# Appearance of 1-Hydroxychlordene in Soil, Crops, and Fish

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A 4-year study in an area of eastern Iowa revealed the presence of a metabolite of heptachlor, 1-hydroxychlordene, in soil, various crops, and fish. Tissues and fluids obtained from human subjects from this area did not reveal the presence of this metabolite. The concentration of 1-hydroxychlordene found in soils in conjunction with heptachlor is consistent with the short half-life reported by Bowman. The appearance of this metabolite in fish is associated with heptachlor and heptachlor epoxide. Translocation of heptachlor and metabolism in crops account for the appearance of the compound in produce.

Since the advent of organochlorine insecticides compounds, there has been widespread use on agricultural lands in Iowa. Heptachlor was one of the earliest and most important of these chemicals. Such use causes concern that these compounds or their metabolites will accumulate in the environment.

Heptachlor has been shown to have a wide variety of metabolic end products. Miles *et al.* (1969) have shown that heptachlor epoxide, chlordene, chlordene epoxide, 1-hydroxychlordene (1-HC), and 1-hydroxy-2,3-epoxychlordene are all products of microorganism degradation of heptachlor. Bowman *et al.* (1965) demonstrated that $\gamma$-chlordane is the principle impurity in stored material. These reports indicate that monitoring heptachlor and its epoxide either in the environment or in humans may not give an adequate reflection of its ultimate impact. For these reasons, orderly analyses, using appropriate detection and confirmatory techniques, should be done on each particular metabolite to establish a library of information upon which rational judgments can be made about their effect on human health and environment.

This is a report of such an orderly study on the metabolite 1-HC. Included is an estimation of usage by farm (where the metabolite of heptachlor and/or 1-HC occurred) and the analyses of environmental samples taken from the same geographical area.

## METHODS

**Gas Chromatography (Quantitative).** Three Micro-tek (Tracor) MT-220 gas chromatographic (glc) systems each fitted with four 6-ft × $^1/_8$-in. i.d. glass U tube columns were used for all analyses. The detection systems consisted of $Ni^{63}$, tritium and microcoulometric (C200, Dohrman Inst.) detectors.

The liquid phases employed were QF-1 (1.95%)/OV-17 (1.5%), a diethylene glycol succinate (3%), an OV-1 (3%), and an SE-30 (4%)/QF-1 (6%). The QF-1/OV-17 column utilized 80/100 mesh Gas Chrom Q, the diethylene glycol succinate utilized Gas Chrom P 80/100 mesh, the OV-1 utilized Chromosorb W 100/120 mesh, and the QF-1/SE-30 utilized Anakrom 80/90 mesh as supports.

The column ovens were maintained at 195°C except for the oven containing the OV-1, which was maintained at 185°C. The nitrogen carrier gas flow was 60 ml/min for the QF-1/OV-17. The diethylene glycol succinate and the QF-1/SE-30 columns used a carrier flow of 90 ml/min. The tritium detectors were operated at 205°C, and the $Ni^{63}$ was operated at 250°C. The instrument injection ports were at 215°C, and the transfer lines were at 225°C.

Chau and Cochrane (1969) have discussed the difficulties of the glc analysis of 1-HC. Cochrane (1969) has also presented methods to confirm the identity of 1-HC by oxidation to 1-ketochlordene. Cochrane and Chau (1968) have devised a derivatization procedure to enhance the chromatographability of 1-HC by making the corresponding silyl ether. The columns and glc employed here chromatographed the compound with adequate sensitivity, underivatized. Thus, the problems outlined by Cochrane were avoided.

Analytical grade 1-HC of 99.7% purity (Velsicol) was used for all identification and quantitation. A Fortran IV least squares regression line program, using instrument response *vs.* concentration and an IBM 360/65 computer, was utilized for computing the concentration of all samples.

**Thin-Layer Chromatography (Qualitative).** Thin-layer (tlc) plates, applicator, board, and developing equipment were purchased from Brinkman. Aluminum oxide plates (E. Merck A.G.) 250 $\mu$ thick, were prepared, ari-dried 24 hr, and activated at 150°C for 2 hr before use. The plates were developed for 10 cm, with hexane saturated with methanol, air-dried, and sprayed with 0.01% Rhodamine B (K&K Laboratories) in ethanol to produce blue spots against a pink background. Although uv irradiation is not required to produce spots with Rhodamine B, the colors are intensified under uv light. The dye is effective for visualization of a wide range of organochlorine compounds.

An $R_f$ of 0.089 was obtained for 1-HC using the above system. Duffy and Wong (1967) have described a tlc system involving hexane/ethyl acetate (7:3) to chromatograph 1-HC. For our purposes better removal of the interferences was obtained with the above system. We further confirmed the tlc analysis by extracting the spot and reanalyzing by glc, the criteria for identification being positive $R_f$ and RRt obtained on the two systems.

**Partitioning (P) Values.** (Bowman and Beroza, 1965). A 5.0-ml aliquot of the sample (and/or standard) in isooctane was analyzed, the lower phase of the solvent pair (5.0 ml) was then added, and the contents were spun on a Vortex mixer (Scientific Products) for 1 min. The phases were allowed to separate and the upper phase was again analyzed. The ratio of the second analyses to the first was considered the P value (Table I).

**Sample Preparation (Preparative).** Soil samples were prepared by the method of Burchfield and Schuldt (1957). The samples were concentrated to a moist residue and made up in isooctane for glc analysis. Crops were extracted with ethyl

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acetate and purified, using sweep codistillation (Watts and Storherr, 1965). Ethyl acetate was used for the distillation as specified by Storherr and Watts (1965). Fish were weighed frozen, the total specimen ground in a meat grinder, and a sub-aliquot of the blended sample (100 g) was taken and prepared for analysis by the method of Mills (1961).

Samples were blended with acetonitrile in a Waring blender three times. The extracts were combined. A sub-aliquot equal to 5.0 g of tissue was taken and the acetonitrile was diluted with water and partitioned three times with fresh hexane. The hexane was reduced in volume with a flash evaporator to 2.5–5.0 ml. This was applied to a Florisil column and the column was eluted with 6% diethyl ether in petroleum ether (200 ml) and 15% diethyl ether in petroleum ether (200 ml). Heptachlor elutes in the 6% fraction, and heptachlor epoxide and 1-HC elute in the 15% fraction. The volumes were reduced to 2.5–5.0 ml by flash evaporation and the aliquots combined in a glass-stoppered centrifuge tube. The contents were reduced to a moist residue with nitrogen and diluted in the isooctane for final analysis by glc. Results of recovery studies for the above methods are given in Table II.

All the samples reported were subjected to two-column analysis using electron capture detection. In addition, samples with exceptionally high levels (*e.g.*, Fa 61, 102, and 242) were analyzed by microcoulometry. The presence of 1-HC in fish samples was additionally confirmed by tlc and P value determinations.

## HISTORY OF PESTICIDE USAGE

A continuing evaluation of randomly selected farmers of Johnson County provided the basis for this study. Long *et al.* (1969) surveyed 155 randomly selected farmers of Johnson County, Iowa, to determine pesticide usage during 1966. These investigators found heptachlor was second in degree of usage in the area. Information on the kinds and amounts of pesticides used was obtained throughout the area from personal interviews for the period 1966–1970. Questions were designed to provide answers concerning the kinds of pesticides employed, how much was used, and where they were used.

The raw data were tabulated, key punched, and presented to an IBM 360/65 computer in Fortran IV for tabulation, normalization with respect to active ingredient, and composition of materials used, and for statistical evaluation with respect to residue data that were being generated simultaneously.

All environmental samples were taken from 50 of these farms. The samples were taken from the fields randomly, three times a year, to represent early spring, midsummer, and harvest or late fall. Soils were taken with a 1-in. probe to a depth of 8 in. in an "X" pattern of five samplings. The area of sampling in the field varied from time to time and each "X" covered about 10 $yd^2$. The samples were combined and blended.

Garden plots in Iowa occasionally are found at the "dead furrows" or ends of field areas. Because of insecticidal application techniques, these areas occasionally have a greater application of pesticides than crop areas. This is so because the spraying operation is not always ceased at rows' end, but may be continued while the vehicle is positioned for further application. The result is that garden soils and garden produce can have higher pesticide concentrations than adjacent fields.

Frequently, interrogation of farm operators failed to reveal adequate insecticide use records. This was due to faulty

**Table I. Partitioning Values and Column Responses for 1-Hydroxychlordene**

| 1-OH-Chlordene | P Values DMF[a] | Acetone[b] |
|---|---|---|
| Bowman and Beroza (1965) | 0.026 | 0.56 |
| ICPS Std. | 0.052 | 0.579 |
| Fish #90 | 0.060 | 0.63 |

**Relative Retention Times (RRT) and Relative Peak Heights (RPH)[c]**

| Column | 1-OH-Chlordene RRT | RPH | Flow rate, ml/min | Column temperature, °C |
|---|---|---|---|---|
| 4% SE-30/6% QF-1 | 1.15 | 0.31 | 90 | 195 |
| 3% OV-1 | 1.65 | 0.069 | 60 | 185 |
| 1.95% QF-1/1.5% OV-17 | 1.17 | 0.66 | 60 | 195 |
| 3% DEGS | 4.12 | 0.33 | 90 | 195 |

[a] Refers to binary system isooctane/dimethyl formamide. [b] Refers to binary system isooctane/80% acetone–water. [c] All data were obtained using aldrin for basis of comparison utilizing a parallel plate tritium foil detector.

**Table II. Methodology Recovery Study of 1-Hydroxychlordene[a]**

| Test | Loading level | Percent recovery |
|---|---|---|
| Soil[b] (Burchfield and Schuldt, 1967) | 1 | 81 |
| | 10 | 93 |
| | 100 | 81 |
| | 1000 | 79 |
| Vegetables[c] (Storherr and Watts, 1965) | 1 | *d* |
| | 10 | 94 |
| | 100 | 57 |
| | 1000 | 47 |
| Tissues[e] (Mills, 1961) | 1 | *f* |
| | 10 | *f* |
| | 100 | 92 |
| | 1000 | 112 |

[a] A QF-1/OV-17 column was used for this study. The values reported are averages of triplicate analysis. All residue values are in PP $10^9$. [b] Soxhlet-extracted soil was used for substrate; the blank was subtracted from the results. [c] Vegetable oil was used at 3% v/v to simulate vegetable extract since various vegetables were analyzed. [d] Below limits of detection for this test. [e] Corn oil was used in lieu of tissue lipids. [f] Partially obscured by artifacts in reagent blank.

**Table III. 1-Hydroxychlordene in Soil**

| Sample | 1-OH-Chlordene PP ($10^9$) | Heptachlor PP ($10^9$) | Kind of agricultural use | lb 100% heptachlor used 1967–1969[a] |
|---|---|---|---|---|
| Ca 29 | 10 | 0 | Corn | 94 |
| Ca 29[b] | 12 | 0 | Garden | 94 |
| Ca 326 | 28 | 25 | Garden | None reported |
| Fa 61 | 107 | 626 | Corn | Not known |
| Fa 61[b] | 2 (qual) | 2 (qual) | Garden | Not known |
| Fa 102 | 4 (qual) | 0 | Corn | 440 |
| Fa 102[c] | 29 | 31 | Corn | 440 |
| Fa 230 | 7 (qual) | 3 (qual) | Corn | 100 |
| Fa 242 | 18 | 110 | Corn | 140 |
| Fa 263 | 5 (qual) | 0 | Corn | 3 |
| Fa 363 | 17 | 3 (qual) | Garden | 3 |
| Fa 384 | 9 (qual) | 4 (qual) | Corn | 330 |

Results below 10 PP ($10^9$) are subject to some error. However, qualitation was possible on two columns. The values reported are estimates of the true value.

[a] Represents field data corrected to lb 100% heptachlor by computer using the percentage of pure heptachlor contained in the various commercial products. [b] Adjacent. [c] Same field.

Table IV. 1-Hydroxychlordene in Crops

| Sample | 1-OH-Chlordene PP ($10^9$)[a] | Kind of crop[b] | lb 100% heptachlor used in 1967–1969 |
|---|---|---|---|
| Ca 21 | 8 (qual)[c] | Soybeans | None |
| Ca 42 | 12 | Soybeans | None |
| Ca 44 | 28 | Soybeans | None |
| Ca 47 | 3 (qual)[c] | Tomato | None |
| Ca 282 | 3 (qual)[c] | Cucumber | None |
| Ca 347 | 53 | Field corn | None |
| Fa 49 | 13 | Ground cob meal | 200 |
| Fa 61 | 22 | Turnip | Not known |
| Fa 102 | 14 | Field corn | 440 |
| Fa 230 | 29 | Field corn | 100 |
| Fa 285 | 46 | Field corn | 200 |
| Fa 363 | 5 (qual)[c] | Sweet corn | 3 |

[a] Quantitation done on either QF-1/SE-30 or QF-1/OV-17. Confirmatory analyses were all done on a diethylene glycol succinate column. [b] Heptachlor is rarely noted in these crops; heptachlor epoxide occurs occasionally. [c] Results below 10 PP ($10^9$) are subject to some error. However, qualitation was possible on two columns. The values reported are an estimate of the true value.

Table V. 1-Hydroxychlordene in Fish

| Kind of fish | 1-OH-Chlordene PP ($10^9$) | Heptachlor PP ($10^9$) | Heptachlor epoxide PP ($10^9$) |
|---|---|---|---|
| *Ictiobus niger* (buffalo) | 13 | 0 | 78 |
| *Pomoxis nigromaculatus* (sunfish/crappy) | 14 | 10 | 33 |
| *Pomoxis nigromaculatus* (sunfish/crappy) | 14 | 3[a] | 23 |
| *Lepomis macrochirus* (blue gill) | 22 | 0 | 0 |
| *Ictalurus melas* (catfish) | 57 | 0 | 20 |
| *Ictiobus niger* (buffalo) | 35 | 35 | 58 |
| *Ictalurus melas* (catfish) | 78 | 0 | 18 |
| *Cyprinus carpio* (carp) | 40 | 11 | 95 |
| *Pomoxis nigro maculatus* (sunfish/crappy) | 20 | 15 | 55 |
| *Carpiodes cyrinus* (carp) | 14 | 9[a] | 9[a] |
| *Lepomis macrochirus* (blue gill) | 15 | 0 | 16 |
| *Lepomis macrochirus* (blue gill) | 16 | 13 | 9[a] |
| *Ictalurus melas* (catfish) | 14 | 0 | 0 |

Quantitation done on either QF-1/SE-30 or QF-1/OV-17. Confirmatory analyses were all done on a diethylene glycol succinate column.

[a] Results below 10 ($PP^9$) are subject to some error. However, qualitation was possible on two columns. The values reported are an estimate of the true value.

records, faulty memory, or land transfer. In some cases, the usage record was shown to be inadequate or erroneous by actual analysis of samples from these farms.

Soil analysis, especially surface soil of plowed fields, can be a misleading indicator of pesticide application. Iowa encounters severe wind erosion of fall-plowed fields during dry seasons. The wind-blown soil is high in pesticide content. This means very few soils of "blank" or no pesticide content were found, despite a known negative use history.

## RESULTS

The analyses of the 1500 soils, 400 crops, and 75 fish samples produced 12 soils (Table III), 12 crop (Table IV) samples, and 13 fish (Table V) of varying species that were positive for 1-HC. Additionally, a few positive samples were encountered before the identity of the peak had been established beyond question. Some fish, algae, and bottom muds taken from the same area have been reported as positive for this metabolite by Mehta (1969).

## DISCUSSION

Metabolism of heptachlor to 1-HC by hydrolysis in soil has been demonstrated by Miles *et al.* (1969). Furthermore, Bowman *et al.* (1965) have reported degradation of heptachlor to 1-HC in insecticidal dusts. Bowman *et al.* (1965) have also demonstrated that the metabolite is reduced about tenfold in 99 days at an application level of 0.3 lb/3 in.-acre. These findings suggest 1-HC is applied as a contaminant resulting from the breakdown of heptachlor, heptachlor is metabolized to 1-HC by hydrolysis, and this metabolite is further acted upon to reduce its concentration with a half-life of about 3 weeks.

The results one might expect to find for soils treated with heptachlor would be similar to radioactive decay with short half-life daughter formation. The process begins with initially high heptachlor and 1-HC, and the 1-HC is reduced to some low level that is sustained by the slower process of heptachlor hydrolysis.

Our findings are in keeping with this hypothesis. Due to the time duration following exposure, almost all the samples we present are the limiting case of total or near depletion of heptachlor and the low or diminished levels of 1-HC. The two exceptions are Fa 61 corn soil and Fa 242 corn soil. Unfortunately, records could not be obtained describing the use history of Fa 61. The soil from farm Fa 242 had had a heptachlor treatment the year of the analysis, which explains the higher value for both heptachlor and 1-HC.

The appearance of 1-HC in crops must represent a case of translocation with metabolism. In all cases heptachlor or the epoxide was found in the associated soil. The only case where the 1-HC metabolite, in conjunction with its appearance in crops, was found in the soil was Fa 363. The non-use of heptachlor reported by application records covers through the period of 1967–1969. The highest use of heptachlor in this study area occurred previous to that time. In our experience heptachlor and its metabolites, as a result of this historic use, appear in a great many soils where no further application has occurred. Such a case is soil Ca 326, which is positive for heptachlor with no application during the 3 years when records were examined.

The finding of 1-HC in fish taken from the river and lakes fed by the runoff of the Johnson County agricultural area was interesting, in that it appears to be the first reported case of 1-HC being found in vertebrate tissue. The presence of 1-HC was almost always closely related to the appearance of heptachlor and its epoxide. The low toxicity of 1-HC in rats ($LD_{50}$ 2400 to 4200 mg/kg) compared to that of heptachlor (90 to 135 mg/kg) or its epoxide (60 mg/kg) (Polen, 1971) indicates that this metabolite is the product of a detoxification reaction. The discovery of this metabolite in tissue prompted us to examine autopsy tissue, blood, and other environmental samples (air and water) for this metabolite. The only occurrence noted was in a rendered human adipose tissue provided as a quality control specimen by the National Communicable Disease Center, Primates Research Center, Perrine, Fla. With human examinations (living and postmortem) numbering in the thousands, no other human or environmental specimens containing 1-HC were obtained. Therefore, it would seem that *in vivo* conversion to 1-HC or storage of 1-HC is not important in humans.

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# Sorption and Recovery of Phosphine

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Minute quantities of phosphine were added to several cereal products and inert materials to investigate if temperature, duration of contact, and various aeration conditions would alter recovery. Previous experiments had shown that phosphine could diffuse through porous materials quite readily, so diffusion studies were also conducted at room temperature and elevated temperature conditions. Loss of added phosphine and poor recovery were shown to occur at elevated temperatures from systems containing cereal products or inert materials when proper precautions were not taken to insure an airtight system. Loss of phosphine was prevented and recovery greatly improved when an airtight system was used in the experiment.

Phosphine has gained a continuously rising importance as a fumigant for the last few years, initially being used as a grain fumigant and later as a fumigant for a vast number of human food products, animal feeds, and tobacco.

The development of Phostoxin tablets and pellets has provided a safe means of fumigating with phosphine, and registration of this product was previously based on the assumption that following adequate aeration no residual phosphine would be found in the final manufactured commodity.

Various experiments reported by Dieterich *et al.* (1967) have demonstrated that a residue can exist if adequate aeration has not been employed. This paper also reported the analysis of a large number of commodities which showed that, following proper aeration, no residue can be detected. Sullivan *et al.* (1969a,b) conducted additional studies on tobacco and a series of whole in-shell nuts and nut meats which showed that, immediately following fumigation, a residue can be detected but falls off rapidly following proper aeration. Robinson and Bond (1970) used radioactive phosphine ($^{32}PH_3$) in studying recovery of the applied fumigant from wheat. They found that small residues would exist and showed that the residue consisted of oxyacids of phosphorus and that oxygen was necessary for this reaction. Bond (1971) reported similar results, and again oxygen was necessary for the formation of the oxyacids of phosphorus. The formation of these acids is greatly decreased in a nitrogen atmosphere. Hilton (1971) also confirmed the results reported by Robinson and Bond (1970).

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Additional studies are being conducted at the present time to determine if the reaction rate of radioactive phosphine ($^{32}PH_3$) to oxyacids of phosphorus is faster than nonradioactive phosphine, and if exchange is possible in both insect systems and various commodities. These studies will be reported at a later date.

Two papers were published by Berck (1968a,b) describing recovery experiments following fumigation of raw and processed commodities, which reported a different result than those found by Dieterich *et al.* (1967), but which also stated that no organoleptic change between treated and untreated commodities could be detected and no evidence of residues could be found in the products.

The analytical procedure used by Berck (1968a) was perfectly acceptable and has also been reported by White and Bushey (1944). The authors of these papers found similar results as far as recovery from an empty vessel was concerned, and also reported similar solubility of phosphine in water.

As a result of the differences reported by Berck (1968a) and Dieterich *et al.* (1967), a study was conducted to establish if different methods were necessary to show that added phosphine could be recovered. The major differences that existed between the two series of studies reported were the volume of the vessels employed for fumigation, the method of aeration, and the dosage rates. Dieterich *et al.* (1967) used either an 8- or an 11-l. desiccator with a tight-fitting seal as a fumigation vessel and an accelerated aeration with warm nitrogen and mechanical stirring of the commodity, and a dosage rate of 1.5 to 5.7 mg/l. Berck (1968a) used a flask with a volume of 1.13 l., a dosage rate of 0.1 to 0.6 mg/l., and aerated with nitrogen with occasional shaking.

Since Robinson and Bond (1970) had reported that residues of oxyacids of phosphorus increased as the concentration of