MINIREVIEW

Bioavailability of Bound Pesticide Residues and Potential Toxicologic Consequences

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Increased demands for enhanced food productivity to meet the needs of society have led to the widespread, indiscriminant use of pesticides. It is well recognized that pesticide usage is predominant in agriculture, in which application of these chemicals protects crops from insects, weeds, and disease. In 1985, Hervé (1) estimated that the world agricultural market spent \$2.52 billion to protect plants. In order to gain a perspective of pesticide usage in the world, the relationship between pyrethroid crop application and the use in each continent is presented in Table I. The protection of crops is but one facet of pesticide use. Pesticides also play an important role in veterinary practice, control of great endemics and public health, and the protection of stored grains. In 1980, it was estimated that the nonagricultural use of pesticides amounted to \$675 million (1), and this figure was projected to increase dramatically by 1985. Thus, it is not surprising that there is an intricate relationship between the effects of pesticide exposure on human health and the regulatory agencies responsible for maintaining safety and environmental consequences to society (2). It should be clearly emphasized that the bulk of existing literature has focused attention on the influence of an "easily accessible" parent compound or metabolite on the target organism. However, it is the purpose of this review to concentrate on bound pesticide residues in plants and to a lesser extent on edible animal tissues. Despite the fact that exposure to bound pesticide residues is a daily occurrence, the toxicological impact and significance of these chemicals remain unanswered.

Definition

The recognition that pesticide application and subsequent extraction from crops still left a portion of the chemical bound to the plant created an awareness among scientists that ingestion of processed plant material still exposed the consumer to the agrochemical. Indeed, concern for bound residues has ranged from the belief that unextractable product is of no health relevance to the postulation that this chemical, although bound, is cumulative and potentially toxic (3). In addition to this ongoing debate, it became clear among pesticide chemists that the definition of a bound plant pesticide residue needed to be standardized (4–6). In plant metabolism studies using ^{14}C-labeled pesticides, the terms "bound," "insoluble," "residual," and "unextractable" have been employed interchangeably to identify the fraction of radioactivity present in the plant residuum. Bound pesticide residues in plants are defined as chemical species originating from pesticides, used according to good agricultural practice, and which can not be extracted by methods that do not significantly alter the chemical nature of these residues (7). The chemical species here refer either to the intact pesticide or compounds derived from it. Extraction refers to the exhaustive removal of the chemical species by solvents from a plant matrix. It is important that in each study concerned with bound residues the extraction procedure be described. Bound residues exclude fragments of the chemical species possessing no pesticidal or toxicological activity and recycled into the natural polymeric products through metabolic path-

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ways of a biological system. Furthermore, some ionic chemical species that are not extractable by the ordinary organic solvents should not be regarded as bound residues, since suitable pH and salt regimes can release them unchanged from the substrate (5, 6).

Significance

With the establishment that bound pesticide residues are indeed present in plants and subsequently ingested by mammals, the environmental consequences and human health significance have been the subjects of numerous debates. Huber and Otto (8) stipulated that the following criteria should be applied to evaluate the significance of bound pesticide residues:

1. The distribution of bound pesticide residues within the plant should be determined. It is well known that the binding of pesticide residues into lignin creates an insoluble material that is usually not digestable in animals (9–11). Consequently, it would be anticipated that binding of pesticide to lignin would serve as an inactivation mechanism and not be potentially harmful. However, in a recent study, Sandermann *et al.* (12) clearly demonstrated that the administration of lignin-bound pesticide to rats resulted in pesticide release within the animal. The observed high degree (66%) of pesticide release was suggested to reflect that these residues may possess significant ecotoxicological impact. Although the majority of studies favor the view that the distribution of bound pesticide residues within plants, in particular binding to lignin, can act as a buffer against potential mammalian toxicity (13), it should not be assumed that pesticides bind solely to lignin; hence, knowledge of the distribution is an important consideration.

2. The amount of residue that becomes bound is a concern for human health consequences. At the time of harvest, the degree of bound residue formation varies with plant species and type of pesticide involved (14). In general, the percentage of unextractable residue increases in relation to the duration after treatment (15). It is of interest that Zayed and Farghaly (16) demonstrated that 20–30% of an applied dose of tetrachlorvinphos on fava beans and 30–40% on soya beans was present in bound form and persisted for 30 weeks. Similarly, Khan *et al.* (17) found that a single application of either dieldrin, permethrin, or carbofuran to radishes resulted in 23.5%, 28.6%, or 92.6% bound residue, respectively, which persisted for 21 days after treatment. The persistence of bound pesticide residues in treated corn for periods of 8–12 weeks also has been demonstrated (18, 19). The finding that ingestion of high concentration, Phosvel-treated corn by lactating cows resulted in body-weight gain and decreased milk production, although this phenomenon was not apparent with low pesticide amounts (18), supports the view that the amount of bound pesticide must be taken into account in the evaluation of potential toxic consequences.

3. The chemical nature of the pesticide following incorporation into plants will ultimately contribute toward potential toxicity. The administration of pesticides to plants does not merely result in the incorporation of parent compound into plant tissue and subsequent elimination. It has been well established that plants, like mammals, undergo extensive and complex metabolism of xenobiotics (3, 15). The complexity of plant metabolic reactions indicated that a general classification system was needed to inherently denote certain characteristics of the residues involved and to evaluate their significance (20). Plant metabolites of pesticides may be classified into four categories of residues: (i) free metabolites, (ii) conjugate metabolites, (iii) natural constituents, and (iv) bound pesticide residues (4, 20).

The free metabolites are considered to be the pesticide residues that are derived from the parent molecule by Phase I reactions and that do not react further with natural constituents of mammalian systems. These residues, in general, are apolar, easily separated from each other, extracted with organic solvent, resemble the parent compound structurally, and remain in the organic phase when partitioned against water. It should be noted that the Phase I metabolism involves the production of free metabolites by various reactions, including desulfuration, hydroxylation, dehalogenation, epoxidation, hydrolysis, oxidation, and reduction (21, 22). The generation of free pesticide metabolites yields, in general, less active compounds; however, in certain cases, Phase I metabolism is required for bioactivation (23).

The pesticide conjugate metabolites are products of secondary metabolism in which a pesticide or its metabolite is involved in a reaction with an endogenous substrate such as glucuronic acid, sulfate, or amino acid (24–26). The resultant product is chemically foreign to the natural cellular constituents and extractable from the substrate with polar solvents, and does not partition from water into apolar solvents. In the majority of

Table I. Distribution of Global Pyrethroid Pesticide Use[a]

Continent	Quantity used 1981 (%)	Quantity used 1983 (%)
Europe	15.0	23.0
North America	27.5	16.5
Latin America	24.0	16.5
Africa	18.0	9.5
Asia/Australia	15.5	34.5

[a] Adapted from Hervé (1). The dollar value actually spent was $390 million in 1981 and $630 million in 1983.

cases, conjugation is considered a detoxication mechanism, with the generation of biologically inactive product. However, the finding that ingestion of carbamate pesticide conjugates resulted in cleavage to toxic derivatives (27, 28) suggests that not all pesticide conjugates are biologically inert and, thus, are of little toxicological significance. It is of interest that Weiss (29) found that a significant portion of approximately 40% of lasalocid metabolites in beef liver extract was bioavailable when fed to rats. In an *in vitro* guinea pig atrial strip preparation, the lasalocid metabolites beef liver extract did not produce significant inotropic responses. The consequences and toxicological significance of these bioavailable metabolites were not reported in the rat.

The natural constituents are classified as the residues derived from a pesticide or its metabolite, but they comprise a normal component of the cell (4, 20). The natural constituents derived from the pesticide may be extractable from a substrate or retained in the plant matrix. As shown by Hutson and Hoadley (30), the natural constituents are distinguishable from pesticide metabolites, degraded into carbon dioxide, and incorporated into amino acids, fatty acids, and glycogen. Similarly, the presence of natural constituents has been reported in conditions in which parent pesticide molecules undergo biotransformation into carbon dioxide (31, 32).

The fourth category, which is bound pesticide residues, was defined previously (7). In the context of this chapter, the unextractability of bound pesticide residues from plants has little meaning unless it is related to their bioavailability. It is important to know the availability of bound residues to living organism, particularly to animals and to those plants consumed in the animal diet. Therefore, in the broadest sense, the bound pesticide residues should be categorized as follows: (i) Bioun-available bound residues: (a) those bound residues in soil that are not taken up by plants and/or soil-inhabiting animals, and (b) those bound residues in plants that when administered orally to animals are not absorbed from the gastrointestinal tract and are excreted in the feces (33, 34); and (ii) Bioavailable bound residues: (a) those bound residues in soils that are taken up by plants and/or soil-inhabiting animals, and (b) those bound residues in plants which, when administered orally to animals, are absorbed from the gastrointestinal tract (5, 35).

4. The last criterion needed for evaluation of the significance of bound residues has been alluded to several times and is clearly of utmost toxicological importance. Bound pesticide residues are of toxicological significance if these compounds become systemically bioavailable to the organism that ingests the material (36). It should be emphasized that studies on the bioavailability of bound residues need to be linked with concern for human safety, since current methodology has provided a more precise measure of the actual concentration of pesticides (37, 38). With this enhanced precision of measurement, it is now possible to determine the influence of minute amounts of released bound pesticide on mammalian function as a result of bioavailability.

Methodology for Determination of the Nature and Quantity of Bound Residues

During the past few years, the determination of the nature and quantities of bound pesticide residues in plants has been a challenging problem for research workers. In most of the studies reported in the literature, quantification of ^{14}C-bound residues in plants has been achieved by combustion (39). This method is limited to the determination of total ^{14}C-bound residues and cannot be used to identify the chemical form of the bound residues. Attempts have also been made to extract and/or release bound pesticide residues by the milder to harsher methods. Drastic extractive procedures destroy the structure of plants by solubilizing the materials, and strong acid or base hydrolysis often results in the destruction of the identity of bound residues (5, 8).

A pyrolysis technique for the estimation of bound residues of chloroaniline compounds in plants was reported by Balba *et al.* (40). A similar technique was developed by Khan and Hamilton (41) involving high-temperature distillation of the solvent-extracted plant material to release bound ^{14}C residues. The bound ^{14}C residues that were released were collected in various solvents and analyzed by gas chromatography and gas chromatography-mass spectrometry as shown in Figure 1. In recent years, this technique has been used widely to determine the chemical identity of bound ^{14}C residues of pesticides and/or metabolites in plant samples (42). While the high-temperature distillation technique provides a possible means for the chemical identification of bound ^{14}C residues of certain pesticides, the thermal decomposition of bound ^{14}C residues to $^{14}CO_2$ during distillation often results in considerably lower recoveries of the bound pesticide and/or metabolites.

A technique that involves extraction of insoluble residues by supercritical fluids, such as methanol, was developed by Capriel *et al.* (37). A fluid phase at the supercritical condition is considered neither exclusively liquid-like nor gas-like, but more of an intermediate state. The advantages of using mobile phase at supercritical conditions are low viscosity, high diffusion coefficients, and variation of density and dielectric constant as a function of pressure. The extracts obtained by supercritical fluids were subsequently analyzed by gas chromatography and gas chromatography-mass spectrometry for determining the identity of the bound ^{14}C residues. A comparison between the results obtained with both high temperature distillation and supercritical

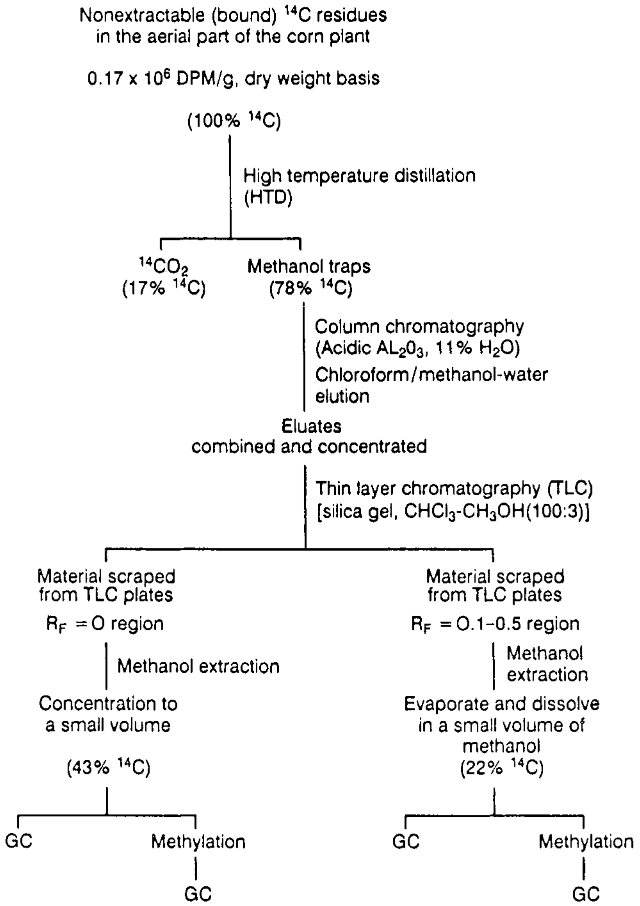

Figure 1. Schematic diagram for the analysis of nonextractable (bound) residues in corn plants treated with ^{14}C-atrazine. Adapted from Ref. 57.

methanol revealed that better recoveries of ^{14}C and higher concentrations of identified residues were obtained by extraction with supercritical methanol (37).

Although this review emphasizes the role of bound plant pesticide residues and their toxicological significance to human health, it should be noted that humans also ingest edible tissues of meat-, milk-, and egg-producing animals. The regulatory agencies are faced with the challenging task of establishing protocols for characterization and toxicological assessment of bound residues. Since 1975, a few studies on characterization and the fate of the bound residues of some important animal pesticides and drugs have been reported (13). As in the case of plant-bound residues, the methodology employed to identify animal tissue-bound residues also requires the combined use of radiolabeled substrates and sophisticated solvent extraction technology, followed by sensitive spectroscopic techniques such as gas chromatography and gas chromatography-mass spectrometry (43, 44). However, the conventional extraction and chromatographic methods were not efficient for the quantitation and structural identification of submicrogram amounts of tissue-bound residues. Chiu *et al.* (45, 46) developed an efficient method for the isolation of bound residue from extremely complex tissue hydrolysates. Livers from sheep or cows containing clorsulon, a potent fasciolicide, were subjected to carbonic anhydrase affinity chromatography and the released bound residue was analyzed by mass spectrometry. The application of this technology for determination of bound tissue residues can have far-reaching implications in light of the sensitivity of the assay. The possibility exists that minute amounts of bound tissue residues are also bioavailable and may pose a health problem. In light of the ever-increasing use of pesticides in agricultural and veterinary practice, the probability is that the exposure of humans to bound tissue residues is enhanced, and consequently, this fraction must be considered in health risk assessment.

Methodology for Determination of Bioavailability

Although bioavailability has been examined in several species, Gallo-Torres (47) in a recent review clearly pointed out a number of distinct advantages of using the rat in this type of research. Aside from the fact that the experimental rat is the most commonly used species in biological research, the requirement for large numbers, the increased resistance to trauma and postoperative infection, and greater control over interspecies variation are all factors that contribute to the choice of the rat for bioavailability studies. It should be noted that the anatomic, physiologic, and nutritional requirements of the rat digestive system are well known and that surgical manipulations for gastrointestinal function studies have been specifically adapted for this species. After consideration of various approaches to assess the human safety of bound residues, Lu *et al.* (48) concluded that the *in vivo* bioavailability study remains the most sensible way to evaluate the toxicological potential of bound residues. The bioavailability approach is based on sound scientific rationale and can be easily conducted and data are reproducible.

The bioavailability of bound residues has generally been determined by studying intestinal absorption of the residues in animals (38). The bound ^{14}C residues are mixed with regular diet and the contaminated feed is compressed into pellets of adequate size, when possible. Animals are fed the fortified diet for 2 or 3 consecutive days to allow sufficient accumulation of radiocarbon in various tissues and organs when absorbed. Feces, bile, and urine are collected during the treatment period and for 2 or 3 days after the treatment has stopped (49). Major organs, such as the kidney, liver, lung, and intestine, are removed and analyzed for radioactivity as presented in Figure 2. The presence of radioactivity in urine, bile, carcass, and liver, kidney and intestinal wall tissues indicates absorption and bioavailability (38). Usually, the complete elimination of ingested radiocarbon in the feces provides evidence of no absorption and, thus, nonbioavailability. The presence of radioactivity in the feces may also be due to the excretion of radiocarbon (i) in bile, pancreatic, and gastric juice, (ii) by lungs and into saliva and swallowing, and (iii) into intestinal lumen, in addition to incomplete absorption of ingested radiocarbon (50).

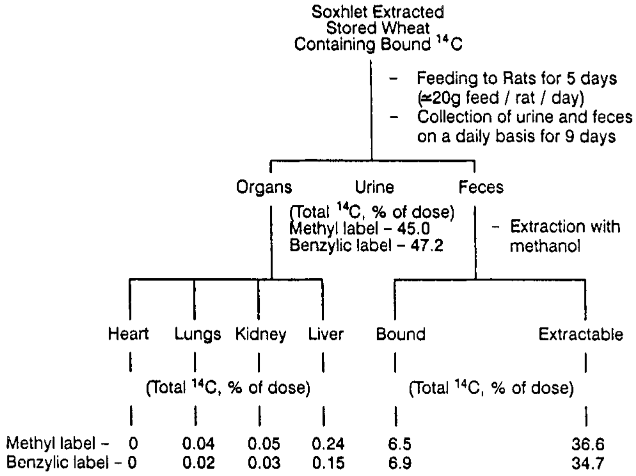

Figure 2. Distribution of ^{14}C in individual organs, urine, and feces of rats fed Soxhlet-extracted stored wheat treated with radioactive deltamethrin. Adapted from Ref. 59.

The amounts of radiocarbon eliminated in the urine, respired as CO_2, and retained in tissues are the guidelines for absorption. The toxicological significance of bound residues is directly proportional to the concentration of radioactivity in the liver, kidney, lung, intestinal wall, and other tissues. Characterization of the ^{14}C-labeled compound(s) is important when the ^{14}C content in urine and tissues is high.

Bioavailability of Bound Plant Residues in Animals

It is known that urinary and/or biliary excretion of a compound or its metabolites signifies that the material is bioavailable. However, quantitative fecal elimination without biliary excretion indicates that a material is not bioavailable. Bakke *et al.* (51) observed that sorghum-containing atrazine bound residues were not bioavailable when fed to either sheep or rats, since 100% and 88–93%, respectively, of the dose was eliminated in the feces. Thus, the bulk of atrazine-bound sorghum residues are not bioavailable and and may not constitute a potential health hazard. In contrast, oral treatment of dichloran in lactating goats resulted in approximately 29%, 48%, and 0.3% of the administered dose excreted in urine, feces, and milk, respectively (52). The presence of one third of the dose of dichloran in urine clearly indicates that this chemical is bioavailable, and, indeed, high concentrations of bound dichloran were present in goat liver and kidney. In a subsequent study (53), bound dichloran residues of goat liver were incorporated into rat feed and administered to rats. Only 8% and 20% of the bound dichloran residue were excreted in rat urine and feces, respectively. Although this may indicate a low degree of bound goat liver dichloran bioavailability, it should be stressed that 70% of the dose was not accounted for and could pose a potential toxicity. It is of interest that ingestion of Phosvel-bound corn residues by cows resulted in approximately 80–90% elimination in feces (18). However, the trace amounts of phosvel present in the cow resulted in body weight gain and decreased milk production. The findings that the majority of bound plant residues are not bioavailable should not be equated with safety, as only trace amounts may be necessary to induce adverse effects.

With the use of animal or fowl tissue extracts, Khan and Akhtar (54) demonstrated that incubation of bovine rumen liquid with bound atrazine corn extracts failed to release the bound plant residues. However, chicken liver homogenate incubation with bound atrazine corn extracts did release the bound plant residues. Thus, the bioavailability of bound plant residues appears to be dependent on the species. Recently, McLean *et al.* (55) found that incorporation of bound febantel cattle liver extract into rat feed diet and ingestion by the rat resulted in 99.9% excretion of the administered dose in feces, an index of no bioavailability. However, the ingestion of beef liver extract fortified with febantel caused an excretion of 52% of the dose in urine. These findings suggest that the ingestion of febantel by cattle results in the formation of bound residues in beef liver that are not readily bioavailable and likely do not present a toxicological risk. In a subsequent study, Lawrence and McLean (56) reported that in rats fed olaquindox-bound swine liver extract, this compound was readily absorbed, with 5.8 and 14.8% present in the bile and urine, respectively. Although the toxicological potential was not examined, a finding that 20.6% of bound residue of olaquindox was bioavailable may be considered as a significant risk factor.

By far, the species of choice for the study of the determination of bioavailability of bound plant pesticide residues has been the rat, in which normal rat feed is mixed with extracted radioactive plant material. As early as 1975, Paulson *et al.* (57) observed that alfalfa plants containing profam-phenyl-^{14}C bound residues were not bioavailable when fed to rats, since 86% of the dose was eliminated in the feces. Sutherland (58) investigated the bioavailability of propanil-phenyl-^{14}C bound residues from rice plants when fed to white albino rats. It was observed that 89% of the dose was eliminated in the feces and 11.5% in the urine. He concluded that because of the low absorption by monogastric animals, the bound propanil residues should be considered to be of little toxicological concern. ^{14}C-Bound residues resulting from the carbaryl-naphthyl-^{14}C treatment of bean plants were given orally to rats (20). It was observed that all the ^{14}C residues were eliminated in feces, thereby indicating the unavailability of bound residues to the animal. Marshall and Dorough (33) investigated the bioavailability in rats of bound plant carbamate-insecticide residues. After 2 days of administering rats the ^{14}C-bound residues of carbofuran and carbaryl from bean plants, 11.5% and 1.3%, respectively, of the bound residues were eliminated in the urine, while 85.1% and 98.1% were voided in the feces. Only 1.4 and 0.4% of the bound residues were detected in the bile. Croneton-bound residues from sorghum exhibited a similar excretion pattern. These data demonstrated that bound residues of the carbamate compounds were very poorly absorbed from the gastrointestinal tract of rats and were not bioavailable.

In subsequent investigations, Zayed *et al.* (59) determined the bioavailability of bound bean residues containing tetrachlorvinphos in rats. Surprisingly, 60% of the bound pesticide form was bioavailable, with 27% eliminated in feces, 7% in urine, and the brain, blood, fat, and liver containing 38% of the administered dose. The significance of these high levels of radioactivity in tissues was not explored further. Mostafa *et al.* (60) also compared the bioavailability of bound carbofuran with methomyl residues in rats. It was observed that sweet potato tubers absorbed about 28% of the total ^{14}C when soaked in a 50-ppm solution of ^{14}C-carbofuran for 1 week. Approximately 23% of the ^{14}C in the tubers was in bound form. Rats fed the bound ^{14}C residues in tubers eliminated ^{14}C as follows: feces, 66%; urine, 9%; and carbon dioxide, 11%. Small amounts of radioactivity were also detected in the brain (0.03%), fat (0.02%), blood (0.09%), liver (0.23%), and kidney (0.08%). The chemical nature of the ^{14}C was not known in either the bound residues or the biological samples. In the case of methomyl, the elimination pattern was as follows: feces, 21%; urine, 24%; carbon dioxide, 9%; brain, 0.1%; fat, 0.6%; blood, 1.0%; liver, 1.9%; and kidney, 0.3%. In comparison to carbofuran, methomyl is more readily bioavailable and the significance of these findings on human health requires further investigation. In a recent study, Lawrence and McLean (56) found that 25% of bound ^{14}C-chloropicrin residues in strawberries were bioavailable in rats, the significance of which is still not known.

The focus of the studies in our laboratory has been to examine the bioavailability of bound pesticide residues in rats given a diet containing extracted plant material. In addition, the distribution of pesticide within the mammalian organism and the potential adverse effects have been studied. Khan *et al.* (61) fed bound ^{14}C-atrazine-related residues in corn plants (mixed in with regular diet) to rats for 2 days and collected the feces and urine during the treatment and 2 days thereafter. Approximately 88% of the total ingested ^{14}C was eliminated in feces and 10% in the urine after 4 days. Almost all of the ^{14}C in feces was bound. Only trace levels of deethylatrazine, its 2-OH analog, and 2-OH ammeline were identified in the urine. When rats were fed extracted corn material fortified with ^{14}C-atrazine, there were appreciable amounts of the ^{14}C in the liver (2.1%) and kidney (0.3%) 4 days after the dosing, whereas extremely small amounts of ^{14}C were found in the liver and kidney of animals fed bound residues. The data show that bound atrazine residues in corn plants have a very low degree of bioavailability and, as such, that their toxicological concern should be minimal. Unlike bound residues, freshly added atrazine to plant material when fed to rats was readily bioavailable. In contrast to corn, Khan *et al.* (62) fed rats extracted bean plant material containing ^{14}C-deltamethrin for 2 days, followed by normal diet for 3 days. The findings that feces contained approximately 50% of the administered dose after 4 days, while 30% was excreted in the urine, demonstrated that bound residues of bean plants treated with deltamethrin were bioavailable. However, no residual levels of radioactivity were detected in the liver, brain, kidney, or lung of rat. The significance of the bioavailable deltamethrin fraction remains to be determined. In a subsequent study, stored wheat containing bound ^{14}C deltamethrin and metabolite residues was fed to rats and shown to be highly bioavailable, with residual radioactivity present in the liver, kidney, and lung (63). During the course of this experiment, rats displayed no apparent toxic manifestations. Based on the high degree of bioavailability, a maximum residue limit for deltamethrin in wheat was recommended to not exceed 1 mg/kg. In contrast to the rat, ingestion of ^{14}C deltamethrin in lactating cows resulted in 95% excretion of radioactivity in feces, with the remainder of labeled compound excreted in milk and urine (64, 65). Thus, it would appear that deltamethrin is not bioavailable in lactating cows.

In a comparative study, Khan *et al.* (66) examined the bioavailability of bound residues from radishes treated with either carbofuran or dieldrin. Rats, when fed bound ^{14}C-carbofuran residues in radishes, excreted about 90% of the ^{14}C activity in feces and less than 2% in the urine during a 4-day period. Only 2% of the ^{14}C in the feces from rats fed the bound ^{14}C carbofuran-related residues in radishes could be extracted. This indicated that almost all of the ^{14}C in the feces was still in the bound form. Carbofuran, 3-hydroxycarbofuran, and 3-ketocarbofuran were detected in the urine and feces of the treated rats. The ^{14}C content in the liver, kidney, and brain of treated rats was very low. In contrast, approximately 16% of the ingested dose of dieldrin was eliminated in urine and 74% was voided in the feces. Higher residual concentrations of dieldrin were present in the rat liver, kidney, and brain compared with carbofuran. Our data demonstrated that bound residues in dieldrin-treated radishes were more bioavailable than those in carbofuran-treated samples. The relationship between the quantities of pesticide bioavailable, even if these are low, and toxicological significance needs to be addressed. In an ongoing study, it was found that ingestion of bound pirimiphos-treated wheat extract by the rat resulted in a fall in body weight gain, suggesting that this pesticide is bioavailable and exerts an adverse effect (unpublished observations). Each bound pesticide residue is unique and these investigations indicate the necessity of bioavailability data.

Conclusions

The use of ^{14}C-labeled pesticides has made us aware of the existence of bound residues in edible tissue and plants. These residues would escape detection by the conventional analytical methods and would result in an underestimation of the edible tissue or plant burden of total pesticide residues. The methodology for the analysis of bound pesticide residues is still in the developmental stage. The total ^{14}C-bound residues in tissues and plants are usually estimated by combustion of the extracted material to yield $^{14}CO_2$. The high temperature distillation technique and supercritical methanol may provide a possible means for the chemical identification of bound residues. However, the application of these techniques to bound residue analysis of other pesticides has not been fully explored.

A major portion of bound pesticide residues in plant tissues may be associated with lignin. Thus, lignin formation in plants may serve as a system for plants to store pesticide residues by incorporating them into insoluble lignin. The feeding of plant materials containing ^{14}C-bound residues to monogastric animals indicates that these residues may be considered to be of little toxicological concern. However, recent studies have shown that even lignin-bound pesticide residues can be bioavailable to a great extent and may possess ecotoxicological significance (12). The binding of pesticide residues in edible tissues of meat also appears to yield fractions of residues that are not readily bioavailable. This need not necessarily be equated with safety, as the minimal levels for toxicity are not known. In addition, these small amounts of pesticide could be cumulative and toxic over time.

Whether bound pesticide residues are of any environmental significance will remain a matter of conjecture until more data are available. However, judging from the limited available information, it appears that bound residues may not present any problem as long as such residues do not accumulate in significant amounts. It is recommended that more coordinated research be undertaken for a better assessment of this complex problem.

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