Egg production was not influenced significantly as a result of the treatments. The only effect observed was a decline in the number of good quality chicks hatched from the fertile eggs of group 4.

In summary, addition of a low level mixture of organochlorine insecticides for a period of over 2 years to broiler breeders food had only a very marginal effect on the performance of these animals.

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Supplementary Material Available: A listing of subgroup figures (4 pages) on egg production, egg shell quality and hatchability. Ordering information is given on any masthead page.

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A Rapid and Simple Method for the Determination of Residues of 2-Chloroethylphosphonic Acid (Ethephon) in Tomatoes, Cherries, and Apples

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A rapid method has been developed for the detection of residues of 2-chloroethylphosphonic acid (ethephon) in tomatoes, cherries, and apples based upon degradation to ethylene at high pH values. The hormone released is determined by gas–solid chromatography. Extraction and purification of 2-chloroethylphosphonic acid from fruit tissue as described in earlier procedures can be omitted. Residue results are consistent with the direct identification of methylated ethephon by gas–liquid chromatography with a flame photometric detector. After a characteristic increase, maximum residues in field-treated crops were found to be 6.8 ppm on tomatoes, 4.1 ppm on cherries, and 1.2 ppm on apples 2 to 8 days after application. The natural content of ethylene in fruit is less than 5% of what can be expected from residues of ethephon.

Since the biological action of ethylene on the development of plants was reported (Neljubow, 1901), a number of physiological responses have been described and reviewed (Abeles, 1973). Fruit growers were unable to take advantage of these responses because of the difficulties in applying gaseous compounds to plants in the field. The search for nongaseous ethylene-releasing chemicals (Gowing and Leeper, 1955) was of little success until 2-chloroethylphosphonic acid (ethephon), described already 17 years earlier (Kabachnik, 1946), was found to be most effective (Bukovac, 1969; Maynard, 1963). Its excellent biological action, especially on the process of fruit ripening, has made it one of the most important plant growth regulators for agricultural purposes. Unfortunately, long-term feeding tests on dog and rat indicate a considerable inhibition of cholinesterases in blood plasma and erythrocytes, as it is known by a number of esters of phosphoric acid used as insecticides. Harmful accumulation of inhibitors of this kind in food can be circumvented by well-considered applications based on residue analysis. The gas chromatographic procedures developed earlier for residue analysis (Amchem Products Inc., 1971; Bache, 1970) determine the methylated phosphonic acid compound by GLC with a flame photometric or alkaliflame ionization detector, respectively. Even simplified extraction and clean-up procedures in recently published papers (Cochrane et al., 1976; Ernst and Anderegg, 1976) are rather time consuming.

In this paper, we described a rapid method suitable for routine analysis of ethephon residues in tomatoes, cherries, and apples, based upon the quantity of ethylene released from ethephon at pH values of 12–14, thus omitting tedious extraction and clean-up steps. This principle has already been successfully applied as a screening method for ethephon residues (Zimmerli, 1974).

MATERIALS AND METHODS

Material. Ethylene (99.5% pure, compressed to 1200 psi in tanks of 0.44 L) was supplied by Merck-Schuchardt, Munich. Ethephon (87% pure, technical grade) was a gift from Amchem Products Inc., Ambler, Pa. Activated alumina F-1 was obtained from Applied Science Laboratories Inc., State College, Pa. Acetone of analytical grade was distilled to remove volatile compounds interfering with the gas chromatographic procedure.

Standard Solution. Ethephon at concentrations of 10–100 mg/L of distilled water was kept in polyethylene bottles and renewed monthly. Ethylene of the tank was trapped in serum bottles and further diluted to 10.0, 1.0, and 0.1 ppm (v/v) in similar bottles containing N_2.

Treatment and Sampling of Fruit. Twenty-four tomato plants of the variety "Montfavet H 63-5" were sprayed by a hand gun with a solution of 0.2% of ETHREL, corresponding to a concentration of 960 ppm of ethephon. (ETHREL is the registered trademark of Amchem Products Inc. for this plant growth regulator

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formulation. The active ingredient consists of 48% (w/v) of ethephon.) The same number of plants was kept as a control. Two hours after treatment, samples of 1 to 2 kg were picked; subsequent harvests were made at intervals of 3 to 4 days. Half of each sample was frozen and lyophilized according to the procedure of Amchem Products Inc. Trees of apple and cherry were treated to the run-off point by gun application with 0.1% of ETHREL. Fruits were harvested in the same manner as for tomatoes.

Preparation of Fruit Samples for Ethylene Determination. Tomatoes, apples, or cherries (800–1000 g, kernel removed) were homogenized in a Waring blender for 30 s within 4 days after picking. In order to obtain representative samples, only quarters of the precut tomatoes and apples were used. Twenty-five grams of the slurry, together with 25 mL of acetone, were put in 120-mL injection bottles. They were sealed tightly immediately after adding 5 mL of 30% (w/v) potassium hydroxide in water, leaving a headspace of 65 mL. In order to achieve reproducible ethylene production, it was important that the reaction bottles were shaken at 180 strokes/min in a water bath at 60 °C for 90 min. After cooling to room temperature, gas samples of 2 mL were removed from the headspace by the use of a gas-tight syringe and investigated gas chromatographically.

Gas Chromatography. Gas chromatography was carried out using a Varian Aerograph Series 2100 gas chromatograph. (1) Ethylene: detector, FID; column, glass, 3.05 m (10 ft), i.d. 1.6 mm ($^1/_{16}$ in.); packing, alumina F-1 activated, 80–100 mesh without liquid phase (Dilley, 1974); carrier gas, N_2, 23 mL/min; temperature, oven 110 °C, inlet 160 °C, detector 170 °C; retention time ethylene: 90 s. (2) Dimethylethephon: detector, FPD, Model 100 AT (Tracor, Inc.), equipped with filter transparent for light of 526 nm; column, glass 3.05 m (10 ft) i.d. 1.6 mm ($^1/_{16}$ in.); packing, 10% carbowax 20 M on Gas-Chrom Q 80/100 mesh; carrier gas, N_2, 30 mL/min; temperatures, oven 160 °C, inlet 190 °C, detector 200 °C; retention time dimethylethephon: 6.25 min.

RESULTS AND DISCUSSION

The degradation of ethephon at pH values above 4.0 to ethylene, phosphate, and chloride ions (Maynard and Swan, 1963; Yang, 1969) offers a convenient possibility for the indirect determination of residues of this growth regulator or for confirming purposes. The reaction efficiency, and thereby the concentration of ethylene available for identification, depends on the completeness of the degradation of the original compound and, to the same extent, on the insolubility of the gas in the liquid phase. Interfering results were to be expected by the natural content of ethylene in fruits and by the ethylene-induced ethylene biosynthesis in plant tissue (Burg and Dijkman, 1967; Kende, 1976).

Influence of Solvent System, Temperature, and Potassium Hydroxide on Ethylene Evolution. These investigations were carried out by adding known amounts of ethylene or ethephon to solvent systems as mentioned in Table I. The efficiency of recovery was determined by analyzing ethylene in the headspace of the injection bottle. Compared to water–KOH, figures clearly represent a loss of 11% of ethylene in the solvent system water–acetone–KOH because of its increased solubility. If this system, however, is supplied with ethephon, the efficiency remains the same, as in the water–KOH system, indicating probably a more complete hydrolysis of the phosphonic acid compound. Investigating spiked samples without acetone in the extraction medium yielded recoveries depending on the nature of fruit tissue. This phenomenon

Table I. Efficiency of Ethylene Recovery from Different Solvent Systems after Supplying Ethylene or Ethephon

	Ethylene in headspace, % of theoretical amount			
	Ethylene[a]		Ethephon[a]	
Solvent system	$\bar{x}$	rel SD	$\bar{x}$	rel SD
50 mL of H_2O + 5 mL of 30% (w/v) KOH	100	1.5	87	6.2
25 g of tomatoes + 25 mL of H_2O + 5 mL of 30% (w/v) KOH	100	7.1	86	2.0
25 mL of H_2O + 25 mL of acetone + 5 mL of 30% (w/v) KOH	89	2.4	84	3.5
25 g of tomatoes + 25 mL of acetone + 5 mL of 30% (w/v) KOH	79	1.9	86	1.7
25 g of cherries + 25 mL of acetone + 5 mL of 30% (w/v) KOH	81	2.3	84	1.6
25 g of apples + 25 mL of acetone + 5 mL of 30% (w/v) KOH	78	5.6	87	4.4

[a] Material supplied; 3.25 mL of nitrogen containing 1000 ppm (v/v) of ethylene (3.25 μL), or 0.25 mL of water containing 100 ppm (w/w) of ethephon (equivalent to 3.89 μL of ethylene), was injected after sealing the 120-mL injection bottles. Incubation took place as mentioned in Materials and Methods. Means ($\bar{x}$) and relative standard deviations (rel SD) are calculated from three independent experiments. Results of ethephon are corrected for 100% pure compound.

disappeared in the presence of acetone as indicated by the calibration curve. It is further assumed that systemically incorporated 2-chloroethylphosphonic acid in field-treated crops becomes more extensively eluted from cell constituents by this solvent system.

To investigate the influence of temperature on the degradation of ethephon at a concentration of 1 ppm in the fruit homogenate, incubation was conducted at 40, 50, and 60 °C for 90 min. The efficiencies of recovery of ethylene equivalents were 48 ± 6.0, 71 ± 5.7, and 85 ± 1.6%, respectively (means and ± standard deviation from three separate results). Incubation temperatures exceeding 60 °C appear inadvisable since losses of ethylene must be considered due to elevated vapor pressure inside the injection bottle. Replacing acetone by water would diminish this phenomenon.

Ethephon becomes increasingly unstable at pH values above 4.0. Therefore, 5 mL of 30% potassium hydroxide, giving pH values of 12 to 14, were sufficient for the degrading reaction. Larger amounts did not change the release of the hormone significantly.

Interference with Endogenously Synthesized Ethylene. Because of its widespread occurrence as an endogenous hormone during the ripening of fruits, the question arises how far the natural content of this gas in fruit interferes with the measurement of the residues of ethephon. Results in Table II indicate a concentration of 2.7 to 3.8 nL of ethylene/g of untreated fruit, simulating 0.017 to 0.024 ppm of ethephon. This represents less than 5% of the residual amount to be expected after an ETHREL application on tomatoes, apples, or cherries.

Investigations on bean petiole explants indicate an enhanced biosynthesis of ethylene by ethephon (Suzuki et al., 1971). Observations on ethylene-induced ethylene synthesis have been made on vanda orchid blossoms (Burg and Dijkman, 1967) and on senescent flower tissues of

Table II. Ethylene Detected in Treated and Nontreated Fruit

	Control fruit analyzed with KOH, nL of ethylene/g of fruit (naturally occuring)	Treated fruit analyzed, nL of ethylene/g of fruit	
		Without KOH[a]	With KOH
Tomatoes	2.7 ± 0.5[b]	15.7 ± 5.3[b] (0.10)[c]	610 ± 80.5[b] (3.93)[c]
Apples	3.4 ± 0.4	2.7 ± 0.4 (0.02)	127 ± 9.7 (0.82)
Cherries	3.8 ± 1.0	4.5 ± 0.6 (0.03)	76 ± 5.9 (0.49)

[a] Five milliliters of 30% KOH was replaced by water. The pH value of the homogenate was 3.5 to 3.7. [b] Standard deviation calculated from three independent determinations on the same sample. [c] Number in parentheses represent parts per million of ethephon equivalents.

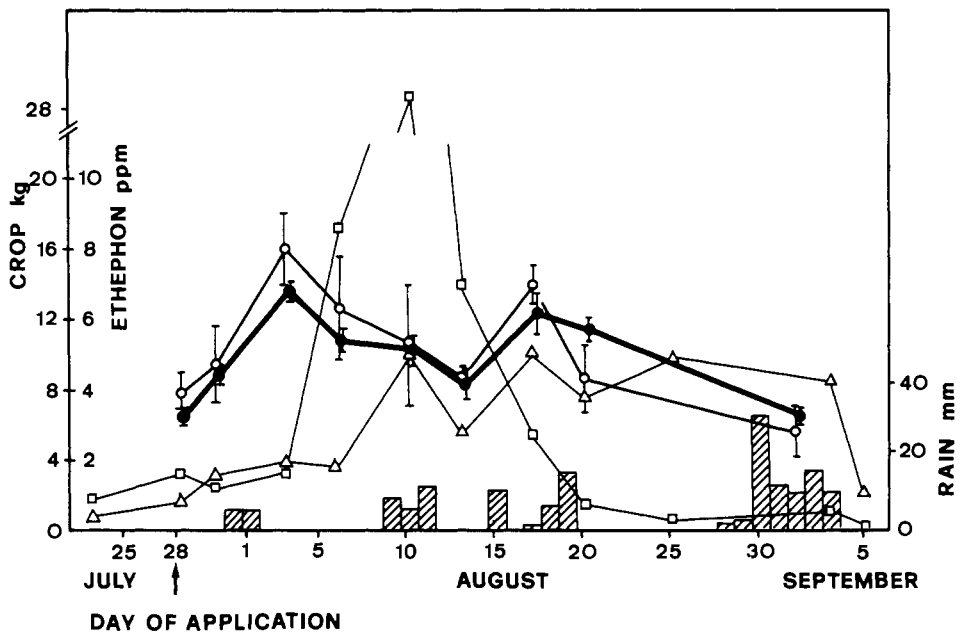

Figure 1. Residues of ethephon in tomatoes: Residue data represent means and standard deviations from three independent analyses on the same sample. (●—●) Residues of ethephon based upon ethylene determination by GSC-FID. (○—○) Residues of ethephon based upon dimethylethephon determination by GLC-FPD. (△—△) Tomato crop untreated. (□—□) Tomato crop treated with a solution of 0.2% of ETHREL (980 ppm of ethephon). Twenty-four plants were sprayed when fruits of the first cluster turned from green to yellow. The same number of plants was kept as a control. Fruits were picked after the red color had developed completely.

morning glory (Kende and Hanson, 1976). To examine whether this phenomenon interfered with the measurements, samples containing residues were analyzed, omitting the addition of potassium hydroxide. The concentration of ethylene in the headspace of the reaction bottle was in the same range as for samples of control fruits, whereas the presence of potassium hydroxide increased this value 16 to 47 times. It may be assumed, therefore, that most of the autocatalytically synthesized ethylene was lost during the homogenation process of the fruit.

Calibration Curve. Recovery experiments of ethylene from tomatoes, apples, and cherries, fortified with ethephon, have been conducted in the range of 0.2 to 10.0 ppm using acetone in the incubation medium. The quantities of ethylene evolved are calculated from three independent experiments for each fruit sample, considering six different concentrations of the growth regulator. The linear regression through the origin (results are corrected for blank values) appear almost identical: tomatoes $y = (3316 \pm 53)x$, cherries $y = (3360 \pm 114)x$, apples $y = (3294 \pm 107)x$, where y = nL of ethylene measured, x = ppm of ethephon added, ± = 95% confidential limits of the slope. Corresponding data from all three fruit samples were therefore averaged and used for the calibration curve in Table III.

Minimum Detectable Amount. With no special modifications, the gas chromatographic system has the capability to identify easily 0.2 nL of ethylene in an injected sample size of 2 mL of nitrogen. Considering headspace and sample weight of the injection bottle, the minimum detectable residue of ethephon is 0.002 ppm. However, as indicated in Table II, concentrations of ethylene smaller than the amount equivalent to 0.1 ppm of ethephon should not be taken as residues without simultaneously identifying dimethylethephon.

Table III. Calibration Curve for the Detection of Ethephon in Tomatoes, Apples, and Cherries[a]

Ethephon in fruit homogenate, ppm	Ethylene developed, nL	
	$\bar{y}$	Rel SD
0.2	610	10.4
0.5	1650	5.9
1.0	3230	11.8
3.0	10'100	7.6
5.0	16'600	5.5
10.0	33'200	5.9

[a] Linear regression: $y = 3323x$ calculated upon 54 determinations of ethylene, 95% confidential limit of the slope ±52. Data represent means ($\bar{y}$) and relative standard deviation (rel SD) of nine independent results (three experiments on each of tomatoes, cherries, and apples).

Residues of Ethephon in Tomatoes, Cherries, and Apples. Residue determinations by the proposed method on field-treated tomatoes show, within exprimental error, the same level as those found by analyzing dimethylethephon (Figure 1). The reliability is further demon-

strated by smaller relative standard deviations of 1.9 to 10.1% for ethylene analysis, compared to 7.9 to 33.0% for dimethylethephon determination. The steady increase of ethephon concentration in treated tomatoes confirms earlier observations (Hurter, 1975). It is unusual for pesticides, in general, but represents the systemic transport of this growth regulator from leaves into fruit (Yamaguchi, 1971). The incorporation into fruit tissue explains the fact that residues of this highly water-soluble growth regulator are not washed off by rainfall. The maximum residue of 6.8 ppm appears to be surprisingly high at an applied concentration of 0.2% of ETHREL. Reducing the active ingredient to 0.15 and 0.10% diminished these figures to 5.3 and 3.4 ppm, respectively. However, the biological response declined markedly as the fruits only colored slightly.

The characteristic increase of ethephon residues was found also in apples and cherries within 2 and 8 days after application. Maximum residues were found to be 1.2 ppm in apples and 4.1 ppm in cherries. The corresponding flame photometric determination yielded 2.0 and 4.3 ppm.

It is concluded that the analytical procedure proposed is a satisfactory method for determining ethephon. It allows to avoid time-consuming lyophilization of crop material and methylation of the phosphonic acid. Esterification in the presence of fruit extracts, using diazomethane proposed by Schlenk and Gellerman (1960), converted only 38–65% of the growth regulator into dimethylethephon. As methylation in general, the success of this procedure may depend on the solvent system. Further experiments to investigate this point have not been conducted.

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Trifluoroacetylation of Pesticides and Metabolites Containing a Sulfoxide Moiety for Quantitation by Gas Chromatography and Chemical Confirmatory Purposes

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The Pummerer reaction was evaluated as a means of derivatizing pesticides and their metabolites, which contain a S→O moiety for analysis by gas chromatography. Dasanit, dasanit oxon, and the sulfoxides of Mesurol, Nemacur, and aldicarb all readily reacted with trifluoroacetic anhydride at RT/15 min to give trifluoroacetoxymethyl sulfide analogues. For compounds possessing an NH moiety, more vigorous reaction conditions were required in order to form the di-trifluoroacetyl derivative as a single product in high yield. Oxydemeton-methyl, oxycarboxin, Counter, and phorate sulfoxides also reacted with trifluoroacetic anhydride, but gave anomalous products. The secondary trifluoracetoxy moiety of oxydemeton-methyl thermally decomposed on-column to give *cis*- and *trans*-dehydrooxydemeton-methyl. Oxycarboxin underwent both an additive and transannular Pummerer reaction, depending on the reaction conditions, while phorate and Counter sulfoxides were transformed to their respective oxons.

Pesticides containing a sulfide group readily undergo oxidation to their sulfoxide and sulfone analogues. These metabolites normally exhibit some pesticidal activity and must therefore be considered in any residue analysis study. Generally, sulfoxides do not gas chromatograph (GC) well due to their polar nature and are analyzed by conversion to their sulfones.

Perfluoroacylation, which is frequently employed to enhance the thermal stability of carbamate insecticides (Khalifa and Mumma, 1972; Seiber, 1972; Wong and Fisher, 1975), has been used to quantitate Mesurol and its metabolites (Greenhalgh et al., 1976). Reaction with trifluoroacetic anhydride (TFAA) has revealed that the Mesurol sulfoxide forms a di-trifluoroacetyl (TFA) de-

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