Purity of Reagent Grade *p*- and *o*-Chlorophenoxyacetic Acids and Its Biological Implications

Reagent grade *p*- and *o*-chlorophenoxyacetic acids contain phenolic (<0.2%) and 2,4-dichlorophenoxyacetic acid (2,4-D) impurities (1–5%). The level of 2,4-D may account for the biological activity of both acids.

The herbicidal properties of halogenated phenoxyacetic acids are well known. Most of the papers comparing the biological effects of these acids were published in the late 1940's and in the 1950's before sensitive gas–liquid chromatographic techniques were available to test the purity of the original starting compounds.

Thompson *et al.* (1946) showed that *p*-chlorophenoxyacetate, methyl *p*-chlorophenoxyacetate, and *o*-chlorophenoxyacetate had 85, between 50 and 79, and 29%, respectively, of 2,4-dichlorophenoxyacetic acid (2,4-D) activity toward corn seedlings when applied in the same concentration in aqueous solution. This was interpreted to mean that the *p*-chloro acid was at least twice as phytotoxic as the corresponding *o*-chloro compound. However, whether this is really so depends on the relative water solubility of the acids, and on their purity. Solubility cannot explain the results as the methyl ester of the *p*-chloro acid is less water soluble than the *o*-chloro acid but is still more active.

Phenoxyacetic acids are usually synthesized using the appropriate halophenol in alkaline aqueous haloacetate (Pokorny, 1941; Crowdy and Wain, 1951). The halophenol is the major cause of impurities as recrystallization, sublimation, or distillation does not completely separate mixtures of similar phenols. Impurities in the haloacetate could also contribute, but acid mixtures are much easier to purify than mixtures of similar phenols, and the corresponding phenoxybutyric acids are not as herbicidal as phenoxyacetic acids. The biological activity of α-halohalophenoxyacetic acids has not been studied.

If the original *o*- or *p*-chlorophenols contained some 2,4-dichlorophenol, then 2,4-D would also be formed along with the monohalo acid. All or part of the observed phytotoxicity of the monohalophenoxyacetic acid could be caused by the 2,4-D impurity. If this were so, much of the early data on the biological effects of these acids would be suspect, and some theories of the mode of action of halogenated phenoxyalkanoic acids would have to be revised.

EXPERIMENTAL SECTION

Commercial reagent grade *p*-chlorophenoxyacetic acid (Aldrich) and *o*-chlorophenoxyacetic acid (Pfaltz and Bauer) were examined.

High (70 eV) and low (7 eV) mass spectra and melting points of the commercial acids were measured.

A known mass of acid was esterified with BF_3–*n*-butyl alcohol reagent according to the method of Horner *et al.* (1974). The yields of *n*-butyl ester were approximately 95 ± 5% for acids. Experiments were done in triplicate.

Aliquots of esters dissolved in a known volume of hexane were injected onto a 6 ft × 3.5 mm i.d. Pyrex U-tube column packed with 10% SE-30 impregnated 60–80 mesh Chromosorb W (AW-DMCS) at injector, column, and ^{63}Ni electron capture detector temperatures of 232, 187, and 222°, respectively. The pulse interval was 15. The flow rate of 19:1 argon–methane carrier was 27 ml/min. The 2,4-D content was obtained by the external standard method, using known masses of *n*-butyl ester.

Glc–mass spectrometry was done with a flame ionization glc and a single focusing MS-12 mass spectrometer. The glc column was a 6 ft × ⅛ in. i.d. stainless steel tube, packed with 10% SE-30 impregnated 60–80 mesh Chromosorb W (AW-DMCS). The injector and detector temperatures were 232 and 350°, respectively. The temperature program involved holding the column at 70° for 6 min after injection and heating the column to 200° at 4°/min, the column then being held at 200° until all compounds eluted. The flow rates of compressed air, hydrogen, and helium carrier were 500, 35, and 20 ml/min, respectively.

RESULTS AND DISCUSSION

The mass spectra of the two acids at both high and low electron volts indicated the presence of 2,4-D by the existence of *m/e* 220 and 222 peaks, their ratio implying two chlorines. In addition, small parents of *m/e* 162 and 128 containing two and one chlorine atoms, respectively, were also visible on low electron volt spectra.

On butylation, traces of *o*-, *p*-, and 2,4-dichlorophenols as well as 2,4-D butyl ester were detected using isothermal glc and matching retention times of standards. Glc–mass spectral examination also confirmed the 2,4-D ester, but the concentrations of the phenols were too low for quantitation as they comprised <0.2% of the total monochlorophenoxyacetic acid. As the biological activity of the phenols is also low (Thompson *et al.*, 1946) these will probably not contribute very highly to the observed biological activity.

Table I compares the melting points observed, stated on the bottle, and in the literature (as given by the "Handbook of Chemistry and Physics") as well as the amount of 2,4-D impurity expressed as the percentage of the true weight of monochlorophenoxyacetic acid.

It will be noted that the *o*-chloro compound has a wider observed melting point range than the *p*-chloro acid and a greater 2,4-D content.

It is well known that herbicidal activity is not linear with concentration, and that synergistic effects may occur when two herbicides are mixed. Thus, the amount of observed impurity could well account for the phytotoxicity observed by Thompson *et al.* (1946) at least for the comparatively less toxic *o*-chloro compound. It is unlikely that the strong biological effect of the para isomer could be explained by the presence of 2,4-D at the levels found experimentally (Table I). However, it is unknown how pure Thompson's starting acids were (Newman *et al.*, 1947). In this regard, melting point data are not very meaningful as it will be noted from Table I that the literature value of the melting point for the *p*-chloro acid is 2.5° below that claimed for by the manufacturer. The observed melting point was 6° below that given on the bottle.

We recommend that pure esters could be collected after glc separation, thus providing quantitation and purification in one step, and then the experiments of Thompson *et al.* (1946) repeated so that the actual biological effects of changing substituents can be found and interpreted meaningfully.

This problem is general for all pesticide chemistry, in that pesticides may contain small amounts of highly toxic impurities which may cause much of the biological activity. If the pesticide contains small amounts of material which are as or less toxic than itself, then probably these impurities will be unimportant in biological effect. How-

Table I. Melting Points and 2,4-D Content of Reagent Grade *o*- and *p*-Chlorophenoxyacetic Acids

Phenoxy-acetic acid	Melting points, °C			% 2,4-D
	Lit.	Bottle	Obsd	
o-Chloro	145.5 ± 0.5		136 ± 3	4.9 ± 0.5
p-Chloro	155.5 ± 0.5	158 ± 1	152 ± 2	1.0 ± 0.1

ever, quantitation of these impurities is still essential so that the true biological activity of the pesticide under examination can be found. As most biological scientists usually use pesticidal formulations of uncertain concentration and content, this problem is compounded even more, and it is not surprising that confusion in interpretation of experimental results has resulted. This paper has shown that chemical analysis and quantitation of a pesticide, and related impurities, are necessary before unambiguous interpretation of biological experiments.

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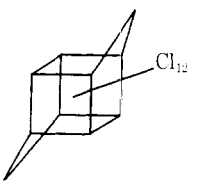
Photoreduction of Mirex in Aliphatic Amines

Solutions of dodecachloropentacyclo[5.3.0.$0^{2,6}$.$0^{3,9}$.$0^{4,8}$]decane (Mirex) in aliphatic amines were irradiated with sunlight. Mass spectral, infrared, and nmr data were used to establish that the major photoproducts are 1,2,3,4,5,5,6,7,8,9,10-undecachloropentacyclo[5.3.0.$0^{2,6}$.$0^{3,9}$.$0^{4,8}$]decane and 1,2,3,4,5,6,7,8,9,-10-decachloropentacyclo[5.3.0.$0^{2,6}$.$0^{3,9}$.$0^{4,8}$]decane. Spectroscopic evidence is reported which indicates a charge transfer complex is formed between the amine and Mirex.

The insecticide Mirex (**1**) (dodecachloropentacyclo[5.3.0.$0^{2,6}$.$0^{3,9}$.$0^{4,8}$]decane) is utilized extensively in the southeastern United States to control the imported fire ants *Solenopsis invicta and Solenopsis richterii*. This chlorocarbon is thermally stable (Eaton *et al.*, 1960) and resistant to chemical degradation even by strong acids and bases as well as oxidizing and reducing agents (McBee *et al.*, 1956). Its photochemistry in hydrocarbon solvents with radiation down to 200 nm and its very slight decomposition by sunlight when adsorbed to silica have been described (Alley *et al.*, 1973; Gibson *et al.*, 1972). The major monodechlorination product in both cases is 1,2,3,4,5,5,6,7,9,10,10-undecachloropentacyclo[5.3.0.-$0^{2,6}$.$0^{3,9}$.$0^{4,8}$]decane (**2**) (Alley *et al.*, 1974). Mirex shows



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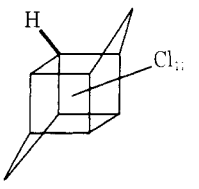

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virtually no absorption at wavelengths greater than 250 nm (Zijp and Gerding, 1958), and thus very little photochemical degradation by sunlight would be expected. The subject of this report is the facile degradation of Mirex by sunlight *via* its interaction with aliphatic amines.

EXPERIMENTAL SECTION

Technical Mirex (**1**), Allied Chemical Corp., was recrystallized from benzene. Cyclohexane (Phillips, 98%) was fractionally frozen and then distilled. Reagent grade triethylamine (Aldrich Chemical Co.) was distilled under nitrogen. The purity of the triethylamine was checked by glpc using a 1.5-m Chromosorb 103 column at 150° and was found to be greater than 99.9%.

The reactions were monitored with a Varian 1400 gas chromatograph equipped with a hydrogen flame detector. The mass spectra were obtained with a Perkin-Elmer Model 270 gas chromatograph–mass spectrometer. The following gas chromatographic columns were used for the analyses: OV-1, 16-m support-coated, open-tubular (SCOT); 3-m 5% SE-30 on 80–90 Anakrom ABS; and a 1.5-m 0.35% diethylene glycol succinate (DEGS) on 100–120 mesh textured glass beads.

Spectra were obtained with a Perkin-Elmer Model 457 infrared spectrophotometer and a JEOL Model MH-60II nuclear magnetic resonance spectrometer. Elemental analyses were performed by Galbraith Laboratories, Knoxville, Tenn.

Photolysis of Mirex (1) in Triethylamine. A solution of Mirex (5 g) in 100 ml of triethylamine was irradiated in a Pyrex tube with a 275-W GE sunlamp. After 50 hr, glpc analysis indicated that 80% of the Mirex had reacted and three major products were formed with retention times (SE-30, 230°) relative to Mirex of 0.75, 0.70, and 0.60 with peak area ratios 30:3:20, respectively. Cyclohexane (100 ml) was added to this solution and it was then neutralized with 6 *N* hydrochloric acid. The aqueous portion was extracted twice with 100 ml of cyclohexane and the extracts were combined, dried, and concentrated. The resulting material was chromatographed on alumina. Elution with cyclohexane gave some fractions containing a small quantity of the product with a relative glpc retention time of 0.70. The infrared, nmr, and mass spectra of this compound were identical with those reported for **2** (Alley *et al.*, 1973).

Further elution of the alumina column with cyclohexane gave fractions that were mixtures of the other two photoproducts. These mixtures were further separated by