

above, IV (major peak from packed column GLC) gave only a single peak using a high-resolution capillary GLC column (ca. 50 000 plates) and is therefore very likely a single diastereoisomer. Factors involved in determining the stereochemistry include (1) the use of optically active *l*-isopulegol which would probably fix the stereochemistry of the 4-position methyl group (cadinane numbering), (2) the direction of approach of the methyl acrylate in the Diels-Alder condensation, and (3) the use of NaOCH₃ to produce the more stable configuration of III.

ACKNOWLEDGMENT

The authors would like to thank Geraldine Secor for carbon and hydrogen microanalyses and Robert E. Lundin and Mabry E. Benson for ¹H NMR spectra.

LITERATURE CITED

Gerber, N. N., *Phytochemistry* 10, 185 (1971).

- Gerber, N. N., *Phytochemistry* 11, 385 (1972).
 Kartha, C. C., Kalsi, P. S., Shaligram, A. M., Chakravarti, K. K., Bhattacharyya, S. C., *Tetrahedron* 19, 241 (1963).
 Martin, J. G., Hill, R. K., *Chem. Rev.* 61, 537 (1961).
 Mitzner, B. M., Theimer, F. T., Freeman, S. K., *Appl. Spectrosc.* 19, 169 (1965).
 Pliva, J., Horak, M., Herout, V., Sorm, F., "Die Terpene I. Sesquiterpenes", Akademie-Verlag, West Berlin, 1960.
 Vig, O. P., Singh, G., Chugh, O. P., Matta, K. L., *Indian J. Chem.* 7, 434 (1969).
 von Rudloff, E., *Can. J. Chem.* 39, 1860 (1961).
 Wintersteiner, O., Moore, M., *J. Am. Chem. Soc.* 65, 1507 (1943).

Received for review August 27, 1976. Accepted November 29, 1976. Reference to a company and/or product named by the Department is only for purposes of information and does not imply approval or recommendation of the product to the exclusion of others which may also be suitable.

Effects of Dietary Feeding of Organocadmium to Cattle and Sheep

Fred C. Wright,* Jack S. Palmer, Jayme C. Riner, Maurice Haufler, John A. Miller, and Clyde A. McBeth

Adult cattle and sheep were fed a cadmium fungicide, Cadminate, in the diet at levels of 50, 100, 200, 300, and 500 ppm of cadmium. The cattle study continued for 49 weeks and the sheep study for 41 weeks. The toxic effects included abortions, dead or short-lived calves and lambs, and birth defects. The animals fed cadmium at levels greater than 200 ppm in feed developed anemia as detected by decreased RBC counts, decreased PCV, and lowered Hb levels. Greatest residues of cadmium were in the kidney and liver of both cattle and sheep. In sheep, there was a significant difference in feed conversion efficiency due to the level of cadmium in feed. Cadmium, fed as Cadminate in feed, is detrimental to both cattle and sheep.

Many researchers have studied the effects of cadmium on different species of animals but only a few will be mentioned here.

Wilson et al. (1941) observed anemia in rats after they were fed a diet containing 31 ppm of cadmium for 2 months. Friberg (1950) also observed anemia in rabbits exposed to cadmium oxide dust and in rabbits injected with cadmium sulfate (0.65 mg of cadmium/kg for 6 days a week) for 2 months. Decker et al. (1958) observed that hemoglobin (Hb) levels were reduced in rats given 50 ppm of cadmium in drinking water for 2 weeks, but reduced Hb levels were not observed in rats given 10 ppm of cadmium in drinking water for 1 year.

Friberg et al. (1971) noted abortions, damage to reproductive organs, anemia, and neonatal deaths in laboratory animals exposed to cadmium-containing compounds.

Residues of cadmium accumulate most rapidly in the kidney and liver of animals exposed to cadmium-containing compounds. This was observed by Cousins et al. (1973) in swine, by Nordberg (1972) in rats, and by Powell et al. (1964) in calves.

Since very little information could be found on the effect of cadmium-containing fungicides on cattle and sheep, the following experimentation was undertaken.

The plant fungicide, Cadminate, is marketed as a 60% wettable powder. Metallic cadmium accounts for 29% of

the total formulation. This fungicide is used to treat turf grasses for copper spot, dollar spot, and red thread. Before 1973, the grazing of treated areas by livestock was not restricted. In 1973, additional restrictions were placed on the product: namely, do not graze treated areas and do not feed clippings to livestock.

However, even though these restrictions are on the label of the product, accidental exposures can occur. Therefore, we exposed adult cattle and sheep to cadmium as Cadminate in feed for the following objectives: (1) to determine the clinical signs of toxicity in cattle and sheep given cadmium at different levels in their feed; (2) to determine the effect on hemopoietic systems; (3) to determine the amount of cadmium present in blood, urine, and hair at various times during the study; and (4) to determine the accumulation of residues of cadmium in various tissues of cattle and sheep after long-term exposures.

The levels of the fungicide fed in feed to cattle and sheep were based on the cadmium content of the formulation.

MATERIALS AND METHODS

Reagents. Cadminate, 60% active ingredient of cadmium succinate (29% cadmium equivalent), was obtained from Mallinckrodt Chemical Works, St. Louis, Mo. Dipotassium ethylenediaminetetraacetate (K₂EDTA) was obtained from Eastman Kodak Co., Rochester, N.Y. Isoton and Zap-Isoton were obtained from Coulter Diagnostics, Inc., Hialeah, Fla. Cyanmethemoglobin was obtained from Hycel, Inc., Houston, Tex. The reagents for the Echols-ultra-micro method were obtained from Echols Professional Products Co., Houston, Tex.

*U.S. Livestock Insects Laboratory, Agricultural Research Service, U.S. Department of Agriculture, Kerrville, Texas 78028.

Apparatus. A Model 403 atomic absorption spectrophotometer (Perkin-Elmer Corp., Norwalk, Conn.) set at 228.8 nm and equipped with a cadmium hollow cathode lamp, HGA-2000 Graphite Furnace, and deuterium background corrector was used to determine cadmium. A Coulter Counter Model B (Coulter Electronics, Hialeah, Fla.) was used to obtain erythrocyte and leucocyte counts. An Adams Autocrit Centrifuge (Clay Adams, Inc., New York, N.Y.) was used to determine the packed cell volumes. A Spectronic 20 (Bausch and Lomb, Rochester, N.Y.) was used in the hemoglobin and blood urea nitrogen determinations.

Treatment of Animals. Twelve mature cows of mixed breed and 12 mature ewes of mixed breed in clinically normal condition were used in this study. The average initial weight of the cows was 270 kg, and that of the ewes was 44 kg. Two of the cows and two of the sheep served as controls. The other ten animals of each species were divided into five groups of two each. Feed containing 50, 100, 200, 300, and 500 ppm of cadmium (as Cadminate) was fed to the treated animals. These levels of cadmium were selected because the 50- and 100-ppm levels are equivalent to what an animal might receive if exposed to treated turf. The levels greater than 100 ppm were selected primarily for toxicological study. An average mix of the feed ration consisted of 28.15% ground corn, 28.15% crimped oats, 10.3% cottonseed meal, and 33.4% rough ground hegarai hay.

Each test animal was weighed weekly, and the amount of feed and cadmium to be given daily was altered, depending on weight gain or loss. The amount of feed was based on 3% of the animal's body weight and was divided into two equal parts; one part was fed in the morning and the other in the afternoon. Cadmium as Cadminate was added to the feed at the selected level. Any of the feed not consumed at one feeding was added to the next unless it equaled or exceeded one-half of the total ration; then this amount alone was provided at the next feeding. The animals were fed separately in individual stalls but were confined to the same pen after feeding. Total feed consumption, weight gain or loss, and exposure to cadmium were recorded for each animal. The data on feed conversion (grams change/kilogram of feed consumed) were subjected to a two-factor analysis of variance with dosage level and length of time in months of treatment. The cattle study continued for 49 weeks and the sheep study for 41 weeks. Animals were exposed to bulls or rams before and after the study began. The treatment data for the animals are given in Table I.

Sampling and Analysis. Samples of blood, urine, and hair were obtained before the start of the study and at monthly intervals during the study to determine cadmium content. Additional samples of blood were collected in tubes containing K₂EDTA, an anticoagulant, to determine erythrocyte (RBC) and leucocyte (WBC) counts, packed cell volume (PCV), and hemoglobin (Hb). Other samples of blood with heparin as the anticoagulant were collected to determine the blood urea nitrogen (BUN). For hair samples, the same area (upper rib area on left side) of the animal was sheared each time. Samples of tissues were obtained from each animal at slaughter or at death. Tissue samples included liver, kidney, heart, muscle, brain, and bone (femur). Samples of tissues were ground in an electric meat grinder. The bones were cut into small pieces with a bone cutter. All samples were frozen until analyzed.

Cadmium in blood and urine was determined by the method of Wright and Riner (1975). Duplicate samples of blood and urine were diluted with deionized-distilled

Table I. Treatment Data for Animals in Cadminate Study

Animal no.	Dosage, ppm in feed ^a	No. of daily doses	Av dosage of Cd to animal, mg/kg of body wt per day
Cattle			
1	50	343	0.7
2	50	343	1.2
3	100	343	2.3
4	100	343	2.2
5	200	343	4.1
6	200	343	4.2
7	300	273 ^b	5.5
8	300	343	4.9
9	500	343	4.4
10	500	140 ^c	6.0
11-12	0	0	0
Sheep			
1	50	287	1.1
2	50	287	0.9
3	100	287	2.1
4	100	287	1.4
5	200	287	4.2
6	200	287	2.7
7	300	287	4.3
8	300	287	4.6
9	500	182 ^b	8.0
10	500	274	4.7
11-12	0	0	0

^a Dosage calculated as cadmium and administered as Cadminate (cadmium succinate, 29% cadmium). ^b Died after last dose. ^c Slaughtered.

water, and aliquots were pipetted into the graphite furnace of the atomic absorption spectrophotometer for quantitation.

Samples of hair (5 g) were digested with 10 ml of nitric acid (HNO₃) until the brown fumes stopped generating during refluxing. After cooling, the digested samples were filtered through glass wool and made to volume with deionized-distilled water. Then these samples were analyzed the same as blood and urine.

Wool samples were washed in hot Kwip (Kwip Industries, Santa Monica, Calif.) solution twice to remove dirt and grease, and then rinsed with tap water and with deionized-distilled water. Samples were air-dried, and duplicate 0.5-g samples were digested with HNO₃ in an 80 °C water bath according to our modification of the procedure of Ullucci and Hwang (1973). Duplicate 1-g samples of tissues, including bone, were digested similarly. Final analysis was by atomic absorption spectrophotometry with the flame technique. The average recovery of cadmium from tissues of both cattle and sheep fortified with cadmium as Cadminate was 101.9%.

The RBC counts on the samples of blood were made with a Coulter Counter after appropriate dilutions with Isoton. Blood of cattle required a 1:50 000 dilution, and blood of sheep required a 1:100 000 dilution. The WBC counts in blood of both cattle and sheep were made after a 1:500 dilution of blood with Isoton and the addition of the hemolyzing agent, Zap-Isoton. The Hb contents of the samples of blood were determined by the cyanmethemoglobin method, with the Spectronic 20. The Echols-ultra-micro method for urea nitrogen was used to analyze for BUN in the plasma from heparinized blood.

RESULTS AND DISCUSSION

Hematologic. In cattle, dietary concentrations greater than 200 ppm of cadmium as Cadminate in feed decreased the RBC, PCV, and Hb values. In sheep, levels greater than 100 ppm of cadmium in feed decreased RBC, PCV, and Hb. The 200-ppm level in feed was an exception. Because the RBC decreased, the decreases in both the

Table II. Effects of Chronic Exposures to Cadmium^a on the Reproduction of Cattle Dosed Daily in Feed for 49 Weeks

Animal no.	Dosage, ^b ppm	Wt change, kg	Total, 49 weeks or less		Results
			Feed, kg	Cd, g	
1	50	163.2	2427.8	121.39	Abnormal calf born; calf slaughtered 3 days later
2	50	75.5	1749.8	87.49	Dead calf born with difficult delivery
3	100	136.4	2250.1	225.01	Dead calf
4	100	60.4	2279.4	227.94	Dead calf
5	200	196.0	2753.5	550.70	Dead premature calf
6	200	19.0	1272.6	254.52	Aborted fetus ^c
7	300	-7.7	1436.2	430.86	Dead immature calf; cow died 14 days later (40th week)
8	300	59.1	1522.2	456.66	Aborted fetus ^c
9	500	-12.7	1396.7	698.35	Two aborted fetuses, ^c from cow within 10-month span
10	500	-60.4	302.0	151.0	Aborted fetus ^c ; cow slaughtered 25 days later
11	0	231.3	2596.2	0	Aborted fetus
12	0	192.7	2422.7	0	Viable calf; calf died 20 days later

^a Cadmium (Cd) administered as Cadminate (cadmium succinate, 29% cadmium) in feed. ^b Daily dose based on average weekly weight of animal. ^c Fetuses in second trimester of gestation or less.

Table III. Effects of Chronic Exposures to Cadmium^a on the Reproduction of Sheep Dosed Daily in Feed for 41 Weeks

Animal no.	Dosage, ^b ppm	Wt change, kg	Total, 41 weeks or less		Results
			Feed, kg	Cd, g	
1	50	3.9	233.9	11.89	Normal lamb born with difficult delivery and died
2	50	12.7	275.8	14.12	Ewe infertile
3	100	-1.4	266.3	27.32	Normal lamb born but died after 15 weeks because of urinary calculi complications
4	100	-4.8	195.4	19.80	Viable immature lamb with malformation but survived
5	200	-9.5	278.8	56.59	Viable immature lamb with malformation but survived
6	200	8.7	239.6	48.48	Dead lamb with corneal opacity
7	300	1.1	226.0	69.45	Aborted fetus ^c with keratitis
8	300	-4.5	176.6	53.88	Ewe infertile
9	500	-21.6	32.7	17.45	Aborted fetus ^c with keratitis; ewe died 7 weeks later because of parturition (26 weeks of exposure)
10	500	-20.2	81.7	42.4	Aborted fetus ^c with keratitis; ewe died from nonutilization of feed in 39th week of exposure
11	0	9.0	341.7	0	Viable normal lamb
12	0	11.8	341.7	0	Viable normal lamb

^a Cadmium (Cd) administered as Cadminate (cadmium succinate, 29% cadmium) in feed. ^b Daily dose based on average weekly weight of animal. ^c Fetuses in second trimester of gestation or less.

PCV and Hb were expected. This anemia has been previously observed by researchers in other species exposed to cadmium-containing compounds (Wilson et al., 1941; Friberg, 1950; Decker et al., 1958).

Only one cow (500 ppm) had WBC counts that decreased during the study. The rest of the cattle and sheep had WBC counts similar to those of the control animals.

At dietary concentrations greater than 200 ppm, both cattle and sheep had BUN levels that increased near the end of the study. The BUN levels increased gradually in the blood of cattle but increased sharply in blood of sheep fed dietary concentrations greater than 200 ppm.

Toxicologic Effects. The toxic effects included aborted fetuses, damage to reproductive organs, anemia, neonatal deaths, and calves and lambs with birth defects. These effects had been reported previously in laboratory animals (Friberg et al., 1971) but had not been reported in cattle and sheep.

Cattle. Four cows aborted five times in the first or second trimester of gestation (Table II). All of these abortions were from cows fed levels greater than 100 ppm; one cow given 500 ppm aborted twice. Complications after an abortion in the other cow given 500 ppm caused prostration, and the cow was slaughtered.

One viable calf was born from the treated cows, but it could not stand without assistance and was unable to nurse; subsequently, it was slaughtered. Five cows delivered dead calves; one of the cows died from complications at parturition. Another cow, after embryotomy and

chemotherapy, survived with the assistance of appropriate chemotherapeutic agents to counteract mechanical damage and subsequent infection from the operation.

One control cow aborted a fetus, whereas the other produced a viable (apparently normal) calf that survived only 20 days before an unexpected death.

At necropsy of the treated cow that died and the one that was slaughtered, gross pathological effects consisted of swollen uteri with reddened endothelium, swollen orange-tinted livers, firm spleens with black matrix, and light-pink bone marrow. No outstanding lesions were observed in the dead fetuses and calves or in the other cattle at the end of the trials.

We found no significant difference in feed conversion efficiency due to the dosage level, the length of time in months of treatment, or an interaction between the two. Although the trend was toward poorer feed conversion efficiencies as the dosage level increased, the variation between the two cattle in each dosage level was too great to detect true dosage level differences.

Sheep. Two of the treated ewes were never observed to be bred and showed no signs of pregnancy during the trials; therefore, these animals were considered infertile (Table III). Three viable lambs were born. One of these died at 15 weeks of age because of uremic toxicity resulting from the rupture of the urethra caused by urinary calculi. In earlier experimentation with laboratory animals, Friberg et al. (1971) showed that this toxic condition resulted from increased urinary excretion of minerals, caused by the

action of the cadmium. The other two viable lambs were malformed; one had partial lateral rotation of both forelimbs, and the other had a spinal malformation. Both survived with normal weight gains to the end of the trials.

Of the sheep fed 200, 300, or 500 ppm, three aborted fetuses in the second trimester of gestation and one ewe delivered a dead lamb. Keratitis was present in three of five offspring.

The two control ewes produced two normal viable lambs that developed with expected weight gains to the end of trials.

The two ewes fed 500 ppm died. At necropsy, the bone marrow had a white jelly-like consistency and only a slight reddish tinge. The ewe that aborted 7 weeks before death had an enlarged uterus with a thickened, brownish endothelium. The other ewe became severely emaciated during the last 4 weeks before death although feed was still being consumed at approximately the same rate as earlier. The condition was considered to be due to the improper utilization of feed that developed because of the exposures. No significant lesions were observed at necropsy in the ewes fed 300 ppm or less, or in the dead lambs and fetuses at the end of the trials or at necropsy.

The difference in feed conversion efficiency due to the dietary level of cadmium in the feed was significant ($p \leq 0.05$). As the dietary level of cadmium in feed increased, the feed conversion efficiency decreased. The mean grams of change per kilogram of feed consumed were 67.5, 64.5, 32.0, 27.0, 16.0, and -306.0 for dosage levels of 0, 50, 100, 200, 300, and 500 ppm, respectively. The difference due to the length of time of treatment in relation to feed conversion was significant ($p \leq 0.05$). We observed a general trend toward increased feed conversion efficiencies with increased length of time the sheep were on the treated feed. Also, an interaction between feed conversion and both the dietary level and length of time on treatment was significant. The interaction is represented by a difference in response over time between particular dosage levels. Sheep given feed with 500 ppm of cadmium showed a more rapid rate of increase in feed conversion efficiency over time than sheep given feed with lower levels of cadmium because, in initial stages of the test, the sheep refused to eat the feed with 500 ppm of cadmium and were consuming at a level below maintenance diet. They began to consume more feed as the length of time of treatment progressed. (This change in consumption is probably the reason for the difference observed in both factors—dietary level at time 0 and for the interaction between dosage and time.)

Residues. Cattle. The cadmium found in the blood of treated and control cattle remained about the same throughout the study and averaged 0.04 ppm.

The residues of cadmium found in the urine of the cattle on test were inconsistent. The cadmium levels of two animals that died increased to 0.7 ppm just before death. The average pretreatment level of cadmium in urine was <0.15 ppm, which indicated an increase of cadmium in urine. Total urine was not collected, so we were unable to determine the exact amount of cadmium excreted within a 24-h collection period. Variability of water intake and urinary output might explain the inconsistent results obtained.

The cadmium found in the hair of cattle before treatment averaged about 0.6 ppm. Although the animals were individually fed, they were allowed to share the same pen and water trough. The average residue of cadmium in the controls increased to 16 ppm, and the average residue of cadmium in dosed cattle increased to 46 ppm. Therefore,

Table IV. Residues of Cadmium in Tissues of Cattle and Sheep Dosed Daily in Feed

Animal no.	Dosage, ppm in feed	Cadmium, ppm ^a in indicated tissue		
		Liver	Kidney cortex	Kidney medulla
Cattle				
1	50	34.0	228.3	125.0
2	50	18.0	117.0	71.5
3	100	58.8	218.5	77.0
4	100	61.3	210.0	119.0
5	200	61.3	232.5	135.0
6	200	97.5	160.0	70.0
7	300	41.8	170.0	79.5
8	300	85.0	227.5	44.5
9	500	160.0	200.0	73.0
10	500	35.5	115.0	72.0
11	0	0 ^b	1.5	0
12	0	0	1.5 ^c	d
Sheep				
			Total kidney	
1	50	39.5	139.0	
2	50	147.5	227.5	
3	100	145.0	209.0	
4	100	107.5	207.5	
5	200	240.0	236.5	
6	200	170.0	389.0	
7	300	492.5	118.0	
8	300	462.5	52.5	
9	500	550.0	184.5	
10	500	600.0	96.5	
11	0	2.0	4.3	
12	0	d	4.3	

^a Figures not corrected for percentage of recovery.

^b Zero indicates residues of cadmium <0.5 ppm. ^c Total kidney (cortex and medulla combined) was used. ^d No sample available.

contamination due to dirt, feces, and other contaminants could have been responsible for part of the increase in the residues of cadmium in the hair of the control cattle. Residues of cadmium in hair of treated cattle increased as the dietary level increased, and in the animals that died, the level reached 100 ppm before death. The average residues of cadmium in hair for the 50-, 100-, 200-, 300-, and 500-ppm dietary levels were 15, 21, 57, 63, and 88 ppm, respectively.

Table IV shows the residues of cadmium in the tissues and bone of cattle in the study. The greatest residues of cadmium were in the kidney cortex, then in kidney medulla, and then in liver. This finding agrees with previous work (Cousins et al., 1973; Nordberg, 1972; Powell et al., 1964). Muscle, brain, and bone had no detectable residues of cadmium, and heart had detectable amounts (<1.0 ppm) in some samples. No residues were detected in tissues of calves born to the cattle on test. According to Friberg et al. (1971), the placenta is a barrier to the transfer of cadmium from the mother to the fetus when low doses of cadmium are given, but high doses of cadmium can overcome the placental barrier and enter the fetus and thus result in the deposition of cadmium.

Sheep. The amount of cadmium present in the blood of treated sheep increased to 0.1 ppm, and levels remained near 0.02 ppm in the control sheep. At 500 ppm, one ewe that died after 6 months had 0.2 ppm of cadmium in the blood at death; the other one survived until slaughter, when the residues of cadmium in blood had increased to >2.0 ppm.

As with cattle, the cadmium content in the urine was inconsistent. Most of the higher residues of cadmium were found during the latter months of the study. The two sheep given 500 ppm had residues that increased to about

1.0 ppm just before death or at the last sampling period. The residues of cadmium in the urine of control sheep were generally <0.01 ppm, but occasionally levels of >0.03 were found.

The pretreatment level of cadmium in the wool of both treated and control sheep was <1.0 ppm. The level of cadmium increased in the control sheep as well as in the treated sheep. The greatest residue of cadmium in the wool of the treated sheep was >20.0 ppm and in the control sheep was >10.0 ppm. Again the residues were inconsistent. Because the wool samples were washed and dried before analysis, the external contamination of the wool due to dirt, feces, urine, and other contaminants should have been eliminated. Possibly the cadmium from the external contamination absorbed into the hair of the control sheep.

The residues of cadmium found in the tissues and bone of sheep are summarized in Table IV. Residues were greatest in the liver and kidney of the treated sheep. At the lower dosages of 50 and 100 ppm, the residues of cadmium were greater in kidney than in liver, whereas, at the higher dosages (300 and 500 ppm), the residues in the liver were much greater. This difference may be due to renal damage that resulted in poorer excretion of cadmium and greater deposition in the liver. Increased BUN's at the end of the study in the sheep given the greatest dosages indicate possible kidney damage. Of the other tissues, heart had the greatest residues followed by muscle, brain, and bone. Liver and kidney of control animals had residues of >2.0 ppm. Liver and kidney samples of three lambs born during the study to ewes no. 1, 3, and 9 were analyzed. The lamb from no. 1 had no detectable residues. The lamb from no. 3 had residues of 1.3 ppm in the liver and 2.0 ppm in the kidney. The lamb from no. 9 had no detectable residues in the kidney but had 1.0 ppm in the

liver. As mentioned earlier, residues of cadmium can cross the placental barrier from mother to fetus if doses are high (Friberg et al., 1971).

This research has shown that residues of cadmium will accumulate in tissues of both cattle and sheep given a cadmium fungicide in their feed. Cadmium fed at dietary concentrations of greater than 200 ppm causes possible kidney damage as indicated by the increase in BUN levels. The placenta, which serves as a barrier to low doses of cadmium, can be overcome with high doses of cadmium, and residues of cadmium will appear in the tissues of fetuses.

LITERATURE CITED

- Cousins, R. J., Barber, A. K., Trout, J. R., *J. Nutr.* 103, 964 (1973).
 Decker, L. E., Byerrum, R. U., Decker, C. F., Hoppert, C. A., Langham, R. F., *AMA Arch. Ind. Health* 18, 22 (1958).
 Friberg, L. T., *Acta Med. Scand.* 138, 240 (1950).
 Friberg, L. T., Piscator, M., Norberg, G. F., "Cadmium in the Environment", CRC Press, Cleveland, Ohio, 1971.
 Nordberg, G. F., *Environ. Phys. Biochem.* 2, 7 (1972).
 Powell, G. W., Miller, W. J., Morton, J. D., Clifton, C. M., *J. Nutr.* 84, 205 (1964).
 Ullucci, P. A., Hwang, J. Y., Pittsburg Conference on Analytical Chemistry and Applied Spectroscopy, Cleveland, Ohio, March 1973.
 Wilson, R. H., DeEds, F., Cox, A. J., *J. Pharmacol. Exp. Ther.* 71, 22 (1941).
 Wright, F. C., Riner, J. C., *At. Absorpt. Newsl.* 14, 103 (1975).

Received for review June 25, 1976. Accepted November 29, 1976. This paper reflects the results of research only. Mention of a pesticide or a proprietary product in this paper does not constitute a recommendation or an endorsement of this product by the U.S. Department of Agriculture.

Vanadium Content of Selected Foods as Determined by Flameless Atomic Absorption Spectroscopy

Duane R. Myron, Samuel H. Givand, and Forrest H. Nielsen*

The vanadium contents of seven classes of foodstuffs were determined by atomic absorption spectrometry by use of a graphite furnace atomizer with deuterium arc background correction. Beverages, fats and oils, and fresh fruits and vegetables contained the least vanadium, ranging from <1 to 5 ng/g. Whole grains, seafood, meats, and dairy products were generally within a range of 5 to 30 ng/g. Prepared foods ranged from 11 to 93 ng/g while dill seed and black pepper contained 431 and 987 ng/g, respectively.

Studies by four laboratories have provided evidence that vanadium is an essential nutrient for both mammals and birds (Hopkins and Mohr, 1971; Schwarz and Milne, 1971; Strasia, 1971; Nielsen and Ollerich, 1973). The probability that vanadium is also essential in human nutrition has renewed interest in the dietary availability of this element. Information is scarce on the vanadium content of foodstuffs. For evaluation of vanadium nutriture, such information would be required, and a single reliable method for determining vanadium in a wide variety of foodstuffs would be a critical prerequisite.

Vanadium qualifies as a "micro" trace element in that it is present in biological specimens at a range from less than 1 to several ng/g (Bertrand, 1941; Schroeder et al., 1963; Söremark, 1967; Christian, 1971; Welch and Allaway, 1972). Thus, a sensitive method is necessary. The wide range of vanadium contents reported for similar materials (e.g., the colorimetric procedure of Schroeder et al. (1963) compared to the neutron activation method of Söremark (1967)) emphasizes the need for a precise and accurate method of analysis.

Atomic absorbance spectroscopy, with the flameless graphite furnace technique, provides an increase in sensitivity of approximately three orders of magnitude compared to conventional flame methods for vanadium. The benefits of the graphite furnace also include a potential for the elimination of interfering substances through selective atomization. This technique is particularly

*United States Department of Agriculture, Agricultural Research Service, Human Nutrition Laboratory (F.H.N), and Department of Biochemistry, University of North Dakota, Grand Forks, North Dakota 58201.