Heavy-Metal Absorption by Soybean on Sewage Sludge Treated Soil

Muchha R. Reddy* and Samuel J. Dunn

Studies were conducted to determine absorption of Cd, Ni, Cu, and Zn by soybean [*Glycine max* (L.) Merr.] grown on Enon soil treated with sewage sludge. The sludge rates were 0, 40, 80, and 120 Mg ha^{-1}. Bragg and Ransom varieties of soybeans were grown in pots to maturity. Cadmium concentration in seeds was found to be 0.08 and 0.20 μg g^{-1} for the control and 120 Mg ha^{-1} sludge treatments, respectively. Bragg and Ransom showed significant difference in the absorption of Cd and Cu. There were significant differences in the concentrations of the metals among various tissues of soybeans. Concentrations of Cu, Ni, and Zn in seeds were found to be 4, 3, and 52 μg g^{-1}, respectively, for the control and 11, 20, 63 μg g^{-1}, respectively, for 120 Mg ha^{-1} sludge treatment. Relative to background levels, Cd in soybeans increased more than Zn.

INTRODUCTION

Absorption and distribution of heavy metals within a plant such as soybean from sludge-treated soil requires a better understanding. Accumulation of heavy metals by some crops grown on sewage sludge treated soils has been reported by earlier workers (Page et al., 1972; Hinesly et al., 1977; Dowdy et al., 1978; King and Dunlap, 1982). The extent of plant tissue accumulation appeared to be affected by many soil-, plant-, and sludge-related factors (Chaney et al., 1977; Chang et al., 1981). The rate of release of heavy metals and form of the metal species in soil solution will have a strong influence on the rate and extent of accumulation of the metals and will possibly also influence mobility and toxicity in the plants and animals that consume them (Cataldo and Wildung, 1978). Increased accumulation of heavy metals could result in phytotoxicity (Soane and Saunders, 1959; Lee and Page, 1967; Chaney and Hornick, 1978). Elevated concentrations of heavy metals in plant tissue could expose consumers to excessive levels of potentially hazardous chemical elements (CAST, 1976).

Boggess and Koeppe (1977) stated that soybean varieties exhibited a range of susceptibility to Cd toxicity when the metal was added to soil as an inorganic salt. It has long been recognized that many varieties exhibit different uptakes of plant nutrients (Epstein and Jeffries, 1964). Varietal differences in plant response to deficiencies and excesses of certain nutrient elements have been reported by Milliken (1961). Boggess et al. (1978) reported that soybean varieties grown on sludge-treated soil accumulated different amounts of Cd. Findings of Chaney and Feder (CAST, 1980) suggest that Cd and Zn contents of nine varieties of lettuce (*Lactuca sativa* L.) varied considerably. John and Van Laerhoven (1976) also found significantly different levels of Cd accumulation in several varieties of lettuce. Differential accumulation of Cd by inbred lines of corn (*Zea mays* L.) has been observed by Hinesly et al. (1978). Peterson (1977) demonstrated that Cd uptakes by several barley (*Hordeum vulgare* L.) varieties cultivated in Sweden were not significantly different; data collected by other researchers showed the contrary (CAST, 1980).

There is limited information on heavy-metal absorption by soybeans from sludge-treated soil. Since soybeans are of great economic significance, it is important to investigate its absorption of heavy metals. The objective of this investigation was to examine the absorption and distribution of Cd, Ni, Zn, and Cu by Bragg and Ransom varieties of soybeans from sludge-amended soil.

Department of Plant Science and Technology, North Carolina Agricultural and Technical State University, Greensboro, North Carolina 27411.

EXPERIMENTAL MATERIALS AND PROCEDURES

For the greenhouse experiment, an Enon sandy loam (fine, mixed, thermic Hapludalfs) was collected (0–15 cm), air-dried, crushed, and passed through a 2-mm stainless-steel sieve. The soil was characterized with respect to texture, pH, cation-exchange capacity (CEC), and organic matter. Soil texture analysis was conducted by using the hydrometer method, CEC with NaOAC method, organic matter with Walkley and Black method, and soil pH with 1:1 soil–water paste, as given in Black (1965). Characteristics of the Enon soil: pH 6.2; organic matter, 1.85%; CEC, 8.78 cmol kg^{-1}; clay, 13.5%; silt, 7.2%; sand, 79.3%. Anaerobically digested sludge from a waste water treatment plant in Winston-Salem, NC, was air-dried, crushed, and passed through a 2-mm sieve before use. The concentrations of Cd, Ni, Cu, and Zn in the sludge were 15, 70, 250, and 305 μg g^{-1}, respectively. Eight kilograms of soil was placed in each pot (25.4-cm diameter) and sludge added at the rates of 0, 40, 80, and 120 Mg ha^{-1} of dry (383 K) sludge. The soil and sludge in the pots were mixed well and brought to field capacity with deionized water as given in Black (1965). Five inoculated seeds of either Bragg or Ransom cultivar were planted in each pot. The moisture in the pots was maintained at a desired level with deionized water by monitoring with tensiometers in the pots, throughout the experiment.

After germination, the plants were thinned to two plants per pot and allowed to grow to the dry pod stage. Plants were harvested at ground level, and pods and stems were collected separately for determination of heavy-metal concentration. Since the leaves were quite dry and had fallen onto soil surface, they were not collected. Pods and stems were washed with deionized water and dried in a forced-draft oven at 343 K for 24 h, and seeds were separated from pods. Seeds, stems, and pod walls were ground separately in a Wiley mill containing stainless-steel blades. Samples were stored in vials until analysis.

For Cd and Ni in the plant tissue, 1 g of each sample was digested with 10 mL of concentrated HNO_3 and 3 mL of perchloric acid for 3 h (AOAC, 1980). The residue was dissolved in 5 mL of 16 M HCl and diluted to 50 mL with deionized water. The extracts were then assayed for the respective metals by inductively coupled plasma (Dahlquist, 1978). For Cu and Zn, 1 g of each tissue sample was dry ashed in a muffle furnace at 773 K for 4 h. The ashed material was dissolved in 5 mL of 2.25 M HNO_3 and brought to a 50-mL volume with deionized water (Tucker, 1983). The extracts were analyzed for Cu and Zn by at-

0021-8561/86/1434-0750$01.50/0

Table I. Variables Related to Tissue Concentration and Absorption of Cd, Ni, Cu, and Zn in Soybean Tissue

variables[a]		regression anal.[b]	
dependent	independent	equation	r^2
		Bragg	
seed Cd	sludge-appl Cd	$y = 0.070 + 0.0667x$	0.92**
pod wall Cd	sludge-appl Cd	$y = 0.046 + 0.0766x$	0.96**
stem Cd	sludge-appl Cd	$y = 0.095 + 0.1333x$	0.99**
seed Ni	sludge-appl Ni	$y = 8.6 + 0.9107x$	0.90**
pod wall Ni	sludge-appl Ni	$y = 2.0 + 0.167x$	0.71**
seed Cu	sludge-appl Cu	$y = 5.90 + 0.0401x$	0.88**
pod wall Cu	sludge-appl Cu	$y = 1.77 + 0.0115x$	0.56**
seed Zn	sludge-appl Zn	$y = 56.1 + 0.0558x$	0.42*
		Ransom	
seed Cd	sludge-appl Cd	$y = 0.120 + 0.0330x$	0.88**
pod wall Cd	sludge-appl Cd	$y = 0.085 + 0.0500x$	0.92**
stem Cd	sludge-appl Cd	$y = 0.158 + 0.0944x$	0.98**
seed Ni	sludge-appl Ni	$y = 3.4 + 2.004x$	0.95**
pod wall Ni	sludge-appl Ni	$y = 0.67 + 0.3452x$	0.83**
seed Cu	sludge-appl Cu	$y = 4.2 + 0.0268x$	0.86**
stem Cu	sludge-appl Cu	$y = 3.9 - 0.0128x$	0.40**
seed Zn	sludge-appl Zn	$y = 52.5 + 0.0842x$	0.77**
pod wall Zn	sludge-appl Zn	$y = 16.7 \pm 0.0417x$	0.51**

[a] Heavy-metal levels were expressed as $\mu g\ g^{-1}$ in tissue and kg ha^{-1} sludge applied. [b] Key: *, ** significant at the 0.05 and 0.01 levels of probability, respectively.

Table II. ANOVA Values for the Model Used To Analyze the Data[a]

		concentration, F value			
effect	df	Cd	Ni	Cu	Zn
		Seed			
sludge rate	3	92.0	80.2	48.0	30.0
variety	1	24.0	1.0	101.2	5.9
sludge rate × variety	3	12.0	11.4	1.9	3.7
errors	16	0.0001	2.08	0.39	3.42
		Pod Wall			
sludge rate	3	144.5	30.3	5.4	4.88
variety	1	13.5	11.6	6.3	11.24
sludge rate × variety	3	8.5	5.5	1.8	6.0
errors	16	0.0001	0.17	0.32	4.54
		Stem			
sludge rate	3	561.3	1.5	1.8	3.1
variety	1	57.8	8.0	40.5	1.3
sludge rate × variety	3	17.3	1.9	3.3	1.5
errors[b]	16	0.0001	1.59	0.35	5.23

[a] F values are reported for all effects other than error, which is represented by the mean square value. [b] Error term included all effects involved replication.

omic absorption spectrophotometry. All analyses were conducted in triplicate.

Experimental design was a 4 (sludge level) × 2 (variety) with three replications completely randomized experiment. Data were analyzed by using SPSS[x] Batch system procedures (1983). Two-way ANOVA was conducted for each plant part separately as given in Table II. Student's t-test was used to compare the means between seed, pod wall, and stem within each data group. Data were also analyzed by the linear regression technique.

RESULTS AND DISCUSSION

Seed Yield. There was an increase in Bragg and Ransom seed yield with increased rate of sludge application as compared to control (Figure 1). The 120 Mg ha^{-1} sludge rate gave the highest seed yield. Regression equations included in the figures explained 92 and 70% of variation in the yield for Bragg and Ransom, respectively. A significant ($p = 0.01$) difference in seed yield between Bragg and Ransom soybeans occurred for all the treatments combined (Bragg, 15.53 g/pot Ransom, 17.30 g/pot). Ransom gave a higher yield of seed than Bragg.

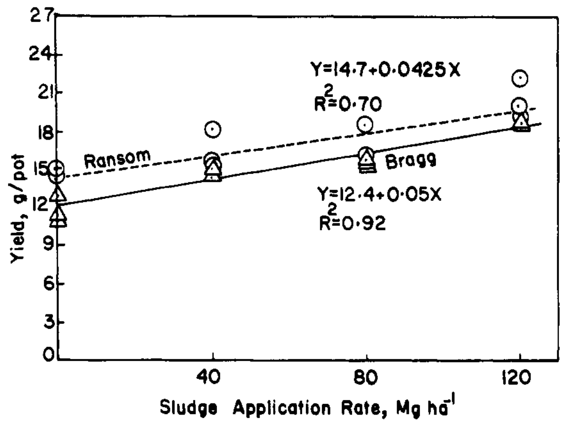

Figure 1. Effect of application rate of sludge on soybean seed yield.

Table III. Effects of Sludge-Borne Heavy Metals on Cd, Ni, Cu, and Zn Concentrations in Soybean Varieties

sludge-borne heavy-metal rate, kg ha^{-1}	amount, $\mu g\ g^{-1}$					
	Bragg			Ransom		
	seed	pod wall	stem	seed	pod wall	stem
			Cd			
control	0.08	0.05	0.10	0.12	0.08	0.15
0.6	0.10	0.09	0.17	0.14	0.12	0.22
1.2	0.14	0.13	0.25	0.16	0.15	0.26
1.8	0.20	0.19	0.34	0.18	0.17	0.33
			Ni			
control	8.10	1.90	1.00	3.30	0.93	1.56
2.8	11.83	2.56	1.00	9.30	1.13	4.56
5.6	13.83	3.06	1.46	14.50	2.80	2.23
8.4	15.93	3.23	1.66	20.26	3.60	2.60
			Cu			
control	5.66	1.86	1.53	3.80	1.00	3.76
10	7.93	2.00	1.43	5.73	2.30	3.76
20	8.96	2.90	1.83	6.33	2.26	2.43
30	10.66	3.10	1.53	7.16	2.00	2.50
			Zn			
control	53.33	23.00	15.66	52.00	16.00	13.66
12.2	62.33	20.33	10.66	57.66	19.00	12.66
24.4	60.66	26.33	11.50	57.33	20.66	12.33
36.6	61.3	18.66	12.93	63.33	21.00	16.33

Table IV. Mean Concentration of Heavy Metals in Different Tissues of Soybeans[a]

	concentration, $\mu g/g$			
tissue	Cd	Ni	Cu	Zn
		Bragg		
seed	0.130 a	12.43 a	8.31 a	59.42 a
pod wall	0.115 b	2.69 b	2.47 b	22.08 b
stem	0.215 c	1.28 c	1.58 c	12.69 c
		Ransom		
seed	0.150 a	11.84 a	5.76 a	57.58 a
pod wall	0.130 b	2.12 b	1.89 b	19.17 b
stem	0.243 c	2.74 b	3.12 c	13.75 c

[a] Means followed with different letter in the columns are significantly different at <0.01 level of probability according to Student's t-test.

Soybean Tissue. Cd, Ni, Cu, and Zn. Linear relationships were observed between sludge-borne Cd rate and Cd concentration in seed, pod wall, and stem of the two varieties ($r^2 = 0.88$–0.99) (Figure 2; Table I). All effects as determined by analysis of variance are presented in Table II. There was a significant difference in concen-

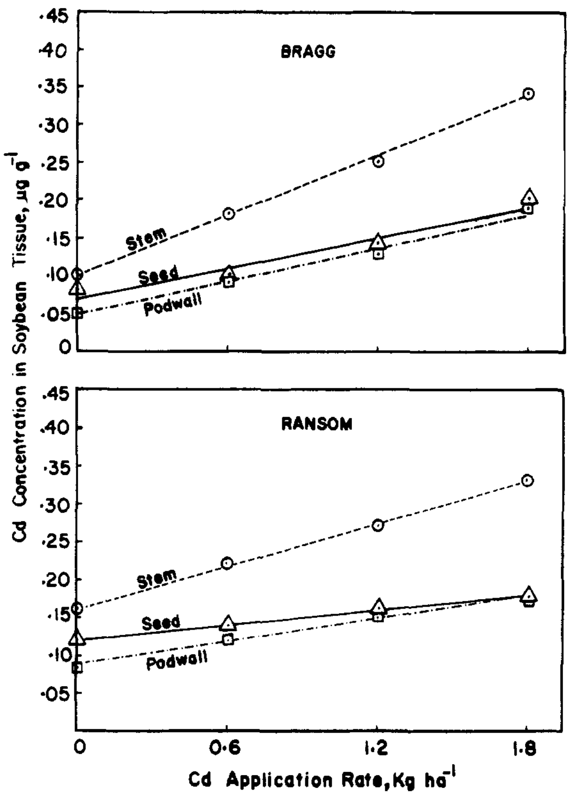

Figure 2. Effect of sludge-applied Cd rate on Cd concentrations in Bragg and Ransom soybean tissue.

tration of Cd between the two varieties. The interaction between sludge-borne Cd and varieties and tissue was significant. The concentration of Cd in various soybean tissues was significantly different (Tables III and IV). There was greater concentration of Cd in stem than other tissues, in both of the varieties. Cadmium concentrations in the plant parts were in the order stem > seed ≈ pod wall. Higher rate of sludge-borne Cd resulted in greater concentration of Cd in the soybean tissues.

Nickel concentration increased linearly in seed and pod wall of both varieties as the rate of sludge-applied Ni increased (r^2 = 0.71–0.95) (Table I). There was no significant difference between the varieties as for the concentration of Ni in seed (Table II). Nickel concentration in pod wall and stem was significantly different between the varieties. The interaction between the sludge-borne Ni and varieties was significant in case of seed and pod wall. The concentration of Ni in various tissues of Bragg was significantly different. Concentration of Ni was significantly different only between seed and other tissues (Table IV) in Ransom; there was no significant difference between pod wall and stem. Seed was more responsive to Ni in sludge than other tissues (Table III). There was a significant increase in concentration of Ni in Ransom stem at 2.8 kg ha^{-1} of Ni and then a decrease. The concentration of Ni in Bragg and Ransom seeds was not significantly different.

There was linear increase (Table I and III) of Cu concentration in seed (r^2 = 0.88) and pod wall (r^2 = 0.56) of Bragg as the rate of sludge applied Cu increased. Ransom showed an increase of Cu concentration in seed (r^2 = 0.86) and a decrease in stem (r^2 = 0.40). The varieties showed significant difference in the concentration of Cu (Table II). The interaction between sludge-applied Cu and varieties was significant in the case of the stem. The concentration of Cu in various tissues differed significantly. Seed was more sensitive to Cu in sludge than other tissue, and there was a greater concentration of Cu in the seed than stem and pod wall (Tables III and IV).

Zinc concentration increased (Tables I and III) in the seed of Bragg as the rate of sludge-applied Zn increased (r^2 = 0.42). There was no significantly linear effect of rate of sludge-applied Zn on the concentration of Zn in Bragg stem and pod wall. In the case of Ransom, Zn concentration increased significantly in seed (r^2 = 0.77) and pod wall (r^2 = 0.51) as the rate of sludge-applied Zn increased. Zinc concentration of Ransom stem did not show a linear increase with increased rate of sludge-applied Zn. The concentrations of Zn in the varieties were significantly different (Table II) in seed and pod wall. The concentrations of Zn were in the order seed > pod wall > stem (Table IV). The interaction between sludge-applied Zn and varieties was significant, for seed and pod wall.

As for the change in concentrations of the heavy metals in soybeans by sludge-applied metals, Ni was increased to the greatest extent. Relative to background levels, Cd concentrations in soybeans increased more than Zn concentrations. Such a trend may portend an animal health problem, as Cd is reported to be harmful (Sittig, 1981). In this study, the concentrations of Cd, Ni, Cu, and Zn observed in seed were below the recommended limit in livestock (National Research Council, 1980). The different absorption pattern of heavy metals observed among Bragg and Ransom varieties could be attributed to individual plant characteristics. It was suggested that genetically controlled features and morphoological and anatomical differences may be responsible for the resulting deviation (Chang et al., 1982).

CONCLUSION

There were significant differences in the concentrations of the metals among different tissues of soybean. Cadmium concentrations in soybeans increased more than Zn concentrations, on the basis of background levels. Nickel concentration in soybeans was increased to the greatest extent by sludge-applied metals. Concentrations of the heavy metals in the soybean seed were below the recommended limit in livestock.

ACKNOWLEDGMENT

Gratitude is extended to D. Lameck, D. Person, and M. S. Burrell for their technical help and to Dr. Fields Gunsett for statistical analysis.

Registry No. Cd, 7440-43-9; Ni, 7440-02-0; Cu, 7440-50-8; Zn, 7440-66-6.

LITERATURE CITED

Association of Official Analytical Chemists *Official Methods of Analysis*; AOAC: Washington, D.C., 1980 AOAC.3.007B, p 31.

Banin, A.; Navrot, J.; Noi, Y.; Yoles, D. *J. Environ. Qual.* 1981, *10*, 536–540.

Black, C. A., Ed. *Methods of Soil Analysis: Physical and Mineralogical Properties, Including Statistics of Measurement and Sampling* (Part 1), *Chemical and Microbiological Properties* (Part 2); American Society of Agronomy: Madison, WI, 1965.

Boggess, S. F.; Keoppe, D. E. *Differences in the Susceptibility of Soybean Varieties to Soil Cadmium*; Ann Arbor Science: Ann Arbor, MI, 1977; pp 229–238.

Boggess, S. F.; Willavize, S.; Koeppe, D. E. *Agron. J.* 1978, *70*, 756–760.

Cataldo, D. A.; Wildung, R. E. *Environ. Health Persp.* 1978, *27*, 149–159.

Chaney, R. L.; Hundeman, P. T.; Palmer, W. T.; Small, R. J.; White, M. C.; Decker, A. M. In Proceedings of a National Conference on Composting Municipal Residues and Sludge, Washington, DC, 23–25 Aug 1977; Information Transfer Inc.: Rockville, MD, 1978; pp 86–97.

Chaney, R. L.; Hornick, S. B. In Proceedings of the 1st International Cadmium Conference Metals Bulletin, Ltd.: London, 1978; pp 125–140.

Chang, A. C.; Page, A. L.; Bingham, F. T. *J.—Water Pollut. Control Fed.* **1981**, *53*, 237–245.
Chang, A. C.; Page, A. L.; Foster, K. W.; Jones, T. E. *J. Environ. Qual.* **1982**, 11, 409–412.
Council for Agricultural Science and Technology *Application of Sewage Sludge to Cropland: Appraisal of Potential Hazards of Heavy Metal to Plants and Animals*, Report No. 64 CAST: Ames, IA, Nov 1976.
Council for Agricultural Science and Technology *Effects of Sewage Sludge on the Cadmium and Zinc Content of Crops*, Report No. 84, 1980.
Dahlquist, R. L. *Appl. Spectrosc.* **1978**, *32*, 1–30.
Dowdy, R. H. Larson, W. E.; Titrud, J. M.; Laterrell, J. J. *J. Environ. Qual.* **1978**, *7*, 252–257.
Epstein, E.; Jeffries, R. L. *Annu. Rev. Plant Physiol.* **1964**, *15*, 169–184.
Hinesly, T. D.; Jones, R. L.; Ziegler, E. L.; Tyler, J. J. *Environ. Sci. Technol.* **1977**, *11*, 182–188.
Hinesly, T. D.; Alexander, D. E.; Ziegler, E. W.; Barrett, G. L. *Agron. J.* **1978**, *70*, 425–428.
John, M. K.; Van Laerhoven, C. J. *Environ Pollut.* **1976**, *10*, 163–173.
King, L. D.; Dunlap, W. R. *J. Environ. Qual.* **1982**, *11*, 608–616.
Lee, C. R.; Page, H. R. *Agron. J.* **1967**, *59*, 237–240.
Milliken, C. R. *J. Aust. Inst. Agric. Sci.* **1961**, *26*, 220–233.
National Research Council, National Academy of Sciences *Mineral Tolerance of Domestic Animals*; NRC: Washington, DC, 1980.
Page, A. L.; Bingham, F. T.; Nelson, C. *J. Environ. Qual.* **1972**, *1*, 288–291.
Peterson, O. *Swed. J. Agric. Res.* **1977**, *7*, 21–24.
Sittig, M *Handboook of Toxic and Hazardous Chemicals*; Noyes Publications: Park Ridge, NJ, 1981; pp 119–122.
Soane, B.D.; Saunders, D. H. *Soil Sci.* **1959**, *42*, 203–216.
*SPSS*ˣ: *A Complete Guide to the SPSS*ˣ *Language and Operations* SPSS Inc.: Chicago, Il, 1983.
Tucker, M. R. North Carolina Department of Agriculture, Raleigh, NC, personnel communication, 1983.

Received for review February 27, 1985. Revised manuscript received September 13, 1985. Accepted April 7, 1986. The financial support to conduct this work was granted by USDA-CSRS through the office of Agricultural Research, North Carolina Agricultural and Technical State University, and is very much appreciated.

Fate of [^{14}C]Deltamethrin in Lactating Dairy Cows

M. Humayoun Akhtar,* Kenneth E. Hartin, and H. Locksley Trenholm

Fate and residues of radiocarbon- (^{14}C-) labeled (*gem*-dimethyl or benzyl) deltamethrin were determined in lactating dairy cows after oral administration for 3 consecutive days and slaughtering the animals 24 h after the last dose. Orally administered deltamethrin (10 mg/kg of body weight) appeared to be poorly absorbed, but the deltamethrin that was absorbed was extensively metabolized and excreted in the bile and urine with very little accumulation in major edible tissues. Approximately 36–43% of the total administered radiocarbon was eliminated in feces within 24 h after the last dose. The major portion (78–82%) of ^{14}C compound in feces was deltamethrin. Only 4–6% of the administered ^{14}C was eliminated in urine, and 0.42–1.62% was secreted in the milk. Radiocarbon secreted into milk and was higher for the *gem*-dimethyl portion (0.69 μg/g) than for the benzyl moiety (0.36 μg/g). Deltamethrin was the major identifiable product in milk (0.10–0.14 μg/g). Radiocarbon content in various tissues was generally very low (0.1 μg/g) with the exception of liver, kidney, udder, abdominal, and subcutaneous fats which were higher.

Synthetic pyrethroids belong to a new generation of pesticides introduced as agricultural insecticides about 10 years ago that have been gaining acceptability very rapidly. One of the important members of this class of compounds is deltamethrin, (*s*)-α-cyano-3-phenoxybenzyl (*1R,3R*)-*cis*-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropanecarboxylate, also known as RU-22974, NRDC-161, OMS-168, decamethrin, Decis, and K-Orthin. It has been registered throughout the world to control insect pests that attack a variety of crops (FAO, 1981). While it has been developed to control pests of livestock and man, very little is known about its metabolism and residues in milk and meat of farm animals.

The metabolic fate of deltamethrin has been reported for rats (Ruzo et al., 1978), mice (Ruzo et al., 1979), mouse liver microsomes (Shono et al., 1979), and cow and chicken liver homogenates (Akhtar, 1984). Recently Akhtar et al. (1985) have carried out a detailed study on the fate of deltamethrin in laying hens and reported that the insecticide was efficiently absorbed and rapidly metabolized. In the present study the objective was to determine the fate of orally dosed ^{14}C-labeled (*gem*-dimethyl or benzyl) deltamethrin in lactating dairy cows with regard to the nature of ^{14}C residues in milk, fat, muscle, urine, and feces.

Animal Research Centre, Agriculture Canada, Ottawa, Ontario K1A 0C6, Canada.

MATERIALS AND METHODS

Chemicals. Radiocarbon- (^{14}C-) labeled and unlabeled deltamethrin were supplied by Hoechst of Canada through Roussel Uclaf of France. The two forms of [^{14}C]deltamethrin preparations used in the study were [^{14}C]-*gem*-dimethyl (>98% radiochemical purity), and [^{14}C]benzyl (>95% radiochemical purity, *m*-phenoxybenzaldehyde and *m*-phenoxybenzoic acid were the major radioactive impurities). Authentic metabolites and spectral data for positive identification of other metabolites were available from a previous study (Akhtar et al., 1985). The abbreviations used in the text, tables, and figures as structural designations for the products are the same as used in a recent publication (Akhtar et al., 1985).

Preparation of [^{14}C]Deltamethrin for Oral Administration. To a solution of a known amount of unlabeled deltamethrin in acetone was added a calculated quantity of individual preparations of [^{14}C]deltamethrin (*gem*-dimethyl, 55.6 mCi/mmol; benzyl, 59.3 mCi/mmol). The solution was thoroughly mixed, and the solvent was allowed to stand at room temperature in a fumehood to