Analysis of Bidrin as Iodoform by Electron Capture—Gas-Liquid Chromatography

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A procedure is presented for determining nanogram quantities of Bidrin as iodoform in crop samples. Extraction and cleanup were accomplished by blending cabbage in methylene chloride with activated charcoal and magnesium sulfate. Interfering extractables were removed with sodium bisulfite. Iodoform, produced from the reaction of Bidrin with sodium hypoiodite, was extracted into petroleum ether and analyzed by gas chromatography with an electron capture detector. Bidrin residues were detected on crop extracts at concentrations as low as 0.01 p.p.m. in 100-gram samples.

ELECTRON CAPTURE (EC)–gas-liquid chromatography (GLC) is routinely applied in determining many chlorine-containing pesticides. However, some organophosphorus and many of the carbamate pesticides do not exhibit sufficient electron-capturing capacity to permit direct analysis in the parts-per-million range. For certain pesticides, modification of the basic structure followed by EC-GLC may prove superior to other procedures with respect to specificity and sensitivity in residue analysis. Such modification would involve addition of one or more appropriately electronegative atoms to the pesticide structure or some fragment thereof. Ideally the modification product would constitute a single, stoichiometrically related compound which would respond to electron capture in a manner sufficiently different from residual extracts of plant and animal origin as to not be masked, reinforced, or in any way altered by interferences.

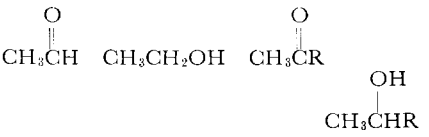
Previous investigators (*1*) found Bidrin [3 - (dimethoxyphosphinyloxy) - *N*,*N*-dimethyl - *cis* - crotonamide] and dibrominated derivatives of Bidrin to be unstable to gas-liquid chromatography. The authors found it possible to chromatograph and detect quantities equal to or greater than 300 nanograms of unreacted technical Bidrin. However, when 1 mg. of Bidrin was added to 100 grams of cabbage, it was impossible to distinguish the response due to Bidrin from the crop background and attempts to improve cleanup did not alleviate this difficulty.

Bidrin can be detected in crops and animal products using the acetylcholinesterase inhibition procedure. However, this method lacks specificity and, therefore, is not generally considered suitable as an enforcement technique for residue analysis (*5*).

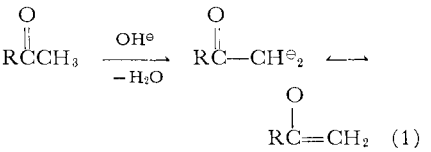
Recently, a colorimetric method (*4*) was presented for analyzing as little as 0.02 p.p.m. Bidrin in the presence of benzene-extractable substances from various crops. This colorimetric method is specific for dialkylamines. Therefore, the reported highly toxic metabolites (*3*)—3 - (dimethoxyphosphinyloxy) - *N*-methyl - *cis* - crotonamide (SD 9129), 3 - (dimethoxyphosphinyloxy) - *N* - hydroxymethyl - *cis* - crotonamide, and 3 - (dimethoxyphosphinyloxy) - *cis* - crotonamide—formed from Bidrin as the result of oxidative *N*-demethylation, are not detected.

The electron capture–GLC procedure described, in which Bidrin is converted to iodoform, is sensitive to 0.01 p.p.m. Bidrin, and to its *N*-substituted demethylated metabolites as well. This method is specific for methyl-vinyl phosphates and is, therefore, more selective than the anticholinesterase method. Related methyl-vinyl phosphates currently in use which can be detected by the described iodoform method are Phosdrin [3-(dimethoxyphosphinyloxy) methyl - *cis* - crotonate] and Phosphamidon [3-(dimethoxyphosphinyloxy) - *N*,*N* - diethyl - 2 - chlorocrotonamide].

The procedure reported here is based on the iodoform reaction which is specific for the methyl ketones, ethanol, and secondary alcohols oxidizable to methyl ketones. Thus, the following types of compound give the iodoform test:

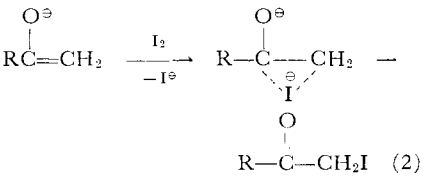


The initial step in the iodoform reaction is formulated as involving formation of the enolate anion (*2*):



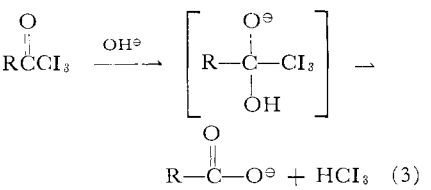
(1)

Electrophilic attack on the anion follows:



(2)

Steps 1 and 2 are repeated producing the triiodo derivative which undergoes alkaline fission:



(3)

The mechanism by which iodoform is produced from Bidrin was not specifically

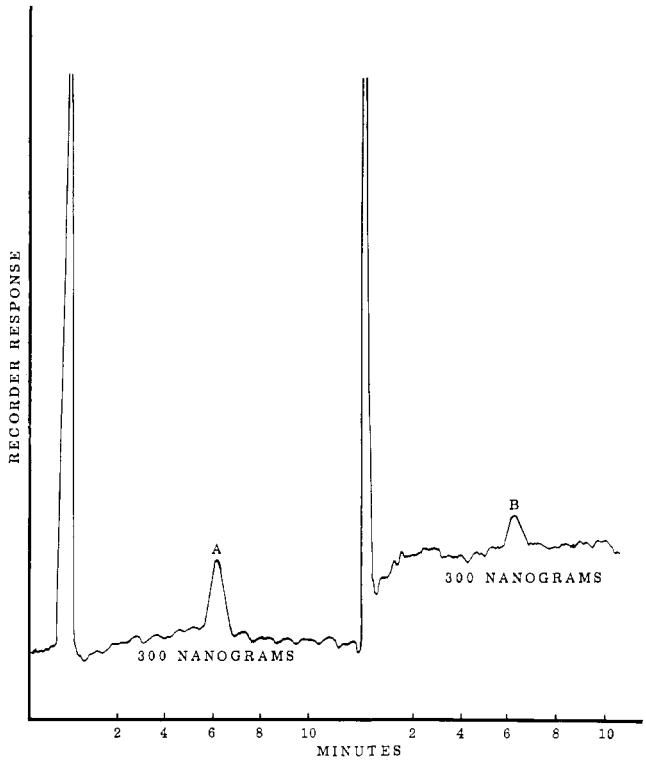


Figure 1. Representative chromatograms of Bidrin

A. Without crop B. Plus cabbage extract

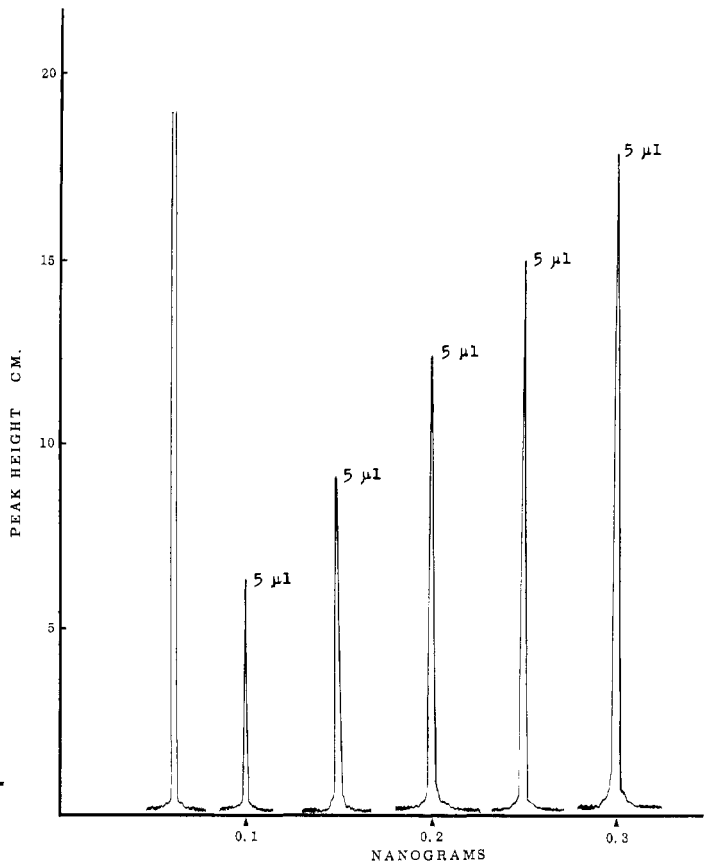


Figure 2. Iodoform analytical curve

investigated; however, production of iodoform from Bidrin is consistent with the observation that Bidrin hydrolyzes first to *N,N*-dimethylacetoacetamide and then is further cleaved to acetone (*1*). The reaction would then be expected to proceed as formulated in steps 1 through 3.

Analytical Procedure

Special Apparatus. A Jarrell-Ash Model 26-705 gas chromatograph equipped with a d.c. electron capture ionization detector and a 4-foot × $^1/_4$-inch stainless steel column packed with 5% DC-11 silicone grease on 80–100 mesh Chromosorb W was used throughout the experimental work. Optimum linearity and reproducibility were obtained at nitrogen flow rates of approximately 140 ml. per minute and at a column temperature of 130° C. The detector and down-stream splitter were maintained at 210° C., and the injector was set at 300° C. Thirty-eight volts was applied to the detector with the electrometer set at 4×10^{-9} ampere.

Procedure. Standard iodoform was prepared by treating 2 ml. of acetone with excess sodium hypoiodite. The resulting yellow solid (m.p. 130° C.) was collected by filtration, dried overnight, and recrystallized three times from boiling petroleum ether.

When technical Bidrin was treated in exactly the manner described above, a yellow solid (m.p. 130° C.) was obtained. No change in melting point was observed when the two yellow solids were subjected to a mixed melting point determination. Additionally, both compounds had identical chromatographic retention times and exhibited a single peak of increased area when mixed and chromatographed simultaneously.

Iodoform Analytical Curve. Iodoform (0.1238 gram) was weighed into a tared flask and transferred with the aid of several portions of petroleum ether into a 100-ml. volumetric flask. The solution was diluted to the mark and 1-, 2-, 3-, 4-, and 5-ml. aliquots were pipeted into separate 100-ml. volumetric flasks which were made to volume with petroleum ether. Each solution was further diluted 1 to 500, producing solutions in the 0.02 to 0.1 nanogram per µl. range. Typical iodoform chromatograms are illustrated in Figure 2.

Bidrin Analytical Curve. Bidrin (0.1000 gram) was weighed, transferred to a 100-ml. volumetric flask, and diluted to volume with distilled water. Aliquots, 1- to 5-ml., were pipetted into separate 50-ml. reaction flasks, and treated with 1 ml. of 10% sodium hydroxide followed by 10 ml. of 0.5*N* iodine–potassium iodide reagent. Each flask was fitted with a Snyder condenser and heated at reflux for 15 minutes. After the solutions were cooled and the iodine color had been removed by addition of a few drops of 10% sodium hydroxide, the reaction mixtures were transferred to 125-ml. separatory funnels. The aqueous phases were extracted with 50- and 25-ml. portions of petroleum ether. Extracts of each reaction solution were combined, washed with 20-ml. portions of 10% aqueous potassium iodide, transferred to 125-ml. Erlenmeyer flasks, and dried over approximately 5 grams of magnesium sulfate. When the petroleum ether solutions were thoroughly dried, each solution was filtered through a small plug of cotton into a 100-ml. volumetric flask and then diluted to volume with petroleum ether. Additional dilutions of 1 to 500 and 1 to 100, respectively, produced solutions in the 0.02 to 0.1 nanogram per µl. range. The Bidrin analytical curve (Figure 3) is linear between 0.1 and 0.4 nanogram and has the same slope as the iodoform analytical curve. Approximately 84% theoretical yield of iodoform was obtained.

Bidrin Recovery. To each of five Mason pint jars were added 100 grams of finely chopped cabbage, 300 ml. of methylene chloride, 10 grams of Darco G-60 activated carbon, 30 grams of anhydrous magnesium sulfate, and 0, 200, 300, 400, and 500 µg. of standard Bidrin. The samples were blended for 10 minutes, and the resultant macerates filtered through Celite 545. Extracts were transferred to 125-ml. Erlenmeyer flasks containing 15 ml. of 40% sodium bisulfite. All flasks were equipped with Snyder condensers, and the reaction mixtures were refluxed for an additional 10 minutes following evaporation of all the methylene chloride. When the solutions had cooled, they were transferred to 125-ml. separatory funnels and extracted with 30- and 20-ml. portions of methylene chloride. The methylene chloride extracts were combined and transferred to 125-ml. Erlenmeyer flasks to which had been added 1 ml. of 4*N* sodium hydroxide and 10-ml. of 0.5*N*

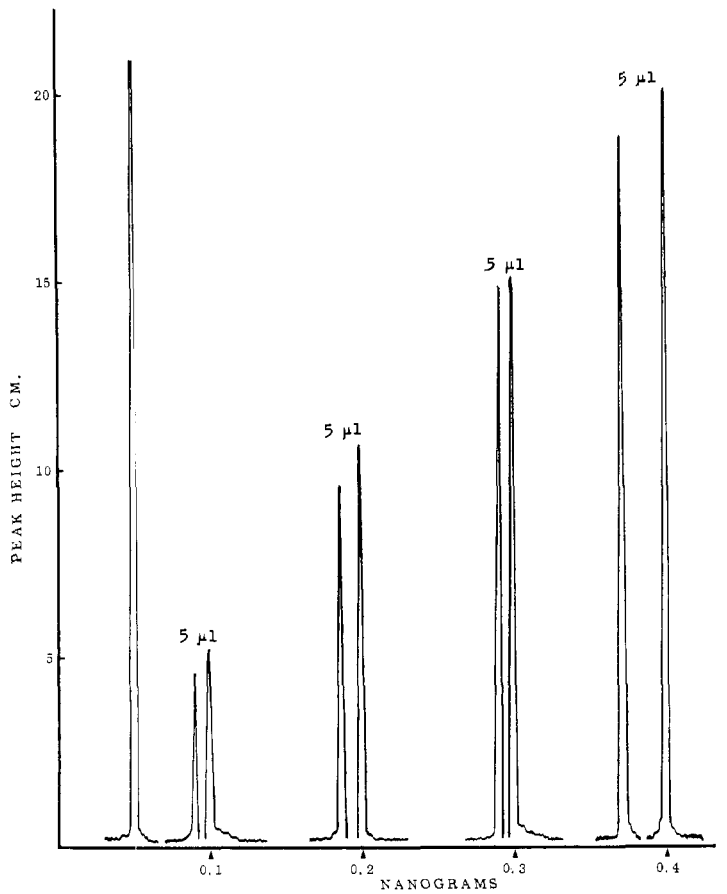


Figure 3. Bidrin recoveries from cabbage extracts

iodine–potassium iodide solution. These solutions were heated to reflux until all methylene chloride had evaporated and then stirred for an additional 10 minutes without further heating. The reaction solutions were returned to 125-ml. separatory funnels and extracted with 50- and 25-ml. portions of petroleum ether. Each petroleum ether solution was made to 100 ml. and further diluted 1 to 50 with petroleum ether, producing solutions in the 0.04 to 0.10 nanogram per µl. range. A 10-µl. Hamilton syringe was used to inject each 5-µl. sample into the gas chromatograph.

Results

Figure 1, *A* and *B*, are representative chromatograms of 300 nanograms of unreacted Bidrin. Figure 1*A* illustrates unreacted Bidrin without crop; Figure 1*B* shows the background effect when 3 mg. of unreacted Bidrin was added to a 100-gram cabbage extract equivalent which had not been subjected to any prior cleanup. When 0.1 to 0.5 mg. of Bidrin was reacted with excess iodoform reagent, a range of 83 to 85% of the theoretical yield of iodoform, based on the iodoform analytical curve (Figure 2), was obtained.

After the raw cabbage extract had been subjected to the described preliminary cleanup with activated charcoal, the colorless filtrate was treated with sodium bisulfite and then sodium hypoiodite. Following this second cleanup step, negligible instrumental background attributable to the crop extract was detectable in the working range of this procedure. In Figure 3, the shorter of the peak pairs covering the range of 0.1 to 0.4 nanogram represents a point on the reacted Bidrin recovery (with crop) curve. Each second peak is a point on the reacted Bidrin analytical (no crop) curve. Per cent recovery values were computed from relative peak heights, and these data are presented in Table I. The Bidrin (iodoform) recovery curve rather than the standard curve was used to determine field residue data.

Table I. Bidrin Recovery from Methylene Chloride–Extracted Cabbage

Sample, Grams	Added, µg.	Recovered, µg.	Recovered, %
100	0	0	
100	200	180	89
100	300	270	91
100	400	395	98
100	500	450	90

Bidrin residue data selected from field-treated, replicated research plots are shown in Table II. These results were obtained by the procedure previously described under Bidrin Recovery, including the final cleanup with sodium bisulfite and conversion of Bidrin to iodoform. Following a 0.25-pound active per acre application, the Bidrin residues on cabbage decreased from 8.4 to 3.6 p.p.m. in one week. When the dosage rate was increased to 0.50 pound of active Bidrin, the initial residues were 14.0 p.p.m. and decreased to 6.0 p.p.m. after 7 days of weathering.

Discussion

Since Bidrin could not be determined directly in the presence of crop extractables, it was necessary to develop a new analytical approach. Simple addition of bromine across the double bond produced a compound that was not sufficiently stable for analysis by gas chromatography. At column temperatures of less than 160° C., no response was obtained in 30 minutes after injection of the dibromo derivative. At temperatures higher than 160° C., the dibrominated compound decomposed on the column.

Bidrin reportedly (*7*) undergoes the Zeisel reaction as formulated below:

$$\text{Bidrin} \xrightarrow[H_2O]{HI} 2CH_3I + CH_3\overset{\overset{O}{\|}}{C}CH_3 + CO_2 + HN(CH_3)_2 + H_3PO_4 \quad (4)$$

However, when Bidrin was treated with aqueous hydrogen iodide, a very complex mixture of hydrolytic products which could not be quantitatively related to the parent compound resulted.

In a preliminary investigation, conversion of Bidrin to iodoform occurred rapidly. Iodoform, in addition to being detectable in subnanogram quantities, can be chromatographed at column temperatures as low as 110° C. The effect of crop impurities on the quantitative nature of the reaction proved troublesome. An extract from 100 grams of cabbage check was treated with sodium hypoiodite and a positive iodoform response was obtained; however, when the extract was refluxed with excess sodium bisulfite, prior to treatment with iodo-

Table II. Bidrin Residues on Cabbage Determined as Iodoform, P.P.M.

Lb. Active per Acre	Field Replication	Interval Since Last Application, Days: 1 Day	3 Days	7 Days
0.25	A	8.6	5.9	3.4
	B	8.1	5.4	3.7
	Av.	8.4	5.7	3.6
0.50	A	14.2	9.8	6.1
	B	13.8	9.2	5.8
	Av.	14.0	9.5	6.0

form reagent, no iodoform response was observed. Bidrin was unaffected by similar treatment with sodium bisulfite.

Increasing the sensitivity of Bidrin or its derivatives to EC detection was the primary objective of this research. This was accomplished by converting Bidrin to iodoform which was approximately 3000 times more responsive to EC detection than Bidrin.

A second objective, the development of a suitable and practical cleanup procedure was accomplished by conversion of Bidrin to iodoform resulting in no discernable background from cabbage extracts (Figure 3).

To date, proposed tolerances for Bidrin on several crops are under FDA consideration. It is anticipated that a tolerance of 1.0 p.p.m. will eventually be requested on leafy crops such as cabbage. Therefore, no attempt was made to analyze for crop residues at the 0.01-p.p.m. level, although data from spiked crops indicated that field residues at this level could have been determined.

The described iodoform procedure should be readily adaptable to routine analysis. Cleanup for 12 crop extract samples should not require more than 4 hours and conversion to iodoform plus preparation prior to injection, not more than an additional 2 hours. The iodoform retention time ($1^1/_2$ minutes compared with 12 minutes for Bidrin under the described conditions) is sufficiently short to allow determination of 12 samples in 2 hours and, therefore, a dozen or more Bidrin samples could be analyzed in a normal working day. Unlike Bidrin, iodoform produces well-defined and symmetrical chromatograms which makes possible a direct relationship between peak heights and quantity of pesticide injected.

Of the common solvents investigated, methylene chloride consistently produced the highest recovery values for extracting Bidrin from cabbage. Hexane, acetone, and isopropyl alcohol were unsuitable because Bidrin selectively partitions from hexane into water, whereas acetone and isopropyl alcohol undergo the iodoform reaction. In the extraction step, magnesium sulfate was added to remove water from the macerate and, thereby, reduce the amount of Bidrin lost to the aqueous phase. Activated charcoal was added directly to crop samples, which were then blended and filtered through Celite producing a colorless filtrate. When the identical macerate, minus charcoal, was chromatographed on a charcoal column, plant pigments passed through the column with the eluent. Addition of charcoal before blending proved to be more convenient as well as more effective than column chromatography in the cleanup.

Acknowledgment

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RESIDUE DETERMINATION

Colorimetric Determination of Abate Residues from Several Environmental Conditions

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A colorimetric method for residues of Abate insecticide, O,O,O′,O′-tetramethyl-O,O′-thiodi-p-phenylene phosphorothioate, is based on its hydrolysis to 4,4′-thiodiphenol and subsequent determination at 485 mμ after reaction with 4-aminoantipyrine and periodate. Procedures are described for natural waters, mud, oysters, rice grain, and rice foliage. Recoveries and apparent Abate insecticide concentrations in control tissues are given.

THE *O,O,O′,O′* - tetramethyl - *O,O′*-thiodi-*p*-phenylene ester of phosphorothioic acid (I) (Abate insecticide, compound 52,160, American Cyanamid Co.) is registered for the large-scale control of mosquito larvae. Before this compound could be used commercially, data concerning its residual behavior in natural waters and adjacent mud were required for registration purposes. Because of the possibility of exposure of oysters and rice during or following normal applications for mosquito control, information about residues on and in these tissues was also of interest.

Since the validity of a residue method depends upon its response to major toxic metabolites as well as to the parent compound, preliminary studies were conducted to investigate the extent and nature of metabolic degradation of this insecticide in simulated natural waters, mud, and bean plants. These studies showed evidence of only minor, if any, residues of postulated metabolic products; therefore, an analytical method responding to parent Abate insecticide should allow a realistic residue evaluation.

The analytical procedure used for the preliminary residue study incorporated thin-layer chromatography for separation of Abate insecticide from postulated metabolic products and either infrared spectrophotometry or total phosphorus determination by Schöniger flask combustion for quantitation. These procedures possessed adequate sensitivity and specificity for collecting the required residue data, but were not suited for mass-production efficiency so necessary for a large residue program. Abate insecticide and its hydrolysis product, 4,4′-thiodiphenol (II), possess low vapor pressure and lack of fluorescence, thus negating the utilization of the attractive features of vapor-phase chromatography and fluorescent spectrophotometry. Although Abate insecticide does absorb strongly in the ultraviolet, the very difficult inherent cleanup problems of ultraviolet residue procedures made this approach undesirable.

Parent Abate insecticide does not possess chemical properties suitable for the more conventional colorimetric pro-