Residues in Crops Receiving Pre-emergence Treatment with Isopropyl *N*-(3-Chlorophenyl)carbamate

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It is important to determine the amount of isopropyl *N*-(3-chlorophenyl)carbamate in specific food crops at harvest, which were grown in experimental field plots receiving pre-emergence treatment with the herbicide. Food crops examined include head lettuce, spinach, sugar beets, onions, cotton seeds, and peanuts. The test method involved extraction of isopropyl *N*-(3-chlorophenyl)carbamate with methylene dichloride, concentration by evaporation, hydrolysis of the herbicide to 3-chloroaniline with sulfuric acid, and colorimetric measurement of the 3-chloroaniline with a photoelectric colorimeter. The crops receiving pre-emergence treatments ranging between 2.5 and 8.0 pounds of isopropyl *N*-(3-chlorophenyl)carbamate per acre did not contain herbicidal residues in excess of 0.05 p.p.m. of isopropyl *N*-(3-chlorophenyl)carbamate, which is the low limit of sensitivity of the method.

ISOPROPYL *N*-(3-CHLOROPHENYL)CARBAMATE (CIPC) when applied to the soil under specified conditions has been recommended as a selective herbicide for the control of the growth of certain narrow-leafed plants such as crab grass (*Digitaria sanguinalis*), wild oats (*Avena fatua*), and witch grass (*Panicum capillare*) (*2*). As this chemical is used for both pre-emergence and post-emergence treatment in the production of certain food crops, it was considered important to determine the residue of isopropyl *N*-(3-chlorophenyl)carbamate that might be present in the harvested crop.

Experiments by Bissinger and Fredenburg (*1*) concerning the determination of isopropyl *N*-phenylcarbamate (IPC) in head lettuce have shown that acidic hydrolysis of this carbamate to aniline and its measurement colorimetrically provide an excellent means of determining the herbicide in this crop. Gard (*3*) and Shaw (*6*) have shown that acidic hydrolysis of isopropyl *n*-phenylcarbamate and isopropyl *N*-(3-chlorophenyl)carbamate, followed by the absorption and measurement of the carbon dioxide evolved, is an effective means of determining the purity of these commercial compounds. Gard and Rudd (*4*) have developed and applied a colorimetric method to determine isopropyl *N*-(3-chlorophenyl)carbamate in specific crops which included head lettuce, sugar beets (roots and foliage), onions, cotton seeds, and peanuts. Similar tests with spinach, conducted recently, showed satisfactory recovery of known added amounts of isopropyl *N*-(3-chlorophenyl)carbamate from the untreated crop. The analytical method involves maceration of the crop, extraction of the herbicide, acidic hydrolysis to 3-chloroaniline, steam distillation of the 3-chloroaniline, and its colorimetric measurement. The calculated accuracy of this method (*4*) was shown to be about 90% when the herbicide was added in the range of 0.05 to 0.5 p.p.m. of isopropyl *N*-(3-chlorophenyl)carbamate, and the precision, based on 95% confidence limits, determined by statistical methods, was ± 0.016 p.p.m. of isopropyl *N*-(3-chlorophenyl)carbamate.

The principal concern of the experiments reported herein is the application of the Gard-Rudd method to the analysis of actual crops which have been grown in soil that received pre-emergence treatment with isopropyl *N*-(3-chlorophenyl)carbamate, and comparison of these results with control crops in which no herbicide was employed.

Source of Crops

The crops analyzed in this study were supplied by the following individuals and organizations, and their contributions are gratefully acknowledged. In all cases pre-emergence applications of emulsifiable formulations of isopropyl *N*-(3-chlorophenyl)carbamate were made in experimental field plots shortly after planting.

Head Lettuce and Spinach. William Richards, Veg-Acre Farms, Forestdale, Cape Cod, Mass.

Sugar Beets. R. T. Nelson, Great Western Sugar Co., Longmont, Colo.

Onions. F. L. Timmons, senior agronomist, United States Department of Agriculture, Logan, Utah.

Cotton Seeds. C. M. Gates and E. A. Behr, Chapman Chemical Co., Memphis, Tenn.

Peanuts. W. B. Ennis, Mississippi Agricultural Experiment Station, State College, Miss.

All harvested, perishable crops associated with this investigation were transported under refrigerated conditions to this laboratory and stored in the refrigerator at 20° F. in order to preserve them and to minimize loss of the herbicide through volatilization.

Interferences with Analytical Method

When untreated specimens of the various crops were tested, it was found that a very small amount of some unknown compound was present which gave an interfering blue color. This interference indicated an apparent isopropyl *N*-(3-chlorophenyl)carbamate content which was at least 50% of the small gross amount of the herbicide found in crops that received herbicidal treatment at the various levels. Even when this control value was not applied, the isopropyl *N*-(3-chlorophenyl)carbamate residue found in the treated crops was always below the 0.05 p.p.m. level, which is given as the lower practical limit of identification by the method under the sample size and conditions prescribed.

If, however, it is desired to obtain approximations of the isopropyl *N*-(3-chlorophenyl)carbamate residues in samples which fall below the practical identification range of the method, calculated average control analyses based on replicate tests may be subtracted from the calculated average gross analyses of the residue found in the samples receiving treatment. The difference between these average analyses may therefore be attributed to isopropyl *N*-(3-

chlorophenyl)carbamate remaining with the treated crop at harvest.

An alternative procedure, aimed at residue measurements at concentration levels of the herbicide substantially below 0.05 p.p.m., was to increase the size of the sample tested from 200 to 1000 grams. While this increase in the sample size probably resulted in lower limits of identification, the limited supply of most of the crops prevented the extension of this study. In one case (head lettuce) where the supply of the crop was adequate, two tests involving 1000-gram samples were conducted and the analytical processing and handling of these samples required modification to accommodate the larger bulks involved.

Methods of Analysis

The analyses given in Table I were obtained by the colorimetric method of Gard and Rudd (*4*), which details maceration of 200-gram specimens of the crop sample in a Waring Blendor with methylene dichloride, separation of the nonaqueous extract from the pulp with a centrifuge, and gentle evaporation of the extract to concentrate the isopropyl *N*-(3-chlorophenyl)carbamate. After evaporation of the extract, the isopropyl *N*-(3-chlorophenyl)carbamate is hydrolyzed with dilute (1 to 1) sulfuric acid to form 3-chloroaniline, which is subsequently steam-distilled from the solution which has been rendered strongly alkaline with caustic soda. The amount of 3-chloroaniline in the distillate is measured colorimetrically by the phenol-hypochlorite method, utilizing a photoelectric colorimeter. Two-hundred-gram specimens of the various crops were processed for the analysis in every case except two, where 1000-gram samples were used. Head lettuce, spinach, sugar beets (roots and foliage), and onions were tested by the procedure described for crops, while cotton seeds and peanuts were tested by the procedure described for cotton seeds, which involves a supplementary extraction and separation of the herbicide from the oily extract with acetonitrile (*5*), prior to acidic hydrolysis of the carbamate.

The analytical processing of 1000-gram specimens of head lettuce was accomplished by extracting four successive 250-gram portions of the crop with the same methylene dichloride. In this instance, however, it was necessary to use additional amounts of methylene dichloride to compensate for evaporative and other losses inherent in the extraction operation. Appropriate reagent blanks were also determined under these same conditions and applied to the analytical results.

Analytical Results

The results of replicate testing, utilizing 200-gram specimens, are given for the various specified crops in Table I. To obtain the apparent net isopropyl *N*-(3-chlorophenyl)carbamate residue which remains with the treated crops at harvest, the control analyses represented by crops receiving no treatment must be subtracted from corresponding values obtained from crops receiving the various levels of herbicidal treatment. These net analyses are shown in Table I. In some cases the control analyses are slightly larger than the values for treated crops, which indicates the uncertainty that any isopropyl *N*-(3-chlorophenyl)-carbamate is present. In any event, the net analyses are considerably below the practical limit of identification of the method.

The analysis of the 1000-gram samples of head lettuce included a control sample grown in soil receiving no herbicidal application, and a treated sample grown in soil receiving 5.0 pounds of isopropyl *N*-(3-chlorophenyl)carbamate per acre. This special testing gave results, when corrected for the blank, of 0.0085 p.p.m. of the herbicide for the control sample and 0.0127 p.p.m. for the crop grown in soil receiving the herbicide application. Correction of the treated sample analysis for the control value shows an apparent herbicidal residue of 0.0042 p.p.m. which remains with the crop at harvest. This is about one tenth of the average residue indicated by analysis of the 200-gram samples of the same head lettuce. This indicates that the residue, if any is present in the 200-gram treated samples, is actually much lower than given by the analyses of these samples; the interferences in the 200-gram analyses thus appear to reduce the lower practical limit of identification by the method to the value of about 0.05 p.p.m. previously noted. It is possible, however, that the herbicide may be detoxified or otherwise metabolized during the growth process of the plant and not be detected as such by the analytical method.

Summary

The isopropyl *N*-(3-chlorophenyl)-carbamate residues found at harvest in crops receiving pre-emergence treatment, after correction for interference, ranged from apparent slightly negative values in the case of sugar beet roots and peanuts, to a maximum of 0.03 p.p.m. of isopropyl *N*-(3-chlorophenyl)carbamate in the case of head lettuce. More

Table I. Isopropyl *N*-(3-Chlorophenyl)carbamate Residue in Crops Receiving Pre-emergence Treatment

Crops	Pre-emergence Treatment, Lb. CIPC/Acre	CIPC Found, P.P.M. Replicate Tests 1	2	3	4	5	Av.	Net
Head lettuce	None	0.03	0.02	0.02	0.03	0.02	0.024	...
	2.5	0.05	0.04	0.02	0.055	0.05	0.043	0.019
	5.0	0.055	0.05	0.04	0.05	[a]	0.049	0.025
Sugar beets (roots)	None	0.02	0.02	0.02	0.03	0.01	0.020	...
	3.0	0.01	0.01	0.01	0.02	0.01	0.012	−0.008
	6.0	0.01	0.01	0.02	0.02	0.02	0.016	−0.004
Sugar beets (foliage)	None	0.00	0.01	0.02	0.01	0.02	0.012	...
	3.0	0.04	0.01	0.02	0.04	0.01	0.024	0.012
	6.0	0.04	0.02	0.03	0.04	0.01	0.028	0.016
Onions	None	0.02	0.01	0.01	0.01	0.00	0.010	...
	4.0	0.02	0.02	0.01	0.01	0.00	0.012	0.002
	8.0	0.03	0.03	0.03	0.02	0.01	0.024	0.014
Cotton seeds	None	0.01	0.02	0.02	0.03	0.03	0.022	...
	3.0[b]	0.02	0.04	0.02	0.04	0.00	0.024	0.002
Peanuts	None	0.00	0.01	0.03	0.01	0.03	0.016	...
	8.0	0.03	0.01	0.00	0.01	0.02	0.014	−0.002
Spinach	None	0.00	0.01	0.03	0.02	0.01	0.014	...
	4.0	0.03	0.03	0.01	0.01	0.01	0.018	0.004

[a] Insufficient sample to conduct fifth replicate test. Average value based on four tests.
[b] Also 2 lb. CIPC/acre applied during postemergence in bands, so that actual treatment was three times as large per unit of surface.

accurate measurements to show the presence of any residue at this extremely low concentration level—i.e., below 0.05 p.p.m.—in crops receiving treatment would require considerable modification of the method and perhaps an entirely different approach to the analysis.

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RODENT REPELLENTS

Preparation and Properties of Thiouronium Compounds and Cyclic Imides

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Syntheses and bioassays of cyclic imides and thiouronium compounds were carried out as part of a search for materials capable of preventing rodent damage to packaged commodities. Previous studies had shown that repellent activity was associated with functional groups containing nitrogen and sulfur, and was enhanced by the presence of ionic linkages. Twenty-seven thiouronium compounds and 40 imides, including 10 compounds not described previously, were prepared for these tests. Ten imides and 26 thiouronium compounds were repellent under the conditions of test. Information obtained in these studies will be utilized in the development and selection of more effective materials for prevention of rodent damage to foods and other commodities.

IN STUDIES OF RODENT DAMAGE to packaged articles and other commodities, the Fish and Wildlife Service has examined more than 5000 chemicals for repellency to rats and mice. Test procedures have been divided into three phases: a preliminary screening operation in which candidate compounds were incorporated in diets fed laboratory rats (*3*); more advanced laboratory studies in which paperboard panels were treated with promising materials for determination of relative resistance to gnawing attacks (*4*); and simulated warehouse studies in which cartons were treated with candidate repellents and exposed to gnawing attacks by wild rodents (*20*).

The preliminary screening (food acceptance) tests have been utilized as a means of selecting promising materials for the more laborious and time-consuming barrier and warehouse studies, and have furnished valuable information on possible relationships between chemical composition and repellent activity. Classification of candidate materials according to structure and composition has shown that repellency may be correlated with certain functional groups, such as $-NH_2$ and $-NO_2$, attached to alkyl, aryl, or heterocyclic nuclei (*5*). Activity of any group may be enhanced or negated by introduction of other substituents, or by changes in molecular weight, spatial configuration, or unsaturation in the nucleus.

The majority of active repellents contain nitrogen, sulfur, or halogen; amines and their derivatives form one of the most active classes. Some free amines are repellent, but activity seems to be enhanced by formation of salts, complexes, or quaternary halides. This enhanced activity appears to be a function of ionic or other linkages:

$$(R—N—)^+(X)^- \text{ or } (R—N—)—(R—X)$$

In addition to the quaternary ammonium and pyridinium compounds, many sulfonium, arsonium, boronium, and phosphonium compounds were found to be active repellents. These materials all contain ionic linkages similar to those of the quaternary ammonium halides, and it appeared that other materials having ionic structures should be investigated. One of the most promising groups in this category was the thiouronium, or isothiourea salts, whose structure can be written:

$$[(H_2NC(SR)=NH)]^+ (X)^-$$

A number of thiouronium compounds were prepared and tested by the food acceptance technique to investigate possible relationships between structure and repellent activity. The list of materials includes some compounds not previously described in the literature, in addition to several compounds developed by other investigators.

Preparation of Thiouronium Compounds

One-tenth mole of thiourea and 0.10 mole of alkyl halide were dissolved in 10 to 25 ml. of ethyl alcohol and refluxed until the solution no longer gave a positive test for =S when treated with ammoniacal silver nitrate. The lower (volatile) halides were obtained by removal of the alcohol and traces of alkyl halide in vacuo, followed by cooling of the residual oil. The higher halides were prepared by precipitation with ether and recrystallization from alcohol-ether. Substituted benzyl thiouronium salts