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Literature Cited

- (1) Fink, R. M., Dent, C. E., Fink, K., *Nature* **160**, 801 (1947).
- (2) Henriques, F. C., Jr., Kistiakowsky, G. B., Margnetti, C., Schneider, W. G., *Ind. Eng. Chem. Anal. Ed.* **18**, 399 (1946).
- (3) Henriques, F. C., Jr., Margnetti, C., *Ibid.*, pp. 915-21.
- (4) Kelly, R. G., Peets, E. A., Gordon, S., Buyske, D. A., *Anal. Biochem.* **2**, 267 (1961).
- (5) Leary, J. B., Koons, J. R., Scroggs, R. E., Holzer, F. J., Division of Agricultural and Food Chemistry, 150th Meeting, ACS, Atlantic City, N. J., September 1965.
- (6) McMahon, R. E., Sullivan, H. R., *Biochem. Pharmacol.* **14**, 1085 (1965).
- (7) Paneth, F. A., "Radioelements as Indicators," McGraw-Hill, New York, 1928.
- (8) Pohland, A. (to Eli Lilly and Co.), U. S. Patent **3,120,434** (February 4, 1964).
- (9) Schmeiser, K., "Radioactive Isotope, ihre Herstellung und Anwendung," Berlin-Göttingen-Heidelberg, Springer-Verlag, 1957.
- (10) Stahl, E., *Pharmazie* **11**, 633 (1956).
- (11) Stahl, E., *Chemiker-Ztg.* **82**, 323 (1958).
- (12) Weiler, H., *Intern. J. Appl. Radiation Isotopes* **12**, 49-52 (1961).

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HERBICIDES

Photodecomposition of 2,4-Dichlorophenoxyacetic Acid

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2,4-Dichlorophenoxyacetic acid decomposes rapidly in the presence of water and ultraviolet light. This decomposition results in the formation of 2,4-dichlorophenol, 4-chlorocatechol, 2-hydroxy-4-chlorophenoxyacetic acid, 1,2,4-benzenetriol, and, finally, polymeric humic acids. The results with artificial light and with sunlight are essentially identical.

ULTRAVIOLET LIGHT has been shown to exert drastic changes in many pesticides under laboratory conditions (7). If similar action were to take place in sunlight under field conditions, the result would be of major importance to the environmental stability and practical use of these substances, and the detailed chemistry and toxicology of the resulting decomposition products would be significant to both agriculture and public health.

The effect of ultraviolet light on the widely used herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) has been examined by several investigators. Although Mitchell (7) did not observe any breakdown, Payne and Fults (8) reported that irradiation of 2,4-D solutions increased their stimulation of cell elongation in the pea slit-stem test, and Bell (3) noted that 2,4-D solutions were decomposed to unidentified "phenolic" substances. Aly and Faust (2) examined the effect of ultraviolet light on aqueous solutions of 2,4-D salts and esters, demonstrated the rapid disappearance of the herbicide, and provided colorimetric evidence for the presence of phenols in the irradiated solutions. The effect of riboflavin on this decomposition also was studied by Bell (3) and others (5, 6), but no conclusive evidence for the chemical identity of any of the breakdown products has been reported.

Practical tests also indicate that sunlight has an effect on 2,4-D in the field. Penfound and Minyard (9) investigated

the relationship of light intensity to the effect of the herbicide on water hyacinth and kidney bean and observed more necrosis and greater epinasty in shaded plants than in those receiving full sun. The purpose of the present experiments was to compare the effect of sunlight and laboratory ultraviolet light on aqueous 2,4-D solutions and to identify any major decomposition products.

Experimental

Materials. 2,4-Dichlorophenoxyacetic acid was recrystallized several times from water and from benzene until a constant melting point (138.2-138.8° C.) and homogeneity to gas chromatography and thin-layer chromatograph were attained.

4-Chlorocatechol, commercially available (Aldrich Chemical Co.) as a dark powder, was crystallized from benzene to remove insoluble resin and then sublimed at 40° to 50° C. (0.1 mm.) to provide the pure compound as white crystals.

2-Chlorohydroquinone (J. T. Baker Chemical Co.) was purified in the same way.

2,4-Dichlorophenol, 1,2,4-triacetoxybenzene, and other chemicals were used in commercially available form.

2-Chloro-4-hydroxyphenoxyacetic acid was prepared from *p*-methoxyphenol by the method of Brown and McCall (4) and exhibited a melting point and infrared spectrum identical with those reported in the literature.

2-Hydroxy-4-chlorophenoxyacetic acid was prepared by reaction of 4-chloro-

catechol with chloroacetic acid in aqueous sodium hydroxide solution followed by a clean separation of the two resulting isomers by crystallization from water. The melting point and infrared spectrum were identical with the literature value (4), but an attempt to repeat the purification of the crude isomer mixture failed to provide adequate resolution of the constituents.

Standard humic acid was prepared by stirring a mixture of 2.5 grams of 1,2,4-triacetoxybenzene and 40 ml. of 10% aqueous sodium hydroxide solution for 30 minutes. The dark mixture was poured into 500 ml. of 20% hydrochloric acid, allowed to stand overnight, and evaporated to dryness (filtration proved to be very difficult). The residue was extracted with acetone, the extract was filtered to remove salt, and the filtrate was evaporated to dryness. The residue was dried under vacuum at 50° C. over solid sodium hydroxide and phosphorus pentoxide to provide a good yield of fine, almost black powder.

Irradiation. Standard solutions for irradiation were prepared by dissolving 440 mg. of 2,4-D and 170 mg. of sodium bicarbonate per liter of distilled water to provide a concentration of 2×10^{-3} M. The solution (10 liters) was stirred vigorously in a large glass tank and externally cooled in ice to maintain a temperature of 20° to 25° C. Irradiation time varied from 5 to 12 hours, although the rate of photodecomposition slowed markedly after about 8 hours. Solution depth in these instances was 8.5 cm.

Laboratory irradiations employed mercury arc lamps which produced light

principally at a wavelength of 254 $m\mu$. Quantities of solution less than 1 liter were irradiated with a Multiray Short Wavelength Laboratory Lamp (G. W. Gates and Co., Franklin Square, N. Y.) positioned about 2 cm. above the liquid surface, while a 360-watt Raymaster high-pressure lamp (G. W. Gates and Co.) at a distance of 22 cm. was employed with larger volumes.

Irradiations in sunlight were carried out in 8- by 12-inch borosilicate glass baking dishes or 9.5-cm. Petri dishes. Each solution was exposed for about 5 hours at noon on each of 10 days in early October or late March; water was added daily to replace that lost by evaporation. Solutions in baking dishes were stirred continuously with a magnetic stirrer, but those in Petri dishes remained unstirred.

A few experiments were conducted by exposing to sunlight a 15-cm. diameter circle of Whatman No. 1 filter paper soaked in a solution of 2,4-D sodium salt and kept continuously wet by capillary action from reservoirs attached to opposite edges of the paper. After irradiation, the reaction products were eluted with acetone.

Extraction and Separation. Two general methods for extraction and separation were employed. In the first, the irradiated solution (pH 3.5) was exactly neutralized with dilute aqueous sodium hydroxide solution and extracted three times with ether to provide an aqueous phase A and a combined ether extract B. Extract B was washed with water and then re-extracted with 2*N* aqueous sodium hydroxide solution to provide a basic solution C and ether solution D. Washing with water, drying over sodium sulfate, and evaporating D at approximately ambient temperature gave a "neutral fraction" E.

Solution C was acidified with dilute hydrochloric acid to pH 2 and extracted with ether; the extract was washed with water, dried, and evaporated to give the "phenolic fraction" F. The original aqueous phase A was treated in an identical manner to provide the "acidic fraction" G.

The second method did not involve a chemical separation. The irradiated solution was acidified to pH 2 with dilute hydrochloric acid and continuously extracted with ether for 24 hours in a liquid-liquid extractor; the ether extract was washed with a small volume of water, dried over sodium sulfate, and evaporated under reduced pressure.

Gas-chromatographic separations were accomplished with F and M Model 500 and Model 720 temperature-programmed instruments equipped with thermal conductivity detectors. Most separations employed a 2-foot by 1/4-inch column containing 20% Dow 11 silicone oil on acid-washed 60- to 80-mesh Chromosorb W and a linear temperature increase of 10° C. per minute. Collections were made by inserting the small end of a Pasteur pipet into the exit port as the desired fraction emerged; in general, detector filaments were turned off briefly at this point to avoid pyrolysis on the hot wire detector.

Thin-layer chromatography was carried out on glass strips or plates coated with a 0.5-mm. thickness of Brinkmann silica gel G. The following solvents were used:

Solvent A. 1-Propanol-concd. ammonium hydroxide (7:3).

Solvent B. 1-Butanol-ethanol-concd. ammonium hydroxide (4:1:5).

Solvent C. Ethyl ether-petroleum ether-glacial acetic acid (20:5:1).

Solvent D. Chloroform-methanol (10:3).

The separated substances were detected by means of color, fluorescence under ultraviolet light, and response to any of a number of common reagents including iodine vapor, *p*-nitrobenzenediazonium fluoborate reagent, silver nitrate, and ferric chloride.

Vacuum sublimation was accomplished in a microsublimator (Ace Glass, Inc., Vineland, N. J.) equipped with a cold-finger condenser, electric heating block, and trap cooled with solid carbon dioxide. Pressure during the sublimations was reduced to less than 0.01 torr. Infrared spectra were measured in 1.5-mm. KBr pellets with a Perkin-Elmer Model 221 infrared spectrophotometer equipped with a 4X beam condenser. Melting points were measured in an Autemp block and were corrected.

Results and Discussion

Neutral Fraction E. Gas chromatography of this fraction indicated the presence of one major and at least five minor volatile constituents. However, the total amount of these was only about 100 μ g. from each 5 grams of irradiated 2,4-D, and at least a part of this quantity may have resulted from impurities in the ether employed as extraction solvent. They eluted at 200° to 260° C., while 2,4-D eluted at 210° C. under the same conditions. No attempt was made to identify the constituents of the neutral fraction at this time.

Phenolic Fraction F. Gas chromatography of this fraction revealed only a single volatile substance. The compound, collected from the chromatograph in crystalline form, was shown to be 2,4-dichlorophenol by comparison of its melting point, infrared spectrum, and chromatographic characteristics with those of an authentic specimen.

Thin-layer chromatography in solvent D resolved the phenolic fraction into nine components as detected in visible light or by their fluorescence under 254 $m\mu$ ultraviolet light. Repeated attempts to obtain these substances in pure form by elution from chromatoplates and subsequent crystallization were unsuccessful. For example, the compound at R_f 0.69 appeared yellow in visible light and exhibited a yellow-green fluorescence under ultraviolet light. It was eluted from the chromatoplate with ether, and the combined eluates from several plates were evaporated. In each instance, when the solution was con-

centrated to about 0.2 ml., it suddenly became dark brown and a strong phenolic odor was noted. Thin-layer chromatography indicated that almost all of the yellow substance had been converted to a dark brown, immobile material. Attempted diazomethane etherification prior to gas chromatography resulted in violent explosions when the diazomethane-free solution was injected into the hot inlet port.

When an aqueous solution of 2,4-dichlorophenol was irradiated and processed in a manner identical to 2,4-D, the corresponding phenolic fraction was found to exhibit exactly these same fluorescent and colored bands on thin-layer chromatography.

Acidic Fraction G. In addition to unchanged 2,4-D and a trace of 2,4-dichlorophenol, gas chromatography indicated that three other volatile substances were present in this fraction. The major one was collected from the chromatograph and recrystallized from water. Its melting point (110°; 127° C.), infrared spectrum, and elemental analysis (C, 47.6%, H, 3.4%) compared closely with those of 2-hydroxy-4-chlorophenoxyacetic acid (*d*) (m.p. 110°, 124-131° C. Calcd. for $C_8H_7ClO_4$: C, 47.42%; H, 3.48%). Comparison with an authentic specimen confirmed the identity.

Thin-layer chromatography of the acidic fraction in solvent C resulted in detection of eight fluorescent zones and seven iodine-absorbing zones (four of which coincided in R_f values). Treatment with *p*-nitrobenzenediazonium fluoborate reagent revealed a major reactive constituent at R_f 0.25 which, upon isolation, proved again to be the 2-hydroxy-4-chlorophenoxyacetic acid (m.p. 127-128° C.). Three other acids also were isolated; they melted at 122-123° C., 92-93° C., and 85-86° C., respectively. At present, their identities have not been established.

In those experiments which employed a chemical separation into fractions, addition of base always resulted in the formation of quantities of dark brown precipitate. This unsatisfactory result finally was circumvented by use of the second, low-pH procedure, and fractionation was achieved by sublimation as described in the next section.

Unfractionated Extract. Vacuum sublimation of the residue resulting from evaporation of the dried initial ether extract permitted 2,4-dichlorophenol to be collected in a dry-ice cooled trap. The phenol sublimed easily at about 30° C., and at least 50 mg. could be obtained from a single experiment. Identity was confirmed by melting point and infrared spectrum.

Between 45° and 60° C., a crystalline fraction was obtained which, upon thin-layer chromatography in each of solvents

A, B, and C, gave a spot corresponding exactly with that from authentic 4-chlorocatechol. Characteristic color reactions were produced with ferric chloride, *p*-nitrobenzenediazonium fluoborate and ammonium hydroxide, the latter was apparently due to formation of a characteristic purple ammonium salt. Examination of this fraction by both gas and thin-layer chromatography failed to indicate even traces of 2-chloro-4-hydroxyquinone.

At 80° C., vacuum sublimation provided a solid acidic fraction. Thin-layer chromatography with solvent A and spraying with *p*-nitrobenzenediazonium fluoborate reagent showed clearly the presence of both the 2-hydroxy-4-chlorophenoxyacetic acid, noted previously, and 2-chloro-4-hydroxyphenoxyacetic acid. However, the amount of this new product was so small, and it proved so difficult to elute from the adsorbent, that spectral confirmation of the structure was not obtained.

At 90° to 100° C., 2,4-D sublimed smoothly and was identified by melting point, infrared spectrum, and chromatographic characteristics. There remained in the sublimator a dark brown residue similar to, or identical with, that encountered after treatment of irradiated 2,4-D solutions with alkali. This material even precipitated during extractions at pH 2.5 and could be separated by filtration when the aqueous irradiation mixture was saturated with ether. On drying, it appeared chocolate-brown and amorphous.

The substance was soluble in dilute alkali and in ethanol but insoluble in water and nonpolar organic solvents. It softened with heat but did not melt; when exposed to flame, it burned with a shower of bright sparks. When chromatographed on a column of silicic acid

with a developing solvent composed of methanol-water-acetic acid (1:1:3), a black band remained at the origin while two brown bands were resolved on the column. The infrared spectrum of the apparently polymeric mixture was found to be very similar to that reported for a standard sample of humic acid prepared from 2-hydroxybenzoquinone (10) and to that of an authentic specimen prepared from 1,2,4-triacetoxybenzene (Figure 1). The near identity of this and the other properties of the substance with those reported for humic acids (10) indicated that the brown residue from photolysis experiments was indeed this type of polymer.

The fate of the aliphatic side chain remains in doubt. A specific search for glycolic, oxalic, and succinic acids was fruitless, and it is quite possible that such compounds would be photooxidized rapidly to carbon dioxide under these experimental conditions (7).

Inhibition of Oxidation. The facile conversion of 1,2,4-benzenetriol (as its acetate) to humic acids in dilute alkaline solution (Figure 2) suggested that its oxidation to hydroxybenzoquinone and subsequent polymerization did not involve photoenergized reactions. Inability to detect the anticipated 1,2,4-benzenetriol could be explained by its oxidative conversion to polymer as rapidly as it formed. To inhibit this oxidation, an irradiation experiment was conducted in which excess sodium bisulfite was present in the aqueous 2,4-D solution. Whereas the solution normally became colored upon irradiation, almost no discoloration was observed in this instance. After partial evaporation of water at the conclusion of the irradiation, the solution was extracted with ether, and the residue, after removal of the solvent, was acetylated by boiling

with excess acetic anhydride and subjected to temperature-programmed gas chromatography. The major product was isolated at 208° C., and its melting point and infrared spectrum were found to be identical with those of an authentic specimen of 1,2,4-triacetoxybenzene.

Sunlight Irradiation. The effects observed from irradiation in sunlight were qualitatively very similar to results from laboratory experiments. Comparison of thin-layer chromatograms showed the presence, in both instances, of identical bands. Although 2,4-dichlorophenol, 2-hydroxy-4-chlorophenoxyacetic acid, and several unknown substances appeared on both chromatograms, 4-chlorocatechol was not detectable as a photodecomposition product formed in sunlight. Humic acids appeared rapidly during sunlight irradiation; when the photodecomposition was conducted on wet filter paper, conversion to the polymer appeared to be essentially complete within a few minutes.

Conclusions

If the amount of recovered starting material is taken into consideration, all, or almost all, of the 2,4-D photolyzed in aqueous solution under laboratory conditions eventually is converted into humic acid. As demonstrated by Aly and Faust (2), the over-all photodecomposition rate of 2,4-D sodium salt in aqueous solution is fairly rapid (50% loss in 50 minutes at pH 7.0), while 2,4-dichlorophenol is even more photolabile (50% loss in 5 minutes at pH 7.0).

From the present evidence, a mechanism may be deduced for the photodecomposition of 2,4-D in dilute aqueous solution (Figure 3). The major reaction is cleavage of the ether bond to produce 2,4-dichlorophenol; this is then dehalogenated to 4-chlorocatechol and, presumably, to 1,2,4-benzenetriol under the influence of ultraviolet light. Dehalogenation of 2,4-D to 2-hydroxy-4-chlorophenoxyacetic acid also occurs to some extent, but very little 2-chloro-4-hydroxyphenoxyacetic acid is evident. Finally, the benzenetriol is rapidly air oxidized and polymerized to a mixture of polyquinoid humic acids by a light-independent process.

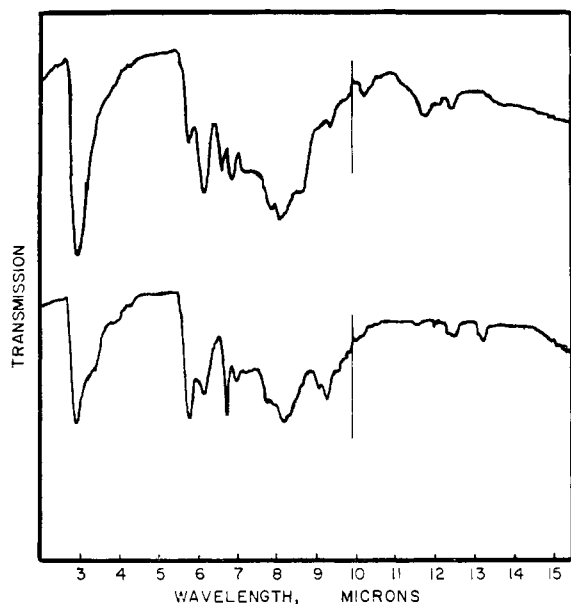


Figure 1. Infrared spectra of humic acids from triacetoxybenzene (upper line) and 2,4-D (lower line)

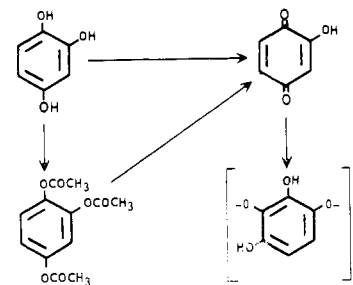


Figure 2. Conversion of 1,2,4-benzenetriol to humic acid

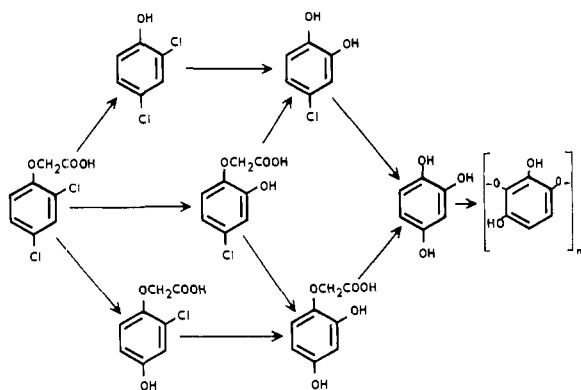


Figure 3. Proposed mechanism of 2,4-D photodecomposition

The reported decrease in pH of the 2,4-D solution upon irradiation (2) may be explained by the formation of 2 moles of hydrochloric acid for each mole of 2,4-D decomposed. Although one of these is neutralized by reaction with the sodium salt of the organic acid, the carboxyl group thus liberated also contributes to the total acidity. Furthermore, the apparent oxidation of the glycolic acid resulting from cleavage of the ether bond would provide further basis for the low observed pH if oxalic acid were assumed to be a final product.

Sunlight appears to produce many of the same qualitative effects as does ultraviolet light in the laboratory, although the number of detectable products is greater under the artificial illumination. The failure to detect 2,4-dichlorophenol may be due to rapid volatilization under outdoor conditions, or it may indicate that less energy is required for

replacement of halogen by hydroxyl than for rupture of the aralkyl ether linkage. However, the fact that humic acid also is observed in the sunlight-irradiated mixture suggests that the ether bond, too, eventually is broken. Based on the amount of recovered 2,4-D and 2,4-dichlorophenol, at least a significant part of the herbicide degraded by sunlight is converted to the brown polymer.

At very low initial concentrations of 2,4-D, such as those to be expected in polluted lakes and streams, the polymerization of 2-hydroxybenzoquinone probably would become much less important than combination with other substances dissolved in or contacting the solution. In its tautomeric form, 4-hydroxy-1,2-benzoquinone, the compound, also would be susceptible to a competing, ring-opening oxidation. However, the present experiments in-

dicating the general course of the photodecomposition of this widely used herbicide and demonstrate the formation of one type of insoluble end product, the polyquinoid humic acids.

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Literature Cited

- (1) Allan, A. O., "The Radiation Chemistry of Water and Aqueous Solutions," p. 117 ff., Van Nostrand, Princeton, 1961.
- (2) Aly, O. M., Faust, S. D., *J. Agr. Food Chem.* **12**, 541 (1964).
- (3) Bell, G. R., *Botan. Gaz.* **118**, 133 (1965).
- (4) Brown, G. P., McCall, E. B., *J. Chem. Soc.* **1955**, p. 3681.
- (5) Evans, W. C., Smith, B. S. W., *Biochem. J.* **57**, xx (1954).
- (6) Hansen, J. R., Buchholtz, K. P., *Weeds* **1**, 237 (1952).
- (7) Mitchell, L. C., *J. Assoc. Offic. Agr. Chemists* **44**, 643 (1961).
- (8) Payne, M. G., Fults, J. L., *Science* **106**, 37 (1947).
- (9) Penfound, W. T., Minyard, V., *Botan. Gaz.* **109**, 231 (1947).
- (10) Ziechmann, W., Scholz, H., *Naturwissenschaften* **47**, 193 (1960).

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HERBICIDES IN PEEL

A Heat-Labile Insoluble Conjugated Form of 2,4-Dichlorophenoxyacetic Acid and 2-(2,4,5-Trichlorophenoxy)propionic Acid in Citrus Peel

IN FLORIDA, dilute sprays of 2,4-dichlorophenoxyacetic acid (2,4-D) and 2-(2,4,5-trichlorophenoxy)propionic acid (2,4,5-TP) have been found to control preharvest fruit drop of midseason oranges. In an investigation of residues of these growth regulators in oranges, a portion of the peel was converted to citrus feed. Total growth regulator residues in this product were approximately three times higher than could be accounted for by the apparent residues in the fresh fruit.

In the commercial manufacture of citrus feed, following extraction of juice,

the peel is mixed with a small amount of lime, comminuted, and fed into a gas-fired dryer blast at approximately 600° F. The primary purpose of adding lime is to release water from pectin on the surface of the peel particles. The over-all pH of the peel is not significantly affected.

Nearly all of the residue occurs in the peel and since heating was the only major additional treatment the peel received, it was suspected that a form of the growth regulators normally not extractable was being liberated. A series of experiments was performed to investigate this apparent release of the growth

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regulator fractions under laboratory conditions.

Experimental

Methods and Materials. Samples of fresh citrus peel were prepared by compositing peel samples obtained from oranges from trees sprayed with either 20 p.p.m. (free acid basis) of 2,4-D isopropyl ester or the propylene glycol butyl ether ester of 2,4,5-TP 1 to 2 months prior to harvesting. The mixtures used varied in composition, so that actual residues reported in separate investigations are not directly comparable.