An Improved Method for the Analysis of Residues of Isopropyl *N*-(3-Chlorophenyl)carbamate (Chlorpropham) in Alfalfa

Charles D. Ercegovich* and Sujit Witkonton

Variable, and often excessive, amounts of interfering plant materials in alfalfa render conventional colorimetric methods of analysis for chlorpropham residues ineffective for this crop. An improved colorimetric method for the analysis of chlorpropham residues in alfalfa is presented. The herbicide residue is directly hydrolyzed in the crop sample by alkali to 3-chloroaniline, which is simultaneously extracted into a nonpolar solvent by means of steam distillation through the use of a Bleidner apparatus. Quantitative determination of the recovered 3-chloroaniline is accomplished by spectrophotometric measurement of its colored complex with *N*-ethyl-1-naphthylamine after it has been separated from interfering colored complexes derived from plant materials by means of cellulose column chromatography. The sensitivity of this method is equivalent to 0.02 ppm for a 25-g sample of alfalfa.

Formulations of isopropyl *N*-(3-chlorophenyl)carbamate, whose common name is chlorpropham, are widely used as herbicides for the preemergence and postemergence control of weeds in agricultural croplands (Klingman, 1961). This aryl carbamate herbicide is especially useful when applied to the soil to control a large variety of economically harmful grasses and broadleafed weeds, *e.g.*, annual bluegrass (*Poa annua* L.), barnyard grass (*Echinochloa crus-galli* (L.) Beauv.), purslane (*Portulaca oleracea* L.), chickweed (*Stellaria media* (L.) Cyrillo), smartweed (*Polygonum pensylvanicum* L.) and crabgrass (*Digitaria* spp.) (Gard and Ferguson, 1964). Chlorpropham is also used to inhibit sprout formation on potatoes in storage (Marth and Schultz, 1952).

The residual life of chlorpropham in many instances is too short to render it an effective herbicide because it is readily degraded in the soil by microorganisms (Kaufman, 1970). Kaufman and coworkers (1970) recently reported that the microbial degradation of chlorpropham in the soil was inhibited in the presence of several methylcarbamate insecticides. Their investigations showed that methylated carbamate compounds are competitive inhibitors of the phenylcarbamate hydrolyzing enzymes produced by soil microorganisms. The practical significance of these findings led to the deliberate combination of *p*-chlorophenyl *N*-methylcarbamate (PPG-124) with chlorpropham to improve its weed control efficacy by prolonging its residual life in the soil. Several years of field testing and development of commercial formulations of the combined inhibitor and herbicide, designated as Furloe by the manufacturer, have demonstrated the potential usefulness of such combinations for improved weed control in many agricultural practices.

Some of the most important applications for the modified chlorpropham formulations appear to be for the control of weeds in forage crops, specifically alfalfa and clover. In order to obtain the necessary residue data required to register the new pesticide combinations for sale, it became essential to develop a more suitable procedure for the analysis of chlorpropham in these plants. This investigation, therefore, was undertaken to develop a more reliable method for the determination of chlorpropham residues in alfalfa in order to meet the stringent requirements of the regulatory agencies.

A number of methods have been reported for the analysis of chlorpropham in crop plants (Bissinger and Fredenburg, 1951; Ferguson and Gard, 1969; Gard and Rudd, 1953; Gard *et al.*, 1954; Gard and Reynolds, 1957; Gard *et al.*, 1959; Gard and Ferguson, 1964; Hardon *et al.*, 1961; Koivistoinen and Karinpaa, 1965; Kroller, 1962; Merz and Kammerer, 1958; Montgomery and Freed, 1959) and in stored potatoes (Ferguson *et al.*, 1963; Gard, 1959; Gard and Ferguson, 1963; Gutenmann and Lisk, 1964; Nultsch, 1959; Van Vliet and Hertog, 1966). These methods are of two general types: those which involve hydrolysis techniques in either case where the chlorpropham is extracted with an appropriate solvent such as methylene chloride or petroleum ether (Bissinger and Fredenburg, 1951; Gard and Rudd, 1953; Gard *et al.*, 1954; Gard and Reynolds, 1957; Gard *et al.*, 1959; Gard, 1959; Hardon *et al.*, 1961; Koivistoinen and Karinpaa, 1965; Kroller, 1962; Merz and Kammerer, 1958) or by the direct hydrolysis treatment of the bulk of the sample without prior extraction (Ferguson and Gard, 1969; Gard and Ferguson, 1964; Montgomery and Freed, 1959); and those which involve instrumental measurement of the chlorpropham directly without hydrolysis (Ferguson *et al.*, 1963; Gutenmann and Lisk, 1964; Nultsch, 1959; Van Vleit and Hertog, 1966). In the first group of methods the primary hydrolytic product of chlorpropham, 3-chloroaniline, is reacted with chemical reagents or coupled with a chromophore to yield a colored complex which is quantitatively measured with a spectrophotometer. The instrumental methods employ either infrared spectroscopy (Ferguson *et al.*, 1963; Nultsch, 1959) or gas–liquid chromatography (Gutenmann and Lisk, 1964; Van Vleit and Hertog, 1966). The infrared methods are less sensitive than the colorimetric methods and are applied exclusively to the analysis of samples which contain chlorpropham in amounts exceeding 0.1 mg in a 200-g sample. The gas chromatographic procedures as described by Gutenmann and Lisk (1964) and Van Vleit and Hertog (1966) are somewhat more sensitive, having a reported sensitivity of 0.02 and 0.30 ppm in potatoes using the electron capture and flame ionization detectors, respectively.

The colorimetric methods, however, have been preferred for the detection of microgram quantities of chlorpropham for routine residue sample analysis. In 1959 Montgomery and Freed described a new technique for determining propham residues in strawberries, which is also applicable for chlorpropham. Since then modifications of this procedure have been most generally used for the analysis of chlorpropham residues. In this method the sample is hydrolyzed with alkali

Department of Entomology and The Pesticide Research Laboratory, The Pennsylvania State University, University Park, Pennsylvania 16802.

without prior solvent extraction of the tissue. After hydrolysis the sample is steam-distilled to collect the volatile aniline hydrolytic product in dilute hydrochloric acid solution. 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. The main features of this method which recommend it for propham and chlorpropham residue analysis are the improvement in the color development steps and the direct hydrolysis of the sample without first recovering the herbicides by means of solvent extraction. The last point is particularly important because by this technique the herbicide, which may be chemically bound to plant tissue, may be recovered.

The above described procedure, however, was found lacking for the analysis of alfalfa and clover because it does not provide for the separation of interfering plant materials. The interfering substances result in excessively high values for untreated check samples and reflect in unreliable and misleading values for the actual amount of chlorpropham residues in these forage crops. This report, therefore, describes a new procedure employing the basic principles of existing colorimetric methods for the analysis of chlorpropham, but which is more easily and efficiently performed and is superior in yielding more absolute values.

EXPERIMENTAL

Principle. Residues of chlorpropham in crop samples are determined by the simultaneous hydrolysis of the herbicide to 3-chloroaniline and its extraction into a nonpolar solvent by steam distillation through the use of a Bleidner apparatus. Quantitative determinations of the recovered 3-chloroaniline are accomplished by spectrophotometric measurement of its colored complex with *N*-ethyl-1-naphthylamine after it has been separated from interfering colored complexes derived from plant material by means of cellulose column chromatography.

Apparatus. Bleidner distillation/extraction apparatus as described by Bleidner *et al.* (1954), Dalton and Pease (1962), and modified by Heizler (Geissbuhler *et al.*, 1971). Chromatographic columns, fritted glass disc, ⅎ 14/35, 300 × 10 mm.

Chemicals and Reagents. Standard solutions for the preparation of standard curve and recovery studies. Ten milligrams of purified 3-chloroaniline in 100 ml of ethanol (100 μg/ml). *N*-Ethyl-1-naphthylamine, reagent grade, 0.2% ethanolic solution. Sulfamic acid, reagent grade, 10% aqueous solution. Isooctane, ACS certified. Antifoam agent, Napco NXZ or Dow Corning A. Cellulose powder, Schleicher and Schull No. 286 pulp.

Procedure. Approximately a 500-g subsample of alfalfa was macerated in a Hobart food chopper. Large bulky samples of plant tissue such as grasses, alfalfa, and clover are most readily and conveniently macerated and thoroughly mixed in preparation for analysis by chopping them in a Hobart food chopper while they are still in their frozen state. It is therefore recommended that such samples be maintained in a freezer until they are placed in the food chopper. This technique is much faster and more efficient than the normally used procedures because it eliminates the need to thaw the tissue and to cut it into smaller pieces with a knife or scissors prior to subsequent maceration in a blender. An appropriate aliquot of the macerate, between 5 and 25 g depending upon the amount of chlorpropham which may be present in the tissue, was transferred into a 500-ml heavy-duty round-bottomed borosilicate glass flask after the macerate equilibrated to room temperature. To this flask were added 100 ml of distilled water, 100 ml of 5 *N* sodium hydroxide, and 3–4 ml of antifoam agent. The flask was then connected to the aqueous side of the Bleidner apparatus. One-hundred milliliters of isooctane (*n*-hexane is equally useful and can be used in place of isooctane) were placed in a 250-ml round-bottomed flask, which was then connected to the receiving side of the Bleidner apparatus. The heating mantles and water condenser were positioned and then the sample was digested and extracted under reflux conditions at 200–210°C for 4 hr. The isooctane fraction was allowed to cool to room temperature and then transferred into a 250-ml separatory funnel for extraction with 10 ml of 1 *N* hydrochloric acid to recover the 3-chloroaniline. The isooctane was extracted two additional times with 5-ml portions of the acid solution.

The hydrochloric acid extracts were collected into a 50-ml flask to which 2 ml of 1% sodium nitrate solution were added to diazotize the 3-chloroaniline at room temperature. After 15 min the excess sodium nitrite was decomposed by adding 2 ml of 10% sulfamic acid solution. The flask was shaken vigorously during the addition of the sulfamic acid and then occasionally during the next 5 min. The solution was then buffered to pH 3.5–4.0, as determined by Hydrion test paper, by adding an appropriate amount of 4 *N* sodium acetate, *ca.* 7 ml. The chromophore was next coupled to the diazotized 3-chloroaniline by adding 1.0 ml of 2% *N*-ethyl-1-naphthylamine solution. The mixture was allowed to stand for 15 min, the period for maximum color formation, during which time the flask was occasionally shaken. Final color adjustment, from orange to reddish-purple, was then made by acidifying with 2 ml of 5 *N* hydrochloric acid.

The colored solution was then transferred to a cellulose chromatographic column fitted with a 250-ml separatory funnel as a reservoir. The column was prepared by suspending the cellulose in approximately two volumes of 1 *N* hydrochloric acid in an Erlenmeyer flask and kept under vacuum for approximately 5 min to remove air bubbles. The suspension was then quickly transferred into the chromatographic column, and the cellulose was allowed to settle and pack without the use of pressure or vacuum. The final height of the cellulose in the column was approximately 12 cm.

The liquid phase was allowed to drain from the column at a rate of about 2 ml per min. After all of the original solution had drained from the column, the dyes were retained in the upper part of the column. The interfering dyes were then eluted from the column with approximately 100 ml of a solution of hydrochloric and glacial acetic acid (9 parts of 1 *N* hydrochloric acid:1 part glacial acetic acid, v/v). By the time most of the interfering dyes were removed from the column, the chlorpropham-derived dye moved about halfway down the column. Elution of the column with 9:1 solution was continued until all of the interfering dyes were removed and the band containing 3-chloroaniline dye reached the lower end of the column. This band was then eluted into a 100-ml volumetric flask with the same acid solution. The flask was then filled to the volume mark with glacial acetic acid. Turbid eluates cleared after the addition of glacial acetic acid.

In some cases with crop material in which the interfering dyes are not as abundant as with alfalfa and clover, the chlorpropham-derived dye can be eluted more quickly by using 30 ml of a solution of hydrochloric:glacial acetic acid (1:2, v/v) into 50-ml volumetric flasks. However, separation of the interfering dyes from the desired dye in extracts of alfalfa and

clover is much superior, though slower, with the 9:1 acid solution. The dye solution was stable for more than 8 hr.

Extinction of the dye solution was measured at 535 nm in a Bausch & Lomb Spectronic 600 spectrophotometer. The reading was taken against an appropriate reagent blank. The amount of chlorpropham in the sample was determined by comparing the respective extinction value with a standard curve established with 3-chloroaniline and multiplying this value by 1.67 (1 μg of 3-chloroaniline is equivalent to 1.67 μg of chlorpropham).

A standard curve was prepared by plotting on a linear scale the absorbance values *vs.* known amounts of 3-chloroaniline which had been diazotized and coupled in the manner described above. Pure 3-chloroaniline, obtained by vacuum distillation of reagent grade chemical and authenticated by boiling point, thin-layer chromatography, infrared and mass spectroscopy, was used for this purpose. The resulting curve followed Beer's law for all concentrations used up to 100 μg of 3-chloroaniline and corresponded to an absorption coefficient ϵ of 3.91×10^4 l. $\times$ mol^{-1} $\times$ cm^{-1}. One absorbance unit, therefore, was equivalent to 0.163 μg of 3-chloroaniline for solutions made up to 50-ml volume and measured in a 1-cm light path.

The absorbance values for untreated check samples, if not contaminated, ranged from 0.000 to 0.005. An absorbance value of 0.001 was considered as the significant limit of detection by the instrument. Therefore absorbance readings twice as high as this were regarded to be the sensitivity limit of this method which was equivalent to 0.54 μg of chlorpropham, or 0.02 ppm for a 25.0-g sample.

Calculation.

$$\text{ppm chlorpropham} = \frac{[\text{Absorbance}_{(\text{sample})} - \text{Absorbance}_{(\text{check})}] \times 163 \times 1.67 \times 1000}{\text{Gram weight of sample} \times \text{Recovery}}$$

RESULTS AND DISCUSSION

The accuracy and reliability of this modified procedure were compared to those of the standard method commonly used for the analysis of chlorpropham residues in plants by analyzing similar untreated, fortified, and field-sprayed samples of alfalfa. Recovery values obtained from check samples fortified with known amounts of 3-chloroaniline or chlorpropham ranged from 93.9 $\pm$ 9.6% for the modified method, whereas the conventional method yielded values of 89.8 $\pm$ 12.4%. The average value for 20 recovery analyses was 94.7% for the former method and 88.8% for the latter method. The statistical analyses of the results for the 20 analyses for Student t-distribution reveal that the modified method is significantly more efficient and reliable at the 5% level. The better recovery of chlorpropham from fortified samples by the modified procedure is due to the greater efficiency with which the alkaline hydrolysis and steam distillation process are performed. By exerting special care and fastidious control during the hydrolysis and steam distillation steps, it is possible to achieve recovery of chlorpropham by the conventional method comparable in value to that of the modified method. However, on a continuing routine basis for the analysis of a large number of plant samples, the manipulation of the Bleidner apparatus, as used in the new procedure, appears consistently more reliable because of the ease of manipulation and its inherent characteristics of being a virtually closed distillation and extraction system.

Table I. Interfering Plant Substances Appearing as Chlorpropham (CIPC) in Untreated Alfalfa Samples as Detected by Two Different Methods of Analysis

Days after application	Conventional method			Modified method		
	o.d.	Apparent μg CIPC	ppm as CIPC	o.d.	Apparent μg CIPC	ppm CIPC
30	0.376	102.35	38.33	0.026	7.08	2.65
38	0.140	38.11	14.27	0.010	2.72	1.02
42	0.255	69.41	26.00	0.006	1.63	0.61
61	0.224	60.98	22.84	0.004	1.09	0.41
91	0.360	98.00	36.70	0.006	1.63	0.61
119	0.360	98.00	36.70	0.002	0.54	0.20
147	0.380	103.44	38.74	0.000	0.00	0.00

One of the primary disadvantages of the conventional colorimetric method for the analysis of chlorpropham in plant tissue is that the determination is based on the difference of color development between a check and experimental sample. When this method is used, however, there usually is an uncontrollable great difference in color development due to interfering plant constituents among checks collected from different areas of a field or at different stages of growth. This is clearly demonstrated by the results of the analyses which were conducted in triplicate on composited check samples collected at seven different intervals after the herbicide was applied to other plots in the experimental field. The averages of these results summarized in Table I readily illustrate that there is no valid correlation between the nature of the plant sample and the amount of interfering substances which give rise to color complexes measured at 535 nm. These data show that the amount of interfering material measured as apparent chlorpropham by the conventional method of analysis varies from 38.11 μg at the 38-day interval to 103.44 μg when sampled at the 147-day interval. The values obtained through the use of the modified procedure show a consistent and uniform decline in the amount of material measured as chlorpropham. The data in this section of Table I reveal that the sample may have been contaminated. Analysis of the four individual check samples which were used to make up the composite sample proved this to be the case. Of the four, one was highly contaminated and another moderately, but the remaining two showed no contamination, as evidenced by absorbance values ranging from 0.000 to 0.005 for alfalfa samples analyzed from fields having a history of no exposure to chlorpropham or other halogenated aniline derivatives.

It is not necessarily true that the variability found among the different check samples will cancel itself out by subtracting the appropriate check value from the experimental sample value. Factual evidence to prove this is presented in Table II, which summarizes the average results of three analyses performed by both procedures on sample composites of four individual plots receiving three different series of treatment, 0, 4, or 8 lb (ai)/A of chlorpropham emulsifiable concentrate, 4 lb/gal, and collected at three different intervals after the application was made. A composite of noncontaminated check samples was used for this series of analyses. These data clearly reveal that all of the checks contained variable and high amounts of interfering materials when analyzed by the conventional method and that subtracting these values from those of the experimental ones did not compensate for these differences. On the other hand, the modified procedure yielded check values equivalent to only trace amounts of chlorpropham, thus making this modified method

Table II. Comparison of Conventional and Modified Methods for the Analysis of Residual Amounts of Chlorpropham (CIPC) in Field-Treated Alfalfa Samples After Various Intervals of Application

Sample	Conventional method: Apparent μg CIPC	Conventional method: Adjusted[a] μg CIPC	Conventional method: ppm as CIPC	Modified method: Apparent μg CIPC	Modified method: Adjusted[a] μg CIPC	Modified method: ppm as CIPC
42 Days after application						
Check	57.71		24.53	0.54		0.23
4 lb/A	118.63	60.92	22.56	38.45	37.91	14.04
8 lb/A	161.92	104.21	37.09	79.34	78.80	28.04
Recovery[b]	100.17	42.46	84.9%	45.17	44.64	89.2%
61 Days after application						
Check	50.36		22.28	0.96		0.36
4 lb/A	65.34	14.91	6.29	21.66	14.81	5.55
8 lb/A	80.55	30.19	11.93	30.78	24.43	9.15
Recovery[b]	99.36	48.99	98.0%	48.45	42.46	84.9%
91 Days after application						
Check	68.05		26.69	0.54		0.21
4 lb/A	83.68	15.63	6.43	3.54	3.00	1.18
8 lb/A	87.21	19.16	7.54	4.57	4.03	1.60
Recovery[b]	110.52	42.47	84.9%	48.18	47.64	95.3%

[a] Corrected for interfering plant material and recovery. [b] 50 μg of CIPC added.

of analysis for chlorpropham residues in alfalfa more reliable. In Table II the data obtained through the use of the modified method of analysis demonstrate a more realistic and typical disappearance of the herbicide during the 49-day period considered, whereas the data obtained by the other method of analysis would indicate a much longer and atypical period for the persistence of chlorpropham in alfalfa. It is therefore apparent that the results obtained with the modified procedure would be much more meaningful when used to assist in establishing registered label recommendations and for other regulatory purposes.

The superiority of the modified procedure is due chiefly to the additional step which incorporates the clean-up of the colored solution by means of cellulose column chromatography. This procedure has the additional advantage of revealing when check samples are contaminated, usually due to drift during spray operations or to mislabeling. It does not, however, detect any dechlorinated degradation products of chlorpropham, if, in fact, they do occur in the alfalfa samples. This procedure also will not detect the major *in vitro* or *in vivo* hydrolytic product of PPG-124, 4-chlorophenol (Chrzanowski and Ercegovich, 1971), since this chemical will not react with *N*-ethyl-1-naphthylamine to produce an interfering dye. This has been confirmed through the analysis of samples which were fortified with both chemicals and numerous samples of alfalfa from fields which had been treated with varying rates of Furloe.

LITERATURE CITED

Bissinger, W. E., Fredenburg, R. H., *J. Ass. Offic. Agr. Chem.* **34,** 813 (1951).
Bleidner, W. E., Baker, H. M., Levitsky, M., Lowen, W. K., J. AGR. FOOD CHEM. **2,** 476 (1954).
Chrzanowski, R. L., Ercegovich, C. D., Pesticide Research Laboratory, Penn State University, University Park, Pa., unpublished data, 1971.
Dalton, R. L., Pease, H. L., *J. Ass. Offic. Agr. Chem.* **45,** 377 (1962).
Ferguson, C. E., Gard, L. N., J. AGR. FOOD CHEM. **17,** 1063 (1969).
Ferguson, C. E., Gard, L. N., Shupe, R. H., Dress, K. S., J. AGR. FOOD CHEM. **11,** 428 (1963).
Gard, L. N., J. AGR. FOOD CHEM. **7,** 339 (1959).
Gard, L. N., Ferguson, C. E., Analytical Methods for Pesticides, Plant Growth Regulators and Food Additives, Zweig, G., Ed., Vol. IV, Academic Press, New York, 1964, pp 49–65.
Gard, L. N., Ferguson, C. E., J. AGR. FOOD CHEM. **11,** 234 (1963).
Gard, L. N., Reynolds, J. L., J. AGR. FOOD CHEM. **5,** 39 (1957).
Gard, L. N., Rudd, N. G., J. AGR. FOOD CHEM. **1,** 630 (1953).
Gard, L. N., Ferguson, C. E., Reynolds, J. L., J. AGR. FOOD CHEM. **7,** 335 (1959).
Gard, L. N., Pray, B. O., Rudd, N. G., J. AGR. FOOD CHEM. **2,** 1174 (1954).
Geissbühler, W. H., Kossman, K., Baunok, I., Boyd, V. F., J. AGR. FOOD CHEM. **19,** 365 (1971).
Gutenmann, W. H., Lisk, D. J., J. AGR. FOOD CHEM. **12,** 47 (1964).
Hardon, H. J., Brunik, H., Besemer, A. F. H., *Deut. Lebensm.-Rundschau* **57,** 31 (1961).
Kaufman, D. D., Pesticides in the Soil: Ecology, Degradation and Movement, International Symposium on Pesticides in the Soil, 1970, Michigan State University, Pesticide Research Center, East Lansing, 73, 1970.
Kaufman, D. D., Kearney, P. C., Von Endt, D. W., Miller, D. E., J. AGR. FOOD CHEM. **10,** 513 (1970).
Klingman, G. C., Weed Control as a Science, Wiley, New York, 1961.
Koivistoinen, P., Karinpaa, A., J. AGR. FOOD CHEM. **13,** 459 (1965).
Kroller, E., *Deut. Lebensm.-Rundschau* **58,** 125 (1962).
Marth, P. C., Schultz, E. S., *Amer. Potato J.* **29,** 268 (1952).
Merz, K. W., Kammerer, A., *Arch. Pharm.* **286,** 198 (1958).
Montgomery, M., Freed, V. H., J. AGR. FOOD CHEM. **7,** 617 (1959).
Nultsch, W., *Angew. Botan.* **33,** 10 (1959).
Van Vleit, W. F., Hertog, S., *Eur. Potato J.* **9,** 152 (1966).

Received for review August 9, 1971. Accepted October 21 1971. This study was supported in parts by Agricultural Research Service, U.S. Department Grant No. 12-14-105-8075(34), administered by the Plant Science Research Division, Beltsville, Maryland, and PPG Industries, Pittsburgh, Pennsylvania. Authorized for publication on August 24, 1971, as paper No. 4044 in the journal series of the Pennsylvania Agricultural Experiment Station.