Table VII. Comparison of Mean Cadmium and Lead $Levels^a$ in Five Crops

refer enc e	lettu c e	potatoes	soy- beans	sweet corn	wheat
		Cadmiı	ım		
this study	0.026	0.031	0.059	0.0031	0.043
FDA ^b	0.048	0.037	0.092	0.018	0.065
other	0.048^{c}	0.0499 ^c		0.0065^{d}	0.066 ^e
studies	0.0295^{c}	0.0448^{c}			0.096 ^e
		Lead			
this study	0.013	0.009	0.042	0.0033	0.037
FDA ^b	0.075	0.038	0.095	0.018	0.115
other	0.029^{d}	0.0666		0.22^{d}	
studies	0.033, 0.62	0.0749 ^c			

^a μg/g of wet weight. ^b Compliance Program Evaluation (1977). ^c Kaferstein (1980). ^d Shacklette (1980). ^e Andersson and Pettersson (1981).

in the same soils and geographical area. Other factors that could have contributed to variation in metal concentration were soil, climate, and fertilization practices.

Table VII compares the data from this study and other major surveys conducted by FDA or reported by other workers. Levels of Cd and Pb found in this study are generally much lower than those previously reported. A variety of factors may be responsible for this: selection of relatively uncontaminated fields, careful sampling and handling, and laboratory equipment and practices that reduce contamination of samples during analysis. An important consideration in evaluating the values for Pb found in other studies is whether the Pb in processed foods had been present when the crop was harvested from the field or had been added by canning, handling, packaging, or other food processing or distribution procedures.

ACKNOWLEDGMENT

The contributions of the following individuals, who participated in the planning and evaluation of this program, are gratefully acknowledged: R. Daniels, Soil Conservation Service (SCS), USDA (retired), and R. Chaney, Agricultural Research Service, USDA, Washington, DC; K. Dotson, EPA, Cincinnati, OH; A. L. Page, University of California, Riverside. Soil scientists of the field staff of the SCS selected sites and collected and shipped all crop and soil samples. Registry No. Cadmium, 7440-43-9; lead, 7439-92-1.

Supplementary Material Available: Complete tabulation of individual data (with part 2), additional statistics, histograms, and probability (SAS computer printouts) (12 pages). Ordering information is given on any current masthead page.

LITERATURE CITED

- Andersson, A.; Pettersson, O. Swed. J. Agric. Res. 1981, 11, 49. Compliance Program Evaluation "Compliance Program Report of Findings, FY-77 Pesticides and Metals Program (7320.79)"; U.S. Food and Drug Administration: Washington, DC, 1977.
- Compliance Program Evaluation "Compliance Program Report of Findings, Total Diet Studies-Adult (7305.003)"; U.S. Food and Drug Administration: Washington, DC, 1978.
- Council for Agriculture, Science, and Technology, Ames, IA, 1976, Report No. 64.
- Council for Agriculture, Science, and Technology, Ames, IA, 1980, Report No. 83.
- Falkoff, A. D.; Iverson, K. E. IBM J. Res. Dev. 1973, 17, 324.
- Fed. Regist. 1979a, 44 (179), 53438.
- Fed. Regist. 1979b, 44 (148), 44940.
- Holmgren, G. G. S.; Chaney, R. L.; Meyer, M. W.; Kubota, J.; Fricke, F. L. "Soil Survey Investigations Report"; Soil Conservation Service, U.S. Department of Agriculture: Washington, DC, 1983.
- Jelinek, C. F.; Braude, G. L. J. Food Prot. 1978, 41, 476.
- Kaferstein, F. K. Zentralbl. Bakteriol., Parasitenkd., Infektionskr. Hyg., Abt. 1: Orig., Reihe B 1980, 171, 352.
- Kienholz, E. W.; Ward, G. M.; Johnson, D. E.; Daxter, J.; Braude, G.; Stern, G. J. Anim. Sci. 1979, 48, 735.
- Pfeilsticker, K.; Maskard, C. Z. Lebensm.-Unters. -Forsch. 1975, 158, 129.
- Reith, J. F.; Engelsma, J.; Van Ditmarsch, M. Z. Lebensm.-Unters. -Forsch. 1974, 156, 271.
- SAS "SAS User's Guide"; SAS Institute: Cary, NC, 1979.
- SAS "SAS Technical Report P-115"; SAS Institute: Cary, NC, 1981.
- Satzger, R. D.; Clow, C. S.; Bonnin, E.; Fricke, F. L. J. Assoc. Off. Anal. Chem. 1982, 65, 987.
- Shacklette, H. T. "U.S. Geological Survey, Professional Paper 1178"; U.S. Government Printing Office: Washington, DC, 1980.
- Thomas, B.; Roughan, J. A.; Watters, E. D. J. Sci. Food Agric. 1972, 23, 1493.

Received for review March 18, 1983. Accepted July 13, 1983. The mention of proprietary products, materials, or trade names does not imply endorsement by the U.S. Government.

Elements in Major Raw Agricultural Crops in the United States. 2. Other Elements in Lettuce, Peanuts, Potatoes, Soybeans, Sweet Corn, and Wheat

Karen A. Wolnik, Fred L. Fricke, Stephen G. Capar,* George L. Braude, Milton W. Meyer, R. Duane Satzger, and Roy W. Kuennen

Six raw agricultural crops (lettuce, peanuts, potatoes, soybeans, sweet corn, and wheat) were collected from fields in major U.S. growing areas and were analyzed for Ca, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, P, Se, and Zn. Statistical frequency distributions of some of the major elements were normal.

In part 1 (Wolnik et al., 1983), the Pb and Cd content of six major agricultural crops was reported based on a large sampling and carefully controlled analytical program. While Cd and Pb are toxic and obviously undesirable in food crops, other major and minor elements are of interest for different reasons. Several elements, such as Ca, Fe, K, Mo, Na, Ni, and P, are essential for human and animal health, and knowledge about their levels in different raw foods will provide information on the nutritional adequacy of diets. Others, such as Cu, Se, and Zn, though essential,

Food and Drug Administration, Cincinnati, Ohio 45202 (K.A.W., F.L.F., R.D.S., and R.W.K.), and Washington, DC 20204 (S.G.C. and G.L.B.), and Soil Conservation Service, U.S. Department of Agriculture, Washington, DC 20013 (M.W.M.).

Table I. Multielement Recovery and Results for Standard Reference Materials^a

	swe	et c o rn	pea	nuts				
ele-	amount	recovery	amount added	recovery.	NBS Sp	inach 1570	NBS Whea	at Flour 1567
ment	μg/g	$\%, \pm \mathrm{SD}^{b}$	μg/g	$\%, \pm \mathrm{SD}^c$	certified, $\mu g/g$	results, $\mu g/g^d$	certified, $\mu g/g$	results, $\mu g/g^e$
Ca	500	100 ± 2	1000	101 ± 2	13 500 ± 300	12900 ± 300	190 ± 10	196 ± 3
Cu	10	98 ± 2	10	101 ± 3	12 ± 2	11.8 ± 2	2.0 ± 0.3	2.03 ± 0.05
Fe	10	100 ± 5	10	99 ± 7	550 ± 30	530 ± 11	18.3 ± 1.0	18.6 ± 1.5
K	5000	97 ± 5	5000	103 ± 3	35600 ± 300	35200 ± 1000	1360 ± 40	1320 ± 40
Mg	500	97 ± 5	1000	101 ± 4	f	8600 ± 200	f	401 ± 8
Mn	10	99 ± 2	10	98 ± 4	165 ± 6	165 ± 3	8.5 ± 0.5	8.42 ± 0.23
Mo	10	99 ± 2	10	96 ± 3	f	< 0.25	0.4^{g}	0.396 ± 0.05
Na	400	96 ± 2	f	f	f	f	8.0 ± 1.5	f
Ni	10	99 ± 2	10	100 ± 2	6 ^g	5.51 ± 0.32	0.18^{g}	f
Р	1000	100 ± 4	2000	103 ± 3	5500 ± 200	5300 ± 70	f	1390 ± 30
\mathbf{Se}	0.05	99 ± 16	0.05	99 ± 16	f	f	1.1 ± 0.2	0.90 ± 0.05
\mathbf{Zn}	10	101 ± 4	10	102 ± 7	50 ± 2	49.8 ± 1.3	10.6 ± 1.0	11.0 ± 0.3

^a Based on dry weight. ^b Mean of 29-30 recovery determinations. ^c Mean of 21-30 recovery determinations. ^d Mean of 14 determinations. ^e Mean of 29 determinations, except for Se, where mean of 50 determinations. ^f Not determined. ^g Noncertified value.

Table II. Elements in Lettuce^a

	<u></u>					
element	mean	median	minimum	maximum	CV	$normality^b$
Са	210	170	110	610	48.1	< 0.01 S
Cu	0.26	0.24	0.065	0.76	47.2	<0.01 S?
Fe	3.0	2.2	1.3	30	100.7	$< 0.01 \ S$
K	1700	1500	900	4500	37.7	<0.01 S?
Mg	83	71.5	42.0	210	39.8	<0.01 S?
Mn	1.8	1.25	0.23	11	99.8	<0.01 S
Mo^{c}	0.013	0.0067	< 0.0039	0.19	202.3	<0.01 S
Na^d	74	66	14	410	83.2	< 0.01
P	220	215	130	490	23.7	<0.01 N1
Se ^e	0.0016	0.00066	< 0.0004	0.011	134.4	<0.01 S
Zn	1.9	1.8	0.51	5.1	43.8	<0.01 N1
\mathbf{wwf}^{f}	0.0415	0.040	0.026	0.084	19.0	

^a n = 150. ^b P value for the Kolomogorov-Smirnov-D statistic and frequency distribution as judged by visual observation of the normal probability plot (S = skewed; S? = questionable skewed; N1 = normal except for < 1% of the data). ^c Sixtythree results below the detection limit. ^d Thirty-four results below the quantitation limit. ^e Fifty-three results below the quantitation limit. ^f Wet weight factor.

have a limited range between required and toxic levels. Other concerns are the interactions between elements, which have been postulated as affecting the toxicity, the level of absorption, and possibly the retention of Cd and Pb by plants (Welch, 1978) and animals (Fox, 1974).

The Food and Drug Administration (Compliance Program Evaluation, 1975, 1978) has determined levels of As, Cd, Hg, Pb, Se, and Zn in a variety of foods. A recent paper from the U.S. Geological Survey (Erdman and Moul, 1982) reported on the analysis of several cultivars of small grains for many elements. An earlier report from the same organization (Shacklette, 1980) presented extensive data on many elements in vegetables, fruits, and soils collected in 1972–1973. In a few other studies, crops (usually a small number of samples) were analyzed for selected elements (Schlettwein-Gsell and Mommsen-Straub, 1970; Lorenz and Loewe, 1977; Thomas et al., 1974; Pfeilsticker and Maskard, 1975; Reith et al., 1974). In none of the studies reported, however, was an attempt made to systematically exclude the effect of human activities to obtain "background" values for these elements. Most work included a limited number of samples of each crop variety so that meaningful statistical data could not be developed. The purpose of this work was to obtain accurate background values for major crops and (later) to investigate correlations with soil element levels, crop species, and other factors that may influence mineral uptake.

MATERIALS AND METHODS

Selection of the sampling sites, techniques for the collection of crop samples, and sample preparation were described in part 1 (Wolnik et al., 1983). Experimental details given below are specific for the determination of Se by hydride generation and for other elements by inductively coupled plasma atomic emission spectroscopy (IC-PAES).

Selenium Determination. Portions of the sample composite were digested with a mixture of HNO_3 , $HClO_4$, H_2SO_4 , and NH_4VO_3 catalyst, and Se was determined by hydride generation/condensation-flame atomic absorption spectrometry according to the method of Hahn et al. (1981). A duplicate sample and spiked sample recovery were carried through the method with every set of 10 samples. Typical recoveries are summarized in Table I.

Multielement Determination. An HNO_3 , $HClO_4$, and H_2SO_4 digestion was used for the ICPAES determination of 11 elements (Ca, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, P, and Zn) in composites of lettuce, potatoes, soybeans, sweet corn, and wheat (Wolnik et al., 1981). For multielement analysis of peanuts, HCl pressure dissolution and ICPAES (Kuennen et al., 1982) were used.

For all six crops, a blank, duplicate sample, and spiked sample recovery were run with every 10 samples. When appropriate, a National Bureau of Standards (NBS) Standard Reference Material was analyzed instead of a spiked sample recovery. A summary of these results is

Table	ш	Elements	in	Peanuts ^a
rabic	777+	LICINCIIUS		i canuto

		μg/g wet					
element	mean	median	minimum	maximum	CV	normality ^b	
Ca	400	380	110	890	40.4	<0.01 N	
Cu	7.6	7.25	0.80	19	38.8	<0.01 N1	
Fe	18	18	7.8	29	22.8	<0.01 N	
K	6600	6500	3300	9900	23.3	>0.15 N	
Mg	1600	1700	800	2300	19.7	<0.01 N2	
Mn	16	16	3.8	52	28.8	<0.01 N1	
Mo^c	0.43	0.25	< 0.032	3.95	129.1	<0.01 S	
Ni^d	2.0	1.4	< 0.15	14	107.0	<0.01 S	
Р	3400	3500	1600	4900	20.7	<0.01 N2	
Se	0.057	0.036	0.002	0.91	130.8	<0.01 S	
Zn	28	28	12	56	25.7	0.025 N1	
\mathbf{wwf}^{e}	0.8795	0.9515	0.428	0.999	16.0		

^a n = 320. ^b P value for the Kolomogorov-Smirnov-D statistic and frequency distribution as judged by visual observation of the normal probability plot (N = normal; S = skewed; N1 = normal except for <1% of the data; N2 = normal with slight negative skewness). ^c Forty results below the detection limit. ^d Seven results below the detection limit. ^e Wet weight factor.

Table IV.	Elements in	Potatoes ^a
-----------	-------------	-----------------------

		µg/g wet				
element	mean	median	minimum	maximum	CV	normality ^b
Са	60	55	17	180	44.9	< 0.01 N1
Cu	0.96	0.86	0.14	2.7	52.1	< 0.01 S2
Fe	3.85	3.9	1.3	6.6	21.4	0.01 N1
K	4100	4100	2500	5500	11.1	<0.01 N
Mg	200	200	100	290	16.2	<0.01 N
Mn	1.4	1.3	0.72	3.7	34.2	<0.01 S
Mo^c	0.036	0.027	< 0.011	0.27	18.3	< 0.01 S
Р	500	480	270	890	21.0	< 0.01 S?
\mathbf{Se}^d	0.0030	0.0011	< 0.002	0.055	176.7	< 0.01 S
Zn	3.1	3.0	1.1	7.0	33.0	<0.01 N1
wwf ^e	0.193	0.193	0.126	0.259	12.7	

^a n = 297. ^b P value for the Kolomogorov-Smirnov-D statistic and frequency distribution as judged by visual observation of the normal probability plot (N = normal; S = skewed; S? = questionable skewed; N1 = normal except for <1% of the data). ^c Seventy-three results below the detection limit. ^d One hundred fifty-three results below the quantitation limit. ^e Wet weight factor.

	μ g/g wet weight					
element	mean	median	minimum	maximum	CV	$normality^b$
Ca	2000	1900	1000	3600	20.7	< 0.01 N1
Cu	12	12	3.5	29	30.4	<0.01 N
Fe	66	65	48	110	14.7	< 0.01 S?
K	18000	18000	14000	21000	6.3	<0.01 N2
Mg	2200	2200	1600	2600	8.7	<0.01 N
Mn	27	24	14	90	30.0	<0.01 S ?
Mo^{c}	2.1	1.1	< 0.06	18	125.7	< 0.01
Ni	4.8	4.5	0.35	29	70.2	<0.01 S
Р	5500	5500	2900	7900	13.6	<0.01 N
Se	0.19	0.075	0.010	2.5	160.3	<0.01 S
Zn	42	41	29	67	15.5	<0.01 N1
\mathbf{wwf}^d	0.996	0.918	0.816	0.953	1.9	

Table V. Elements in Soybeans^a

^a n = 322 except for Se, where n = 316. ^b P value for the Kolomogorov-Smirnov-D statistic and frequency distribution as judged by visual observation of the normal probability plot (N = normal; S = skewed; S? = questionable skewed; N1 = normal except for <1% of the data; N2 = normal with slight negative skewness). ^c Nine results below the detection limit. ^d Wet weight factor.

presented in Table I. The recovery values listed for sweet corn are representative of those obtained for potatoes and soybeans. NBS Spinach 1570 and Wheat Flour 1567 were used as controls for the analysis of lettuce and wheat samples, respectively.

Data Processing. The data output from the ICPAES computer was logged onto a magnetic tape data cartridge (Capar and Dusold, 1982). This data tape was read into an IBM 370/168 computer by using the interactive computer language APL (Capar and Dusold, 1978).

Using APL programs, the results were checked for errors and formated onto a disk file. The files were then processed by using the procedure UNIVARIATE of the Statistical Analysis System (SAS) Release 79.5 (SAS, 1979, 1981). SAS was accessed by way of batch processing with job submission through APL. The SAS UNIVARIATE procedure was applied to each crop for each element. This procedure produces a number of simple descriptive statistics including quantiles, vertical bar charts, normal probability plots, skewness, and median. A test for nor-

Table VI.	Elements	in Sweet	Corna
-----------	----------	----------	-------

	$\mu g/g$ wet weight					$\mu g/g$ wet weight	weight			
element	mean	median	minimum	maximum	CV	\mathbf{n} ormality b				
Ca	22	17	6.4	91	66.6	<0.01 S				
Cu	0.45	0.41	0.19	0.92	36.3	<0.01 S?				
Fe	4.0	3.8	1.3	7.5	30.0	<0.01 N				
K	2900	2800	2100	4800	10.3	<0.01 N1				
Mg	270	270	190	440	18.5	<0.01 N1				
Mn	1.6	1,5	0.71	5.6	39.8	<0.01 S				
Mo ^c	0.048	0.031	< 0.009	0.45	130.1	<0.01 S				
Ni^d	0.062	0.039	< 0.026	0.35	82.0	<0.01 S				
Р	830	820	460	1300	15.5	<0.01 N				
Se^{e}	0.0064	0.0028	< 0.002	0.086	164.7	<0.01 S				
Zn	5.6	5.3	2.6	11	28.7	<0.01 S?				
\mathbf{wwf}^{f}	0.214	0.2145	0.083	0.468	28.4					

^a n = 268. ^b P value for the Kolomogorov-Smirnov-D statistic and frequency distribution as judged by visual observation of the normal probability plot (N = normal; S = skewed; S? = questionable skewed; N1 = normal except for <1% of the data). ^c Fifty-six results below the detection limit. ^d One hundred thirty-two results below the detection limit. ^e Fiftyeight results below the quantitation limit. ^f Wet weight factor.

Figure 1. Histogram and probability plot for peanuts.

mality using the Kolomogorov-Smirnov-D statistics (SAS, 1979) was also performed.

The wet weight factor (wwf) reported in the tables can be used to calculate dry weight results (divide values for elements by wwf) or to calculate moisture content $(1 - wwf \times 100)$.

RESULTS AND DISCUSSION

Data obtained on 10–11 elements in the six crops are presented in Tables II–VII. Nickel was not determined in lettuce, potatoes, and wheat. Statistical information such as means, medians, and coefficients of variation show differences not only in absolute levels but also in variability. The number of samples for each crop ranged from 150 for lettuce to 332 for soybeans. However, a few elements, especially Mo and Ni, were present at levels below detection limits in some of the crops. For Se, 0.01 $\mu g/g$ (dry weight basis) was defined as the quantitation level. In the statistical calculations, half of the detection limit value was substituted for sample results that were below the detection or quantitation limit. ICPAES parameters for Na were selected to provide accuracy at high concentration levels. Consequently, except for lettuce, results for Na were below the quantitation limit in the majority of

Table VII. Elements in Wheat^a

		µg/g we				
element	mean	median	minimum	maximum	CV	normality ^b
 Ca	380	370	180	740	23.7	<0.01 N1
Cu	4.4	4.3	2.2	8.7	28.5	$< 0.01 \ S$
Fe	32.5	32	17	60	22.2	0.07 N1
K	3800	3700	2600	7300	18.5	<0.01 S?
Mg	1400	1400	700	2800	18.3	<0.01 N1
Mn	38	37	13	67	25.5	>0.15 N1
Mo^c	0.40	0.38	< 0.06	1.85	71.2	$< 0.01 \ S$
Р	3400	3400	1400	6000	20.8	0.024 N1
\mathbf{Se}^d	0.37	0.16	< 0.010	5.3	163.1	$< 0.01 \ S$
Zn	27	26.5	9.3	67	35.7	<0.01 N1
\mathbf{wwf}^{e}	0.881	0.883	0.722	0.988	6.3	

^a n = 290 except for Cu, where n = 284. ^b P value for the Kolomogorov-Smirnov-D statistic and frequency distribution as judged by visual observation of the normal probability plot (S = skewed; S? = questionable skewed; N1 = normal except for <1% of the data). ^c Twenty-five results below the detection limit. ^d Twenty-three results below the quantitation limit. ^e Wet weight factor.

samples and therefore are not reported.

As shown in Tables II-VII, data for most major elements such as Ca, K, Mg, and P fall within a fairly narrow range, while for trace elements greater differences exist between minimum and maximum values. Although the absolute concentrations of the elements may vary from sample to sample, crop cultivars display distinct patterns of relative elemental abundance even when grown over wide geographical areas. In each of the crops, except lettuce, the general pattern for the major elements is K > P > Mg >Ca. Lettuce follows a K > P > Ca > Mg pattern, although in 33% of the samples, Ca content exceeds that of P. Thirty-five percent of soybean samples display a pattern similar to that of lettuce, in which Ca > Mg, and in 25% of the wheat samples, P > K.

The minor elements display more variation; however, certain predominant patterns are apparent. Sweet corn and peanuts show a Zn > Fe > Mn > Cu pattern except for 30% of the peanut samples, in which Mn > Fe. Only 15 of 320 peanut samples had Cu > Mn. Those 15 samples came from the same state, although the other 49 samples from that state showed the normal pattern.

Soybeans and potatoes give the pattern Fe > Zn > Mn> Cu. Lettuce shows the same general pattern, but in 33% of the samples the pattern changes to Fe > Mn > Zn >Cu, and in 21% of the samples the pattern resembles that in sweet corn and peanuts. The most frequently occurring pattern for wheat is Mn > Fe > Zn > Cu, although 20% of wheat samples show Fe > Mn and 17% show a Mn >Zn > Fe > Cu pattern.

The general trend for trace elements in these crops is Ni > Mo > Se. Nickel was determined only in sweet corn, peanuts, and soybeans, and oftentimes in those crops the Ni > Mo relationship is reversed. In wheat, Se > Mo in 28% of the samples.

The frequency distribution of elements in plant material has been shown to be positively skewed (Shacklette, 1980). This was also true in the present study for some of the elements, especially for trace elements. Some other elements, especially major nutrients such as Ca, K, and P, as well as Fe, Mg, and occasionally Cu and Zn, showed perfect or near perfect linear statistical distributions. This is expressed by the term "normality" in Tables II-VII and is visually presented for Fe and K in peanuts in Figure 1. These graphs were prepared by using an SAS program (SAS, 1981). [Additional examples are provided as supplementary material (see paragraph at end of paper regarding supplementary material)]. As an additional test, plotting some of the data on arithmetic probability paper also resulted in nearly straight lines. It is not clear what is responsible for the normality of this distribution, which, to our knowledge, has not been reported before. It is possible that the large number of samples collected, prepared, and analyzed by standardized, uniform procedures and the absence of extraneous environmental and other contaminants eliminated the skewing effects observed in other studies. The elements that often showed the most normal distribution were generally those with a relatively narrow range between minimum and maximum values. This limited normal distribution indicates that crop genetic factors are more important than fertility, soil, and environment (Chaney, 1983).

ACKNOWLEDGMENT

The contributions of the following individuals, who participated in the planning and evaluation of this program, are gratefully acknowledged: R. Daniels, Soil Conservation Service (SCS), U.S. Department of Agriculture (USDA) (retired), and R. Chaney, Agricultural Research Service, USDA, Washington, DC; K. Dotson, Environmental Protection Agency, Cincinnati, OH; A. L. Page, University of California, Riverside. Soil scientists of the field staff of the SCS selected sites and collected and shipped all crop and soil samples.

Registry No. Ca, 7440-70-2; Cu, 7440-50-8; Fe, 7439-89-6; K, 7440-09-7; Mg, 7439-95-4; Mn, 7439-96-5; Mo, 7439-98-7; Na, 7440-23-5; Ni, 7440-02-0; P, 7723-14-0; Se, 7782-49-2; Zn, 7440-66-6.

Supplementary Material Available: Computer printouts containing data tabulations on elements, including Cd and Pb in six crops, and statistical calculations, histograms, and normality plots (119 pages). Ordering information is given on any current masthead page.

LITERATURE CITED

- Capar, S. G.; Dusold, L. R. Am. Lab. (Fairfield, Conn.) 1978, 10 (9), 17–28, passim.
- Capar, S. G.; Dusold, L. R. J. Assoc. Off. Anal. Chem. 1982, 65, 1259.
- Chaney, R., U.S. Department of Agriculture, Washington, DC, personal communication, 1983.
- Compliance Program Evaluation "FY-75 Metals in Foods Survey (7320.13)"; U.S. Food and Drug Administration: Washington, DC, 1975.
- Compliance Program Evaluation "Total Diet Studies-Adult"; U.S. Food and Drug Administration: Washington, DC, 1978.

Erdman, J. A.; Moul, R. C. J. Agric. Food Chem. 1982, 30, 169. Fox, M. R. S. J. Food Sci. 1974, 39, 321.

- Hahn, M. H.; Kuennen, R. W.; Caruso, J. A.; Fricke, F. L. J. Agric. Food Chem. 1981, 29, 792.
- Kuennen, R. W.; Wolnik, K. A.; Fricke, F. L.; Caruso, J. A. Anal. Chem. 1982, 54, 2146.
- Lorenz, K.; Loewe, R. J. Agric. Food Chem. 1977, 25, 866.

- Pfeilsticker, K.; Maskard, C. Z. Lebensm.-Unters. -Forsch. 1975, 158, 129.
- Reith, J. F.; Engelsma, J.; Van Ditmarsch, M. Z. Lebensm.-Unters. -Forsch. 1974, 156, 271.
- SAS "SAS User's Guide"; SAS Institute: Cary, NC, 1979.
- SAS "SAS Technical Report P-115"; SAS Institute: Cary, NC, 1981.
- Schlettwein-Gsell, D.; Mommsen-Straub, S. Int. Z. Vitaminforsch. 1970, 40, 658.
- Shacklette, H. T. "U.S. Geological Survey, Professional Paper 1178"; U.S. Government Printing Office: Washington, DC, 1980.
- Thomas, B.; Roughan, J. A.; Watters, E. D. J. Sci. Food Agric. 1974, 25, 771.

- Welch, R. M. In "Cadmium Seminar"; The Fertilizer Institute: Washington, DC, 1978.
- Wolnik, K. A.; Fricke, F. L.; Capar, S. G.; Braude, G. L.; Meyer, M. W.; Satzger, R. D.; Bonnin, E. J. Agric. Food Chem. 1983, preceding paper in this issue.
- Wolnik, K. A.; Fricke, F. L.; Kuennen, R. W. In "Development in Atomic Plasma Spectrochemical Analysis"; Barnes, R. M., Ed.; Heyden: London, 1981; pp 685–696.

Received for review March 18, 1983. Accepted July 13, 1983. The mention of proprietary products, materials, or trade names does not imply endorsement by the U.S. Government.

Radioimmunoassay for Naringin and Related Flavanone 7-Neohesperidosides Using a Tritiated Tracer

Pablo S. Jourdan,¹ Elmar W. Weiler, and Richard L. Mansell*

A radioimmunoassay (RIA) that can be used for the quantification of naringin in grapefruit tissues is described. The assay utilizes antisera raised against a naringin 4-O-(carboxymethyl)oxime hapten. The tracer used was a ³H derivative of naringin that was stable for at least 8-12 months. The detection limit of the assay is 0.2 ng or 2 ppb of naringin. Only flavanone 7-neohesperidosides are detected by the assay and naringin is the most reactive compound; the isomeric 7-rutinosides do not interfere. The RIA has a high sample throughput and requires only dilution of crude extracts. The reliability and reproducibility of the assay are demonstrated by intra- and interassay variability of 2.3% and 5.0% cv, respectively.

Naringin $(5,7,4'-trihydroxyflavanone 7-O-\beta-neo$ hesperidoside) is an intensely bitter compound that occurs in the Rutaceae, primarily in Citrus paradisi Macf. (grapefruit), Citrus grandis Osbeck (pummelo), Citrus aurantium L. (sour orange), Poncirus trifoliata L. (Raf.) (trifoliate orange), and Fortunella margarita Swing (kumouat) (Horowitz and Gentili, 1977). In the processing of citrus fruits, various juice parameters are routinely measured in order to maintain proper quality control (McAllister, 1980), and it is therefore important to be able to accurately monitor the levels of bitter principles (Attaway, 1977). The most widely used method for naringin quantification is the Davis test (McAllister, 1980), which is a very simple and inexpensive technique. Unfortunately, this test is highly unspecific since it does not discriminate between other flavanone 7-neohesperidosides and the nonbitter, isomeric flavanone 7-rutinosides. A highly specific HPLC method for the detection and quantification of naringin and related bitter flavanone 7-neohesperidosides has been developed (Fisher and Wheaton, 1976), but this technique is limited because of high cost, complexity of instrumentation, and, most importantly, small sample throughput-making it impractical for routine analysis of large numbers of samples. Thus, a rapid, simple, and specific procedure to measure naringin would be of potential importance to citrus processors.

Recently we reported the development of a ¹²⁵I-based radioimmunoassay (RIA) for naringin and related flavonoid neohesperidosides (Jourdan et al., 1982). In the present paper we report on an improved RIA for naringin that uses a tritiated tracer of long shelf life and is characterized by greater selectivity and sensitivity than the ¹²⁵I RIA. The assay is being used routinely in our laboratory for the analysis of naringin in citrus juices and fruits.

MATERIALS AND METHODS

Chemicals. Naringin and narirutin (naringenin 7-Orutinoside) were a gift from Dr. James Fisher, Florida Department of Citrus. Fortunellin, poncirin, and neohesperidin were kindly provided by Prof. Dr. H. Wagner, Munich. Phloroacetophenone 4-neohesperidoside was prepared by base hydrolysis of naringin after the method of Horowitz and Gentili (1961). Prunin was prepared by specific hydrolysis of naringin using naringinase (Sigma), following the methods of Versteeg et al. (1977). Other compounds tested for cross-reactivity (cf. Table I) were purchased from Roth, Karlsruhe, FRG. (Aminooxy)acetic acid and 5-aminolevulinic acid were purchased from Sigma, St. Louis, MO. Calf serum was supplied by Mediapharm, Aschaffenburg, FRG, and bovine serum albumin (BSA) was purchased from Serva, Heildeberg, FRG. Sodium [³H]borohydride, specific activity 50-60 Ci/mmol, came from New England Nuclear, Boston, MA. Phosphatebuffered saline (PBS, 0.01 M phosphate, 0.15 M NaCl, pH 7.4) was used as the RIA buffer. Saturated ammonium sulfate solution was prepared by stirring 800 g of ammonium sulfate and 1000 mL of water overnight, filtering, and adjusting the pH to 7.0 with 4 N NaOH. Naringin standards were prepared from a stock solution of 1 g/mL in MeOH which was stored at -18 °C; serial dilutions were done with water and the standards were stored at 4 °C. Under these conditions, they remained stable for 2 weeks. The scintillation cocktail was Minisolve (Zinsser), and all other solvents were of the highest purity available.

Synthesis of Naringin 4-O-(Carboxymethyl)oxime. The oxime of naringin was prepared by refluxing 500 mg

Lehrstuhl fur Pflanzenphysiologie, Ruhr Universitaet, D-4630 Bochum 1, West Germany (P.S.J. and E.W.W.), and Department of Biology, University of South Florida, Tampa, Florida 33620 (R.L.M.).

¹Present address: Department of Biology, University of South Florida, Tampa, FL 33620.