ISOTOPE-LABELED INSECTICIDES

Synthesis of Alpha-Methylbenzyl 3-(Dimethoxyphosphinyloxy)-Crotonate Labeled with Phosphorus-32 and Carbon-14

J. C. POTTER and W. B. BURTON
Shell Development Co.,
A Division of Shell Oil Co.,
Modesto, Calif.

The cis and trans isomers of alpha-methylbenzyl 3-(dimethoxyphosphinyloxy)-crotonate labeled with phosphorus-32 and carbon-14 have been prepared. The carbon-14 preparation was made by the exchange of methanol-C^{14} with the methoxy groups in trimethyl phosphite. The trimethyl-C^{14} phosphite was then reacted with α-methylbenzyl 2-chloroacetoacetate to form α-methylbenzyl 3-(dimeth-C^{14}-oxyphosphinyloxy)-crotonate. The phosphorus-32 preparation was made by the reaction of phosphorus-32 trichloride and methanol. The resulting trimethylphosphite-P^{32} was reacted to form α-methylbenzyl 3-(dimethoxyphosphinyl-P^{32}-oxy)-crotonate. The mixtures of cis and trans crotonates so prepared were separated from each other by columnar chromatography. The purity of the cis isomer was estimated to be 98 to 99%. The yield of the purified cis isomer was 32% in the carbon-14 preparation and 41% in the phosphorus-32 preparation.

ALPHA-methylbenzyl 3-(dimethoxyphosphinyloxy) - cis-crotonate (Ciodrin insecticide, trademark of Shell Chemical Co., a Division of Shell Oil Co.) controls a number of important insect pests when used as a spray on dairy cattle. To facilitate measurements of the residues of Ciodrin insecticide and its metabolites, the compound has been labeled with phosphorus-32 and carbon-14.

The reactions used in the synthesis are summarized in Figure 1. The first step in the preparation was the synthesis of trimethyl phosphite-P^{32} from phosphorus-P^{32} trichloride and methanol. N,N-diethylaniline was employed as the hydrogen chloride acceptor. This procedure is a modification of the one previously published (1, 3, 4, 9, 10). The crude trimethyl phosphite-P^{32} was reacted with potassium to destroy dimethyl hydrogen phosphite-P^{32} which was present as an impurity. The trimethyl phosphite was next reacted with α-methylbenzyl 2-chloroacetoacetate to form α-methylbenzyl 3-(dimethoxyphosphinyloxy)-crotonate.

Ciodrin insecticide labeled with carbon-14 can be prepared from methanol-C^{14} by the series of reactions indicated by route 1, Figure 1. Because the yields and purity obtained by this method were low, trimethyl-C^{14} phosphite was prepared by exchange with methanol-C^{14}, route 2, Figure 1. After the exchange reaction, the reaction mixture was treated with potassium and the trimethyl-C^{14} phosphite separated from the potassium methoxide-C^{14} by distillation. The trimethyl-C^{14} phosphite was reacted with α-methylbenzyl 2-chloroacetoacetate to form Ciodrin insecticide.

Alpha-methylbenzyl 3-(dimethoxyphosphinyloxy)-crotonate prepared as described above is a mixture of two geometric isomers as shown in Figure 2. Ciodrin insecticide is defined as the cis-crotonate or α-isomer.

The two isomers can be separated from each other by partition chromatography because their partition coefficients between immiscible polar and nonpolar solvents are different. The partition coefficients in various solvent systems are given in Table I. The ethylene glycol-

Route 1:

$$P^{32}Cl_3 + 3CH_3OH + 3B \longrightarrow$$

$$(CH_3O)_3P^{32} + 3B \cdot HCl$$

$$(CH_3O)_3P^{32} + CH_3\overset{\overset{O}{\|}}{C}-CHCl\overset{\overset{O}{\|}}{C}O\overset{\overset{H}{|}}{C}\underset{CH_3}{\big\langle\!\!\!\big\rangle}$$

$$\downarrow$$

$$(CH_3O)_2P^{32}\!\!-\!\!O-\overset{\overset{CH_3}{|}}{C}\!\!=\!\!CH\overset{\overset{O}{\|}}{C}-O-\overset{\overset{H}{|}}{\underset{CH_3}{C}}\!\big\langle\!\!\!\big\rangle$$

$$+$$

$$CH_3Cl$$

Route 2:

$$C^{14}H_3OH + (C^{12}H_3O)_3P \rightleftharpoons$$

$$(C^{14}H_3O)_3P + C^{12}H_3OH$$

$$(C^{14}H_3O)_3P + CH_3\overset{\overset{O}{\|}}{C}-CHCl\overset{\overset{O}{\|}}{C}O\overset{\overset{H}{|}}{C}\underset{CH_3}{\big\langle\!\!\!\big\rangle}$$

$$\downarrow$$

$$(C^{14}H_3O)_2P-O-\overset{\overset{CH_3}{|}}{C}\!\!=\!\!CH\overset{\overset{O}{\|}}{C}-O-\overset{\overset{H}{|}}{\underset{CH_3}{C}}\!\big\langle\!\!\!\big\rangle$$

$$+$$

$$C^{14}H_3Cl$$

Figure 1. Outline of synthesis

cyclohexane system gave the best separations.

The radiochemical purity and the proportion of cis and trans isomers were determined by paper chromatography. The chemical purity was determined by infrared analysis.

Experimental

Purification of Starting Materials.
N,N-DIETHYLANILINE. The diethylaniline was purified by the method described by Fieser (2) and stored in a glass-stoppered bottle sealed with paraffin wax.

METHANOL. The methanol was dried by the method of Riddich and Toops (7). The methanol-C^{14} was purchased from the Research Specialties Co., Richmond, Calif. It was used as received without further purification.

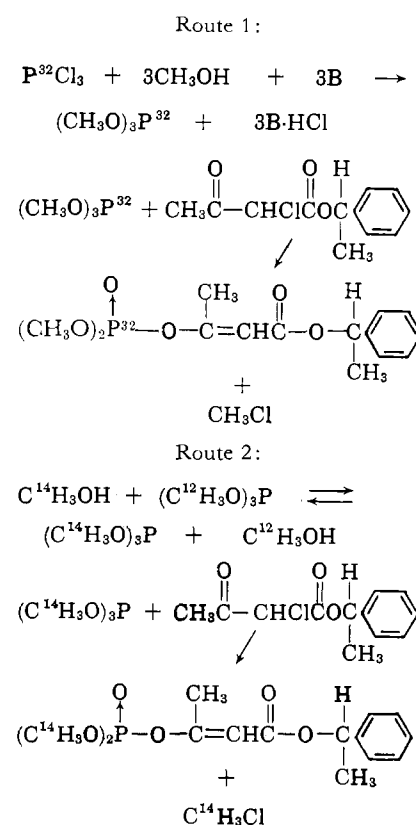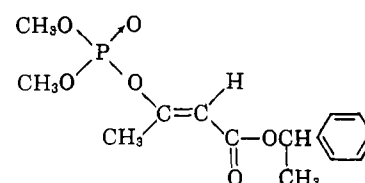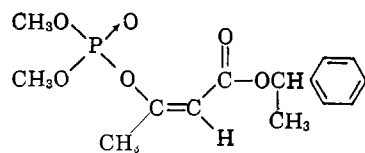PHOSPHORUS TRICHLORIDE. The phosphorus trichloride used in the trial

Alpha-methylbenzyl 3-(dimethoxyphosphinyloxy)-cis-crotonate

Alpha-methylbenzyl 3-(dimethoxyphosphinyloxy)-trans-crotonate

Figure 2. Cis and trans isomers

runs was purified by distillation through a 12-inch long Vigreaux column at a reflux ratio of five to one and stored in glass-stoppered flasks sealed with paraffin wax. Phosphorus-P³² trichloride was purchased from Volk Radiochemical Co., Chicago, Ill., and the New England Nuclear Corp., Boston, Mass., and used as received without further purification.

TRIMETHYL PHOSPHITE. The trimethyl phosphite was purified by distillation through a 20-bubble plate column from potassium at a reflux ratio of 10 to one. It was stored in glass-stoppered flasks sealed with paraffin wax.

RADIATION PROTECTION. Because of the radiation hazard from phosphorus-32, reaction flasks, storage vessels, etc., were shielded with 1 cm. of Lucite or 5 mm. of glass. Unshielded glass vessels containing phosphorus-32 were handled with long-handled tongs. All manipulations of carbon-14 and phosphorus-32 were carried out in vacuum manifold in a hood. Vapors from the manifold were collected in a liquid nitrogen trap.

Synthesis of Phosphorus-32–Labeled Preparation. A small, two-necked reaction flask, fitted with a rubber-sealed inlet, magnetic stirrer, and cold water condenser, was dried by continuous pumping on the vacuum manifold with a diffusion pump for several hours. Air was then admitted to the reaction flask. Next 1.0 gram (31.32 mmoles) of anhydrous methanol and 4.91 grams (32.88 mmoles) of N,N-diethylaniline were weighed out in a dry box and transferred to the reaction flask by means of hypodermic syringes. The flasks which had contained the methanol and N,N-diethylaniline were rinsed with phenylcyclohexane and the rinsings added to the reaction flask. The mixture of methanol, N,N-diethylaniline, and phenylcyclohexane was then cooled in an ice bath.

Next 1.43 grams (10.4 mmoles) of phosphorus-32 trichloride was diluted with 1 ml. of phenylcyclohexane and the solution taken up in a shielded syringe under anhydrous conditions. The needle

of the syringe was then inserted through the rubber seal into the reaction flask. The magnetic stirrer was started and the phosphorus-32 trichloride added to the reaction mixture over a period of 45 minutes. The mixture was then stirred for 30 minutes at 0° C., warmed to room temperature, and allowed to stand an additional 30 minutes. The reaction mixture was then cooled in liquid nitrogen, the reaction flask evacuated, and the trimethyl phosphite-P³² (TMP-P³²) distilled into a round-bottomed flask fitted with a side arm. A water-ice bath was then placed around the flask containing the TMP-P³² and dry air was admitted to the flask. Several small pieces (50 mg. each) of freshly cut potassium were added to the flask over a period of 1¼ hours. The TMP-P³² was then distilled into a graduated tube fitted with a stopcock and the volume of TMP-P³² measured. The weight of TMP-P³² was then calculated from its volume and the density (1.05 grams per ml.) of TMP.

One and one-tenth mmoles of α-methylbenzyl-2-chloroacetoacetate per mmole of TMP-P³² was weighed into a microreaction flask, fitted with a water-cooled cold finger. The flask was connected to the vacuum manifold and the TMP-P³² was distilled into the flask. Dry air was admitted to the flask and the mixture was then heated to 115° C. for 2 hours with a Glas-Col heating mantle. The temperature was controlled with a Celect-Ray temperature controller. After 2 hours, the mixture was topped at room temperature at 0.1-micron pressure.

Synthesis of Carbon-14–Labeled Preparation. Thirty-three mmoles of trimethyl phosphite were weighed into a round-bottomed flask. The TMP was treated with a few pieces of freshly cut potassium at room temperature, the flask was then connected to the vacuum

manifold and the TMP distilled into a two-necked reaction flask. Next, 8.3 mmoles of anhydrous methanol-C¹⁴ (specific activity 6.0 mc. per mmole) were distilled into the flask containing the TMP. After all the methanol-C¹⁴ had been added, the mixture was allowed to come to room temperature, and the flask removed from the manifold. The flask was then fitted with a spiral water condenser, drying tube, and cold trap, followed by another drying tube. The reaction mixture was heated to 95° C. with a Glas-Col heating mantle for 2 hours. The temperature was then increased to 105° C. and the mixture heated at this temperature for an additional 2 hours. The temperature was controlled with a Celect-Ray temperature controller. The apparatus was connected to the vacuum manifold, and the TMP-C¹⁴ and methanol-C¹⁴ were distilled into a flask with a side arm for potassium treatment. A slight residue remaining from the distillation was determined by infrared analysis to be 70% trimethyl phosphate, 20% dimethyl phosphite, and 10% dimethyl methyl phosphonate. The distilled TMP-C¹⁴ was then cooled in a water-ice mixture and freshly cut potassium added. One mmole of potassium was added per mmole of methanol-C¹⁴. The TMP-C¹⁴ was then distilled into a tared receiver tube fitted with a stopcock and weighed. The TMP-C¹⁴ was then reacted with α-methylbenzyl-2-chloroacetoacetate as described in the preparation of Ciodrin insecticide labeled with phosphorus-32.

Purification by Partition Chromatography. Two hundred to 240 grams of crushed firebrick (Johns Manville Silocel C-22 firebrick, 60–80 mesh) were weighed to the nearest gram, and ethylene glycol saturated with cyclohexane was then added to the firebrick in 10-

Table I. Partition Coefficients of the Cis and Trans Isomers

Polar Solvent	Hydrocarbon Solvent	Partition Coefficient[a]	
		Cis-crotonate[b]	Trans-crotonate
Ethylene glycol	Cyclohexane	0.49	0.23
Ethylene glycol	10%[c] benzene–90%[c] cyclohexane	0.93	0.44
Ethylene glycol	20%[c] Benzene–80%[c] cyclohexane	1.62	0.73
65% Water–35% ethanol	n-Hexane	2.81	1.01

[a] Partition coefficient = $\dfrac{\text{concentration in hydrocarbon solvent}}{\text{concentration in polar solvent}}$.

[b] Ciodrin insecticide.

[c] Per cent by volume.

Table II. Yields of Cis and Trans Isomers

Synthesis of P³²-Labeled Material

	Chemical Yields, %			
	Trimethyl phosphite[a]	Mixed isomers[b]	Cis-crotonate[b]	Trans-crotonate[b]
Trial 1P	62	98	..	..
2P	38	97	..	..
3P	26	96	..	..
4P	32	96	..	..
P³² preparation	70	95	74	21
Trial 5P	38	95	..	..
P³² preparation	40	87	67	20

Synthesis of C¹⁴-Labeled Material

	Chemical Yields, %		Radiochemical Yields, %	
	Trimethyl phosphite[c]	Mixed isomers[c]	Cis crotonate[d]	Trans crotonate[d]
Trial 1C	100	99	..	..
2C	97	97	..	..
C¹⁴ preparation	83	82	41	15

[a] Based on phosphorus trichloride.

[b] Based on trimethyl phosphite.

[c] Based on trimethyl phosphite before exchange reaction.

[d] Based on methanol-C¹⁴. See text for discussion.

Table III. Purity of Labeled Preparations

| | Proportion of Cis-Crotonate Isomer[a] (%) | |
	Before chroma-tography	After chroma-tography
P³² preparation	54[b]	98[b]
C¹⁴ preparation	64[c]	98.3[c]

[a] α-methylbenzyl 3-(dimethoxyphos-phinyloxy)-cis-crotonate.
[b] Determined by infrared analysis.
[c] Determined by paper chromatography.

ml. portions at a ratio of 0.95 ml. per gram of firebrick. The firebrick ethyl-ene glycol mixture was tumbled for 4 hours and then added in small portions to a glass column (2-cm. i.d.) fitted with a stainless steel needle valve at the bottom. The length of the packed section was 175 cm. Cyclohexane was then passed through the column for 12 hours at a rate of 400 ml. per hour. The sample of Ciodrin insecticide, not exceeding 4.0 grams, dissolved in 100 ml. of cyclohexane was added to the column. Twenty-milliliter fractions were collected. Aliquots of the fractions were counted in a liquid scintillation counter (Tracerlab CE-1). The appropriate fractions containing the cis or trans isomer were combined, and the solution was concentrated to about 50 ml. with a rotary evaporator and washed twice with distilled water. The washed solution was then dried with anhydrous sodium sulfate and the remainder of the solvent removed.

Paper Chromatography. The presence of radioactive impurities and their relative amounts in the preparations before and after partition chromatography were determined by paper chromatography. Isopropanol-concentrated ammonium hydroxide (2.5:1 v./v.) as the mobile phase and Whatman No. 1 paper were used to separate the polar impurities from the cis and trans isomers (6). Silicone-treated Whatman No. 1 paper as the stationary phase, and water-ethanol-chloroform (6:4:0.2 v./v./v.) were used to separate the cis and trans isomers (5).

The presence of radioactive spots on the paper chromatogram and the amounts of radioactivity were determined as follows. An autoradiogram of the paper chromatogram was first made. With the autoradiogram as a guide, the areas of the paper containing carbon-14 or phosphorus-32 were then cut out and counted with a Nuclear-Chicago gas flow GM counter.

The knowns were located by the ammonium molybdate reagent of Smith (8). Impurities were identified by co-chromatographing mixtures of 10 to 20 μg. of the radioactive samples and 50 to 100 μg. of a known sample.

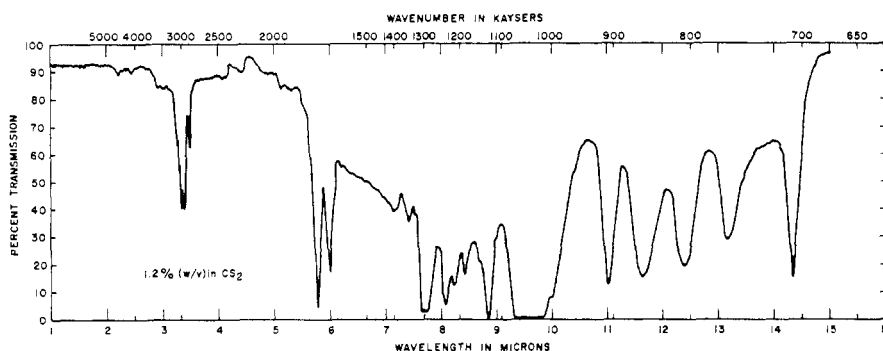Infrared analyses were performed by dissolving 10 μl. of the samples in 1 ml. of

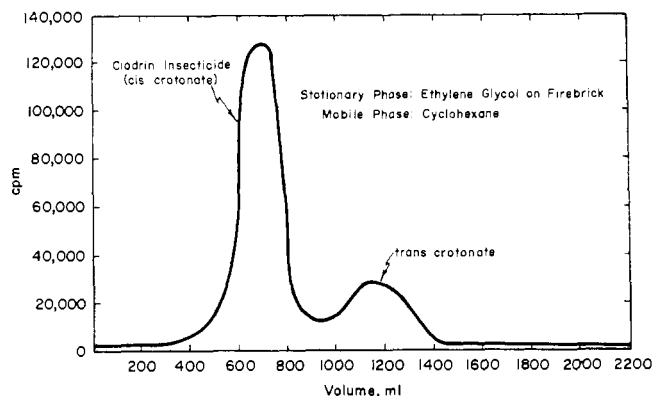Figure 3. Infrared scan of carbon-14 preparation

Figure 4. Partition chromatography of carbon-14 preparation

carbon disulfide. Infrared spectra of the solutions were made on a Beckman IR-4 spectrophotometer with a cell with 1-mm. pathlength. Chromatographi-cally pure samples of the cis and trans isomers were used as standards. The infrared scan of a typical preparation is shown in Figure 3.

Results

The yields without chromatographic purification of the cis and trans isomers and of the total isomers are given in Table II. The variable yield of trimethyl phosphite from phosphorus trichloride is believed to be caused by difficulties in mixing the phosphorus trichloride and methanol on a microscale. The low yield of the carbon-14 preparation is caused by the fact that one third of the carbon-14 is lost as methyl-C¹⁴ chloride.

The results of a typical columnar chromatographic separation of the cis-and trans-crotonate are shown in Figure 4. The separation of the two isomers is not complete. The cis-crotonate peak is symmetrical. The trailing of the trans-crotonate peak is believed to be due to a minor impurity. In two chromatographic separations, yields of the cis isomer were 80 and 78%. The losses are believed to be caused by catalytic decomposition of the organic phosphate by the crushed firebrick.

After chromatographic purification, the yield of the cis-crotonate isomer labeled with phosphorus-32 was 41%

based on phosphorus-P³² trichloride, and the yield of the cis-crotonate isomer labeled with carbon-14 was 32% based on methanol-C¹⁴.

The proportion of the cis-crotonate isomer before and after chromatography is summarized in Table III. As these data show, the columnar chromatography increases the purities of the isomers. The impurities in the sample before chromatography are α-methylbenzyl 3-(dimethoxyphosphinyl-oxy)-trans-crotonate, monomethyl hydrogen phosphate, dimethyl hydrogen phosphate, and a trace of the chloro analog of Ciodrin insecticide, α-methylbenzyl 2-chloro-3-(dimethoxy-phosphinyloxy)-crotonate.

The amount of exchange of the meth-anol-C¹⁴ with the methoxy groups can be estimated as follows. A total of 23.4 mc. of carbon-14 was found in the final preparation of the mixed isomers. Since one molecule of methyl-C¹⁴ chloride is produced for each molecule of the crotonate, and each crotonate molecule contains two methoxy groups, the number of millicuries of carbon-14 in the trimethyl phosphite must have been 23.4 × 1.5. The chemical yield of the alpha plus beta isomers was 83.4%, and 50 mc. of methanol-C¹⁴ were used. The per cent of the carbon-14 exchanged was therefore

$$\text{Exchange} = \frac{(23.4)(1.5)(100)}{(0.834)(50)} = 84.2\%$$

The proportion of carbon-14 in the trimethyl phosphite in a mixture of 33 mmoles of trimethyl phosphite and 8.3 mmoles of methanol at equilibrium is

$$\frac{(3)(33)(100)}{(3)(33) + (8.3)} = 92.3\% \text{ of the carbon-14}$$

This calculation is based on the assumption that all the methoxyl groups in the trimethyl are equivalent and that the isotope effect is negligible. The factor of 3 is in the above equation because each molecule of trimethyl phosphite contains 3 methoxyl groups.

The results obtained indicate that exchange was 91% of the equilibrium value.

Literature Cited

(1) Casida, J. E., Gotterdam, P. E., Getzin, L. W., Jr., Chapman, R. K., J. AGR. FOOD CHEM. 4, 236–43 (1956).
(2) Fieser, L. F., "Experiments in Organic Chemistry," 2nd ed., Part II, p. 382, D. C. Heath and Co., New York, 1941.
(3) Kosolapoff, G. M., "Organophosphorus Compounds," 1st ed., p. 184, Wiley, New York, 1950.
(4) Marshall, W. W. (to Monsanto Chemical Corp.), U. S. Patent 2,848,-474 (August 19, 1958).
(5) Metcalf, R. L., March, R. B., Science 117, 527 (1953).
(6) Plapp, F. W., Casida, J. E., Anal. Chem. 30, 1622 (1958).
(7) Riddich, J. A., Toops, E. E., Jr., in "Techniques of Organic Chemistry," Vol. III, 2nd ed., Interscience, New York, 1955.
(8) Smith, I., "Chromatographic Techniques, Clinical and Biochemical Applications," p. 162, Interscience, New York, 1958.
(9) Spencer, E. Y., Robinson, J. R., J. AGR. FOOD CHEM. 8, 293 (1960).
(10) Whetstone, R. R., Stiles, A. R. (to Shell Oil Co.), U. S. Patent 2,982,-686 (May 2, 1961).

INSECTICIDE FORMULATION

Petroleum Fractions as DDT Solvents

A. M. THOMAS, Jr.

Esso Research and Engineering Co., Linden, N. J.

The solubility of DDT in a variety of aromatic petroleum fractions used to prepare emulsifiable concentrates of pesticides has been measured. Both the low boiling, alkylbenzene-type solvents and high boiling, condensed ring aromatic solvents were investigated. These studies show that total aromatics content, molecular weight, and the structure of aromatic compounds determine solvency for DDT. The solubility of DDT decreases as the molecular weight of the alkylbenzenes increases for aromatic isomers of the same type of configuration. For the same molecular weight, the isomers with the ortho arrangement of substituted alkyl groups have the highest solvency for DDT. Naphthalenes are better solvents for DDT than alkylbenzenes. The solvency of pure aromatic compounds for DDT is best described using solubility parameter concepts. Solubility parameter, which considers the solvency effects of both solvent and DDT, qualitatively predicts DDT solubility much better than Kauri Butanol Value or Mixed Aniline Point. Solubility parameter can also be used to describe the solubility of other insecticides. However, additional development is required to make solubility parameter more of a quantitative measure and to provide means for estimating it correctly for commercial solvents.

THE USE of hydrocarbon solvents in the application of pesticides has increased greatly in the past 15 years. Solvents dissolve the pesticide along with an emulsifier to form an emulsifiable concentrate. This concentrate is mixed with water prior to application. In addition to dissolving sufficient quantities of insecticide for effective use, good solvents must also retain the insecticide in solution at low temperatures, since emulsion concentrates may be manufactured during cold weather or carried over from year to year stored in unheated warehouses. Since the solvent comes in direct contact with plants, it must be chosen so as to cause minimum plant damage. This can be accomplished by choosing a solvent with a relatively high evaporation rate so that the time of contact with the plant is minimized, or by choosing a solvent containing hydrocarbon types that have low phytotoxic activity. The solvent should also have reasonably low odor, good color, and a flash point above 80° F. to minimize flammability (5, 12).

The type of solvent required varies with the insecticide employed. While some toxicants are soluble in practically all petroleum solvents, others such as DDT require selected solvents such as aromatics or ketones. More DDT is produced annually than any other single insecticide. This large and growing use of DDT, coupled with the difficulty of dissolving it, makes specific knowledge of the solubility characteristics of this insecticide particularly important.

There has been a great deal of interest in determining which solvents are most suitable for use in the application of insecticides. Studies of the solubility of DDT (2, 8) in several pure chemicals and some petroleum fractions of varying aromatic content showed that nitrocyclohexane and cyclohexanone dissolve the most DDT. Paraffinic hydrocarbon fractions are very poor solvents. Aromatic hydrocarbon fractions are intermediate. Some other ketones are only slightly better than aromatics. Examination of the data on an economic basis (dollars worth of solvent per pound of DDT dissolved) shows that aromatic petroleum fractions are the most economical solvents for DDT.

Two types of aromatic petroleum solvents are available to insecticide formulators. The low boiling or alkylbenzene type has an initial boiling point of from 260° to 325° F. and a final boiling point no greater than 450° F.