potatoes may be attributed to the difference in sample thickness and lack of further protection by aluminum foil during frying, resulting in more volatilization of O_2 in this case.

Effects of Boiling on P_t. Determinations of P_t residues in both the boiled potatoes and boiled water are presented in Table VII. Taking the P_t quantities determined in fresh potatoes as 100.0%, the P_t in the boiled potatoes chopped in the form of fries reduced to an average of 37.7%, and in the boiled potato cubes, 69.1%. On the other hand, the P_t residues reduced in the boiled potatoes were found nearly quantitatively in the boiled water, indicating that water extraction is the major way for reducing the P_t residues in boiled potatoes. These data also show that in a given boiling period, the P_t residues extracted from potatoes chopped in smaller pieces, as in the form of fries, are more than those in larger pieces, as in the form of cubes. This is due to the fact that smaller pieces have a larger surface area to contact with water. The hydrolysis of P_t during the 10-min boiling was insignificant because the sum of P_t determined in the boiled potatoes and water was very close to the P_t levels in the original fresh potatoes.

Cooking Effects on P_t. Based on the results obtained, it is concluded that cooking potatoes may reduce or detoxify the anticholinesterase carbamate residues derived from thiofanox in two ways: (1) Baking and frying potatoes at or above 200 °C may reduce the anticholinesterase residues of thiofanox 50 to 90% through the chemical hydrolysis of P_2 to O_2 which is 386 times less toxic than P_2 (Chin et al., 1975). (2) Boiling potatoes at 100 °C may reduce the anticholinesterase residues of thiofanox 30 to 60% through physical extraction of P_2 from the boiled potatoes by the boiling water.

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Fate of Chlorothalonil in Apple Foliage and Fruit

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Chlorothalonil, a fungicide that enhances the growth regulating activity of ethephon, was applied to apple foliage and fruit under field and laboratory conditions. No evidence of chemical breakdown products of chlorothalonil was obtained. In addition, chlorothalonil apparently did not alter ethephon metabolism when used in combination with the growth regulating chemical. It is theorized that the increased fruit maturation effects of ethephon in the presence of chlorothalonil may be related to increased foliar uptake of ethephon.

Chlorothalonil (tetrachloroisothalonitrile) is produced by the Diamond Shamrock Corporation under the trade name Bravo and is an effective fungicide against a broad spectrum of plant pathogens. Studies have recently demonstrated additional biological activities of this chemical in addition to its well-known fungicidal action. Thus, chlorothalonil can dramatically enhance the plant growth regulating action of ethephon (2-chloroethylphosphonic acid) resulting in more rapid apple fruit maturation expressed by such growth parameters as increased color and soluble solids and decreased pull force and fruit firmness in apples and cherries (Edgerton and Hatch, 1972; Holm and Edgerton, 1976). The present study is concerned with the possible translocation and degradation of chlorothalonil when used alone and in combination with ethephon on the fruit and foliage of apple, *Malus domestica*.

EXPERIMENTAL SECTION

Translocation Studies. The possible translocation of ring-labeled [^{14}C]chlorothalonil was studied using 4-month old McIntosh seedlings raised under greenhouse conditions. The top and bottom surfaces of selected leaves were liberally painted with a radioactive mixture (0.94 μCi/ml) containing 500 ppm of [^{14}C]chlorothalonil (formulation code DS-28052B, Diamond Shamrock Corporation) and 250 ppm of ethephon (Ethrel, Amchem Products, Inc.). Seedlings were harvested after 3 days and covered with Kodak Royal X-Omat medical x-ray film for 2 weeks at 4 °C in a dark room.

Fruit and Leaf Treatment. Leaves and fruit of mature McIntosh trees growing in the Cornell University orchard were analyzed for radioactive residues of chlorothalonil by a liquid scintillation counter (lsc) and by gas–liquid chromatography (GLC). Treatment consisted of applying either a 500-ppm radioactive chlorothalonil formulation (12 μCi/ml) or an aqueous mixture of 500 ppm of [^{14}C]chlorothalonil (195 μCi/ml) and 250 ppm of ethephon. The leaves and apples were painted to the point of run-off with the aid of a small brush.

In additional experiments, fruit bearing branches on orchard McIntosh trees were thoroughly covered with 250 ppm of [^{14}C]ethephon by applying the solution with a brush. Additional branches were painted to run-off with an aqueous formulation mixture of 250 ppm of radioactive ethephon (255 μCi/ml) and 500 ppm of chlorothalonil.

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Samples of fruit and leaves were harvested over a 12-day sampling period.

Extraction and Analysis of Chlorothalonil. All glassware used in this investigation was thoroughly washed to prevent possible loss of polar derivatives of chlorothalonil such as 4-hydroxy-2,5,6-trichloroisothalonitrile. The cleaning procedure consisted of rinsing in turn with technical acetone, hot tap water, distilled water, 6 N hydrochloric acid, tap water, distilled water, and acetone.

Leaf samples consisting of 10 to 20 leaves and weighing 2.5 to 5 g were rinsed for 30 s with 75 ml of acetone–50% H_2SO_4 (acidic acetone) (100:1, v/v) using a wrist action shaker. Several five-gram portions from two chopped apples were rinsed in a similar fashion. After rinsing, 90 ml of 0.4 N $NaHCO_3$ was added to 10-ml aliquots of the fruit and leaf rinse mixtures. The pH adjustment and a subsequent hexane partitioning step facilitated the separation of chlorothalonil from more acidic molecules, such as the 4-hydroxy derivative of chlorothalonil, that may possibly be present in the extraction mixtures. The alkaline mixtures were partitioned with three 5-ml portions of hexane. The three hexane solutions were combined and adjusted to 15 ml prior to drying over sodium sulfate.

Tissue residue levels were studied by extraction with two 25-ml portions of acidic acetone (95:5, v/v) in an Omni tissue blender. These extract mixtures were then concentrated to 10 ml before adding 90 ml of $NaHCO_3$ (0.4 N) and partitioning with hexane in the manner previously described. Following the hexane extraction, the aqueous fractions were acidified with 2.2 ml of 50% H_2SO_4 and partioned four times with 5 ml of diisopropyl ether. The volume of the combined solution was adjusted to 20 ml.

The hexane and ether samples were analyzed using a Varian Aerograph Model 705 gas–liquid chromatograph (GLC) containing a nickel-63 electron capture detector. The glass column was 1.2 m × 2 mm packed with 3% OV-17 on 100–120 mesh Gas-Chrom Q. The injector, column, and detector were operated at 195, 160, and 255 °C, respectively. Ten-milliliter aliquots of the ether samples were also assayed for the 4-hydroxy derivative of chlorothalonil by GLC after evaporation just to dryness and derivitization with diazomethane by the procedure of Schlenk and Gellerman (1960). The GLC data were quantitated by measuring appropriate gas chromatographic peak heights in the sample chromatograms and then referring to calibration curves prepared with authentic chlorothalonil and the methylated derivative of 4-hydroxychlorothalonil.

The organic fractions were assayed for radioactivity by a Packard Model 3310 Tri-Carb liquid scintillation counter (lsc) using 0.5 to 1 ml of the appropriate organic solution in 20 ml of toluene containing 2,5-diphenyloxazole (PPO) (0.5% w/v) and 1,4-bis[2-(5-phenyloxazolyl)]benzene (POPOP) (0.01% w/v). Aqueous fractions were measured for activity in 15 ml of Aquasol (New England Nuclear, Boston, Mass.). Tissue remaining after extraction was assayed by lsc after blending 0.5 g of residue with 2 ml of water and 7 ml of Protosol tissue solubilizer (New England Nuclear). This mixture was heated overnight at 70 °C in a tightly sealed tube. A 0.5-ml aliquot of the hydrolysate was added to 15 ml of Aquasol and allowed to stand overnight at 5 °C in the dark prior to lsc.

Extraction and Detection of [^{14}C]Ethephon. Because ethephon is readily absorbed by apple leaves and fruit (Edgerton and Hatch, 1972), only total tissue levels of radioactivity were determined. Radioactive residue in surface rinses was not studied. Chopped apples (5 g) and 10 to 20 leaves (2.5–5 g) were extracted twice with 25-ml portions of acetone–50% H_2SO_4 (100:1 v/v). The apple and foliage extracts were evaporated to 10 ml and replicate 5-μl portions of these solutions were streaked on 5 × 20 cm Sil G-25 plates produced by Brinkmann Instruments Inc. (Westbury, N.Y.). Plates were developed in methanol–2-propanol–water–ammonia (9:6:3:1, v/v) (Gilbert et al., 1975) until the front had developed about 5 cm. After locating radioactivity with a Packard radiochromatogram scanner appropriate regions of the silica gel were scraped into 20 ml of toluene scintillation mixture and assayed by lsc. In additional experiments, autoradiographs were prepared by covering the developed plates with x-ray film for 3–4 weeks in a dark room at 4 °C.

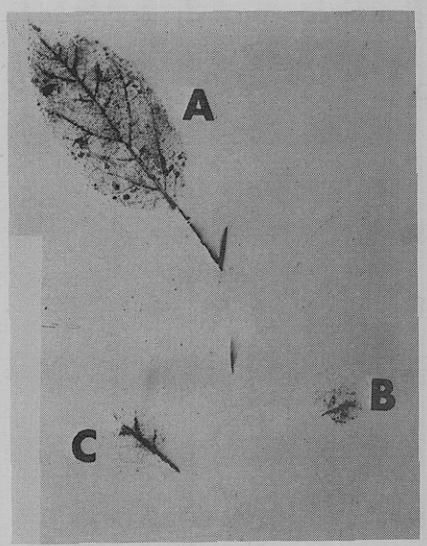

Figure 1. Autoradiograph of treated apple seedling. [^{14}C]Chlorothalonil applied to whole leaf (A) or basal portion of leaf (B, C).

Table I. Recovery of Radioactivity[a] from Control Leaf Extracts Spiked with 1 ppm of Appropriate Compound

Compound	% recovery ± std dev	No. of replicates
[^{14}C]Chlorothalonil	101 ± 7	5
[^{14}C]-4-Hydroxy-2,5,6-trichloroisothalonitrile	101 ± 2	2
[^{14}C]Ethephon	100 ± 4	6

[a] Compound extracted as described in the Experimental Section.

RESULTS AND DISCUSSION

Figure 1 represents an autoradiograph prepared from an apple seedling harvested 3 days after treatment with [^{14}C]chlorothalonil and ethephon. Evidence of radioactivity is only observed in the painted leaf areas including a whole treated leaf (Figure 1A) and two partially painted leaves (Figures 1B and C). In a similar experiment, radiographs of seedling leaves receiving [^{14}C]chlorothalonil, without ethephon, also demonstrated the absence of chlorothalonil translocation.

Studies were subsequently undertaken to determine the location and chemical nature of radioactive residues in samples of apple leaves harvested at 0, 1, 5, and 12 days after spraying to run-off with either [^{14}C]chlorothalonil or a mixture of radioactive chlorothalonil and ethephon. [^{14}C]Chlorothalonil is readily recovered from control leaf extracts spiked with this chemical as indicated by lsc data (Table I) or GLC analysis. From 95 to 99% of the total radioactivity in each harvest leaf sample was detected in

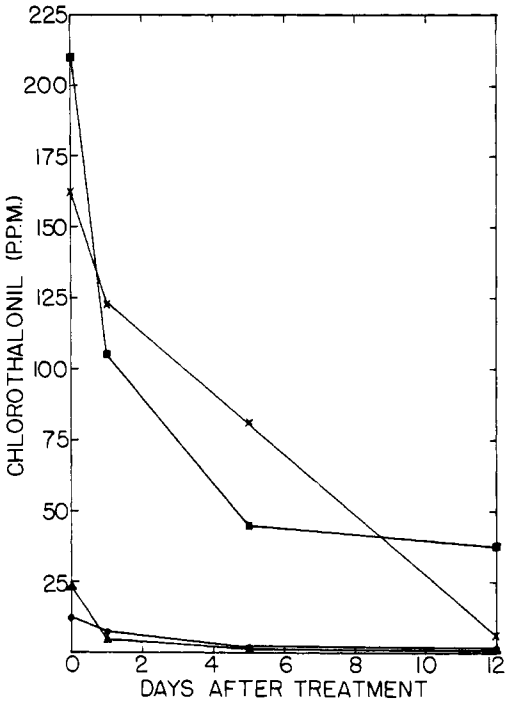

Figure 2. Leaf residue levels of chlorothalonil, GLC determination. Residues prepared after leaf treatment with (X) [^{14}C]chlorothalonil or (■) [^{14}C]chlorothalonil + ethephon. Total blend extracts of above leaves after receiving (●) [^{14}C]chlorothalonil or (▲) [^{14}C]chlorothalonil + ethephon.

the hexane fraction prepared from the acidic acetone rinse of the treated leaves. The remaining activity in these leaf samples was primarily found in the hexane fractions partitioned from the acetone leaf extract mixtures. Trace quantities of radioactivity were found in the isopropyl ether fractions obtained from either the leaf rinse or leaf extract solutions. The 4-hydroxy-2,5,6-trichloroisothalonil, if present, would have been readily extracted by isopropyl ether under these conditions (Table I). This acidic compound is a major metabolite of chlorothalonil in soil and a minor residue component in certain types of vegetable and orchard crops (Ballee, 1976). This chlorothalonil hydroxy derivative was not, however, detected by GLC in the ether mixtures after treatment with diazomethane. Trace levels of radioactivity were also observed in the Protosol hydrolysis mixtures prepared from the leaf tissue samples remaining after acetone extraction.

Figure 2 shows the level of chlorothalonil residue in the various organic fractions of the leaf harvest samples as determined by GLC. The distribution of chlorothalonil in the rinse and extract fractions prepared from each leaf sample (Figure 2) closely resembled the corresponding distribution of radioactivity as previously described. Thus, about 90–99% of the chlorothalonil harvest residues were recovered by briefly rinsing the leaf samples with acidic acetone (Figure 2) suggesting that chlorothalonil was primarily present in the outer waxy layers of the apple leaves. These observations, obtained with the aid of GLC and lsc, provide evidence for the contention that chlorothalonil was not chemically degraded when applied to apple leaf tissue either alone or in combination with ethephon. Gas chromatographic and lsc data from a similar experiment with young apple seedlings conducted in a laboratory in the absence of field-weathering conditions also supported this contention.

The fate of [^{14}C]chlorothalonil in laboratory apples treated in combination with and in the absence of ethephon is represented in the data of Figure 3. As in the case of apple foliage, no significant evidence of radioactivity was detected in either the isopropyl ether fractions or in the tissue hydrolysis mixtures remaining after rinsing and extracting the samples. Liquid scintillation detection and GLC analysis indicated that 84–99% of the radioactive residue in each apple sample was recovered by a 30-s rinse of the apple peelings. As shown in Figure 3, the remaining quantities of residue were detected in extracts of the washed peelings (0.16–1.3 ppm) and in extracts of the peeled apple (0.18–0.22 ppm of chlorothalonil). The reason for the apparent increase in residue levels in the 1-day apple peeling samples is not known. The higher chlorothalonil residue levels in the 1-day samples may, however, be related to inadequate sampling of the peeling tissue since the chlorothalonil formulation poorly adhered to the

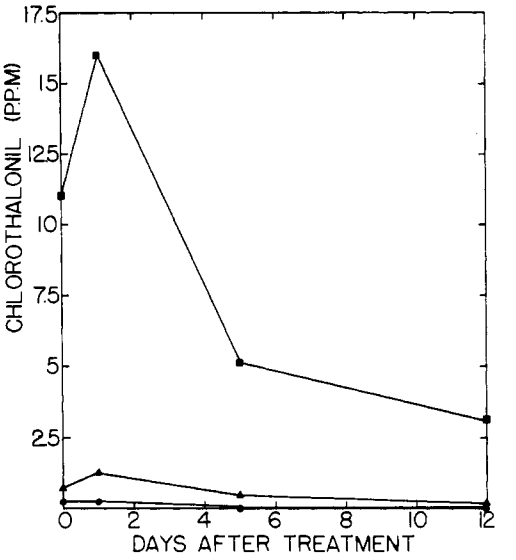

Figure 3. Chlorothalonil levels in treated apple fruit. Hexane fractions analyzed by GLC after partitioning (■) peeling rinse; (▲) blended extract of peeling after rinsing; and (●) extract of fruit with peelings removed.

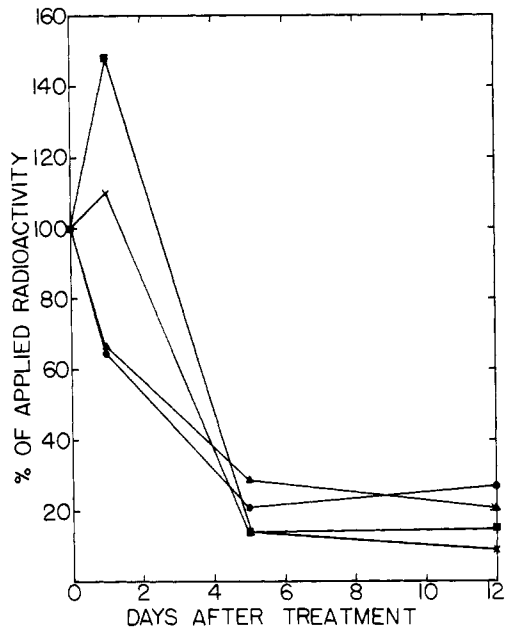

Figure 4. Levels of radioactivity in total extracts of treated apple leaves and fruit. Fruit treated with (●) [^{14}C]ethephon or (▲) [^{14}C]ethephon + chlorothalonil. Foliage treated with (■) [^{14}C]ethephon + chlorothalonil or (X) [^{14}C]ethephon.

waxy tissue of the apple fruit.

Experiments were conducted to evaluate the possible effect of chlorothalonil on ethephon metabolism in view of the suggestion that chlorothalonil can increase the uptake of ethephon by apple foliage in the first 3 to 4 days after chlorothalonil treatment (Holm and Edgerton, 1976). Consequently, the increased level of foliar [^{14}C]ethephon found on the first day in the presence of chlorothalonil (Figure 4) may represent chlorothalonil-enhanced ethephon uptake. Edgerton and Hatch (1972) found that leaf uptake of [^{14}C]ethephon was important to subsequent levels of [^{14}C]ethephon in the fruit and to production of ethylene in fruit. Thus, chlorothalonil may enhance the fruit ripening response of ethephon by increasing foliar ethephon uptake. The radioactive residue remaining in either the fruit or leaf total extracts in the 4- to 12-day harvest interval was not significantly affected by the presence of chlorothalonil (Figure 4). Examination of autoradiographs of TLC plates developed from the apple or leaf total extracts indicated only one spot that had the same corresponding R_f value as that of authentic [^{14}C]-ethephon. These observations indicate that chlorothalonil apparently affects neither the chemical breakdown nor the rate of disappearance of [^{14}C]ethephon from either McIntosh apple or leaf tissues.

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Metabolism of the Herbicide Methazole in Lactating Cows and Laying Hens

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[*phenyl*-^{14}C]Methazole [2-(3,4-dichlorophenyl)-4-methyl-1,2,4-oxadiazolidine-3,5-dione] was fed to hens at 0.3, 1.0, and 3.0 ppm in the diet for 14 days, and administered daily to cows via gelatin capsule at dosages equivalent to 0.5, 2.5, and 10 ppm in the diet for the same period. Maximum residues in the body after 14 days were located in the liver and were 0.026, 0.101, and 0.201 ppm of [^{14}C]methazole equivalents for hens at the three feeding levels; for cows, the residues in liver were 0.016, 0.065, and 0.406 ppm. Eggs contained ^{14}C-labeled residues at 3% the dietary parts per million levels and milk at about 0.3% that in the diet. Radiocarbon in tissues, eggs, milk, and excreta was characterized by extraction and partitioning behavior, and by TLC analysis of metabolites extractable into organic solvent. Major components of the latter were 3-(3,4-dichlorophenyl)-1-methylurea, 3,4-dichlorophenylurea, and *N*-(2-hydroxy-3,4-dichlorophenyl)urea and its 4,5-dichlorophenyl analogue.

The herbicide methazole [2-(3,4-dichlorophenyl)-4-methyl-1,2,4-oxadiazolidine-3,5-dione] has shown promise for control of broadleaf and grassy weeds in several crops and has recently been registered for use on cotton (Whitacre and Whitehead, 1976). The metabolic fate of methazole has been studied in several crop plants (Butts and Foy, 1974; Dorough et al., 1973; Dorough, 1974; Jones and Foy, 1972). These studies show methazole to be transformed largely to 3-(3,4-dichlorophenyl)-1-methylurea (DCPMU) and 3,4-dichlorophenylurea (DCPU) which may be present in the free and/or conjugated form.

A study of the metabolism of methazole in rats (Dorough et al., 1974) demonstrated that *N*-(2-hydroxy-4,5-dichlorophenyl)urea was the major urinary metabolite and was present almost entirely in the glucuronide form. The glucuronide of *N*-(2-hydroxy-3,4-dichlorophenyl)urea was also detected in the urine. DCPU was the major metabolite in the feces and existed almost completely in the free form. The highest levels of methazole equivalents were in the kidney, liver, and fat.

Table I. [^{14}C]Methazole Administered in the Diet of Lactating Cows and Laying Hens

ppm of methazole in diet		No. of animals		mg/animal per day	
Cows	Hens	Cows	Hens	Cows	Hens
0.0	0.0	1	6	0	0
0.5	0.3	1	6	11	0.03
2.5	1.0	1	12	55	0.10
10.0	3.0	1	6	220	0.30

Feeding studies with methazole in a dairy cow (Gutenmann et al., 1972) at 5 ppm in the diet for 4 days indicated that there were no detectable residues of the parent compound or its metabolites in the milk, urine, or feces. Because these findings were inconsistent with those reported for rats, a more thorough investigation of the metabolic fate of [^{14}C]methazole in dairy cows was warranted.

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