MINIREVIEW

Bioavailability of Bound Pesticide Residues and Potential Toxicologic Consequences—An Update (43952)

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Abstract. **The growing demand for enhanced food productivity to meet the needs of the global population has led farmers to use sophisticated agricultural technology in which pesticides play a crucial role. Pesticide use has a positive and dramatic impact on agricultural production through protection of crops against insects, pests, and disease, but every effort must be made to ensure that application is safe and, more importantly, to assure safety for human and environmental health. Since our initial review (1), global usage of pesticides has increased and knowledge regarding the biological significance of bound pesticide residues has expanded. The fact that more reports are appearing in the literature signifies an increased awareness of the presence of bound pesticide residues and indicates that a greater number of scientists are attempting to establish the potential toxicologic consequences of this pesticide residue fraction. Rico (2), in a review in 1990, concluded that covalently bound residues in edible animal tissues were not carcinogenic, were not readily bioavailable, and hence produced little, if any, adverse effects. Whether this conclusion is applicable to the presence and consequences of bound pesticide residues in plants and food commodities remains unanswered. Thus, the aim of this updated review is not simply to list studies on bioavailability of bound pesticide residues in grains subsequent to the review in 1992 (1) but also to establish the toxicological impact of this chemical fraction on health.**

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Although it may seem somewhat repetitious, it is important to define clearly the role of bound pesticide residues. The recognition that a portion of the chemical remained bound to the plant even when the pesticide was extracted from crops subsequent to application made scientists aware that even processed plant material exposed the consumer to the agrochemical. 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 origi-

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nating from pesticides, used according to good agricultural practice and which cannot 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 pathways 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 as 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 are subsequently ingested by mammals, the environmental consequences and human health significance have been the subject 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 binding of pesticide residues into lignin creates an insoluble material which is usually not digestible 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 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 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; and, 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). 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%, and 92.6% bound residue, respectively, which persisted for 21 days post treatment. The persistence of bound pesticide residues in treated corn for periods of 8 to 12 weeks also has been demonstrated (18, 19). The finding that ingestion of high concentration of 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 evaluation of potential toxic consequences.

3. The chemical nature of the pesticide following incorporation into plants will ultimately contribute to potential toxicity. The administration of pesticides to plants does not merely result in incorporation of the 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 denote inherently certain characteristics of the residues involved and evaluate their significance (20). Residues of pesticides and/or metabolites in plants may be classified into three categories of residues: (i) freely extractable residues, (ii) extractable conjugates, and (iii) unextractable residues bound to natural constituents of plants (4, 20).

The free metabolites are considered 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, hydoxylation, dehalogenation, epoxidation, hydrolysis, oxidation, and reduction (21, 22). The free pesticides metabolites are, in general, less active compounds; and are produced by Phase I activation (involvement) (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 rarely partitions from water into apolar solvents. In the majority of 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 (a veterinary drug) 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 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 previously defined (7). In the context of this paper, 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) Biounavailable 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 that, 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.

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. 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.* (39) 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, atrazine freshly added to plant material was readily bioavailable when fed to rats. In contrast to corn, Khan *et al.* (40) 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 (41, 42). 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 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 (43, 44). Thus, it would appear that deltamethrin is not bioavailable in lactating cows.

In a comparative study, Khan *et al.* (45) 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 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% voided in the feces. Higher residual concentrations of dieldrin were present in 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. Indeed, Mostafa *et al.* (46) in a recent study demonstrated bound fava bean residues of carbofuran were highly bioavailable (46% excreted in urine) and produced adverse effects in liver and kidney as manifested by inhibition of erythrocyte cholinesterase activity and increase in blood urea nitrogen (BUN), serum glutamic oxaloacetic transaminase (SGOT), and serum glutamic pyruvic transaminase (SGPT).

Based on our findings that bound plant pesticide residues are bioavailable and may pose a potential health risk to human health (1), various investigators have examined whether this phenomenon is common to other pesticide compounds. This review summarizes the current knowledge on bioavailability of bound plant pesticide residues and their toxic consequences. Pirimiphos-methyl (0-2-diethylamino-6-methyl-pyrimidin-4-yl 0,0-dimethyl phophorothioate), "Actellic" is a broad spectrum insecticide used to control a wide range of stored grain pests. It has low mammalian toxicity and is applied directly to grains, where it controls pests by both contact and vapor activity. The studies presented in Table I emphasize that the bound ^{14}C residues of pirimiphos methyl in stored wheat are highly bioavailable to the rat. Although bound pirimiphos-methyl is metabolized to a certain extent by hydrolytic processes involving dealkylation and demethylation, the major excretory form is the parent compound (47). Hence, the adverse effects described in Table I may be attributable to pirimiphos-methyl. Data thus suggest that bound pirimiphos-methyl residues in stored grains are bioavailable and exert toxicological manifestations in rats.

Malathion is an important selective organophosphorous insecticide being used for the control of pests in stored products. Based on its relatively low mammalian toxicity (53, 54), malathion is applied directly to grains prior to storage. Data in Table II indicate that malathion is highly bioavailable when bound plant residues are fed to mice or rats. Furthermore, the pesti-

Table I. Bioavailability of Plant Bound Pirimiphos-Methyl Residues and Toxic Consequences in the Rat

Grain	Percentage of C^{14}		Parameters examined	Reference
	Urine[a]	Feces		
Wheat	72	17.9	↓ body weight gain No histopathological alterations	47
Wheat	29	24.6	↓ serum AChE, lymphocytes and monocytes ↓ alkaline phosphatase and BUN	48
Wheat	30	40	Body weight not affected ↓ Spleen weight ↑ RBC count and hemoglobin ↓ plasma and brain AChE	49
Maize	30	55	↓ body weight gain ↓ serum AChE ↑ WBC count	50
Wheat	82	18	↓ whole blood AChE ↑ serum alkaline phosphatase and SGOT	51
Wheat	ND	ND	↑ SGOT ↑ BUN	52
Rice	ND	ND	↑ SGOT ↑ SGPT	52

Note. ↑, increase; ↓, decrease or inhibition; ND, not determined; AChE, acetylcholinesterase; BUN, blood urea nitrogen; RBC, red blood cells; WBC, white blood cells; SGOT, serum glutamic oxaloacetic transaminase.
[a] Quantity in urine denotes degree of bioavailability.

Table II. Bioavailability and Toxic Consequences of Grain Bound Malathion Residues in Rodents

Grain	Percentage of ^{14}C excreted		Parameter examined	Reference
	Urine[a]	Feces		
Wheat	63	16	↓ brain and serum AChE ↓ SGOT and SGPT ↑ BUN ↓ RBC and hemoglobin ↑ alkaline phosphatase	53, 55–57
Wheat	49	2	↑ SGPT ↓ body weight gain ↓ blood AChE ↑ hemoglobin	54–58
Wheat	ND	ND	No maternal adverse effects Present in fetus, thus placental transfer	59
Lentil	35	26	↑ BUN ↑ WBC ↓ serum AChE	60
Rice	7	75	↓ serum AChE ↑ BUN	61
	14	78	Not studied	62
Maize	36	47	↑ BUN	61
	8	80	Not studied	62
Beans	60	3	↓ body weight gain ↓ serum AChE ↑ SGOT and alkaline phosphatase	63
Corn	ND	ND	↓ body weight gain ↑ SGOT, SGPT, alkaline phosphatase and BUN	64
Chickpea	29	49	ND	65

Note. ↑, increase; ↓, decrease or inhibition; ND, not determined. For abbreviations, see Table I.
[a] Quantity in urine denotes degree of bioavailability.

cide appears to produce hepatic and renal dysfunction as evidenced by an increase in SGPT, SGOT, and BUN (54–57). Although teratogenicity was not evident under the experimental conditions of Bitsi *et al.* (59), malathion readily crossed the placental barrier. It should be noted that a fetus is more vulnerable to functional and histopathological damage than an adult and hence further study is warranted. The bioavailability of a number of other bound pesticide residues has been determined, and data are given in Table III. Evidence indicates that these compounds, although bound to grains, are bioavailable when fed to rodents and may exert toxicological effects.

Increased awareness of the existence of bound

Table III. Bioavailability of Various Grain Bound Pesticides and Potential Toxic Consequences in Rodents

Pesticide	Grain	Percentage of ^{14}C excreted		Parameters examined	Reference
		Urine[a]	Feces		
Chlorpyrifos-methyl	Wheat	75	8	↓ plasma AChE ↑ SGPT ↑ RBC and hemoglobin	66
	Wheat	70	2	No signs of toxicity in clinical chemistries No effect on body weight gain No effect on histopathology	67
3,4-Dichloroaniline	Carrot	10	51	ND	68
	Wheat	12	85	ND	69
Methyl bromide	Bean	37	24	↓ WBC ↑ SGOT and SGPT ↑ BUN	70
Lindane	Wheat	4	26	ND	71
Diflubenzuron	Wheat	47	50	ND	72
Fenvalerate	Wheat	40	60	ND	73

Note. ↑, increase; ↓, decrease or inhibition; ND, not determined. For abbreviations, see Table I.
[a] Quantity in urine denotes degree of bioavailability.

residues in edible tissue and plants is now possible through the development of analytical methods. Based on the limited amount of data available, it would appear that bound residues exert adverse effects in rodents. The effects of humans ingesting equal amounts of bound pesticide residues are at present not known; however, it is evident that a quantity of bound pesticide residues would be bioavailable. The significance of the released bound form of plant pesticide residues in human requires further study.

This manuscript is an update of a previously published minireview (see Ref. 1).

1. Akhtar MH, Khan SU, Kacew S. Bioavailability of bound pesticide residues and potential toxicologic consequences. Proc Soc Exp Biol Med **199**:13–21, 1992.
2. Rico AG. Biological models for assessing bound residues-comments. Drug Metab Rev **22**:803–806, 1990.
3. Kaufman DD. Bound and conjugated pesticide residues. In: Kaufman D, Still G, Paulson G, Bandal S, Eds. Bound and Conjugated Pesticide Residues. Washington, DC: American Chemical Society Symposium Series, Vol **29**:pp1–10, 1976.
4. Dorough HW. Classification of radioactive pesticide residues in food-producing animals. J Environ Pathol Toxicol **3**:11–19, 1980.
5. Khan SU. Bound pesticide residues in soil and plants. Residue Rev **84**:1–25, 1982.
6. Pillmoor JB, Roberts TR. Approaches to the study of non-extractable (bound) pesticide residues in plants. In: Hutson DH, Roberts TR, Eds. Progress in Pesticide Biochemistry and Toxicology. New York: John Wiley and Sons, Vol **4**:pp85–101, 1985.
7. Kearney PC. Definition of non-extractable pesticide residues in plants and soils. J Assoc Off Anal Chem **65**:1031–1032, 1982.
8. Huber R, Otto S. Bound pesticide residues in plants. In: Miyamoto J, Kearney PC, Eds. Pesticide Chemistry. Human Welfare and the Environment. Oxford: Pergamon Press, Vol. **3**:pp357–362, 1983.
9. Zeikus JG. Lignin metabolism and the carbon cycle. Polymer biosynthesis, biodegradation and environmental recalcitrance. Adv Microb Ecol **5**:211–243, 1981.
10. Khan SU, Dupont S. Bound pesticide residues and their bioavailability. In: Greenhalgh R, Roberts TR, Eds. Pesticide Science and Technology. Oxford: Blackwell Scientific Publications, pp417–420, 1987.
11. Edwards VT, Hutson DH. The disposition of plant xenobiotic conjugates in animals. In: Paulson GD, Caldwell J, Hutson DH, Menn JJ, Eds. Xenobiotic Conjugation Chemistry. Washington, DC: American Chemical Society Publication, pp322–340, 1986.
12. Sandermann H Jr., Arjmand M, Gennity I, Winkler R, Struble CB, Aschbacher PW. Animal bioavailability of defined xenobiotic lignin metabolites. J Agric Food Chem **38**:1877–1880, 1990.
13. Akhtar MH. The disposition of bound ^{14}C agrochemical residues in plants and animal tissues when consumed by animals. In: Greenhalgh R, Roberts TR, Eds. Pesticide Science and Technology. Oxford: Blackwell Scientific Publications, pp509–512, 1987.
14. Dorough HW. Bioavailability and potential toxicological significance of bound pesticide residues using nuclear techniques. Report from Consultants Group Meeting, North Carolina State University, Raleigh, North Carolina, October, 1985.
15. Harvey J Jr. The fate of pesticide plant metabolites in vertebrates. In: Miyamoto J, Kearney PC, Eds. Pesticide Chemistry. Human Welfare and the Environment. Oxford: Pergamon Press, Vol **3**:pp369–374, 1983.
16. Zayed SMAD, Farghaly M. Distribution and degradation of tetrachlorvinphos on stored faba beans and soya beans. J Stored Prod Res **21**:199–205, 1985.
17. Khan SU, Stratton GD Jr., Wheeler WB. Characterization of bound (nonextractable) residues of dieldrin, permethrin and carbofuran in radishes. J Agric Food Chem **32**:1189–1191, 1984.
18. Johnson JC Jr., Bowman MC, Leuck DB, Knox FE. Persistence of phosvel in corn silage and effects of feeding dairy cows the treated silage. J Dairy Sci **54**:1840–1847, 1972.
19. Johnson JC Jr., Jones RL, Leuck DB, Bowman MC, Knox FE. Persistence of chlorpyrifos-methyl in corn silage and effects of feeding dairy cows the treated silage. J Dairy Sci **57**:1467–1473, 1974.
20. Dorough HW. Biological activity of pesticide conjugates. In: Kaufman D, Still G, Paulson G, Bandal S, Eds. Bound and Conjugated Pesticide Residues. Washington, DC: American Chemical Society Symposium Series, Vol **29**:pp10–34, 1976.
21. Parke DV. Development of detoxication mechanisms in the neonate. In: Kacew S, Reasor MJ, Eds. Toxicology and the Newborn. Amsterdam: Elsevier Science Publishers, pp3–31, 1984.
22. Juchau MR. Fetal and neonatal drug biotransformation. In: Kacew S, Ed. Drug Toxicity and Metabolism in Pediatrics. Boca Raton, FL: CRC Press, pp15–34, 1990.
23. Casida JE. Mode of action of carbamates. Annu Rev Entomol **8**:39–58, 1963.
24. Still GG, Mansager ER. Alfalfa metabolism of propham. Pest Biochem Physiol **5**:515–522, 1975.
25. Paulson GD. Sulfate ester conjugates—Their synthesis, purification, hydrolysis, and chemical and spectral properties. In: Kaufman D, Still G, Paulson G, Bandal S, Eds. Bound and Conjugated Pesticide Residues. Washington, DC: American Chemical Society Symposium Series, Vol **29**:pp86–102, 1976.
26. Mumma RO, Hamilton RH. Amino acid conjugates. In: Kaufman D, Still G, Paulson G, Bandal S, Eds. Bound and Conjugated Pesticide Residues. Washington, DC: American Chemical Society Symposium Series, Vol **29**:pp68–85, 1976.
27. Dorough HW, Casida JE. Nature of certain carbamate metabolites of the insecticide Sevin. J Agric Food Chem **12**:294–304, 1964.
28. Leeling NC, Casida JE. Metabolites of carbaryl(1-naphthyl N-methylcarbamate) in mammals and enzymatic systems for their formation. J Agric Food Chem **14**:281–290, 1966.
29. Weiss G. The integration of pharmacological and toxicological testing of tissue residues in the evaluation of their human food safety. Drug Metab Rev **22**:829–848, 1990.
30. Hutson DH, Hoadley EC. The comparative metabolism of ^{14}C-vinyl-dichlorovos in animals and man. Arch Toxicol **30**:9–18, 1972.
31. Baron RL. Radioactive lactose in skim milk following administration of carbonyl-^{14}C-carbaryl to a lactating cow. J Assoc Off Anal Chem **51**:1046–1048, 1968.
32. Dorough HW, Ivie GW. Carbon-^{14}C milk constituents resulting from feeding carbonyl-^{14}C carbamate to cows. Science **159**:732–733, 1968.
33. Marshall TC, Dorough HW. Bioavailability in rats of bound and conjugated plant carbamate insecticide residues. J Agric Food Chem **25**:1003–1009, 1977.
34. Stratton GD Jr., Wheeler WB. Fate of dieldrin in radishes. J Agric Food Chem **31**:1076–1079, 1983.
35. Khan SU. Plant uptake of unextracted (bound) residues from an organic soil treated with prometryn. J Agric Food Chem **30**:1096–1098, 1980.
36. Weber NE. Bioavailability of bound residues. Toxicol Eur Res **4**:271–275, 1982.

37. Capriel P, Haisch A, Khan SU. Supercritical methanol: An efficacious technique for the extraction of bound pesticide residues from soil and plant samples. J Agric Food Chem **34**:70–73, 1986.
38. Gallo-Torres HE. Methodology for the determination of bioavailability of labeled residues. J Toxicol Environ Health **2**:827–845, 1977.
39. Khan SU, Kacew S, Molnar SJ. Bioavailability in rats of bound ^{14}C residues from corn plants treated with [^{14}C]atrazine. J Agric Food Chem **33**:712–717, 1985.
40. Khan SU, Kacew S, Akhtar MH. Bioavailability of bound ^{14}C residues in rats from bean plants treated with ^{14}C-deltamethrin. Chemosphere **15**:923–927, 1986.
41. Khan SU, Kacew S, Akhtar MH. Formation of bound residues of [^{14}C]-deltamethrin in stored wheat and their bioavailability in rats. J Environ Sci Health **B27**:477–478, 1992.
42. Khan SU, Kacew S, Akhtar MH. Bound ^{14}C residues in stored wheat treated with [^{14}C]deltamethrin and their bioavailability in rats. J Agric Food Chem **38**:1077–1082, 1990.
43. Akhtar MH, Hartin KE, Trenholm MHL. Fate of ^{14}C-deltamethrin in lactating dairy cows. J Agric Food Chem **34**:753–758, 1986.
44. Venant A, Belli P, Borrel S, Mallet J. Excretion of deltamethrin in lactating dairy cows. Food Addit Contamin **7**:535–543, 1990.
45. Khan SU, Kacew S, Dupont S, Stratton GD Jr., Wheeler WB. Bioavailability in rats of bound residues from radishes treated with either radiolabeled dieldrin or carbofuran. J Agric Food Chem **35**:89–93, 1987.
46. Mostafa IY, Zayed SMAD, Farghaly M, Mahdy F. Bioavailability to rats and toxicity in mice of carbofuran residues to fava beans. J Environ Sci Health **B27**:399–405, 1992.
47. Khan SU, Kacew S, Matthews W. Bioavailability to rats of bound [C^{14}] pirimiphos-methyl in stored wheat. J Environ Sci Health **B27**:355–367, 1992.
48. Akay MT, Yilmazoglu G, Yasacan S, Turk H, Kolanyaka D. Bioavailability and toxicological potential of wheat-bound pirimiphos-methyl residues in rats. Xenobiotica **22**:293–302, 1992.
49. Qureshi MJ, Jamil FF, Haq A, Naqvi SHM. Bioavailability and toxicity to rats of bound residues of ^{14}C-pirimiphos-methyl in stored wheat. J Environ Sci Health **B27**:369–375, 1992.
50. Yeboah PO, Semanhyia CB, Melfah PT. Bioavailability and biological activity of bound residues of radiolabelled pirimiphos-methyl in maize grains. J Environ Sci Health **B27**:377–386, 1992.
51. Neskovic NK, Karan VZ, Sabovijevic V, Vitorovic SL. Bioavailability and toxicological potential of wheat bound ^{14}C-pirimiphos-methyl residues. J Environ Sci Health **B27**:471–473, 1992.
52. Ramos-Ocampo VE. Biological activity of wheat and rice-bound pirimiphos-methyl residues in the rat. J Environ Sci Health **B27**:475–476, 1992.
53. Akay MT, Kolankaya D. Bioavailability of bound residues of ^{14}C-malathion in stored wheat grains to rats. DOGA Turkish J Zool **13**:47–53, 1989.
54. Akay MT, Yilmazoglu G, Gokce D, Kolankaya D. The effects of bound residues of malathion in stored wheat grains on rat brain acetylcholinesterase and pseudocholinesterase. DOGA Turkish J Zool **14**:162–166, 1990.
55. Akay MT, Yilmazoglu G, Keskin P, Kolankaya D. Biological activity in rats of bound residues of malathion in stored wheat grains. DOGA Turkish J Zool **14**:167–173, 1990.
56. Akay MT, Kolankaya D, Yilmazoglu G. Bioavailability and toxicological potential of wheat-bound ^{14}C-malathion residues. J Environ Sci Health **B27**:457–458, 1992.
57. Neskovic NK, Karan VZ, Vitorovic SL, Vojinovic VD. Toxic effects of bound malathion residues in rats. Biomed Environ Sci **4**:423–432, 1991.
58. Neskovic NK, Karan Z, Vitorovic SL, Vojinovic VD. Bioavailability and biological activity of wheat bound ^{14}C-malathion residues. J Environ Sci Health **B27**:459–461, 1992.
59. Bitsi GA, Singh K, Khan SU, Akhtar MH, Kacew S, White NDG. Fate of wheat bound malathion residues in rats during gestation. Chemosphere **29**:451–455, 1994.
60. Akay MT, Elcuman A, Nurcan M, Kolankaya D, Yilmazoglu G. Bioavailability and toxicological potential of lentil-bound residues of malathion in rats. J Environ Sci Health **B27**:325–340, 1992.
61. Syed MA, Arshad JH, Mat S. Biological activity and bioavailability of grain bound ^{14}C-malathion residues in rats. J Environ Sci Health **B27**:347–354, 1992.
62. Dumrugs B, Veerakul N, Tayaputch N. Bioavailability to rats of bound residues of ^{14}C-pirimiphos-methyl or ^{14}C-malathion in maize and rice. J Environ Sci Health **B27**:469–470, 1992.
63. Zayed SMAD, Farghaly M, Mostafa IY. Bioavailability to rats and toxicological potential in mice of bound residues of malathion in beans. J Environ Sci Health **B27**:341–346, 1992.
64. Ramos-Ocampo VE. Toxicological potential of corn-bound malathion residues in the rat. J Environ Sci Health **B27**:465–467, 1992.
65. Akay MT. Bioavailability to rats of chickpea-bound ^{14}C-malathion residues. J Environ Sci Health **B27**:463–464, 1992.
66. Neskovic NK, Karan V, Budimir M, Vojinovic V, Gasic S, Vitorovic SL. Bioavailability and biological activity of wheat-bound chlorpyrifos-methyl residues in rats. J Environ Sci Health **B27**:387–397, 1992.
67. Singh K, Khan SU, Akhtar MH, Kacew S, White NDG. Nature and bioavailability of nonextractable (bound) residues in stored wheat treated with chlorpyrifos-methyl. J Agric Food Chem **41**:2421–2425, 1993.
68. Worobey BL, Shields JB. Preliminary studies on the bioavailability and disposition of bioincurred carrot residues of [^{14}C]linuron and [^{14}C]3,4-dichloroaniline in rats. Food Addit Contamin **8**:193–200, 1991.
69. Sandermann H Jr., Musick TJ, Aschbacher PW. Animal bioavailability of a 3,4-dichloroaniline-lignin metabolite fraction from wheat. J Agric Food Chem **40**:2001–2007, 1992.
70. Mostafa IY, Zayed SMAD, Hazzaa NI, Hegazi B. Bioavailability to rats and toxicity of bound residues in bean seeds fumigated with ^{14}C-methyl bromide. J Environ Sci Health **B27**:407–417, 1992.
71. Verma A, Pillai MKK. Bound residues of DDT and HCH in wheat and their bioavailability to rats. Chemosphere **21**:1315–1320, 1990.
72. Aly MAS, Dauterman WC. Bioavailability, biological activity and characterization of bound residues of diflubenzuron in wheat. J Environ Sci Health **B27**:481–482, 1992.
73. Aly MAS, Dauterman WC. Bioavailability, biological activity and characterization of bound residues of fenvalerate in wheat. J Environ Sci Health **B27**:483–484, 1992.