**Table II. Recovery of Dimefox from Crops**

| Crop | Method | Dimefox, P.P.M. Added | Dimefox, P.P.M. Recovered | % Recovery |
|---|---|---|---|---|
| Potatoes (stored) | 1 | 0.75 | 0.57 | 76 |
| Potatoes (new) | 1 | 1.41 | 1.03 | 72 |
| Pineapples | 1 abbv. | 1.50 | 1.38 | 93 |
| Oranges (fruit) | 1 | 0.30 | 0.21, 0.22, 0.23 | 73 |
| Sugar mangolds (roots) | 1 | 0.95 | 0.92, 0.89 | 85 |
| | | 0.39 | 0.36, 0.37 | 94 |
| Mangolds (roots) | 1 | 0.93 | 0.69 | 80 |
| | | 0.67 | 0.59, 0.62 | 90 |
| | | 0.37 | 0.33, 0.35 | 92 |
| Cocoa beans | 2 | 200 | 144.0, 150.0, 150.0, 136.0 | 73 |
| | | 1.40 | 1.06, 1.06, 1.00 | 74 |
| | | 1.00 | 0.81, 0.76, 0.82, 0.68 | 77 |
| | | 0.20 | 0.20 | 100 |
| Coffee beans | 2 | 0.25 | 0.17 | 68 |
| | | 0.10 | 0.10 | 100 |
| | | 0.05 | 0.04 | 80 |
| Brussels sprouts | 2 | 1.00 | 0.73, 0.70 | 71 |

The accuracy of the final determination of phosphate limits the accuracy of any method to ±0.02 p.p.m. In only one instance are replicates not within ±5% of the mean.

have not been performed on all crops for which blanks were obtained.

Recoveries from different crops are probably significantly different. To obtain the accuracy required for research purposes, it would be necessary to find the percentage recovery more accurately than has been attempted here. It appears from the good reproducibility that this should not prove difficult. The authors have, however, used these methods only to assess the hazards to consumers of the crops. For this, high accuracy is not required, but it is essential that the results quoted should not be low. Therefore a recovery of 67% can be assumed, and the results multiplied by 1.5 will give an estimate of the dimefox. This is justified by the results in the table; no result is significantly below 70% and only one is significantly above 90%.

The methods described give satisfactory and reproducible recoveries and satisfactorily low blanks. The method which starts with macerating the sample in oil or glycerol does not, however, seem to be generally applicable, several crops giving high blanks if they are treated in this way. Distillation from oil is the least satisfactory in practice, because it is difficult to stop carry-over from the distilling flask to the condenser, and frothing is always a serious problem. This method may eventually be superseded entirely by methods using other solvents, but it is at present the best method for oily crops.


### Acknowledgment

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**PESTICIDE RESIDUES**

# Residual Properties of the Systemic Insecticide *O,O*-Dimethyl 1-Carbomethoxy-1-propen-2-yl Phosphate


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SUBSTITUTED-VINYL PHOSPHATES have been of considerable current interest to both entomologists and chemists because of their high insecticidal activity (*2, 5–7, 10, 11, 14, 20, 25–27, 32, 33, 36–38*). Their adaptability as insecticides may be somewhat limited by a mammalian toxicity in the range of parathion (*22–24, 27, 32*), whereas other organophosphate insecticides of lower mammalian toxicity have been recently introduced (*12, 13*). However, their very high insecticidal toxicity and the almost unique systemic properties of certain of these compounds may lead to commercially feasible substituted-vinyl phosphate insecticides.

*O,O*-Dimethyl 1-carbomethoxy-1-propen-2-yl phosphate (Compound OS-2046, Shell Development Co., Denver, Colo.) (*38*) has shown promise as a short residual systemic insecticide. Its high biological activity was first noted by Corey and others (*11*), and excellent systemic, contact, and fumigant insecticidal properties were demonstrated. Translocation studies reported by the same workers (*10*) indicated that the toxicant entered the plant to the full extent in the early minutes after application and was rapidly translocated throughout the plant. They proposed that the significant reduction in residues within 24 hours following application was a function of the volatility of the compound.

The studies reported here utilized bioassay, antiesterase, radiotracer, and chromatographic determinations to evaluate further the residual properties of compound 2046.

O,O-Dimethyl 1-carbomethoxy-1-propen-2-yl phosphate (compound 2046) offers considerable promise as a short-residual systemic insecticide. The potential hazard of residues in crop plants was investigated. Compound 2046 consists of about two thirds cis and one third trans isomers. The cis is about 100 times more toxic than the trans to insects and mammals. Despite the greater residual persistence of the trans isomer within the plant, its residual hazard was negligible compared to the less stable but more toxic cis material. The initial enzymatic attack on both isomers within the plant appeared to be on the carboxylic ester group, followed by hydrolysis of the vinyl phosphate bond. Foliage application to vegetable crops in the field resulted in a 90% residual loss in less than 2 days and over 99% loss in 4 days based on anticholinesterase determinations. The toxic 2046 residues in crop plants treated at dosage levels used for insect control were essentially dissipated within 2 days following insecticide application.

### Methods

**Synthesis of Radioactive *O,O*-Dimethyl 1-Carbomethoxy-1-propen-2-yl Phosphate.** Red phosphorus-32 (service irradiation at Atomic Energy Commission, Oak Ridge, Tenn.) was chlorinated to yield phosphorus-32 trichloride (*16*, *35*). This was converted to trimethyl phosphite-32 (*3*), which when added to methyl 2-chloroacetoacetate (*38*) yielded a pale yellowish orange technical radioactive 2046 in 33% overall yield with a specific activity of 2.6 millicuries per gram. Partition chromatography of this technical product with silica gel and carbon tetrachloride yielded 66% of the $\alpha$ fraction or cis isomer and 34% of the trans isomer (*7*). The identical composition of the radioactive fractions with those from the industrial sample was indicated by the same isomeric ratio in the two preparations and ascertained by identical infrared spectrograms, anticholinesterase activity, and behavior on chromatography.

**Radioactive Measurements.** Radioactive determinations were made with a Geiger-Müller counter, using the whole tissue where samples of less than 10 mg. were involved or counting an aliquot of an aqueous homogenate (*34*) with larger plant samples. Radioautograms of treated plants were made by exposing Kodak Blue Brand x-ray film with the radioactive plant for 24 hours prior to developing and comparing the developed films from radioactive-treated and control plants.

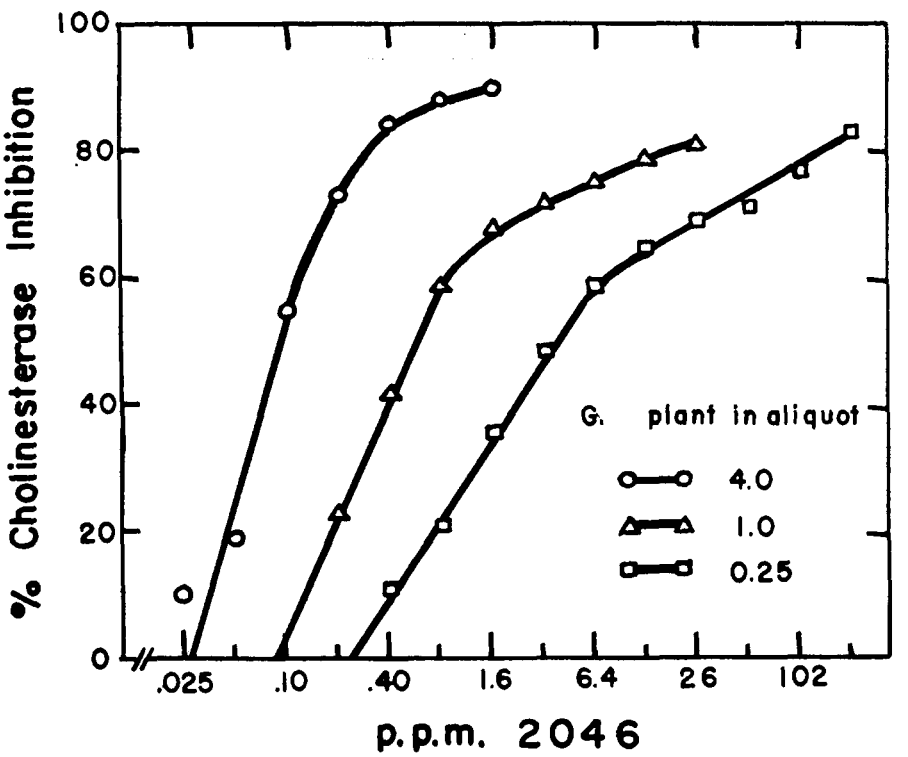
**Anticholinesterase Determinations.** Anticholinesterase determinations were made by a modification of the method of Hensel and others (*19*). The plant tissue was homogenized in cold water and adjusted to pH 8.0, and immediately serial dilutions of the brei (2.0-fold dilution factor) were added to 30% whole human blood at pH 8.0 and incubated for 2 hours at 38° C. in order to allow the 2046 to combine with the esterase. Following this preincubation of the inhibitor and enzyme together, an aliquot of the blood was added to an acetylcholine solution in a barbiturate-phosphate buffer at pH 8.0 and allowed to incubate for an additional 2 hours. The change in pH due to acetic acid formation from acetylcholine hydrolysis was then determined with the Beckman Model G pH meter. Human plasma was found to contain enzymes other than cholinesterases, which readily hydrolyze and thus detoxify both the *cis*- and *trans*-2046. Accordingly, the method requires careful standardization, since the measurement is based on competition of plasma esterases to decompose 2046 and the combination of the residual 2046 with the blood cholinesterases.

A standard curve for technical 2046 in the presence of varying amounts of pea tissue is shown in Figure 1.

**Bioassays.** Systemic bioassays for studying the rate of *cis*-2046 decomposition (Table I) were made with Perfection variety pea plants 2 to 4 inches tall and fourth instar pea aphids, *Macrosiphum pisi* (Harr.). Twenty pea aphids on each of four replicates per treatment were used for these time-mortality determinations. Where systemic $LD_{50}$ values were approximated, a 2.0-fold dilution factor was used with two replicates of 10 aphids each per dilution. In the residual study on peas and cabbage (Table IV), 30 aphids were confined in lantern chimneys on two treated plants with three such replicates per treatment. The per cent mortality was recorded 24 hours after the aphids were introduced. The cabbage aphids used were *Brevicoryne brassicae* (L.).

Acute mammalian toxicity was determined by intraperitoneal injection into adult white rats (150 to 200 grams) and

Figure 1. Standard curve for determination of 2046 residues in peas by anticholinesterase assay



the results served only as an approximation of the $LD_{50}$ value, as the dosages assayed differed by a factor of 2.0. The contact toxicity to houseflies was determined by topical application to 4-day-old adult female *Musca domestica* L. (see Table V). The insecticide was applied in 1.3 μl. of acetone using a twofold factor between the various dosages; 24-hour mortality counts were made on the three replicates of 10 flies each.

**Use of Partition Coefficients to Determine Stability of 2046 to Hydrolysis.** Compound 2046 partitions over 97% into an equal volume of chloroform, but after alkaline hydrolysis the phosphorus from 2046 appears completely in the aqueous layer. The hydrolyzed material would presumably be a salt of *0,0*-dimethyl phosphoric acid. This solubility difference was used to determine the 2046 as differentiated from its more polar degradation products. Partitioning properties of compounds or biological fractions were routinely determined by extracting 2.0 ml. of water with 2.0 ml. of chloroform or other organic solvent in a 15-ml. centrifuge tube at 28° C. Following separation of the layers (centrifugation often necessary with biological materials) a 1.0-ml. aliquot of each layer was analyzed for total phosphorus colorimetrically (*8*) or radiometrically.

The figures for the hydrolytic half life in days at the pH of the plant sap (see Table II) were based on a pH-stability curve established at 28° C. by determining loss in partitioning properties of technical 2046 into chloroform as the phosphorus was converted to an ionized form on hydrolysis. The half life of the vinyl phosphate bond to hydrolysis for the materials reported in Table V was determined at 28° C. and pH 11.6 (0.1*M* sodium carbonate) and based on the loss of partitioning properties into chloroform.

**Volatility Half-Life Determinations.** The volatility of the vinyl phosphates (Table V) was based on the loss of phosphorus-containing materials at 28° C. from a coarse filter paper to which there had been applied in acetone the organophosphate with 1.0 mg. impregnating an area of about 4 sq. cm. The initial rapid loss for the first 4 hours was discarded and volatility curves were plotted from the more uniform period of loss from 4 to 24 hours.

**Field Application of 2046.** Technical 2046 was formulated in water with 1 ml. of emulsifying agent (provided by the Shell Development Co. for use with 2046) per gallon of water. The insecticide was used at 1.8 ml. per gallon for the 0.25-pound-per-acre treatments and 7.2 ml. per gallon for the 1-pound-per-acre treatment. This material was applied at the rate of 50 gallons per acre with a knapsack sprayer. For the foliage application three swaths were made with the largest vegetables (corn, tomatoes, cucumbers, and potatoes), with two swaths for the beans and radish, and a single swath for the remaining plants because of their smaller size. The material adhered very poorly to the onions, possibly accounting for the very low initial residue observed. For the soil application, the nozzle was removed and the insecticide solution dribbled on the ground near the base of the plants, with care not to touch the stems or leaves of the plants with the insecticide solution. With transplant water treatment of cabbage, a quarter pint of solution was added to the soil with each plant.

Plants were treated on July 22, 1954, in a single large plot in the Kenosha vegetable growing area of Wisconsin. The 3 days following application were sunny and warm (mean temperature 70° F.), a 2.5-inch rain occurred on the fourth day, and the remainder of the experimental period was sunny and warm. At the time of application, there was some dew on the plants. All plants were young and actively growing, with the exception of the cabbage which had just been transplanted, the spinach which was near to the blossom stage, the pea which was just starting to form pods, and the radish on which seeds had already formed.

About 200 grams of plant leaves and stem were cut at the indicated interval following treatment, quick frozen, and held at −20° C. for subsequent analysis. Duplicate anticholinesterase determinations were made on all samples within the week and the results compared to standard

**Table I. Decomposition of *cis*-2046 in Pea and Bryophyllum Plants**

| | Perfection Peas | | | | | Bryophyllum-P[23] | |
|---|---|---|---|---|---|---|---|
| Days | Hr. for 50% aphid kill | γ/G. in dead aphids (2046 + decomp. prod.) | P.p.m. 2046 AntiChE | P.p.m. 2046 $P^{32}$ | % 2046 undecomposed[a] | P.p.m. 2046 | % 2046 undecomposed[a] |
| 0[b] | 0.14 | < 0.3 | 24 | 25 | 22 | 154 | 79 |
| 0.5 | 0.53 | < 0.3 | 22 | 14 | 19 | 118 | 72 |
| 1.0 | 1.07 | < 0.3 | 12 | 8.3 | 14 | 107 | 70 |
| 2.0 | 2.16 | 1.0 | 2.2 | 5.3 | 9.5 | 79 | 44 |
| 3.0 | 2.42 | 1.0 | 2.0 | 2.9 | 6.3 | 74 | 46 |
| 5.0 | 10.2 | 14.2 | 0.13 | 1.1 | 3.6 | 28 | 33 |
| 7.0 | 24.0 | 18.2 | 0.09 | 0.08 | 0.4 | 15 | 23 |

[a] Obtained by dividing $P^{32}$ in plant due to 2046 by total $P^{32}$ in plant and multiplying by 100.
[b] At this time 2046 source was removed from roots after 24-hour absorption.

**Table II. 2046 Residues on Vegetable Foliage Following Field Treatment with 0.25 Pound per Acre (Anticholinesterase Assay)**

| Plant | Av. pH[a] Plant Sap | Hydrolytic Half Life, Days at pH of Plant Sap | Initial P.P.M. | Days until % Residue Loss Indicated[b] 90% | 99% |
|---|---|---|---|---|---|
| Bean | | | | | |
| Lima | 5.9 | 8.8 | 3.3 | 1.4 | 4.2 |
| Snap | 6.1 | 7.5 | 2.9 | 1.3 | 4.0 |
| Beet | 6.7 | 4.2 | 4.2 | 2.5 | 3.6 |
| Cabbage | 6.0 | 7.9 | 2.6 | 1.1 | 3.2 |
| Carrot | 6.2 | 7.1 | 2.9 | 0.8 | 3.2 |
| Corn | 6.0 | 7.9 | 0.9 | 1.8 | [c] |
| Cucumber | 7.9 | 0.7 | 0.6 | 1.7 | [c] |
| Onion | 6.3 | 6.2 | 0.8 | 1.4 | [c] |
| Pea | 6.3 | 6.7 | 0.5 | 1.6 | [c] |
| Potato | 5.8 | 9.2 | 5.1 | 2.2 | 3.9 |
| Radish | 6.0 | 7.9 | 2.6 | 4.2 | 8.0 |
| Spinach | 6.6 | 4.6 | 4.8 | 1.7 | 3.8 |
| Tomato | 6.0 | 7.9 | 9.6 | 0.8 | 1.6 |
| Av. | | | 3.1 | 1.7 | 3.9 |

[a] Based on 10 readings of tissue brei prior to adjusting pH for assay.
[b] Estimate based on graphing logarithm of residual versus time on arithmetic scale.
[c] Initial residue too low to estimate time of 99% residual loss.

**Table III. Residual Properties of 2046 in Cabbage and Snap Bean Following Application in Field (Anticholinesterase Assay)**

| | | P.P.M. Residue after Days Indicated | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Plant | Treatment | 0 | 0.5 | 1.0 | 2.0 | 4.0 | 8.0 | 16 |
| Cabbage | 1/4 lb. foliage | 2.6 | 2.8 | 0.29 | 0.06 | <0.04 | <0.04 | <0.04 |
| | 1/4 lb. soil | 0.56 | 1.6 | 0.07 | <0.04 | <0.04 | <0.04 | <0.04 |
| | 1/4 lb. transplant water | 1.8 | 9.3 | 4.3 | 2.0 | 0.88 | 0.07 | <0.04 |
| Snap beans | 1/4 lb. foliage | 2.9 | 1.1 | 0.50 | <0.04 | <0.04 | <0.04 | <0.04 |
| | 1 lb. foliage | 9.9 | 4.7 | 1.0 | 0.44 | 0.07 | <0.04 | <0.04 |
| | 1/4 lb. soil | 1.7 | 0.60 | 0.38 | 0.09 | <0.04 | <0.04 | <0.04 |
| | 1 lb. soil | 3.2 | 2.9 | 1.9 | 0.10 | <0.04 | <0.04 | <0.04 |

curves prepared by adding known amounts of 2046 to untreated plant materials from the same field plot. The times to achieve 90 and 99% residual loss were determined by plotting the logarithm of the residues at 0, 0.5, 1.0, 2.0, 4.0, 8.0, and 16 days against the time after application on an arithmetic basis. Such curves were nearly linear after a rapid initial loss, apparently due to volatilization. These field residue results are given in Tables II and III.

### Experimental

**Distribution and Decomposition of 2046 in Pea Plant.** A group of 4-inch-tall pea plants was allowed to absorb *cis*-2046-$P^{32}$ for 12 hours by immersing the roots in a 1 to 1000 solution. The total amount of 2046 absorbed was almost twice as great after a 12-hour root exposure as after a 6-hour period. After 12 hours the peas were transplanted in white sand, with no further source of insecticide. Plants were removed after the appropriate intervals, homogenized, and extracted with chloroform to separate 2046 from decomposition products, and an aliquot was taken for radioactivity determination. The total radioactivity declined, apparently because of volatilization or excretion of some organophosphorus materials from the plant. The proportion of phosphorus-32 appearing in the different plant regions is shown in Figure 2. It is evident that during the period of effective insecticidal toxicity there is a continual redistribution of 2046 toward the actively growing leaf and tip regions.

Radioautographs showed a marginal leaf accumulation of 2046 following root absorption by peas, cucumbers, and bryophyllum.

When the percentage of phosphorus-32 in the pea plant remaining as undecomposed *cis-0,0*-dimethyl 1-carbomethoxy-1-propen-2-yl phosphate (chloroform-extractable) was studied, the decomposition curve shown in Figure 3 was obtained. Each point in the graph represents three replicates of six plant parts, as there was no significant difference between plant parts in decomposition rate. The kinetics of decomposition are characteristic of a second-order reaction. The half life of *cis*-2046 in these 4-inch-tall pea plants where loss by volatilization was experimentally eliminated was about 1 day.

**Loss of 2046 from Treated Peas and Bryophyllum Plants.** Groups of young pea and bryophyllum plants were allowed to absorb *cis*-2046-$P^{32}$ from a 1 to 1000 solution through the roots for 24 hours and then transplanted into insecticide-free white sand. After the appropriate time interval, aphids were placed on the pea plants for bioassay and as soon as the mortality was about 90%, the plants were removed and assayed for

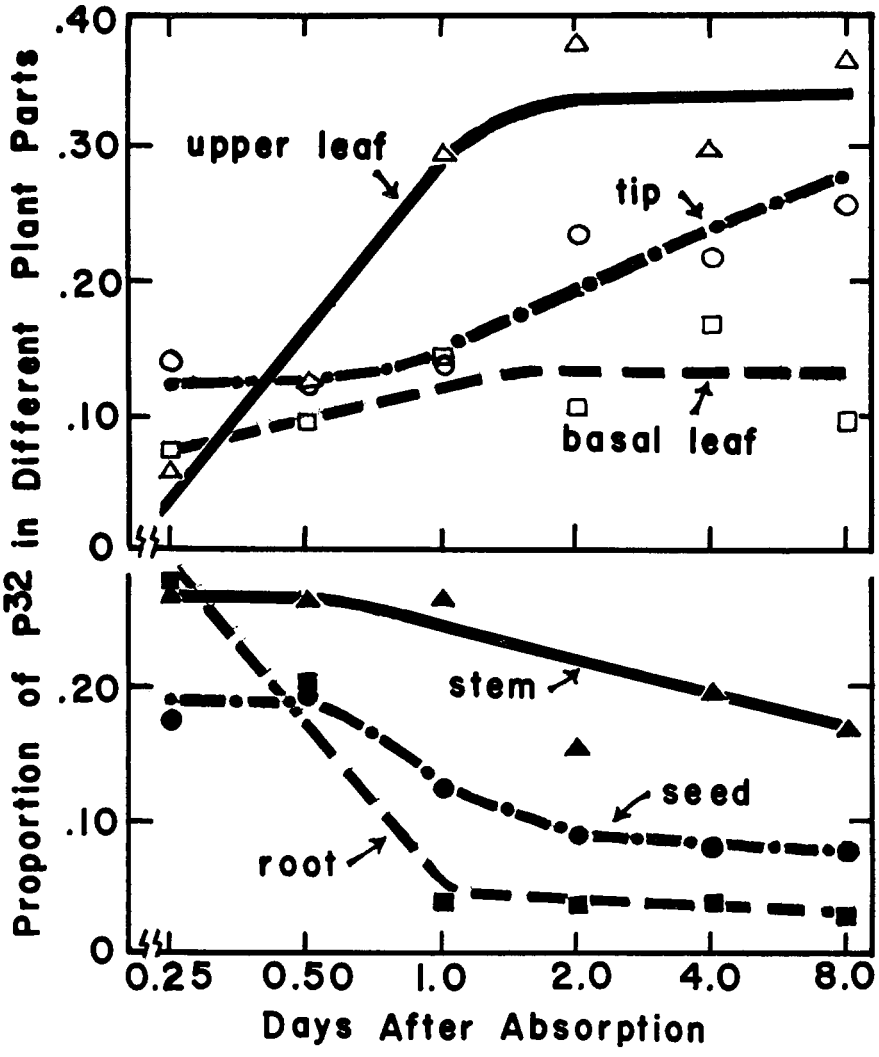



Figure 2. Redistribution of phosphorus-32 in young pea plants following root absorption of *cis*-2046-$P^{32}$

2046 by radiotracer and anticholinesterase analyses. The results (Table I) show that 2046 decomposed more rapidly in pea than in bryophyllum plants. Both 2046 and some decomposition products either volatilized or were excreted from the plants, but the 2046 was lost much more rapidly than its phosphorus-containing decomposition products. Immediately after treatment, the volatility loss of 2046 appeared to be the predominant method of loss, but after about a half day, the more significant factor was the decomposition within the plant.

The rate of residual loss of 2046 from plants based on radiotracer and anticholinesterase methods was similar to that based on aphid bioassay. These data indicate that 2046 acts itself as a systemic insecticide and that metabolism within the plant is not required to activate this chemical. Further evidence of this was obtained by introducing 2046 into a variety of biological systems, all of which either left the 2046 unaltered (based on solubility characteristics) or lowered its anticholinesterase activity. Similarly chemical alteration with halogens, hydrohalogens, and various oxidizing agents seemed only to reduce the anticholinesterase activity. Heat effected a thermal decomposition to a lower boiling vinyl phosphate or formed polymers. Prolonged exposure to intense ultraviolet light effected an isomerization of cis to trans and trans to cis forms.

**Relative in vivo Decomposition Rates for cis and trans Isomers of 2046.** The stability of *cis*- and *trans*-2046 was studied with radioactive materials in young bean, corn, cucumber, and pea plants. Degradation of 2046 was more rapid in beans than in the other three crops, which showed almost identical degradation rates. The trans isomer, being more stable to alkaline hydrolysis, was also more stable to decomposition within the plant. Figure 4 shows the relative rates of cis and trans loss due to degradation and volatility from pea and bean plants, together with the loss of total radioactive phosphorus. The loss of total phosphorus was essentially the same for both isomers, and so the results for the two isomers were averaged before plotting. Figure 5 shows the average

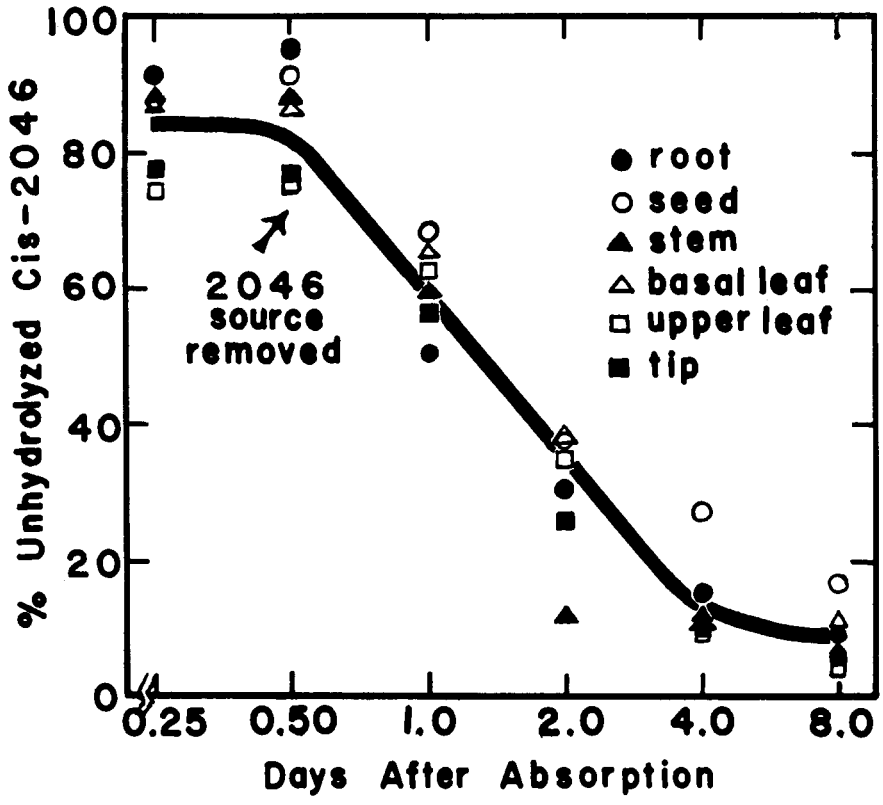


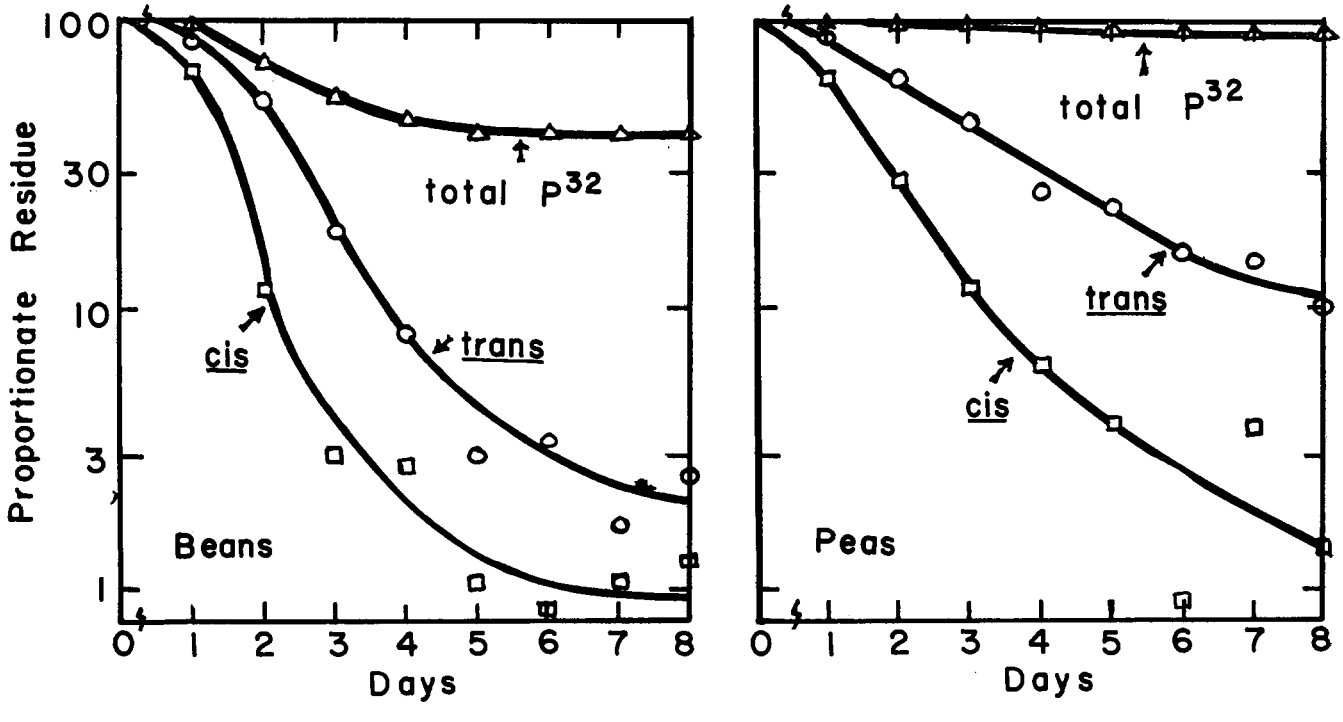

Figure 3. In vivo decomposition rate of *cis*-2046 in different parts of pea plant

residual picture which appeared with the isomers in the four crops along with the half life and fifth life of the isomers under these conditions. The isomers are of essentially the same volatility (Table V) and were apparently absorbed by plants at about the same rate (based on relative rates of uptake from nutrient solutions). Therefore application of the technical material would yield an initial isomer ratio of 2 parts of cis per part of trans in the plant. The ultraviolet of sunlight would not appear to be sufficiently intense to isomerize (7) any of the material on the surface of the plant before absorption, owing to the volatility of the isomers. However, if isomerization did occur it would probably tend to increase the amount of trans isomer at the expense of the cis form. Once in the plant, the cis decomposed more rapidly than the trans, so that in less than 2 days there was more trans than cis, and after 6 days there were over 3 parts of trans per part of cis isomer remaining in the plant.

Attempts to detect a shifting of isomers within the plant proved unsuccessful. Based on column chromatography and infrared analysis of plants during the first 3 days after treatment, there was merely a selective decomposition of the cis isomer rather than a transformation of isomers within the plant. Whether the trans isomer might over longer periods of time have served as a reservoir for the formation of the more toxic and less stable cis form is not known, but the hydrolytic instability of these materials makes a conversion of isomers within the plant appear unlikely.

**Residual Properties of 2046 in Field on Vegetable Crops.** Following foliage treatment with 0.25 pound per acre, the vegetables in the field had an average initial residue of 3.1 p.p.m. (Table II). A 90% residual loss occurred in less than 2 days, and less than 1% was left on the average after 4 days. An apparent longer residual persistence on radish could possibly be explained by the low relative physiological activity of these plants, upon which seeds had already been formed. The results with different application methods (Table III) show that even at a higher dosage level, the residues are essentially eliminated in 4

Figure 4. In vivo decomposition of *cis*- and *trans*-2046 in pea and bean plants



days, with the single exception of the cabbage transplant water treatment.

**Comparative Residues of Organophosphorus Insecticides in Greenhouse Based on Aphid Bioassay.** The substituted-vinyl phosphorus insecticides 2046 and Shell compound 1808 (*0,0*-diethyl 1-carbethoxy-1-propen-2-yl phosphate) were compared by bioassay for residual properties in the greenhouse with other systemic insecticides and three contact organophosphates. The two vinyl phosphates offered the shortest residual protection of the systemic materials, whether applied on the foliage or in the soil (Table IV). Both materials were ineffective following the first day with soil application and after the second day with foliage application. By treating cabbage and pea seeds by soaking in 2046 solutions or by coating with 2046 in charcoal, the residual action could be extended so that 100% aphid control was still effected after about 10 days in the greenhouse.

### *Mechanism of 2046 Degradation in Plants*

Several mechanisms must be considered for 2046 decomposition in plants. Residue losses are due to enzymatic attack which ultimately forms materials not extractable from water by chloroform. The rate of degradation in plants is too rapid to be accounted for by nonenzymatic cleavage of the vinyl phosphate bond (see Table III). Furthermore, enzyme preparations of plant mitochondria and human serum were found to form more polar derivatives from the 2046 isomers.

Enzymatic oxidation of vinyl groups is known to occur, and the substituted vinyl phosphates are very susceptible to oxidation, the cis isomer being more susceptible than the trans (*7*). Oxidation of

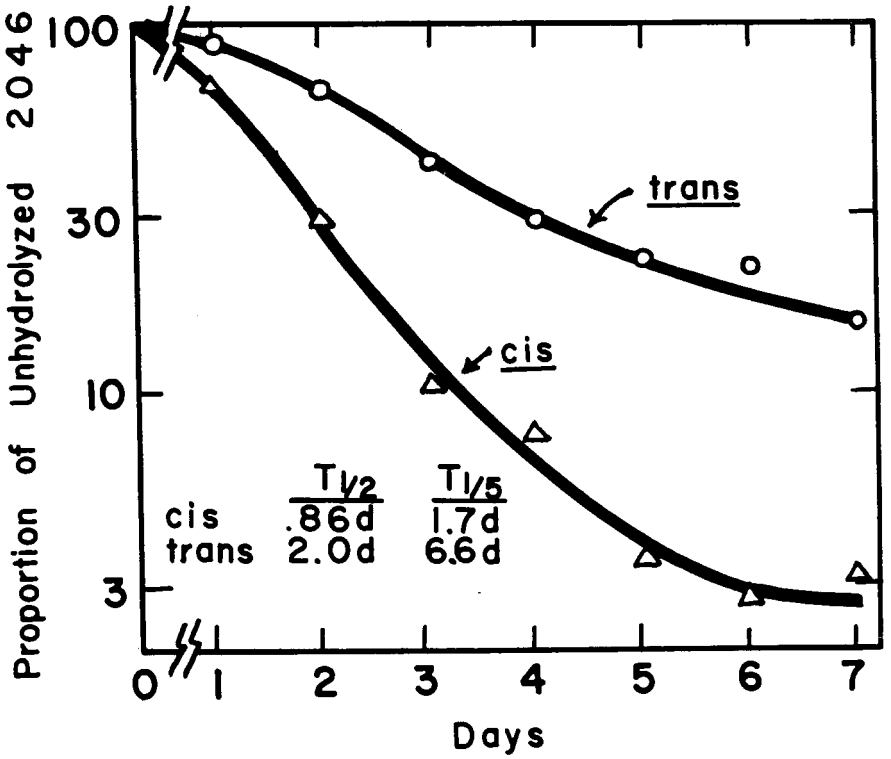



**Figure 5. Average in vivo decomposition curves for *cis*- and *trans*-2046 in beans, corn, cucumbers, and peas**

2046 under a variety of conditions with permanganate, peracetic acid, and hydrogen peroxide yielded only less active anticholinesterase agents in the reaction mixtures. No evidence was obtained of oxidation intermediates which would partition into chloroform, as the chloroform-soluble materials from the reaction mixtures proved to consist only of residual *cis*- and *trans*-2046 based on infrared spectrograms. Contrary evidence to an enzymatic oxidation hypothesis appeared from the demonstration within 2046-treated plants of materials other than 2046, which yielded acetone on oxidation with dichromate (*17*). An enzymatic oxidation to form an epoxide structure or to cleave the double bond would yield only derivatives which would not hydrolyze to form acetone. On analogy with acetoacetic acid metabolism (*39*), a hydrolytic mechanism with or without a decarboxylation appears more likely than an oxidative degradation.

Possible sites of hydrolytic attack are at the phosphate methoxyl, the carboxylic ester methoxyl, and the vinyl phosphate bond as follows:

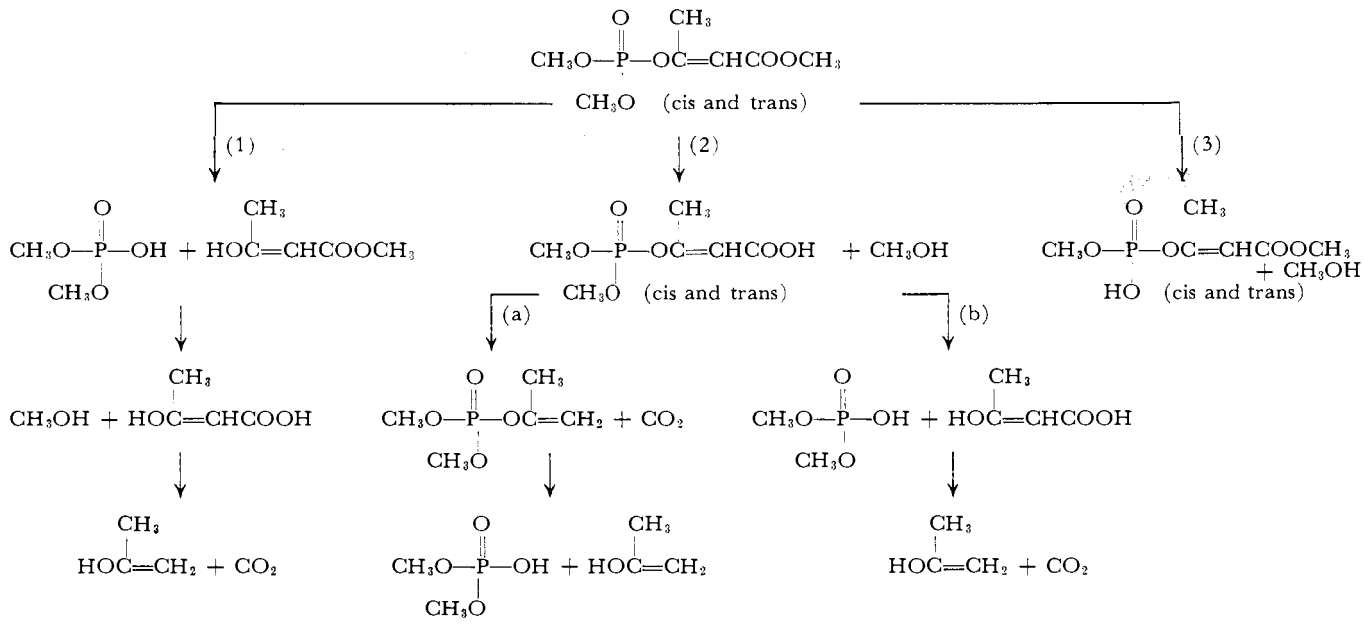


**Table IV. Residual Properties of Various Contact and Systemic Organophosphorus Insecticides on Cabbage and Peas in Greenhouse**

| Insecticide | Dilution Actual Toxicant[a] | Effective Residual Period[b] | | | |
|---|---|---|---|---|---|
| | | Soil Applic. | | Foliage Applic. | |
| | | Cabbage | Peas | Cabbage | Peas |
| Systemic | | | | | |
| Shell 2046 | 1:8000 sol. | < 1 | 0.8 | 1.3 | 1.5 |
| Shell 1808 | 1:8000 sol. | < 0.5 | 0.5 | 1.6 | 0.8 |
| Demeton | 1:8000 emul. | 21 | 29 | 6.7 | 3.4 |
| Metasystox | 1:8000 emul. | 4.5 | 15 | 2.7 | 4.7 |
| Am. Cyan. 12008 | 1:4000 emul. | 50 | >48 | 2.7 | 3.6 |
| Schradan | 1:4000 sol. | 1.2 | 50 | 1.4 | 1.4 |
| Isolan | 1:4000 sol. | 40 | >48 | 2.1 | 5.0 |
| Contact | | | | | |
| TEPP | 1:8000 sol. | ... | ... | 0.6 | 0.5 |
| Parathion | 1:8000 emul. | ... | ... | 3.1 | 2.8 |
| Malathion | 1:2000 emul. | ... | ... | 1.1 | 0.8 |

[a] This concentration was used for soil application with twice this concentration used for foliage application.
[b] Defined as number of days following treatment that 50% or greater aphid kill resulted in 24 hours following placement of aphids on plants.

The end products of the first two degradation routes would be dimethyl phosphoric acid, acetone, carbon dioxide, and methanol. With the initial cleavage of the vinyl phosphate bond (route 1), methyl acetoacetate and acetoacetic acid might appear as intermediates; whereas if the carboxylic ester were to hydrolyze first (route 2), there might be certain other vinyl phosphates formed as intermediates. Therefore the demonstration of a substituted-vinyl intermediate in the decomposition would eliminate the first route. Both carboxylic ester and vinyl phosphate groups should be less stable to hydrolysis in cis than in trans forms.

Repeated attempts were made to differentiate between these routes by selective degradation and colorimetric acetone analysis (*17*), but 2046 readily formed acetone under these colorimetric reaction conditions and a method of adequately differentiating 2046, methyl acetoacetate, acetoacetic acid, and acetone with a sensitive colorimetric reaction could not be found. The hydrolysis of 2046 with either equimolar acid or alkali produced a material in low yield which approached unity in partitioning between chloroform and water. When homogenates of plants which had been metabolizing either cis or trans radioactive 2046 for about 30 hours were fractionated by successive chloroform and water extractions, the radioactivity was found to partition as though three labeled compounds were present. The first extracted in an identical manner to the original isomer, a second as a completely water-soluble dimethyl phosphate hydrolytic product, and the third approached a partition coefficient of unity as it was purified on repeated extraction. Where the plant homogenate was allowed to stand at room temperature for about a day in aqueous medium, this third fraction of intermediate solubility was not recovered. Demonstration of this intermediate rules out route 1 for 2046 degradation in plants.

This material partitioning about unity between chloroform and water might be either the free carboxylic acid corresponding to 2046 shown in route 2, or the material formed on hydrolysis of the methoxyl phosphate group as shown in route 3. The latter route seems unlikely, because the methoxyl phosphate groups should be far more stable to acid and alkali hydrolysis than the methyl carboxylic ester group, and yet an intermediate of similar solubility appeared on enzymatic, acid, and alkali hydrolysis of 2046. Enzyme preparations attacking diisopropyl phosphorofluoridate (*9*, *21*, *28*, *31*), *O*-ethyl *N*-dimethyl phosphoramidic fluoridate (*4*), tetraethyl pyrophosphate (TEPP) (*15*, *30*), and paraoxon (diethyl *p*-nitrophenyl ester of phosphoric acid) (*1*, *18*) do not hydrolyze the alkoxyl phosphate groups. Further, the hydrolysis of the methoxyl phosphate as in route 3 might be expected to yield a product more water-soluble than the intermediate demonstrated. A more probable nature of the material partitioning about unity would appear to be the free carboxylic acid corresponding to 2046 as shown in route 2. The instability of this product might have been due to decarboxylation to form *0,0*-dimethyl 1-methylvinyl phosphate (route 2a), or to vinyl phosphate hydrolysis to yield dimethyl phosphoric acid (route 2b).

As can be seen from Table V, the methylvinyl material would have solubility properties close enough to those of the 2046 isomers that it could not be differentiated on the basis of chloroform and water partitioning. Where a set of 2046-treated plants were extracted with chloroform after about 30-hour metabolism and the chloroform-extractives chromatographed on a silica gel column with carbon tetrachloride, only the two peaks corresponding in position to those of *cis*- and *trans*-2046 appeared. Each of these peaks was divided into 12 fractions and infrared spectrograms of the fractions were compared with those from *cis*- and *trans*-2046 and from known *0,0*-dimethyl 1-methylvinyl phosphate. No indications of any material other than the two isomers were found. The infrared bands of the methyl vinyl phosphate are sufficiently characteristic to be detected even in low concentrations and its partitioning properties between carbon tetrachloride and water are such that if present it should have appeared as a contaminant between the *cis*- and *trans*-2046 fractions. The methylvinyl phosphate is more stable to hydrolysis than either *cis*- or *trans*-2046 (see Table V) and as such, if it were formed in plants as an intermediate in degradation, it might be expected to accumulate to some extent. The appearance of a 2046 derivative partitioning about unity between chloroform and water formed from both isomers, and the inability to demonstrate *0,0*-dimethyl 1-methylvinyl phosphate as an intermediate, tend to favor route 2b for in vivo degradation.

**Table V. Properties of *cis*- and *trans*-0,0-Dimethyl 1-Carbomethoxy-1-propen-2-yl Phosphate and 0,0-Dimethyl 1-Methyl Vinyl Phosphate**

| | *cis* | *trans* | Isopropenyl[a] |
|---|---|---|---|
| Partition coefficients | | | |
| $CHCl_3/H_2O$ | 53 | 49 | 50 |
| $CCl_4/H_2O$ | 4.22 | 0.83 | 1.18 |
| *n*-Hexane/$H_2O$ | 0.32 | 0.059 | 0.13 |
| Hydrolytic stability, $T^1/_2$, hours (pH 11.6) | 1.8 | 3.0 | >48 |
| Volatility, $T^1/_2$, hours | 21 | 24 | 4.8 |
| Biological activity | | | |
| $pI_{50}$ ChE[b] | 6.08 | 4.41 | < 2.7 |
| $LD_{50}$ flies (topical) | 0.27 | 23 | 960 |

[a] *0,0*-dimethyl 1-methylvinyl phosphate, b.p. 111–112° (33 mm.), generously provided by Shell Development Co., Denver, Colo.
[b] Negative logarithm of molar concentration required to effect 50% blood cholinesterase inhibition with assay conditions indicated in methods section.

With limited information available it would thus appear that 2046 is decomposed in plants by an initial enzymatic hydrolysis of the carboxylic ester group to form the free carboxylic acid corresponding to 2046, and that the hydrolysis of this carboxylic ester is quickly followed by a cleavage of the vinyl phosphate bond. The data in Table V show that only *cis*-2046 of the materials discussed shows appreciable insect and mammalian toxicity or anticholinesterase activity.

### Discussion

*0,0*-Dimethyl 2-carbomethoxy-1-propen-2-yl phosphate (Shell compound

2046) offers promise as a short-residual systemic insecticide. It serves to control many economic insect pests efficiently by systemic, contact, and fumigant action at low dosage levels (*6, 10, 11, 25*). The material is at least as effective on a dosage basis as tetraethyl pyrophosphate and demeton. 2046 is somewhat comparable to tetraethyl pyrophosphate in residual insecticidal properties on plants in the field, and much less residual than the current systemic materials. Both tetraethyl pyrophosphate and 2046 rely for their effectiveness on a near-complete initial insect mortality. 2046 has the advantage over tetraethyl pyrophosphate and the amido- and thiophosphates now being tested as systemic insecticides of being very rapidly absorbed and translocated to give very rapid insect eradication. Foliage application of 2046 will destroy many chewing as well as sucking insect forms. This lack of specificity might create problems due to possible destruction of the insect predators and parasites.

Under field conditions 2046 would not normally be applied to plant foliage to yield a concentration greater than 10 p.p.m. This would amount to about 6.7 p.p.m. of cis and 3.3 p.p.m. of trans isomer. Much more than half of the material would be lost by volatilization. (Volatility half life of 2046 from a glass slide was 3.2 hours and from filter paper was about 21 hours, following a more rapid loss in the first 4 hours when determined at about 30° C.) The isomers are of essentially the same volatility and both appear to be absorbed by plants to almost the same extent. Therefore, a maximum estimate of the amount of insecticide that would be absorbed by the plant would be 3.35 p.p.m. of the cis and 1.65 p.p.m. of the trans isomer. Any isomerization occurring on the plant surface due to the ultraviolet of sunlight would probably increase the proportion of trans isomer. The half life of the cis isomer in the plant is approximately 20 hours, while that of the trans is about 48 hours. After 2-day decomposition within the plant, there would be a maximum residual of 0.52 p.p.m. of the cis and 0.79 p.p.m. of the trans isomer. An isomerization within the plant would appear unlikely, owing to the hydrolytic instability of the materials. Under actual field conditions, where both the volatility and enzymatic degradation were taken into account, there was found to be a 90% loss of 2046 residues in the first 1.7 days and a 99% loss in less than 4 days following field application. The presence of 0.1 p.p.m. residues is readily detectable by the anticholinesterase analysis method.

Kodama and others (*22–24, 29*) have investigated the toxicology of technical 2046. As an in vivo cholinesterase inhibitor in rats it was less effective and of shorter duration than parathion (*29*). The acute and subacute toxicity did not differ significantly from parathion (*23, 29*). With a 60-day feeding period, the cumulative $LD_{50}$ to female rats for 2046 was between 50 and 100 p.p.m., but levels as low as 6.3 p.p.m. caused some shivering, ear twitching, and cholinesterase inhibition of plasma and erythrocyte (*23*). This is about the maximum amount of cis isomer that would appear on the plants immediately following foliage application in the field. Within 2 days the loss due to volatility and enzymatic breakdown would reduce the residue of the cis isomer to less than 0.5 p.p.m.

The trans isomer was found to be only about 1/100th as toxic to rats as the cis isomer (intraperitoneal). Insect bioassays showed that the toxicity of *cis*-2046 was not affected by its isomeride in ratios as high as 10 parts of trans to 1 part of cis. Systemic bioassays with pea aphids showed the cis to be 30 to 100 times more toxic than the trans. Several of the possible degradation products were compared with *cis*-2046 in bioassays with rats (intraperitoneal), houseflies (contact), and pea aphids (systemic); these included *0,0*-dimethyl 1-methylvinyl phosphate, dimethyl sodium phosphate, methyl acetoacetate, and acetone. All of these possible degradation products were less than 0.00025 as toxic in the bioassays or as active in inhibiting cholinesterase as *cis*-2046.

The toxic 2046 residues in crop plants treated at dosage levels used for insect control appeared to be essentially dissipated within 2 days following insecticide application.

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