Bound ^{14}C Residues in Stored Wheat Treated with [^{14}C]Deltamethrin and Their Bioavailability in Rats†

Shahamat U. Khan,*,‡ Sam Kacew,§ and M. Humayoun Akhtar⊥

Land Resource Research Centre and Animal Research Centre, Research Branch, Agriculture Canada, Ottawa, Ontario, Canada K1A 0C6, and Department of Pharmacology, School of Medicine, University of Ottawa, Ottawa, Ontario, Canada K1H 8M5

Wheat grains treated with radiolabeled deltamethrin [(*S*)-α-cyano-3-phenoxybenzyl (1*R*,3*R*)-*cis*-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropanecarboxylate] and stored in the laboratory for 168 days formed bound (nonextractable) ^{14}C residues. The amount of bound ^{14}C residues formed was about 11% of the total ^{14}C in stored grain. Br_2CA [3-(2,2-dibromovinyl)-2,2-dimethylcyclopropanecarboxylic acid] and 3-PBacid (3-phenoxybenzoic acid) were present in the form of bound ^{14}C residues in addition to some radiolabeled product of unknown composition. The stored wheat containing bound ^{14}C was fed to rats. The ^{14}C residues were excreted in urine and feces in nearly equal proportion. The ^{14}C residues identified in urine were Br_2CA, 3-PBacid, and conjugated compounds of 4′-OH-3-PBacid [3-(4-hydroxyphenoxy)benzoic acid]. Most of the ^{14}C residues excreted in feces were extractable with methanol. Trace amounts of ^{14}C residues were also present in lungs, kidney, and liver. The results suggest that bound residues in stored wheat treated with deltamethrin when fed to rats are highly bioavailable.

In recent years several synthetic insecticides have been used on stored grains to control insects. However, the number approved by regulatory agencies for application to stored grain and for which maximum residue levels are established is limited. Deltamethrin [(*S*)-α-cyano-3-phenoxybenzyl (1*R*,3*R*)-*cis*-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropanecarboxylate], a synthetic pyrethroid, is considered to be potent against the whole spectrum of stored product pests. The insecticide is effective at low dose levels in the range of 1–2 mg/kg of cereal grains, is stable on grain, and shows little or no tendency to penetrate the individual grains (Nobel et al., 1982, Soderlund and Casida, 1977; Hargreaves et al., 1982). It has been shown that over a 9-month storage period deltamethrin was not degraded on wheat (Halls and Periam, 1980) and is considered highly persistent on stored wheat (Bengston et al., 1983; Hargreaves et al., 1982).

Deltamethrin residue levels in stored grain have been determined by using conventional techniques involving solvent extraction of the insecticide from the substrate (Halls and Periam, 1980; Nobel et al., 1982; Hargreaves et al., 1982). However, no information is available on the extent of bound (nonextractable) residues formation in stored grains treated with deltamethrin. The presence of these bound, usually undetected, and chemically unidentified residues in significant concentration may, however, pose problems in determining the total residue levels in stored grains and in assessing their toxicological significance. Therefore, information concerning the amounts and nature of bound deltamethrin residues on grain under controlled storage conditions is also important in managing their use. These grain-bound residues are of unknown toxicological potential.

The joint FAO/IAEA Division of Isotope and Radiation Application of Atomic Energy for Food and Agricultural Development initiated a program in 1986 on biological activity and bioavailability of bound pesticide residues, using nuclear techniques. The major thrust of the program has emphasized studies on grain-bound residues, their bioavailability to experimental animals, and their toxicological potential. The current work is a part of this program and reports on the formation of bound ^{14}C residues in stored wheat treated with radiolabeled deltamethrin. The potential bioavailability in rats of bound ^{14}C residues in stored wheat was also determined.

EXPERIMENTAL SECTION

Chemicals. Deltamethrin (^{14}C-labeled and unlabeled) was a gift from Roussel-Uclaf through its subsidiary Hoescht of Canada Ltd. The position of labeling, radiochemical purity (as determined by thin-layer chromatography), and specific activity were as follows: methyl-labeled, 96%, 60 mCi/mmol; benzyl-labeled, 96%, 59 mCi/mmol. A portion of methyl- and benzyl-labeled material (50 μCi) was individually mixed with purified deltamethrin and dissolved in diethyl ether to give a concentration of 10 μg/mL. Base-catalyzed hydrolysis of deltamethrin followed by purification by thin-layer chromatography gave pure 3-(2,2-dibromovinyl)-2,2-dimethylcyclopropanecarboxylic acid (Br_2CA). 3-Phenoxybenzaldehyde (3-PBald), 3-phenoxybenzoic acid (3-PBacid), and 3-phenoxybenzyl alcohol (3-PBalc) were purchased from Aldrich Chemical Co. (Milwaukee, WI); 3-(4-hydroxyphenoxy) benzoic acid (4′-HO-PBacid) and 3-(4-hydroxyphenoxy)benzyl alcohol (4′-HO-PBalc) were prepared following the procedure of Unai and Casida (1977).

All solvents were of pesticide grade and were used as received.

Grain Treatment and Storage. Spring wheat of the variety Glenlea with a moisture content of 15.2% was obtained from Experimental Farm, Department of Agriculture, Ottawa. Analysis of wheat samples, as described later, indicated that it was free of deltamethrin and/or metabolite residues. Batches (1 kg) of wheat were placed in brown glass jars coated with Sigmacoat in order to avoid the adsorption of the insecticide on

† LRRC Contribution No. 89-71; ARC Contribution No. 1627.

‡ Land Resource Research Centre, Agriculture Canada.

§ Animal Research Centre, Agriculture Canada.

⊥ University of Ottawa.

0021-8561/90/1438-1077$02.50/0

the glass surface. Ether solution of methyl- or benzyl-labeled deltamethrin was applied to grain with continuous stirring to achieve the insecticide concentration of 5.0 ppm. The glass jar was sealed immediately, shaken vigorously by hand, and then tumbled on a mechanical tumbler for 1 h. The treated grain was then spread in a thin layer on a tray in a fume hood draught for about 30 min. After the ether was evaporated, the grain was returned to the jar, sealed, and tumbled for an additional 1 h. This procedure resulted in deltamethrin-treated grain (5 ppm) of 15% moisture content. The treated grain was then stored at room temperature in sealed jars for 168 days. The grain used as control was handled similarly except that no deltamethrin was added to the carrier.

Generation and Determination of Bound Residues. The wheat grains were grounded in a Retsch grinder and then Soxhlet-extracted with methanol for 24 h. The extracted material dried at room temperature for 24 h contained about 6.0% H_2O. One part was combusted to $^{14}CO_2$ to determine the total bound ^{14}C. The other part was used to determine the nature of ^{14}C-bound residues by utilizing the high-temperature distillation technique (HTD) and supercritical fluid extraction (SFE).

(*i*) *HTD.* This technique has been described by Khan and Hamilton (1980) and was used in our earlier studies dealing with bound residues of deltamethrin in bean plants (Khan et al., 1984). In the present investigation the material released from the Soxhlet-extracted wheat grains by HTD was collected in four traps containing hexane, acetone, methanol, and Oxisorb, respectively. The solution in the last trap was used to trap any released $^{14}CO_2$. The distillates in hexane, acetone, and methanol were concentrated to small volume and radioassayed for ^{14}C. They were then subjected to thin-layer chromatographic (TLC) separation and finally analyzed by gas chromatography (GC).

(*ii*) *SFE.* The supercritical fluid extractor (Suprex Model SFE-50; Suprex Corp., Pittsburgh, PA) used consisted of a 250-mL syringe pump, a control module for control of the SFE system, an extraction oven, a 12-mL extraction vessel containing 2 g of Soxhlet-extracted wheat grains, and a four-port valve connected with the outlet restriction (fused silica tubing, 50-μm i.d.) that were inserted into glass tubes containing methanol. Carbon dioxide was used as a supercritical fluid, and the extractions were performed at a pressure of 215 atm and temperature 110 °C. The extraction was carried out for 2 h. The extracted material in methanol was relatively clean and required no TLC sparation prior to GC analysis.

The remaining portion of the Soxhlet-extracted material was used for feeding to rats as described below.

Treatment of Rats and Collection of Samples. Sprague–Dawley male rats each weighing approximately 200 g were housed (one animal/cage) in metabolism cages that permitted the separate collection of urine and feces. They were conditioned for 4 days to a daily diet consisting of a mixture of rat feed (Purina Laboratory Chow). The feed was then replaced for 5 days with Soxhlet-extracted material containing bound ^{14}C residues (methyl label, 1.64×10^4 dpm/g; benzyl label, 1.90×10^4 dpm/g). Six rats were used in each treatment group. The control rats were fed the Soxhlet-extracted untreated wheat material. Food consumed was approximately 15 g/day per rat. The rats were then maintained on regular rat feed for an additional 4 days. At the end of the experiment, the animals were sacrificed by decapitation, selected organs were excised and weighed, and a portion of these organs was frozen in liquid nitrogen for radioassay for ^{14}C content. The urine and feces were collected daily after the start of treatment, and collection was continued on a daily basis until the end of the experiment.

Analysis of ^{14}C Residues in Feces and Urine. (*i*) *Feces.* The feces of the rats were extracted with methanol until further extraction did not result in any extractable ^{14}C. The methanol extract was evaporated to a small volume, an aliquot was radioassayed for ^{14}C content, and the remaining portion was analyzed by HPLC. The insoluble feces material containing only bound ^{14}C residues was air-dried and combusted to $^{14}CO_2$ for bound ^{14}C residues. Total ^{14}C content of feces was obtained by the summation of the two values.

(*ii*) *Urine.* An aliquot of urine was radioassayed by scintillation counting. The remaining portion was brought to just dryness under a stream of air, dissolved in methanol, and analyzed by HPLC.

Determination of Radioactivity. Liquid (e.g., urine, solvent extracts, and HTD distillates) were assayed in a Packard Model 3320 scintillation spectrometer, and solids (ground wheat material, feces, animal tissues) were assayed by combustion in a Packard sample oxidizer, Model 306, followed by liquid scintillation counting (LSC) with an external standard and correcting data for quenching.

Chromatography and Analysis. (*i*) *TLC.* The concentrated solutions from the HTD distillates were chromatographed on silica gel GF plates as described earlier (Khan et al., 1984). The plate was developed with a toluene–hexane–acetic acid (15:3:2) solvent system. The plate was then photographed by a Berthold Beta camera LB292 to detect radioactive regions. The radioactive regions at R_f 0.7 and 0.5 (methyl label) and R_f 0.7 and 0.4 (benzyl label) were scraped off the plates and placed directly into the vials for LSC counting to determine the total radioactivity of the TLC region. The remaining portion of the HTD distillates was applied on a preparative chromatoplate (20 × 20 cm, 1.0-mm gel thickness), and various radioactive regions as described above were extracted with methanol and analyzed by LSC to determine the total extractable radioactivity. The extractable material was then further analyzed by GC.

(*ii*) *GC.* The gas chromatograph was a Varian Model 6500 fitted with a ^{63}Ni detector. The capillary column was a 15 m × 0.524 mm (i.d.) megabore column coated with DB-17 (1.5 μm). The oven temperature was programed at 3 °C min^{-1} from 100 to 240 °C. The detector and injector port temperatures were 320 and 140 °C, respectively. Nitrogen was used as a carrier gas at a flow rate of 15 $mL.min^{-1}$. Under the GC condition described, the pentafluorobenzyl ester of 3-PBacid, the methyl ester of Br_2CA, and deltamethrin showed peaks at retention times of 5.3, 8.9, and 34.3 min, respectively.

(*iii*) *HPLC.* A Waters Associates HPLC instrument equipped with a Model U6K loop injector and a Model 450 variable-wavelength UV detector connected to Berthold HPLC radioactive monitor LB504 was used for the analysis of the methanol extracts from the feces or urine. A 300 × 3.9 mm (i.d.) μBondapak C_{18} liquid chromatography column (10 μm) protected by a 70 × 2.1 mm (i.d.) guard column packed with C_{18} was used. Chromatographic conditions used for the analyses: (i) acetonitrile–buffer (3:7, v/v) Na_2HPO_4, 0.05 M, pH 4.5, flow rate 3.5 mL/min; (ii) water–methanol (1:1, v/v) plus 0.1 M ammonium acetate, flow rate 0.5 mL/min. In both cases the ultraviolet detection was set at λ = 243 mm, LB 504 detector sensitivity 500 dpm full-scale deflection.

Confirmation. The identities of the compounds were confirmed by comparing the TLC R_f values, GC, and HPLC retention times with those of authentic samples, cochromatography, and finally gas chromatography–mass spectrometry (GC–MS).

RESULTS AND DISCUSSION

Bound residues remaining in the Soxhlet-extracted stored wheat amounted to 11.5% (methyl label) and 11.9% (benzyl label) of the total ^{14}C applied (Figure 1). The extractable fractions from both treatments were found to contain primarily the parent compound. The identity of the bound ^{14}C residues in stored wheat was determined by a combination of HTD and SFE techniques and chromatographic analyses as described earlier. In preliminary experiments it was observed that HTD and SFE of the Soxhlet-extracted control wheat fortified with the reference standard of [^{14}C]deltamethrin resulted in a recovery of 87–91% and 94–96%, respectively, of ^{14}C residues in the solvents used. HTD of the Soxhlet-extracted stored wheat containing bound ^{14}C residues showed that the amount of radioactivity in the combined solutions from the three traps (hexane–acetone–methanol) was 71.2% (methyl label) and 73.8% (benzyl label) of the total bound ^{14}C residues (Figure 1). The corresponding values in methanol from SFE were 83.3% (methyl

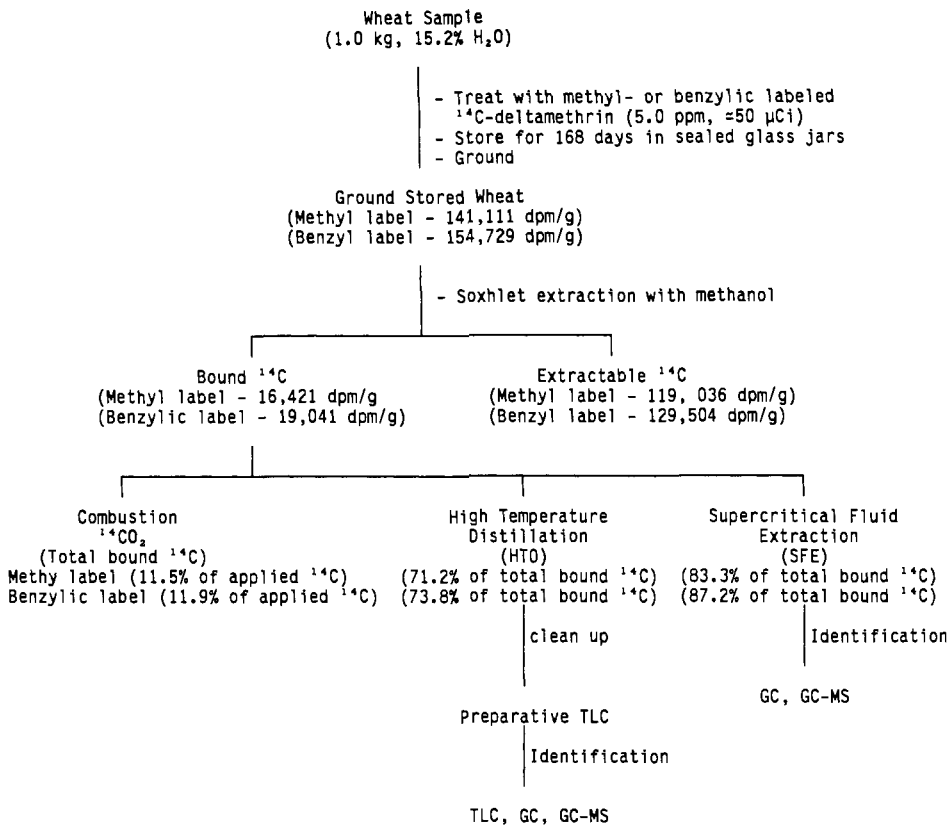


Figure 1. Schematic diagram for the analysis of bound (nonextractable) ^{14}C residues in stored wheat treated with [^{14}C]deltamethrin.

label) and 87.2% (benzyl label). It was also observed that during HTD of the material about 2% bound ^{14}C residues was thermally decomposed to $^{14}CO_2$ whereas no such loss of ^{14}C residues was observed by SFE. It is obvious, therefore, that the extraction efficiency for bound ^{14}C residues from wheat samples was higher by SFE than HTD.

The nature of the released bound ^{14}C residues in the HTD distillates was determined by a combination of TLC and GC techniques. Analysis of the distillates by TLC followed by photographing the plates by a Berthold Beta camera showed two radioactive regions at R_f 0.7 and 0.5 (methyl label) and R_f 0.7 and 0.4 ((benzyl label). In both cases the radioactive zones at R_f 0.7 were very poorly resolved and occurred near the expected region of unchanged deltamethrin. Authentic standards were also chromatographed on the same TLC plate for preliminary identification. The radioactive region at R_f 0.7 was extracted and, after ^{14}C measurement (12.2% ^{14}C and 23.6% ^{14}C of the total bound ^{14}C in stored wheat for methyl and benzyl labels, respectively), was analyzed by GC. However, we could not confirm the identity of the compound(s) present in the extracts (R_f 0.7) because of their very small amounts and the presence of interfering coextractives. The radioactivity of the extracts at R_f 0.5 (methyl label) and R_f 0.4 (benzyl label) amounted to 46.7% and 28.9%, respectively, of the total bound ^{14}C residues (Figure 2). The compounds with R_f 0.5 (methyl label) and R_f 0.4 (benzyl label) were identified as Br_2CA and 3-PBacid, respectively, by comparing with the TLC R_f values of the authentic standards. The extracts obtained from regions at R_f 0.5 (methyl label) and R_f 0.4 (benzyl label) were derivatized and analyzed by GC. Major peaks at retention times of 8.9 min (R_f 0.5) and 5.3 min (R_f 0.4) were observed, indicating the presence of esters of Br_2CA and 3-PBacid, respectively. Similarly, GC analysis of the derivatized extract from SFE showed two peaks at retention times of 5.3 and 8.9 min, confirming the presence of esters of 3-PBacid and Br_2CA, respectively. Furthermore, the amounts of 3-PBacid and Br_2CA calculated from the peak heights were about 0.49 and 0.43 ppm in the stored grains. In addition, a very small single peak at 34.3 min was noted, indicating the presence of a trace amount of deltamethrin. Further identity of these compounds was confirmed by cochromatography of the pentafluorobenzyl ester and methyl ester of authentic 3-PBacid and Br_2CA, respectively, and finally by GC–MS. A GC–MS of the peak with a GC retention time of 5.3 min exhibited major peaks at m/z 394 (base peak, $M^{\bullet+}$, $C_{20}H_{11}F_5O_3$), 197 ($M^{\bullet+} - C_7H_2F_5O$), and 181 ($M^{\bullet+} - C_{13}H_9O_3$). The spectrum was consistent with the mass spectrum of authentic pentafluorobenzyl derivative of 3-PBacid. The mass spectra of the compound represented by GC peak at 8.9 min exhibited molecular ions at m/z 314, 312, and 310 in a 1:2:1 ratio, indicating a distinct pattern for two bromine atoms (McLafferty, 1980) due to various isotopes of bromine atoms. Other major ions were at m/z 283, 281, 279 ($M^{\bullet+} - OCH_3$), 255, 253 (base peak), 251 ($M^{\bullet+} - COOCH_3$), 231 and 233 ($M^{\bullet+}$ – Br). This pattern is in agreement with the mass spectrum obtained for the methyl ester of Br_2CA. The amount represented by GC peak at 34.3 min (deltamethrin) was too small to be confirmed by GC–MS. Thus, from the foregoing it is obvious that the stored wheat treated with [^{14}C]deltamethrin contained primarily Br_2CA and 3-PBacid in the form of bound (nonextractable) residues. These types of residues are not detected by conventional analytical procedures, thereby resulting in an underestimation of stored grain burden of total pesticide residues.

It has been suggested that urinary and/or biliary excretion of the radioactivity from animals fed radiolabeled compounds signifies that the compound is bioavailable. However, quantitative excretion of radioactivity in feces

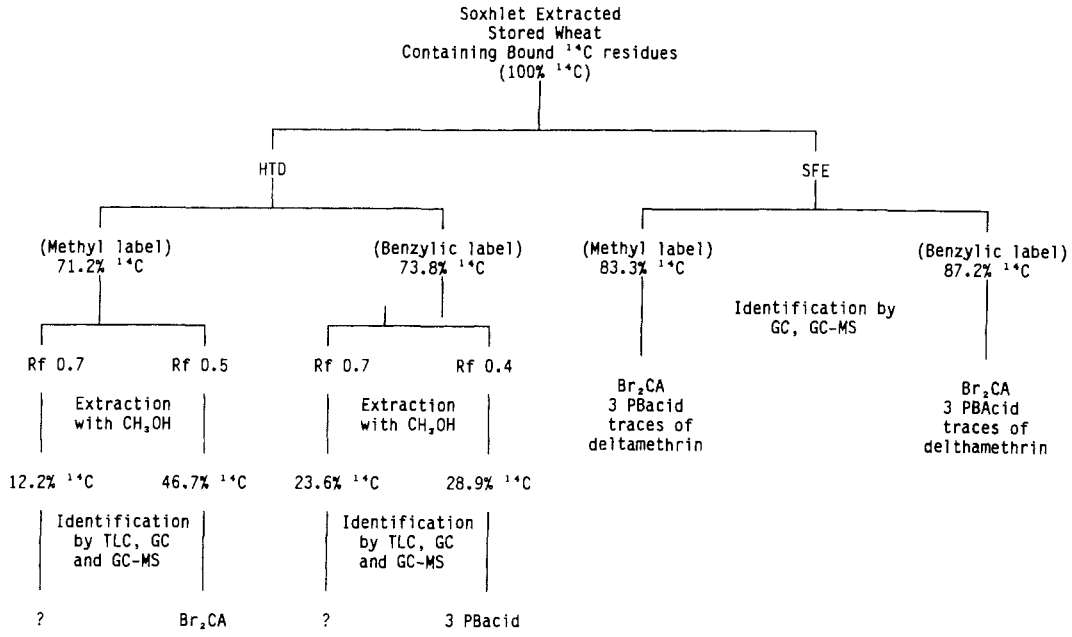


Figure 2. Schematic diagram for the identification of bound ^{14}C residues in stored wheat treated with [^{14}C]deltamethrin.

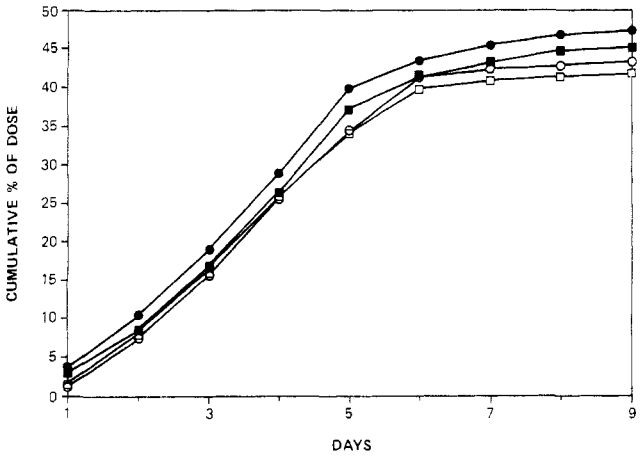


Figure 3. Elimination of ^{14}C from rats fed Soxhlet-extracted stored wheat treated with [^{14}C]deltamethrin. Feces: ○, methyl label; □, benzyl label. Urine: ■, methyl label; ●, benzyl label.

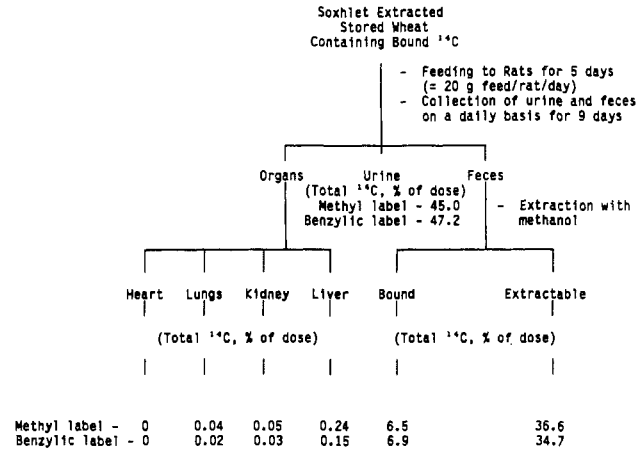


Figure 4. Distribution of ^{14}C in individual organs and feces of rats fed Soxhlet-extracted stored wheat treated with [^{14}C]-deltamethrin.

signifies that the material is not bioavailable. In previous studies measurement of excreted ^{14}C from the animals fed a diet containing bound ^{14}C residues indicated that a majority of bound residues were excreted through the feces (Paulson et al., 1975; Sutherland, 1976; Dorough, 1976; Marshall and Dorough, 1977; Khan et al., 1985, 1987). Thus, it was suggested that bound residues in crop materials treated with different pesticides have a low degree of bioavailability in rats. Figure 3 shows the cumulative excretion of radioactivity in feces and urine by rats fed the Soxhlet-extracted stored wheat containing bound ^{14}C residues. After 9 days, cumulative excretions of radioactivity in the urine were 45.0% (methyl label) and 47.2% (benzyl label) of the total administered bound ^{14}C in stored wheat. Similarly, the cumulative fecal excretions of radioactivity amounted to 43.1% (methyl label) and 41.6% (benzyl label). The ^{14}C excretion pattern demonstrated that much of the bound ^{14}C residues in stored wheat are unquestionably bioavailable in rats. Cumulative urinary excretion was evident at slightly higher levels than those in feces (Figure 3).

It has been suggested that relative amounts of fecal and urinary excretion of ^{14}C may not accurately indicate the degree of absorption of ^{14}C residues from the gut (Marshall and Dorough, 1977). Thus, fecal elimination of ^{14}C does not necessarily mean that the bound ^{14}C residues were not absorbed by gastrointestinal tract. No attempt was made in this study to monitor biliary radiocarbon. However, it has been suggested that the ^{14}C residues, which may undergo biliary excretion, will eventually be eliminated in the feces (Boyland and Sims, 1964). Extraction of the feces with methanol extracted 36.6% (methyl label) and 34.7% benzyl label) of the total bound ^{14}C in stored grain. Furthermore, 6.5% ^{14}C (methyl label) and 6.9% ^{14}C (benzylic label) remained tightly bound in the feces (Figure 4). It would appear, therefore, that biliary radiocarbon was partly responsible for the extractable radiocarbon in feces while the radiocarbon remaining in the stored wheat solids was excreted in the feces.

It was suspected that Soxhlet extraction of the stored wheat treated with [^{14}C]deltametrin may not have effectively removed all the extractable ^{14}C residues. This, in turn, may partly be responsible for the relatively higher amounts of extractable radiocarbon in the feces. Aliquots of the Soxhlet-extracted stored wheat containing bound ^{14}C residues were further extracted (3×) with methanol by mechanical shaking. It was observed that only a small amount ranging from 1.2% ^{14}C to 1.4% ^{14}C of the total ^{14}C was extracted in methanol. This obviously will not account for the observed higher amounts of extract-

Table I. High-Performance Liquid Chromatography (HPLC) Properties of ^{14}C-Labeled Reference Standards and Metabolites in the Urine and Feces of Rats Fed Bound ^{14}C Residues in Stored Wheat Treated with Methyl- or Benzyl-Labeled Deltamethrin

compound	retention time, min		
	solvent system i[a]	solvent system ii[b]	
3-PBacid metabolites derived from benzyl-labeled deltamethrin	6.9	7.4	30.5[b]
urine	2.9 (major), 5.2, 6.9	4.1 (major), 5.1, 7.3	9.0 (major), 12.0, 30.8
ether extract of acid-hydrolyzed urine			8.2, 11.7 (major), 29.7
feces		3.9 (major), 7.4, 11.2	
Br_2CA metabolites derived from methyl-labeled deltamethrin	15.0	11.1	
urine	2.7, 3.7, 15.0 (major)	4.0, 10.9 (major)	
feces		4.0, 11.4 (major)	

[a] Solvent systems: i, acetonitrile buffer (Na_2HPO_4, 0.05 M, pH 4.5) (3:7, v/v), flow rate 3.5 mL/min: ii, water–methanol (1:1, v/v) plus 0.1 M NH_4OAc, flow rate 1.5 mL plus/min. [b] Values when flow rate was 0.5 mL/min.

able ^{14}C residues excreted in feces. In view of the foregoing, the excretion of extractable ^{14}C residues in feces must be considered bioavailable. These data demonstrate that bound ^{14}C residues in stored wheat treated with [^{14}C]deltamethrin are highly bioavailable.

The nature of ^{14}C residues excreted in urine was determined by HPLC (Table I). The urine samples were evaporated to dryness, and the residue was solubilized in methanol. Analysis (condition i) of sample (methyl label) exhibited two minor radioactive peaks at retention times 2.7 and 3.7 min. However, a major radioactive peak (95–98% of the applied ^{14}C) appeared at a retention time of 15.0 min, which was identified as Br_2CA. Analyses of urine samples from rats (benzyl label) exhibited a major radioactive peak (90% of the applied ^{14}C) at a retention time of 2.9 min and smaller radioactive peaks at 5.2 and 6.9 min. A similar pattern was observed when samples were analyzed under condition ii. The identity of the later peak (R_t = 6.9 min) was confirmed as 3-PBacid. The material representing the major radioactive peak (R_t = 2.9 min, solvent system i; or R_t = 4.1, 9.0 min, solvent system ii) was hydrolyzed with 3 N HCl at 110–115 °C for 2 h. Ether extract of the hydrolyzed material contained about 79% ^{14}C of the total ^{14}C in the urine sample before hydrolysis. The material obtained after acid hydrolysis exhibited a radioactive peak with a retention time at 11.7 min (solvent system ii). The material represented by this peak was identical with that of an authentic reference standard of 4′-OH-3-PBacid. Further hydrolysis of the polar metabolite(s) with β-glucuronidase liberated only 2–5% of extractable ^{14}C. This suggested that the major conjugating moiety was not a glucuronide. The identity of the methyl derivative of this compound was further confirmed by GC–MS. The mass spectrum of the methyl derivative consisted of peaks at m/z 258 ($M^{\bullet+}$), 243 ($M^{\bullet+} - CH_3$), and 227 ($M^{\bullet+} - OCH_3$) with further fragmentation analogous to that observed for authentic methyl 4′-OCH_3-PBacid. These data indicate that rats fed bound ^{14}C residues in stored wheat excreted primarily Br_2CA (methyl label) and 4′-OH-3-PBacid and 3-PBacid (benzyl label) in the urine.

A major portion of methanol-extractable ^{14}C residues from feces remained unidentified by the techniques used in this study. However, presence of small amounts of Br_2CA (methyl label) and 3-PBacid and conjugates of 4′-OH-3-PBacid (benzyl label) were noted in the methanol extract of feces.

The residual radioactivity in various organs was determined following the sacrifice of rats fed stored wheat containing bound ^{14}C residues (Figure 4). Radiocarbon was detected in all the organs analyzed except the heart. Liver contained the highest ^{14}C residues. During the course of the experiment none of the rats showed any sign of toxic manifestations and no apparent histopathological alterations.

This study suggests that information concerning the formation of bound insecticide residues in stored grains is essential for the proper assessment of their safety to consumers. Bioavailability tests on laboratory animals could be extrapolated to predict potential effects on man and livestock at a much lower dose. On the basis of toxicological evaluation and the extensive residue data on deltamethrin, a maximum residue limit for the insecticide in wheat of 1 mg/kg has been recommended (FAO/WHO, 1983). The likely formation of bound residues and their bioavailability at this recommended level of deltametrin may therefore not present any potential hazard.

ACKNOWLEDGMENT

The skilled technical assistance of W. R. McDowell and C. Danis is much appreciated. This study was part of a coordinated program of research under the sponsorship of the International Atomic Energy Agency, Vienna, Austria.

LITERATURE CITED

Bengston, M.; Davies, R. A. H.; Desmarchelier, J. M.; Hemming, R.; Murray, W.; Simpson, B. W.; Snelson, J. T.; Stocka, R.; Wallbank, B. E. Organophosphorothioates and synergized synthetic pyrethroids as grain protectants on bulk wheat. *Pestic. Sci.* **1983**, *14*, 373–384.

Boyland, E.; Sims, P. Metabolism of Polycyclic Compounds. 23. The metabolism of pyrene in rats and rabbits. *Biochem. J.* **1964**, *90*, 391–398.

Dorough, H. W. Biological activity of pesticide conjugates, in Bound and Conjugated Pesticide Residues. *ACS Symp. Ser.* **1976**, *No. 29*, 11–34.

FAO/WHO. 1982 evaluation of some pesticide residues in food; FAO Plant Production and Protection Paper 49; FAO/WHO: Rome, 1983.

Halls, G. R. H.; Periam, A. W. The fate of residues of deltamethrin on wheat during storage and after milling and baking – Report after 9 months storage. Wellcome Research Laboratories Report HEFH80-4; Wellcome Research Laboratories: Nov 1980.

Hargreaves, P.; Bengston, M.; Alder, J. Inactivation of deltamethrin in stored wheat. *Pestic. Sci.* **1982**, *13*, 639–646.

Khan, S. U.; Hamilton, H. A. Extractable and bound (non-extractable) residues of prometryn and its metabolites in an organic soil. *J. Agric. Food Chem.* **1980**, *28*, 126–132.

Khan, S. U.; Zhan, L. Z.; Akhtar, M. H. Bound residues of deltamethrin in bean plants. *J. Agric. Food Chem.* **1984**, *32*, 1141–1144.

Khan, S. U.; Kacew, S.; Molnar, S. J. Bioavailability in rats of bound ^{14}C residues from corn plants treated with [^{14}C]atrazine. *J. Agric. Food Chem.* **1985**, *33*, 712–717.

Khan, S. U.; Kacew, S.; Dupont, S.; Stratton, G. D.; Stratton, G. D.; Wheeler, W. B. Bioavailability in rats of bound residues from radishes treated with either radiolabeled dieldrin or carbofuran. *J. Agric. Food Chem.* **1987**, *35*, 89–93.

Marshall, T. C.; Dorough, H. W. Bioavailability in rats of bound and conjugated plant carbamate insecticide residues. *J. Agric. Food Chem.* **1977**, *25*, 1003–1009.
McLafferty, F. W. *Interpretation of mass spectra*, 3rd ed.; University Science Books: Mill Valley, CA, 1980.
Nobel, R. M.; Hamilton, D. J.; Osborne, W. J. Stability of pyrethroids on wheat in storage. *Pestic. Sci.* **1982**, *13*, 246–252.
Paulson, G. D.; Jackobsen, A. M.; Still, G. G. Animal metabolism of prophan (isopropyl carbamilate), the fate of residues in alfalfa when consumed by the rat and sheep. *Pestic. Biochem. Physiol.* **1975**, *5*, 523–528.
Soderlund, D. M.; Casida, J. E. *Substrate specificity of mouse-liver microsomal enzymes in pyrethroids metabolism in Synthetic Pyrethroids*; ACS Symposium Series 42; American Chemical Society: Washington, DC, 1977; pp 162–172.
Sutherland, M. L. Nature of propanil bound residues in rice plants as measured by plant fractionation and animal bioavailability experiment, in Bound and Conjugated Pesticide Residues. *ACS Symp. Ser.* **1976**, *No. 29*, 153–165.
Unai, T.; Casida, J. E. Synthesis of isomeric 3-(2,2-dichlorovinyl)-2-hydroxymethyl-2-methylcyclopropanecarboxylic acids and other permethrin metabolites. *J. Agric. Food Chem.* **1977**, *25*, 979–987.

Received for review August 10, 1989. Accepted December 14, 1989.

[3+2] Cycloaddition of 1,3,4-Oxadiazol-2-ylhydrazones to Aryl Isothiocyanates and Fungitoxicity of the Resulting Thiadiazolidines

Atma R. Misra,* Lal Dhar S. Yadav, Harendra Singh, and Jagdamba P. Misra

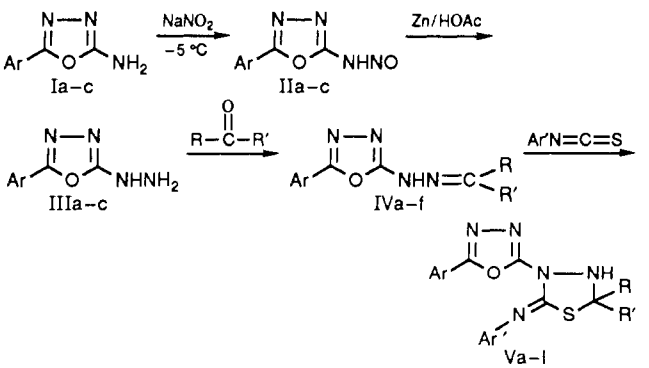
Department of Chemistry, S. D. J. Post-Graduate College Chandesar, Azamgarh 276128, India

[3+2] cycloaddition of 1,3,4-oxadiazol-2-ylhydrazones IVa–f to aryl isothiocyanates yielded a novel class of compounds, 2-(arylimino)-3-(5-aryl-1,3,4-oxadiazol-2-yl)-5,5-dimethyl-1,3,4-thiadiazolidines, and the corresponding 5-methyl-5-phenyl derivatives Va–l. Condensation of 5-aryl-2-hydrazino-1,3,4-oxadiazoles IIIa–c with the appropriate ketone in equimolar quantity furnished the requisite hydrazones IVa–f. The required 5-aryl-2-hydrazino-1,3,4-oxadiazoles IIIa–c were prepared by diazotization of 2-amino-1,3,4-oxadiazoles Ia–c, followed by treatment with Zn and glacial acetic acid. Compounds Va–l have been compared with a standard commercial fungicide Dithane M-45 (manganous ethylene bis(dithiocarbamate) with zinc ions), for their fungitoxic action against *Aspergillus niger* and *Fusarium oxysporium*, and results have been correlated with the structural features of the tested compounds.

Many 1,3,4-oxadiazoles are known to display a broad spectrum of useful pesticidal activity (Meek, 1972; Stachler and Sachse, 1977; Rhone-Poulenc, 1968). Similarly, a number of 1,3,4-thiadiazoline and -thiadiazolidine derivatives, especially those bearing 2-substituted imino groups, are known to display important pesticidal activity (Paul et al., 1981; Dahle, 1977; Nuesslein and Arndt, 1977; Russo and Santagati, 1976). In view of these factors and with the hope of achieving antifungal compounds of high potency, the biolabile 1,3,4-oxadiazole and 1,3,4-thiadiazolidine nuclei have been combined to probe how this combination could enhance antifungal action. The investigation appeared quite interesting as the 2-(arylimino)-3-(5-aryl-1,3,4-oxadiazol-2-yl)-5,5-dimethyl-1,3,4-thiadiazolidines and the corresponding 5-methyl-5-phenyl derivatives Va–l reported herein have been synthesized for the first time.

The reaction sequence leading to the formation of Va–l is given in Scheme I. In the synthesis of Va–l, a novel class of heterocycles, aryl isothiocyanates act as dienophiles and the hydrazones IVa–f as dienes (aza dienes). This is an interesting example of hetero-Diels–Alder synthesis. The [3+2] cycloaddition of hydrazones IVa–f to aryl isothiocyanates was carried out at 50–60 °C in acetone to yield Va–l in 67–81% yields (Table I). The required 2-amino-5-aryl-1,3,4-oxadiazoles Ia–c were prepared by oxidative cyclization of aldehyde semicarbazones with bromine (Gibson, 1962). Diazotization of compound I followed by treatment with Zn and glacial acetic acid resulted in the formation of the precursor hydrazines IIIa–c, which, when treated with ketones, afforded the requisite hydrazones IVa–f.

Scheme I



0021-8561/90/1438-1082$02.50/0