at the end of this time, 10 to 15 tons of roots per acre are left to decompose whether the new crop is grown from stubble (ratooned) or completely replanted. Further, the sugar cane leaves, discarded at the rate of one leaf in approximately 14 days as a new leaf is formed, provide a mat, part of which (depending on climate) decomposes or leaches into the soil. Wet oxidation of this fraction with hydrogen peroxide has shown that the soil adsorption capacity for monuron decreased nearly to the subsoil values, provided that no free carbon was present in the soil (Table II, Hilo, A).

True carbon provides a major source of herbicide adsorptive capacity in Hawaiian sugar cane soils. Just prior to harvest, sugar cane fields are burned to facilitate mechanical harvesting by removal of as much of the leaf trash and tops as possible. In dry areas or in dry seasons, the burns will be relatively complete but will supply a considerable quantity of particulate matter, more or less completely carbonized. The total quantity may not be large in comparison with soil volume, but the adsorptive capacity is high and the pre-emergence herbicides are applied for the new crop generally within 2 to 3 weeks after harvesting. Areas with greater rainfall have less carbon due to poor burning of wet trash and in limited areas there is no attempt to burn unless conditions are very favorable.

Unfortunately, conventional wet methods for determining organic matter in soil do not oxidize carbon and combustion methods affect soil water, bicarbonates, and other minerals, and do not distinguish between organic forms. Further, it is doubtful that organic residues in soils of different climates decompose at the same rate or even to the same products.

Table II summarizes a series of oxidations with H_2O_2 and ignition on several horizons of three soils. HC&S-715 comes from a dry climate with good burns; its topsoil adsorption remained high even after peroxide treatment. Hilo-50 soil is from a wet climate with poor burns; its topsoil adsorption was reduced markedly with peroxide and completely on ignition. Honokaa-5a is from an intermediate climate with fair burning conditions for part of the year; its properties were apparently intermediate with complete removal of adsorption on ignition.

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Literature Cited

(1) Jackson, M. L., "Soil Chemical Analysis," p. 222, Prentice-Hall, New York, 1958.
(2) Wiklander, L., Kouther-Andersson, E., *Acta Agr. Scand.* **5,** 215 (1955).
(3) Young, H. Y., Gortner, W. A., *Anal. Chem.* **25,** 800 (1953).
(4) Yuen, Q. H., Hilton, H. W., J. Agr. Food Chem., **10,** 386 (1962).

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HERBICIDE RESIDUES

Determination of Micro Amounts of Isopropyl *N*-(3-Chlorophenyl)carbamate (CIPC) in Milk and Urine Excreted from Dairy Cows

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Sensitive analytical methods for determining micro amounts of the herbicide CIPC in milk and urine from dairy cows were needed in connection with feeding tests. The developed analytical methods entail extraction, hydrolysis, and spectrophotometric techniques as a basis of measuring CIPC residues in the samples. Results from fortified and aged samples show recovery of CIPC ranging from 75 to 80% at the 0.1 p.p.m. and 0.025 p.p.m. concentration levels, respectively. The methods are applicable to analysis of milk produced by dairy cows who have eaten forage crops treated with CIPC for weed control.

During the past several years, isopropyl *N* - (3 - chlorophenyl)-carbamate (CIPC) has been used experimentally as a pre-emergence and postemergence herbicide to control the growth of narrow-leaved plants, such as crabgrass, wild oats, and witch grass (*3*). This herbicide was first used in the production of food crops such as lettuce, sugar beets, onions, tomatoes, spinach, and carrots. Success in these applications encouraged its experimental use in the production of forage crops, such as alfalfa, with good results.

It is very important to determine the amount of CIPC or its metabolite, 3-chloroaniline, in milk produced by dairy cows consuming forage crops treated with this chemical. Simultaneously with this determination in the milk, it is of interest to determine the amount of CIPC or its metabolite, which is excreted in the urine.

During May and June, 1961, a cooperative program was instituted between the Pittsburgh Plate Glass Co., Chemical Division, and the Ohio Agricultural Experiment Station, Wooster, Ohio, to conduct feeding studies (*5*) wherein known amounts of CIPC were introduced in the daily rations of grain given to dairy cows. These feeding studies were conducted as a part of a program wherein the selection of the test animals, administration of the fortified diet, and the sampling and analytical procedures were carried out according to accepted practices. The results of this feeding study will be reported elsewhere.

Basic spectrophotometric procedures for determining micro amounts of

isopropyl *N*-phenylcarbamate (IPC) and CIPC in various crops are described by Gard and Rudd (*2*) and by Montgomery and Freed (*4*). Modifications and improvements in the Gard-Rudd original method were made by Gard, Ferguson, and Reynolds (*1*) in 1959. The basic method details extraction of the macerated crop with methylene dichloride to separate the CIPC from the sample, after which the evaporated extract is treated with dilute sulfuric acid to hydrolyze the CIPC to 3-chloroaniline. The resulting 3-chloroaniline is then steam-distilled from the solution and determined spectrophotometrically by the phenol-ammonia-hypochlorite method. Modification and improvement of this method incorporates the use of methanol as extractant for the CIPC with subsequent re-extraction of the methanol solution with petroleum ether, and the treatment of the distillate with Celite as a means of reducing interferences in the method.

The Montgomery-Freed method details a direct alkaline hydrolysis treatment of the macerated sample without extractive separation of the CIPC with an immiscible solvent. Separation of the 3-chloroaniline hydrolysis product is effected by steam distillation, and the product is collected in the distillate. Measurement is made spectrophotometrically by a diazotization and dye-coupling reaction with *N*-1-naphthylethylenediamine dihydrochloride.

Certain technical features of both methods outlined above were incorporated into procedures for analyzing milk and urine samples. The resulting methods yielded nominal interferences and provided satisfactory recovery values for CIPC when added in known amounts to the samples.

Experimental Work

Experimental work involved in developing methods for milk and urine samples was commenced by hydrolyzing the entire analytical samples without extraction with solvents to separate the CIPC. Direct techniques of this type eliminate possibilities of incomplete separation which potentially exist when solvent extraction processes are employed.

Application of the Montgomery-Freed method involving direct alkaline hydrolysis of milk yielded very satisfactory and reproducible blank or interference values for control samples and gave satisfactory recovery values of CIPC fortified samples of milk.

This direct hydrolysis procedure when applied to urine samples produced excessive and erratic amounts of some component which interfered seriously with the analytical method. After considerable experimentation along this direct line with unsatisfactory results, the authors resorted to solvent extractive techniques.

Analysis of urine samples employing solvent extraction technique and acid hydrolysis yielded nominal and reproducible control values for unfortified urine and satisfactory recovery of CIPC from fortified samples. The fortified samples of both milk and urine were prepared by adding appropriate volumes of a petroleum ether solution of CIPC (1 ml. equivalent to 0.005 mg. of pure CIPC) to the sample prior to the direct hydrolysis in the case of milk and prior to the extractive process in the case of urine.

Aging and Source of Samples

It is unlikely that analysis of the milk and urine samples would be commenced directly after collection. Consequently, it is important to know how CIPC residue results for these materials are influenced as a result of storage periods prior to analysis. This aspect was appraised experimentally for both materials by analyzing freshly collected samples, and then two days later, after storage, re-analyzing portions of the same sample. A similar experiment was conducted with samples fortified in the laboratory with known amounts of CIPC to evaluate any adverse effects on the recovery resulting from storage.

The results involving whole milk indicated that the interferences and precision of the analysis for the samples were not influenced adversely as a result of refrigerated storage extending 2 days and that the recovery of CIPC from the fortified samples was the same as that obtained with the samples analyzed immediately after collection.

Analysis of the control and fortified samples of urine, however, revealed that the storage period influenced the results significantly and that storage at room temperature during a 2-day period was necessary for precision and reproducibility of results. Data from unfortified urine samples given in Table I provide comparison and illustrate the effects of sample-aging on the results from the same samples. Analyses obtained from unaged samples are sufficiently randomized and do not justify grouping for calculating an average interference factor as a correction for recovery and sample analysis. On the other hand, the data obtained from the aged samples are satisfactorily grouped and related, and justify calculation of a valid interference or correction factor.

The methods described were developed on the basis of composite samples from several cows and do not include any individual variations in the interference which may exist. Prior to any feeding tests, it was necessary to conduct repetitive analyses of milk and urine from the individual cows fed diets as close as possible to those used during the feeding tests, in order that valid values for interference could be established on an individual basis. The evaluation of this variable was necessary in computing the net amount of CIPC which may be present in the milk and urine as a result of the feeding experiments.

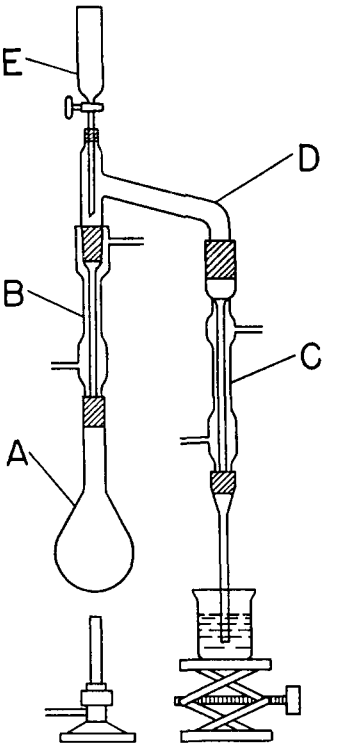

Figure 1. Hydrolysis and distillation apparatus

(A) 500-ml. Kjeldahl-type hydrolysis flask; (B,C) West-type condenser, 12 inches in length; (D) side arm distilling head; (E) 125-ml. dropping funnel

Table I. Analysis of Urine Excreted from Dairy Cows for Interferences Expressed as CIPC

Fresh Samples[a]		*Aged Samples*[b]	
Transmittance, % (540 mμ)	*Found, p.p.m. as CIPC*	*Transmittance, % (540 mμ)*	*Found, p.p.m. as CIPC*
69	0.033	79	0.019
39	0.158	77	0.021
62	0.045	77.5	0.021
50	0.067	77.5	0.021
59	0.050	78	0.020

[a] Samples analyzed immediately after collection.
[b] Samples stored 2 days at room temperature before analysis.

Apparatus

The apparatus required for the analysis includes a distillation assembly, shown in Figure 1, and a spectrophotometer which accommodates 5-cm. comparison cells.

Reagents

Dow Corning Antifoam, Type A.

Sodium nitrite, 2% solution, prepared fresh daily.

Sulfamic acid, 10% solution, prepared fresh daily.

N-1-naphthylethylenediamine dihydrochloride, 2% solution.

Preparation of Standard Curve

A standard calibration curve was prepared using 3-chloroaniline specially purified in the laboratory by distillation at reduced pressure. Only the water-white, middle fraction with n_D^{20} 1.5937 was used for this work, the calculated purity of which was 99.5% based on total chlorine and infrared analysis. CIPC may be calculated from the 3-chloroaniline.

Weigh 0.1000 gram of the purified 3-chloroaniline and dissolve in 1*M* hydrochloric acid. Dilute to 1000 ml. with additional 1.0*M* acid and mix thoroughly. Dilute 10 ml. of this solution to 1000 ml. with the acid. One milliliter of the resulting solution is equivalent to 0.001 mg. of 3-chloroaniline or 0.0017 mg. of CIPC.

Measure 0.0, 2.0, 4.0, 6.0, 8.0, 10.0, and 12.0 ml. of the standard 3-chloroaniline solution into a series of 50-ml. volumetric flasks. Dilute each standard to approximately 40 ml. with 1*M* hydrochloric acid and mix thoroughly. Add 1 ml. of the sodium nitrite solution, mix thoroughly, and allow 20 minutes for diazotization. Add 1 ml. of the sulfamic acid solution, mix thoroughly, and allow 15 minutes for the complete destruction of the excess nitrite. After the decomposition of the nitrite is complete, add 5 ml. of the *N*-1-naphthylethylenediamine dihydrochloride solution, dilute to 50 ml. with 1*M* hydrochloric acid, and mix thoroughly.

After 90 minutes, transfer a portion of the developed standard solution to the 5-cm. comparison cell of the spectrophotometer and measure the transmittance at 540 mμ as compared with distilled water in the reference cell.

Data for the standard curve employing a Beckman Model B Spectrophotometer, 5-cm. comparison cell, and 540-mμ wavelength are shown in Table II.

Table II. Calibration of Spectrophotometer

3-Chloroaniline, mg.	Transmittance, % 540 mμ[a]
0.000	95.0
0.002	80.0
0.004	68.5
0.006	58.0
0.008	49.0
0.010	40.0
0.012	36.0

[a] Spectrophotometric readings *vs.* distilled water in reference cell.

Methods

The analytical method for milk involves direct treatment with strong alkali to effect hydrolysis of CIPC to 3-chloroaniline followed by steam distillation. Urine samples require solvent extraction to separate CIPC prior to an acidic hydrolysis treatment, and steam distillation of the 3-chloroaniline. The spectrophotometric method for determining 3-chloroaniline in the distillates is common to both samples and directs the use of *N*-1-naphthylethylenediamine dihydrochloride.

Milk, Alkaline Hydrolysis. A direct alkaline hydrolysis of the entire sample was followed according to the technique described by Montgomery and Freed (*4*).

Weigh 100 grams of raw, whole milk into the 500-ml. Kjeldahl flask, add 6 to 8 drops of Dow Corning Antifoam, Type A, and attach to the hydrolysis and distillation assembly. Cautiously add 100 ml. of 50% sodium hydroxide solution (prepared from reagent-grade pellets) through the dispensing funnel, rinse the funnel into the flask with 25 ml. of distilled water, and mix as much as possible by rocking the assembly gently.

Cautiously heat and reflux the solution in the flask for 3 hours to effect hydrolysis of the CIPC. At the end of the hydrolysis period, discontinue the flow of cooling water through the reflux condenser. Place 5 ml. of diluted (3 to 2) hydrochloric acid in the receiver beaker, and position at the exhaust of the downward condenser in such a fashion that the delivery tip is beneath the surface of the liquid in the beaker.

Continue to heat the solution gently and collect 30 ml. of distillate in the receiver beaker. After collection of the distillate, add 0.2 gram of Celite filter aid (Johns-Manville) to clear the distillate and remove some of the interfering materials which may have distilled. After treatment, filter the solution through a Whatman No. 42 filter paper and collect the clear filtrate in a 50-ml. volumetric flask. Wash the residue on the filter paper sparingly with distilled water to an extent not exceeding 40 ml. of volume in the flask. The color development and measurement is conducted in the same manner as indicated for the calibration.

Urine, Sample Preparation and Extraction. At the time of sample collection, add 0.5 ml. of formalin per pint as preservative. Allow the samples to age at room temperature for about 48 hours before commencing the analysis.

Weigh 200 grams of the aged sample into a separatory funnel and render it slightly alkaline by addition of a few pellets of sodium hydroxide. Commence the extraction by adding 150 ml. of ethanol and 75 ml. of petroleum ether. Shake and extract the mixture vigorously for 1 minute, and then allow the phases to separate.

Drain the aqueous bottom phase into another separatory funnel and retain the ether phase in the initial funnel. Re-extract the aqueous portion a second and third time with petroleum ether. At the conclusion of the third extraction, discard the aqueous phase, and retain the petroleum ether portions in the three respective funnels. Wash these extracts progressively three times with water-alcohol mixture diluted 3 to 1 and finally discard these water-alcohol washes.

Combine the ether phases in the 500-ml. hydrolysis and distillation flask, add four to five glass beads to prevent bumping and evaporate the petroleum ether to apparent dryness under reduced pressure furnished by a water aspirator pump.

Acidic Hydrolysis. Add 20 ml. of dilute (1 to 1) sulfuric acid solution to the residue remaining in the flask and attach to the hydrolysis and distillation assembly. Heat and boil the solution gently under reflux conditions for 1.5 hours to effect hydrolysis of the CIPC to 3-chloroaniline sulfate. After hydrolysis, cool to room temperature and rinse the reflux condenser into the flask with 100 ml. of distilled water introduced through the dispensing funnel at the top of the assembly.

Discontinue the flow of cooling water through the reflux condenser and place 5 ml. of diluted (3 to 2) hydrochloric acid in the receiver beaker and position at the exhaust of the downward condenser.

Cautiously add 50 ml. of 50% sodium hydroxide solution through the dispensing funnel, mixing as much as possible, and proceed with the distillation, color development, and measurement as indicated for the milk analysis and preparation of the standard curve.

Calculation.

$$\text{p.p.m. of CIPC} = \frac{\text{mg. of 3-chloroaniline from std. curve} \times 1.67 \times 1000}{\text{sample weight}}$$

Recovery of CIPC

The blank measurement of the reagents used in the method which involved no CIPC or 3-chloroaniline is of a low order but must be verified from time to time, particularly when supplies of reagents are replenished.

The analyses of samples of cows' milk and urine shown in Table III indicate the presence in the samples of

Table III. Recovery of Isopropyl *N*-(3-Chlorophenyl)carbamate

CIPC Added		Transmittance,	CIPC Found			
			Total		Net	
Mg.	P.p.m.	%, 540 mμ	Mg.	P.p.m.	P.p.m.	% Recovery
		MILK (100-GRAM SAMPLES)				
0.000	0.000	67	0.0072	0.072	...	...
	0.000	70	0.0063	0.063	...	...
	0.000	66.5	0.0073	0.073	...	...
	0.000	70	0.0063	0.063	...	...
0.010	0.100	45	0.0155	0.155	0.087	87
	0.100	48	0.0142	0.142	0.074	74
	0.100	51	0.0130	0.130	0.062	62
	0.100	42	0.0170	0.170	0.102	102
	0.100	47	0.0147	0.147	0.079	79
					Av.	81
		URINE (200-GRAM SAMPLES)				
0.000	0.000	78	0.0040	0.020	...	...
	0.000	76	0.0047	0.024	...	...
	0.000	76.5	0.0044	0.022	...	...
	0.000	79	0.0038	0.019	...	...
0.005	0.025	65	0.0080	0.040	0.019	76
	0.025	64	0.0084	0.042	0.021	84
	0.025	66.5	0.0073	0.037	0.016	64
	0.025	63.5	0.0085	0.043	0.022	88
	0.025	68	0.0070	0.035	0.014	56
					Av.	74

some components which respond to the analytical methods and thus cause interference. The calculated average interference for the milk samples is 0.068 p.p.m. expressed as CIPC; and for urine is 0.021 p.p.m. The precision of the methods based on 95% confidence limits is $\pm 19.91/\sqrt{n}\%$ of the average of n determinations. This precision applies to levels ranging from 0.02 to 0.15 p.p.m. of CIPC. The calculated average recovery of CIPC from the samples of milk fortified at the 0.1 p.p.m. concentration level in the laboratory is 81%. Similarly, samples of aged urine fortified at the 0.025 p.p.m. concentration level gave a calculated average recovery of 74%.

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Literature Cited

(1) Gard, L. N., Reynolds, J. L., Ferguson, C. E., Jr., J. AGR. FOOD CHEM. **7**, 335 (1959).
(2) Gard, L. N., Rudd, N. G., *Ibid.*, **1**, 630 (1953).
(3) Klingman, G. C., "Weed Control as a Science," Wiley, New York, 1961.
(4) Montgomery, M., Freed, V. H., J. AGR. FOOD CHEM. **7**, 617 (1959).
(5) Ware, G. W., Brakel, W. J., unpublished report prepared jointly with the Department of Zoology and Entomology, and Department of Dairy Science, The Ohio State University, Columbus, Ohio, 1961.

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INSECTICIDE SCREENING

Synthesis and Insecticidal Activity of *O*-Methyl *O*-(2,4,5-Trichlorophenyl) Phosphoramidothioates and Related Compounds

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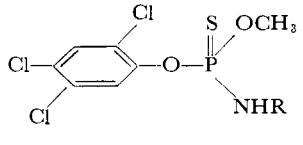
PRACTICALLY all the work reported on the synthesis and biological activity (*7*, *10*) of organic phosphorus compounds has been done on triesters containing two methyl groups or two ethyl groups. The investigations described in this publication are concerned with the synthesis and insecticidal activity of a series of *O*-methyl *O*-(2,4,5-trichlorophenyl) phosphoramidothioates,



where R is hydrogen or an aliphatic hydrocarbon radical containing one to four carbon atoms.

Different phosphorus acid chlorides can be used to prepare *O*-alkyl *O*-aryl phosphoramidothioates. Alkylphosphoramidodichloridothioates (*9*), *O*-alkyl phosphorodichloridothioates (*6*, *9*), and *O*-aryl phosphorodichloridothioates (*4*, *6*) have been used for such purposes. The dichloridothioates are converted to monochloridothioates by esterification with an alcohol or a phenol in the presence of hydrogen chloride acceptors. However, the purity and yields of many of these intermediates as well as the final products have not been entirely satisfactory due to the formation of other phosphorus derivatives. An improved method for the preparation of various thiophosphoric acid chlorides was therefore desirable.

Chemical Studies

O-(2,4,5-Trichlorophenyl) phosphorodichloridothioate (I), available in high purity (*12*), appeared to be an ideal starting material. Since the authors were interested in preparing *O*-alkyl *O*-aryl phosphoramidothioates in which the two ester groups were constant constituents(III), *O*-methyl *O*-(2,4,5-trichlorophenyl) phosphorochloridothioate (II) was prepared for use as an intermediate (*13*).