lation, either in plotting or in actual operation with the same scale.

The apparatus has been so designed that it can be constructed easily in the ordinary laboratory from commonly available parts, with no significant amount of labor other than simple trimming, fitting, assembly, and calibration. A list of suitable components has been prepared, specifying aluminum and stainless steel as dominant materials; a sturdy preformed sheet-metal base; and adequate controls, including panel-mounted switch and pilot lamp (Figure 1). The total cost of parts is approximately $40.

Performance

A good illustration of performance is the record of the first three series of replicate sieve runs made with the sampler. The materials were granulated heptachlor formulations, one each of low, medium, and high dust content. All samples were repeated to exhaustion, the first yielding 16 runs and the others seven each. The mean values for dust content (fraction under 250 microns) were 1.5, 4.6, and 8.6%; the mean deviations were 0.1, 0.2, and 0.3%, respectively, of the sample weight. Thus the mean deviations were roughly proportional to the dust content; and near the critical dust content of 5%, the mean deviation was only 0.2% of the sample weight.

The original tests by the designer have been supplemented with large numbers of analyses by another observer, on formulations of various insecticides with attapulgite and on several other types of carriers without toxicants. The improvement in reproducibility effected by the turntable was generally about threefold, and the mean deviation with materials of normal quality was seldom more than 0.2% of the sample weight.

In many instances, the same sample was subjected to two nearly simultaneous series of analyses, one with turntable and one without. Examples for direct comparison of the two methods are an analytical series on five samples, each of which was divided into two portions, one for testing with turntable and one without; the observations were repeated until the material was exhausted, each portion yielding either seven or eight runs. The results are summarized in Table I.

The advantages of this sampling procedure, in addition to their direct relationship to dust determination, have an important bearing on other kinds of tests, especially where dust content may be a factor. The possibilities in grain-breakdown determination as prescribed in Interim Federal Specifications for granulated insecticides (*3*) have been investigated, and it has been found that there is often a worthwhile improvement in reproducibility when the turntable is used. The first five pairs of comparative test series comprised a total of 98 observations divided approximately equally between use and omission of turntable with the same samples. For the four samples (out of five) for which the mean deviations by the usual procedure were as much as 0.5% of the sample weight, the mean deviations were from one third to two thirds as great with turntable as without.

Possible Extensions

The sampling apparatus and procedure as described are intended specifically for use in taking the ultimate test sample from the laboratory stock sample of moderate size as ordinarily received for analysis. Beyond the scope of the analyst's operation, but equally important, is the drawing of the laboratory sample in such a way that it will be truly representative of the lot from which it is taken. The same principle may be followed, with any necessary modification in dimensions of equipment, for sampling on a larger scale, as in drawing a laboratory stock sample from a 50-pound bag, or for dividing an original sample into subsamples. Two or more test samples may be taken simultaneously from the stock sample by using a separate cup for each test sample.

Acknowledgment

Test runs to confirm the effectiveness of the sampler were made by James R. G. Jones, Entomology Research Division, U. S. Department of Agriculture.

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FUNGICIDE RESIDUES

Extraction and Determination of 2,6-Dichloro-4-nitroaniline in Processed Fruits

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Interest in the use of 2,6-dichloro-4-nitroaniline (dicloran) (Botran, The Upjohn Co.) in California as a control for postharvest fungus rots on stone fruits has created a need for a suitable method of analysis and residue studies in processed fruits. For effective control of the diseases, fruits are treated with the chemical after harvesting, but before storing for ripening (*5*). Once ripened, they are processed as usual for canning (*4*). Postharvest application places this fungicide in the category with food additives, making it imperative that residue data be obtained with the canned products.

The existing method for the determination of dicloran residues is based on the reduction of the parent structure to the corresponding phenylenediamine by zinc and acid (*1*, *2*). Although useful for some crops (*2*), this procedure was not sensitive enough nor readily adapted for our purposes.

In the present study, a procedure is described for the extraction and colorimetric determination of dicloran residues in processed fruits and their sirups. The colorimetric procedure is based on the development of an intense yellow color characteristic of some mononitro aromatic compounds in the presence of strong alkali and acetone. It is a modification of the method previously described by Porter (*6*) for aromatic nitro compounds, and employs the Janovsky reaction (*3*).

Treatment of Fruit

The peaches (Vivian, clingstone; Fay Elberta, freestone) and apricots (Royal) were dipped for 2 minutes in suspensions containing 250, 500, 750, and 1000 p.p.m. of dicloran and stored for 24 hours at 72° F., 80% relative humidity. After storing, the fruits were canned as halves by the Department of Food

2,6-Dichloro-4-nitroaniline (dicloran) is effective as a postharvest fungicide in controlling fungus rots on stone fruits. Therefore, a method of analysis was developed for microdetermination of the fungicide in extracts of processed fruits and their sirups. The residue is suitably extracted, purified by column chromatography, evaporated to dryness, and taken up in acetone. Following the addition of strong alkali, the color which develops is read at 464 mμ. Recovery experiments from fortified samples, as well as data obtained from commercially processed samples, are presented.

Science and Technology, University of California, Davis, using standard commercial processing procedures. Peaches (Peak, clingstone) to be canned as purée were dipped for 2 minutes in a suspension containing 750 p.p.m. of dicloran, stored outside overnight, and processed in the pilot plant of Gerber Baby Foods, Oakland, Calif.

Peaches (Indian Red, freestone) to be processed as frozen fresh fruit with sirup were dipped for 2 minutes in a suspension containing 1000 p.p.m. of dicloran and stored in an open shed for 48 hours. The fruit was then canned by the Frosted Fruit Company, Sanger, Calif. In one experiment, untreated fruit was processed in the normal manner except the lye peeling solution contained 41 p.p.m. of dicloran. In another experiment, the treated fruit was also peeled in a lye solution containing 41 p.p.m. of dicloran. All other processing conditions were unchanged.

Table I. Recovery of Dicloran from Processed Fruits and Sirups[a]

Material Analyzed	Dicloran, p. p. m. Added	Dicloran, p. p. m. Found	Recovery, %
Clingstone peach, Vivian			
Fruit	0.1	0.09	90
	0.2	0.19	95
	0.4	0.36	90
Sirup	0.1	0.08	80
	0.2	0.17	85
	0.4	0.38	95
Freestone peach, Fay Elberta			
Fruit	0.1	0.08	80
	0.2	0.18	90
	0.4	0.35	88
Sirup	0.1	0.08	80
	0.2	0.17	85
	0.4	0.36	90
Apricot, Royal			
Fruit	0.1	0.08	80
	0.2	0.18	90
	0.4	0.35	88
Sirup	0.1	0.08	80
	0.2	0.17	85
	0.4	0.34	85
Peach purée, Peak			
Sugared	0.1	0.10	100
	0.2	0.16	80
	0.4	0.34	85
Unsugared	0.1	0.08	80
	0.2	0.15	75
	0.4	0.32	80
Frozen peach, Indian Red			
Fruit	0.1	0.09	90
	0.2	0.16	80
	0.4	0.31	78
Sirup	0.1	0.09	90
	0.2	0.17	85
	0.4	0.37	93
		Av.	86%

[a] 50 grams of fruit and 25 grams of sirup were used in each determination.

Procedure

Preparation of Standard Curve. Prepare a calibration curve by diluting aliquots of a stock solution (100 μg. of dicloran per ml. of acetone) with acetone to contain from 2.5 to 20 μg. of dicloran in a final volume of 5.0 ml. Add 0.15 ml. of 4% aqueous KOH and determine the absorption at 464 mμ.

Following the addition of aqueous KOH to acetone solutions containing dicloran, an intense yellow color develops which has a peak absorption at 464 mμ. Maximum color development is obtained almost immediately, and the color which is formed remains stable for about 30 minutes. Calibration curves prepared by the procedure obey Beer's law up to at least 20 μg. of dicloran in 5 ml. of acetone. Under these conditions, standard solutions containing 2.5, 5, 10, 15, and 20 μg. of dicloran have absorbances of 0.080, 0.155, 0.310, 0.455, and 0.630, respectively, when measured in $\frac{1}{2} \times$ 4-inch round cuvettes with a Bausch and Lomb Spectronic 20 colorimeter.

Extraction Method. Open samples of the canned fruit, pour over $\frac{1}{8}$-inch standard screens, and drain for exactly 2 minutes, the sirups being collected in separate containers. Thaw the canned frozen fresh peaches at 5° C. for 12 hours before opening.

Chop the drained fruit into small pieces and place 400 grams of the material in a 1-gallon tin can equipped with a metal baffle for mixing purposes. Add 800 ml. of benzene, seal tightly, and roll on a mechanical roller (35 r.p.m.) for 30 minutes. Decant the supernatant fluid into a beaker containing approximately 100 grams of sodium sulfate, mix thoroughly, and filter through fluted filter paper. Store the filtrate in a tightly capped bottle until analyzed.

Extract 25 to 50 grams of sirup, previously diluted ten-fold with distilled water, with 100 ml. of ethyl ether for 1 minute in a 500-ml. separatory funnel. Allow to stand for 10 to 15 minutes, then drain off the lower aqueous layer and transfer the remaining upper layer to a 200-ml. beaker containing 20 grams of sodium sulfate. Filter and store these extracts as previously described for the fruits.

Pass a 100-ml. aliquot of the sample (extractives from 50 grams of fruit or 25 grams of sirup) through a 2.5 × 25 cm. column containing 25 grams of florisil (60–100 mesh) previously rinsed with 50 ml. of benzene. The florisil is not reactivated, being used as obtained from the Floridin Co., Tallahassee, Fla. Discard the first 50-ml. effluent. Collect the solvent remaining in the column and the subsequent washings, two 100-ml. portions of 1% ethyl ether in benzene, in a 500-ml. evaporation flask.

Evaporation and Colorimetric Determination. Evaporate the solvent under reduced pressure with a rotating flash evaporator, the evaporation flask being partially immersed in a 60° C. water bath. Remove the flask from the evaporator approximately 1 minute after the last traces of solvent have disappeared and blow air into the flask until no benzene vapors are evident. Rinse down the sides of the flask with 3 ml. of acetone, and filter the contents through glass wool. Rinse the flask successively with 2-ml. and 1.0-ml. aliquots of acetone, filtering after each addition. Combine the filtrates in a test tube, adjust the volume to 5 ml. with acetone, and add 0.15 ml. of 4% aqueous KOH. Transfer the solution to a $\frac{1}{2} \times$ 4 inch round cuvette previously rinsed with acetone and determine the absorption of the resulting yellow color against a reagent blank with a Bausch and Lomb Spectronic 20 colorimeter at 464 mμ within 30 minutes.

Results and Discussion

Although dicloran is soluble in several organic solvents, benzene was selected as the solvent most useful for the extraction of residues in processed fruits, particularly because it extracts a very small amount of interfering substances. Extraction of fruits for 30 minutes by the tumbling procedure was sufficient to ensure complete removal of the residue. Longer periods of extraction only increased the blank values.

The sirups of the canned fruits presented a special problem because of their high sugar content. Benzene did not prove to be a satisfactory extracting solvent for the sirup, since the emulsion which formed was not readily broken.

Table II. Dicloran Residues in Processed Fruits and Sirups

Kinds of Fruits	Variety	Material Analyzed[a]	Dicloran Dipping Solution, P.P.M. 250	500	750	1000
			Residue present after processing, p.p.m.[b]			
Clingstone peach	Vivian	Fruit	<0.05	<0.05	<0.05	<0.05
		Sirup	<0.1	<0.1	<0.1	<0.1
Freestone peach	Fay Elberta	Fruit	<0.05	<0.05	0.09	0.10
		Sirup	<0.1	<0.1	<0.1	<0.1
Apricot[c]	Royal	Fruit	<0.05	<0.05	<0.05	<0.05
		Sirup	...	...	<0.1	<0.1
Peach purée	Peak	Sugared	...	...	0.85	...
		Unsugared	...	...	1.00	...
Frozen peach	Indian Red	Fruit	...	...	...	2.17
		Sirup	...	...	...	0.8
Frozen peach (peeled in lye solution)[d]	Indian Red	Fruit	...	...	...	2.21
		Sirup	...	...	...	1.0

[a] 50 grams of fruit and 25 grams of sirup were analyzed.
[b] The figures are uncorrected for the average recovery of 86%. Each value represents the average of 3 to 6 determinations.
[c] Unpeeled and rinsed.
[d] The lye solution contained 41 p.p.m. of dicloran. Untreated fruit dipped in a lye solution containing 41 p.p.m. of dicloran had no detectable residue.

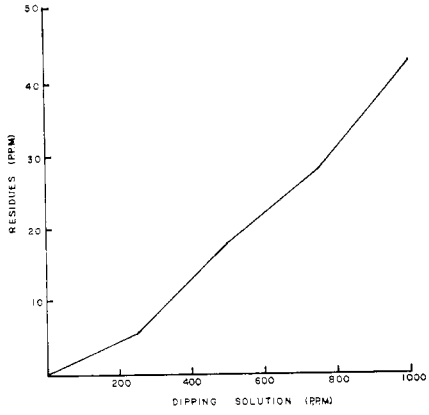

Figure 1. Dicloran residues on peaches after dipping

However, it was found that the residues could be extracted with good recoveries by using ethyl ether. Very little emulsion was formed with this solvent, and that which did form broke on standing.

Of the several column materials used, florisil proved to be the most useful for removing interfering substance from both fruits and sirups. The elution patterns of dicloran fr m reactivated florisil were erratic and often overlapped the interfering materials which produced variable results. On the other hand, florisil which was not reactivated retained almost all the undesirable components, permitting excellent recoveries of the residues.

Excellent recoveries of dicloran were obtained when the extracting solvent was removed under reduced pressure at 60° C. with the flash evaporator. Since dicloran is of low volatility and stable to heat, the flask can remain on the evaporator for several minutes after the last visible traces of solvent have been removed with no significant loss of the residue. It is important, however, to be sure that all extracting solvent, as well as the vapors, is removed from the flask. A slightly turbid solution will develop on addition of acetone, the color developing solvent, if extracting solvent is present in the flask. This occurs because a small quantity of wax which is soluble in benzene and ethyl ether, but not in acetone, is eluted from the florisil column along with the dicloran residues. Blanks from unfortified cheek samples varied from 0.02 to 0.03 absorbance after color development. With the sample size (50 grams of fruit) used for total extraction, these values correspond to about 0.01 to 0.02 p.p.m. on an absolute basis.

Table I presents data showing recovery of dicloran added to processed fruits and their sirups. The data in Figure 1 illustrate the amount of dicloran which is retained by the fruit after dipping but prior to canning. Table II shows the amount of residue remaining in the canned products. Frozen fresh peaches with sirup, as well as the peach purée, showed significant quanitities of residues. This may be caused by differences in peach varieties, or possibly differences in canning procedures used by various processors. In most instances, however, residues were below the sensitivity of the method.

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