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THE USE OF CHLORINE AND OZONE AS A POSTHARVEST WASH IN THE REMOVAL OF PESTICIDES ON APPLE FRUIT

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THE USE OF CHLORINE AND OZONE AS A POSTHARVEST WASH IN THE REMOVAL OF PESTICIDES ON APPLE FRUIT

By

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ABSTRACT

USE OF CHLORINE AND OZONE AS POSTHARVEST WASH IN THE REMOVAL OF PESTICIDES ON APPLE FRUIT

By

Kheng-Chuan Peter Ong

The objective of this study was to determine the effectiveness of chlorinated and ozonated washes in the dissipation of pesticides in solution and on and in fresh and processed apples.

Laboratory studies were conducted in a model system to determine the effects of calcium hypochlorite (50 and 500 ppm) and ozone (0.25 ppm) at pH 4.5, 7.0, 10.7 and at 21°C and 44°C on the degradation of each pesticide in solution over a 30 minute period. Apple fruits spiked with the three pesticides were also used to determine the effectiveness of chlorine and ozone washes on the removal and degradation of the pesticide residues. All samples were analyzed for residues by gas chromatography or high performance liquid chromatography.

Chlorination and ozonation were effective in degrading azinphos-methyl, captan and formetanate-hydrochlorite in solution. Rate of degradation generally increased at higher pH and temperature. Pesticide residues on apple fruits and in processed products were reduced by the chlorine and ozone washes. The 500 ppm chlorine wash was the most effective wash treatment.

DEDICATED TO

My parents, Robert & Annie Ong,

for their love and nurture through the years ...

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INTRODUCTION

The demand for produce with good sensory quality by the consumer has continued to sustain the use of pesticides in the control of insects and diseases in apple fruits. As a result, there is a need to develop methods for removing or reducing the levels of pesticide residues on fresh and processed apples after harvest. Such methods would alleviate concerns that these chemicals are hazardous to humans and the environment. Postharvest treatments such as the postharvest water wash and scrub that have been traditionally employed to remove debris and dirt, have been shown to reduce residues (El-Hadidi, 1993). The use of postharvest chlorine dips has also shown potential as an effective postharvest treatment in the reduction of pesticide residues on apple fruits (Hendrix, 1991). The use of ozonated water dips shows similar potential as an alternative postharvest treatment method.

Apple (*Malus* x *domestica* Borkh.) is considered to be a major agricultural product with substantial economic value. In terms of annual tonnage produced, apples are the third most important fruit crop grown in the United States (Downing, 1989). Michigan is one of the nation's most important apple producing states, with 9% of the total U.S. production in 1987-1990 (Ricks and Hull, 1992). As a result of its high economic value as well as the large number of plant diseases (apple scab, powdery mildew and sooty blotch), insects (codling moth, apple

maggot, scales and apple aphids), and mites (spider mites) that infest apples during their growth, significant quantities of pesticides are often necessary for the protection of this crop. This leads to residues on (or in) the fruit at harvest. Although these residue levels are generally below established tolerances, consumer wariness warrants efforts to further reduce pesticide residues.

Three pesticides, azinphos-methyl, captan and formetanate hydrochloride, were selected in this study and used in the spray schedules of apple fruits. These three pesticides are used in the control of the major diseases, insects and mites that affect apples. Azinphos-methyl (Guthion®) is a non-systemic organophosphrous insecticide that acts both by contact and ingestion. Captan is used widely as a non-systemic organosulphur fungicide in the prevention of fungal diseases of pome fruits and grapes. Formetanate-hydrochloride (Carzol®) is an insecticide/acaricide that is characterized by its ability to evoke a variety of behavioral and other effects in several plant insects, beet fly, mites and thrips. Application of these pesticides before harvest is often necessary for the protection of fruits during the preharvest period. Only pesticides with no evidence of carcinogenicity according to Environmental Protection Agency (EPA) standards were selected for this study.

The objective of the present study was to determine the effectiveness of chlorine and ozone as postharvest washes used in the dissipation of pesticide residues in a solution and on fresh and processed apple fruits. Such treatments can be used in conjunction with an Integrated Pest Management (IPM) program to ensure

undetectable or negligible residues of the applied pesticides on fresh and processed apple fruits.

LITERATURE REVIEW

A. Pesticide Use And Monitoring

Between 1964 to 1982, pesticide usage increased from 225 million to 558 million pounds (Osteen and Szmedra, 1989). Over the years, organophosphates, carbamates, and pyrethoids have gradually replaced the more persistent organochlorines. These insecticides, especially the pyrethoids, are safer and more effective, thereby requiring less active ingredients (Osteen and Szmedra, 1989). The use of fungicides has also increased ten fold since 1964 (Osteen and Szmedra, 1989).

The increased use of pesticides has been a direct consequence of the substantial loss of fruit crops caused primarily by insects and diseases. In the U.S., a third of all crops is lost to pests prior to harvest, and an additional 9% is lost to pests after harvest (Pimental, 1976). A major portion of these losses are due to the demand by the consumer for cosmetically perfect produce of high quality standards.

With the increased usage of pesticides, consumer concern about pesticide residues on produce has increased. This concern initiated increased monitoring of pesticide residues on fresh produce and processed foods to reassure the public about the safety of the food supply. The National Food Processors Association Protective Screening

Program was formed in 1960 to evaluate pesticides in processed foods. The goal of the program was to prevent illegal pesticide residues from contaminating processed foods (Elkins, 1989). During the same period, the Food and Drug Administration (FDA) started a large scale monitoring program for pesticide residues on fresh produce. The program was divided into two areas of monitoring: (i) regulatory or commodities monitoring, and (ii) the Total Diet Study, which evaluates pesticides in prepared food where the original ingredients were purchased from retail stores. The Total Diet Study evaluates 234 foods for over 100 pesticides based on the diets of males and females from various age groups. This study is carried out four times a year in three cities in each of four geographical regions (Lombardo, 1989). The levels of most pesticides found were orders of magnitude lower than EPA-established residue tolerances, and less than 1% were in violation. However, Pimentel (1983) estimated that at least 50% of the food items contain detectable pesticide residues.

B. Pesticides Involved

Three pesticides, an insecticide, a fungicide and an acaricide/insecticide, were selected for use in this study. These pesticides are classified as non-carcinogenic and are able to control the major disease, insect and mite pests that damage apples.

(i) Azinphos-methyl: It's chemical name is O,O-dimethyl-S-[(4-oxo-1,2,3-benzotrizazin-3(4H)-yl)-methyl]-phosphorodithioate and is commercially known as Guthion®.

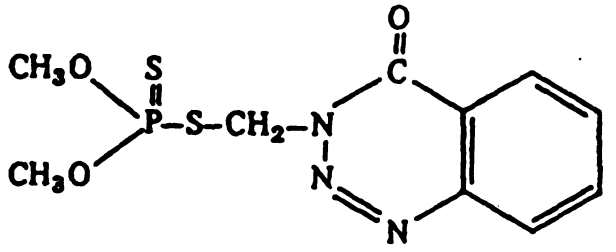


Figure 1 : Structure of Azinphos-methyl

Azinphos-methyl is an insecticide marketed for the control of many insect pests on a wide range of crops such as fruits, nuts, vegetables, field crops and ornamentals. It is generally applied in ultra-low volume for control of various insect pests on field, fruit and forage crops. This pesticide has a broad spectrum of activity, especially against lepidopterous larvae, bugs, sawfly larvae, fleas, scale insects and aphids (Worthing and Hance, 1991). Its solubility in water is 28 mg/L at 20°C.

(ii) Captan: Captan is the common name adopted for N-trichloromethylthio-4-cyclohexene-1,2-dicarboximide.

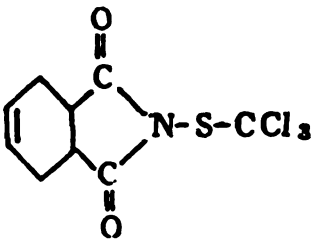


Figure 2 : Structure of Captan

Captan is widely used as a surface fungicide in the control of scabs, blotches, rots, mildew, and other diseases on fruits, vegetables, nut and ornamental crops at a typical rate of 1.2 gram active ingredient (a.i.) per liter (Worthing and Hance, 1991). It is also used in general purpose pesticide mixes. The major diseases of apple, pear and grape which captan is effective against are apple scab, pear scab, downy mildew, and Botrytis bunch rot, respectively. The solubility of captan in water is 3.3 mg/L at 25°C.

(iii) Formetanate-hydrochloride: The chemical name is [m-[[(dimethylamino)methylene]amino]phenylmethylcarbamate] hydrochloride.

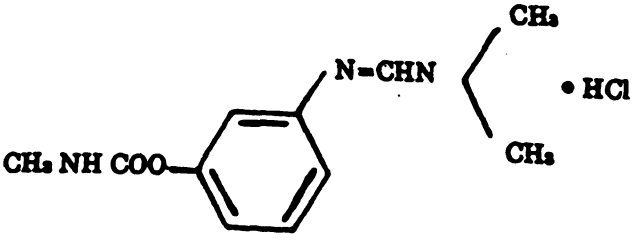


Figure 3 : Structure of Formetanate-hydrochloride

Formetanate-hydrochloride, which is marketed under the tradename Carzol®, is used as an acaricide and insecticide for the control of spider mites, rust mites, certain aphids, thrips, lygus bugs, leaf hoppers, slugs and snails on a variety of orchard fruit (Jenny and Kossmann, 1978). It is applied on citrus, pome and stone fruits (Worthing and Hance, 1991). Formetanate-HCl is especially effective against organophosphate resistant mites. Formetanate-HCl is completely soluble in water (> 500 g/L), while the solubility of formetanate is < 1 g/L at room temperature.

C. Pesticide Residues in Fruits and Vegetables

With concerns focused on the potential risk of the pesticides used, information regarding residue on fruit crops has been sought over the years on many pesticides. Most data were gathered from controlled field trial studies. Data on the effect of processing on the residue levels on various fruits and vegetables have been reported in numerous studies. Literature on the three pesticides used in this study were readily available in some instances, and limited in others.

In general, residue levels in crops are dependent on a number of factors such as rate and frequency of application, nature of the plant surface, and weather conditions such as rainfall, temperature, humidity, sunlight, and wind (Anderson *et al.*, 1974).

The contact and ingestion insecticide, azinphos-methyl was first field-evaluated against cotton insects in Mexico in 1954 and registered

for use on cotton in the U.S. in 1956 (The Chemargo Division Research Staff, 1974). Following the introduction of azinphos-methyl to American agriculture on cotton, the compound has come into widespread use on fruits, field crops such as potatoes and apples, forage crops such as alfaalfa and clover and many vegetables. The maximum residue limit for azinphos-methyl on apples in the U.S. is 2 ppm (Code of Federal Regulations, USA, 1990).

The half-life for azinphos-methyl on vegetables and forage crops grown under field conditions ranges from three to five days. Azinphos-methyl persists on tree fruits somewhat longer than on field crops (Anderson *et al.*, 1974). The average half-life on apples is six days, and is considerably longer on citrus fruit due probably to the high oil and acid content of the rind.

Several supervised residue trials were carried out on apples in Europe and the U.S. (FAO Plant and Protection Paper, 1991). A wet powder (WP) formulation was applied to the crop, with dosage rates of 0.51-1.68 kg a.i. ha^{-1} (4-6 applications). Residues found in U.S. trials 7 days after the last application on apples were 0.14-1.8 ppm.

Belanger *et al.* (1991) determined azinphos-methyl residues on apples in Canada, where the trees were sprayed with the insecticide at different plant stages. Residue analysis revealed detectable residues on the foliage until mid season. However, negligible residue levels were found on the peel and the whole fruit at harvest. In another study, Winterlin *et al.* (1974) found azinphos-methyl residues on grape leaves at approximately 20% of their original levels at harvest 42 days after the

initial application. Residue levels on the fruit were 12, 14 and 4.4 ppm at 28, 31 and 42 days after application, respectively.

The effect of processing has been shown to reduce azinphos-methyl residues in food products. Gunther *et al.* (1963) determined that 71-94% of the azinphos-methyl on orange rind is removed by normal washing procedures. No azinphos-methyl was detected in the pulp (edible portion). In another study, Anderson *et al.* (1963) reported that standard washing procedure removed 30% of azinphos-methyl residues from oranges. Processing of grapes into juice also resulted in a reduction of azinphos-methyl residues (The Chemargo Division Research Staff, 1974). When whole grapes were fortified with azinphos-methyl at 5 ppm, the juice pressed at room temperature contained 2.6 ppm of azinphos-methyl. Juice pressed from grapes subjected to heating contained about 1.71 ppm of the pesticide.

The fungicide captan is widely used for the prevention of fungal diseases of pome fruits and grapes around the world. On a global scale, official maximum residue limits for captan range from 3 to 50 ppm. The maximum residue limits for captan on apples in the U.S. is 25 ppm (Frank *et al.*, 1985).

Frank *et al.* (1985) studied the persistence of captan on apples, grapes and pears in Canada between 1981-1983 over a 14-day period following the last application. Residues declined significantly in seven of nine experiments, with levels on apples below 5 ppm after only 3 days following the last spray. Correlation between rainfall and captan residues were also observed. Captan residues on cherry and peach fruits, where the trees were sprayed with captan at 2.4-4.5 kg ha^{-1}, were

determined by Northover *et al.* (1986). Residue analysis showed residue levels of approximately 5 ppm of captan, and greatly decreased with increased rainfall.

Experiments conducted on the degradation behavior of captan on greenhouse tomatoes revealed that the maximum concentration (initial deposit) of captan on the tomatoes were 3.4 ppm after the last application (El-Zemaity, 1988). The percent dissipation of captan residues after 7 days from application were 11.5-55.0% and 37.3-59.5% when sprayed at 7 and 15 days intervals, respectively.

Several postharvest treatments have been reported to remove captan residues on some fruits. Following a 20 minute cold water rinse, 14% of residues were removed from strawberries and 95% reduction after a 5 minutes cook (Ritcey *et al.*, 1984). Northover *et al.* (1986) determined that captan was easily removed by washing, reducing residues on sweet cherry by 70-74%. The same study also reported that 10 seconds of hand washing removed a maximum of 50% from the initial deposit of captan in peaches. Vigorous washing with a stiff bristle brush removed about 70% of the residue from peaches. Household washing using running tap water reduced captan residues by 97.7-98.9% on tomatoes (El-Zemaity, 1988). Similar levels of reduction were also obtained by cooking tomatoes for 15 minutes at 100°C without washing. Hendrix (1991) showed that captan residues on apples were reduced to less than detectable levels with a chlorine wash compared to water washed and brushed fruits.

Frank *et al.* (1983) determined that captan residues in apples were reduced by washing, peeling, boiling and cooking or a combination of

these procedures. Washing reduced residues of captan by 43-94%, boiling by 70-98%, and a combination of washing and cooking gave almost 100% removal.

Formetanate-HCl residues on fruits crops have been studied by few researchers. In 1985, Hadjidemetriou *et al.* studied the dissipation of formetanate-HCl and several other pesticides on citrus foliage. Formetanate-HCl as carzol® were sprayed at a rate of 1.1 kg a.i. ha^{-1}. Formetanate-HCl residue on the citrus did not appear to dissipate at all, except in the presence of rainfall. The solubility of formetanate-HCl in water is reported to be 500 ppm (Worthing and Hance, 1991), and as such is greatly affected by rainfall.

Residues of formetanate-HCl on and in orange fruits were also studied by Iwata *et al.* (1985). Test plots of orange trees were treated with formetanate-HCl at the maximum permitted dose rates (1.03 kg a.i. ha^{-1}) with a low volume airblast sprayer (940 liters ha^{-1}). Mature fruit samples were collected 5, 7, 10, 13, 17 and 31 days after the spray application. Residues on and in the rind were 0.96, 1.0, 1.2, 1.0, 1.2 and 0.85 ppm, respectively. The residue over the entire sampling period remained essentially constant at 1.0 ppm. Similar fruits, sampled on day 7 and washed under tap water had a residue of only 0.03 ppm.

During 1990-91, a study was conducted at Michigan State University by El-Hadidi (1993) to determine pesticide residues in fresh and processed apple fruits under certain developed pest control programs. A correlation was found between the postharvest intervals (PHIs) and the residue levels of formetanate-HCl and azinphos-methyl. In general, the longer the PHIs the lower the total residue levels. Of

several pesticides applied in the study, azinphos-methyl showed the highest residue levels on fruits (0.3-0.4 ppm). However, the residues detected were much lower than the 2.0 ppm tolerance level. Formetanate-HCl total residues in the whole fruit ranged from 0.07 to 0.13 ppm. These levels were also lower than the established tolerance of this pesticide on or in apples.

In the same study by El-Hadidi (1993), washing/grading of apples were shown to significantly reduce the residue levels of formetanate-HCl and azinphos-methyl. Processing of the apples into apple products such as apple slices, sauce and juice were also significantly effective in reducing the pesticide residue levels to non-detectable amounts in some and residues lower than those on fresh fruits in others.

D. Degradation of Pesticides In Solution

The fate of pesticides in solution, due to hydrolysis, ozonation and chlorination must be understood in order to understand its degradation behavior during a wash treatment, after it is washed off the fruit and its safe disposal as pesticide waste water.

(I) Hydrolysis

Laboratory studies on the effect of pH and temperature on the breakdown of pesticides in aqueous solution have been conducted to provide information on their relative persistence.

The hydrolysis of azinphos-methyl may proceed under acid, neutral and alkaline pH, but is generally more persistent under acidic conditions (Faust & Gomma, 1972). Azinphos-methyl was observed in this study to have its greatest stability under acidic conditions with increasing rate of hydrolysis at higher pH. At pH 3.0, 7.0 and 9.0, the $t_{1/2}$ values for azinphos-methyl were 9, 4.8 and 0.6 hours respectively. In another study, Liang and Lichtenstein (1972) found that azinphos-methyl was relatively stable in water below pH 10. At pH 11, 97% of the pesticide was converted to degradation products such as anthranilic acid, benzazimide and 3 unidentified compounds.

Faust and Gomma (1972) also studied the effect of temperature on the degradation of azinphos-methyl, and showed the compound to be less stable as temperature increased. Liang and Lichtenstein (1972) showed that rapid degradation of azinphos-methyl occurred above 37°C.

Captan readily undergoes hydrolysis in water with a maximum half-life of about half a day (Wolfe *et al.*, 1976). At pH 6, 7 and 8.25, the half life of captan in water was 250, 175 and 10 minutes, respectively. No half life data were available for captan at pH 10 and above. In the same study, the authors determined the products of captan hydrolysis as 4-cyclohexene-1,2-dicarboximide, carbon dioxide, hydrochloric acid and sulfur (Figure 4).

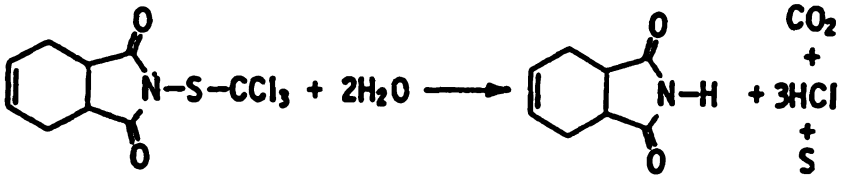


Figure 4 : Hydrolysis of Captan

As anticipated, the rate of hydrolysis of captan increased with increasing temperature in both neutral and alkaline (pH 9) conditions.

Formetanate-HCl is a weak basic compound which hydrolyses slowly in acidic media but considerably faster in neutral and basic aqueous solutions, especially at higher temperature (Jenny and Kossmann, 1978). It was found that formetanate at 1 ppm in aqueous solution at pH 8 decomposed by 20% after one day at room temperature (Lawrence *et al.*, 1981). Hydrolysis of formetanate-HCl at pH 9 reached 50% in 100 minutes (Su and Zabik, 1972).

(II) Chemical Oxidation

Chlorine, chlorine dioxide, potassium permanganate, and ozone have been employed historically for the oxidation of organic compounds at water treatment plants, and were consequently investigated for their capacity to degrade organic pesticides (Gomma and Faust, 1974). The present study is focused on the use of ozone and chlorine in the degradation of pesticides. Besides a study on the effect of chlorination of captan (Suzumoto *et al.*, 1983), no other literature is available on the effect of ozonation or chlorination on azinphos-methyl, captan and formetanate-HCl in solution. However, there are numerous studies that have investigated the effect of ozone and/or chlorine on a wide range of other pesticides.

As a strong oxidant and an electrophile (Kirk and Mitchell, 1980), chlorine as hypochlorous acid can oxidize various organic compounds

(Dychdala, 1977). In their study on the effect of residual chlorine on the degradation of pesticides in water, Suzumoto *et al.* (1983) showed that thirteen kinds of pesticides were easily degraded by chlorine at concentrations of 1 ppm. It was also shown that pesticides containing sulfur within their chemical structure seemed to be easier to degrade. Among the pesticides studied, benthiocarb showed rapid degradation in solution containing 0.2 ppm residual chlorine where only 6% of the original concentration remained after 3 hours. Captan at 0.04 ppm in a solution containing 1 ppm chlorine degraded by only 5% after 24 hours.

Hilden *et al.* (1979) studied the effectiveness of chlorine bleach in the removal of some pesticides from clothing fabrics, and determined that chemical structure and water solubility were two factors that explained the differences in percent removal of the pesticides. The study showed that the organophosphate and carbamate insecticides (parathion, diazinon and carbofuran) were easily degraded by oxidation due to the weak phosphoric and carbamic acid ester linkages of those pesticides. However, the pesticide lindane was not susceptible to oxidation due to its strong saturated cyclic bonding of cyclohexane and the chlorine-carbon bonding.

An increase in pH from 7 to 8 decreased the half-lives of carbaryl, 1-naphthol, and propoxur from 6- to 162-fold, while chlorinated water (10 ppm hypochlorite solution) shortened the half-lives of the pesticides from 3- to 62-fold (Miles *et al.*, 1988). The authors suggested that the effect of chlorination on half-life was greater at pH 8 than pH 7, and was due to the importance of chlorine speciation ($HOCl/OCl^-$; pK_a=7.5) in the degradation of the pesticides in chlorinated water.

Other fate studies of pesticides in chlorinated water have shown that paraquat and diquat (Gomma and Faust, 1971), phenylureas, phenylamides and phenylcarbamates (El-Dib and Aly, 1977), thiobencarb (Au *et al.*, 1984) and diazinon (Dennis *et al.*, 1979) degraded faster in the presence of chlorine, and that pH was an important factor in the degradation rate. In general, all the above studies showed that oxidation was a major effect of chlorination and that other mechanisms such as hydrolysis and chlorination occurred when organic compounds react with chlorine in water.

Robeck *et al.* (1965) ozonated aqueous solutions of lindane, dieldrin, DDT and parathion and found that dosages of 10 to 38 mg/L of ozone were required to destroy these pesticides to acceptable levels. These dosages were considered to be too high to be practical.

Aqueous solutions containing 10 mg/L of malathion were ozonized at ozone dosages of 3.5 mg/L, reducing the concentration of malathion to 2 mg/L (Gabovich *et al.*, 1980). Increasing the ozone dosage to 9.8 mg/L reduced malathion to 1 mg/L. A dosage of 26 mg/L of ozone caused 100% destruction of malathion.

Mallevialle *et al.* (1978) ozonated aqueous solutions of aldrin, and found this compound to be easily degraded by ozone. Prengle and Mauk (1978) showed that ozonation of DDT in water proceeds very slowly, but the oxidation rate is accelerated by combining UV radiation with ozonation.

The use of ozone alone as an oxidant for treatment for several herbicides has been compared to combined UV-O_3 for 11 major pesticides and gave comparable rates of oxidation (Kearney *et al.*, 1987).

The pesticides that were used in the study included 9 formulated herbicides (alachor, atrazine, butylate, cyanazine, 2,4-D, metolachlor, metribuzin and trifluralin) and two formulated insecticides (carbofuran and malathion). The time required for 90% destruction was dependent on the concentration and increased as the concentration of pesticide increased. The average degradation time for all 11 pesticides at concentrations between 10 and 100 ppm was about 60 minutes.

According to Laplanche and Martin (1982), organochlorous pesticides are not easily oxidized and need exhaustive ozonation conditions. On the other hand, organophosphorous pesticides are very sensitive to ozonation.

E. Chemistry of Chlorine and Ozone

(I) Chlorine Chemistry

Assessment and prediction of chlorination effects on organic compounds present in waters and food systems requires a knowledge of chlorine reaction mechanisms, concentration of reactants and the concentration of reaction products.

When chlorine as calcium hypochlorite is added to water, a mixture of hypochlorous acid (HOCl) and hydrochloric (HCl) acids is formed:

$$Cl_2 + H_2O \longrightarrow HOCl + H^+ + Cl^- \qquad \text{Equation 1}$$

This reaction is essentially complete within a few seconds. In dilute solution and at pH levels above 4, the equilibrium shown in Equation 1 is displaced to the right and very little Cl_2 exists in solution (Laubusch, 1962). Hypochlorous acid is a weak acid (Equation 2) with a dissociation constant at 0°C to 25°C of 1.6 to 3.2 $x10^{-8}$ and a pKa of 7.8 to 7.5 (Morris, 1966).

$$HOCl \longrightarrow H^+ + OCl^- \qquad \text{Equation 2}$$

As a result, the chlorine species present in the pH range 3.0-8.0 (the range for most foods) would be HOCl and the hypochlorite ion. At pH 5.0, the species distribution would be 99.7% HOCl vs. 0.03% OCl^- for a 10^{-2} M chlorine solution at 20°C. At pH 8.0, species distribution shifts to 23.2% HOCl vs. 76.8% OCl^- for the same 10^{-2} solution (Figure 5). Other species besides HOCl including the hypochlorous hydronium ion, H_2OCl^+, the chloronium ion, Cl^+ and Cl_3^+ may be present in very low concentrations and/or have very low specific reactivities (Laubusch,1962).

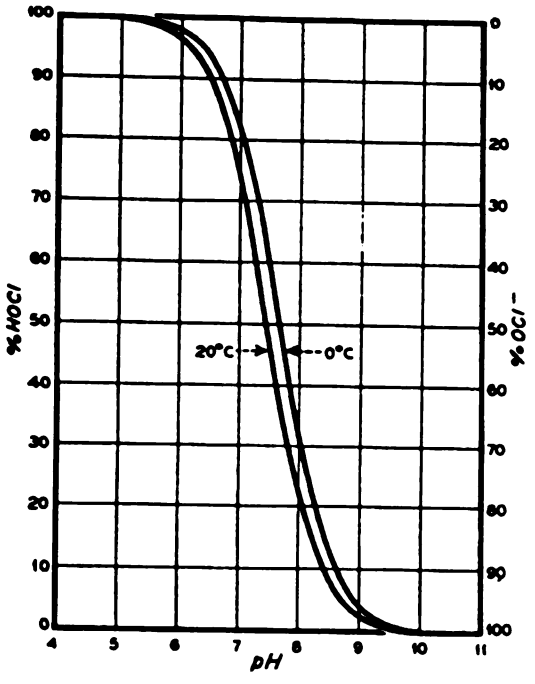


Figure 5 : Relative Amounts of HOCl and OCl^- Formed At Various pH Levels (Fair *et al.*, 1948)

The tendency for chlorine to acquire electrons is so strong that it may split from the molecule and form the reduced chloride ion by displacement (Wei *et al.*, 1985). This is the basis for the oxidation reactions of HOCl with organic compounds. The antibacterial efficiency and sporicidal effectiveness of chlorine solution has been shown to decrease with increasing pH (Dychdala, 1977). An increase in temperature will decrease the percent of HOCl, and consequently its reactivity with organic compounds (Wei *et al.*, 1985).

The capability of one substance to oxidize another is measured by its Oxidation Potential, normally expressed in volts of electrical energy.

The oxidation potential is a measure of the relative ease by an atom, ion, molecule or compound to lose electrons, thereby being converted to a higher state of oxidation. In general, the higher the oxidation potential, the stronger it is as an oxidant. As indicated in Table 1, HOCl is a stronger oxidizing agent (1.49 V) than is free chlorine (1.36 V), so that HOCl is actually more desirable when using chlorine as an oxidant in aqueous solution.

Table 1 : Oxidation-Reduction Potentials Of Various Compounds

Reactions	Potential In Volts (E°) 25°C
$F_2 + 2e \longrightarrow 2F^-$	2.87
$O_3 + 2H^+ + 2e \longrightarrow O_2 + H_2O$	2.07
$H_2O_2 + 2H^+ + 2e \longrightarrow 2H_2O$ (acid)	1.76
$MnO_4^- + 4H^+ + 3e \longrightarrow MnO_2 + 2H_2O$	1.68
$HClO_2 + 3H^+ + 4e \longrightarrow Cl^- + 2H2O$	1.57
$MnO_4^- + 8H^+ + 5e \longrightarrow Mn^{2+} + 4H_2O$	1.49
$HOCl + H^+ + 2e \longrightarrow Cl^- + H_2O$	1.49
$Cl_2 + 2e \longrightarrow 2Cl^-$	1.36
$HOBr + H^+ + 2e \longrightarrow Br^- + H_2O$	1.33
$O_3 + H_2O + 2e \longrightarrow O_2 + 2OH^-$	1.24
ClO_2 (gas) $+ e \longrightarrow ClO_2^-$	1.15
$Br_2 + 2e \longrightarrow 2Br^-$	1.07
$HOI + H^+ + 2e \longrightarrow I^- + H_2O$	0.99
ClO_2 (aq) $+ e \longrightarrow ClO_2^-$	0.95
$ClO^- + 2H_2O + 2e \longrightarrow Cl^- + 2OH^-$	0.90
$H_2O_2 + 2H_3O^+ + 2e \longrightarrow 4H_2O$ (basic)	0.87
$ClO_2^- + 2H_2O + 4e \longrightarrow Cl^- + 4OH^-$	0.78
$OBr^- + H_2O + 2e \longrightarrow Br^- + 4OH^-$	0.70
$I_2 + 2e \longrightarrow 2I^-$	0.54
$I_3 + 3e \longrightarrow 3I^-$	0.53
$OI^- + H_2O + 2e \longrightarrow I^- + 2OH^-$	0.49
$O_2 + 2H_2O + 4e \longrightarrow 4OH^-$	0.40

Handbook of Chemistry & Physics, 56th Ed. (1975-76)

(II) Ozone Chemistry

Ozone is an unstable gas which is partially soluble in water (about 10 times the solubility of oxygen) and has a characteristic penetrating odor, readily detectable at concentrations as low as 0.01 to 0.05 ppm (Katz, 1980). It is a powerful oxidant, having an oxidation potential (2.07 V) higher than HOCl and free chlorine (Table 1).

Ozone is thought to decompose in water according to Equation 3-6 as a cyclic chain mechanism as shown in Figure 6.

$$O_3 + H_2O \longrightarrow HO_3^+ + OH^- \qquad \text{Equation 3}$$

$$HO_3^+ + OH^- \longrightarrow 2HO_2^{\cdot} \qquad \text{Equation 4}$$

$$O_3 + HO_2^{\cdot} \longrightarrow HO^{\cdot} + 2O_2 \qquad \text{Equation 5}$$

$$HO^{\cdot} + HO_2^{\cdot} \longrightarrow H_2O + O_2 \qquad \text{Equation 6}$$

The overall stoichiometry is shown in Equation 7:

$$2O_3 \longrightarrow 3O_2 \qquad \text{Equation 7}$$

Ozone reacts with organic compounds in four pathways as depicted in Figure 6: (1) ozone with hydroxyl ions (OH^-) via intermediate radicals to $HO^{\cdot}$; (2) ozone with organic molecules (M) via intermediate radicals to $HO^{\cdot}$; (3) ozone with M to H_2O_2 ; (4) ozone with $HO_2^{\cdot}$ to $HO^{\cdot}$ (Stockinger *et al.*, 1994)

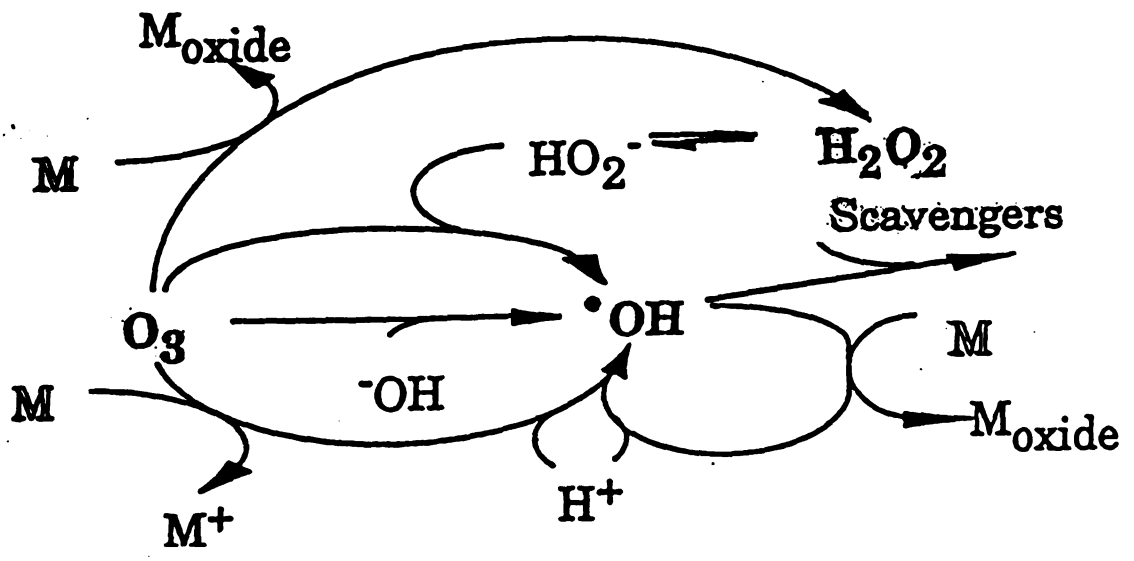


Figure 6 : Simplified Reaction Model of Ozone With Organic Compounds M in Water (Stockinger *et al.*, 1994)

Decomposition of ozone can be initiated by hydroxide ions, formate ions, or a variety of other species, and in pure water the chain ends (Glaze, 1987). A single initiation step can cause the decomposition of hundreds of molecules of ozone before the chain ends. The electrophilic direct ozonolyses by molecular ozone of double or triple bonds and the reactions with HO˙ radicals are the two most important steps (Stockinger *et al.*, 1994). High formation rates of HO˙ radicals in water by ozone and low direct ozonolyses rates occurs at high pH and vice versa at low pH values.

As such, oxidation mechanisms differ highly with pH. In general, low pH and high inorganic carbon concentration encourage the direct

molecular attack which is more selective and less hindered by competitive reactions (i.e. oxidant-demanding substances). On the other hand, indirect radical attack is favored by high pH, low concentration of carbonates, and presence of activating substances, such as hydrogen peroxide.

Under practical conditions, the dose of ozone is never enough to satisfy the ultimate demand, and the reaction with organic compounds will generally stop when the supply of ozone is depleted. Typically, this will be long before the organic substances have become mineralized to carbon dioxide (Glaze, 1987). However, the use of ozone creates a distinct advantage over the use of chlorine as the danger of the formation of harmful degradation products such as chlorinated by-products can be eliminated.

F. Other Uses of Chlorine and Ozone Treatment

(I) Chlorine

Aqueous chlorine is used extensively in the food industry to sanitize food processing equipment and food containers (100-200 ppm), to rinse and convey raw fruits and vegetables (1-5 ppm), and to cool heat-sterilized canned foods (1-2 ppm) (Foegeding, 1983). Chlorine is also widely used in the fishing industry, in washing nutmeats, and in the processing of seafood, poultry, and red meats (Wei *et al.*, 1985).

Chlorine gas is used in the flour industry as an oxidizing and bleaching agent to improve the quality of flour (Johnson *et al.*, 1980).

The use of chlorine as a postharvest chlorine dip has also been shown to be effective in the control of decay in apples (Baker and Heald, 1932) and d'Anjou pears (Spotts and Peters, 1980) as well as to remove sooty blotch from oranges (Vanderplank,1945) and apples (Hendrix, 1991).

In the United States, chlorine and hypochlorites are acceptable for use in food processing and for bottled water as prescribed by the 1958 amendment to the Federal Food, Drug and Cosmetic Act of 1938 (FD&C Act). This amendment allows for the continuing use of generally recognized as safe (GRAS) substances that were commonly used in the United States before 1958.

(II) Ozone

The ability of ozone to disinfect polluted water has been recognized for many decades, and has become an integral part of many water treatment facilities around the world. The most common applications of ozone are for disinfection by-product control and biological stabilization, or minimization of the microbiological growth potential of the water (Brink *et al.*, 1990).

Manifold possibilities for using ozone in the food industry and agriculture are also possible due to the bactericidal and germicidal activity of ozone, as well as its spore killing abilities (Horváth *et al.*,

1985). Utilization of these properties has made ozone suitable for increasing the storage life of perishable foods in refrigerated premises. This practice first started in Europe in the early 1900s, where ozone is used in the sterilization of air entering the storage room. During storage, ozone exerts a three-fold effect by destroying the microorganisms, oxidizing the odors and affecting the processes of metabolism. Fruits and foodstuffs exposed to ozone can undergo changes in its metabolism by inactivating their metabolic products. At the same time it reacts with other materials present that can be oxidized and thereby it destroys fragrances and odors (Horváth *et al.*, 1985).

Although few publications or research reports have been made available, the use of ozone is increasing in several major cold storage plants in Europe, and even in the U.S. The storage life of fruits, vegetables, and meats have been shown to increase when kept in an atmosphere of ozone gas (Horváth *et al.*, 1985). Other successful applications of ozone include uses in the beverage and milk industry.

MATERIALS AND METHODS

MATERIALS

A. Apple Samples

Mature Golden Delicious apples were harvested from the Botany Research Field Laboratory at Michigan State University, East Lansing, one day after the last pesticide application. The fruits were hand picked randomly from various regions of the treated trees, thoroughly mixed, and representative samples of 8-9 fruits were set aside for each replication. The samples were stored at -20°C for approximately 10 days until they were prepared for residue analysis.

B. Reagents

(I) Solvents

All organic solvents used for preparation of pesticide stock solutions, in sample extraction, cleanup and high performance liquid chromatography (HPLC) were distilled-in-glass grade. Acetone and

methylene chloride were obtained from Mallinckrodt, Co. (Paris, KY) and hexane and acetonitrile were obtained from EM Science, Inc. (Gibbstown, NJ).

(II) Chemicals

Azinphos-methyl and captan standards were obtained from AccuStandard, Inc. (New Haven, CT). Formetanate-HCl standard was obtained from Chem Services, Inc. (West Chester, PA). The stock solutions of azinphos-methyl and captan were prepared in hexane at a concentration of 50 μg/100 ml, while formetanate-HCl was prepared in acetonitrile at a concentration of 50 μg/100 ml. The standards were protected from light and stored in a refrigerator.

Chlorine solutions at 50 and 500 μg/ml were prepared from calcium hypochlorite (Mallinckrodt, Co., Paris, KY) as a source of chlorine. Sodium thiosulfate, sodium sulfate, potassium iodide, potassium indigo trisulfonate, sulfuric acid and hydrochloric acid were all reagent grade.

C. Glassware

All glassware was thoroughly washed with detergent and warm water then rinsed with distilled water. The glassware was then rinsed

twice with acetone and hexane before being placed in an oven overnight before use.

METHODS

A. Study on Degradation of Pesticides In Solution

(I) Sample Preparation

Laboratory studies were conducted in a model system to determine the effects of: (i) Calcium hypochlorite at two concentrations (50 and 500 ppm) and Ozone (0.25 ppm); (ii) three pH's (4.5-4.8, 7.0, 10.7); and (iii) 2 temperatures [ambient (21°C) and elevated (44°C)] on each of the three pesticide over a 30 minute period. There were three replications per treatment. Aqueous solutions were unbuffered for the control and ozone treatments and buffered (0.2 M citrate-phosphate) for the chlorine treatments at pH 4.5. Aqueous solutions buffered at pH 7.0 (0.2 M sodium phosphate) and pH 10.7 (0.2 M carbonate-bicarbonate) were also prepared. The pH values were selected so as to aid in understanding the degradation behavior of the 3 pesticides in acidic, neutral and alkaline medium. Degradation of the pesticides was studied over a 30 minute period because the typical dip time for apples in a commercial plant is 10-15 minutes and would rarely exceed 30 minutes. Temperatures of 21±1°C and 44±1°C were maintained in a water bath.

For the chlorination study, an appropriate amount of calcium hypochlorite stock solution (5000 ppm) was added to each pH solution to bring the final chlorine concentration to 50 or 500 ppm. Each pH solution (pH 4.5, 7.0 and 10.7) was spiked with 4 ml of the pesticide stock solution (500 ppm) to give a final concentration of 2 ppm. Total available chlorine was determined by sodium thiosulfate titration method (Standard Methods for Examination of Water and Wastewater, 1987) before and after each sampling run. The 1 L sample solution was placed in a 2 L glass beaker with a magnetic stirrer to ensure thorough mixing. A 40 ml aliquot of the sample was transferred at 0, 5, 15 and 30 minutes intervals into 200 ml French square glass bottles. A 0.5% 0.1M sodium thiosulfate solution (Segall, 1968) and 40ml methylene chloride were immediately added to the samples to quenched the reaction. Approximately one minute elasped between transfering the sample to the bottle and the quenching of the reaction. The samples were stored at -20°C for subsequent pesticide residue analysis.

For the ozonation study, 1 L of sample solution was pumped from a 2 L glass beaker through a Lifex EV 200 ozone water system (Lifex Corporation, Birmingham, MI) using a Variostatic pump (Manostat, NY, NY) at a flow rate of 1.4-1.5 ml/minutes (Figure 7). The solution was recirculated during the entire sampling period and 40 ml aliquots were pipetted from the glass beaker at 0, 5, 15 and 30 minute intervals into 200 ml French square glass bottles. 40ml methylene chloride was immediately added to the sample to quenched the reaction. Approximately one minute then elasped between transfering the sample to the bottle and the quenching of the reaction. Ozone concentration in

the solution was monitored before and after the sampling period using the indigo colorimetric method (Standard Methods for the Examination of Water and Wastewater, 1987). The samples were stored at -20°C for subsequent pesticide residue analysis.

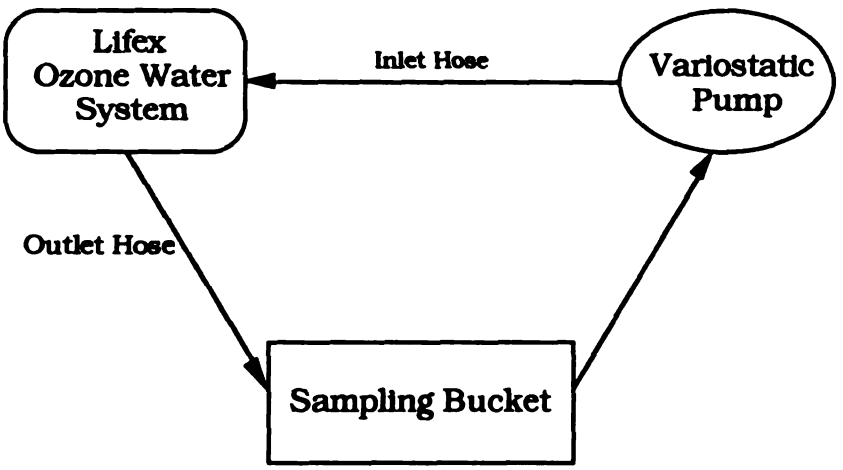


Figure 7: Schematic Diagram of Ozone Treatment

(II) Chlorine Determination

Total residual chlorine was measured using the iodometric method. Ten ml and 100 ml samples from the 500 ppm and 50 ppm chlorine sample solution, respectively, were pipetted to Erlenmeyer flasks containing 5 ml acetic acid and 1 gram potassium iodide. The

stirred samples were titrated with 0.01 N sodium thiosulfate, $Na_2S_2O_3$, until the endpoints were reached.

The total residual Cl_2 was determined using the formula:

$$\text{mg } Cl_2/L = [(A \pm B) \times N \times 35450] / \text{ ml of sample}$$

where A was the amount $Na_2S_2O_3$ titrated for the sample (in ml), B was the amount $Na_2S_2O_3$ titrated for the blank (in ml) and N was the normality of $Na_2S_2O_3$ (0.01 N).

(III) Ozone Determination

Ozone detection and monitoring were performed using the indigo colorimetric method as described in Standard Methods for the Examination of Water and Wastewater (1987). All reagents were prepared just prior to use. The ozone concentration was monitored before and after each sampling run. The ozonated water was collected directly from the outlet hose leading from the ozonator into a 100 ml volumetric flask containing 10 ml of the indigo reagent to minimize loss of ozone. A separate volumetric flask was filled with distilled water containing 10 ml indigo reagent to serve as a blank. The solutions were mixed thoroughly and the absorbance of each solution was immediately measured at 600 nm in a 10 cm cell. A Spectronic-70 spectrophotometer (Milton Roy Co., Rochester, NY) was used to monitor the change in absorbance.

The concentration of ozone, in mg/L, was calculated using the formula:

$$mg\ O_3/L = (100 \times \Delta A) / (f \times b \times V)$$

where ΔA is the difference in absorbance between sample and blank solution, b is the path length (10 cm), V is the volume of the sample (90 ml), and f is a constant of 0.42.

(IV) pH Determination

The pH of the sample solutions were determined in duplicate using a pH meter model 601A (Corning Glass Works, Medfield, MA) before and after each 30 minutes sampling period to ensure the pH of the solution did not deviate from the desired pH conditions. Appendix I shows the pH readings of samples before and after each sampling period.

B. Studies On Fresh and Processed Apples

(I) Pesticide Application and Spray Schedule

Golden Delicious apples were grown at the Botany Research Field Laboratory at Michigan State University in East Lansing. Maintenance sprays of pesticides were applied throughout the growing season, and a final application of guthion®, captan and carzol® at 5X the

Table 2 : 1994 Spray Schedule for Golden Delicious Apples

Dates	Chemical	Formulation	Rate	Purpose
April 30	Polyram	80DF	2.4 lbs/A	maintenance spray
	Rubigan	1EC	0.1 lbs/A	
	Spray Oil	6E	2%/1.6gal	
May 4	Asana	XL 0.66EC	0.04 lbs/A	maintenance spray
May 10	Polyram	80DF	2.4 lbs/A	maintenance spray
	Rubigan	1EC	0.07 lbs/A	
May 12	Streptomycin	17%	0.34 lbs/A	maintenance spray
May 18	Streptomycin	17%	0.34 lbs/A	maintenance spray
May 19	Polyram	80DF	2.4 lbs/A	maintenance spray
	Rubigan	1EC	0.1 lbs/A	
May 23	Mycoshield	17%	0.68 lbs/A	maintenance spray
May 27	Guthion	50W	0.75 lbs/A	maintenance spray
	Polyram	80DF	2.4 lbs/A	
	Rubigan	1EC	0.07 lbs/A	
June 9	Captan	50W	9 lbs/A	maintenance spray
	Guthion	50W	0.75 lbs/A	
June 16	Captan	50W	9 lbs/A	maintenance spray
June 23	Captan	50W	9 lbs/A	maintenance spray
	Guthion	50W	0.75 lbs/A	
July 11	Guthion	50W	0.75 lbs/A	maintenance spray
July 28	Guthion	50W	0.75 lbs/A	maintenance spray
August 17	Captan	50W	9 lbs/A	maintenance spray
	Guthion	50W	0.75 lbs/A	
October 3	Captan	50W	2 lbs/A	treatment spray
	Carzol	92SP	1.25 lbs/A	
	Guthion	50W	6 lbs/A	

Harvest date : October 4, 1994

recommended label rates were applied just before harvest. Table 2 shows the spray schedule for 1994.

Pesticides were applied with an airblast sprayer at 80 gallons/acre and 300 psi. For the final application, all three products (Captan 50W, Carzol 92SP and Guthion 50W) were tank mixed and applied as a single application. The apples were harvested by hand the following day (1 day PHI) to ensure the maximum amount of residue on the fruits.

(II) Wash Treatments

While the laboratory model study attempted to show the degradation patterns of the three pesticides in aqueous solution, apple fruits spiked with the three pesticides were used to determine the effectiveness of chlorine and ozone dip washes on the removal and degradation of the pesticides residues found on the actual fruits. Eight apples were used per replication (3 replications per treatment) and placed in a 15 L bucket containing 5 L of water. The five treatments were: (1) No wash, (2) Water wash, (3) Ozone wash @ 0.25 ppm, (4) Chlorine wash @ 50 ppm, and (5) Chlorine wash @ 500 ppm. The apples were agitated every minute to maximize contact between the water and the surface of the apples. The temperature, pH, chlorine and ozone concentration were monitored before and after each wash treatment (Appendix II).

A preliminary study was carried out to determine the appropriate dip time that would be sufficient for an effective wash. The preliminary

study utilized 5, 10 and 15 minute dip times for the 5 treatments with no replications. These three dip times were determined to be the typical range for apples in a commercial facility. A 15 minute dip time was subsequently chosen for the actual study. All 5 treatments were used for the apple washes with three replications per treatment.

(III) Sample Preparation

After the wash treatments, the eight apples in each replication were divided into two batches. One batch (4 fruits) was chopped in a Horbart food chopper (Hobart MFG. Co., Troy, OH) and thoroughly mixed to ensure homogeneity. The chopped apples were transferred into plastic Ziplock bags, weighed, and stored at -20°C. These samples were used for analysis of pesticides on and in the unprocessed apple fruits.

The remaining 4 apples were processed into apple sauce. The apples were processed at the Fruit and Vegetable Processing Laboratory, Department of Food Science and Human Nutrition, Michigan State University, East Lansing, MI. The apples were first sliced with a Sunkist slicer and subsequently blanched in a steam blancher for 10 minutes at approximately 110°C. Once the apples were cooked, they were passed through a Langsencamp finisher with a 0.033-0.045 inch screen to remove coarse fibers, seeds, stems, and peel particles. The applesauce samples were transferred into plastic Ziplock bags immediately after finishing, weighed and stored at -20°C for residue analysis.

The water used for the wash treatments was saved and 400 mls from each wash were pipetted into clean 8 oz. French square glass bottles. Analysis of the wash water was carried out to determine the amount of pesticides that were washed off the fruits and did not undergo degradation either on the fruits or in solution. The samples were stored at -20°C for residue analysis.

C. Pesticide Residue Analyses

(I) Extraction and Cleanup of Azinphos-methyl And Captan

Both azinphos-methyl and captan were extracted from water and apple samples with a modification of the method described by Liang and Lichtenstein (1976). Water samples from the model studies were transferred quantitatively into 250 ml separatory funnels and extracted with 3 x 30 ml of methylene chloride. The methylene chloride extracts were collected through a glass funnel containing a glass wool plug and anhydrous sodium sulfate into a turbo vap tube. The extract was evaporated to dryness with a turbo vap evaporator. The dried sample was flushed with a gentle stream of nitrogen gas to remove any traces of methylene chloride which may interfere with the GC analysis. The residue was redissolved in hexane and adjusted to an appropriate volume for GC analysis.

Azinphos-methyl and captan were similarly extracted from chopped apple and applesauce samples according to a modified method

of Liang and Lichtenstein (1976). Fifty grams of the apple sample were blended with 100 ml of acetone with a homogenizer for 3 minutes. The sample was filtered under vacuum through a ceramic buchner funnel with a Whatman #1 filter paper. The filter cake was rinsed twice with 10 ml acetone and transferred to a separatory funnel. The filtrate was extracted twice with 140 ml and 35 ml of methylene chloride, and the methylene chloride layers (lower layer) were collected through anhydrous sodium sulfate in a turbo vap tube. The extract was evaporated to dryness, redissolved in 50 ml of hexane, and transferred through a glass funnel containing sodium sulfate to a separatory funnel. The turbo vap tube was rinsed with an additional 50 ml of hexane and transferred to the separatory funnel. The hexane was partitioned with 3 x 25 ml portions of acetonitrile. The acetonitrile extracts were combined and evaporated to complete dryness under vacuum at 40°C. The residue was redissolved with hexane and brought to an appropriate volume for GC analysis.

Extraction of the water samples from the wash treatments were carried out according to the method used for the extraction of the water samples from the model study, but in proportionally larger amounts. The 100 ml samples were extracted with 3 x 70 ml of methylene chloride. After the methylene chloride extract was dried, the sample was redissolved in 2 ml of hexane for GC analysis.

(II) Extraction and Cleanup of Formetanate-HCl

Extraction and cleanup of both the water and apple samples containing formetanate-HCl was carried out according to modified methods of Lawrence *et al.* (1981). Water samples from the model study were transferred quantitatively into 250 ml separatory funnels. Ten ml of saturated sodium chloride and 3 grams of sodium bicarbonate were added to the samples to adjust the pH of the solution to pH 8-9. The samples were immediately extracted with 3 x 30 ml of methylene chloride. The methylene chloride layer was collected through a glass funnel containing a glass wool plug and approximately 4 grams of anhydrous sodium sulfate into a turbo-vap tube. The combined extract was evaporated to dryness at 30°C in a Zymark Turbo vap evaporator (Hopkin, MA) using nitrogen gas. The dried extract was then flushed with a gentle stream of nitrogen gas to remove any traces of methylene chloride that could interfere with the HPLC analysis. The residue was redissolved in an appropriate volume of acetonitrile for HPLC analysis.

Formetanate-HCl was extracted from the chopped apples and applesauce samples with acidified acetonitrile and cleaned up using acidic and basic aqueous-organic partitioning. Twenty-five grams of the chopped apple or applesauce were blended with 70 ml of acetonitrile containing 0.5% concentrated HCl, using a Pro-200 handheld homogenizer (Pro Scientific, Inc.) for about 3-5 min. The mixture was filtered with suction through a ceramic buchner funnel with a Whatman #1 medium filter paper. The filtrate was transferred into a turbo vap tube with an additional 20 ml of acidified acetonitrile, and evaporated to

an aqueous residue (approximately 3-4 ml). The aqueous residue was transferred to a 250 ml separatory funnel where 20 ml of 0.2 N sulfuric acid and 40 ml of methylene chloride were added to it. The funnel was shaken for 1 minute then allowed to separate into layers. The lower organic layer was discarded. Five grams of sodium bicarbonate and 20 ml of saturated sodium chloride were added to the aqueous solution in the funnel and the solution was extracted with 3 x 60 ml of methylene chloride. The methylene chloride layer was collected through a glass funnel containing a glass wool plug and anhydrous sodium sulfate into a turbo vap tube. The combined extract was evaporated to dryness and flushed with a gentle stream of nitrogen gas to remove any traces of methylene chloride. The residue was redissolved in an appropriate volume of acetonitrile for HPLC analysis.

Extraction of the water samples from the wash treatments was the same as the method used for extraction of water samples from the model study, but in proportionally larger amounts. The 100 ml samples were mixed with 25 ml of NaCl, 5 grams of sodium bicarbonate and extracted with 3 x 70 ml of methylene chloride. After the methylene chloride extract was dried, the sample was redissolved in 2 ml of acetonitrile for HPLC analysis.

D. Recovery Studies

Recovery studies for azinphos-methyl, captan and formetanate-HCl were carried out by spiking water and apple samples with 0.5 ppm

and 5.0 ppm of the respective pesticide standards. Both the water and apple samples were extracted as described above in the extraction and cleanup methods for the three pesticides.

E. Chromatographic Analyses

The final extracts of captan and azinphos-methyl were dissolved in hexane, while formetanate-HCl was dissolved in acetonitrile in known volumes and subsequently analyzed using either the GC or HPLC.

(I) Azinphos-methyl (Guthion®)

Residues of this pesticide were detected by a Hewlett Packard Series II 5890 gas chromatograph equipped with a nitrogen phosphorus detector (NPD). The GC was equipped with a DB-5 fused silica capillary column (30 m x 0.25 mm i.d.) with a film thickness of 0.25 micron (J.W. Scientific, Folsom, CA). The oven temperature was programmed isothermally at 230°C, while the injector and detector temperatures were set at 250°C and 250°C, respectively. The injection volume was 3 μl and samples were injected with a HP 7673 automatic sampler linked to the GC. Integration was carried out with HP Chemstation software interfaced to the GC.

(II) Captan

Residues of this pesticide were detected by a Hewlett Packard 5890 Series II gas chromatograph equipped with a Ni^{63} electron capture detector (ECD). The GC was equipped with a DB-5 fused capillary (60 m x 0.25 mm i.d.) with a film thickness of 0.25 micron (J.W. Scientific, Folsom, CA). The oven temperature was programmed isothermally at 180°C, while the injector and detector temperatures were 220°C and 275°C respectively. The injection volume was 2 μl. Integration was carried out with HP Chemstation software interfaced to the GC.

Gas chromatography-mass spectrometry was used to confirm the presence of oxidative degradation products from the reaction of calcium hypochlorite with captan. Degradation products from oxidation reactions of captan with ozone were not analyzed. The Delsi Di 700 gas chromatograph was equipped with a capillary column DB-1 (30m x 0.25 mm i.d.) with a film thickness of 0.25 micron (J.W. Scientific, Gibbstown, NJ). The GC was programmed in a splitless mode with an initial oven temperature set at 40°C for 1 minute, and then raised to 180°C for 40 minutes at a rate of 5°C per minute. The injector temperature was set at 210°C, and the heated interface temperature between the GC and the mass spectrometer was maintained at 250°C. The Nermag R10-10C mass spectrometer was set in an electron ionization (EI), positive ion mode, with the following parameters: primary pressure = 1.3×10^{-3} Torr; secondary pressure = 1.6×10^{-7} Torr; IE = 0,208 amps; e^- = 70.6 eV; focal = -0.01 ; ions = -96; external =

16.3 and multiplier = -3.02. The quadrapoles were scanned from 50 to 500 u at a rate of 2 scans per second.

(III) Formetanate-HCl (Carzol®)

Formetanate-HCl residues were analyzed by HPLC. A Milton Roy Spectrophotometer 3100 variable wavelength UV-visible detector, set at a wavelength of 254 n.m. (maximum absorbance for formetanate), 0.05 absorbance unit full scale (AUFS), and 0.1 response time was used. The column used was a Brownlee Spheri-5, RP-18 (5 micron, 4.6 mm i.d. x 220 mm). The mobile phase was 35% acetonitrile in 0.01 N $NH_4H_2PO_4$ (pH 8) filtered through a 0.45 μm filter and degassed prior to use. An Anspec 3113 HPLC pump was used for solvent delivery at a flow rate of 2 ml/minutes. After the system was stabilized (about 30 minutes from initial warm-up), 50 μl samples were injected via a Rheodyne syringe-loop injector (50 μl loop) for analysis. Integration was carried out using Waters 840 data and chromatography control station software.

F. Calculation of Pesticide Residue Concentration

Pesticide residue concentrations in solution and in fresh or processed apples were calculated based on the area of the integrated peaks of the samples compared with known concentrations of analytical standard of the respective pesticide. Standard curves of the pesticide standards were plotted and least square linear regression was obtained

using a Microsoft Excel (Microsoft Corporation, Redmond, WA) software. Appendix II - IV show examples of standard curves for each of the three pesticide standards of known concentration between 0.5-10 ppm.

The concentration of each pesticide residue in solution or in the apple fruit or sauce was calculated based on the following formula:

(a) Residues in solution in $\mu g/ml$ =

$$\frac{\text{Conc. of sample based on std. curve } (\mu g/ml) \times \text{Vol. of final extract (ml)}}{\text{Volume of sample analyzed (40 ml)} \times \text{\% Recovery}}$$

(b) Residues in apple fruit or sauce in $\mu g/g$ of fruit =

$$\frac{\text{Conc. of sample based on std. curve } (\mu g/ml) \times \text{Vol. of final extract (ml)}}{\text{Weight of sample analyzed (g)} \times \text{\% Recovery}}$$

G. Statistical Analyses

In the model studies, the experiments were designed as a split plot (treatments x pH x temperature) randomized model, split across the 30 minutes duration of the treatment (Gill, 1986). The study on effect of wash treatments on dissipation of pesticides on apple fruits was designed as a two factor (treatments x replication) and (treatments x product) randomized model. All determinations for both studies were made in triplicate. Mean, standard errors, mean square errors, two factor ANOVA, correlation and interaction of main effects were calculated using SuperANOVA computer software (Abacus Corporation, Inc., Berkeley, CA). Bonferroni's t was used to determine significant differences between treatment means.

RESULTS AND DISCUSSION

A. Model Study

(I) Chlorine Monitoring

Chlorine, as calcium hypochlorite, was prepared as a stock solution of 5000 ppm. The stock solution was diluted in proportionate amounts to obtain a final concentration of 50 or 500 ppm in the model solutions. The chlorine concentration of the stock solution was determined to be 5012 ± 8 ppm. The chlorine concentration of each sample solution was monitored, and determined to be within ±2 ppm (for 50 ppm) and ±10 ppm (for 500 ppm) of the intended final concentrations.

(II) Ozone Monitoring

Water ozonated by circulating water through the Lifex® Ozone system was monitored for ozone concentration in the water. The concentration of ozone measured directly from the hose extending from the machine was 0.251 ppm, while the concentration of ozone in the

Table 3 : Ozone Output In Water From Lifex Ozone System

Sample #	Ozone from hose (ppm)	Ozone from bucket (ppm)
1	0.315	0.078
2	0.323	0.157
3	0.246	0.043
4	0.204	0.056
5	0.209	0.110
6	0.188	0.094
7	0.220	0.083
8	0.221	0.080
9	0.214	0.065
10	0.372	0.068
Mean :	**0.251**	**0.083**
Standard Dev. :	**0.062**	**0.032**

1. The temperature of the water was 21 ± 1.2°C

water sampled from the bucket was determined to be 0.083 ppm (Table 3). The ozone concentrations were an average of 10 samples. The difference in the concentration of ozone in the bucket and directly from the hose indicated that ozone in solution was relatively unstable. Katz (1980) indicated that ozone in solution was unstable, having a half-life of about 20-30 minutes in distilled water at 21°C. Also, the half-life of ozone in water is shortened considerably at higher temperatures.

(III) Degradation of Azinphos-methyl (Guthion®)

In the GC analysis, azinphos-methyl appeared as a single sharp peak at a retention time of 9.2 minutes. Figure 8 shows a typical chromatogram of an azinphos-methyl standard at a concentration of 1 ppm, while Figure 9 shows an example of a chromatogram of a sample in a pH 7.0 solution ozonated at 44°C and sampled at 5 minutes. The standard curve shown in Appendix III was representative of the standard curves used to calculated azinphos-methyl concentration in the sample solutions. The correlation coefficients (R^2) for linear regression of the standard curves were between 0.903 and 0.998, showing that the response was linear over the concentration range of 0.5-10 ppm. The percent recovery of azinphos-methyl in solution spiked with 0.5 ppm and 5 ppm pesticide standard was 91.3 ± 7.09%.

Azinphos-methyl was stable in pH 4.5 and 7.0 solutions at both 21°C and 44°C (Figure 10 and 11) with very little degradation of the pesticide due to hydrolysis. Between 96-100% (21°C) and 93-96% (44°C)

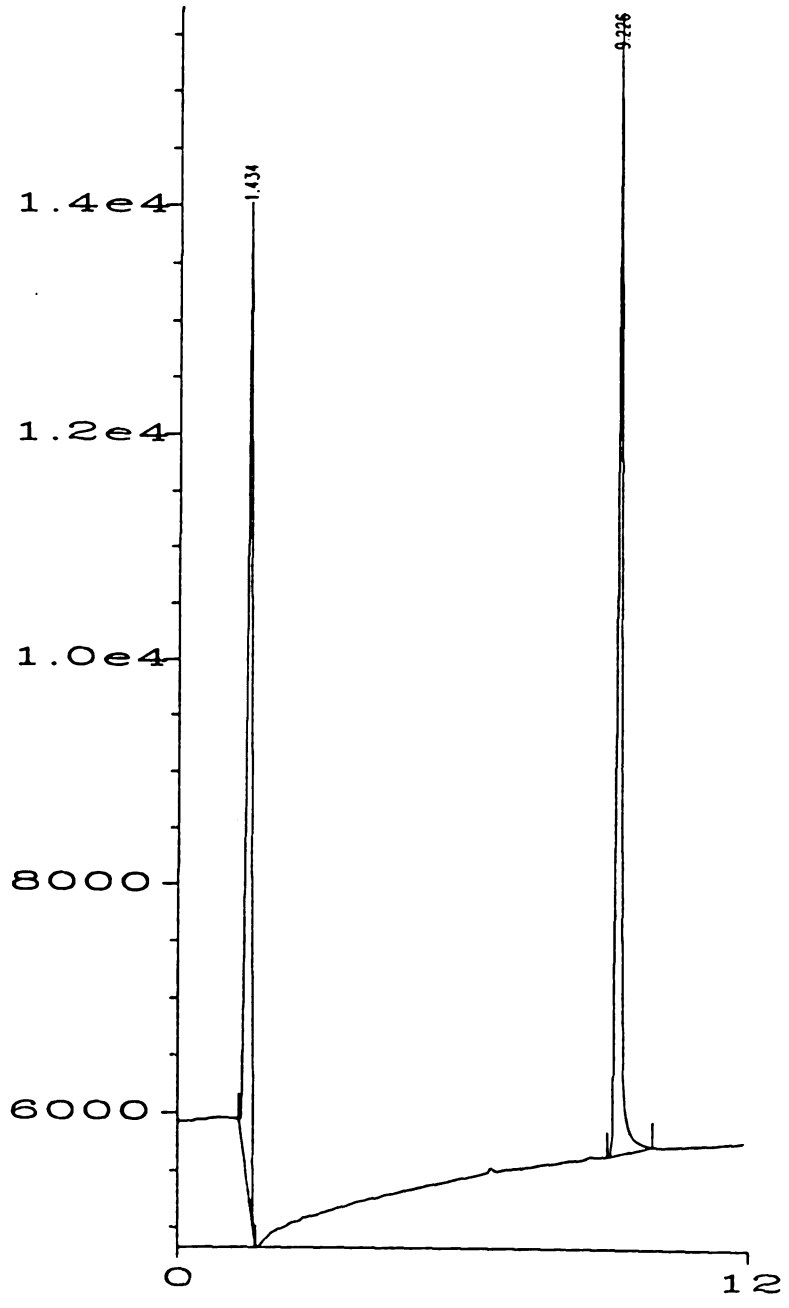


Figure 8 : GC Chromatogram Of An Azinphos-methyl Standard

1. 1.0 ppm
2. Rt = 9.2 mins

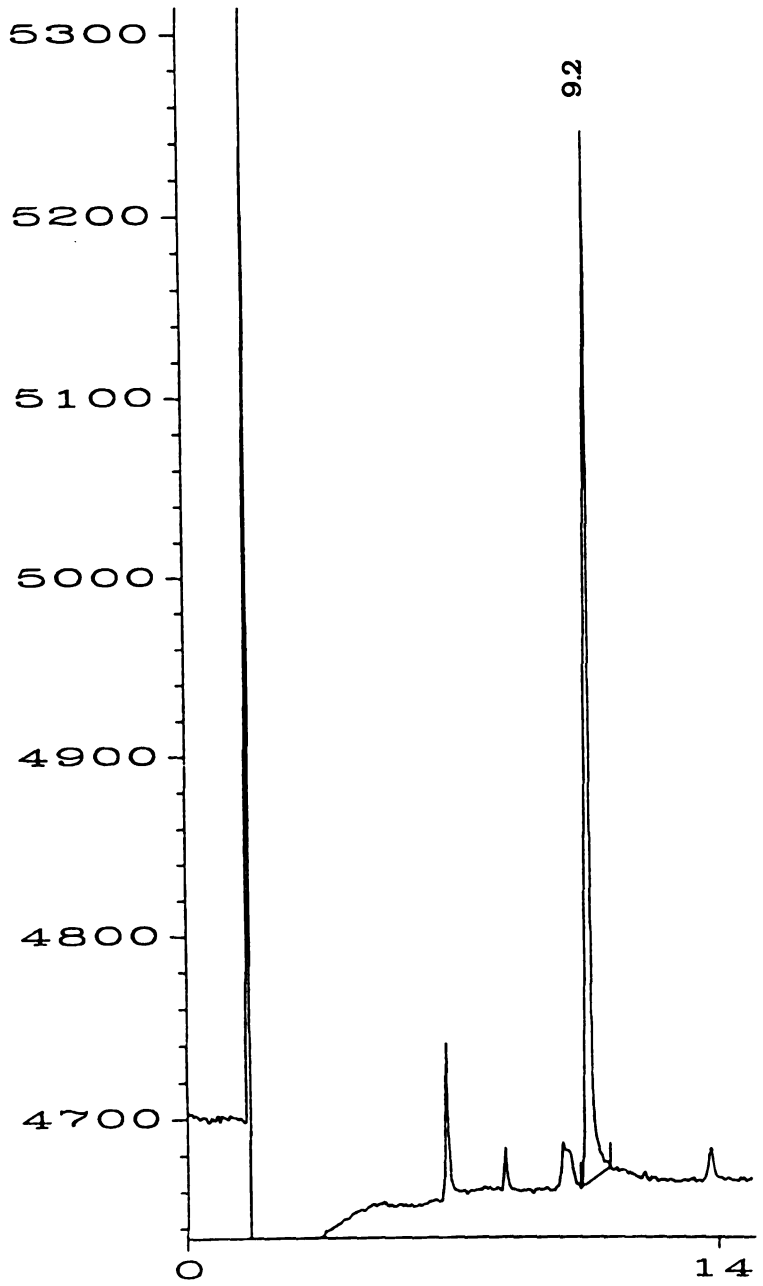


Figure 9 : GC Chromatogram Of An Azinphos-methyl Sample

1. ozonation in pH 7.0 at 21°C.; time = 5 minutes
2. Rt = 9.2 mins

residual pesticide remained after 31 minutes. Azinphos-methyl was relatively less stable at pH 10.7, with about 87% remaining after 6 minutes and 51% remaining after 31 minutes in solution at ambient temperature. At 44°C, azinphos-methyl was even less stable, with about 76% remaining after 6 minutes and 43% after 31 minutes.

These results are in agreement with various studies that have been carried out to show the behavior of azinphos-methyl in aqueous solutions over a wide range of pH and temperature. Faust and Gomaa (1972) have shown that azinphos-methyl was less stable under basic than acidic conditions. The half-lives of azinphos-methyl in buffered solutions at pH 1, 3, 5, 6, 7 and 9 maintained at 21°C were 24, 9, 8.9, 7.5, 4.8 and 0.6 hours, respectively. In another study conducted at temperatures of 30°C and 50°C, the stability of azinphos-methyl was shown to be pH and temperature-dependent (Flint *et al.*, 1970). In buffers at pH 5, 7 and 9 half-life values were 17, 10 and 0.5 days respectively at 30°C and 1.8, 1.3 and 0.08 days at 50°C.

Degradation of azinphos-methyl by ozone was greatest at pH 4.5 and decreased with increasing pH (Figure 10 and 11). Between 17-39% of azinphos-methyl remained after 31 minutes at both 21°C and 44°C for all three pH treatments. As shown in Figure 10, about 60 and 85% of the initial amount was degraded by ozone treatment at 21°C sampled at 5 and 30 minutes, respectively. Compared to the control, it appeared that most of the azinphos-methyl degradation at the low pH was due mainly to ozonation and little due to hydrolysis. Degradation of azinphos-methyl at pH 7.0 at both 21°C and 44°C was significantly different compared to pH 4.5, except at t=16 minutes. The ozone

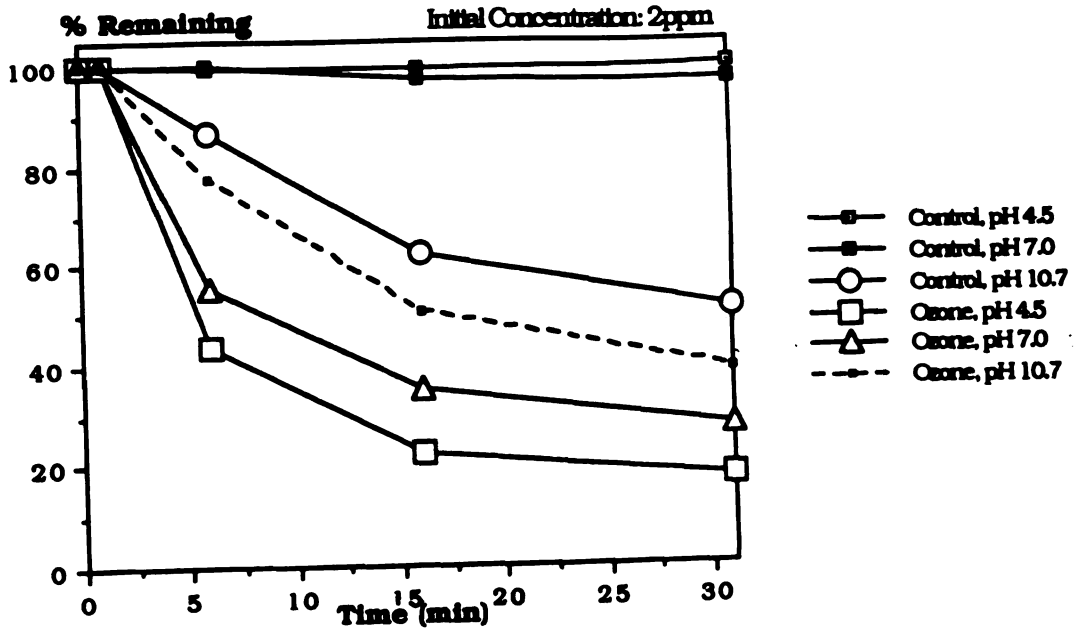


Figure 10 : Effect of Ozone Treatment on the Degradation of Azinphos-methyl at 21°C

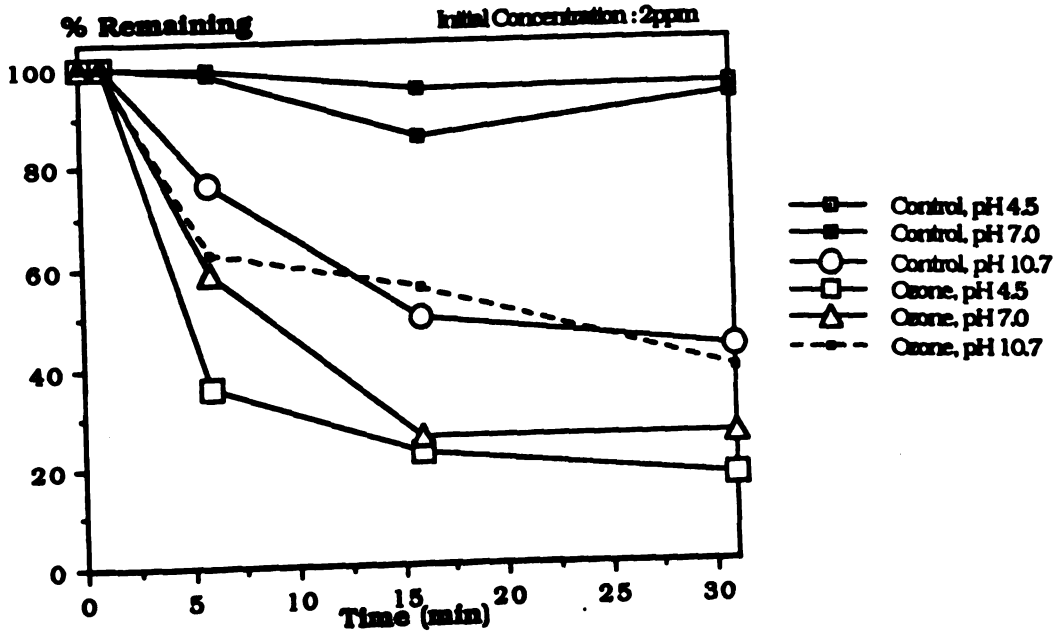


Figure 11 : Effect of Ozone Treatment on the Degradation of Azinphos-methyl at 44°C

treatment at pH 10.7 was the least effective at both 21°C and 44°C. Increased temperatures did not significantly ($p < 0.05$) increase the rate of degradation of azinphos-methyl.

Kearney et al. (1988) found that an increase in pH decreases the stability of ozone in water, due to the catalytic effect of hydroxyl ions on the O_3 decomposition process. As such, pH increases reduce the effect of ozone on the degradation of azinphos-methyl, while the effect of hydrolysis increases.

In 50 ppm hypochlorite solution, azinphos-methyl was completely degraded at pH 4.5 at both 21°C and 44°C (Figure 12 and 13). At pH 7.0, almost 85% of the initial amount of azinphos-methyl was degraded after only 6 minutes in a 50 ppm hypochlorite solution (Figure 12). The 50 ppm chlorine treatment at pH 10.7 was the least effective, where its degradation was only about 15% and 56% after 6 and 31 minutes treatment, respectively. Elevated temperature increased the degradation of azinphos-methyl at both pH 7.0 and 10.7 (Figure 13). At both pH's, higher temperature increased the degradation of azinphos-methyl by about 10% during the entire sampling period.

Chlorination at 500 ppm significantly ($p < 0.05$) increased the rate of degradation of azinphos-methyl in all three pH treatments and at both temperatures. Again, the most effective treatment was chlorination at 500 ppm in the pH 4.5 solution, while pH 10.7 was the least effective treatment (Figure 12 and 13). Also, increased temperatures completely degraded azinphos-methyl sampled at 5 minutes in 500 ppm hypochlorite at both pH 4.5 and 7.0. Only about 4% of the pesticide remained at pH 10.7 after 31 minutes (Figure 13).

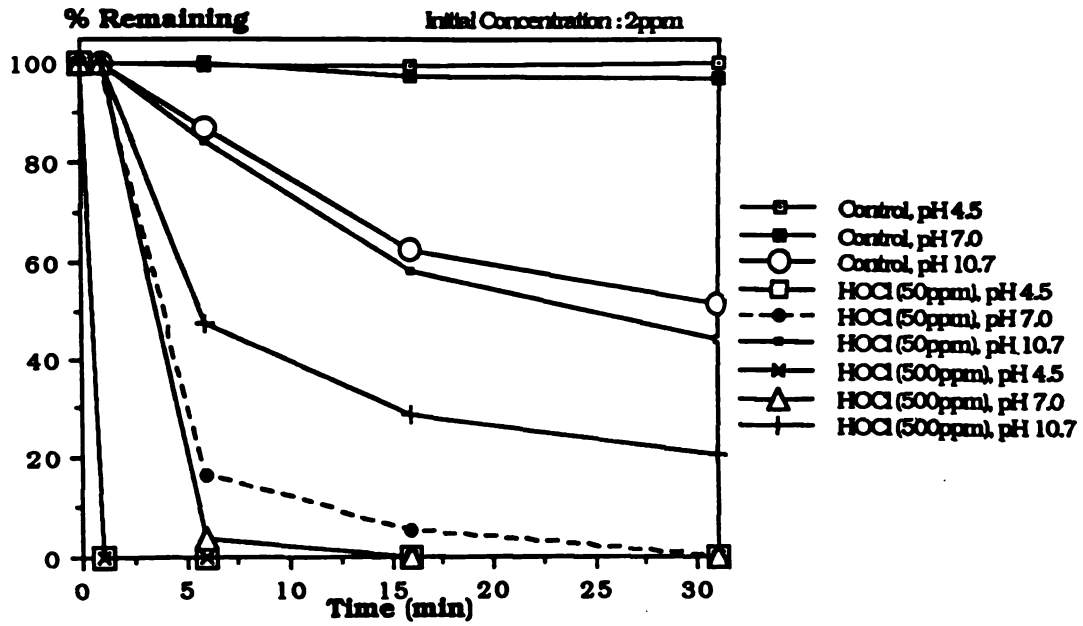


Figure 12 : Effect of Chlorine Treatment on the Degradation of Azinphos-methyl at 21°C

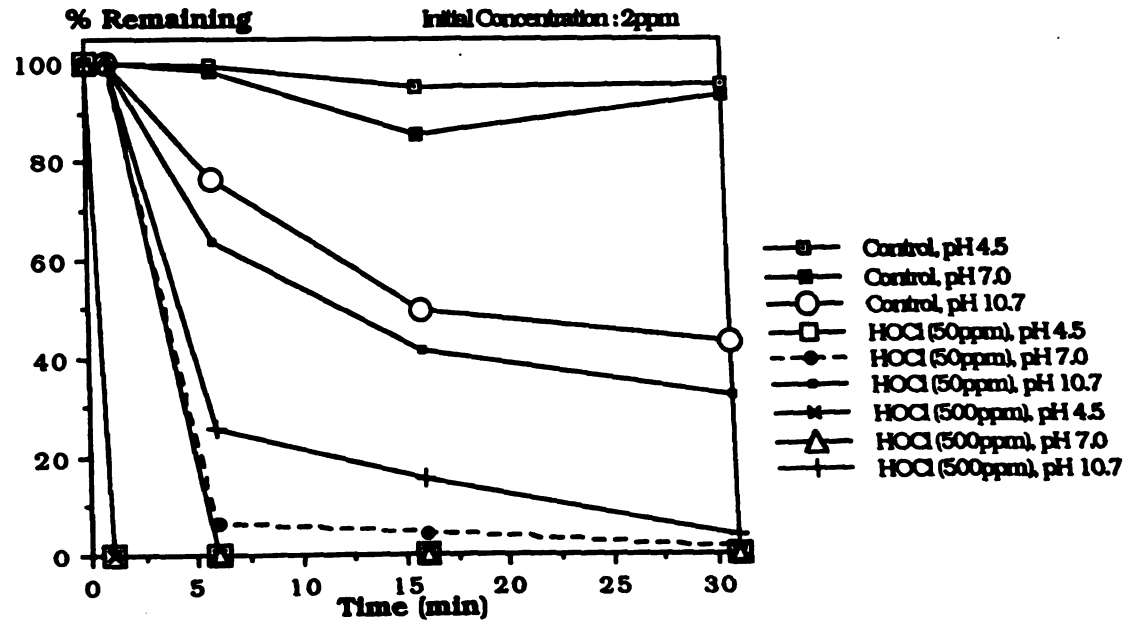


Figure 13 : Effect of Chlorine Treatment on the Degradation of Azinphos-methyl at 44°C

The percent of HOCl is greatest at low pH and decreases as the pH and temperature of a solution increase (Wei *et al.*, 1985). Under these circumstances the oxidation reaction of HOCl with azinphos-methyl should be greatest at pH 4.5 and least at pH 10.7, as was observed in the present study.

Also the organophosphate insecticide used in this experiment contains a phosphoric acid ester linkage that is relatively less stable and more susceptible to oxidation in a strong oxidizing medium. This was shown to be the case because of the strong correlation between the rate of azinphos-methyl degradation and increasing hypochlorite concentration.

In general, all treatments were significantly different ($p < 0.05$), with the 500 ppm chlorine treatment being the most effective. Also, there were significant differences between the pH and temperature treatments, showing that degradation of azinphos-methyl is dependent upon various environmental factors such as pH and temperature.

(IV) Degradation of Captan

In the GC analysis, captan appeared as a sharp peak with a retention of 46 minutes. Figure 14 shows a typical chromatogram of a captan standard at a concentration of 1 ppm, and Figure 15 shows a typical chromatogram of a sample solution sampled after 5 minutes in a pH 7.0 solution treated with 50 ppm calcium hypochlorite at 21°C. The retention time is considerably long due to the length of

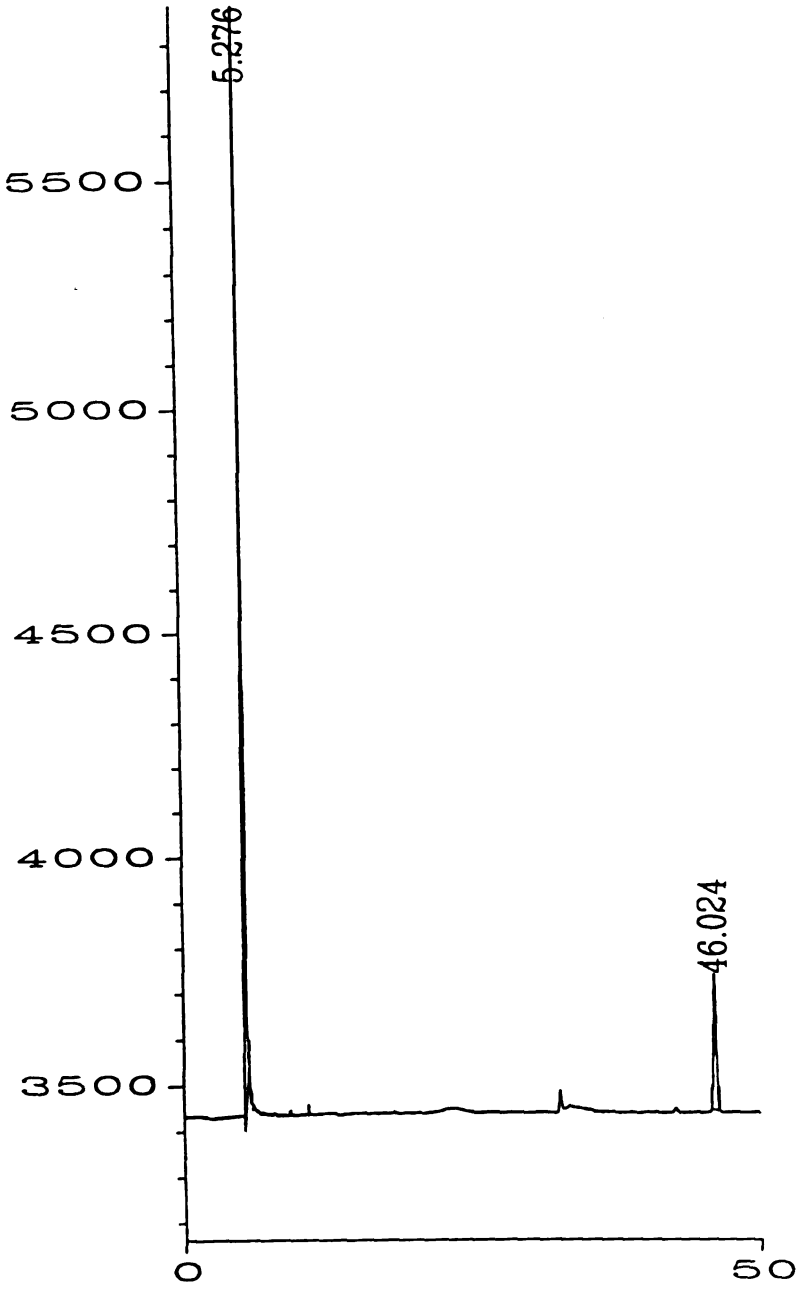


Figure 14 : GC Chromatogram Of A Captan Standard

1. 1 ppm
2. Rt = 46 mins

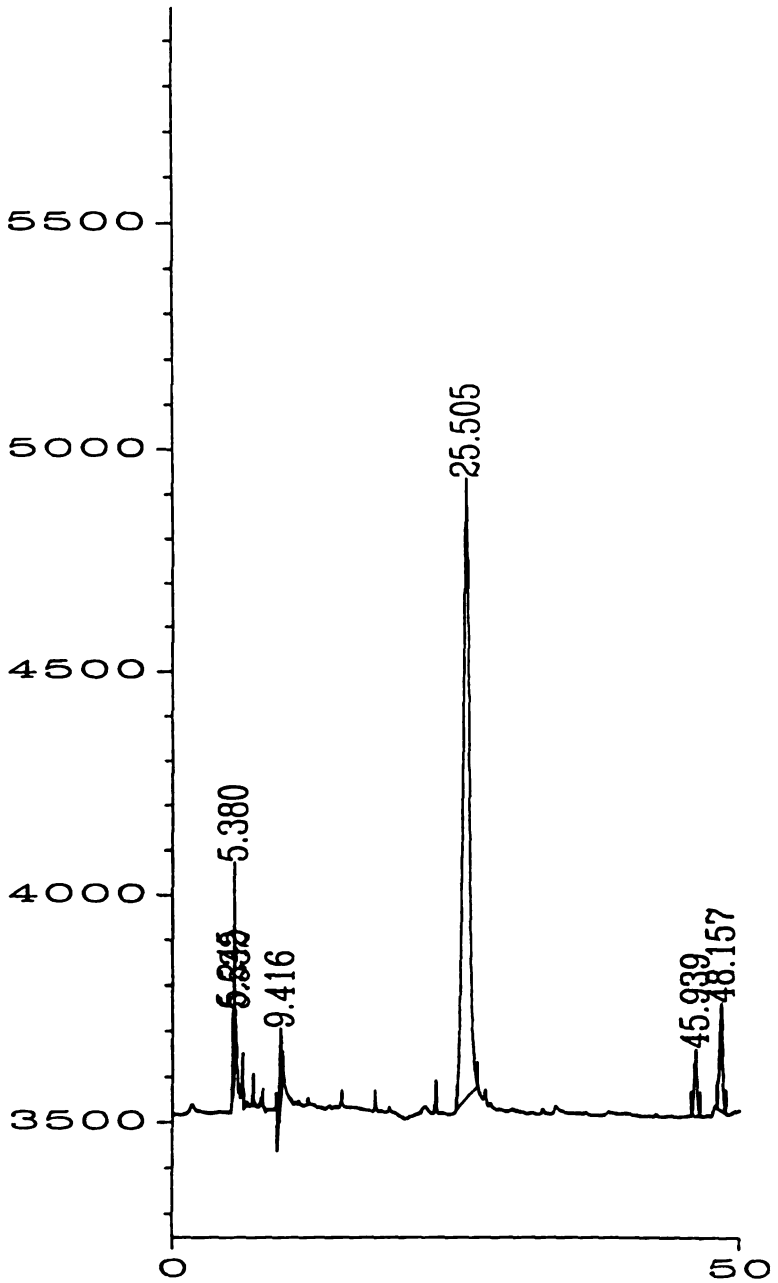


Figure 15 : GC Chromatogram Of A Captan Sample

1. chlorine 50 ppm in pH 7.0 at 21°C; sampling time = 5 mins
2. Rt = 46 mins

the column used (60 m) as well as the low oven temperature (180°C). Setting the oven temperature above 200°C produced a significantly large second peak at 42 minutes. By lowering the temperature to 180°C, the second peak was eliminated up to 2.5 ppm. This second peak was visible at 5 and 10 ppm, but was not large enough to significantly affect the detection of captan. An example of a standard curve for captan standards between 0.5-10 ppm is shown in Appendix IV. The correlation coefficients (R^2) for the linear regression of the curves were between 0.975 and 1.0, showing that the response was linear under the above conditions over the concentration range of 0.5-10 ppm. The recovery of captan spiked with 0.5 and 5 ppm of the standard was 105.9 ± 1.71%.

Wolfe *et al.* (1976) had reported the appearance of a second peak in the chromatogram along with the captan peak above 200°C. They assumed that the two peaks were a result of captan decomposing at such high temperatures. In their work, they used a short column (2 ft) and a column temperature of 160°C to eliminate the anomalous peak.

The degradation of captan (2 ppm) in solutions due to hydrolysis, ozonation and chlorination is shown in Figure 16-19. Due to the insolubility of captan in water, captan was added to the aqueous solution with a carrier solvent (acetonitrile). Wolfe *et al.* (1976) reported that this was necessary because the rate of solution of captan in water was slow compared to the rate of hydrolysis, and that the addition of 1% organic solvent did not affect the rate constant when compared with pure water.

At 21°C, captan degradation due solely to hydrolysis was less significant at pH 4.5 and 7.0, than at pH 10.7 (Figure 16). Captan was

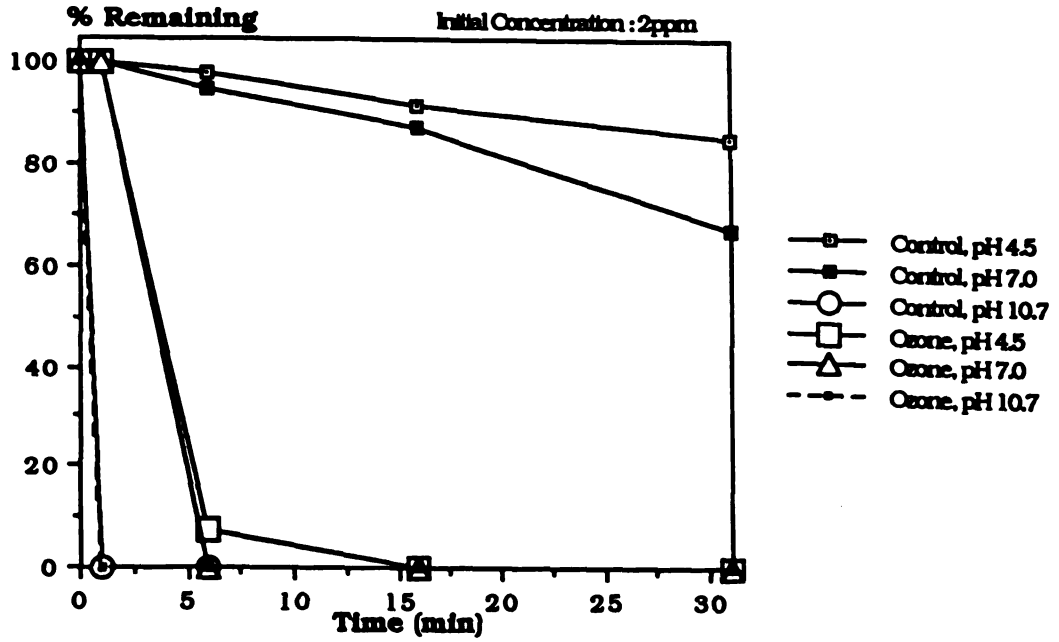


Figure 16 : Effect of Ozone Treatment on the Degradation of Captan at 21°C

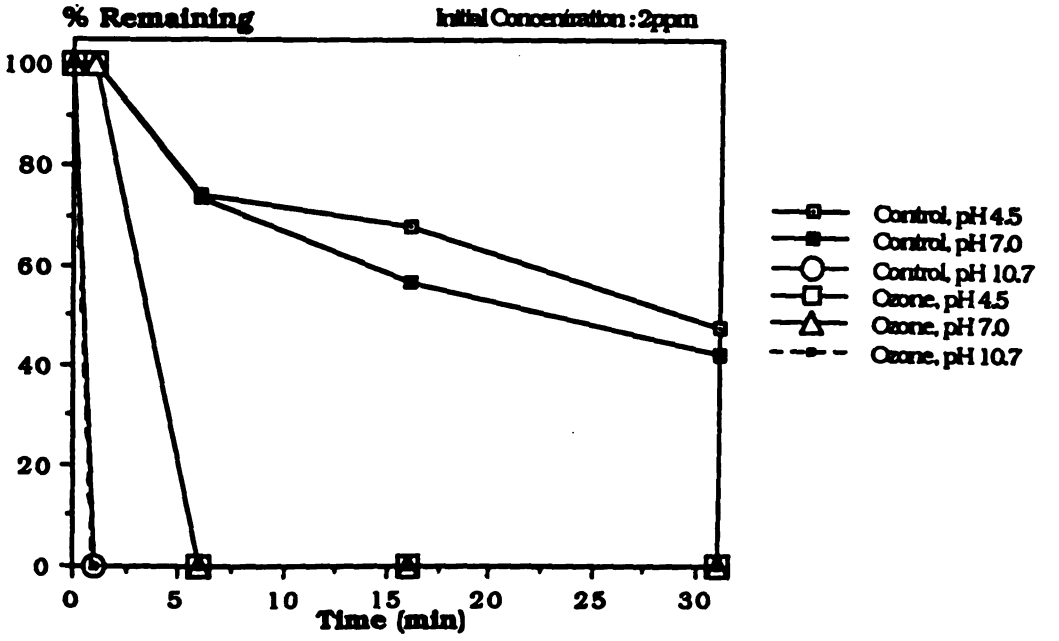


Figure 17 : Effect of Ozone Treatment on the Degradation of Captan at 44°C

completely unstable at pH 10.7 in both the control and ozonated samples, even when sampled at 0 minute. After 6 minutes of ozone treatment at ambient temperature, only 7.3% of captan remained at pH 4.5 and levels were non detectable at pH 7.0. Elevated temperature (44°C) accelerated the degradation of captan in solution (Figure 17). In pH 4.5 and 7.0 solutions, about 47% and 42% of captan remained for approximately 31 minutes in solution, respectively. With ozonation, captan was completely degraded at pH 4.5, 7.0 and 10.7 when sampled at 5 minutes.

In the ozonation study, the degradation of captan at 21°C and 44°C was significantly different ($p < 0.05$). The rate of degradation was significantly greater at the higher temperature (44°C) than at the lower temperature (21°C).

Various investigators have reported the relative instability of captan in solution. Melnikov (1971) reported that captan was hydrolyzed by moisture and the reaction was accelerated by alkali. Von Rümker and Horay (1972) have presented data showing that the half-life of captan decreased with increasing pH at 20°C: at pH 4.0, 4 hours and at pH 10, <2 minutes. The half-life also decreased when the temperature was increased to 40°C. Wolfe *et al.* (1976) reported that the reaction of captan is independent of pH over the pH range 2-6, and pH dependent above pH 7.

The effect of chlorine treatment on the degradation of captan at both ambient and elevated temperatures was significantly greater as compared to the control (hydrolysis only). Captan was unstable in chlorinated solution. Figure 18 shows that captan in a 50 ppm chlorine

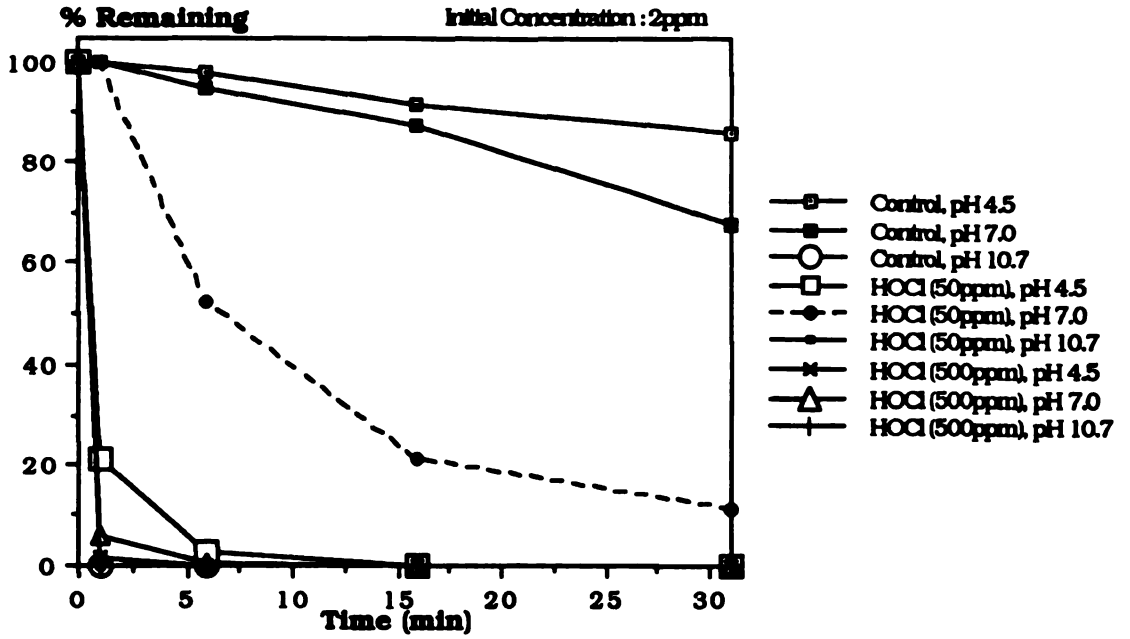


Figure 18 : Effect of Chlorine Treatment on the Degradation of Captan at 21°C

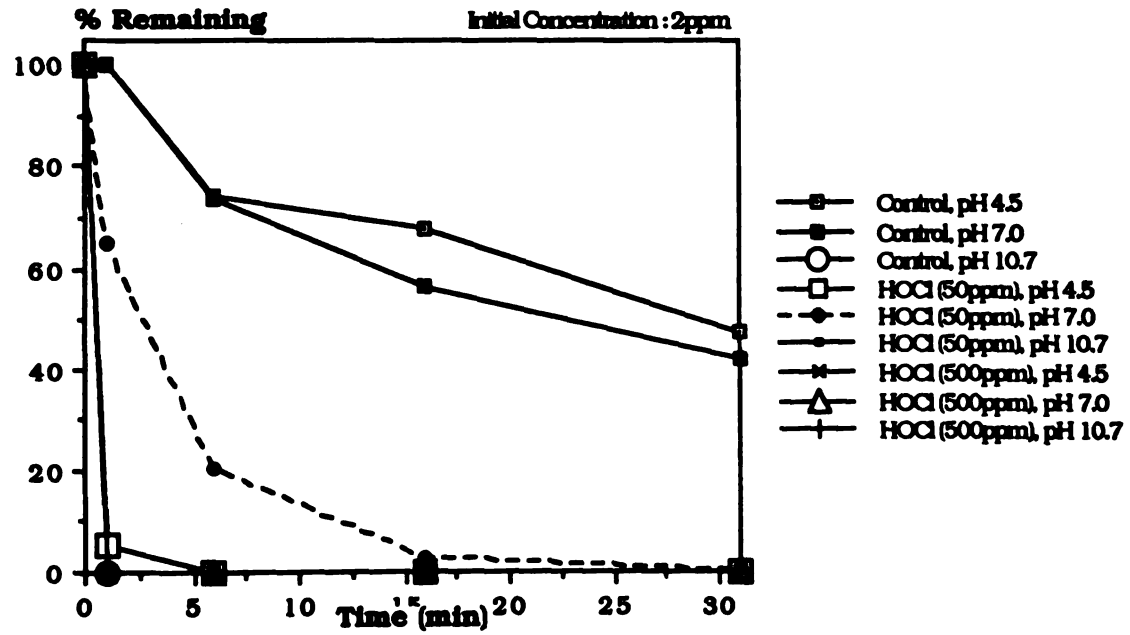


Figure 19 : Effect of Chlorine Treatment on the Degradation of Captan at 44°C

solution was most stable at pH 7.0 and very unstable at pH 4.5 and 10.7. Almost 80% of the pesticide was degraded in a pH 4.5 solution when sampled at 0 minute, with only about 3% remaining when sampled at 5 minutes. Almost all the pesticide was degraded at pH 7.0 and 10.7. However, the degradation of captan at pH 10.7 was due almost entirely to hydrolysis and not as a result of chlorine treatment. On the contrary, almost all the pesticide in the pH 4.5 solutions was degraded due to chlorination and little due to hydrolysis. This was evident by comparing the chlorine treatments with the control treatments. Captan was completely degraded in 50 ppm chlorine solutions at all three pH's (Figure 18 and 19).

Temperature significantly influenced the effect of chlorination on captan in all treatments. A 20-30% increase was observed in the degradation of captan at the higher temperature between the 5 to 30 minutes treatment interval at pH 7.0 (Figure 18 and 19). These results were consistent with those reported in earlier studies showing similar increase in the degradation rate of captan when temperature was increased. Frank *et al.* (1983) reported the half-life of captan in water at pH 8.5 was less than 1 hour at 21°C and 13 hours at 5°C, while at pH 5.5 the half-lives were 13 hours at 21°C and 208 hours at 5°C.

In general, the various treatments (control, ozonation and chlorination @ 50 and 500 ppm) were significantly different ($p < 0.05$). There was also a significant difference ($p < 0.05$) among the pH treatments (4.5, 7.0 and 10.7).

Mass spectrometric analysis showed that the major products of degradation found in the solution treated with calcium hypochlorite

were mainly oxidation products, and that there was no evidence of any chlorinated by-products. This was based on the isotope molecular ion patterns for all degradation products.

(V) Degradation of Formetanate-HCl (Carzol®)

In the HPLC analysis of formetanate-HCl, formetanate appeared as a relatively broad peak with a retention time of 3.4 minutes (Figure 20 and 21). There appeared to be good separation between the peak of interest and the solvent peak. Dissolving the final extract in water as described by Lawrence *et al.* (1981) caused difficulty in obtaining a consistent peak due to the appearance of a second peak near the solvent peak on occasion. This inconsistency was probably attributed to the fact that formetanate-HCl changed between the weakly acidic form and its basic form. This could be due to the fluctuation of the pH of the water. Dissolving the final extract in acetonitrile and using a pH 8.0 buffered mobile phase provided consistent separation of the formetanate form.

Standard curves for formetanate standards between 0.5-10 ppm were plotted, and a typical curve is shown in Appendix V. The correlation coefficients (R^2) for the linear regression of the curves were between 0.95 and 1.0. This showed that the response was linear at the specified conditions over the concentration range of 0.5-10 ppm. The average recovery for formetanate-HCl, spiked at 0.5 and 5 ppm in solution, was 103.2 ± 6.19%.

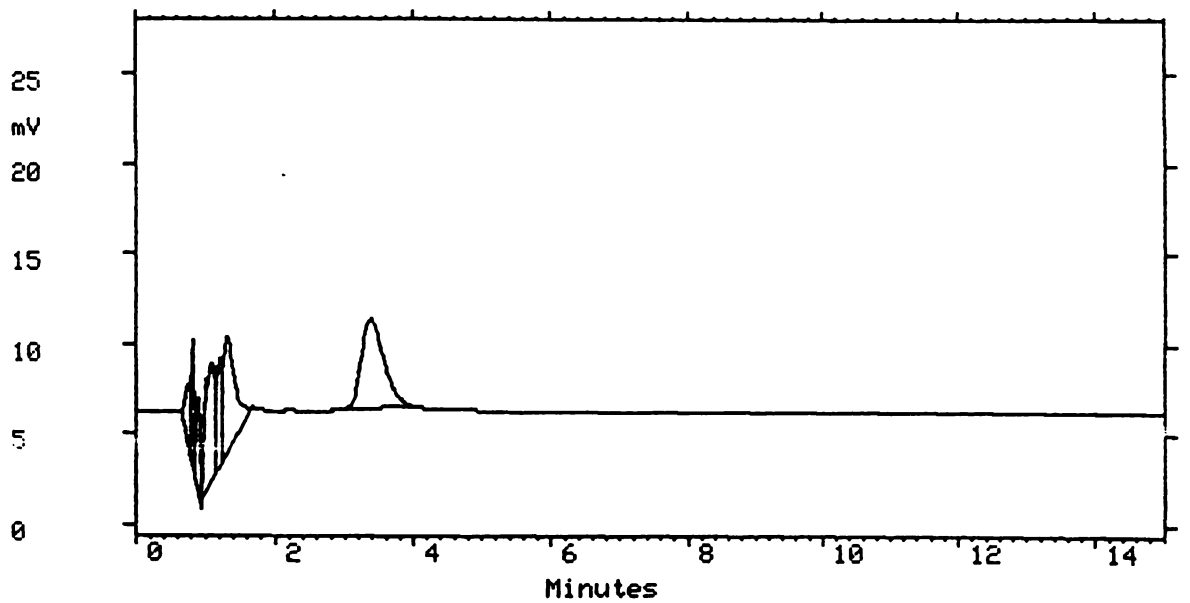


Figure 20 : HPLC Chromatogram Of A Formetanate-HCl Standard

1. 1.0 ppm
2. Rt = 3.4 mins

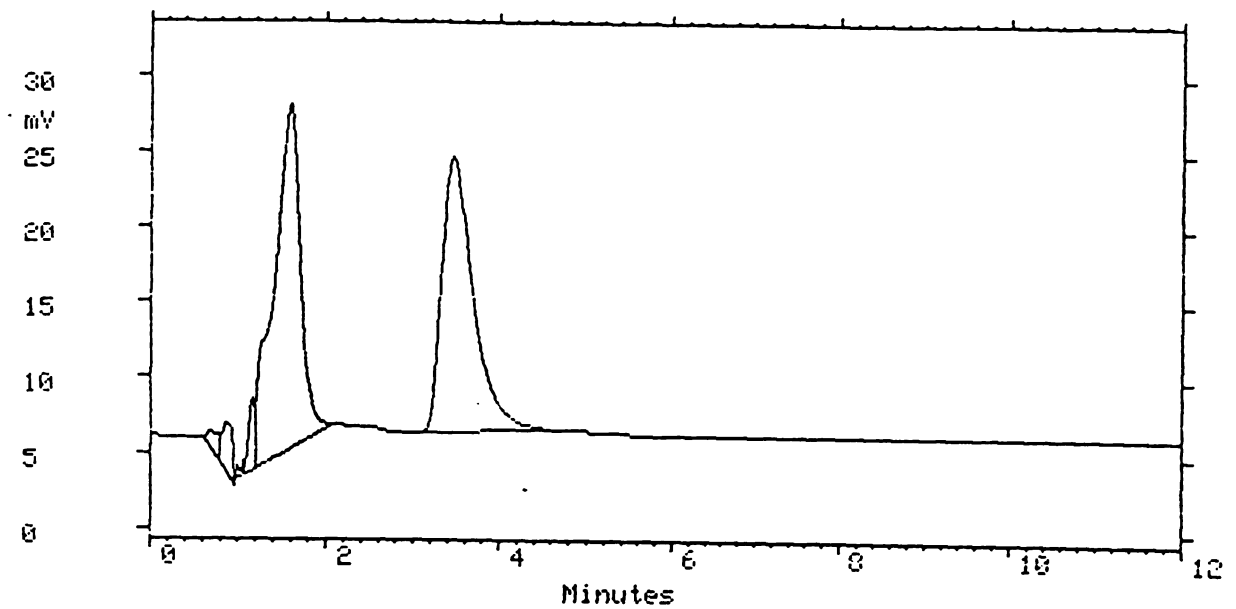


Figure 21 : HPLC Chromatogram Of A Formetanate-HCl Sample

1. chlorine 50 ppm in pH 7.0 at 21°C; sampling time = 10 mins
2. Rt = 3.46 mins

Due to the chemical structure of formetanate-HCl and the form it takes in different pH medium, it appeared that the acidic form was relatively stable while its basic salt was unstable and subjected to both hydrolysis and chemical oxidation. Jenny and Kossmann (1978) reported that formetanate-HCl hydrolyzes slowly in acid medium, and is extremely susceptible to hydrolysis in neutral or basic medium especially at higher temperatures.

The rate of degradation of formetanate-HCl due to hydrolysis generally increased at higher pH and temperature. Formetanate-HCl was relatively stable at low pH in its hydrochloride salt form. Formetanate-HCl as formetanate was unstable and degraded rapidly at alkaline pH (Figure 22 and 23). Almost 100% remained after 30 minutes in solution at pH 4.5 and 7.0 and at both 21°C and 44°C. At pH 10.7, only 14.9% remained after 6 minutes in solution at 21°C, and it was non detectable after 6 minutes at 44°C. Lawrence *et al.* (1981) reported that formetanate at 1 ppm in aqueous solution at pH 8.0 decomposed by 20% after 1 day at room temperature. There are no available data in the literature on the degradation of formetanate-HCl at pH 10 and above.

Figure 22 and 23 show ozonation at pH 4.5 was the least effective. Almost 50% of formetanate-HCl remained after 31 minutes of ozone treatment at both 21°C and 44°C. At pH 7.0, about 50-60% and 3-23% remained after 6 and 31 minutes of ozonation at 21°C and 44°C respectively. Higher temperature did not significantly ($p < 0.05$) accelerate degradation of the pesticides by ozonation. Although formetanate-HCl was degraded to non detectable levels at pH 10.7 at

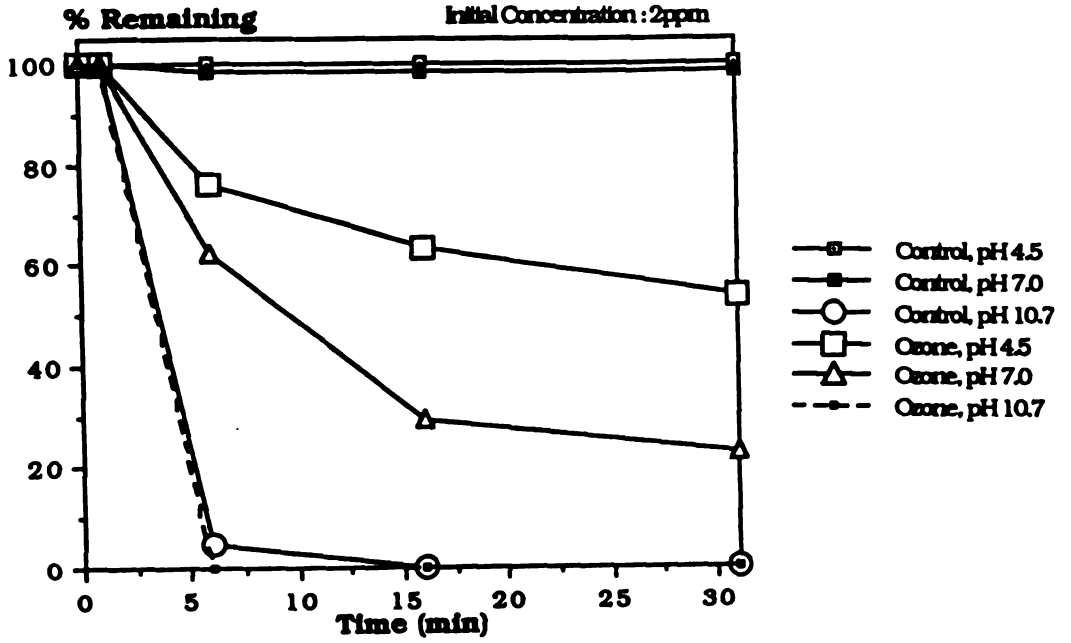


Figure 22 : Effect of Ozone Treatment on the Degradation of Formetanate-HCl at 21°C

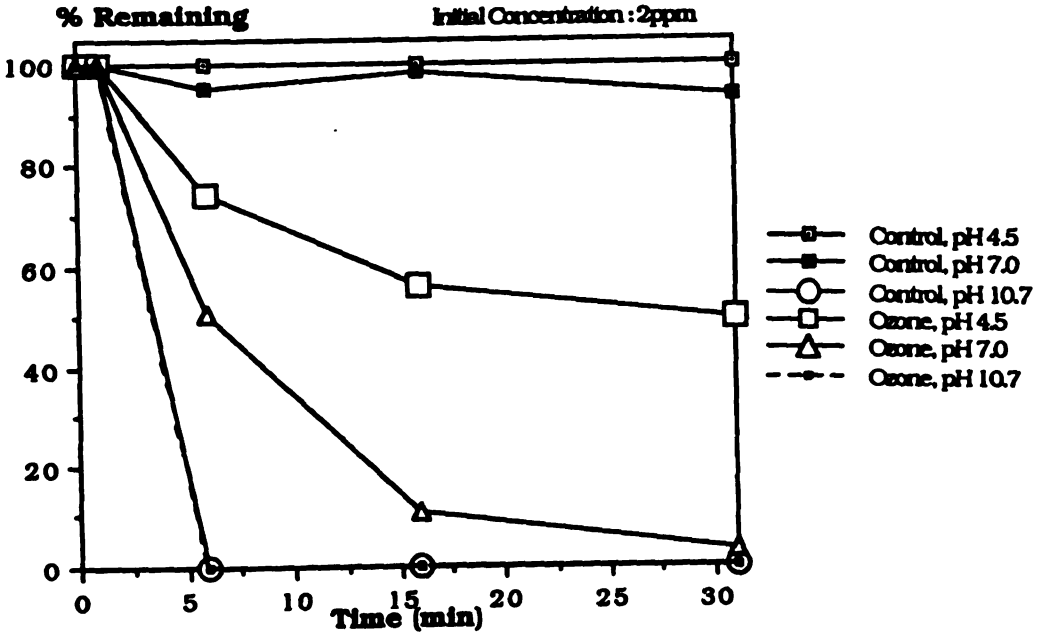


Figure 23 : Effect of Ozone Treatment on the Degradation of Formetanate-HCl at 44°C

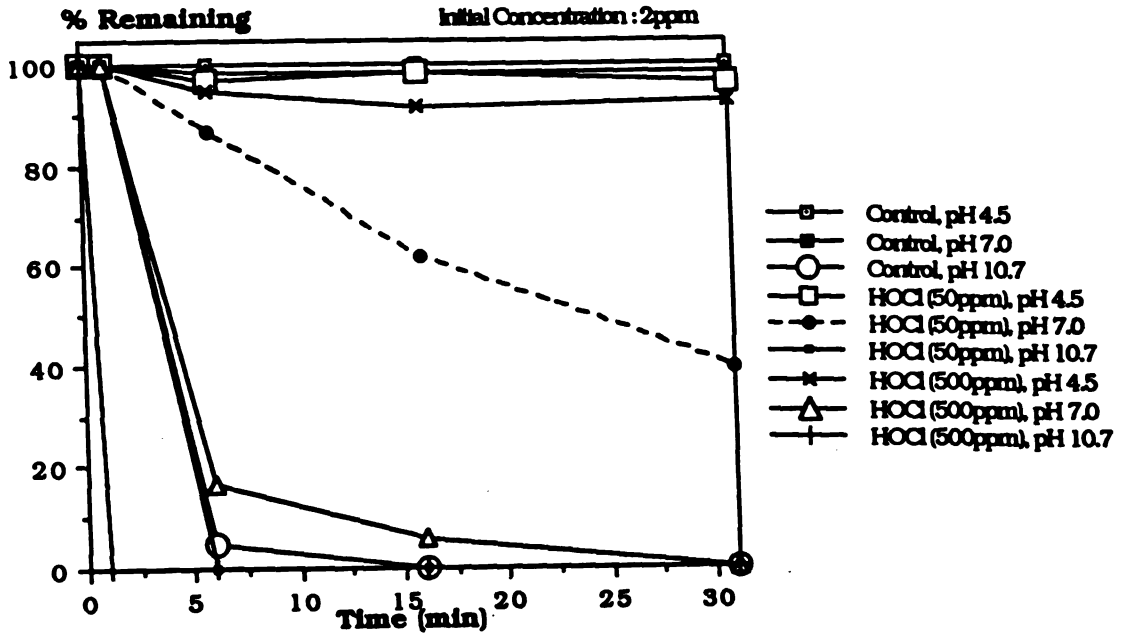


Figure 24 : Effect of Chlorine Treatment on the Degradation of Formetanate-HCl at 21°C

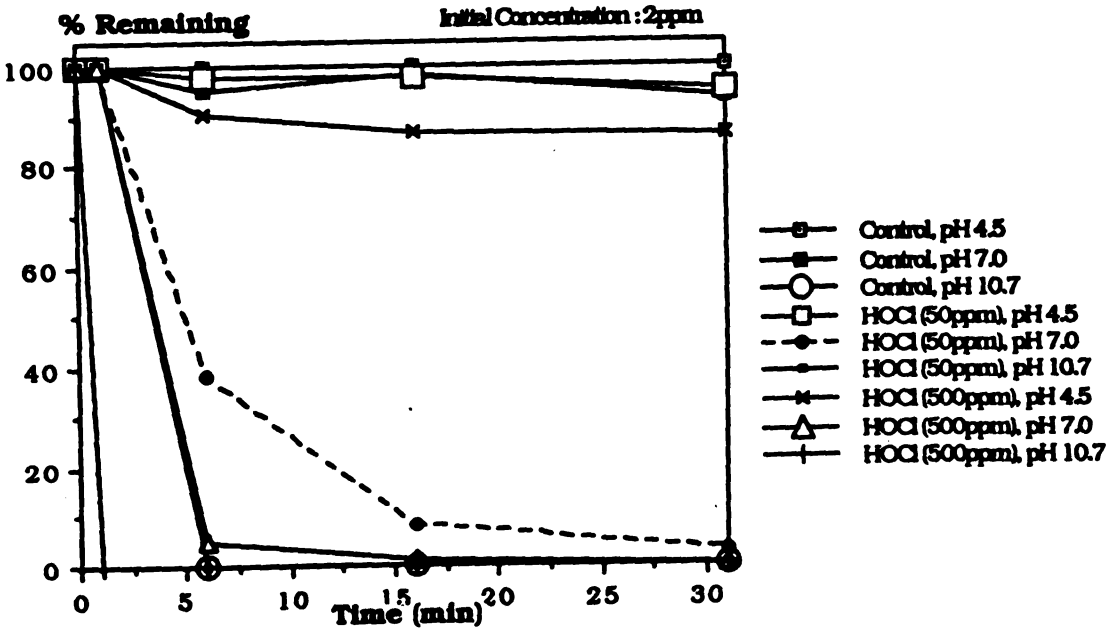


Figure 25 : Effect of Chlorine Treatment on the Degradation of Formetanate-HCl at 44°C

both temperatures when treated with ozone, most of the degradation was due to hydrolysis rather than oxidation by ozone.

Formetanate-HCl in chlorinated solution was relatively stable at low pH, and extremely unstable in its basic form at pH 10.7 (Figure 24 and 25). Formetanate-HCl was degraded by 80-100% in 50 and 500 ppm calcium hypochlorite at pH 10.7. However, this was due mainly to hydrolysis rather than oxidation by the HOCl (Figure 24 and 25). Elevated temperature significantly increased the degradation of the pesticide in chlorinated water at pH 7.0. There was no significant difference between the two temperature treatments at pH 4.5, which indicated the relative stability of the pesticide in its acidic form. Of the three treatments (ozone, 50 and 500 ppm HOCl), chlorination at 500 ppm was the most effective treatment in the degradation of formetanate-HCl.

B. Study on Pesticide Dissipation In Fresh and Processed Apples

(I) Apple Processing

The apples were either prepared by chopping in a Hobart food chopper or processed into a commercial type apple sauce, for analysis on/in the fruit and in the sauce respectively. Appendix II shows raw data for the various wash treatments such as temperature, pH, ozone or chlorine concentration and the weight of the fruits before and after preparation. The average yield of the apple sauce was 31.3 ± 1.66 %.

The low yield was a direct result of processing, where coarse fibers, seeds, stems, and peel particles were removed to obtain the finished apple sauce.

(II) Azinphos-methyl Residues In/On Apple Fruit And Sauce

The data presented in Table 4 and illustrated in Figure 26, show the effects of the various wash treatments on reduction of azinphos-methyl residue in/on apple fruit and sauce. The total amount of residue on the control unwashed fruit was determined to be 0.67 ppm or 401.78 μg. The established tolerance level for azinphos-methyl as published in the Code of Federal Regulations, USA (1990) is 2.0 ppm.

Almost 53% of azinphos-methyl residue was removed from the fruit with the water wash. Apples dipped in ozonated water reduced residue levels by about 75%. Chlorine wash at 50 and 500 ppm removed about 76% and 83% residue, respectively. The various wash treatments significantly reduced residue levels as compared to the unwashed samples. While there appeared to be a decrease in the amount of azinphos-methyl removed from the water washed fruits to those washed with either ozone or chlorine, there was no statistical difference between treatment means (Bonferroni t test).

Processing apples into apple sauce significantly reduced the levels of azinphos-methyl residue (Figure 26). About 96% of azinphos-methyl was removed when the unwashed apples were processed into sauce. Washing the apples followed by processing reduced the amount of

Table 4 : EffectOf Various Treatments On Residual Azinphos-methyl On Apples

Treatments	Mean Wt. of 4 Apples (g)	Sample Conc (μg/g)	Amt. Pesticide (μg)	% Removal
Fruit - No Wash	597 ± 18	0.670 ± 0.1615	401.78 ± 110.352	0.00%
Fruit - Water Wash	575 ± 37	0.249 ± 0.0503	181.34 ± 50.047	52.93%
Fruit - Ozone Wash (0.25 ppm)	582 ± 02	0.170 ± 0.0162	98.76 ± 9.516	74.66%
Fruit - Chlorine Wash (50 ppm)	552 ± 38	0.163 ± 0.0147	89.70 ± 8.760	75.73%
Fruit - Chlorine Wash (500 ppm)	585 ± 13	0.114 ± 0.0014	66.48 ± 3.010	83.04%
Sauce - No Wash	180 ± 07	0.085 ± 0.0041	15.27 ± 1.097	96.30%
Sauce - Water Wash	173 ± 14	0.054 ± 0.0016	9.31 ± 1.130	97.51%
Sauce - Ozone Wash (0.25 ppm)	178 ± 14	0.048 ± 0.0032	8.66 ± 1.356	97.82%
Sauce - Chlorine Wash (50 ppm)	190 ± 13	0.050 ± 0.0026	9.53 ± 1.294	97.48%
Sauce - Chlorine Wash (500 ppm)	185 ± 03	0.032 ± 0.0018	5.88 ± 0.450	98.48%

Note : Washes @ ambient temperature (21°C) for 15 minutes

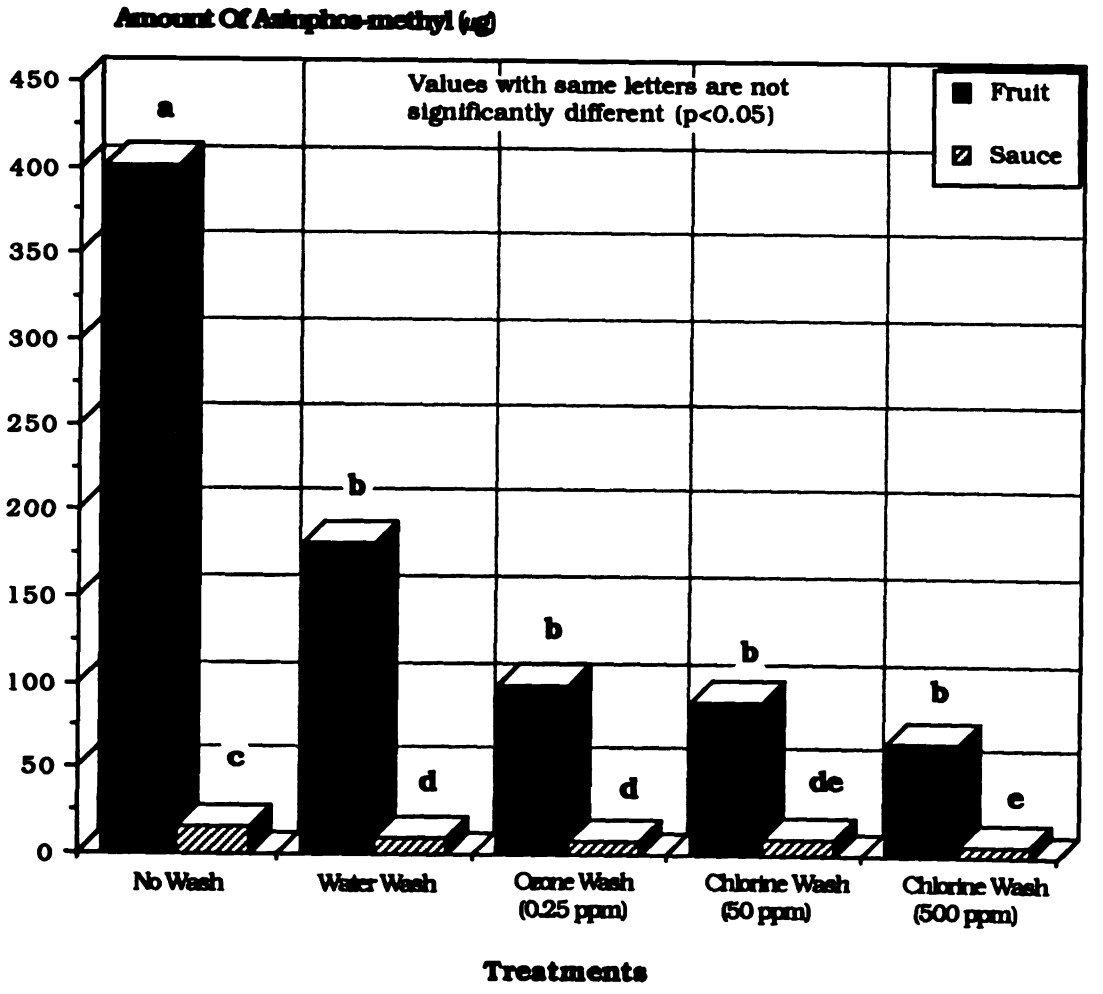


Figure 26 : Amount Of Azinphos-methyl Residue On/In Apple Fruit & Sauce

residue by 98%. The amount of azinphos-methyl remaining in the sauce from fruit washed with either ozonated or chlorinated water was between 2-3%.

Studies have shown that the effect of processing can reduce azinphos-methyl residues in fruits. Gunther *et al.* (1963) determined that 71-94% of the azinphos-methyl on orange rind was removed by normal washing procedures. In a study by El-Hadidi (1993) at Michigan State University, the effects of washing/grading of apples were shown to reduce azinphos-methyl by 60%. Processing of apples into apple products such as apple slices, sauce and juice were also significantly effective in reducing the pesticide residue levels to non-detectable amounts in some and lower than those on fresh fruits in others. Processing the apples into apple sauce reduced the amount of azinphos-methyl from 174.1 to 6.8 ppb or by 96%. The results from the present study were all in agreement with those reported by Gunther *et al.* (1963) and El-Hadidi (1993).

(III) Captan Residues In/On Apple Fruit And Sauce

Figure 27 and Table 5 show the amount of captan residue in and on apple fruits and in apple sauce after various wash treatments. The amount of captan on the control apples (unwashed) was 0.488 ppm or 291.54 μg. This amount was well below the maximum tolerance level of 25 to 100 ppm established in the United States (CFR-US,1990),

considering the apples were harvested only one day after the last pesticide application.

The effect of a simple water wash treatment reduced the amount of captan by about 50%. Ozone wash removed approximately 72% of captan, while the 50 and 500 ppm chlorine wash removed 66% and 77% pesticides, respectively. There was a significant ($p < 0.05$) difference between the unwashed and washed fruits. However, there was no significant difference between the water wash and the ozone or chlorine at 50 ppm wash treatments. Chlorine wash at 500 ppm was the most effective treatment for captan removal.

Frank *et al.* (1983) reported that washing apples with water removed 43% of the captan residue. The washing in that study involved rinsing each apple with 500 ml of water at 25°C. The percent removal by this method was in agreement with the results obtained in this study for the water wash treatment. Hendrix (1991) reported that 0.42 ppm captan remained on apples washed with water, while 500 ppm chlorine dip for 5 minutes reduced residues to less than detectable levels. The percent reduction of residue from the water wash to the 500 ppm chlorine wash in this study was about 56% (from 0.255 to 0.110 ppm). In the study by Hendrix (1991), the apples were brushed in addition to washing, while the apples in the present study were simply dipped in water and agitated every minute during the 15 minute treatment.

Processing the apples into apple sauce significantly ($p < 0.05$) reduced the amount of captan. Almost 97% was removed from the unwashed fruits by processing, while 99% was removed after the fruits were water washed and processed. No detectable amount of captan was

Table 5 : Effect Of Various Treatments On Residual Captan On Apples

Treatments	Mean Wt. of 4 Apples (g)	Sample Conc (μg/g)	Amt. Captan (μg)	% Removal
Fruit - No Wash	597 ± 18	0.488 ± 0.0617	291.54 ± 41.354	0.00%
Fruit - Water Wash	575 ± 37	0.255 ± 0.0338	147.39 ± 27.8378	47.47%
Fruit - Ozone Wash (0.25 ppm)	582 ± 02	0.139 ± 0.0051	80.76 ± 3.348	71.55%
Fruit - Chlorine Wash (50 ppm)	552 ± 38	0.165 ± 0.0222	90.63 ± 10.210	66.33%
Fruit - Chlorine Wash (500 ppm)	585 ± 13	0.110 ± 0.0129	64.48 ± 8.459	77.41%
Sauce - No Wash	180 ± 07	0.062 ± 0.0002	11.22 ± 0.503	96.15%
Sauce - Water Wash	173 ± 14	0.013 ± 0.0231	2.60 ± 4.495	99.05%
Sauce - Ozone Wash (0.25 ppm)	178 ± 14	n.d. *	n.d. *	100.00%
Sauce - Chlorine Wash (50 ppm)	190 ± 13	n.d. *	n.d. *	100.00%
Sauce - Chlorine Wash (500 ppm)	185 ± 03	n.d. *	n.d. *	100.00%

* n.d. = non detectable

Note : Washes @ ambient temperature (21°C) for 15 minutes

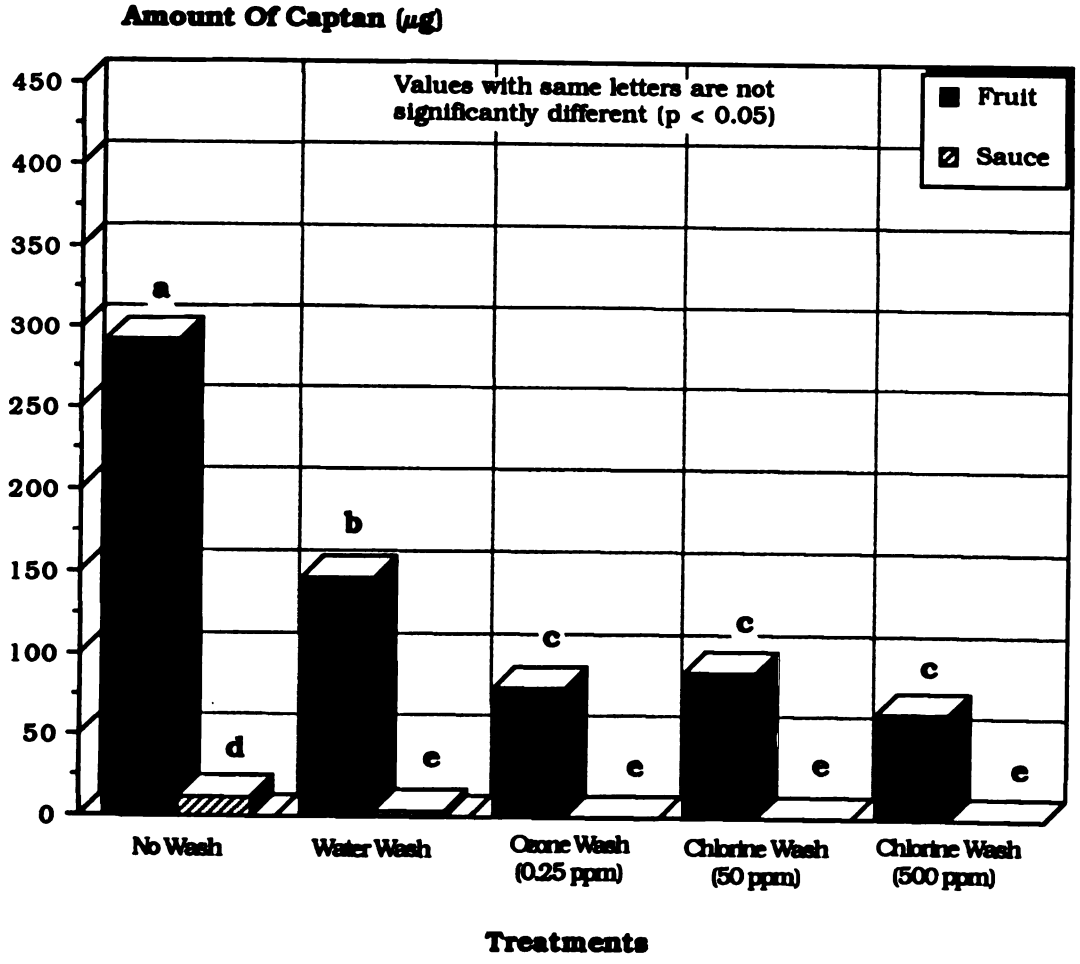


Figure 27 : Amount of Captan Residue On/In Apple Fruit & Sauce

found in the sauces that were first washed with ozonated or chlorinated water.

The significant decrease ($p < 0.05$) in captan residue due to processing could be due to the high heat and pressure generated during the blanching of the apples. According to Worthing and Hance (1991), the melting point of captan is 160-170°C, and it decomposes at or near its melting point. Therefore, it would be expected that most of the residual captan would be degraded during the blanching process where temperature is maintained at 110°C for about 10 minutes.

Frank *et al.* (1983) reported that boiling the whole apple for 5 minutes or cooking the peeled and diced apple removed and/or destroyed 70% to 98% of the residue. They also showed that the combination of thorough washing and cooking gave almost 100% removal. Cooking tomatoes for 15 minutes also reduced captan residues by 97.7-98.9% (El-Zemaity, 1988). Ritchey *et al.* (1984) reported that washing and cooking strawberries for 5 minutes reduced captan by more than 95%. All the studies indicated that a combination of washing and high temperature treatment significantly reduced residues as was the case in this study.

In general, there were significant differences ($p < 0.05$) between the washed fruits compared to the unwashed fruits in terms of captan reduction. However, there was no statistical difference at the 5% level between the water wash apples and the ozone and 50 ppm chlorine washed fruits, although there was a reduction in the residue levels. Increasing the chlorine concentration to 500 ppm did significantly increase the effectiveness of the chlorine wash. It is anticipated that

residue levels would be reduced considerably by the ozone treatment if the concentration of ozone was increased above the 0.25 ppm that was used in this study.

(IV) Formetanate-HCl Residues In/On Apple Fruit And Sauce

The amount of formetanate-HCl found on/in unwashed apples was 392.23 μg or 0.657 ppm (Table 6). The maximum tolerance level for formetanate-HCl in the U.S. is 3.0 ppm (CFR-US, 1990). Reduction in residual formetanate-HCl was significantly ($p < 0.05$) influenced by the effect of various wash treatments. There was significantly less residue in the water washed apples than apples that were unwashed (Figure 28). While water wash fruits reduced levels by 23%, the ozone and chlorine washes reduced formetanate-HCl by 46-58%. The 500 ppm chlorine wash was the most effective treatment. However, there appeared to be no statistical difference between the three washes (ozone, 50 and 500 ppm chlorine) at the 5% level.

The reduction of formetanate-HCl by water wash was not as effective as expected. Studies done by Iwata *et al.* (1985) and Hadjidemetriou *et al.* (1985) showed that simple washing removed a significant amount of the pesticide on the fruit. One possible explanation could be that the pesticides were in the apple fruits rather than on the surface, and would have not been completely washed off by the water dip. El-Hadidi (1993) reported that residues on golden delicious apples were found mainly in the fruits rather than on the

Table 6 : Effect Of Various Treatments On Residual Formetanate-HCl On Apples

Treatments	Mean Wt. of 4 Apples (g)	Sample Conc (μg/g)	Amt. Pesticide (μg)	% Removal
Fruit - No Wash	597 ± 18	0.657 ± 0.061	392.23 ± 51.800	0.00%
Fruit - Water Wash	575 ± 37	0.505 ± 0.018	290.03 ± 23.840	23.23%
Fruit - Ozone Wash (0.25 ppm)	582 ± 02	0.354 ± 0.098	205.83 ± 57.063	46.14%
Fruit - Chlorine Wash (50 ppm)	552 ± 38	0.348 ± 0.060	190.13 ± 14.464	47.54%
Fruit - Chlorine Wash (500 ppm)	585 ± 13	0.278 ± 0.024	162.59 ± 16.575	57.70%
Sauce - No Wash	180 ± 07	0.356 ± 0.034	64.03 ± 5.681	84.20%
Sauce - Water Wash	173 ± 14	0.268 ± 0.024	46.70 ± 8.409	87.27%
Sauce - Ozone Wash (0.25 ppm)	178 ± 14	0.174 ± 0.035	31.16 ± 7.740	91.98%
Sauce - Chlorine Wash (50 ppm)	190 ± 13	0.174 ± 0.015	33.16 ± 5.154	91.07%
Sauce - Chlorine Wash (500 ppm)	185 ± 03	0.092 ± 0.024	17.14 ± 4.868	95.48%

Note : Washes @ ambient temperature (21°C) for 15 minutes

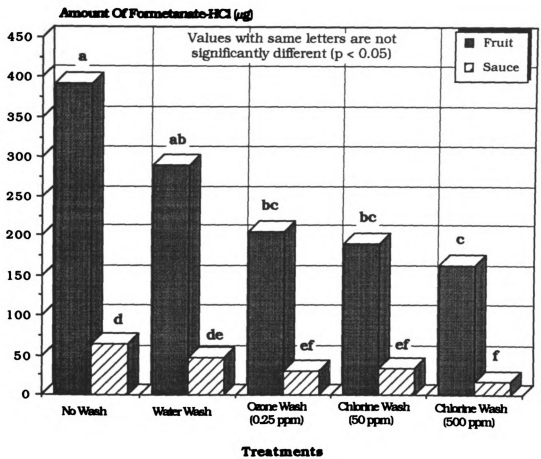


Figure 28 : Amount Of Formetanate-HCl On/In Apple Fruit And Sauce

surface. Of the total residues detected (90.7-122.1 ppb), 1.5-1.0 ppb were detected on the fruit and 90.7-122.1 ppb were found in the fruit.

Processing of apples into sauce significantly ($p < 0.05$) reduced the amount of formetanate-HCl (Figure 28). Unwashed apples that were processed into sauce showed an 84.2% reduction in residue level. Apples that were water washed and subsequently processed into sauce reduced pesticide residue by 87.3%. Ozone and chlorine washed apples processed into sauce reduced the pesticide by 91-96%. The 500 ppm chlorine wash was the most effective treatment, while the ozone treatment was more effective than the 50 ppm chlorine wash treatment.

In the study by El-Hadidi (1993), processing of apples into apple products such as apple slices, sauce and juice were significantly effective in reducing the pesticide residue levels to non-detectable amounts.

(V) Pesticide Residues In Wash Water

Table 7 shows the amount of the three pesticides that were washed off the apples. The amount of azinphos-methyl, formetanate-HCl and captan recovered in the water wash treatment was 420, 253 and 137 μg, respectively. There was significant reduction of the three pesticides in the ozonated water wash treatment as compared to the simple water wash (Figure 29). The reduction of the three pesticides ranged between 29-42%. The 50 and 500 ppm chlorine wash treatment resulted in significantly less pesticide residue in wash water as compared to the water wash treatment. In the 50 ppm chlorine wash

Table 7 : Pesticide Residues In Wash Water After Various Washes

Treatments	Amount of Wash Water (L)	Pesticide Conc (μg/g)	Amt. Carzol (μg)	% Removal
Carzol - Water Wash	5	0.051 ± 0.0082	252.66 ± 40.938	0.00%
Carzol - Ozone Wash (0.25 ppm)	5	0.032 ± 0.0033	161.90 ± 16.470	35.92% *
Carzol - Chlorine Wash (50 ppm)	5	0.012 ± 0.0033	61.17 ± 16.413	75.79% *
Carzol - Chlorine Wash (500 ppm)	5	0.004 ± 0.0066	18.959 ± 32.838	92.50% *
Captan - Water Wash	5	0.027 ± 0.0019	137.48 ± 9.488	0.00%
Captan - Ozone Wash (0.25 ppm)	5	0.016 ± 0.0006	80.27 ± 2.747	41.61% *
Captan - Chlorine Wash (50 ppm)	5	0.003 ± 0.0012	10.08 ± 10.431	92.67% *
Captan - Chlorine Wash (500 ppm)	5	0	0	100.00% *
Guthion - Water Wash	5	0.084 ± 0.0182	419.25 ± 90.755	0.00%
Guthion - Ozone Wash (0.25 ppm)	5	0.060 ± 0.0090	299.63 ± 45.010	28.53% *
Guthion - Chlorine Wash (50 ppm)	5	0.003 ± 0.0009	17.192 ± 4.301	95.90% *
Guthion - Chlorine Wash (500 ppm)	5	0	0	100.00% *

* compared to amount of residue in Wash Water - Water

Note: (1) Carzol = Formetanate-HCl ; (2) Guthion = Azinphos-methyl

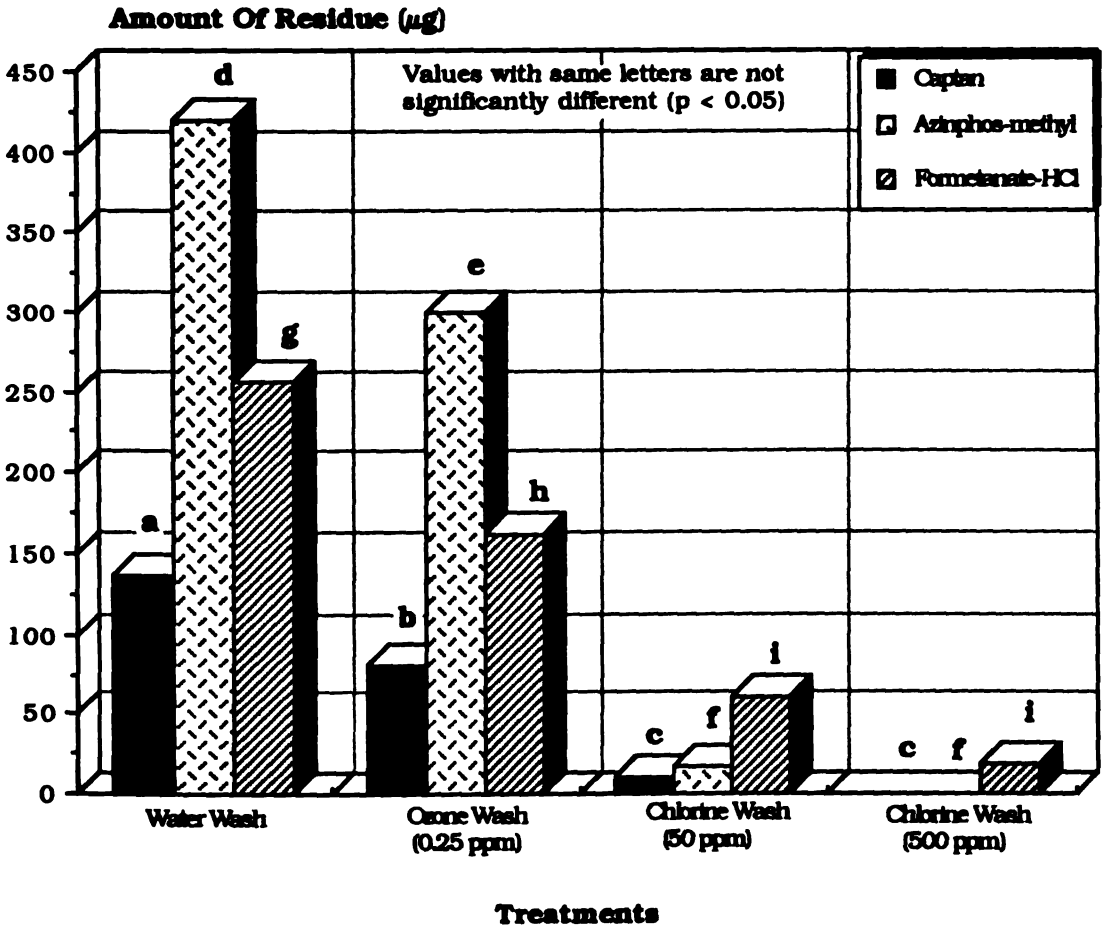


Figure 29 : Pesticide Residues In Wash Water

treatment, 76%, 93% and 96% of formetanate-HCl, captan and azinphos-methyl were removed respectively as compared to the water wash treatment. The 500 ppm chlorine wash removed 93% of formetanate-HCl and all detectable levels of captan and azinphos-methyl (i.e. 100%).

The ozone wash was not as effective as would be expected. In the model studies, each of the three pesticides showed greater rates of degradation in water at the various pH's and temperatures than in the ozone wash treatment at similar pH's and temperatures. One possible explanation could be that the ozone wash at 0.25 ppm was not as effective due to its low concentration, instability in water, and the high organic content in the wash water. The presence of organic materials has been reported by Glaze (1987) to accelerate the decomposition of ozone.

The results indicate that the ozone and chlorine wash treatments were effective in reducing the amount of pesticide residues in the wash water after the pesticides have been rinsed off the apples. This would ensure that the wash water would be 'detoxified' before it is disposed of as waste. This is an advantage in terms of reducing chemical waste and ensuring the safe disposal of pesticide waste.

SUMMARY AND CONCLUSIONS

The objective of the present study was to determine the effectiveness of chlorine and ozone as postharvest washes used in the dissipation of pesticide residues in solution and on fresh and processed apple fruits. A model study was conducted to determine the effects of calcium hypochlorite (50 and 500 ppm) and ozone (0.25 ppm) at pH 4.5, 7.0, 10.7 and ambient (21°C) and elevated (44°C) temperatures on the degradation of azinphos-methyl, captan and formetanate-HCl in solution over a 30 minute period. Aqueous solutions were unbuffered at pH 4.5-4.8 and buffered at pH 7.0 (0.2 M sodium phosphate) and pH 10.7 (0.2 M carbonate-bicarbonate). Samples from the model study were analyzed for residues using either GC (captan and azinphos-methyl) or HPLC (formetanate-HCl).

The rate of degradation of azinphos-methyl, captan and formetanate-HCl due to hydrolysis generally increased at higher pH and temperature. Degradation of azinphos-methyl by ozone was greatest at pH 4.5 and decreased with increasing pH. Between 17-39% of azinphos-methyl remained at both 21 and 44°C in all three pH solutions when sampled at 30 minutes. Compared to the control, it appeared that most of the azinphos-methyl degradation at the low pH was due mainly to ozonation and little due to hydrolysis. Captan was extremely unstable when treated with ozone. After 6 minutes in

ozonated water at 21°C, 7.3% and 0% (i.e. nondetectable levels) of captan remained at pH 4.5 and 7.0, respectively. Captan was unstable even at time zero at pH 10.7. Formetanate-HCl was relatively stable at low pH in its hydrochloride salt form. As formetanate at alkaline pH, it was unstable and degraded rapidly. In general, ozonation at high pH was less effective due to the instability of ozone in solution as pH increases. Elevated temperatures did not significantly accelerate degradation of the pesticides by ozonation, except for captan.

Azinphos-methyl and captan were rapidly degraded in 50 & 500 ppm chlorine solutions at low pH, but degradation decreased at higher pH. Formetanate-HCl was relatively stable at low pH in its hydrochloride salt form, while its basic form as formetanate was unstable. An increase in pH decreases the percent of HOCl, and consequently its reactivity with the pesticides. Chlorination (50 and 500 ppm) at pH 4.5 and 7.0 decreased azinphos-methyl and captan by 80-100% after only 6 minutes. Formetanate-HCl was degraded by 80-100% at 50 and 500 ppm chlorination at pH 10.7. However, this was due mainly to hydrolysis rather than oxidation by HOCl. Elevated temperature increased the degradation of the three pesticides in chlorinated water. Chlorination at 500 ppm was the most effective treatment in the degradation of all three pesticides.

Apples sprayed with the three pesticides were used to determine the effectiveness of chlorine and ozone dip washes on the removal of the pesticides on and in fresh and processed fruits. Eight apples were used per replication (3 replications per treatment) and placed in a 15 L bucket containing 5 L of water. The five treatments were: (1) No wash,

(2) Water wash, (3) Ozone wash @ 0.25 ppm, (4) Chlorine wash @ 50 ppm, and (5) Chlorine wash @ 500 ppm. The apples were dipped for 15 minutes at ambient temperatures with constant agitation. The apples were analyzed for pesticide residues on and in the whole fruit and in apple sauce using either GC or HPLC.

The amount of pesticide residues found on the unwashed fruits were well below the EPA tolerance level. The water wash treatment reduced azinphos-methyl, captan and formetanate-HCl residues on and in the fruit by 53, 48 and 23%, respectively. Ozone wash removed 46-75% on/in the fruit, while the 50 and 500 ppm chlorine wash treatments reduced residue levels by 48-76% and 58-83%, respectively. Processing the apples into sauce significantly reduced all three pesticide residues in all the treatments. Between 84-100% of the three pesticides were removed after processing. The 500 ppm chlorine wash was the most effective wash treatment. Ozone wash at 0.25 ppm was not as effective due to its low concentration, its instability in water, and the high organic content of the wash water.

The ozone and chlorine wash treatments resulted in significantly less pesticide residue in the wash water as compared to the water wash treatment. This is an advantage in terms of minimizing pesticide wastes in the wash water and the safe disposal of the waste water.

To conclude, a laboratory-scaled model system was developed and has proven to be effective in showing rates of degradation and/or disappearance of azinphos-methyl, captan and formetanate-HCl with various pH, temperature, ozone and chlorine treatments. Residue

analysis of fresh and processed fruits showed that postharvest and processing treatments were effective in reducing pesticide residue levels.

FUTURE WORK

Possible future research efforts include:

(1) To elucidate possible degradation pathways of azinphos-methyl, captan and formetanate-HCl during ozonation and chlorination, as well as to identify degradation products as a result of chemical oxidation. Assessment of toxicity should also be carried out on the degradation products, especially as applied to chlorinated products.

(2) The author found no indication that ozonated water has been used as a postharvest wash treatment of fruits before this study. The present research indicates that the use of ozone as a postharvest wash treatment is a promising alternative to chlorine washes that are currently used in commercial facilities. More research should be carried out to study concentrations of ozone that can be used to maximize reduction of pesticide residue levels, while ensuring that the amount used do not exceed current safe levels. Studies should also be carried out to determine the possible use of ozonated water washes in replacing chlorine wash as a method of reducing/eliminating various diseases on fruit crops after harvest.

(3) This study determined that the various wash treatments and processing methods were effective in the degradation/removal of pesticide residues on apples at the pilot plant level. Future work should focus on the possibility of scaling up to a commercial size operation, especially as it applies to the ozone treatment. This would help determine the feasibility of using such methods (e.g. ozone washes) on a commercial scale.

APPENDICES

Appendix I : pH Readings Of Samples From Model Study

Sample	Temperature	Azinphos-methyl (Guthion)		Captan		Formetanate-HCl (Carzol)	
		Before	After	Before	After	Before	After
Control, pH 4.5	Ambient (21°C)	4.55	4.60	4.70	4.70	4.40	4.65
Control, pH 7.0	Ambient (21°C)	6.95	6.95	7.00	7.00	7.00	6.95
Control, pH 10.7	Ambient (21°C)	10.70	10.70	10.60	10.60	10.70	10.70
Control, pH 4.5	Elevated (44°C)	4.50	4.55	4.65	4.70	4.50	4.55
Control, pH 7.0	Elevated (44°C)	7.00	7.00	6.90	6.90	7.00	7.00
Control, pH 10.7	Elevated (44°C)	10.65	10.65	10.70	10.70	10.65	10.65
Ozone, pH 4.5	Ambient (21°C)	4.40	4.75	4.30	4.50	4.90	4.75
Ozone, pH 7.0	Ambient (21°C)	7.00	7.00	7.00	7.00	6.95	6.95
Ozone, pH 10.7	Ambient (21°C)	10.60	10.60	10.70	10.70	10.65	10.70
Ozone, pH 4.5	Elevated (44°C)	4.50	4.40	4.65	4.70	4.50	4.55
Ozone, pH 7.0	Elevated (44°C)	7.00	7.00	7.00	7.00	6.95	6.95
Ozone, pH 10.7	Elevated (44°C)	10.70	10.70	10.70	10.70	10.65	10.65
Chlorine 50ppm, pH 4.5	Ambient (21°C)	4.50	4.50	4.50	4.50	4.55	4.55
Chlorine 50ppm, pH 7.0	Ambient (21°C)	7.00	7.00	7.10	7.00	7.00	7.00
Chlorine 50ppm, pH 10.7	Ambient (21°C)	10.75	10.75	10.70	10.70	10.70	10.70
Chlorine 50ppm, pH 4.5	Elevated (44°C)	4.50	4.50	4.55	4.55	4.50	4.55
Chlorine 50ppm, pH 7.0	Elevated (44°C)	7.00	7.00	6.95	6.90	6.90	6.90
Chlorine 50ppm, pH 10.7	Elevated (44°C)	10.72	10.70	10.60	10.60	10.70	10.65

Chlorine 500ppm, pH 4.5	*Ambient (21°C)*	4.55	4.55	4.50	4.50	4.50	4.50

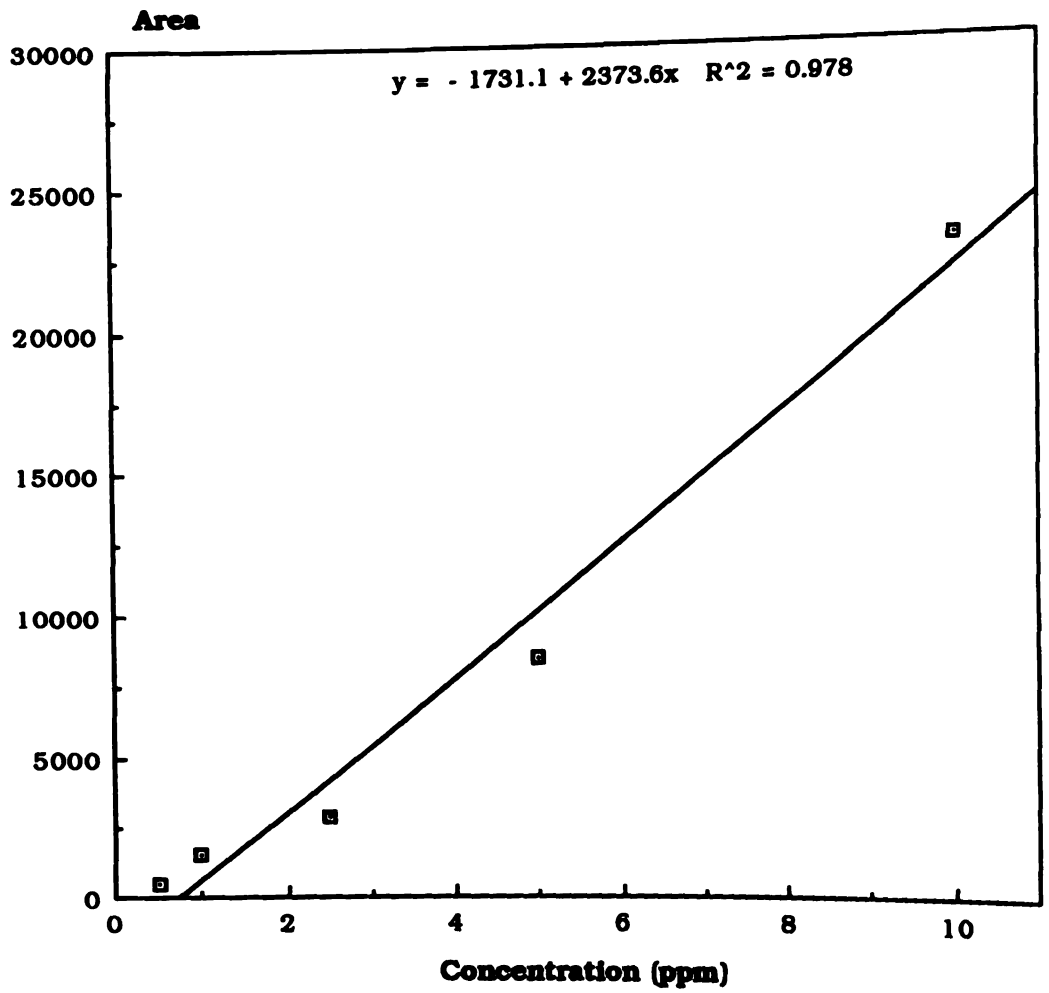
Chlorine 500ppm, pH 4.5	Ambient (21°C)	4.55	4.55	4.50	4.50	4.50	4.50
Chlorine 500ppm, pH 7.0	Ambient (21°C)	7.00	7.00	6.90	7.00	7.00	6.85
Chlorine 500ppm, pH 10.7	Ambient (21°C)	10.60	10.65	10.70	10.70	10.75	10.70
Chlorine 500ppm, pH 4.5	Elevated (44°C)	4.35	4.40	4.50	4.50	4.50	4.55
Chlorine 500ppm, pH 7.0	Elevated (44°C)	7.00	7.10	7.00	6.90	7.00	7.00
Chlorine 500ppm, pH 10.7	Elevated (44°C)	10.70	10.70	10.65	10.65	10.65	10.70

1. Each reading is an average of 3 readings

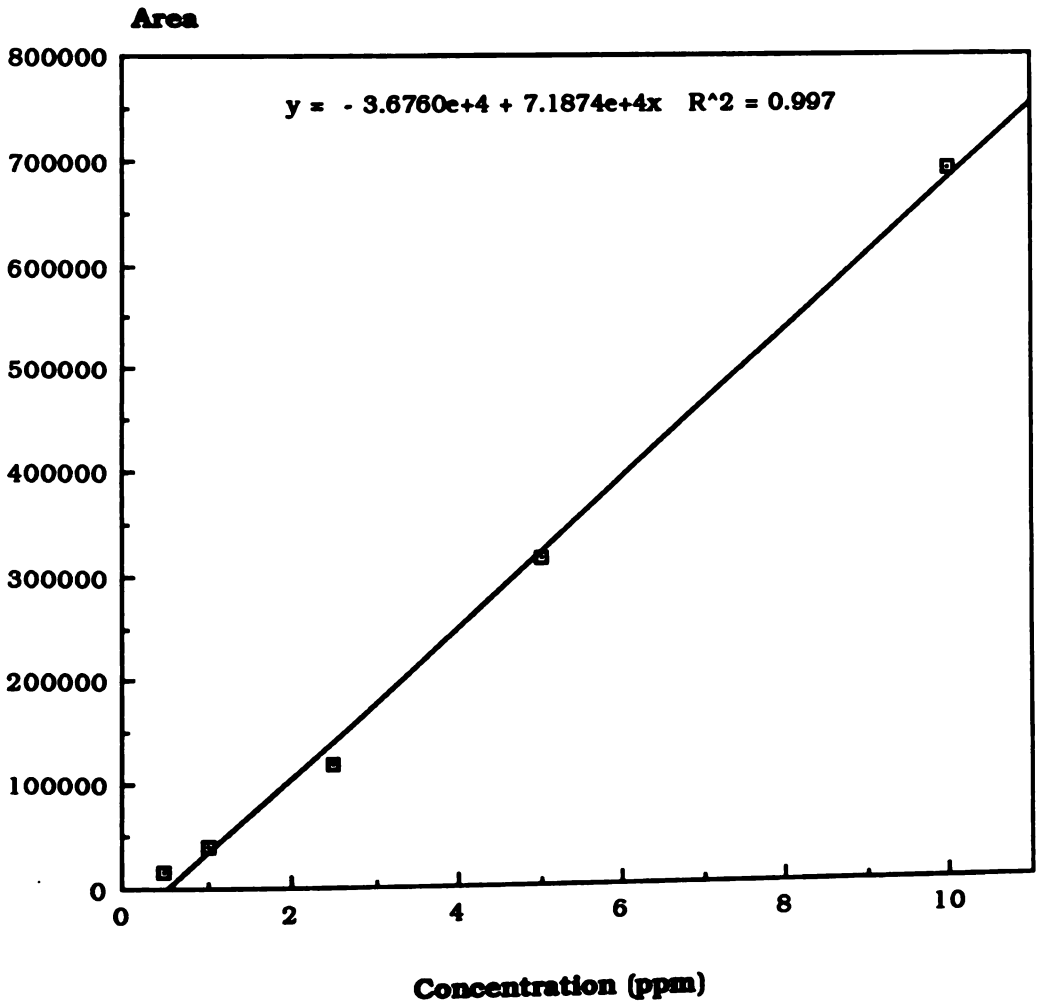
Appendix II : Wash Treatment Raw Data

Sample #		pH		Temperature	Concentration (μg/ml)	Wt. of 4 Apple Fruits (g)				Wt. Apple Sauce (g)			
		Before Wash	After Wash	(°C)	(chlorine or ozone)	Before Process		After Process		Before Process		After Process	
	T1 - R1	-	-	-	-	570		525		570		175	
No Wash	T1 - R2	-	-	-	-	600	597 ± 18	590	602 ± 59	590	617 ± 49	175	180 ± 7
	T1 - R3	-	-	-	-	620		690		690		190	
	T2 - R1	5.50	5.75	21.0	-	600		590		590		195	
Water Wash	T2 - R2	5.40	5.70	21.5	-	520	575 ± 37	500	558 ± 39	500	558 ± 39	170	173 ± 14
	T2 - R3	5.45	6.30	21.4	-	605		585		585		155	
	T3 - R1	5.70	5.50	19.9	0.246	585		610		610		175	
Ozone Wash	T3 - R2	5.80	5.35	21.0	0.257	580	582 ± 2	575	592 ± 12	575	595 ± 12	160	178 ± 14
(0.3 ppm)	T3 - R3	5.50	5.85	22.1	0.238	580		590		590		200	
	T4 - R1	9.35	8.90	21.5	50.50	570		550		550		205	
Chlorine Wash	T4 - R2	9.20	8.80	22.3	49.10	590	552 ± 38	620	565 ± 37	620	565 ± 37	195	190 ± 13
(50 ppm)	T4 - R3	9.35	8.80	21.8	50.00	495		525		525		170	
	T5 - R1	10.00	9.35	21.0	490.00	585		575		575		190	
Chlorine Wash	T5 - R2	10.15	10.00	21.5	492.00	565	585 ± 13	560	577 ± 12	560	577 ± 12	180	185 ± 9
(500 ppm)	T5 - R3	10.20	9.60	22.0	495.00	605		595		595		185	

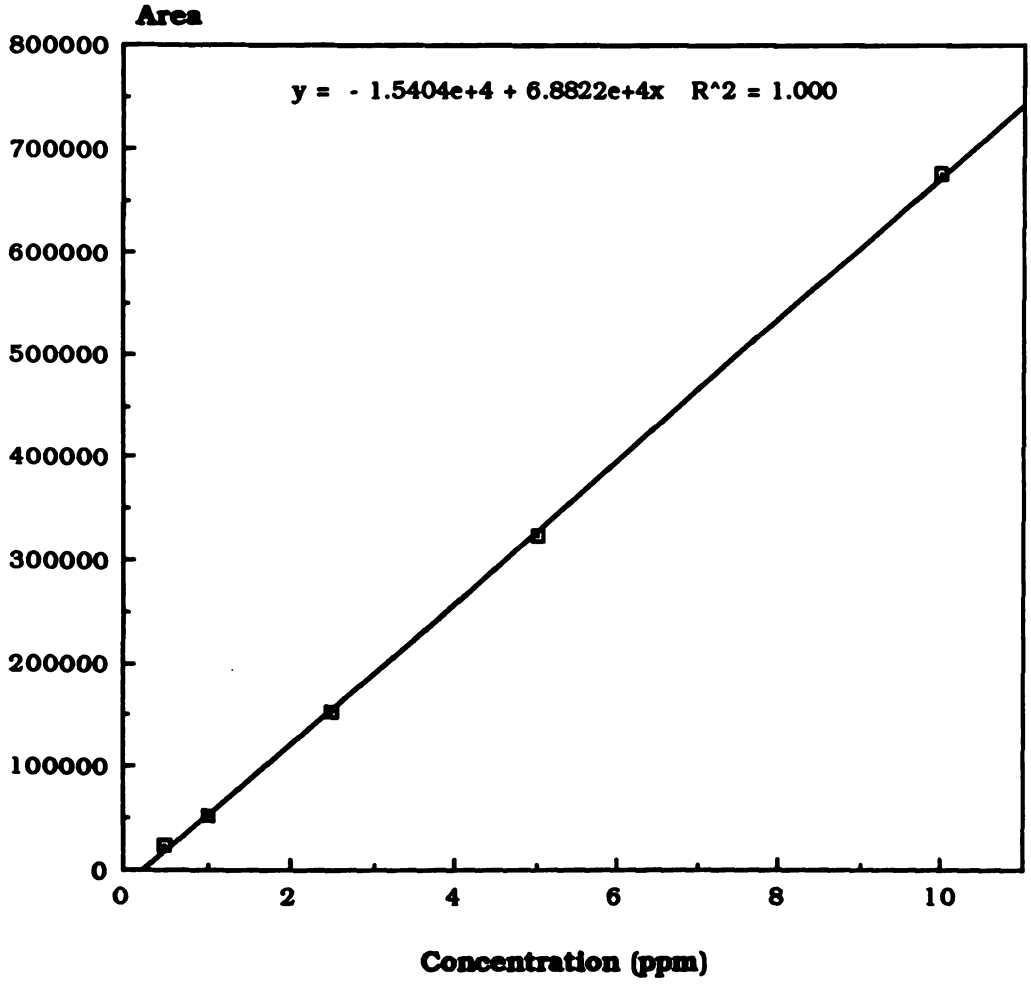
Appendix III : A Typical Standard Curve For Azinphos-methyl Standards



Appendix IV : A Typical Standard Curve For Captan Standards



Appendix V : A Typical Standard Curve For Formetanate-HCl Standards



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