## HERBICIDES DETERMINATION

# Isopropyl *N*-(3-Chlorophenyl)carbamate (CIPC) In Soil and Crops

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**The use of isopropyl *N*-(3-chlorophenyl)carbamate (CIPC) to control the growth of narrow-leafed grasses has created the need for a method to determine micro quantities of the herbicide in soils and crops. The method describes procedures for soil, cotton seeds, head lettuce, onions, and sugar beets and details separation by extraction with methylene dichloride, concentration and acidic hydrolysis to 3-chloroaniline, steam distillation of the aniline, and its measurement photoelectrically utilizing the hypochlorite-phenol method. Particular emphasis is directed toward measurements at the 0.05 p.p.m. level of the herbicide. The calculated average recovery of the method is 89% and the lower practical limit of sensitivity is 0.05 p.p.m. of isopropyl *N*-(3-chlorophenyl)carbamate.**

EXPERIMENTAL USE OF isopropyl *N*-(3-chlorophenyl)carbamate (CIPC) to control the growth of narrow-leafed grasses such as green foxtail, witch grass, crab grass, wild oats, and smart weed (*2, 3*) created the need for a method to determine micro quantities of this herbicide in the soil and certain crops. Determination in soil, head lettuce, sugar beets, onions, and cotton seeds is the principal concern of this paper.

A method by Bissinger and Fredenburg (*1*) for determining micro quantities of isopropyl *N*-phenylcarbamate (IPC) in head lettuce involves separation of the herbicide residue by extraction with methylene dichloride, concentration and hydrolysis to aniline with a phosphoric-hydrochloric-acetic acid mixture, and visual colorimetric measurement of the resulting aniline by the hypochlorite-phenol method (*4*), which depends on the formation of the hypochlorite complex of aniline and its conversion to a permanent deep-blue dye by the addition of ammonia and a dilute solution of phenol. The Bissinger-Fredenburg method was applied experimentally to the current problem. Unpublished results of experiments in this laboratory show that isopropyl *N*-(3-chlorophenyl)carbamate is hydrolyzed quantitatively by heating and refluxing with dilute (1 to 1) sulfuric acid. The Bissinger-Fredenburg method was modified to use this nonvolatile acid mixture for the hydrolysis. It was found desirable also to follow the suggestion of Jacobs (*4*) in calibrating and utilizing a photoelectric colorimeter for measuring the chloroaniline product. The analytical operations concerned with color measurement required careful control and standardization.

### Experimental Work

The general procedure for determining isopropyl *N*-(3-chlorophenyl)carbamate in soils and crops involves intimate mixing and maceration with methylene dichloride in a Waring Blendor to extract the herbicide, concentration of the extract by evaporation, hydrolysis of the isopropyl *N*-(3-chlorophenyl)carbamate with dilute sulfuric acid, separation of the resulting 3-chloroaniline from alkaline solution by steam distillation, and colorimetric measurement of the resulting aniline complex with a photoelectric colorimeter utilizing a red light filter (650 mμ). Crops which contain substantial amounts of oils require a special treatment of the methylene dichloride extract with acetonitrile to separate the herbicide from the oil.

Samples of soils and crops containing known amounts of isopropyl *N*-(3-chlorophenyl)carbamate were analyzed to determine the accuracy, precision, and sensitivity of the method. These samples were prepared by adding appropriate volumes of a methylene dichloride solution of isopropyl *N*-(3-chlorophenyl)-carbamate [1 ml. equivalent to 0.005-mg. of pure isopropyl *N*-(3-chlorophenyl)-carbamate] to the sample just prior to the extraction or blending operation.

### Apparatus and Assembly

Waring Blendor

Basket centrifuge, 5-inch, equipped with a glass-cloth liner.

Centrifuge, conventional type, equipped with 250-ml. sedimentation bottles.

Round-bottomed flask, 500-ml., equipped with a 24/40-joint.

Allihn condenser, 300-mm.

Distillation head and condenser with a 24/40-joint to fit flask.

Photoelectric colorimeter (Cenco-Sheard-Sanford) equipped with 5-cm. comparison cells and red light filter (650 mμ).

The apparatus for hydrolysis consisted of a 500-ml. round-bottomed flask fitted with an Allihn condenser. After hydrolysis the Allihn condenser was rinsed and detached from the flask. For distillation, a one-piece distillation head and condenser assembly was attached directly to the flask through a ground-glass joint. The distillation head was a simple, one-piece design and consisted of a ground-glass joint sealed to a short length of 5-mm. glass tubing bent in an inverted U-shaped fashion, which in turn was sealed to the inner tube of a downward condenser. The delivery tube of the condenser had sufficient length to reach the bottom of the condensate receiver vessel (125-ml. Erlenmeyer flask).

### Reagents

Methylene dichloride.

Sulfuric acid, diluted 1 to 1 with distilled water.

Sodium hydroxide, 50% solution.

Hydrochloric acid, 0.36 *M* solution.

Dow Corning Antifoam, Type A.

Calcium hypochlorite solution was prepared by treating 5 grams of calcium hypochlorite (70% available chlorine) with 95 ml. of distilled water and heating to 60° C. while stirring. The resulting mixture was filtered through a Whatman No. 40 filter paper and the clear filtrate stored in a glass-stoppered bottle for not more than 10 days.

Phenol reagent was prepared fresh each day by diluting 5 ml. of ammonium hydroxide (specific gravity 0.90) with 95 ml. of distilled water and dissolving 5 grams of pure phenol in the solution.

### Calibration of Photoelectric Colorimeter

The photoelectric colorimeter was calibrated in terms of 3-chloroaniline, which may be calculated to isopropyl *N*-(3-chlorophenyl)carbamate. The calibration involved the use of 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 99.6% based on total chlorine analysis.

Weigh 0.1000 gram of the purified 3-chloroaniline and dissolve in 0.36 *M* hydrochloric acid. Dilute to 1000 ml. with additional 0.36 *M* acid and mix thoroughly. Dilute 100 ml. of this solution to 1000 ml. with the acid. One milliliter of the resulting solution is equivalent to 0.01 mg. of 3-chloroaniline or 0.017 mg. of isopropyl *N*-(3-chlorophenyl)carbamate.

Measure 0.0, 1.0, 2.0, 3.0, 4.0, and 5.0 ml. of the standard 3-chloroaniline solution into a series of 125-ml. Erlenmeyer flasks. Add sufficient 0.36 *M* hydrochloric acid solution to each standard so that the final mixture contains 5 ml. of the acid. Dilute each standard to 40 ml. with distilled water and process each standard separately. Add 2 drops of the calcium hypochlorite solution and allow to digest at room temperature for 5 minutes. Heat the solution to boiling on a hot plate and allow to boil vigorously for 0.5 minute, then remove from the hot plate and immediately add 5 ml. of the phenol reagent. Allow the mixture to digest 15 minutes without accelerated cooling, during which time filter through a double thickness of Whatman No. 42 filter papers. Transfer a portion of the clear filtrate to the 5-cm. comparison cell of the photoelectric colorimeter and measure the red light (650 mμ) transmittance of each standard as compared with the standard to which no 3-chloroaniline was added.

The calibration data for 3-chloroaniline employing a Cenco-Sheard-Sanford Photelometer, 5-cm. comparison cell, and red light filter (650 mμ) are shown in Table I. A suitable calibration curve was prepared from these data.

Experiments with the calibration described above showed that erratic colors were obtained if standard conditions were not closely adhered to, such as the order of reagent addition, acidity of the sample solution, time and temperature of digestion, and the elimination of turbidity from the final colored solution.

Distillate containing the 3-chloroaniline was collected in 5 ml. of 0.36 *M* hydrochloric acid; a 5-minute reaction period at room temperature was allowed after adding the hypochlorite solution; the sample mixture was heated and boiled vigorously for 0.5 minute, then removed from the source of heat and digested, during which time it was passed through a double thickness of Whatman No. 42 filter paper to remove turbidity; and the red light (650 mμ) transmittance of the filtrate was measured with a photoelectric colorimeter.

**Table I. Calibration of Photoelectric Colorimeter**

| 3-Chloroaniline, Mg. | Red Light Transmittance, % |
|---|---|
| 0.000 | 100.0 |
| 0.005 | 93.0 |
| 0.010 | 83.0 |
| 0.020 | 67.0 |
| 0.030 | 56.0 |
| 0.040 | 48.5 |
| 0.050 | 38.5 |

### Methods

**Soils, Sample Preparation, and Extraction.** Weigh 200 grams of the soil sample into a 600-ml. beaker and add 200 ml. of methylene dichloride. Mix thoroughly and vigorously for 5 minutes with an air stirrer, then allow the solids to settle. Decant the supernatant liquid through a Whatman No. 40 filter paper into a 500-ml. round-bottomed flask equipped with a 24/40-joint. Repeat the extraction and separation processes three additional times, using 100-ml. portions of methylene dichloride, and combine the extracts in the flask. Proceed with the evaporation, hydrolysis, and color development as for crops.

**Cotton Seeds, Sample Preparation, and Extraction.** Weigh 50 grams of the cottonseed sample into a Waring Blendor and blend dry for 2 to 3 minutes. Add 100 ml. of methylene dichloride and continue blending for 5 minutes. Evaporative losses of the extractant may be minimized by adding chipped ice to the mixture to maintain the temperature at 35° C. or below. Transfer the mixture to a 600-ml. beaker and repeat the operation with additional 50-gram portions of sample until 200 grams have been processed. Combine the blended mixtures in the beaker. Separate the pulp by passing the mixture through glass wool placed in the vertex of a funnel and collect the solvent portion in a 500-ml. round-bottomed flask equipped with a 24/40-joint. Add two boiling chips and evaporate the methylene dichloride. This operation may be accelerated by immersing the flask in hot water and passing a slow stream of pure filtered air over the surface of the liquid. The residue in the flask contains the cottonseed oil and the herbicide.

Treat the oily residue in the flask with 50 ml. of acetonitrile (Eastman organic chemicals, white label, boiling point 80–81.5°) and stir vigorously to extract the isopropyl *N*-(3-chlorophenyl)carbamate from the oil. Allow the phases to separate, pass the nitrile phase (top layer) through a Whatman No. 40 filter paper, and collect the filtrate in another 500-ml. round-bottomed flask equipped with a 24/40-joint. Repeat the acetonitrile extraction process in the flask three additional times with 50-ml. portions of acetonitrile and combine the filtered extracts. Heat and slowly evaporate the nitrile by passing a slow stream of filtered air over the surface of the liquid. Remove the last portion of nitrile by aeration at room temperature. Add dilute sulfuric acid to the residue and proceed with the hydrolysis and color development as for crops.

**Crops.** This procedure has been applied successfully to head lettuce, sugar beets, and onions.

**Sample Preparation, Blending.** Weigh 200 grams of the crop and place in a Waring Blendor. Blend the sample for 3 minutes in its own liquid, then add 400 ml. of methylene dichloride, and continue blending for 5 minutes. Evaporative losses of the extractant may be minimized by adding chipped ice to the mixture to maintain the temperature of the mixture at 35° C. or below.

**Extraction.** Transfer the blended mixture to a 5-inch basket centrifuge equipped with a glass-cloth liner to separate the pulp from the liquid. Process the mixture in this manner three or four times to effect separation of virtually all of the pulp. Transfer the liquids to a conventional-type centrifuge equipped with sedimentation bottles and separate the moisture and any pulp which remains from the nonaqueous phase. This operation requires from 25 to 40 minutes with the centrifuge adjusted between 1200 and 1500 r.p.m., depending on the character of the crop under examination. Separate the nonaqueous extract by decantation and place in a 500-ml. round-bottomed flask equipped with a 24/40-joint. Evaporate the extract to near dryness by immersing the flask in hot water while passing a slow stream of filtered air over the surface of the liquid. Remove the flask from the water bath and continue aeration to volatilize the last portion of solvent.

**Hydrolysis.** Add 2 or 3 boiling chips to the residue in the flask and treat with 20 ml. of dilute (1 to 1) sulfuric acid solution. Attach a water-cooled Allihn condenser and heat the mixture to boiling. Continue to boil and allow the mixture to reflux for 1 hour, effecting hydrolysis of the isopropyl *N*-(3-chlorophenyl)-carbamate to yield 3-chloroaniline. Cool the hydrolyzed mixture to room tempera-

ture, rinse the condenser into the flask with 75 ml. of distilled water, and add 3 drops of Dow Corning Antifoam. Dilute to 100 ml. with distilled water and add 50 ml. of 50% sodium hydroxide solution. Attach the flask immediately to the distillation apparatus. Charge the distillate receiver (125-ml. Erlenmeyer flask) with 5 ml. of 0.36 *M* hydrochloric acid solution and immerse in a chipped ice bath for cooling. Distill and collect 35 ml. of distillate.

**Color Development.** Add 2 drops of the calcium hypochlorite solution to the distillate, mix thoroughly, and allow to digest at room temperature for 5 minutes. Heat the solution to boiling on a hot plate and allow to boil vigorously for 0.5 minute. Remove from the hot plate, immediately add 5 ml. of the phenol reagent, and allow the mixture to digest 15 minutes without accelerated cooling, during which time filter through a double thickness of Whatman No. 42 filter paper.

Transfer a portion of the clear filtrate to the 5-cm. comparison cell of the photoelectric colorimeter and measure the red light (650 mμ) transmittance of the sample as compared with distilled water adjusted to 100% transmittance. Determine the reagent blank each time reagents are renewed and make appropriate corrections in terms of 3-chloroaniline.

### Calculation

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

### Recovery of CIPC

The blank measurement of the reagents associated with the method which involved no isopropyl *N*-(3-chlorophenyl)-carbamate nor 3-chloroaniline was significant although constant within the precision of the test. This blank gave red light transmittance values which ranged between 86 and 88% with a calculated average value of 87% compared with distilled water adjusted to 100% red light transmittance. Frequent checks of this blank were made, especially when supplies of reagents were replenished, and the values always fell within the above range. Since the blank was constant, it was feasible to construct a supplementary calibration curve for the photoelectric colorimeter which compensated for this reagent test. Construction of the supplemental curve involved displacement from 100 to 87% red light transmittance for 0.000 mg. of 3-chloroaniline. The data in Table II were derived from the supplemental curve.

The analysis of the untreated control materials shown in Table II indicates the presence of some compound which responds to the isopropyl *N*-(3-chlorophenyl)carbamate test and ranges from 0.01 p.p.m. of isopropyl *N*-(3-chlorophenyl)carbamate in the case of onions to 0.04 p.p.m. in the case of soils.

The calculated average precision based on 95% confidence limits at the 0.05 p.p.m. level of isopropyl *N*-(3-chlorophenyl)carbamate for all materials tested is ±0.016 p.p.m. The lower practical limit of sensitivity based on 200-gram samples is 0.05 p.p.m. of isopropyl *N*-(3-chlorophenyl)carbamate, because the difference in the transmittance reading between the control material and materials treated with 0.010 mg. of isopropyl *N*-(3-chlorophenyl)carbamate (0.05 p.p.m.) averages about 8%. Differential transmittance readings which are substantially less than 8% derived from this method lead to uncertainty of the analysis. The calculated average recovery of isopropyl *N*-(3-chlorophenyl)-carbamate from the materials tested involving all ranges studied is 89%. Although ranges of 0.5 p.p.m. were not studied for all materials reported, there is no reason to suspect that the recovery at these levels in cotton seeds, onions, and sugar beets would be significantly different from those shown for head lettuce and soil.

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

| CIPC Added | | Red Light Transmittance, | CIPC Found | | | |
|---|---|---|---|---|---|---|
| | | | Total | | Net | |
| Mg. | P.p.m. | % | Mg. | P.p.m. | P.p.m. | % recovery |
| | | | Soils | | | |
| 0.000 | 0.000 | 81 | 0.0063 | 0.032 | ... | ... |
| 0.000 | 0.000 | 80 | 0.0070 | 0.035 | ... | ... |
| 0.000 | 0.000 | 81 | 0.0063 | 0.032 | ... | ... |
| 0.010 | 0.050 | 72 | 0.0167 | 0.084 | 0.051 | 102 |
| 0.010 | 0.050 | 73 | 0.0155 | 0.078 | 0.045 | 90 |
| 0.010 | 0.050 | 71 | 0.0180 | 0.090 | 0.057 | 114 |
| 0.025 | 0.125 | 62 | 0.0297 | 0.149 | 0.116 | 93 |
| 0.050 | 0.250 | 47 | 0.0539 | 0.270 | 0.237 | 95 |
| 0.100 | 0.500 | 28 | 0.1007 | 0.504 | 0.471 | 94 |
| | | | Cotton Seeds | | | |
| 0.000 | 0.000 | 84 | 0.0032 | 0.016 | ... | ... |
| 0.000 | 0.000 | 81 | 0.0063 | 0.032 | ... | ... |
| 0.000 | 0.000 | 82 | 0.0050 | 0.025 | ... | ... |
| 0.010 | 0.050 | 75 | 0.0127 | 0.064 | 0.040 | 80 |
| 0.010 | 0.050 | 73 | 0.0155 | 0.078 | 0.054 | 108 |
| 0.010 | 0.050 | 75 | 0.0127 | 0.064 | 0.040 | 80 |
| | | | Head Lettuce | | | |
| 0.000 | 0.000 | 82 | 0.0050 | 0.025 | ... | ... |
| 0.000 | 0.000 | 84 | 0.0032 | 0.016 | ... | ... |
| 0.000 | 0.000 | 83 | 0.0038 | 0.019 | ... | ... |
| 0.010 | 0.050 | 75 | 0.0127 | 0.064 | 0.044 | 88 |
| 0.010 | 0.050 | 76 | 0.0117 | 0.059 | 0.039 | 78 |
| 0.010 | 0.050 | 74 | 0.0142 | 0.071 | 0.051 | 102 |
| 0.030 | 0.150 | 65 | 0.0257 | 0.123 | 0.103 | 69 |
| 0.040 | 0.200 | 58 | 0.0364 | 0.182 | 0.162 | 81 |
| 0.050 | 0.250 | 53 | 0.0441 | 0.221 | 0.201 | 80 |
| 0.080 | 0.400 | 38 | 0.0735 | 0.368 | 0.342 | 87 |
| 0.100 | 0.500 | 30 | 0.0949 | 0.475 | 0.455 | 91 |
| | | | Onions | | | |
| 0.000 | 0.000 | 85 | 0.0017 | 0.009 | ... | ... |
| 0.000 | 0.000 | 85 | 0.0017 | 0.009 | ... | ... |
| 0.000 | 0.000 | 86 | 0.0013 | 0.007 | ... | ... |
| 0.010 | 0.050 | 75 | 0.0127 | 0.064 | 0.056 | 112 |
| 0.010 | 0.050 | 79 | 0.0084 | 0.042 | 0.040 | 80 |
| 0.010 | 0.050 | 76 | 0.0117 | 0.059 | 0.051 | 102 |
| | | | Sugar Beets (Roots) | | | |
| 0.000 | 0.000 | 83 | 0.0038 | 0.019 | ... | ... |
| 0.000 | 0.000 | 84 | 0.0032 | 0.016 | ... | ... |
| 0.000 | 0.000 | 83 | 0.0038 | 0.019 | ... | ... |
| 0.010 | 0.050 | 77 | 0.0109 | 0.055 | 0.037 | 74 |
| 0.010 | 0.050 | 78 | 0.0097 | 0.049 | 0.031 | 62 |
| 0.010 | 0.050 | 76 | 0.0117 | 0.059 | 0.041 | 82 |


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