Table II. Determination of Bromine in Check Samples by Activation Analysis (Triga Reactor)

Sample	Bromine Content, P.P.M. Chemical analysis	Activation analysis
Banana peel	1	6 ± 3
Pineapple	6	9 ± 2
Carrots	8	12 ± 4
Asparagus	4	9 ± 2
Lemon peel	3	4 ± 1
Corn	...	2 ± 2
Peaches	0	<1

Table III. Determination of Bromine in Skellysolve B Extracts of Check Samples by Activation Analysis (Triga Reactor)

Sample	Bromine Content of Extract, P.P.M. Chemical analysis	Activation analysis
Carrot extract	0.0	<0.1
Asparagus extract	<0.2	0.1 ± 0.1
Lemon peel extract	0.0	<0.1
Peach extract	<0.2	0.07 ± 0.03

study at the time, so no statement can be made as to the extractability of the bromine present in such samples. However, chemical analyses of such samples, and their extracts, have shown that the bromine is similarly present in largely nonextractable form.

A standard nematocide solution in Skellysolve B, made up to contain 10.0 p.p.m. Br, was found by activation analysis to contain 9 ± 1 p.p.m. Br.

Figure 4 shows the Br^{82} spectra resulting from activation of the nematocide standard solution and the NH_4Br reference solution. These are pure Br^{82} spectra. Figure 5 shows the spectra obtained from the low bromine content nematocide-treated sample (pineapple) and the similarly treated high bromine sample (banana peel). The 0.55 and 0.78 m.e.v. photopeaks of Br^{82} are clearly evident. Figure 6 shows the spectrum of a lemon peel check sample containing only a small amount of bromine (4 p.p.m.), but a considerable amount of sodium activity. Figure 7 shows the spectrum of a banana peel check sample containing a small amount of bromine (6 p.p.m.), a little sodium activity, and a larger amount of potassium activity. Reference spectra of neutron-activated sodium and potassium are shown in Figures 8 and 9, respectively. They were obtained by activation of aqueous solutions of pure $NaNO_3$ and KNO_3. Quantitative determinations of the bromine contents of the various crop and crop extract sample studied were carried out by calculations from the analyzer print-out data, of the Br^{82} photopeak area, above the Compton level, of each sample and comparison of this area with the corresponding area of the simultaneously activated NH_4Br reference solution. The photopeak area used in the calculations is shaded in Figure 4.

Sodium is monoisotopic in nature, and Na^{23} captures thermal neutrons to form 15.0-hour Na^{24} with a cross section of 0.53 barn (*2*). Sodium-24 decays entirely by emission of a 1.39 m.e.v. beta and a cascade of 1.37 and 2.75 m.e.v. gammas (*10*). The gamma-ray spectrum of Na^{24} should thus show peaks at 1.37 and 2.75 m.e.v., plus a small summation peak at 4.12 m.e.v. In these studies, only lower energy peaks (<2 m.e.v.) were utilized, so the 1.37 m.e.v. peak of Na^{24} is evident in the spectra, but higher energy ones are off-scale (Figure 8).

The K^{41} isotope of potassium (6.8% abundance) has a neutron capture cross section of 1.1 barns, forming 12.5-hour K^{42} (*2*). This isotope emits only a 3.53 m.e.v. beta in 82% of its disintegrations, but a 2.00 m.e.v. beta and a 1.53 m.e.v. gamma in the other 18% (*10*). The observed spectrum should therefore show only a single peak, at 1.53 m.e.v., as observed (Figure 9).

Literature Cited

(1) Boyd, G. E., *Anal. Chem.* **21,** 335 (1949).
(2) General Electric Co., "Chart of the Nuclides," 1956.
(3) Guinn, V. P., *Intern. Sci. and Tech.*, p. 74, August 1961 (Prototype Issue).
(4) Guinn, V. P., *Nucleonics* **19,** No. 3, 81 (1961).
(5) Guinn, V. P., Wagner, C. D., *Anal. Chem.* **32,** 317 (1960).
(6) Jenkins, E. N., Smales, A. A., *Quart. Revs. London* **10,** 83 (1956).
(7) Leddicotte, G. W., *Nucleonics* **14,** No. 5, 46 (1956).
(8) Mapes, D. A., Shrader, S. A., *J. Assoc. Offic. Agr. Chemists* **40,** 189 (1957).
(9) Meinke, W. W., *Science* **121,** 177 (1955).
(10) Strominger, D., Hollander, J. M., Seaborg, G. T., *Rev. Mod. Phys.* **30,** 585 (1958).

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NEMATOCIDE RESIDUES

Direct Elemental Analysis of Citrus Crops by Instrumental Neutron Activation. A Rapid Method for Total Bromide, Chloride, Manganese, Sodium, and Potassium Residues

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ALIPHATIC ORGANIC HALIDES have proved to be increasingly attractive as combatants of the destructive action of plant-parasitic nematodes. Thus, methyl bromide, ethylene dibromide, propargyl bromide, 1,2-dibromo-3-chloropropane (DBCP), chloropicrin, and 1,3-dichloropropene are now recognized as effective nematocides (*16*). The widespread utilization of these substances as "soil fumigants" demands that analyses of the attendant residues in edible crops be ascertained. Although some information concerning the adsorption of organic halides by soils (*2, 8*) and the possible mode of action (*14*) of these compounds has been reported, the paucity of information relating to the chemical fate of these biocides in soils and in plants complicates the difficulty of obtaining significant residue analyses.

The residue tolerances which have been set for some of the organic bromides noted above are based upon analysis for total bromine. Such analyses require that any organic bromide present in the sample must first be converted to bromide ion by reaction with a suitable base and subsequent lengthy ashing with sodium hydroxide. The composite quantity of this ion is then determined iodometrically (*12, 15*) or potentiometrically (*4*) by employing a Dowex ion exchange column to separate the interfering water-soluble organic compounds from the halides (*5*).

The sensitivity of these methods for bromine (~25 μg.) is not adequate

An entirely instrumental method of neutron activation analysis has been successfully employed to analyze directly the total bromine residues in raw navel orange peel and juice. The inorganic bromide found was the result of fumigation with the nematocide, 1,2-dibromo-3-chloropropane. Simultaneously, the amounts of sodium, potassium, manganese, and chlorine were quantitatively determined from the same set of gamma-ray spectra. No organic bromides were detected in these fruit.

to satisfy possible "zero tolerances." Moreover, by virtue of their chemical nature, the methods are subject to the inherent errors of iodometry (*9*) and those resulting from trace impurities which may plague the electrometric determinations of halide in complex media.

Recent findings have pointed up the wide scope and power of neutron activation analysis (*11*). Most recently, an entirely instrumental method of neutron activation analysis based upon gamma-ray spectra and half-life has been described (*7, 13*). The extreme sensitivity of activation analysis for many elements (*1*) coupled with the availability of high uniform neutron fluxes attainable in reactors such as the General Atomic TRIGA reactor suggested that a direct analysis for bromine in raw agricultural crops might be obtained. This expectation has been fully confirmed by a determination of the bromine residue of DBCP in raw orange peel and juice. In fact, because of the ease of obtaining the information, Na, K, Mn, and Cl were simultaneously determined in these samples from the same set of gamma-ray spectra. Analyses for organic halides are also reported. The nematocide DBCP was selected for study because of the lack of residue data in the open literature concerning this molecule. The crudest possible sample preparation was purposely employed to test the applicability of this technique.

Materials and Methods

All fruit used in this work was obtained from trees that were harvested 8 months after application of the nematocide.

Preparation of Samples for Total Elemental Analysis. Samples of 16 oranges obtained from eight trees were washed with doubly distilled water and halved. The halves were placed on an electric ("Sunkist") juicer for 5 seconds. The rinds remaining constitute the "peel" and were ground in a Hobart food chopper for 10 minutes.

While the batch of juice described above was being vigorously stirred, two 2.5- to 3-gram samples were aliquoted and accurately weighed into 2-dram, snap-cap, polyethylene vials (polyvials). Two 2.5- to 3-gram samples of the peel "homogenate" were similarly handled. In this manner, six samples of juice and six of peel were obtained. The samples represented three plots of eight trees. Duplicates from each composite batch of peel and juice were taken.

In addition, 2.05 μg. of Br as NH_4Br was added to fortify single samples of peel and of juice from the untreated plot to demonstrate categorically the feasibility of neutron activation analysis.

For Organic Bromine Analysis. Samples of 25 grams of the stirred batch of juice described above, which had been previously macerated in a Waring Blendor, were placed in a 500-ml., three-necked Morton flask, and 50 ml. of redistilled Skellysolve B, b.p. 65° to 67° C., was added. The flask was stoppered, and the contents were vigorously stirred for 30 minutes. The contents were rinsed into a separatory funnel with Skellysolve B and subsequently shaken with 100 ml. of saturated sodium sulfate. This procedure hastened the phase separation and aided in driving any possible water-soluble organic bromides into the petroleum ether layer. The light-yellow organic phase was shaken with a second 100-ml. portion of saturated sodium sulfate, separated, and dried over sodium sulfate. The clear yellow solution was filtered into a graduated cylinder, and the sodium sulfate was rinsed three times with Skellysolve B. The resulting solution was made up to 100 ml. Five-milliliter aliquots of this solution, corresponding to 1.25 grams of orange juice, were placed directly into glass-stoppered quartz tubes of approximately 15-ml. capacity.

Samples of organic peel extracts were prepared in the same manner, except that after stirring for 1 hour with Skellysolve B the hydrocarbon extract was decanted from the ground peel. The peels were rinsed three times with the hydrocarbon solvent. The resulting solution was dried over sodium sulfate.

In this way, three samples of juice extracts and three of peel extracts (one juice and one peel sample per plot of eight trees) were obtained.

Checks on the extraction procedure were ascertained by adding DBCP at levels of 1.56 μg. of Br per 1.25 grams of sample and 6.25 μg. of Br per 1.25 grams of sample to the petroleum ether before stirring.

Aqueous solutions of the following standards were employed: 60 μg. of Cl (NH_4Cl), 48 μg. of Br (NH_4Br), 103 μg. of Na (sodium oxalate), 2.02 mg. of K (potassium oxalate), 8.4 μg. of Mn ($MnCl_2$), and 5.34 μg. of Br as DBCP in petroleum ether.

Irradiation and Counting. Samples for total elemental analysis and for organic bromine analysis were irradiated separately. Each set was handled in essentially the same manner.

The sample vials were placed in larger screw-cap polyethylene sample holders and lowered into the rotating circular rack of the TRIGA reactor. Rotating the rack at 1 r.p.m. exposed all of the samples to the same neutron flux. The samples were irradiated at 250 kw. with a thermal neutron flux of 1.8×10^{12} neutrons per sq. cm. per second for 0.5 hour. The samples were removed from the reactor and accurately transferred into unirradiated 2-dram snap-cap polyvials which were then heat-sealed. All liquid samples and standards were made up to approximately the same volume (3 ml.) before irradiation and after transferring. The samples of organic extracts were quantitatively transferred into small screw-cap glass vials for counting.

The counting was accomplished by employing a 3 × 3-inch solid sodium iodide crystal on which was placed an 0.5-inch-thick polystyrene disk. The sample vial was placed on this disk.

Highly energetic gamma-rays are emitted isotropically by the neutron induced radioactive species in the sample. The pertinent species were 37.3-minute Cl^{38}, 36-hour Br^{82}, 15.0-hour Na^{24}, 12.8-hour K^{42}, and 2.56-hour Mn^{56}. Interaction of the gamma rays with the sodium iodide crystal results in light pulse scintillations which in turn knock electrons off of a photocathode. The photocathode is part of a high-gain photomultiplier tube which is attached to the sodium iodide crystal. Electrical pulses, multiplied by $\sim 10^6$ by the photomultiplier are then analyzed in terms of pulse amplitudes by a 256 channel pulse height analyzer (gamma-ray spectrometer, Radiation Counter Laboratory analyzer). Since the amplitudes of the electrical pulses are proportional to the gamma-ray energy, the resultant pulse amplitude spectrum is directly proportional to the gamma-ray spectrum emitted by the neutron irradiated sample.

The gamma-ray spectra which could

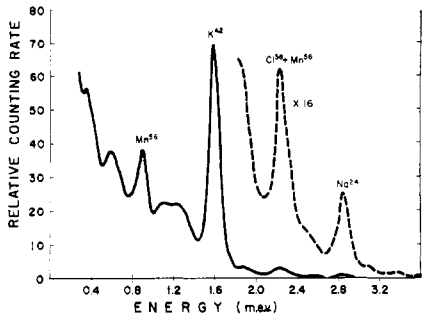

Figure 1. Gamma-ray spectrum of raw orange juice, sample 5, 2.38 hours after irradiation. Count: 494,000 d.p.m.

Table I. Activation Analysis of Raw Orange Juice and Peel for Mn, Na, K, Cl, and Br

Sample No.	Mn	Na	K	Cl	Br
			P.P.M. Juice		
2	1.7 ± .2	1.4 ± .1	2.9 ± .2 × 10^3	30 ± 5	2.0 ± .2
2a	1.9	1.4	3.1 × 10^3	31	2.4
5	1.8 ± .2	...	3.2 ± .2 × 10^3	31 ± 5	4.6 ± .5
5a	1.8	2.2 ± .2	3.3 × 10^3	...	5.2
8	2.0 ± .2	1.6 ± .2	3.2 ± .2 × 10^3	31 ± 5	<0.03
8a	2.1	...	3.2 × 10^3	...	<0.03
			P.P.M. Peel		
2	9.5 ± .9	5.9 ± .4	2.0 ± .2 × 10^3	...	3.3 ± .3
2a	9.1	5.8	2.0 × 10^3	30 ± 5	3.3
5	9.0 ± .9	7.9 ± .4	2.2 ± .2 × 10^3	31 ± 5	5.5 ± .5
5a	9.4	7.1	2.0 × 10^3	...	4.7
8	...	6.7 ± .4	2.0 ± .2 × 10^3	26 ± 5	<0.03
8a	9.3 ± .9	6.7	1.9 × 10^3	24	<0.03

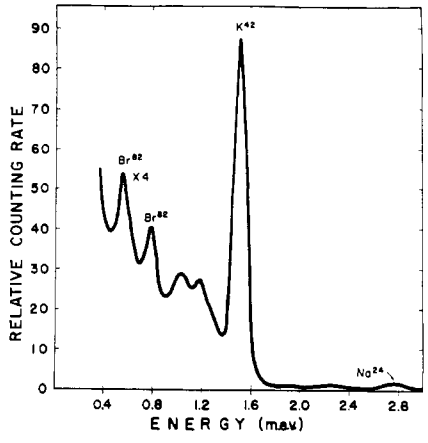

Figure 2. Gamma-ray spectrum of sample 5, 29.72 hours after irradiation. Count: 122,000 d.p.m.

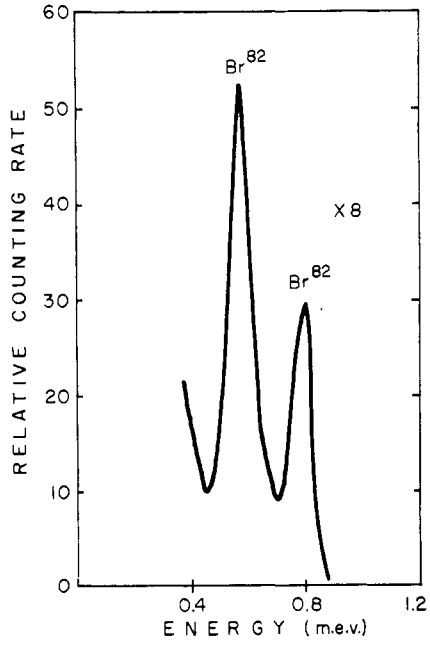

Figure 3. Gamma-ray spectrum of sample 5, 29.72 hours after irradiation, with Na and K subtracted. Count: 22,000 d.p.m.

be observed on the oscilloscope were printed at two magnifications on 10 × 15-inch graph paper with a Moseley X-Y Recorder. All samples were counted at least twice, once soon after irradiation and once approximately 30 hours later. The manganese and chlorine contents were determined from the early count, and the sodium, potassium, and bromine from the latter. The spectrum used for the calculation of bromine content was obtained by "subtracting out" the sodium and potassium contributions to the latter spectrum (*10*). Thus, after the composite spectrum of Na^{24}, K^{42}, and Br^{82} was printed, the "memorized spectrum" retained by the analyzer was "complemented."—that is, inverted on the oscilloscope. Sodium and potassium standards were then placed into the counter and their emissions allowed to "erase" the Na^{24} and K^{42} contributions to the total spectrum. The remaining Br^{82} spectrum was then inverted and printed out.

Calculations. The following radioisotopes were induced in the samples by neutron activation and were used to perform a quantitative analysis of the samples. The radioisotopes, half-life, and energy of the gamma-ray peak used for calculations are: Cl^{38}, 37.3 minutes, 2.15 m.e.v.; Mn^{56}, 2.86 hours, 0.84 m.e.v.; K^{42}, 12.8 hours, 1.52 m.e.v.; Na^{24}, 15.0 hours, 2.76 m.e.v.; and Br^{82}, 36 hours, 0.77 m.e.v.

The peak height of an unknown sample above the Compton background was normalized to the same time after the irradiation as the standard sample, normalized for the duration of counting (usually 60 seconds), and normalized to the same display scale setting to coincide with the counting parameters of the standard. The quantity of element x present in the sample is then given by the expression

$$\text{weight } x \text{ in sample} = \frac{(\text{peak height } x \text{ in sample})}{(\text{peak height } x \text{ in standard})} (\text{weight } x \text{ in standard})$$

The peak heights have provided reliable representations of radioactivities.

Results and Discussion

The gamma-ray spectra of all of the elements in this study have been recorded elsewhere (*3*), hence spectra of the radioisotopes of the standards have been omitted.

Figure 1 shows the gamma-ray spectrum of a sample of orange juice 2.38 hours after irradiation. The spectra in Figures 2 and 3 were taken 29.72 hours after irradiation, the Na^{24} and K^{42} contributions having been "subtracted out" from the spectrum depicted in Figure 3. The magnifications indicated refer to the first lower spectrum depicted in Figure 1. For the analysis of chlorine, it was necessary to correct for the Mn^{56} contribution (2.12 m.e.v.) to the Cl^{38} peak at 2.15-m.e.v. because of the relatively late first count. Thus, the manganese content was first determined from the 0.84-m.e.v. peak. Its contribution to the 2.12-m.e.v. peak of Cl^{38} was calculated from the expression

peak height of Mn^{56} at 2.12 =
(1/34) × (peak height at 0.84)

This ratio was ascertained from the known spectrum of Mn^{56}. The consistency of the results obtained for widely different counting times attests to the validity of this correction.

Results of analyses of raw orange juice and peel are presented in Table I. Samples 2 and 2a represent fruit from a plot which had been treated at 4 gallons per acre with DBCP using a shank injection method. Samples 5 and 5a represent a plot treated by flooding in basins at an application rate of 4 gallons per acre. The amount of pesticide actually used in the former

Table II. Detection of Added NH_4Br to Raw Juice and Peel by Activation Analysis

Sample	Br, μg. Added	Br, μg. Detected
8 juice	2.05	2.3 ± .2
8 peel	2.05	2.3 ± .2

Table III. Recovery of DBCP from Orange Peel and Juice

Sample	Br, μg. Added	Br, μg. Detected by activation analysis
8 juice	1.56	1.5 ± .2
8a juice	6.25	7.0 ± .7
8 peel	1.56	1.8 ± .2
8a peel	6.25	6.3 ± .6

method was approximately 60% of that in the latter. Samples 8 and 8a represent a nonfumigated check plot.

The peels constituted 45% of the weight of the oranges. Thus, averaging the values for duplicate runs and weighing the results accordingly, the total bromide contents of samples 2 and 5 are 2.7 and 5.0 p.p.m., respectively. These results are in good agreement with the relative amounts of pesticide applied to these two plots, and they suggest that a linear relationship exists between the amount of DBCP applied and the quantity of bromide remaining as a residue.

Samples of the untreated check plot were fortified with NH_4Br. The results are presented in Table II.

No bromine could be detected in any of the organic juice or peel extracts, the minimum level detectable being 0.05 μg. or 0.04 p.p.m. The extraction procedure proved adequate by the results presented in Table III. The values obtained for duplicate samples are, in all cases, within the counting errors indicated.

Because of the limited number of plots sampled here, perhaps no general conclusions regarding the residues of DBCP are warranted. At the same time, however, the results clearly demonstrate that essentially no organic bromine residues are present in these fruits. Thus, the bromine found must be present as bromide ion, and it is very definitely a result of the treatment with DBCP. This is evident not only from the fact that no bromine could be found in the untreated check plot, but from the observation that the concentration of other elements in the fruit of all three plots was not markedly different. Thus, the remote possibility that the trees of the check plot were not effective in assimilating substances from the soil can be eliminated.

As a method of residue analysis for bromine, the procedure outlined is nondestructive, the sample preparation required is minimal, and the method is extremely rapid. More than 100 samples for bromine analysis might be irradiated simultaneously in the TRIGA reactor. Because of the favorable 36-hour half-life of Br^{82}, no early counting is necessary. A greater lapse in time between irradiation and counting than employed in the present work would, in fact, enhance the clarity of the Br^{82} spectrum and eliminate a second counting and "subtracting out" step. Furthermore, since the concentration of Br in polyvials is negligible, the transfer step after irradiation could be eliminated. Thus, the amount of work necessary for preparing samples for total Br analysis is reduced to homogenizing the sample and weighing it out.

Of greater importance is the sensitivity and wide scope of instrumental neutron activation depicted in this work. Undoubtedly this extremely simple technique can be applied as a direct method of elemental analysis to a wide range (if not all) of raw agricultural crops (*6*). Furthermore, the facility of the method suggests that it is likely to be of value for the analysis of many plant and animal forms. The authors intend to explore the biochemistry of plant-parasitic nematodes and their host relationships utilizing this technique.

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Literature Cited

(1) Atkins, D. H. F., Smales, A. A., in "Advances in Inorganic and Radiochemistry," H. J. Emeleus and A. G. Sharpe, eds., Vol. **1,** pp. 315–45, Academic Press, New York, 1959.
(2) Call, F., *J. Sci. Food Agr.* **8,** 630 (1957).
(3) Crouthamel, C. E., "Applied Gamma-Ray Spectrometry," pp. 171–314, Pergamon Press, New York, 1960.
(4) DeBrunce, R. E., University of California, Riverside, Calif., private communication, 1960.
(5) DeGeiso, R. C., Rieman, W., III, Lindenbaum, S., *Anal. Chem.*, **26,** 1840 (1954).
(6) Guinn, V. P., Potter, J. C., J. Agr. Food Chem., **10,** 232 (1962).
(7) Guinn, V. P., Wagner, C. D., *Anal. Chem.*, **32,** 317 (1960).
(8) Jurinak, J. J., J. Agr. Food Chem., **5,** 598 (1957); *Ibid.*, **8,** 113 (1960).
(9) Kolthoff, I. M., Sandell, E. B., "Textbook of Quantitative Inorganic Analysis," 3rd ed., p. 587, Macmillan, New York, 1952.
(10) Lee, W., *Anal. Chem.*, **38,** 800 (1959).
(11) Leddicote, G. W., *Nucleonics*, **14,** 46 (1956).
(12) Mapes, D. A., Shrader, S. A., *J. Assoc. Offic. Agr. Chemists*, **40,** 189 (1957).
(13) Meinke, W. W., *Anal. Chem.*, **31,** 686 (1958).
(14) Moje, W., J. Agr. Food Chem., **7,** 702 (1959).
(15) Sinclair, W. B., Crandall, R. P., *J. Econ. Entomol.*, **45,** 882 (1952).
(16) Taylor, A. L., in "The Nature and Fate of Chemicals Applied to Soils, Plants, and Animals," ARS Bull. **20-9,** p. 116, Sept. 1960.

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