Table I. Percentage Recovery of Dieldrin from Butter Fat[a]

After Partitioning Only	Through the Column Only	Net Recovery (Two Steps) Actual	Theoretical
82.32	93.03	69.11	
79.23	87.07	73.42	
79.95	90.99	71.36	
80.32	99.43	81.55	
81.67	94.07	86.02	
Av. 80.70	92.91	76.29	74.89

[a] Each value in the table was obtained on a different sample of butter.

fat which is difficult to remove efficiently. The first 500 ml. of eluate from the column is almost completely free of all traces of this fraction. Trace quantities which may interfere on the chromatogram will elute in the last 500 ml. However, the quantity present in the entire 1 liter of eluate does not interfere in the counting of the C^{14}-labeled material, and it was not toxic to mosquito larvae. Only 2% of the larvae were inactivated after 215 minutes using the Burchfield *et al.* technique (*2*), indicating that this cleanup technique might be useful prior to bioassay work.

The amount of solvent used for the addition of the residue to the column is very important as indicated in Figure 3. If the residue is dissolved in a small volume of solvent, there is coprecipitation of the dieldrin when the temperature on the column is dropped to −70° C. If, however, a volume of 100 ml. of solvent is used for the transfer, the recovery value is increased from 57.0% in the first 500 ml. of eluate to 72.0%. The increase in the amount of dieldrin in the earlier fractions of eluate takes place without any shift of the peak. The lower graph in Figure 3, showing 57.0% recovery for the first 500 ml. was allowed to warm up to room temperature overnight with approximately 25 ml. of solvent above the upper surface of the column. The column was cooled again to −70° C. and another 500 ml. of eluate was collected. Another portion of the dieldrin came off the column with a peak after 300 ml. had passed through the column. This suggests that the dieldrin, which had coprecipitated in the fat during the first drop in temperature with only 25 ml. of solvent present, must have gone back into solution after the column had warmed to room temperature, and a portion of it remained in solution when the temperature dropped again to −70° C.

Columns which were eluted at room temperature retained very little of the residual fat when acetone was used as the eluting solvent. Furthermore, the residual fat remaining after partitioning may be precipitated in acetone at −70° C., but all the fatty material is not removed. These observations demonstrate the need of the low temperature and of the column. Quantitative recoveries were obtained for dieldrin which was added to the residue remaining after partitioning 50 grams of butter oil (Table I). The theoretical value of 74.89% was calculated from the average recovery values from each of the two cleanup steps.

One hundred grams of lettuce containing added C^{14}-labeled dieldrin was extracted by the Klein procedure (*4*) to obtain a large portion of the pigments and waxes. The solvent was removed, and the residue was added to the column described without partitioning and with the column operating at room temperature. Eluates were clean enough for paper chromatographic analyses, and quantitative recoveries were obtained, suggesting that this cleanup procedure may be applicable to extracts of fruit and vegetables as well as butter fat.

Acknowledgment

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NEMATOCIDE RESIDUES

Determination of Total Bromine Residues in Agricultural Crops by Instrumental Neutron Activation Analysis

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VARIOUS ORGANIC BROMO-COMPOUNDS are employed in the field to combat the destructive action of plant-parasitic nematodes. Examples of such soil fumigant nematocides are methyl bromide, ethylene dibromide, propargyl bromide, and 1,2-dibromo-3-chloropropane (DBCP). It is of interest and concern to determine the extent of the resulting nematocide residues in edible crops. Residue tolerances that have been set for some of these organic bromides are based upon analysis for total bromine. Existing chemical methods for such total bromine determinations in crop materials are generally fairly sensitive and accurate, but quite time consuming. The present study was initiated to explore the possibilities of a much faster technique, that of instrumental neutron activation analysis (*3–5*). Check (untreated) plant samples and samples of plants grown in soil treated with DBCP were analyzed. The method appears to be very useful.

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Theory of Method

The theory of neutron activation analysis has been discussed previously (*1, 3–7, 9*). However, the application of the theory to bromine is apropós. Reference is made below to the basic equation of activation,

$$A = Nf\sigma\left(1 - e^{\frac{-0.693t_i}{t_{0.5}}}\right) \qquad (1)$$

The Br^{82} disintegration rate that would result from irradiation of 1 μg. of bromine for 1 hour at a neutron flux of 10^{12} neutrons per sq. cm. per second may be computed as follows. Normal bromine consists of 50.6% Br^{79} and 49.4% of Br^{81} (*2*). The cross section for the $Br^{81} \xrightarrow{n} Br^{82}$ nuclear reaction is 2.6×10^{-24} sq. cm. per nucleus (i.e., 2.6 "barns")

Instrumental neutron activation analysis has been successfully applied to determine bromine in a variety of crop samples and plant extracts. Both untreated plants and plants grown in soil fumigated with the nematocide, 1,2-dibromo-3-chloropropane (DBCP), were investigated. Both accelerator and nuclear reactor neutron sources were employed. Bromine levels as low as 1 p.p.m. are detectable in crop samples, and levels as low as 0.01 p.p.m. in extracts. The method is rapid, nondestructive, and accurate.

(*2*). The half-life of Br^{82} is 36.0 hours (*2*). Using the chemical atomic weight of bromine (79.9), N in Equation 1 becomes 3.72×10^{15} Br^{81} nuclei per μg. of bromine. Values of f and σ to be used in Equation 1 are 10^{12} and 2.6×10^{-24}, respectively. Using t_i = 1 hour and $t_{0.5}$ = 36.0 hours, the parenthetical, or "saturation," term of Equation 1 acquires a value of 0.0190. Hence, A becomes 183 disintegrations per second (or 11,000 disintegrations per minute).

Since Br^{82} emits one or more gamma-ray photons in each disintegration (principally of energies of 0.78, 0.55, 0.62, 0.70, 0.83, 1.03, 1.32, 1.48 m.e.v. (*10*), and a typical sodium iodide well-type crystal scintillation counter will detect these gamma rays with an efficiency of perhaps 70%, the Br^{82} counting rate in this case would be about 7700 c.p.m.—above a lead-shielded background radiation counting rate of only about 300 c.p.m. Thus, under these conditions, one might expect to be able to detect even as little as 0.039 μg. of bromine in a sample (i.e., 300 c.p.m. above a background of 300 c.p.m.) in the absence of appreciable amounts of interfering activities. Hence, in a 1-gram sample, as little as 0.04 p.p.m. Br might be detected. Other induced radioactivities, pertinent to the case of neutron-activated plant samples, are discussed in a later section. This sensitivity of detection could be improved further by the use of a longer irradiation time. Since the saturation term in the activation equation approaches a value of 1 as t_i becomes much larger than $t_{0.5}$, the sensitivity of bromine detection could be increased by a factor of 1/0.0190, or 53-fold, if a very long irradiation period were used. Higher neutron fluxes as well as the use of larger samples increase the sensitivity further. Where interferences limit the sensitivity of detection, chemical separations can be utilized to eliminate them to a high degree, as in the work of Leddicotte (*7*). Such separations, however, may consume considerable time.

Gamma-Ray Spectrometry

In general, samples can be analyzed for certain elements by purely instrumental neutron activation analysis if no major interfering induced radioactivity obscures the activity being sought, and if the induced activity emits gamma rays.

The instrumental method is practicable for crop samples because the major constituents of plant material (C, H, O, N, S, P) are not appreciably activated by slow neutrons (phosphorus is, but it does not form a gamma-emitting radioisotope). Hence, trace metals and the halogens, which generally are activated by thermal neutrons to form gamma-emitters, if present, show up very well in the gamma-ray spectrum of an activated sample. In the present study, Br, Na, Mn, and K were thus detected in crop samples.

Apparatus and Samples

In the first phase of this study, check and DBCP-treated crop samples were activated at a thermal neutron flux of about 8×10^7 in the 3 m.e.v. Van de Graaff electron-accelerator beryllium photoneutron source in the Shell Development Company's Emeryville laboratories. As many as six 10- to 30-gram samples were activated simultaneously, usually for a period of 30 minutes, and then counted for 1 minute each in rapid succession. The counter used was a 3 × 3-inch NaI well-crystal, shielded by 3 inches of lead. A 100-channel Penco analyzer was used with printer and strip-chart recorder. In this phase, bromine was detected by means of the 18-minute Br^{80} activity.

In the second phase of the study, similar samples were activated at a thermal neutron flux of about 10^{12} in a Triga nuclear reactor at the General Atomic laboratories in La Jolla. As many as 12 0.5- to 1.0-gram samples were activated simultaneously (as many as 120 could be activated at one time if desired), usually for 30 to 60 minutes. To minimize the effect of interfering activities, these samples were allowed to decay for 24 to 72 hours before being counted. The bromine was then detected by means of the 36-hour Br^{82} activity. A 3 × 3-inch NaI well-crystal, shielded by 4 inches of lead, and an RCL 256-channel analyzer, with printer and X-Y plotter, were used.

The crop samples studied were grown in large field plots, the check samples being untreated, and the others soil-treated with the fumigant by the usual injection method. One-kilogram representative samples were then taken at intervals, chopped up in a Hobart food chopper, mixed thoroughly, and aliquots taken for activation analysis.

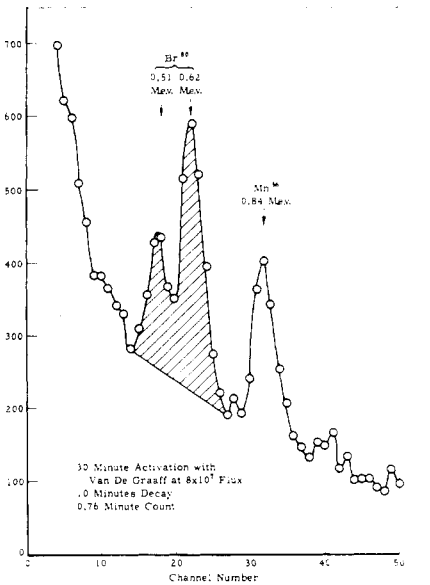

Figure 1. Gamma-ray spectrum of neutron-activated sample of DBCP-treated bush beans

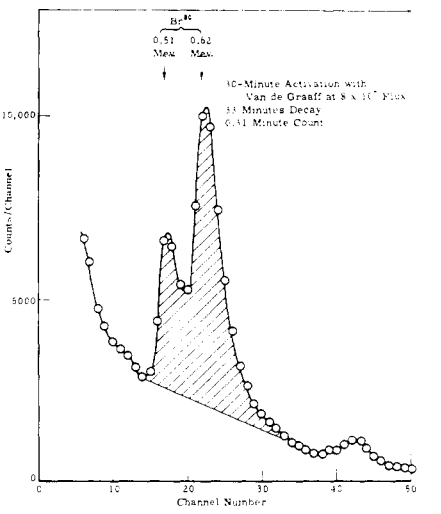

Figure 2. Gamma-ray spectrum of neutron-activated NH_4Br solution

Results

In general, plants grown in untreated soil (i.e., check samples) showed, in the gamma-ray spectra obtained after

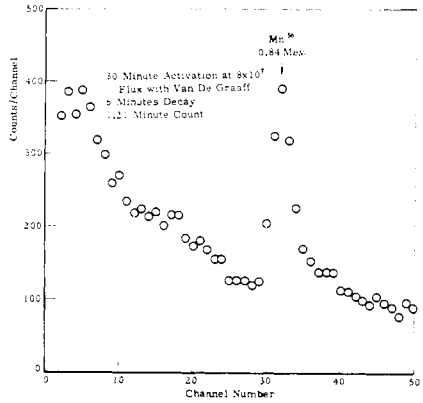

Figure 3. Gamma-ray spectrum of neutron-activated check sample of bush beans

Table I. Determination of Bromine Residues from DBCP by Activation Analysis (Van de Graaff)

Application Rate, Gallons/Acre	Interval, Weeks	Bromine Content, P.P.M. Duplicates	Average
		Bush Beans	
0	...	<10, <10	<10
2.5	2	448, 428	438 ± 10
	4	233, 238	235 ± 10
	6	77, 70	74 ± 10[a]
	6	131, 117	124 ± 10[a]
5.0	1	152, 151	152 ± 10
	2	269, 231	250 ± 20
	4	250, 296	273 ± 20
		Lima Beans	
0	...	<10	<10
2.5	2	25, 25	25 ± 10
	4	21, 15	18 ± 10
	6	21	21 ± 10[a]
	6	21	21 ± 10[a]
5.0	...	41, 39	40 ± 10
	...	47, 63	55 ± 10
	4	55, 59	57 ± 7
	6	75, 80	77 ± 10
		Irish Potatoes	
0	...	<10, <10	<10
2.5	...	51, 55	53 ± 10
	6	53, 51	52 ± 10
	...	40	40 ± 10
	...	47	47 ± 10
5.0	...	94	94 ± 10
	...	49, 49	49 ± 10
	...	64, 64	64 ± 10
	...	43, 58	50 ± 10
		Sweet Potatoes	
0	...	<10	<10
2.5	0	96, 95	96 ± 10
	1	73, 81	77 ± 10
	4	49, 49	49 ± 10
	6	78, 79	78 ± 10
5.0	2	191, 205	198 ± 10

[a] Two different samples.

activation, the presence of small amounts of manganese, sodium, and potassium. In most cases, traces (<10 p.p.m.) of bromine were detected even in such check samples. In crop samples obtained from plants grown in fumigant-treated soil, a higher concentration of bromine was found, generally in the range of 20 to 200 p.p.m.

Van de Graaff Studies. Results of the Van de Graaff accelerator studies employing the induced 18-minute Br^{80} activity are shown in Table I. The duplicate values listed represent bromine values computed at two different decay times from a single activated sample. The uncertainty shown for each average value is estimated from the counting statistics of the observed 0.51 plus 0.62 m.e.v. photopeak areas of Br^{80}, above the underlying Compton level. In the Van de Graaff studies, no bromine was observed in any of the check samples. It is estimated that, in the presence of the other induced activities, this means that less than 10 p.p.m. bromine was present. An illustrative gamma-ray spectrum of one of the activated samples (bush beans, 124 p.p.m. Br) is shown in Figure 1. In this spectrum the 0.51 and 0.62 m.e.v. photopeaks of Br^{80} and the 0.84 m.e.v. photopeak of 2.6-hour Mn^{56} are clearly seen. A pure Br^{80} spectrum, obtained by activation of an aqueous solution of pure NH_4Br, is shown in Figure 2 for comparison. Calculation of the bromine content of each sample was based on comparison of the Br^{80} photopeak area, above the Compton level, of the sample with that of the simultaneously activated NH_4Br standard solution-corrected to the same decay time. The photopeak area used in the calculations is shown as a shaded area in Figures 1 and 2. The spectrum of a bush bean check sample is shown in Figure 3 (corrected to the same decay time as Figure 1). The check sample showed no definite sign of a Br^{80} peak; only Mn^{56} was clearly present.

The reproducibility of the instrumental activation analysis system, using the Van de Graaff accelerator, has been checked extensively in earlier studies for many elements (*5*), including bromine. At levels well above the limit of detection of any given element and in the absence of serious interferences, the reproducibility is typically ±3% of the value. Because of the interfering activities, it is felt that the absolute accuracy of the bromine values shown in Table I is probably about ±5% of the value for Br levels of 200 to 400 p.p.m., and perhaps closer to ±10% of the value for Br levels of 50 to 200 p.p.m. The interfering activities, such as Mn^{56}, Na^{24}, and K^{42}, also raise the limit of detection of Br from a value of about 1 p.p.m., achievable with the Van de Graaff with uncomplicated Br samples, to perhaps 10 p.p.m. in typical crop samples. The effect of the other activities may be illustrated by noting that the counting rate of the bush bean check sample of Figure 3 was about 16,000 c.p.m., as opposed to a counter background rate of only 300 c.p.m. Reproducibility of sampling is probably a more serious problem in crop studies than is reproducibility of the activation method itself.

The 18-minute Br^{80} activity is formed from stable Br^{79} with a thermal neutron cross section of 8.5 barns (*2*). In 79% of its disintegrations, Br^{80} emits only 2.00 m.e.v. beta particles, but it emits a 0.62 m.e.v. gamma ray in 13%, and a positron (resulting in 0.51 m.e.v. annihilation gamma rays) in 3% of its disintegrations (*10*). The remaining 5% of its disintegrations occur via electron capture (*10*). Thus, the observed gamma-ray spectrum should show photopeaks, as observed, at 0.51 and 0.62 m.e.v. At long decays, the Br^{80} peak decays more slowly than the indicated 18-minute half-life, because of concurrent formation of Br^{80} from 4.6-hour Br^{80m} (by isomeric transition). With short irradiation and decay times, however, the Br^{80m} contribution is small.

Manganese is detectable with high efficiency because: it is monoisotopic in nature, the stable Mn^{55} forms Mn^{56} by neutron capture with a high cross section (13.3 barns) (*2*), and Mn^{56} emits one or more gamma-rays in every disintegration. In 50% of its disintegrations, Mn^{56} emits a 2.86 m.e.v. beta and a 0.84 m.e.v. gamma; in 30%, a 1.04 m.e.v. beta and a 1.81 and 0.84 m.e.v. gamma cascade; and in 20%, a 0.72 m.e.v. beta and a 2.14 and 0.84 m.e.v. gamma cascade (*10*). Thus, its observed gamma-ray spectrum should consist primarily of a 0.84 m.e.v. photopeak, as found, with smaller peaks at 1.81 and 2.14 m.e.v. (plus even smaller summation peaks at 2.65 and 2.98 m.e.v.).

Triga Reactor Studies. As shown in Table II, bromine was detected in each of the check samples (except peaches) but only at levels of from 2 to 12 p.p.m. These samples were also analyzed chemically for total bromine by the method of Mapes and Shrader (*8*), and agreement between the two methods is generally fairly good. The chemical values tend to be lower than the activation values, and this may be a real difference, representing a possible loss of bromine during the chemical determination. In all of these samples, sodium and/or potassium were also detected, but not determined quantitatively (although this could be readily done if of interest). Manganese was also undoubtedly present in these samples, too, but the 2.6-hour Mn^{56} had decayed away almost completely during the long decay (24 to 72 hours) which was employed prior to counting for 36-hour Br^{82} activity.

Two samples were from plants grown

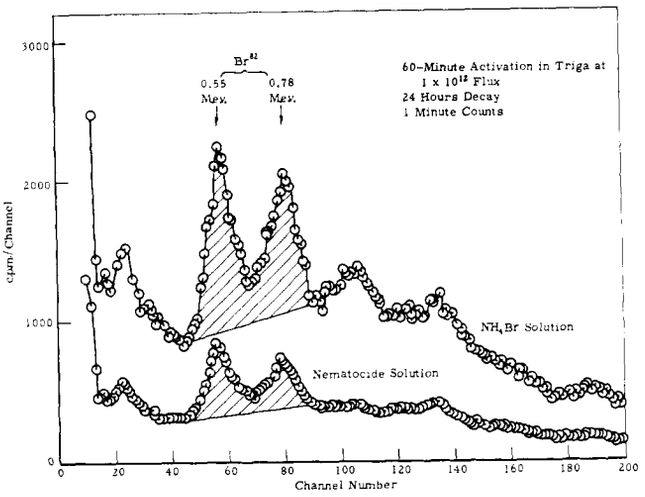

Figure 4. Gamma-ray spectra of neutron-activated DBCP and NH_4Br solutions

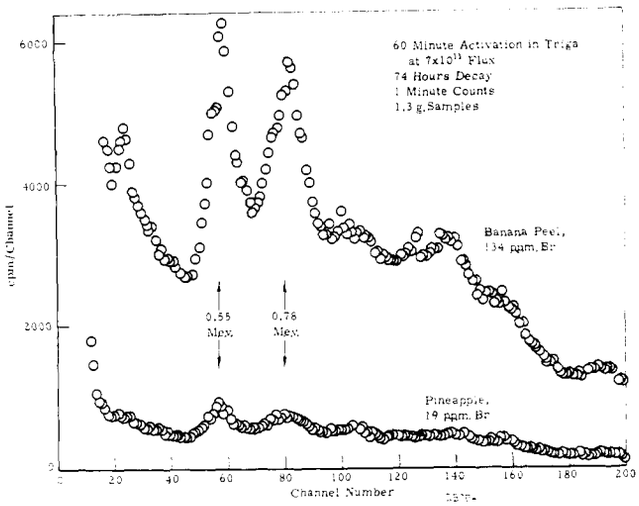

Figure 5. Gamma-ray spectra of neutron-activated DBCP-treated crop samples

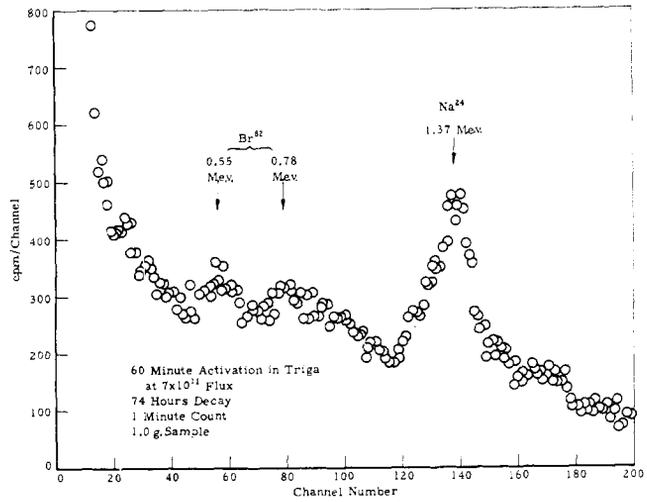

Figure 6. Gamma-ray spectrum of neutron-activated check sample of lemon peel

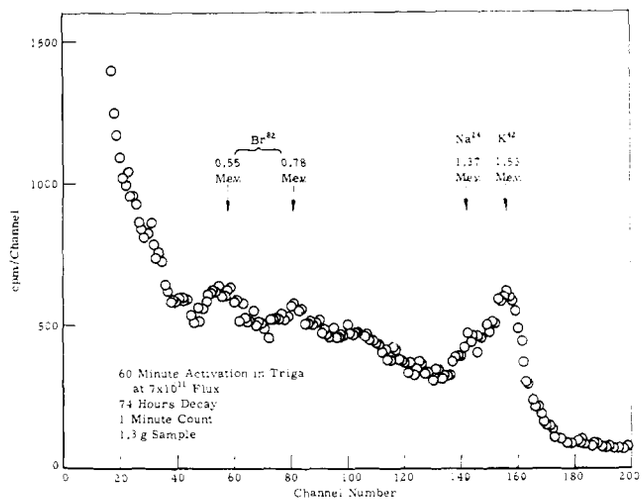

Figure 7. Gamma-ray spectrum of neutron-activated check sample of banana peel

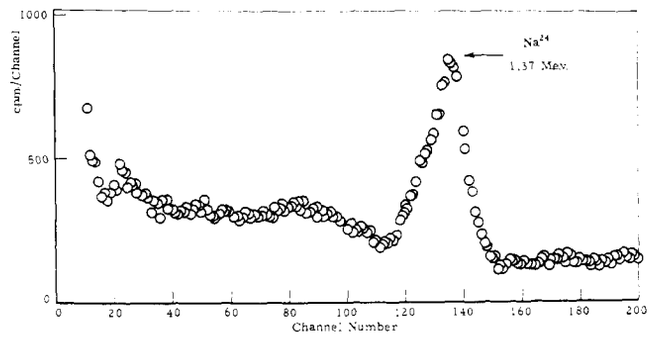

Figure 8. Gamma-ray spectrum of neutron-activated $NaNO_3$

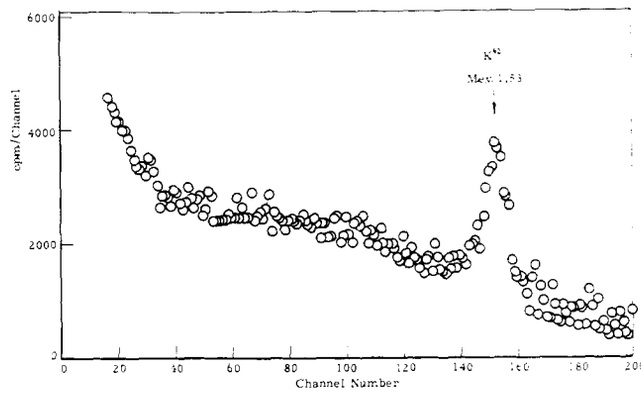

Figure 9. Gamma-ray spectrum of neutron-activated KNO_3

in fumigant-treated soil. One, a banana peel sample, was found by activation to contain 134 ± 4 p.p.m. Br; the chemical analysis value on this sample was 165 p.p.m. Another, a pineapple sample, showed 19 ± 4 p.p.m. Br by activation, 27 p.p.m. by chemical analysis. In these two instances, the chemical values were slightly higher than the activation values, rather than lower.

In addition, several Skellysolve B extracts of check samples were analyzed for bromine by activation. As expected, the extracts showed only bare traces (of the order of 0.1 p.p.m. Br), if any, of bromine. The values obtained, or the upper limits which could be set where none was actually detected, are shown in Table III. The check samples generally contained from 2 to 12 p.p.m. Br (Table II); therefore, the bromine is not present in a hydrocarbon-soluble chemical form. Unfortunately, no extracts of nematocide-treated samples were available for

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).

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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