>4 weeks</td>
<td>0.45</td>
<td>( )</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10 weeks</td>
<td>0.90</td>
<td>( )</td>
</tr>
<tr>
<td>Profifluralin</td>
<td>Soybean</td>
<td>4 weeks</td>
<td>0.58</td>
<td>( )</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10 weeks</td>
<td>0.70</td>
<td>( )</td>
</tr>
<tr>
<td>Trifluralin</td>
<td>Soybean</td>
<td>4 weeks</td>
<td>0.56</td>
<td>( )</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10 weeks</td>
<td>0.59</td>
<td>( )</td>
</tr>
<tr>
<td>Prometryn</td>
<td>Oats</td>
<td>3 weeks</td>
<td>0.53</td>
<td>40</td>
</tr>
<tr>
<td>Methabenzthiazuron</td>
<td>Maize shoots</td>
<td>4 weeks</td>
<td>1.68</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>Maize roots</td>
<td>4 weeks</td>
<td>0.73</td>
<td></td>
</tr>
<tr>
<td>2,4,6-Tri-chlorophenol</td>
<td>Wheat</td>
<td>10 days</td>
<td>&lt;0.1</td>
<td>3</td>
</tr>
</tbody>
</table>

Table 4 gives some examples of the bioavailability of non-extractable pesticide residues to plants (refs. 3,11,40,41). This Table confirms the assumption that, based on the findings on biodegradation of soil complexes, in principle every non-extractable residue can be available to plants. However, the Table shows that the uptake is mostly below 1% of the amount non-extractable in soil. Uptake of soil non-extractable residues by earthworms has also been reported (ref. 17).

Soils are biologically dynamic and plants non-selective in their incorporation of small metabolites. These observations must be considered when the significance of non-extractable residues are part of a regulation process.
4. PESTICIDE RESIDUES BOUND IN PLANTS AND FOOD

It has been established from many studies that plants may incorporate pesticides, pesticide metabolites and fragments from both. Table 5 gives some examples of the occurrence of non-extractable residues in plants, derived from different classes of chemicals (refs. 3-6,8,22-25,42-44). As in the case of non-extractable residues in soil, the chlorinated hydrocarbons form the lowest portion of non-extractable residues and higher percentages are formed from phenols and nitrogen-containing compounds. One difference between soil and plant binding should be noted; in soil the free phenols and anilines form comparatively high concentrations of non-extractable residues, whereas in plants the percentage is considerably lower for phenols than for anilines. It may be concluded that the incorporation rates of phenols into plant macro-molecules (lignin, as discussed below) is low as compared to those for anilines, but the incorporation of anilines into plant material occurs as readily in plants as it does in soils.

4.1 Effect of plant growth

A study with $^{14}$C lindane in lettuce (ref. 45) has shown that plant growth conditions have an important influence on the formation of non-extractable residues in plants. Under good growth conditions for lettuce plants (i.e. relatively high temperatures), high crop yields resulted with high levels of non-extractable lindane residues. Under poor growth conditions (low temperatures) poor crop yields resulted with low lindane binding rates in plants (ref. 45).

4.2 Sequential extraction schemes

To identify the binding sites of pesticide residues in plant material, various schemes for the fractionation may be used. One is described for rice and wheat straw (ref. 46) and the method is designed to isolate the lignin and cellulose fractions. Other separation methods include the use of specific enzymatic degradation of pectin (ref. 47), protein (refs. 44, 48) and starch (ref. 49). Non-extractable residues were detected in all of these fractions.

Since most plant non-extractable pesticide residues were shown to be localized in lignin, model experiments have been carried out to copolymerize chlorinated anilines with a lignin monomer, coniferyl alcohol. 3-Chloroaniline, 3,4-dichloroaniline (refs. 50,51) and 4-chloroaniline (ref. 52) were used as representative anilines. In all cases, copolymers were found. The postulated mechanism is an addition of the aniline to a quinone methide intermediate.
In order to identify the chemical nature of plant non-extractable residues, hydrolytic (ref. 53) or pyrolytic (refs. 50, 54) degradation methods have been developed, which are similar to those used for soils. Simple dissolution of lignin by hot dimethylsulfoxide has been successful for the isolation and identification of lignin non-extractable carboxin residues (ref. 55). In this case, the residue is probably not copolymerized into the lignin but held in the plant matrix by unknown means. Another method for the liberation of non-extractable 3,4-dichloroaniline from plant material uses nitric acid digestion. This procedure resulted in chemical alteration of the liberated pesticide residue (ref. 56).

The anilide, propanil, forms non-extractable lignin complexes and it is believed that the metabolite 3,4-dichloroaniline is actually bound to lignin (ref. 53). The anilide carboxin is bound unchanged as the parent compound or its sulfoxide metabolite (ref. 55). The triazine prometryn forms non-extractable residues from its mono- and didealkylated metabolites (ref. 54).

4.3 Incorporation of $^{14}$C into natural macromolecules

As in soil, plant non-extractable residues may also form natural plant constituents by assimilation of $^{14}$C resulting from pesticide degradation. These radiolabelled natural products may be misinterpreted for xenobiotic residues and to demonstrate that these $^{14}$C residues are really part of a natural polymer, e.g. a polysaccharide, sophisticated procedures are needed often requiring a great deal of experimental effort. These procedures include purification, degradation and derivatization, and characterization in order to exclude the possibility of physical adsorption of the parent radioactive xenobiotic into the polysaccharide. The use of $^{14}$C derived from $^{14}$C-labelled pesticides for the biosynthesis of cellulose can be demonstrated by derivatization of the cellulose hydrolysate to form a glucosazone (ref. 46). Formation of radioactive starch may be similarly demonstrated (ref. 49).

An important aspect of pesticide residues in food plants is the alkylation of food constituents by alkylating pesticides. The reactions of the fumigant methyl bromide with food constituents have been reported by Winteringham (ref. 57). Cereal foods, feeds and small areas of agricultural soils have been intermittently exposed to this alkyl halide under conditions of fumigation for many years. Methyl bromide is a powerful methylating agent and its reaction products in cereals have been characterized (ref. 57). According to this scheme, the oxygen in proteins or carbohydrates, the nitrogen in histidine, the sulphur in cysteine or methionine may be methylated. These methylation products may be regarded as non-extractable methyl bromide and, thus, as residues in food.

4.4 Fate of plant non-extractable residues in animals

Several authors have determined the bioavailability of plant non-extractable ($^{14}$C-labelled) residues in animals and it has been shown that the majority of radioactivity is excreted in the faeces (refs. 59-60). Studies have been carried out with several compounds in rats and sheep. In a recent study, bovine rumen liquor had no effect on plant non-extractable residues from atrazine (ref. 61). From these studies it can be concluded that covalently bound non-extractable residues in plants are not in the main bioavailable to animals.

5. CONCLUSIONS

The chemical identity of non-extractable pesticides covers a large range of products. These range from physically adsorbed xenobiotics to chemically bound parent molecules and metabolites and natural products or normal plant constituents assimilated from pesticide-derived small fragments or CO$_2$. The latter are not included in the definition of non-extractable pesticide residues; in practice, however, it is very difficult to recognize them as such and to determine them separately from real xenobiotic residues.

The soil non-extractable residues investigated thus far seem to be susceptible to microbial attack and therefore may be bioavailable to plants. However, the amounts taken up by plants are small in all cases reported. The few studies available today on the uptake of plant non-extractable residues by animals indicate that they enter the blood and participate in natural metabolism only to a small extent.

The non-extractable residue situation varies from compound to compound. Since bioavailability is more important than chemical identification, a sequential approach to studying these residues based on bioavailability, such as that proposed by Otto and Huber (ref. 59) is recommended.
REFERENCES

31. I. Scheunert; unpublished results.


59. M.L. Sutherland; in Ref. 1 pp 153-155.

