IPC and CIPC Residue Analysis

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Food crops grown in soil treated with IPC (isopropyl-*N*-phenylcarbamate) and CIPC (isopropyl *N*-3-chlorophenylcarbamate) are analyzed for residues by the basic alkaline hydrolysis and colorimetric method of Montgomery and Freed. The method is modified to adapt it to the specific crops examined. Modifications include selection of appropriate sample sizes (10 to 200 grams) and hydrolysis medium (acidic or alkaline) to achieve low and reasonable levels of interference with good recovery of residue and physical performance during analytical processing. Recovery of residue from fortified samples ranges from 80 to 120%. The low limit of residue measurement is 0.05 p.p.m. for cranberries employing 200 grams of sample and 2 p.p.m. for sesame seeds employing 10 grams of sample.

For a number of years, the Chemical Division of PPG Industries has been studying the applications of its selective herbicides IPC (isopropyl *N*-phenylcarbamate) and CIPC (isopropyl *N*-3-chlorophenylcarbamate). Many different crops have been treated successfully for weed control with the IPC or CIPC applied at pre-emergence, post-emergence, or lay-by.

During the early years of the program of study on the uses and applications of IPC and CIPC, the analytical method used for determining residues of these herbicides in harvested crops was that reported by Bissinger and Fredenburg (1951), Gard *et al.* (1954, 1959), Gard and Reynolds (1957), Gard and Rudd (1953), and Zweig (1964). The method depends on extraction of the herbicide from macerated plant tissue using a suitable solvent, evaporation of excess solvent, hydrolysis of the residue with acid or alkali, steam distillation of aniline or chloraniline, and finally determination of the amine by the ammonia-phenol-hypochlorite colorimetric method.

In 1959 Montgomery and Freed described a new technique for determining IPC residues in strawberries. In this method, the entire sample is hydrolyzed in alkali without prior solvent extraction of the tissue. After hydrolysis the sample is steam-distilled, collecting the distilled aniline hydrolysis product in dilute hydrochloric acid. The analysis is completed by diazotizing the aniline with nitrous acid, followed by coupling with *N*-(1-naphthyl) ethylenediamine. The absorbance of the colored complex which results is measured with a suitable spectrophotometer. In 1963 Gard and Ferguson adapted this method to the analysis of cow's milk and urine for possible traces of CIPC.

The main features of this method which recommend it for IPC and CIPC residue analyses are the improvement in the color development steps and the use of the principle of hydrolyzing the entire sample without solvent extraction. This last point is particularly important because by this technique herbicide which may be chemically bound with the plant tissue may be recovered. In addition this technique permits the recovery of possible metabolites with a greater degree of reliability. The basic principles of this method have been used for the analysis of a number of additional samples of IPC and CIPC treated crop samples for possible residues of the herbicide. Results of these analyses and a description of some of the modifications in the method required for specific applications are reported here.

The apparatus previously described by Gard and Ferguson (1963) in their report on milk analyses was used for these residue studies. This apparatus consists of a hydrolysis and distillation flask connected to a West type reflux condenser. At the top of the reflux condenser is attached a distillation adapter connected to a vertically mounted West-type distillation condenser. The drip-tip of the distillation condenser dips beneath the liquid level of dilute acid in a receiver beaker to trap all vapors and minimize losses during the distillation of aniline. The entire apparatus remains assembled throughout the hydrolysis and distillation of a sample.

Because of the widely varying physical character of the many different crops analyzed, various minor modifications in the apparatus were required. In place of the Kjeldahl flask previously used, other types of flasks were often substituted. Glass-stoppered Erlenmeyer flasks with a capacity of 250 to 1000 ml. were most often used. On occasion a 3-neck flask was substituted when a heavy duty stirrer was required for a viscous or woody sample, or when it was necessary to observe and control pH with a high-temperature combination electrode. The most important feature of the arrangement, however, is the fact that hydrolysis under reflux can be immediately followed by the steam distillation of the sample without disassembling the equipment or opening the connections with possible accompanying losses.

The reagents described by Montgomery and Freed (1959) in their paper on IPC residue analysis were used for the diazotization and color development steps. Standard calibration curves for IPC and CIPC (using aniline and 3-chloraniline, respectively) were prepared using aliquots of standard solutions. These standards contained 0.01 mg. of either aniline or 3-chloraniline per ml. Aliquots taken for preparation of the two standard curves ranged from 0.2 ml. to 7.0 ml. The standard calibration data obtained are given in Table I for IPC and CIPC determination.

Sample Preparation. Crop or sample preparation was generally accomplished by maceration or grinding in a Waring Blendor. Dry samples such as safflower seed or dried corn were broken down into a powder very rapidly. Some crops such as berries were chopped and mixed in their own juices. Woody or fibrous samples such as alfalfa or celery were prepared by adding a measured volume of water to a weighed sample and mixing thoroughly in the Blendor.

General Procedure, Sample Analyses. Transfer a suitable sized sample (10 to 200 grams) to the hydrolysis and distillation flask, adding sufficient water as required for adequate mixing and stirring and to prevent charring during hydrolysis and distillation. A magnetic stirring bar, used in conjunction with a magnetic stirrer-hot plate unit, was most often used

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Table I. Standard Calibration Data for IPC and CIPC

Aniline or 3-Chloraniline, Mg.	Transmittance, % 555 Mμ[a] 1-Cm. cell	555 Mμ[a] 5-Cm. cell	540 Mμ[b] 1-Cm. cell	540 Mμ[b] 5-Cm. cell
0.000	100.0	92.5	98.5	98.0
0.002	98.5	82.0	96.0	89.5
0.004	95.0	73.5	95.0	83.5
0.007	91.5	62.0	93.5	73.5
0.010	89.0	53.5	91.5	60.0
0.020	80.5	31.5	84.5	43.0
0.040	67.0	—	71.5	—
0.070	50.0	—	56.0	—

[a] Aniline calibration curve, for IPC analyses.
[b] 3-Chloraniline calibration curve, for CIPC analyses.

for stirring, although on occasion a motor-driven stirrer and paddle arrangement was required for a specific problem.

Attach the flask to the reflux condenser, distillation adapter, and distillation condenser. Through a dropping funnel at the top of the reflux condenser and distillation adapter, add sufficient acid or alkali to the sample flask as required for a particular application to effect complete hydrolysis of the plant tissue and particularly the IPC or CIPC. In most cases the use of 25 to 50 ml. of a 50% (w./v.) NaOH solution for the hydrolysis of a 200-gram sample, slurried with 200 to 300 ml. of water, is recommended. Smaller samples required a proportionally smaller volume of the 50% NaOH solution. For several of the samples reported, an acid hydrolysis, followed by alkaline distillation, was the most satisfactory technique. Acid hydrolysis was accomplished using 25 ml. of 1:1 H_2SO_4 or an H_2SO_4/H_3PO_4 acid mix added to the sample slurry.

Measure 10 ml. of dilute hydrochloric acid (3 parts acid to 2 parts water) into a 150-ml. receiver beaker and place at the exhaust of the downward distillation condenser, with the drip-tip of the condenser immersed below the liquid level of the acid in the beaker. Begin heating the sample under reflux to effect hydrolysis of the tissue and the IPC or CIPC. After an appropriate hydrolysis period, usually about 3 hours, render the sample alkaline, if necessary, by the careful addition of a slight excess of 50% NaOH solution through the dropping funnel. Discontinue the flow of cooling water through the reflux condenser and steam distill the aniline or 3-chloraniline. Collect 60 to 65 ml. of distillate.

Because the distillate is usually cloudy with steam-distilled oils or greases, it must be clarified before color development. Clarification of the distillate was accomplished when necessary by treating the distillate with about 0.2 gram of Celite filter-aid mixed into the distillate thoroughly, filtering through a Whatman No. 40 filter paper. Proceed with the diazotization of the aniline, color formation with the indicator, and spectrophotometric measurement of the sample as previously described by Montgomery and Freed (1959).

Calculations. Calculate the analytical results as follows:

Table II. Blank Values Expressed as IPC and CIPC for Various Food Crops

Crop	Hydrolysis Conditions	Sample Wt., Grams	Transmittance, % 555 Mμ	Interferences or Blank as Aniline, Mg.	as IPC Mg.	as IPC P.p.m.
Crimson clover	Acid	50	84.5	0.016	0.030	0.62
Sugar beets, roots	Alk.	200	95.0	0.004	0.008	0.04
Sugar beets, tops	Alk.	150	87.5	0.013	0.025	0.17
Lettuce, iceberg	Alk.	100	95.0	0.004	0.008	0.08
Alfalfa	Alk.	20	94.5	0.005	0.009	0.47
			540 Mμ	as 3-Chloraniline	as CIPC	
Safflower, seed	Alk.	50	91.5	0.008	0.013	0.26
Celery, Pascal[a]	Alk.	100	83.5	0.018	0.030	0.30
Celery, Pascal[a]	Alk.	100	93.0	0.007	0.011	0.11
Carrots	Alk.	100	94.5	0.005	0.008	0.08
Ladino clover	Acid	50	83.5	0.019	0.031	0.62
Alfalfa	Alk.	20	96.0	0.002	0.003	0.15
Soybeans	Alk.	10	96.5	0.002	0.003	0.25
Soybeans	Alk.	10	97.0	0.002	0.004	0.35
Okra	Alk.	100	97.0	0.001	0.002	0.02
Corn, green foliage	Alk.	50	92.5	0.007	0.012	0.23
Corn, dried	Acid	25	[c]	0.008	0.014	0.60
Sesame seed	Alk.	10	96.0	0.003	0.004	0.42
Peppers, bell	Alk.	100	91.5	0.008	0.014	0.14
Peppers, pimento	Alk.	100	92.0	0.008	0.012	0.12
Peppers, chili	Alk.	100	[c]	0.003	0.005	0.05
Raspberries, purple	Alk.	100	96.0	0.003	0.004	0.04
Raspberries, red	Alk.	50	96.0	0.003	0.004	0.08
Raspberries, black	Alk.	100	96.5	0.002	0.004	0.04
Cranberries	Alk.	200	98.0	0.001	0.002	0.01
Blackberries	Alk.	100	98.0	0.001	0.002	0.02
Lima beans, Fordhook	Acid	100	92.5	0.007	0.012	0.12
White soup beans	Acid	50	92.5	0.007	0.012	0.24
Southern peas	Alk.	20	93.5	0.007	0.012	0.57
Lettuce, iceberg[b]	Alk	100	87.0	0.014	0.023	0.23
Lettuce, iceberg[b]	Alk.	100	95.0	0.004	0.006	0.06

[a] Celery samples submitted from Wisconsin and California.
[b] Lettuce samples submitted from Pennsylvania and California.
[c] Base line correction method for absorbance measurements.

$$\text{IPC, p.p.m.} = \frac{\text{mg. aniline} \times 1.94 \times 1000}{\text{wt. of sample (grams)}}$$

from aniline calibration curve.

$$\text{CIPC, p.p.m.} = \frac{\text{mg. 3-chloraniline} \times 1.67 \times 1000}{\text{wt. of sample (grams)}}$$

from 3-chloraniline calibration curve.

DISCUSSION

Because of the widely variable physical and chemical characteristics of the many different crops analyzed, and because of space limitations, it is not practical to describe specific details of the techniques and reagents used for each crop reported. The basic directions which require a thorough hydrolysis of the sample followed by steam distillation of the aniline from alkaline solution are applicable to all samples. Some minor adjustments in technique, apparatus, or volumes or concentrations of reagents were required for each new crop considered. Previous experience and consideration of the character of a particular crop suggested the successful approach to each new analysis. Specific details for each sample reported here are available from the authors.

Table III. IPC and CIPC Recovery Analyses

Sample	IPC Added Mg.	IPC Added P.p.m.	IPC Recovered Mg.	IPC Recovered P.p.m.	Recovery, %
Crimson clover	0.020	0.40	0.018	0.36	90
	0.050	1.00	0.049	0.98	98
Sugar beets, roots	0.020	0.10	0.019	0.95	95
	0.020	0.10	0.017	0.85	85
Sugar beets, tops or foliage	0.010	0.07	0.010	0.07	100
	0.020	0.13	0.016	0.11	80
Lettuce, iceberg	0.010	0.10	0.010	0.10	97
	0.020	0.20	0.018	0.18	88
Alfalfa	0.010	0.50	0.008	0.40	80
	0.020	1.00	0.017	0.85	85
	CIPC Added Mg.	CIPC Added P.p.m.	CIPC Recovered Mg.	CIPC Recovered P.p.m.	
Safflower seed	0.010	0.20	0.012	0.24	120
Celery, Pascal[a]	0.010	0.10	0.012	0.12	120
Celery, Pascal[a]	0.010	0.10	0.008	0.08	84
Carrots	0.010	0.10	0.010	0.10	99
Ladino clover	0.020	0.40	0.018	0.04	88
Alfalfa	0.010	0.50	0.009	0.44	87
Soybeans	0.010	1.00	0.011	1.09	109
Okra	0.010	0.10	0.009	0.09	94
Corn, green foliage	0.020	0.40	0.016	0.32	80
Corn, dried	0.020	0.80	0.017	0.68	84
Sesame seed	0.020	2.00	0.016	1.59	80
Sesame seed	0.010	1.00	0.007	0.67	67
Peppers, bell	0.010	0.10	0.013	0.13	130
Peppers, pimento	0.020	0.20	0.018	0.18	92
Peppers, chili	0.020	0.20	0.017	0.17	86
Raspberries, purple	0.020	0.20	0.018	0.18	83
Raspberries, red	0.010	0.20	0.010	0.20	102
Raspberries, black	0.020	0.20	0.018	0.18	91
Cranberries	0.010	0.05	0.009	0.05	89
Blackberries	0.012	0.12	0.012	0.12	103
Lima beans, Fordhook	0.020	0.20	0.016	0.16	82
White soup beans	0.014	0.28	0.011	0.22	76
Southern peas	0.010	0.50	0.010	0.51	102
Lettuce, iceberg[b]	0.010	0.10	0.009	0.09	92
Lettuce, iceberg[b]	0.010	0.10	0.008	0.08	80

[a] Celery samples submitted from Wisconsin and California.
[b] Lettuce samples submitted from Pennsylvania and California.

Table IV. IPC and CIPC Residue Analyses

IPC Samples	Treatment Rate Lb./Acre	Transmittance, % 555 Mμ	IPC Found[a] Residue Mg.	IPC Found[a] Residue P.p.m.
Crimson clover	6	84.5	0.001	0.00
Sugar beets, roots		88.5	0.014	0.07
(Location A)	[b]	86.5	0.020	0.10
		94.5	0.002	0.01
		95.0	0.000	0.00
(Location B)	[b]	95.0	0.000	0.00
		94.5	0.000	0.00
		96.0	0.000	0.00
(Location C)	[b]	96.0	0.000	0.00
		96.0	0.000	0.00
Sugar beet, tops		88.0	0.000	0.00
(Location A)	[b]	88.0	0.000	0.00
		88.0	0.000	0.00
		87.5	0.000	0.00
(Location B)	[b]	84.0	0.008	0.05
		81.0	0.014	0.09
Lettuce, iceberg	Unknown	95.0	0.000	0.00
Alfalfa	5	93.0	0.004	0.20
Alfalfa	5	93.5	0.002	0.10
			CIPC Found[a] Residue Mg.	CIPC Found[a] Residue P.p.m.
CIPC Samples		540 Mμ		
Safflower seed	4	91.5	0.000	0.00
Celery, Pascal	8	—	0.386	3.9
Celery, Pascal	6	93.0	0.000	0.00
Celery, Pascal	6	93.0	0.000	0.00
Celery, Pascal	8	93.0	0.000	0.00
Carrots	8	94.5	0.000	0.00
Ladino clover	4	83.5	0.000	0.00
Ladino clover	8	83.5	0.000	0.00
Alfalfa	[c]	95.5	0.000	0.00
Alfalfa	[c]	91.5	0.008	0.38
Alfalfa	[c]	95.0	0.005	0.25
Alfalfa	[c]	95.5	0.000	0.00
Alfalfa	[c]	95.5	0.000	0.00
Soybeans	40	96.0	0.000	0.00
Soybeans	6	97.5	0.000	0.00
Okra	6	96.5	0.000	0.00
Okra	10	96.5	0.000	0.00
Okra	20	96.5	0.000	0.00
Corn, green foliage	6	92.5	0.000	0.00
Corn, dried	6	[d]	0.000	0.00
Corn, dried	4.5	[d]	0.000	0.00
Corn, dried	4.5	[d]	0.004	0.10
Sesame seed	12	95.5	0.000	0.00
Peppers, bell	16	91.5	0.000	0.00
Peppers, pimento	16	92.0	0.000	0.00
Peppers, chili	8	[d]	0.000	0.00
Raspberries, purple	8	98.0	0.000	0.00
Raspberries, red	8	89.0	0.015	0.29
Raspberries, black	8	96.5	0.000	0.00
Cranberries	25	97.5	0.000	0.00
Blackberries	24	97.5	0.000	0.00
Lima beans, Fordhook	6	89.0	0.007	0.07
White soup beans	6	92.5	0.000	0.00
Southern peas	20	93.5	0.000	0.00
Lettuce	4	84.5	0.005	0.05
Lettuce	[c]	95.0	0.000	0.00

[a] Corrected for blank or interference value as IPC or CIPC.
[b] The sugar beet samples (roots and tops) were treated at the rate of 0, 4, 8 lb. per acre at each location. The samples were coded, and not identified as to which sample reported corresponded to which treatment rate.
[c] Coded samples, treatment rate and history unknown.
[d] Base line correction method for absorbance measurements.

On occasion it was observed that there was some variation in the blank or background value for duplicate analyses of the same crop. Because of the character of the sample, it was sometimes difficult to prevent the occurrence of marked and variable discoloration in the distillate which was not due to color differences. However, it was also observed

that the visual appearance of "recovery" samples containing the colored aniline or chloraniline complex was distinctly different from blank or control samples of the same crop, even though the absolute transmittance or absorbance values at a particular wavelength were essentially the same. This effect is due to the variation in background from test to test. When this occurs, resort was taken to the use of the "base line corrected absorbance" technique. This technique, proposed by Heigl *et al.* (1947), compensates for variations in background by permitting measurement of the difference between the total absorbance for a sample and the base line absorbance due only to background. These data are obtained by tracing the visible spectrum of the sample, using a recording spectrophotometer such as the Cary Model 14 M, and then drawing a straight line or base line from the absorbance value at 400 mμ to the value at 700 mμ. The absorbance difference is measured as before at either 555 mμ for IPC samples or 540 mμ for CIPC samples.

ANALYTICAL RESULTS

The results of the most recent IPC and CIPC residue analyses are given in Tables II, III, and IV. For each sample studied blank values, to evalate and determine naturally occurring interferences, recovery analyses to validate the ability of the method for determining the herbicide in the presence of plant tissue, and treated crop analyses were conducted.

Table II lists the results of analysis of untreated control samples for blank or interference values expressed as IPC or CIPC. Table III lists the results of the recovery analyses for these same samples, after fortifying the control or check sample with IPC or CIPC at p.p.m. levels anticipated for treated crops. Table IV reports the actual analysis of these crops or food samples harvested from fields actually treated with the herbicide IPC or CIPC at various application rates. In each case the treated crop analyses were conducted using identically the same analytical conditions as were used for blank and recovery analyses.

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