Environmental Fate of Pesticides Used in Australian Viticulture : A Comparison of the Behaviour of the Fungicides Dithianon and Vinclozolin

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requirements for the degree of

Doctor of Philosophy

by

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Abstract

Dithianon is degraded within twenty-four hours of exposure to both red and white grape juice and wine. Vinclozolin is more stable in both red and white grape juice and wine, with a half-life of over 800 h. in these media. At pH's typical for natural Australian waters both dithianon and vinclozolin underwent hydrolysis readily. At 20 °C, the half-life of dithianon at pH 7.0 was 392 h., while that of vinclozolin was 59 h. The half-life of dithianon in aqueous solution exposed to South Australian summer sunlight was 1008 h., while that of vinclozolin was 17.5 h. The half-life of solid dithianon exposed to South Australian sunlight was 1632 h. There was no significant decrease in dithianon levels on the surface of grapes one week after spraying, suggesting that dithianon is both chemically and thermally stable in the conditions prevalent under the vine canopy. However, there was a significant decrease in vinclozolin levels found on the surface of grapes one week after spraying, suggesting that vinclozolin is both chemically and thermally unstable in the conditions prevalent under the vine canopy. Analysis of wines made from grapes sprayed with dithianon found no detectable dithianon residues, suggesting that none of the residual dithianon found on the grape surfaces persists through the vinification process to the young wine. Analysis of wines made from grapes sprayed with vinclozolin indicated that about 10% of the residual vinclozolin found on grape surfaces persisted through the vinification process to the young wine. Dithianon was both immobilised in the top 2 cm and unstable in both clayey soils extracted from the Rutherglen region of Victoria and alkaline sandy loam soils extracted from the South Australian Riverland (over 90% degrading in six weeks). Vinclozolin was mobile in clayey soils extracted from the Rutherglen region of Victoria (10 % of applied dose leaching in ten days), but unstable in alkaline sandy loam soils extracted from the South Australian Riverland (over 95% of the applied dose degrading in six weeks).

Preface

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

I give consent to this copy of my thesis, when deposited in the University Library, being available for loan and photocopying.

Mayumi Ueoka

Date 19 August 97

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Mayumi UEOKA

1. Introduction

There has been a dramatic increase in public awareness and concern about the state of both the global and their own local environments over the past few decades. This has been to a large extent prompted by the significant body of evidence detailing the way in which introduction of anthropogenic substances into the environment have had adverse effects on natural ecosystems, agricultural production and not least human health. One of the factors that led to this increased public awareness of environmental matters was the publication of a small number of inspiring books. Perhaps the most famous was *Silent Spring* by Rachel Carson (1962), the title of which refers to the chilling scenario of a spring devoid of birdsong due to most songbirds having been killed by pesticides, either directly or through the insidious effects of persistent residues. At that time little attention was being given to the environmental consequences of the widespread use of chemicals in agricultural production, urban and even rural roadside weed control. Carson was perhaps the first author to point out that most environmental pollution is insidious and that its harmful effects often only become apparent after long periods of exposure e.g. delayed onset of cancer in humans, gradual increases in atmospheric pollution can cause chronic effects in trees which do not appear for decades, lakes may become polluted and species therein die out without there being many obvious signs.

Since *Silent Spring* there have been many books on environmetal issues. Problems such as habitat destruction, soil erosion, and species extinction are today recognised by much of the general public as being very important for the future of mankind. However, chemical contamination still often arouses the most passionate consumer

interest. This is because people realise that pollution impacts on them directly through effects on their health, their food, and their living environment. It is perhaps wise at this point to make a distinction between pollution and contamination. Pollution is generally reserved for cases where harmful effects are apparent. Contamination is used for situations in which a substance, whether anthropogenic or natural in origin, is present in the environment but not causing any obvious environmental problem. Although undesirable and costly, environmental contamination by anthropogenic substances is often considered to be invevitable. It is also sometimes seen to be a necessary part of life by many in both the world's industrialised and mechanised nations and also developing nations, with many arguing that where the volumes and rates of contamination are relatively low, environmental processes can degrade or assimilate the contaminants.

The viticulture industry in Australia comprises over 5,000 independent grapegrowers and more than 800 wineries spread across all States and Territories. These viticultural enterprises range from the very small to the large multinational. They operate across an extensive range of soils and climatic conditions, and use a wide range of vine, pest, and vineyard water and soil management practices (GWRDC, 1996). Australia's grape and wine industries are significant contributors to both the domestic economy and export markets. For instance in the twelve months to May 1996, Australia had wine exports worth nearly A$400 million to the USA, New Zealand, Canada, Japan, the United Kingdom and other European countries (AWRI, 1996). This Thesis is primarily concerned with examining the environmental behaviour of two fungicides licensed for use in Australian viticulture, dithianon and vinclozolin, and assessing potential environmental problems associated

with trace residues in aquatic systems, on grapes, in wine and in soil, rather than instances of gross environmental pollution. In such cases there will be :

1. a source of the contaminant (viticultural use of fungicide formulations)
2. the contaminants themselves (dithianon and vinclozolin)
3. the transport medium (water, surface run-off, leachate, grapes, wine)
4. the target (aquatic ecosystems, groundwater, the vine canopy, grape juice, wine)

The Codex Alimentarius - an international body established by the United Nations' Food & Agriculture Organisation and World Health Organisation - sets Maximum Residue Limits (MRLs) for agrochemicals in a range of crops, including grapes. These MRLs are used as benchmarks in Australia where MRLs are set to reflect 'good agricultural practice' (AWRI, 1996). Within Australia's viticulture industry there is a range of management systems, particularly in relation to the use of pesticides (insecticides, herbicides, fungicides), all arguably 'best practice' for the particular combination of soil and climatic conditions under which a vineyard operates (Pers. comm., V. Patrick, Mildara Wines, Coonawarra, S.A.). Even within a region growing conditions can change markedly. For instance, it is estimated that in the Barossa there are *at least* twenty seven different soils used for viticulture (Northcote, 1995). The physical and chemical heterogeneity of the natural environments found in and around Australia's vineyards makes the accurate prediction of the fate of agrochemicals very difficult. Nevertheless, the regulatory authorities and organisations such as the Australian Wine Research Institute and the Grape and Wine Research and Development Corporation are under pressure to guide viticulturists in selecting pesticides with minimal residuals. Since significant pesticide toxicity is often seen at sub-mg/L concentrations only a comparatively small amount

need remain on the surface of table grapes, or survive the vinification process, dissolve in surface water run-off or leach through the soil to cause significant problems. As a result, there may be significant variation in, and under estimation of, the environmental risks from pesticide use across the range of soils and climates experienced by the industry.

In screening and registration programs, it is still common practice to estimate pesticide mobility by simply determining physical and chemical properties of the pesticides e.g., adsorption constants, water solubilities and degradation rates, and predict their environmental fate based on such information. However, the environmental conditions in these tests are quite different from natural soils and field conditions. Pesticides contribute in a major way to the quality of life, but their careless or indiscriminate use can have harmful side effects. Efficient and effective use of pesticides requires knowledge of their distribution and persistence in the environment. The main objective of this study was to compare and contrast the environmental fate and behaviour of two fungicides registered for use in Australian viticulture, namely dithianon and vinclozolin, by :

- developing rapid, robust solid phase extraction methods for their extraction from aqueous, grapes, grape juice, wine and soil matrices.
- developing an accurate and precise analytical method for their detection and determination in extracts from aqueous, grapes, grape juice, wine and soil matrices.
- examining their persistence in aqueous solutions of different temperature and pH.
- examining their thermal stability.

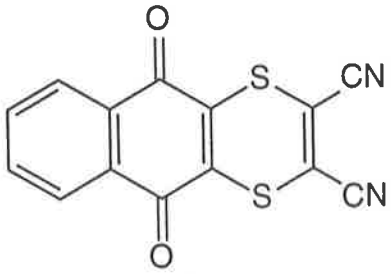
- examining their persistence in grape juice and wine.
- examining their stability towards photo-irrradiation in both the solid and aqueous phases.
- examining their persistence on the surface of grapes under the vine canopy.
- determining their fate during the vinification process.
- examining their transport potential and mobility in soils typically used in Australian viticulture.

Given that environmental contamination may pose one of the greatest threats to the health and food security of the human race, the need for greater understanding of the behaviour of chemicals in the environment becomes even more important. This Thesis introduces some of the key chemical principles to be considered with regard to the behaviour and effects of pesticides in the Australian environment, and describes a series of experimental investigations into the fate and behaviour of two in particular, namely dithianon and vinclozolin.

2. Literature Review

2.1 Dithianon

2.1.1 Introduction



(I)

Figure 2.1 Chemical structure of dithianon

Dithianon (I) [IUPAC name 2,3-dicyano-1,4-dithia-anthraquinone; Chemical Abstracts name 5,10-dihydro-5,10-dioxonaphtho[2,3-b]-1,4-dithiin-2,3-dicarbonitrile or 5,10-di-hydro-5,10-dioxonaphtho[2,3-b]-p-dithiin-2,3-dicarbonitrile] is a member of the quinone family of chemical compounds. This compound has a molecular formula of $C_{14}H_4N_2O_2S_2$ and a molecular weight of 296.33 g/mol. Physically, pure dithianon occurs as dark brown crystals having a coppery lustre, a melting point of 225 °C, vapour pressure of 0.066 mPa (25 °C), and density of 1580 kg/m^3. Dithianon is decomposed by alkaline media, by concentrated acids, and by prolonged heating. Stable up to 80 °C. The concentration of dithianon in an aqueous solution (0.1 mg/L) exposed to artificial sunlight was halved (had DT_{50}) in 19 hour. At 20 °C, dithianon is moderately soluble in chloroform (12 g/l), acetone (10 g/l), and benzene (8 g/l), but only sparingly soluble in water (0.5 mg/l). Its partition coefficient (octanol/water) K_{ow} is 1585 (BCPC/RSC, 1994). Dithianon is manufactured and sold in Australia as Delan® suspension concentrate and wettable powders by Cyanamid.

There are relatively few references to Dithianon in the open literature. Its chemical reactions appear to have been little studied, with the vast majority of citations referring to its uses as an agricultural agent. Indeed, almost all the references to the preparation of dithianon are found in a series of patents dating from the early 1960's. Much useful information concerning the environmental fate of a compound can be garnered by studying the corresponding methods for synthesising the molecule. Historically, the most popular way to prepare dithianon has been to react a derivative of 1,2-dimercapto-1,2-dicyanoethene with one of naphtho-1,4-quinone. van Schoor et al. (1959; 1961) boiled the alkali metal salt of 1,2-dimercapto-1,2-dicyanoethene (II) with 2,3-dichloronaphtho-1,4-quinone (III) in ethanol (Figure 2.2); while others did essentially the same in aqueous media in the presence of surfactants or phase-transfer agents (Kubota et al., 1975; Sanwa, 1982; Kawada et al., 1991).

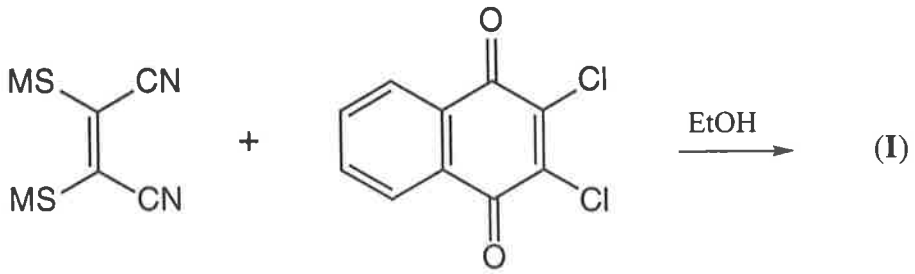


Figure 2.2

A slightly different approach was used by Scheuring et al. (1974), who treated 2-dichloronaphtho-1,4-quinone (IV) with (II) in aqueous acetic acid (Figure 2.3). Treatment with ferric chloride converted the resulting hydroquinone (V) to dithianon.

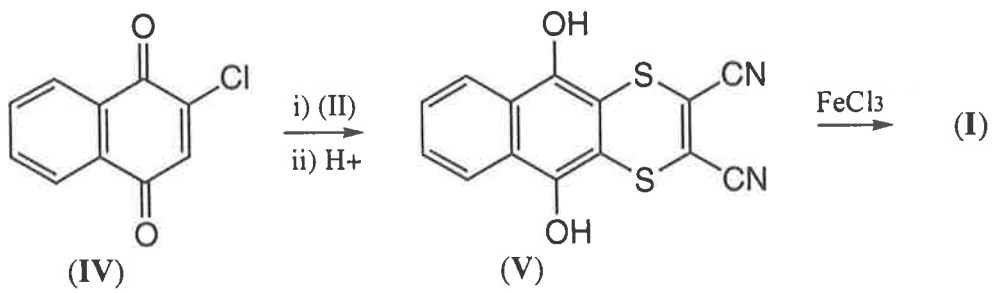
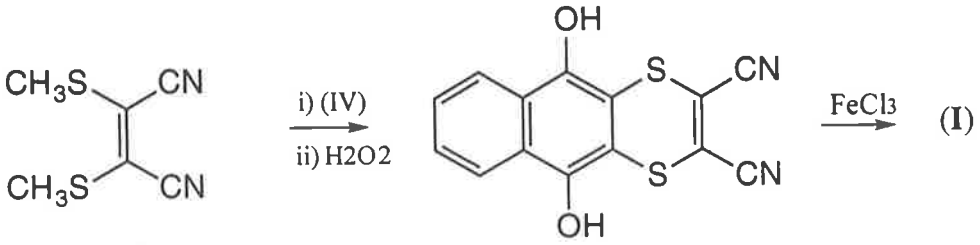


Figure 2.3

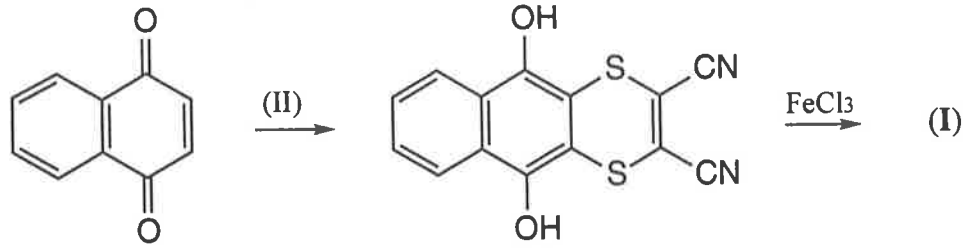
This route through the hydroquinone intermediate was also employed by van Schoor et al. (1963) when they treated 1,2-dicyano-1,2-dimethylmercaptoethene (VI) with (IV) and hydrogen peroxide (Figure 2.4).



(VI) (V)

Figure 2.4

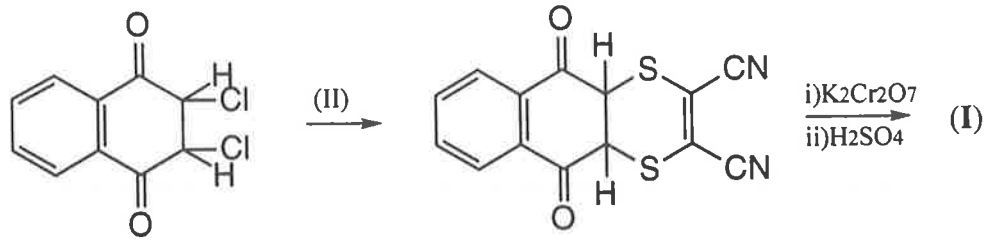
In an alternative patented method, naphtho-1,4-quinone (VII) was coupled with (II) in aqueous media (Jacobi et al., 1962; Kawasaki, 1982a; 1982b) (Figure 2.5).



(VII) (V)

Figure 2.5

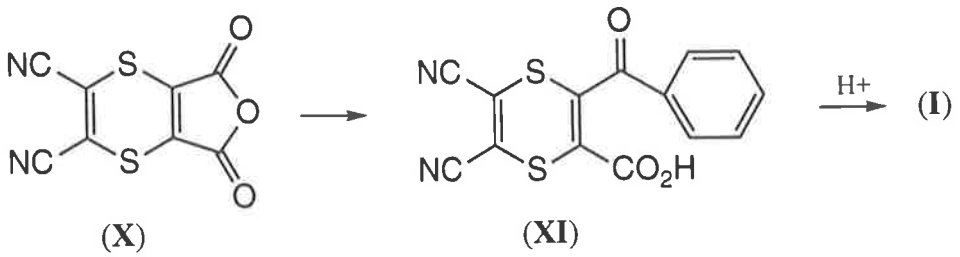
Matsura et al. (1973) condensed 2,3-dichloro-2,3-dihydronaphtho-1,4-quinone (VIII) with (II) and then treated the resulting compound (IX) with potassium dichromate / sulphuric acid (Figure 2.6).



(VIII) (IX)

Figure 2.6

Kobayashi et al. (1969; 1970) prepared the 2,3-dicyano-1,4-dithiine-5,6-carboxylic acid anhydride (X) and then condensed this molecule with benzene. They subjected the resulting compound (XI) to an intramolecular Friedel-Crafts condensation with polyphosphoric acid to give the desired product (Figure 2.7).



(X) (XI)

Figure 2.7

2.1.2 Agricultural Use of Dithianon

Dithianon is used for the control of many foliar diseases, although not against powdery mildews (BCPC/RSC, 1994). Dithianon finds widespread use in the control of fruit diseases, particularly *Venturia spp.*, *Microthriella rubi*, and scab on apples (Byrde and Harper, 1966; Byrde and Melville, 1971; Dancs et al., 1967, 1970; Cartwright, 1967; Kirby and Bennett, 1967-68; Parak, 1970; Wirth and Grimm, 1971; Swinburne et al., 1975; Buchberger and Winsauer, 1980); *Stigmina carpophila*, *Coccomyces hiemalis*, and scab on cherries (RSC, 1993); *Monilia species*, *Taphrina deformans*, *Tranzschelia discolor*, rust, and leaf curl of peaches and apricots (Gottwald and Fiedler, 1987); *Didymella applanatu* on raspberries (O'Riordain, 1969); *Mycosphaerella fragariae* and *Diplocarpon earliarum* on strawberries (Ikeda and Nakamura, 1990); downy mildew and *Pseudoperonospora humili* on hops (Maier, 1988); scab, *Phomopsis citri*, *Elsinoe fawcetti* on citrus fruits (Lee and Liu, 1968; Morita and Nagano, 1987); *Glomerella cingulata* on coffee (Hocking, 1967a,

1967b; Vine et al., 1973). Application rates in most countries are 0.4 - 0.1 kg active ingredient (a.i.) / ha, depending on climatic conditions, infection pressure and agricultural practices. However, application-harvest periods vary considerably, e.g. apples: 35 days (Australia) to 14 days (South Africa); stone fruits: 14 days (Chile) to 21 days (Australia); peaches: 7 days (Japan) to 21 days (Australia) (FAO/WHO, 1992).

Viticultural uses of dithianon include the control against black spot disease (or dead arm disease, *excoriosis*) caused by *Phomopsis viticloa* (Brendel, 1970; Bulit and Bugaret, 1972; Arya, 1988); botrytis bunch or grey mould caused by *Botrytis cinerea* (Chkheidze, 1977); anthracnose caused by *Gloeosporium species* (Sadamatsu and Sanematsu, 1978); and downy mildew caused by *Plasmopara viticolla* (Schuck and Matos, 1985). The recommended application rate in South Australia (1992) is 50 g a.i./100L, with a pre-harvest withholding period of 21 days (Roger, 1992).

2.1.3 Toxicity of Dithianon

Dithianon is classified as a mild skin and eye irritant. This places it in WHO Class III; U.S. EPA toxicity Class III. Mammalian toxicity : acute oral LD_{50} (rat) 638 mg/kg; (guinea pig) 115 mg/kg; acute percutaneous LD_{50} (rat) >2000 mg/kg; acute inhalation LC_{50} (rat, 4 h.) 2.1 mg/l air. In 2-year feeding trials, the no observed effect level (NOEL) for rats was 20 and for dogs 40 mg/kg diet. Other toxicity data ; acute oral LD_{50} for male quail is 280 mg/kg, and for female quail 430 mg/kg. Toxic to fish;

LC_{50} (96h) for common carp 0.1 mg/L. Dithianon, however, is not toxic to bees; contact LD_{50} for bees is >0.1 mg/bee (BPCP/RSC, 1994).

The toxicity of 36 fungicides to tadpoles was examined by Nishiuchi and Yoshida (1972), and dithianon showed comparatively high toxicity. Its LC_{50} against the *Cloeon dipterum* nymph was determined to be 3.8 ppm (48 h-median tolerant limits at 25°C) (Nishiuchi and Asano, 1979). Dithianon showed potent toxicity to the tadpoles of *Rana brevipoda porosa*, LC_{50} <1 ppm (Nishiuchi, 1989).

The effects of pesticides against the aquatic insects *Sigara substriata*, *Micronecta sedula*, *Cloeon dipterum*, *Orthetrum albistylum speciousum*, and *Sympetrum frequens* were determined by Nishiuchi (1981) and these species had median tolerance limits to dithianon of 35 , 120, 3.8, 40, and 40 ppm respectively.

The effect of pH on the toxicity of 14 fungicides on the Japanese Medaka (*Oryzias latipes*) was investigated, again by Nishiuchi (1980a). Dithianon showed decreasing toxicity with increasing pH. Median tolerant limits (LTm) of 0.013 ppm at pH 5.00, 0.020 ppm at pH 6.00, 0.034 ppm at pH 7.00, 0.068 ppm at pH 8.00, 0.52 ppm at pH 9.00, and 1.7 ppm at pH 10.00 were reported.

Nishiuchi (1982a) also investigated the effects of water temperature on the toxic effects of 150 pesticides on *Daphnia pulex*. Dithianon showed 3-h LC_{50} of >40, >40, >40 and 2.5 ppm at 10.0, 17.5, 25.0, and 32.5 °C respectively.

2.1.4 Analytical Methods for Dithianon Determination

Few analytical methods for the detection and determination of dithianon have been reported in the published literature. Traditionally, pesticide residues have been determined by gas chromatography (GC). There have been some reports of the determination of dithianon residues by this technique in the literature (Kadenczki et al., 1992; Suzuki et al., 1974), but also reports that GC methods do not have enough sensitivity for quantitation (Eisenbeis and Sieper, 1973). In addition, dithianon, as a fused quinone derivative, forms a thiophen derivative through extrusion of sulphur on simple heating in a similar manner to that observed with dithiin (Stark and Duke, 1967) (Figure 2.8). The fact that GC methods require the vaporisation of the sample and, therefore, promote sulphur extrusion, has limited the use of this technique for this compound.

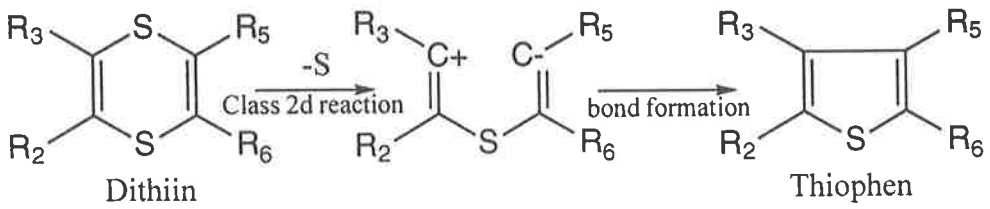


Figure 2.8 Ring-contraction of a 1,4-dithiin to a thiophen

A number of other methods for the analysis of dithianon in fungicide formulations and products, and food and water have been reported, including IR spectrometry (Double, 1969); colorimetry (CIPAC, 1985; Amadori and Haupt, 1978); spectrophotometry (Yuen, 1969; Sieper and Pies, 1968; Goto and Ito, 1967); and thin layer chromatography (TLC) (Korsos and Lantos, 1984; Ambrus et al., 1981; Salo and Salminen, 1966).

Kojima (1980) reported dithianon determinations using high performance liquid chromatography (HPLC) with postcolumn derivatisation. However, Eisenbeis and Sieper (1973) and Baker and Clarke (1984) showed that dithianon can be determined using HPLC without derivatisation. Baker and Clarke (1984) suggested that dithianon standards made up in pure methanol were found to break down rapidly, and that solutions containing less than 20 µg/mL were difficult to chromatograph due to on-column breakdown. These problems were overcome by making up analytical standards in methanol containing 1% v/v acetic acid. Standards made this way were stable over a period of months, and as a result solutions containing less than 1 µg/mL could be chromatographed without any breakdown occurring. Recently, AOAC International adopted liquid chromatography as an official method (AOAC, 1995). Hanks (1995) used their method in a collaborative study to determine dithianon in technical products and fungicide formulations. However, because their standards contained more than 100 µg/mL dithianon, and on-column degradation is negligibleat these levels, the AOAC method uses solvent systems that do not include acetic acid.

2.1.5 Stability of Dithianon

Dithianon is decomposed by alkaline media, by concentrated acids, and by prolonged heating but no data is available regarding its stability to light (RSC, 1993). The rates of hydrolysis of dithianon at different pH has been reported (FAO/WHO, 1992) but no data concerning hydrolysis products was presented. The half-life values reported are shown in Table 2.1. Photodegradation of dithianon was also suggested in the report, but again no details of any degradation products was reported.

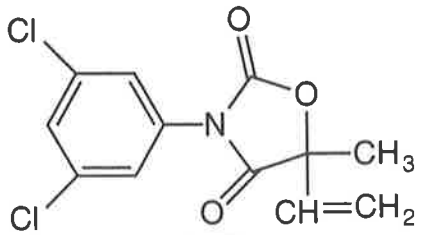
Table 2.1 Dithianon hydrolysis half-life values

FAO/WHO, 1992; *BCPC/RSC, 1994

pH	T (°C)							
	15	22	25	30	40	50	60	70
4						278h	118h	42h
5		295h						
6								
7		15.7h	12.2h*	6.3h	1.50h	0.47h		
8								
9	0.23h	0.15h	0.12h					

2.2 Vinclozolin

2.2.1 Introduction



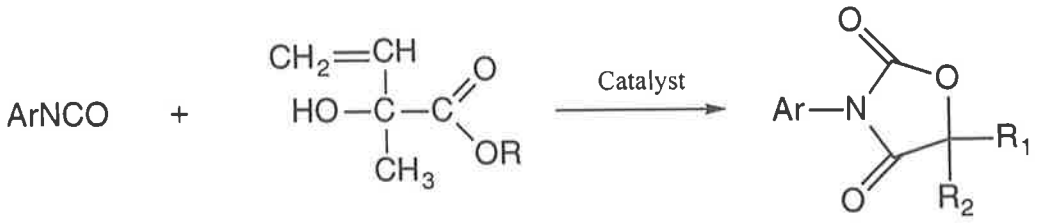
(XII)

Figure 2.9 Chemical structure of vinclozolin

Vinclozolin (**XII**) [IUPAC name 3-(3,5-dichloro-phenyl)-5-methyl-5-vinyl-1,3-oxazolidine-2,4-dione;Chemical Abstracts name 3-(3,5-dichlorophenyl)-5-ethenyl-5-methyl-2,4-oxazolidinedione] has the molecular formula $C_{12}H_9Cl_2NO_3$ and a molecular weight of 286.1 g/mol. Pure vinclozolin forms colourless crystals with a slight aromatic odour, a melting point of 108 °C, and a vapour pressure of 0.016 mPa at 20 °C. At 20 °C, vinclozolin is moderately soluble in water (3.4 mg/l), benzene (8 g/kg), cyclohexane (9 g/kg), and ethanol (14 g/kg), but much more soluble in diethyl ether (63 g/kg), xylene (110 g/kg), ethylacetate (253 g/kg), chloroform (319 g/kg), acetone (435 g/kg), and cyclohexanone (540 g/kg). Vinclozolin is thermally stable up to 50 °C, and also stable in neutral and weakly acidic media Vinclozolin has a partition coefficient (octanol/water) K_{ow} of 1000 at pH7 (BCPC/RSC, 1994). Vinclozolin is manufactured and sold in Australia, and also internationally, as Ronilan® suspension concentrate and wettable powders by BASF.

Compared to dithianon, there are many more references to vinclozolin in the open literature. Its chemical reactions have been quite extensively studied, but again the vast majority of citations refer to its uses as an agricultural agent. Much useful

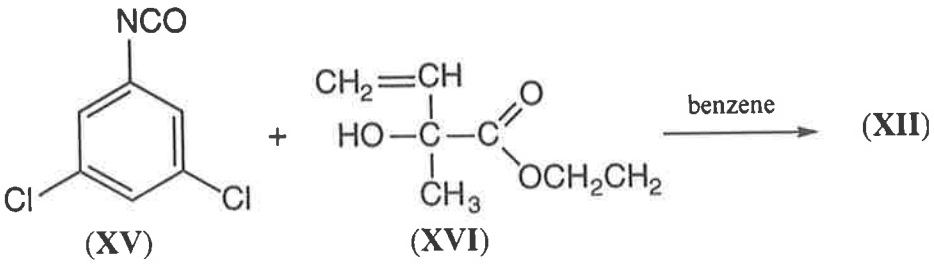
information concerning the environmental fate of a compound can be garnered by studying the corresponding methods for synthesising the molecule. Vinclozolin (XII) can be prepared by a number of different chemical methods. By far the most popular route appears to have been to react an isocyanate (XIII) with the vinyl lactate ester (XIV), often in the presence of a catalyst (Figure 2.10).



(XIII) (XIV) (XII)

Figure 2.10

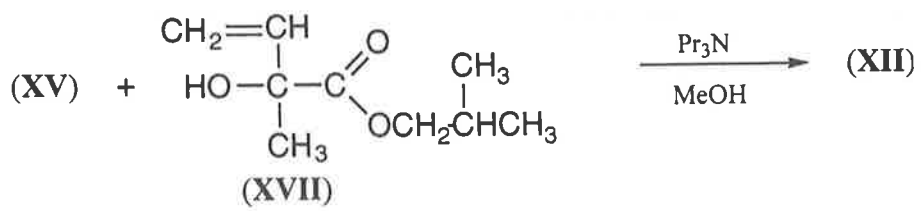
Mangold et al. (1973; 1976a; 1976b) and BASF (1975) refluxed 3,5-dichlorophenyl isocyanate (XV) with ethyl vinyl acetate (XVI) in benzene (Figure 2.11).



(XV) **(XVI)**

Figure 2.11

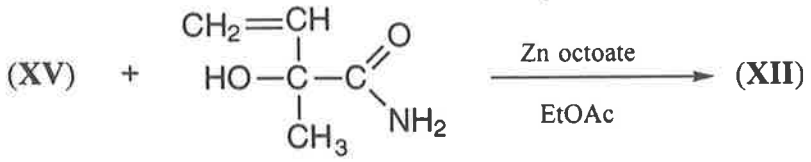
Zanker reacted 3,5-dichlorophenyl isocyanate (XV) with the ester (XVII) in the presence of tripropylamine in methanol (Zanker et al., 1981; Zanker and Ohlinger, 1985) (Figure 2.12).



(XVII)

Figure 2.12

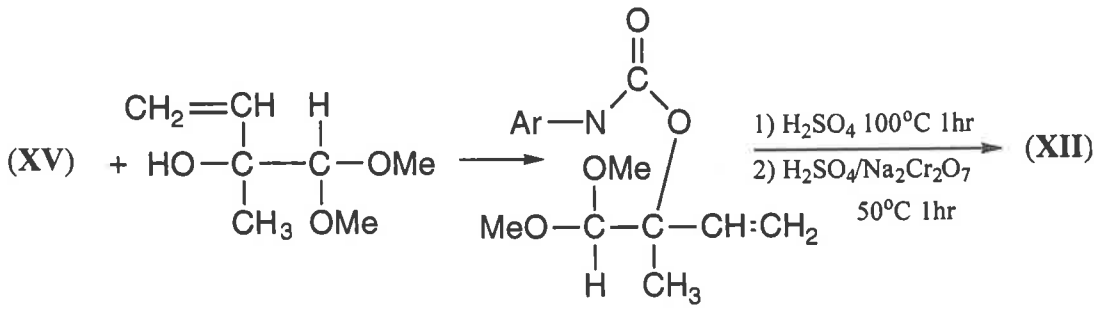
Fauss (1982) reacted 3,5-dichlorophenyl isocyanate (XV) with the amide (XVIII) in the presence of zinc octoate (Figure 2.13).



(XVIII)

Figure 2.13

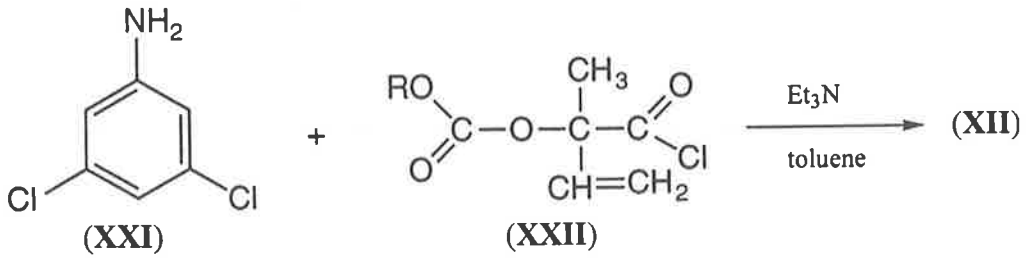
Mangold et al. (1977) also prepared vinclozolin by reacting 3,5-dichlorophenyl isocyanate (XV) with the di-methyl acetal (XIX) and subsequent acid catalysed cyclisation and oxidation of the resulting intermediate (XX) to give the desired product (XII) (Figure 2.14).



(XIX) (XX)

Figure 2.14

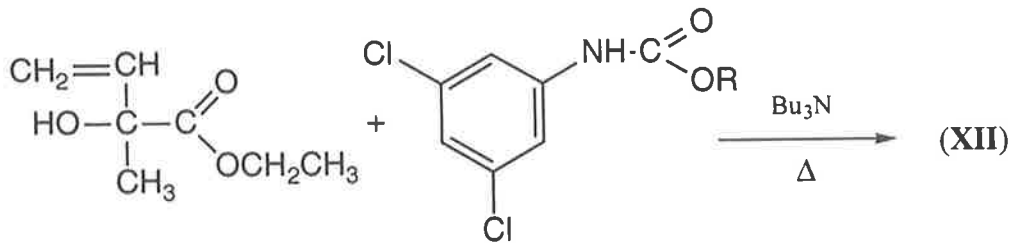
Goetz et al. (1978) prepared vinclozolin (XII) by reacting 3,5-dichloroaniline (XXI) with the acid chloride (XXII) in the presence of triethylamine in toluene (Figure 2.15).



(XXI) (XXII)

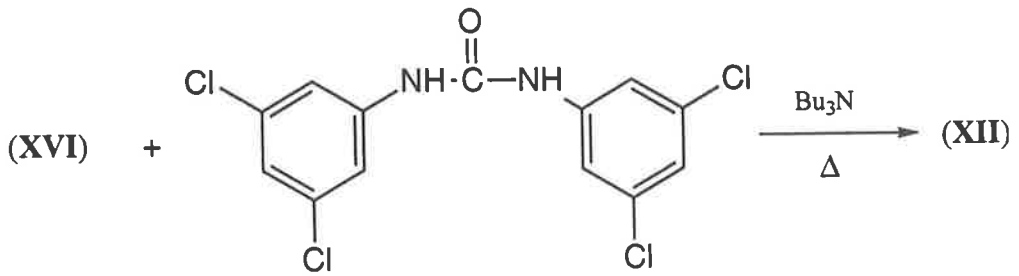
Figure 2.15

Scholz (1979; 1980; 1982) heated ethyl vinyl acetate (XVI) with the amide (XXIII) (Figure 2.16), or the bis-dichlorobenzyl urea derivative (XXIV) in the presence of tributylamine (Figure 2.17). Merger and Towae (1980) reacted ethyl vinyl acetate (XVI) with 3,5-dichloronitrobenzene (XXV) and carbon monoxide at elevated temperatures and pressures in the presence of selenium (Figure 2.18).



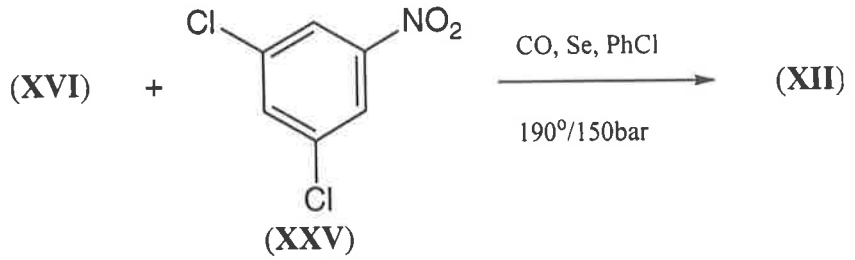
(XVI) (XXIII)

Figure 2.16



(XXIV)

Figure 2.17



(XXV)

Figure 2.18

2.2.2 Agricultural Use of Vinclozolin

Vinclozolin finds many agricultural uses as a contact fungicide. It is used extensively for the control of several noxious fungi, e.g. *Botrytis* spp. (grey mould), on grapes, pome and stone fruits, strawberries, and other berries, on various vegetables (leafy and legume vegetables) and on ornamentals. It is also used against *Sclerotinia* spp. (Sclerotinia rot) on vegetables, oilseeds such as rape and sunflower, red clover and ornamentals. Vinclozolin also finds applications against *Monilia* spp. (blossom blight) and *Sclerotinia* spp. (brown rot) on stone fruit (FAO/WHO, 1986; Directions for Use - Booklet from a Ronilan® 500FL bottle). Application rates in most countries are within a small range, generally 0.5 - 1.5 kg a.i./ha, depending on climatic conditions, infection pressure and agricultural practices. However, application-harvest periods vary considerably, e.g. strawberries : 1 day (Australia) to 21 days (Italy); stone fruits: 1 day (New Zealand) to 21 days (Belgium, Luxembourg); lettuce: 1 day (Australia) to 28 days (The Netherlands, USA); fruiting vegetables, outdoors and under cover (glasshouse or plastic) in most countries: 3-4 days, but 21 days for outdoor tomatoes in Italy (FAO/WHO, 1986).

Perhaps the most important viticultural use of vinclozolin is in the control of Botrytis bunch rot (Botrytis or grey mould) caused by *Botrytis cinerea*. Botrytis exists in all the world's grapegrowing regions, and has the potential to seriously reduce crop quality and quantity. Wine quality is particularly vulnerable because this fungus converts glucose and fructose to glycerol and gluconic acid and produces enzymes that catalyse the oxidation of phenolic compounds. Wines produced from *Botrytis cinerea* infected grapes have off-flavours, and are sensitive to oxidation and

bacterial contamination in the bottle (Bulit, 1988). *Botrytis cinerea* developed resistance to the benzimidazole group of systemic fungicides, such as benomyl and carbendazim, within a few years of their introduction (Cabras et al., 1987). The dicarboximide group of fungicides, of which vinclozolin is a member, have been developed as a substitute for benzimidazoles. In the mid-1970s vinclozolin was reported to control 80% of the infection produced after inoculation with *B. cinerea* suspension (Pommer and Mangold, 1975). In Europe there have since been numerous reports of vinclozolin effectiveness in controlling of grey mould on grapes - in Switzerland (Bolay et al., 1976), Germany (Holz, 1977), Italy (Bisiach et al., 1978; Rumine and Comucci, 1979), Bulgaria (Chelebiev, 1979), Yugoslavia (Matijevic, 1980), and Portugal (Frazao and Alves, 1982). So far, *B. cinerea* has developed less marked resistance to dicarboximide fungicides, including vinclozolin, than to benzimidazoles (Leroux et al., 1982; Gullino et al., 1986; Staneva and Eftimov, 1986). In addition, vinclozolin also has little effect on yeast development during fermentation or wine quality (Sapis-Domercq et al., 1976, 1978; Barbero and Caia, 1979; Stojanovic and Vukmirovic, 1979; Sapis-Domercq et al., 1977; Perez Marin, 1982). For Botrytis control on grapes, the recommended application rate in South Australia (1992) is 100 g a.i./100L, with a pre-harvest withholding period of 7 days (South Australia, 1992).

2.2.3 Toxicity of Vinclozolin

Vinclozolin is classified as a moderate skin irritant and a slight mucous membrane irritant (rabbits). This places it in WHO Table 5; U.S. EPA Toxicity Class IV. Skin

contact should nevertheless be avoided due to a potential for sensitisation and the possibility of limited dermal absorption (Zober, 1995). A Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Expert Group on Pesticide Residues has evaluated vinclozolin toxicology (FAO/WHO, 1987). Their report is based on unpublished, commercial data submitted to the WHO by BASF Aktiengelsellschaft. Vinclozolin is apparently metabolised extensively in rats. The major metabolite excreted in urine, blood, kidneys, and liver is N-(3,5-dichlorophenyl)-2-methyl-2,3,4-trihydroxy-butanoic acid amide (metabolite XXVI, Figure 2.20). Other compounds resulting from further degradation of metabolite XXVI are formed in insignificant amounts. Studies of carcinogenicity, mutagenicity, reproduction, and teratogenicity and reproductive effects were described in the report. Vinclozolin apparently showed negative potential under the test conditions. Mammalian toxicity : acute oral LD_{50} (rat/mice) >10,000 mg/kg; (guinea pig) 8,000 mg/kg; acute percutaneous LD_{50} (rat) >2,500 mg/kg; acute inhalation LC_{50} (rat, 4 h) >29.1 mg/L air. No observed effect level (NOEL) (male rat) 27.1 mg/kg b.w.; (female rat) 28.2 mg/kg b.w.; NOEL (90 day) (rat) 450 mg/kg diet; (dog) 300 mg/kg diet. Other toxicity data : acute oral LD_{50} for male quail is >2,510 mg/kg, LC_{50} for female quail >5,620 mg/kg; LC_{50} (96h) (trout) 23-32 mg/L; (guppy) 32.5 mg/L; (bluegill) 50 mg a.i. (as WP)/L; EC_{50} (48h) (daphnia) 4.0 mg/L. Vinclozolin, however, is not toxic to bees or earthworms (BCPC/RSC, 1994). For humans, the acute toxicity by oral, dermal, and inhalation routes is low. A preliminary estimate of acceptable daily intake for man was 0 - 0.04 mg/kg b.w.

The effects of pesticides on the aquatic insects *Sigara substriata*, *Micronecta sedula*, *Cloeon dipterum*, *Orthetrum albistylum speciousum*, and *Sympetrum frequens* have

been studied, and vinclozolin showed 48-h median tolerant limits (LTm-48 at 25 °C) of 40 ppm respectively (Nishiuchi, 1981).

The effects of water temperatures on the toxicity of 150 pesticides to *Daphnia pulex* were determined, and vinclozolin showed 3-h LC_{50} of > 40 ppm at 10.0, 17.5, 25.0, and 32.5 °C, respectively (Nishiuchi, 1982a).

Vinclozolin was reported as being lethally toxic to tadpoles of *Bufo bufo japonicus* and *Rana brevipoda porosa* at an LC_{50} of 13 and 7.6 ppm, respectively (Nishiuchi, 1982c).

Vinclozolin toxicity to Nishikigoi (*Carrasius auratus*) and Magoi carp (*Cyprinis carpio*) species at 25 °C was examined. Vinclozolin had a 48-hr median LC_{50} of 6.3 and 12 ppm, respectively (Nishiuchi, 1982b). The effect of pH on the toxicity of vinclozolin to *Cyprinus carpio*, and *Oryzias latipes* at 25 °C has also been investigated. Vinclozolin showed decreasing toxicities with increasing pH. The 24-h median LC_{50} of vinclozolin to *Cyprinus carpio* was 11, 12, 13, 20, 30, and >40 ppm at pH 5.0, 6.0, 7.0, 8.0, 9.0, and 10.0, respectively. The 24-h median LC_{50} to *Oryzias latipes* was 5.3, 10, 10, 12, 13, and >40 ppm again at pH 5.0, 6.0, 7.0, 8.0, 9.0, and 10.0, respectively (Nishiuchi, 1982d).

Rankin et al. (1989) studied nephrotoxicity of carboximide fungicides. Vinclozolin is reported to have a negative nephrotoxic potential and induce only minor acute renal effects at the doses tested in rats. Hasegawa and Ito (1992) tested carcinogenecity of vinclozolin using a medium-term liver bioassay. They suggested vinclozolin has the

potential to cause liver cancer even though Ames tests (Salmonella/ microsome test) are negative. However, long-term carcinogenicity tests were equivocal. In addition, in vitro cytotoxicity tests and cell transforming activity suggests carcinogenic potential (Porocco et al., 1993). Vinclozolin may also promote side effects when applied with other pesticides. Treatment of bobwhite quail with vinclozolin produced increased malathion acute *in vivo* toxicity (Ronis and Badger, 1995).

Any assessment of the potential health risks of vinclozolin should include not only the parent compound but also the primary hydrolysis products i.e. 2-[[(3,5-dichlorophenyl)carbamoyl]oxy]-2-methyl-3-butenioic acid (XXVII) and 3',5'-dichloro-2-hydroxy-2-methylbut-3-enanilide (XXVIII) (Figure 2.19). These are found within the soil, the plants, and in the serum of exposed rats (Kelce et al., 1995). Exposure of rats to 100 or 200 mg vinclozolin/kg/day from gestational day 14 (GD-14) to postnatal day 3 (PD-3) results in male pups exhibiting reduced anogenital distance, nipple development, and cleft phallus with hypospadias (Gray et al., 1993). Vinclozolin does not appear to be capable of androgen receptor binding at concentrations that are likely to exist *in vivo*. The primary vinclozolin degradation products XXVII and XXVIII act as pure competitive antagonists of androgen receptor bindings.

Occupational health of vinclozolin exposure to human has also raised concerns. BASF conducted an internal study to examine exposure and targeted health outcomes of 67 employees exposed to vinclozolin for one to 13 years during synthesis and formulation operations through biomonitoring of urinary metabolites that contained a 3,5-dichloroaniline moiety. There was no evidence of any health effects induced by

vinclozolin among employees. In particular, no antiandrogenic effects were found (Zober et al., 1995). Exposure risks after spraying in greenhouses have also studied (Nilsson and Papantoni, 1996). The assumed dermal exposure was higher than the value set by the U.S. EPA but lower than the value given by the WHO. Thus, the use of protective garments during harvesting, especially on the hands, is recommended.

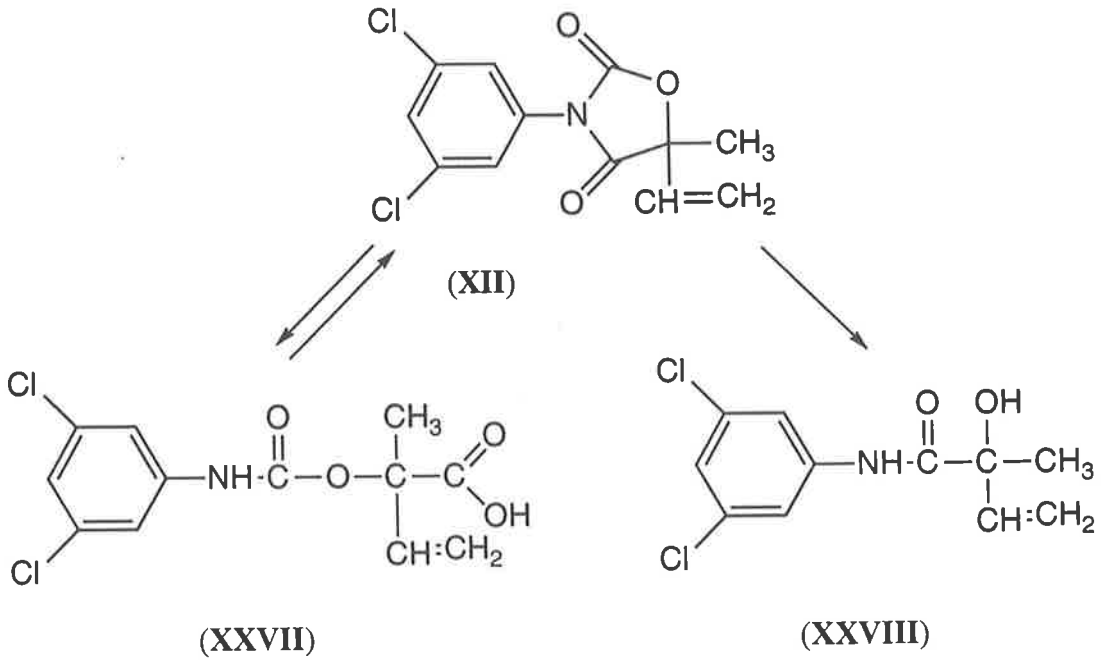


Figure 2.19 Vinclozolin hydrolysis pathways

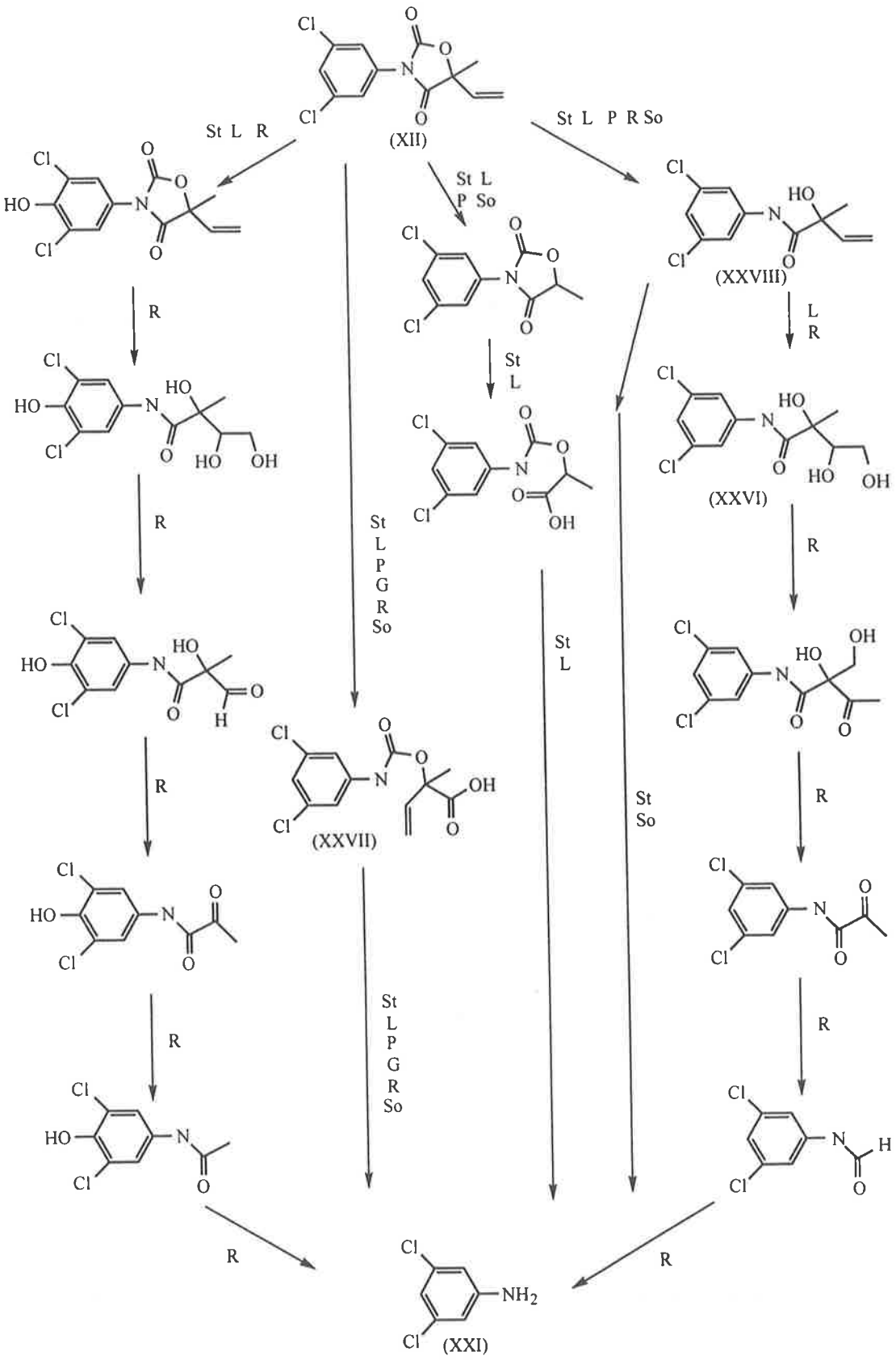


Figure 2.20 Metabolic pathways of vinclozolin (FAO/WHO, 1986)

2.2.4 Analytical Methods for Vinclozolin Determination

Sample Extraction and Clean-up Methods

Pesticide residue analysis often requires sample extraction and clean-up procedures to separate the analyte from interfering compounds in the matrix. Traditionally this has been done using liquid-liquid extraction and partitioning using organic solvents. Cabras et al. (1979) used light petroleum ether to extract vinclozolin from grapes, but the recovery was very low, 20 - 55 %. Acetone extraction is very common in Europe, especially for the extraction of pesticides from food commodities (Loekke, 1979; Zanini et al., 1980; Luke et al., 1981; Bicchi et al., 1985; Gennari et al., 1985; Newsome and Collins, 1989; Stan, 1989; Steinwandter, 1989; Anderson and Paelsheden, 1991; Specht et al., 1995; Roy et al., 1995). Extraction methods using acetonitrile were first developed in the U.S. in 1952, with hexane as the partitioning solvent (Steinwandter, 1989). A number of other researchers have also extracted vinclozolin from a variety of matrices using acetonitrile extraction (Gretch and Rosen, 1987; Ting and Kho, 1991; Hsu, J. P. et al., 1991; Hsu, R. C. et al., 1991; Liao et al., 1991; Schattenberg and Hsu, 1992).

Gel permeation chromatography (GPC) has been used to separate pesticides in food (Steinwandter, 1989; Anderson and Paelsheden, 1991; Stan and Heil, 1991), including grapes (Specht et al., 1995).

Solid phase extraction (SPE) is a more rapid, modern alternative to the aforementioned methods. SPE is based on the principle that the components of interest are retained on a special sorbent contained in a disposable cartridge. By using

SPE one can remove matrix interferences (these either pass through the cartridge or are subsequently washed off) and then isolate with selective enrichment one's target compounds. Multiple extractions can be done simultaneously, rapidly and relatively inexpensively. The final extract from SPE is well suited to chromatographic analysis. Florisil, silica or alumina are among the sorbents that have been used for column chromatography and introduction to SPE (Loekke, 1979; Zanini et al., 1980; Holland and McGhie, 1983). SPE method is primarily used to extract vinclozolin in water samples (Schlett, 1991; de la Vigne et al. 1991; Balinova, 1995; Just et al., 1995; Butz and Stan, 1995; Eisert et al., 1995). SPE has also been reported as a clean-up technique after solvent extraction of vinclozolin from grapes and strawberry (Newsome and Collins, 1989), and from broccoli, carrot, celery, and orange (Hsu, R. C. et al., 1991). Pesticides have been extracted directly from wine using C-18 SPE cartridges (Cabras et al., 1992a; Holland et al., 1994). Table 2.2 summarises the various sample extraction / clean up techniques employed for vinclozolin and published between 1976 - 1996 with the exception of methods contained in the FAO/WHO Pesticide Residues in Food, Reports of JMPR 1986-1992.

Table 2.2 Summary of vinclozolin extraction / sample clean-up methods published 1976-1996

(M = multiresidue analysis)

Reference	Matrix	Extraction/Clean-up Method	Detection Technique	Recovery	M	Residue
1976 Bolay et al.	grape wine					0.46-3.67ppm <0.5ppm
1979 Barbero and Caia	wine					found 5 mo after ferment
1979 Cabras et al.	grapes	light petroleum ether	HPLC	20-55%		
1979 Loekke	strawberries or apples	acetone, dichloromethane, light petroleum alumina column	GC-ECD	66-124%		
1980 Zanini et al.	strawberries	freeze-dried acetone, hexane Florisil column	GC-ECD	91-98%		54.0-145.7 µg/kg 10.8-52.9 µg/kg (after 3 w)
1981 Luke et al.	strawberries	acetone, petroleum ether, methylene chloride, petroleum ether, acetone	GC-HECD-X	98%	M	
1981 Ambrus et al.			TLC		M	
1982 Boccelli et al.	vineyard soil	$Na_4P_2O_7$, benzene	GC	80-119%		0.004-2.10ppm
1982 Cabras et al.	grapes	light petroleum ether benzene	HPLC	95-116% 87-120%		0.24ppm
1982 Lemperle et al.	grapes wine					0.3-4.4ppm 0-0.8ppm
1983 Holland and McGhie	kiwi fruit	methanol, toluene Carbon-cellulose-Florisil column	GC-ECD, AFID	93%	M	
1983 Cabras et al.	must wine	light petroleum	HPLC	103.9±6.5% 100.4±5.2%		
1983 Bufo and Signorile	grapes					1.04-1.15ppm 1.02-1.09ppm (after25-27d)
1983 Fida and Womastek	grapes, must, wine					detected (<MRL)
1983 Gnaegi et al.	grapes, wine					50% of fortified level
1984 Stan and Goebel			GC-ECD/NPD		M	
1984 Flori and Zironi	must, wine					still present in wine
1984 Taccheo et al.	apples and pears	C-18 cartridge	GC-ECD	70.0-89.2%	M	
1985 Bicchi et al.	vine leaves apple strawberries	acetone Florisil cartridge C-18 cartridge	GC-ECD	80.5-90.6% 79.9-89.3%		

Table 2.2 continued

Reference	Matrix	Extraction/Clean-up Method	Detection Technique	Recovery	M	Residue
1985 Gennari et al.	vine leaves grapes	A. acetone/hexane Florisil ctrdge B. benzene Silica ctrdge C. petroleum ether sweep codistilln D. acetone hexane-methylene chloride	GC-ECD	98% 93% 97% 82%		A. 2.5-125µg/g(L) 0.32-0.40µg/g (G) B. 0.06-136.8µg/g (L) 0.30µg/g(G) C. 0.1-201.1 µg/g (L) 0.11-0.94 µg/g (G) D. 0.2-238.9µg/g(L) 0.70-1.20 µg/g (G) (all single dose appl)
1985 Cabras et al.	tomatoes	cyclohexane-benzene mix	HPLC	81.8-16.2%		0.31-5.56ppm
1986 Pirisi et al.	wine	light petroleum	HPLC	87.7-88.2%		
1987 Gretch and Rosen	red peppers	acetonitrile, hexane Florisil column	GC-ECD	93.1-111.1%	M	
1989 Newsome and Collins	grape apple tomato pear cucumber strawberry orange potato	acetone C-18 cartridge	GC-ECD, HPLC	96, 83% 87, 85% 91, 88% 97, 88% 101, 99% 100, 88% 89, 89% 90, 88% (0.5, 5.0 ppm spike)	M	
1989 Stan	vegetables	acetone, dichloromethane gel permeation	GC/MS		M	
1990 Newsome and Collins	grape strawberry	NaOH, distil with H_2SO_4, NaOH, toluene derivatisation to heptafluoro-butyramide of 3,5-dichloroaniline	GC-ECD	88-99% 86-95%		
1991 Schlett	drinking and surface water	C-18 cartridge	HPLC-PDA		M	
1991 de la Vigne et al.	water	C-18 cartridge	HPTLC		M	
1991 Hogendoorn et al.	carrot, fennel, onion	ethyl acetate column switching of HPLC	HPLC	94%	M	
1991 Anderson and Paelsheden	fruits & vegetables	acetone, hexane-methylene chloride, 3-X column ethyl acetate, 3-X column	GC-TSD, FPD GC-ECD		M	

Table 2.2 continued

Reference	Matrix	Extraction/Clean-up Method	Detection Technique	Recovery	M	Residue
1991 Nagayama et al.	green pepper					0.02ppm
1991 Ting and Kho	cabbage redleaf lettuce carrot potato apple nectarine lemon tangerine green onion chill pepper	acetonitrile-dry-isooctane	GC-MIP/AED	116.1% 112.6 107.5 108.9 106.2 116.4 96.5 116.7 127.9 114.5	M	
1991 Garcia et al.	peppers cucumbers	ethylacetate-dry-petroleum ether/acetone Florisil column	GC-ECD	70.6-90.1% 70.6-91.3	M	
1991 Grob and Kalin	lettuce	ethyl acetate automated size exclusion chrom. (SEC)	GC-ECD		M	
1991 Hsu, J.P. et al.	fruits & vegetables	acetonitrile-dry-benzene	GC-ECD	92%	M	
1991 Hsu, R.C. et al.	broccoli carrot celery orange	acetonitrile-dry-benzene-Attagel acetonitrile C-18 SPE acetonitrile-dry-n-hexane-Florisil SPE	GC-ECD	77, 124, 110 80, 116, 111 99, 121, 112 32, 119, 75 % (Attagel, C_{18}, Florisil)	M	
1991 Liao et al.	green bean, lettuce, carrot, bell pepper	acetonitrile-toluene (keeper) concentrated(to 0.5ml by 3 steps)	GC/MS	93%	M	
1991 Stan and Heil	green pepper	extraction, L/L partition gel permeation chrom.	GC-ECD, NPD, FPD column switching		M	
1992a Cabras et al.	wine	C-18 cartridge	HPLC	92-100%	M	
1992 Schattenberg and Hsu	grapes onions peppers strawberries	acetonitrile-dry-benzene (Hsu, J.P. 1991)	GC-ECD		M	detected below EPA tolerance limit
1992a Bernal et al.	non - fat samples fat sample water	hexane/CH_2CL_2 - Florisil column hexane - Florisil	GC - ECD/NPD		M	
1992b Bernal et al.	potato, carrot, olive oil, butter, lyophilised fish tissue (certified ref materials)	hexane - conc sulphuric acid - hexane (acidclean-up)	GC - ECD/NPD	ND	M	

Table 2.2 continued

Reference	Matrix	Extraction/Clean-up Method	Detection Technique	Recovery	M	Residue
1992 Kobayashi et al.	green pepper					0.56ppm
1992 Kadenczky et al.	apples lettuce tomatoes	Florisil powdery sample-chrom., methylene chloride-acetone (or ethyl acetate) extract-dry	GC-ECD	93-112% 103-126 91-97	M	
1994 Holland et al.	wine	C-18 cartridge	GC-ECD, NPD	85-120%	M	
1994 Garcia-Cazorla and Xirau-Vayreda	grapes must wine	cyclohexane	GC-ECD	93.9-96.8% 88.1-91.0 89.0-94.0		0.37mg/kg 0.22 0.08
1995 Papantoni and Mathiasson	leaf surface	ethanol	HPLC	74%		
1995 Balinova	distil. water tap water	C-18 cartridge	TLC - bio-autography	84% 77%	M	
1995 Just et al.	deionised water tap water surface water from wine region	C-18 cartridge	GC-Hall detector	88% 89		not detected
1995 Specht et al.	grapes cucumbers	acetone-ethyl acetate / cyclohexane gel-permeation chrom (Bio Beads S-X3)	GC-ECD	90-101% 86-101	M	
1995a Lehotay and Fller	potato	SFE (supercritical fluid extraction)	GC/MS	86-91%	M	
1995b Lehotay et al.	potato peach	SFE	GC/MS	85-99% 103%	M	
1995 Reuke and Hauck			TLC, HPLC		M	
1995 Cabras et al.	wine					0.02ppm
1995 Butz and Stan	water	C-18 cartridge	HPTLC	LD 10ng	M	
1995 Roy et al.	tomatoes	acetone Florisil column	GC-ECD	82.3-95.2%	M	0.04ppm (EPA tolerance 3ppm)
1995 Eisert et al.	water	C-18 chrom-p	HPLC, GC	97% 71	M	
1996 Matisova et al.	wine	PGCBs cartridge	GC-FID, ECD, MS	80-97%		

Vinclozolin Detection Techniques

Traditionally, pesticide residues have been determined by gas chromatography (GC). GC is also most commonly employed for multiresidue analysis of pesticides since analyte separation is good for thermally stable and volatile organic compounds. There have been a large number of literature reports of the use of GC to determine vinclozolin. Table 2.3 summarises the various GC techniques employed for vinclozolin and published between 1980 - 1995.

Table 2.3 Summary of GC based methods for vinclozolin published 1980 - 1995

Reference	GC detector	Column packing	Carrier gas	Detection limit (Sensitivity)
1980 Zanini et al.	ECD	3% SE52 on 100-120 mesh Chromosorb W	N2	0.05ng
1981 Luke et al.	HECD-X	DEGS / H3PO4, OV-101, SE-30/OV-210 on Chromosorb W	He	
1982 Boccelli et al.	ECD, ECD-FID	OV-11 on Chromosorb W, OV-11/QF-1 on Chromosorb W	N2	0.001ppm
1982 Lemperle et al.	ECD			~0.01ppm
1983 Holland and McGhie	ECD, AFID	OV-225 on Chromosorb W, SE 30 on Gas Chrom Q	N2	
1983 Ripley et al.	NPD	SE-30	He	
1984 Agneessens et al.	ECD	OV1 HTS	H2	0.01-0.05ppm
1984 Stan and Goebel	ECD/NPD	BP-1, BP-10	He	
1985 Bicchi et al.	ECD	OV-1	H2	15pg/µL
1985 Gennari et al.	ECD ECD ECD ECD	SE30 on Chromosorb W OV-1 QF on Chromosorb W OV-17/OV-210 on Varaport	N2 He N2 He	0.02ng 0.02ng 0.05ng 0.02ng
1987 Gretch and Rosen	ECD	OV-101 on Chromosorb W	N2	
1989 Newsome and Collins	ECD	DB-5	He	
1989 Stan	MS	HP-1	He	
1991 Anderson and Paelsheden	TSD, FPD, ECD	SE-30, OV-1701		
1991 Nagayama et al.	ECD, FPD, FTD			
1991 Ting and Kho	MIP/AED	Ultra-2		
1991 Garcia et al.	ECD	HP-1	N2	
1991 Grob and Kalin	ECD	DPT-MDS, PS-255		

Table 2.3 continued

Reference	GC detector	Column packing	Carrier gas	Detection limit (Sensitivity)
1991 Hsu, J. P. et al.	ECD	DB-608, DB-5	He	0.100ppm
1991 Hsu, R. C. et al.	ECD	50% phenyl/methyl silicone		
1991 Liao et al.	MS	HP-1	He	0.10µg/g
1991 Stan and Heil	ECD, FPD, NPD	SE-54, DB-17	H2	
1992 Kobayashi et al.	ECD, FPD, FTD, MS			
1992 Schattenberg and Hsu	ECD	DB-608, DB-5	He	0.100ppm
1992 Bernal et al.	ECD/NPD	DB-5, DB-17	He	9µg/L(ECD) 31µg/L(NPD)
1992 Kadenczki et al.	ECD, NPD	DB-5, DB-1701		0.005mg/kg
1994 Holland et al.	ECD/NPD ECD/FPD	HP-5 DB-17	He He	0.01mg/L
1994 Garcia-Cazorla and Xirau-Vayreda	ECD	SPB-5	He	
1995 Just et al.	Hall detector	DB-1701	He	0.02µg/mL
1995 Specht et al.	ECD			
1995a Lehotay et al.	MS	DB-1701	He	4ng/g
1995b Lehotay et al.	MS, ECD	DB-5		
1995 Matisova et al.	FID, ECD, MS	CP-SIL-5 CB, HP-1	H2, N2	

Vinclozolin residue levels may also be determined by HPLC (Cabras et al., 1979). In comparison to GC, HPLC often requires less extensive sample extraction and clean-up, and can monitor many chemically different groups of pesticides simultaneously e.g. carbamate or organophosphorous or organochlorine (Cabras et al., 1982). Isocratic elution HPLC produces gives a more rapid and simple analysis than gradient elution HPLC or temperature programmed GC analysis. Again, there have been numerous publications detailing the use of HPLC to determine vinclozolin. These are summarised in Table 2.4.

Table 2.4 HPLC conditions for vinclozolin determination

Reference	HPLC column	Injection volume µl	Mobile phase	Flow rate ml/min	Detection wavelength nm
1979 Cabras et al.			CH_3CN:buffer 45:55 (pH4.0) CH_3CN:buffer 45:55 (pH7.0) CH_3CN:buffer 50:50 (pH7.0)	0.6 0.6 0.5	221 (LD 0.05ppm)
1982 Cabras et al.	LiChrosorb RP-18 250x4.0mm	50	CH_3CN:H_2O 45:55	2.0	221
1983 Cabras et al.	Merck Hibar 250x4.0mm	50	CH_3CN:H_2O 55:45	1.0	210
1983 Faticheniti et al.	MerckRP-18 250x4.0mm		CH_3CN:H_2O 65:35	1.0	200
1985 Cabras et al.	Merck Hibar RP-8 250x4.0mm	50	CH_3CN:H_2O 50:50	1.5	200
1989a Szeto et al.	Varian MicroPak 300x4.0mm	20	MeOH:buffer 0.05Mphosphate 72:28 (pH3.3)	1.0	212±2
1989 Newsome and Collins	µBondpack C18	100	MeOH:aq Trimethylamine buffer(pH7) 60:40	1.0	254
1991 Hogendoorn et al.	RP-18, Hypersil ODS (column switching HPLC)	150	CH_3CN:H_2O (20-50%)		229 (LD 50ppb)
1991 Schlett	Hypersyl ODS	50	CH_3CN:CH_3COONa (0.002M) gradient	0.80	PDA (max 200)
1992a Cabras et al.	Spherisob S5-ODS-1 250x4.6mm	100	CH_3CN:H_2O 50:50	1.0	200
1995 Papantoni and Mathiasson	Spherisob S10W 200x4.6mm	20	1-chlorobutane	0.5	240
1995 Eisert et al.	RP-select B Lichrospher 60	20	MeOH:H_2O gradient		PDA

Other reported methods are bioautography based on the application of fungispore suspensions as spray reagents in TLC of fungicides (Balinova, 1995), and high performance thin layer chromatography with automated multiple development (HPTLC-AMD) (de la Vinge et al., 1991; Butz and Stan, 1995).

Vinclozolin residues in grapes and wines

There have been a number of reports suggesting that vinclozolin may be detected not only in treated grapes but also in wines. In 1974 and 1975, residues of fungicides used to control grey mould on various grape varieties from vineyards in Switzerland were examined. The fungicides were applied 3 to 5 times and the grapes were harvested 43 to 58 days after the last spraying. Residues were 0.46 to 3.67 ppm for vinclozolin in grapes and < 0.5 ppm in wine (Bolay et al., 1976). Residues of vinclozolin were found in the wines five months after fermentation in Italy (Barbero and Caia, 1979). Vinclozolin residues found on grapes 28 days after the last spraying were in the range 0.6 to 1.5 ppm, and 0 - 0.8 ppm in wine made from the grapes (Lemperle et al., 1982). Vinclozolin residues on Italian field-sprayed grapes were analysed in 1978 and a level of 0.24 ppm was found (Cabras et al., 1982). Vinclozolin concentrations in the range 1.04 - 1.15ppm were detected on grapes harvested 17 -18 days after treatment, and 1.02 - 1.09 ppm 25 - 27 days after treatment (Bufo and Signorile, 1983). Gennari et al. (1985) did a comparative study of the vinclozolin residues on leaves and grapes in four different areas in Italy. On leaves the values of vinclozolin right after spraying varied in the range 64.0 - 238.9 µg/g for three successive sprayings within two months. At harvest time the values were 0.1 - 2.8 µg/g on leaves and 0.11 - 0.7 µg/g on grapes. In the U.S., 111 pesticides were screened in 6,970 food commodities from 1989 to 1991. Vinclozolin residues were determined to be below EPA tolerance limit (Schattengerget al., 1992). In Spain Garcia-Cazorla and Xirau-Vayreda (1994) studied residues of dicarboximide fungicides in grapes, must, and wines. Vinclozolin residues of 0.37, 0.22, and 0.08 mg/kg were found in the grapes, must and wine, respectively. The average

vinclozolin residue level in sixty-four Italian wine samples was 0.02 ppm (Cabras et al., 1995).

Joint Meetings of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Expert Group on Pesticide Residues have reported vinclozolin residues since 1986 (FAO/WHO, 1986). In these reports, extensive information was provided on residues from supervised trials in many food commodities (77 fruits and vegetables) in 23 countries, including Australia, USA, several European countries and Japan. Vinclozolin residues on grapes were in the range 0.5 - 4.8 mg/kg, and in wine 0.03 - 0.8 mg/kg in Germany, Australia, and France. In the 1987 report (FAO/WHO, 1988), New Zealand data was added. Residues from pre-harvest applications to grapes in 1975 were 1.5 to 0.03 mg/kg (1 to 21 days after application). Vinclozolin residues were calculated as total residue including metabolites containing 3,5-dichloroaniline.

Table 2.5 Fate of vinclozolin residues from grapes to wine (FAO/WHO, 1986)

Country	Trial year	Application rate (kg a.i./ha)	Grape berries (mg/kg)	Must (mg/kg)	Young wine (mg/kg)	Wine (mg/kg)
Germany	1984	0.6-0.75	1.70	0.20	0.20	0.19
Austria	1984	50 g/100L	0.42	0.12	0.06	0.03
Switzerland		0.6-0.7	1.45	0.30		

Vinclozolin residues in soil

Most fungicide treatments are directed to the aerial part of plants. However, no matter what the application method it is not possible to avoid fallout of pesticides onto soil. Moreover, rains, dews, and wind wash away and remove physically the

residues from leaves and fruits to the soil, where both the active ingredient and some transformation products can accumulate (Boccelli et al., 1982). Boccelli et al. analysed vineyard soil samples in Italy, and found vinclozolin residues in the range 0.004 - 2.10ppm. Walker et al. (1986) studied enhanced degradation of iprodione and vinclozolin in sandy loam soil samples after its direct application onto onion and potato crops. They found vinclozolin residues in soil 50 days after application. The degradation rate of vinclozolin increased after repeat treatment of vinclozolin. Walker (1987a,b) compared vinclozolin degradation rates in soils of different pH. Higher pH soils degraded vinclozolin more quickly than lower pH soils. The half-life of vinclozolin in soil with pH6.5 was 30 days. Soil bacteria also caused degradation of vinclozolin. Vinclozolin was converted to 3,5-dichloroaniline within 30 hours in fungicide-degrading bacteria enriched media (Head et al., 1988). In Russia, Golovleva et al. (1991) studied microbial conversion of vinclozolin in soil. Vinclozolin half-life was 23 days. Residues after 12 months were 6 - 12% of the applied dose. Five vinclozolin metabolites were caused by microorganisms in soil, including 3,5-dichloroaniline. Slade et al. (1992) studied vinclozolin degradation in clay loam soil in New Zealand. Vinclozolin half-life was 22 days at soil pH 5.8 - 6.1. Soil underneath of turf was analysed and the half-life of vinclozolin determined to be 7 to 10 days (Frederick et al., 1994). Polettini et al. (1993) determined adsorption-desorption isotherms in a sandy soil of vinclozolin and its metabolite, 3,5-dichloroaniline. They suggested 3,5-dichloroaniline in groundwater may derive from the degradation of vinclozolin in deep soil horizons rather than from the direct migration of 3,5-dichloroaniline from top soil because vinclozolin shows a greater mobility than 3,5-dichloroaniline in the soil-water system.

Vinclozolin residues in water

There are few reports of vinclozolin residues in actual water samples. The surface run off from vineyards has been analysed and vinclozolin residues, albeit at low concentration (≤ 8 mg/L) were found in all samples (von Aufsess, et al., 1989). Just et al. (1995) reported that vinclozolin is easily metabolised and therefore the analysis of vinclozolin residues in tap and surface water is circumstantial and problematic. They analysed surface water samples from a wine growing region in Germany, but did not detect any vinclozolin.

2.2.5 Stability of Vinclozolin

Vinclozolin is unstable in methanolic and ethanolic solutions. After 24 hours 23% had decomposed in methanol, and 25% in ethanol (Clark, 1983). Vinclozolin is more susceptible to hydrolysis in basic solutions than in acidic ones (Melkebeke et al., 1986). The kinetics of hydrolysis of vinclozolin in aqueous buffers of pH 4.5 - 8.3 at 13 - 35 °C was studied and the reaction reported as being base-catalysed and the rate proportional to pH (Szeto et al., 1989a). Szeto et al. (1989a,b) also isolated and identified three hydrolytic degradation products of vinclozolin, namely 2-[[(3,5-dichlorophenyl)carbamoyl]oxy]-2-methyl-3-butenoic acid (XXVII), 3',5'-dichloro-2-hydroxy-2-methylbut-3-enanilide (XXVIII), and 3,5-dichloroaniline (XXI) (Figure 2.19, 2.20). 3,5-Dichloroaniline (XXI) was detected only as a minor degradation product. FAO/WHO (1986) reported that vinclozolin was hydrolysed very rapidly at pH9 and was much more stable at pH3. The compounds XXVII and XXVIII were identified as hydrolysis products (Figure 2.19). At pH3 XXVIII appeared almost exclusively, but at pH9 XXVII and XXVIII were both formed.

Table 2.6 Vinclozolin hydrolysis half-life values

1 FAO/WHO 1986 ; 2 Szeto et al., 1989a

pH	T (°C)						
	13	20	25	30	35	40	45
3 3.5			70d[1]				12d[1]
4 4.5					529h[2], 541h[2]		
5 5.5					56h[2],57.5h[2]		
6 6.5			61h[1]		15h[2], 15.6h[2]		9.5h[1]
7 7.5	140h[2]	25.9h[2]	19.7h[2]		6.45h[2] 2.8h[2]		
8 8.5					1.16h[2]		
9			15.4m[1]				1.4m[1]

The photodegradation of vinclozolin has been studied in methanol (Clark and Watkins, 1984), in 2-propanol (Schwack and Beate, 1989), in 2-propanol, n-propanol, and cyclohexane (Schwack et al., 1995). However there have been no photodegradation studies in aqueous phase under natural sunlight.

Cabras et al. (1984) studied vinclozolin degradation in wine at pH 3.0 and 4.0 at 30 °C. The kinetic data obtained by observing the disappearance of the active ingredient (3.0 mg/kg) showed the pseudo first-order rate constants to be higher at pH 4.0 than at 3.0. The degradation products were isolated and identified as 3',5'-dichloro-2-hydroxy-2-methylbut-3-enanilide (XXVIII) (Pirisi et al., 1986). Szeto et al. (1989c) studied persistence of vinclozolin on pea leaves surface and the dissipation of the compound was reported to be linear with a half-life of 33.1 days. They found no degradation products in the treated plants, but XXVII, XXVIII and XXI were

detected when pea and bean were grown in nutrient solution containing either vinclozolin or its degradation product XXVII.

The poor solubility of many pesticides in water means that they are often used in micellar solutions. Villedieu et al. (1995) reported the effects of such media on kinetics of hydrolysis of dicarboximide fungicides including vinclozolin. The rate constants observed were slightly reduced by sodium dodecyl sulphate micellar media, slightly increased by cetyltrimethylammonium bromide micellar media, but unaffected by nonionic micelles. These results can be explained by means of the pseudophase kinetic model coupled with the mechanisms of hydrolysis of these fungicides in water solution.

3. Analytical Method Development

3.1 Introduction

Two major experimental parameters must be established prior to any study of pesticide residues in environmental samples, namely the method by which the sample is prepared prior to analysis, and the method by which analyte levels are determined. For most laboratories the latter is limited to those instruments commonly employed within the laboratory. For this study there was a clear cut choice - gas chromatography (GC) or high performance liquid chromatography (HPLC). However, although traditionally pesticide residues have been determined by GC, and this is particularly so for thermally stable and volatile analytes, and while there were some reports of the determination of dithianon residues by GC (Kadenczki et al., 1992; Suzuki et al., 1974), there were also suggestions that GC methods did not have enough sensitivity for quantitation (Eisenbeis and Sieper, 1973). In addition, it was suggested that dithianon forms a thiophen derivative through extrusion of sulphur on simple heating in a similar manner to that observed with dithiin (Stark and Duke, 1967) (Figure 3.1). This latter point does not seem to have been addressed by authors determining dithianon residues by GC, and there remains some doubt as to whether the analyte determined by them using ECD or NPD detection methods is indeed dithianon or in fact an extruded product.

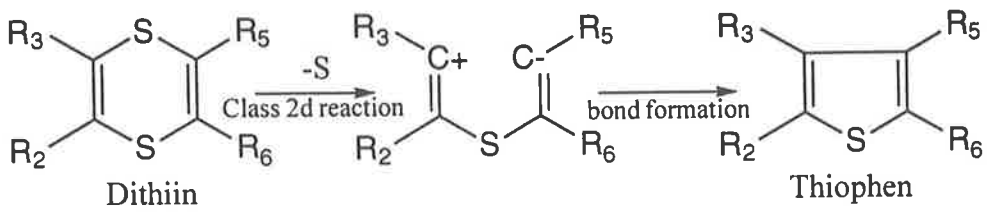


Figure 3.1 Ring-contraction of a 1,4-dithiin to a thiophen

Although, there were also a large number of literature reports of the use of GC to determine vinclozolin residues (see Table 2.3) the previous arguments relating to thermal lability of dithianon, and the requirement for a single instrumental method to determine residues of both chemicals essentially ruled out GC as the routine method of choice for these studies.

HPLC is often seen as the method of choice for polar, involatile and thermally labile compounds. However, many compounds that have historically been determined by GC may also be determined by HPLC e.g. vinclozolin, numerous reports of the determination of this compound by GC, but also numerous publications detailing the use of HPLC (see Table 2.4). However, again there has been very limited reporting of HPLC methods for dithianon (Kojima,1980; Eisenbeis and Sieper, 1973; Baker and Clarke, 1984; AOAC, 1995; Hanks, 1995). This may in part have been due to a lack of interest in this compound among the analytical chemistry community, but perhaps also in part also due to the fact that dithianon standards made up in the traditional HPLC standard solvent, pure methanol, degrade rapidly, and that solutions containing less than 20 μg/mL were difficult to chromatograph due to on-column breakdown (Baker and Clarke, 1984). These problems may be overcome by making up analytical standards in methanol containing 1% v/v acetic acid.

This chapter discusses some of the early investigative work undertaken to assess the optimum analytical conditions for the target compounds, and presents simple methods for dithianon, vinclozolin, their degradation products and also some of the ancillary chemicals used in these studies based on modified versions of the methods reported by Baker and Clarke (1984) and Hanks (1995) for the direct determination

of dithianon by reversed-phase HPLC using an ultraviolet spectrophotometric detector.

3.2 Materials

(a) *Chemicals* : acetonitrile, methanol, and acetic acid glacial (HPLC grade; BDH Laboratory Supplies). 3,5-dichloroaniline and p-nitroacetophenone (Aldrich Chemicals Company, Inc.) standard solutions prepared in acetonitrile. Distilled water was purified using an ion exchange-activated carbon filter system (MilliQ, Millipore Corp.).

(b) *Fungicide standards* : dithianon (95%, Riedel-de Haen AG., Germany) standard solutions prepared in 99:1 acetonitrile : acetic acid solution; vinclozolin (99%, Chem Service PA, USA) standard solutions prepared in acetonitrile.

(c) *Spectrophotometer* : Varian Cary1 UV-visible spectrophotometer.

(d) *Solvent degassing* : mobile phases were degassed and filtered through GVWP filter papers in a Millipore filtration set.

(e) *Liquid chromatograph* : Waters Model 510 HPLC pump equipped with a U6K manual injector and Waters Model 490 UV detector. Quantitation by peak area integration. Operating conditions: isocratic mobile phase; temperature 22 ± 3 °C.

(f) *HPLC columns* : 150 x 3.9 mm i.d., stainless steel, packed with reversed-phase dimethylphenylpropylsilyl bonded amorphous silica (Nova-Pak® Phenyl 60Å 4µm Millipore Corporation, MA, USA) and dimethyloctadecylsilyl bonded amorphous silica (Nova-Pak® C18 60Å 4µm Millipore Corporation, MA, USA).

(g) *Gas chromatograph* : Varian 3300 equipped with a flame ionisation detector; interfaced to DB-5 column; hydrogen gas. Operating conditions: injector 280 °C, detector 300 °C, temperature program, column 150 °C, increase to 300 °C at 5 °C/min.

(h) *Gas chromatograph / mass spectrometry* : Varian 3400 with DB-5 column interfaced to a Finigan MAT TSQ70 mass spectrometry using electron impact ionisation source (EI). GC operating conditions were the same as (g).

(i) *Mass spectrometry* : Finigan MAT TSQ70 using direct electron impact mode (70 eV).

3.3 Method Development : Results and Discussion

The measurement of UV-visible absorption spectra

The UV absorbance detector is by far the most popular detector for HPLC systems. At the heart of these detectors is a flow cell held in the beam of a UV/visible photometer. The absorption of radiation by solutes is governed by the Beer-Lambert law

$$A = Ec\ell \qquad (3.1)$$

where A = absorbance, ℓ = path length, c = analyte concentration, and E = the molar extinction coefficient. Essentially, the analyte concentration is proportional to the absorption of radiation at any given wavelength. Most UV/visible detectors provide a rather narrow band of wavelengths around the selected wavelength. As a result, analytical chemists seek to operate the detector in flat regions of a molecule's

absorbance spectrum i.e. maxima, minima or shoulders (Lindsay, 1992). In order to determine the most appropriate operating wavelength for the HPLC determination of both dithianon and vinclozolin, their UV-visible absorption spectra were determined. Dithianon absorbance maxima were found at around 330 and 250 nm (Figure 3.2), and vinclozolin absorbance maxima at around 210 nm (Figure 3.3). These results suggested that the optimum UV/vis spectrophotometer settings would be 254 nm for the detection of dithianon, and 210 nm for the determination of vinclozolin.

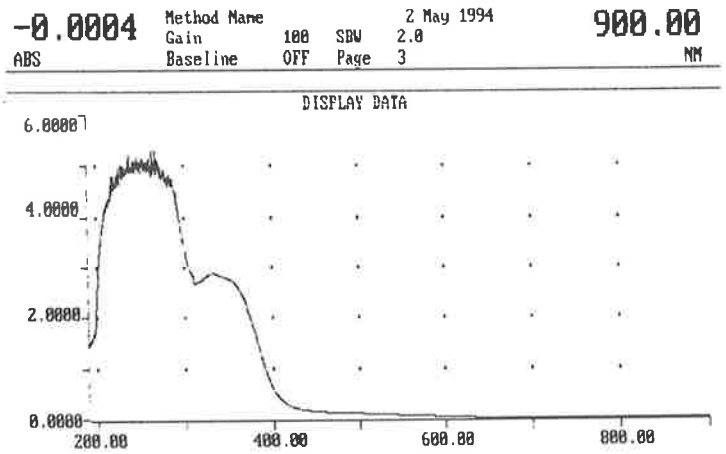


Figure 3.2 Dithianon UV-visible absorbance spectrum

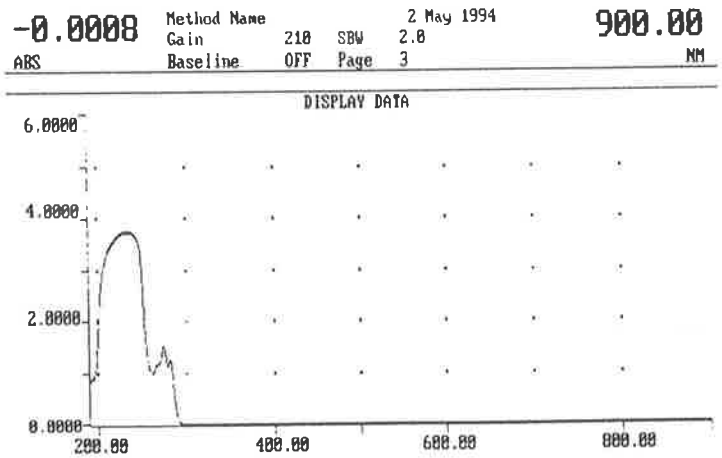


Figure 3.3 Vinclozolin UV-visible absorbance spectrum

Development of the HPLC conditions

Hanks (1995) used a reversed-phase C18 column, an acetonitrile-water mobile phase and standards prepared in dioxane-acetonitrile. Baker and Clarke (1984) also used a C18 column, but with a methanol-water mobile phase and standards prepared in methanol-1% v/v acetic acid. These operational differences and concerns over the stability of dithianon at low concentrations prevented the adoption of one of these methods as an 'off the shelf' published method but rather suggested that some preliminary investigations into the optimum HPLC conditions for the determination of both dithianon and vinclozolin were warranted.

The HPLC system operational parameters and conditions i.e. column type, combinations of solvent mixture, pump speeds, were initially varied to ascertain the optimum HPLC analytical conditions for dithianon. Dithianon standard solutions were prepared in acetonitrile-1% acetic acid. The effect of column, mobile phase and flow rate on analyte retention time and peak shape on a 20 ppm dithianon standard solution were investigated using two columns (Nova-Pak® C18 stainless steel and Nova-Pak® Phenyl stainless steel), and six solvent systems (80:20, 70:30, and 60:40 methanol : water, and 80:20, 70:30, and 60:40 acetonitrile : water) with the detector set at 254 nm. The results of these investigations are shown in Tables 3.1 and 3.2. In general, retention times were shorter when using the Nova-Pak® C18 stainless steel column compared to the Nova-Pak® Phenyl stainless steel column. The greatest separation between solvent and dithianon peaks was observed using the Nova-Pak® Phenyl stainless steel column with an 80 : 20 methanol : water mobile phase at a flow rate of 1.0 mL/min. However, the broadness of the dithianon peaks

observed when using methanol : water mobile phases compared to the sharp peaks observed with an acetonitrile : water mobile phase suggested that the latter was the most appropriate mobile phase. However, bearing in mind the results of Baker and Clarke (1984), 1% v/v acetic acid was added to the mobile phase to prevent dithianon breakdown during chromatography. Finally, it was determined that the combination of sharp peaks, analyte retention time and solvent / dithianon peak separation observed with a 50:50:1 acetonitrile : water : acetic acid mobile phase in combination with the Nova-Pak® Phenyl stainless steel column was the most appropriate system to use (Figures 3.4 and 3.5).

There have been no reports of vinclozolin standard stability problems, so analytical standards were prepared in neat acetonitrile. From the UV spectral data obtained, the appropriate wavelength setting for the UV/vis detector was determined to be 210 nm. However, acetic acid interferes with UV absorbance below 220 nm. This is not a problem during dithianon determination - the detector is set at 254 nm - but prevents the use of the same mobile phase for vinclozolin determination. However, no spectral interference is observed when the detector setting is 210 nm and acetonitrile : water is the mobile phase. The sharp peaks, analyte retention time and solvent / vinclozolin peak separation observed with a 50:50 acetonitrile : water mobile phase in combination with the Nova-Pak® Phenyl stainless steel column was determined to be the most appropriate system to use (Figures 3.6 and 3.7).

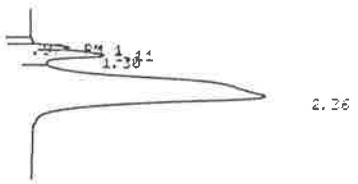
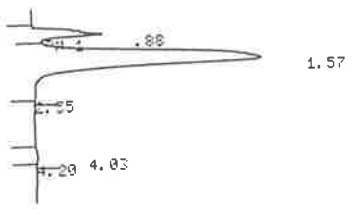
In addition to dithianon and vinclozolin, it was envisaged that a number of other compounds would have to be analysed on a routine basis e.g. vinclozolin degradation products identified by Szeto et al. (1989a), and actinometer constituents (Chapter 5).

Of the former compounds, only 3,5-dichloroaniline could be obtained commercially. Fortunately, both 3,5-dichloroaniline and p-nitroacetophenone (the monitored constituent of the actinometer system (see Chapter 5, section 5.5)) are both stable in acetonitrile. Therefore, analytical standards for both compounds were made up in neat acetonitrile. From the UV spectral data obtained, the appropriate wavelength setting for the UV/vis detector was determined to be 210 nm for 3,5-dichloroaniline, 280 nm for p-nitroacetophenone. In both cases, initial HPLC investigations confirmed the supposition that the vinclozolin mobile-phase / column / LC conditions would be suitable for the routine determination of these compounds. Without access to MS equipment routine determination of fungicide residues was by isocratic elution using the external standard method. The calibration curve used for dithianon determination throughout most of these studies is shown in Figure 3.4 (R^2=0.9999). A typical dithianon standard is shown in Figure 3.5. The calibration curve used for routine vinclozolin determination is illustrated in Figure 3.6 (R^2=0.9993), a typical standard chromatogram is in Figure 3.7. 3,5-dichloroaniline was also to be determined by the external standard in Figures 3.8 (R^2=0.9986) and 3.9, similarly for p-nitroacetophenone, Figures 3.10 (R^2= 0.9987) and 3.11.

Table 3.1 Dithianon 20 ppm at 250 nm with the column Nova-Pak® C18

(RT = retention time, PH = peak height)

CH_3OH : H_2O (flow rate)

80 : 20 1.0 mL/min		RT 2.36 min PH 6.2 cm Area 87.7 %
80 : 20 1.5 mL/min		RT 1.57 min PH 6.0 cm Area 87.5 %

CH_3CN : H_2O (flow rate)

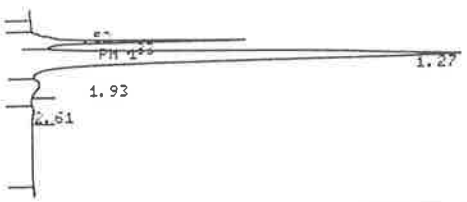
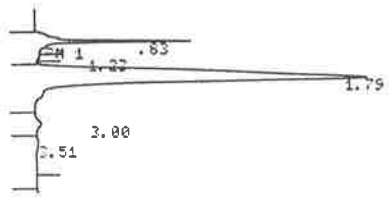
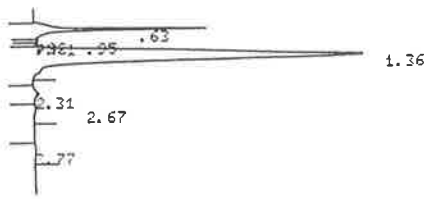
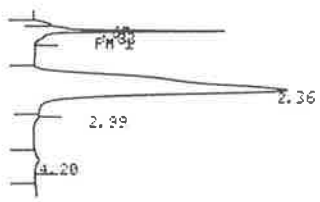
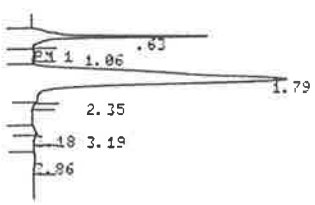
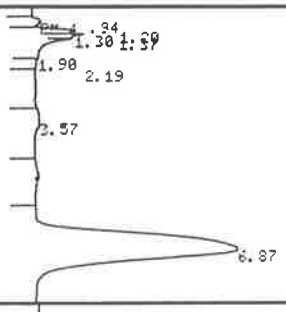
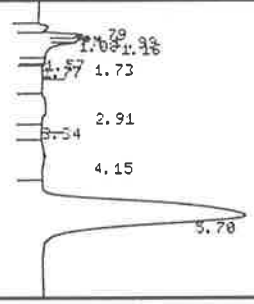
80 : 20 1.5 mL/min		RT 1.27 min PH 11.4 cm Area 84.7 %
70 : 30 1.5 mL/min		RT 1.79 min PH 8.8 cm Area 86.5 %
70 : 30 2.0 mL/min		RT 1.36 min PH 8.7 cm Area 84.2 %
60 : 40 1.5 mL/min		RT 2.36 min PH 6.7 cm Area 85.0 %
60 : 40 2.0 mL/min		RT 1.79 min PH 6.7 cm Area 84.2 %

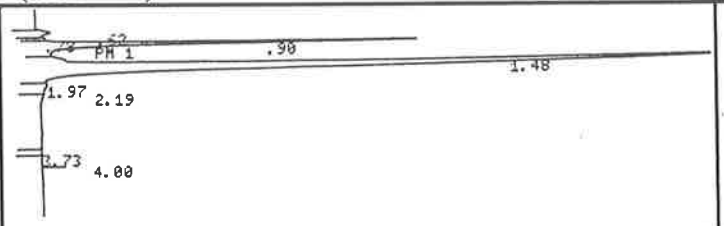
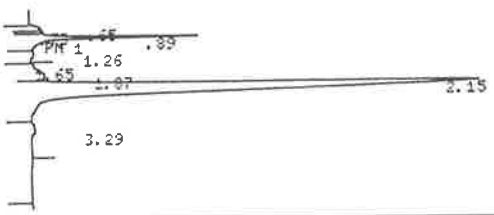
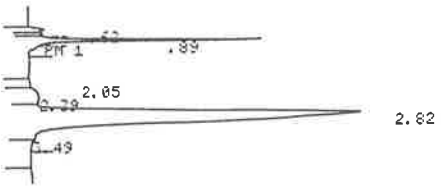
Table 3.2 Dithianon 20 ppm at 250 nm with the column Nova-Pak® Phenyl

(RT = retention time, PH = peak height)

CH_3OH : H_2O (flow rate)

80 : 20 1.0 mL/min		RT 6.87 min PH 5.4 cm Area 83.8 %
80 : 20 1.5 mL/min		RT 5.70 min PH 5.4 cm Area 86.7 %

CH_3CN : H_2O (flow rate)

80 : 20 1.5 mL/min		RT 1.48 min PH >18 cm Area 73.0 %
70 : 30 1.5 mL/min		RT 2.15 min PH 11.9 cm Area 83.9 %
60 : 40 1.5 mL/min		RT 2.82 min PH 8.8 cm Area 81.7 %

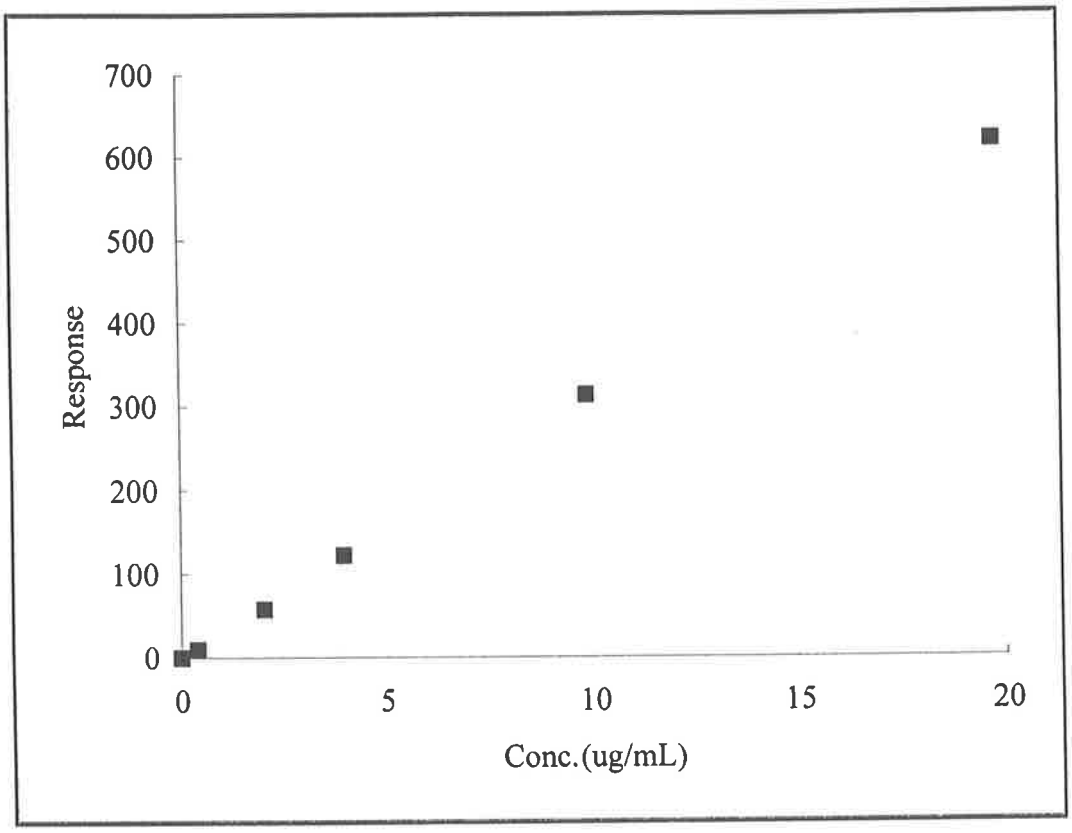


Figure 3.4 Calibration curve of dithianon standard (254nm)(R^2=0.9999)

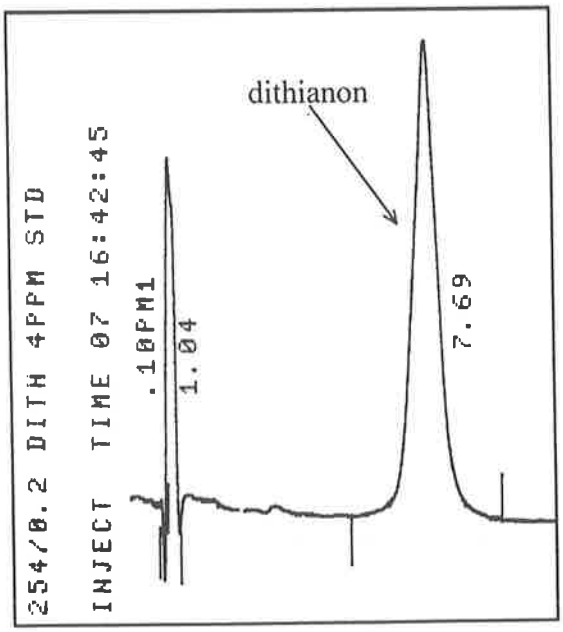


Figure 3.5 Typical chromatogram of dithianon standard

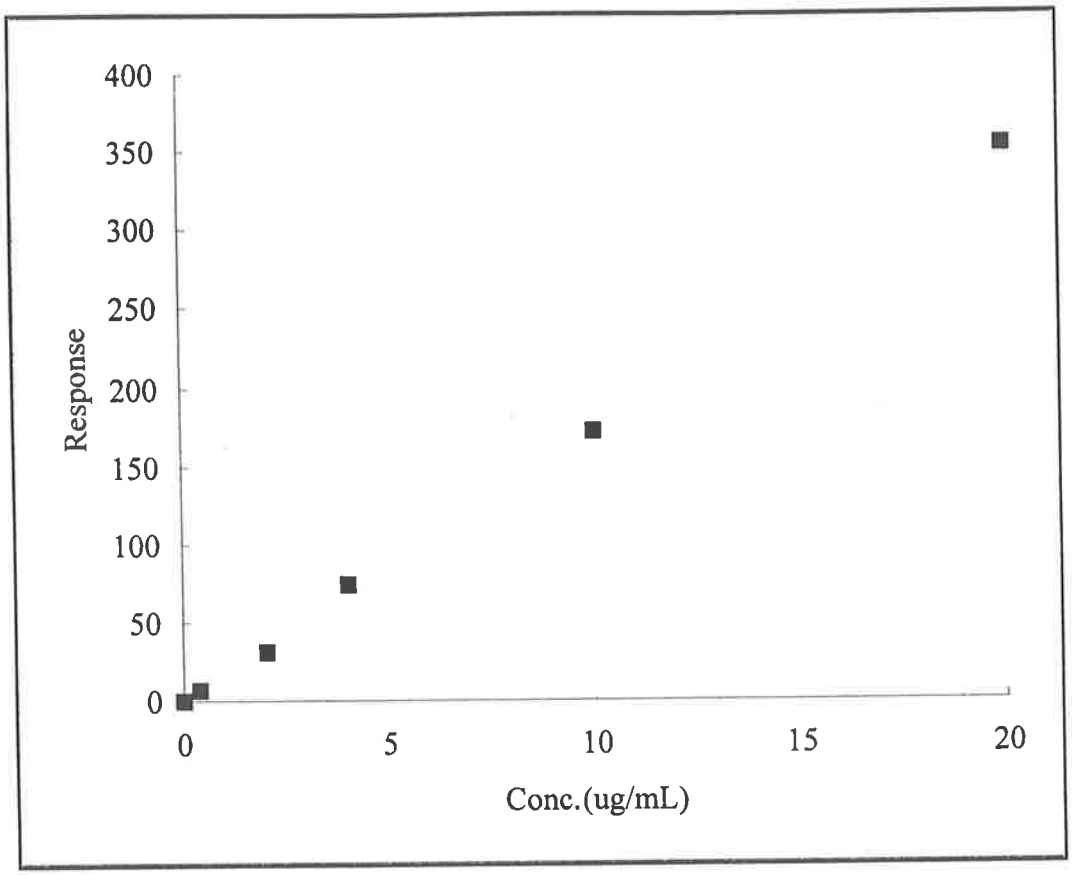


Figure 3.6 Calibration curve of vinclozolin standard (210nm)(R^2=0.9993)

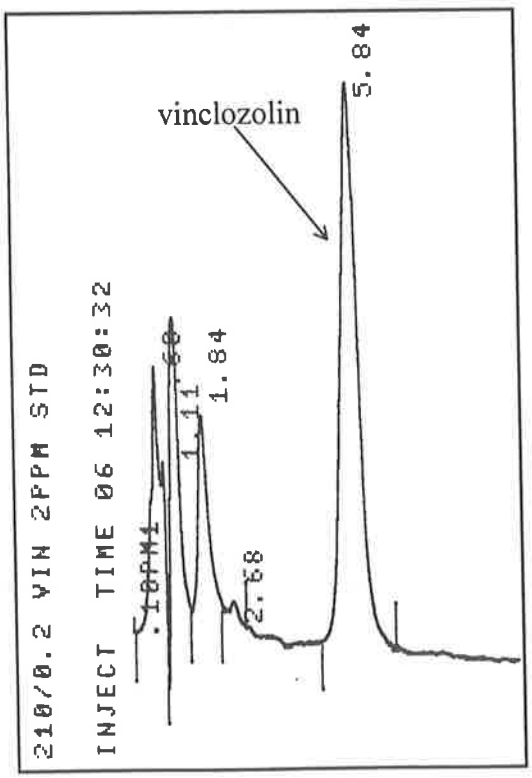


Figure 3.7 Typical chromatogram of vinclozolin standard

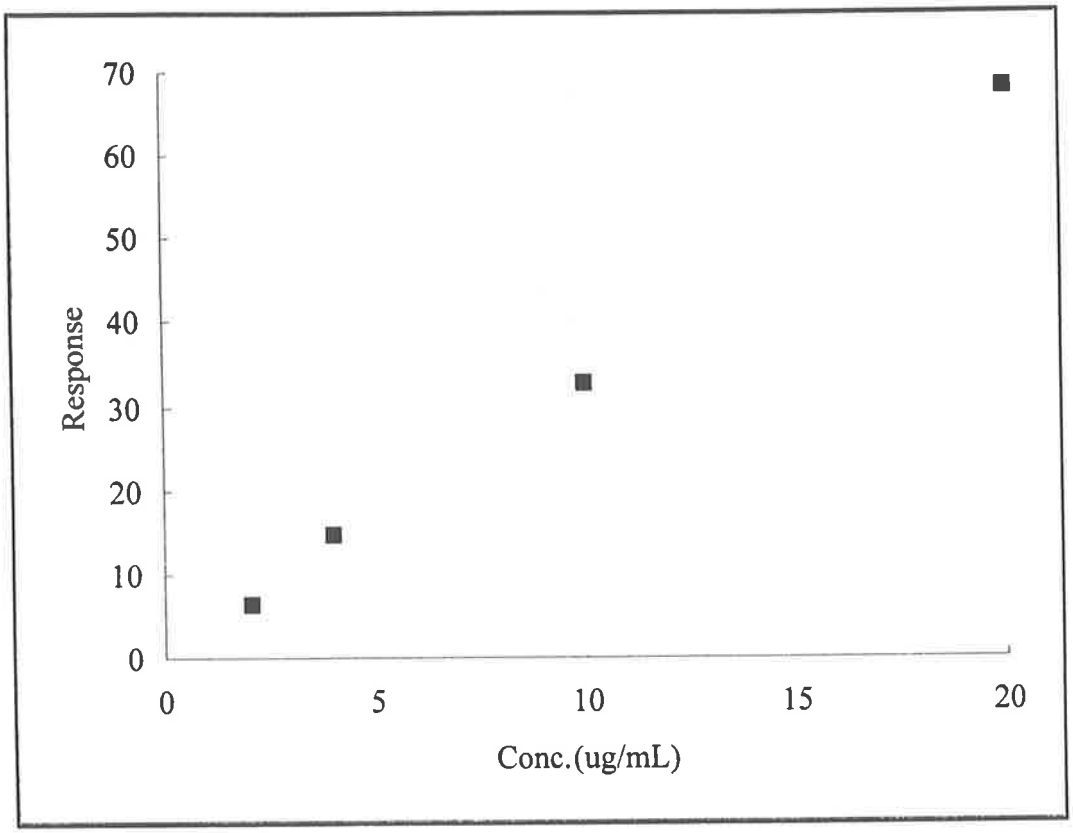


Figure 3.8 Calibration curve of 3,5-dichloroaniline standard (210nm) (R^2=0.9986)

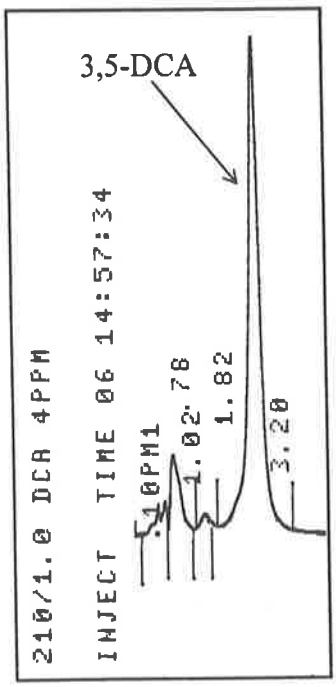


Figure 3.9 Typical chromatogram of 3,5-dichloroaniline standard

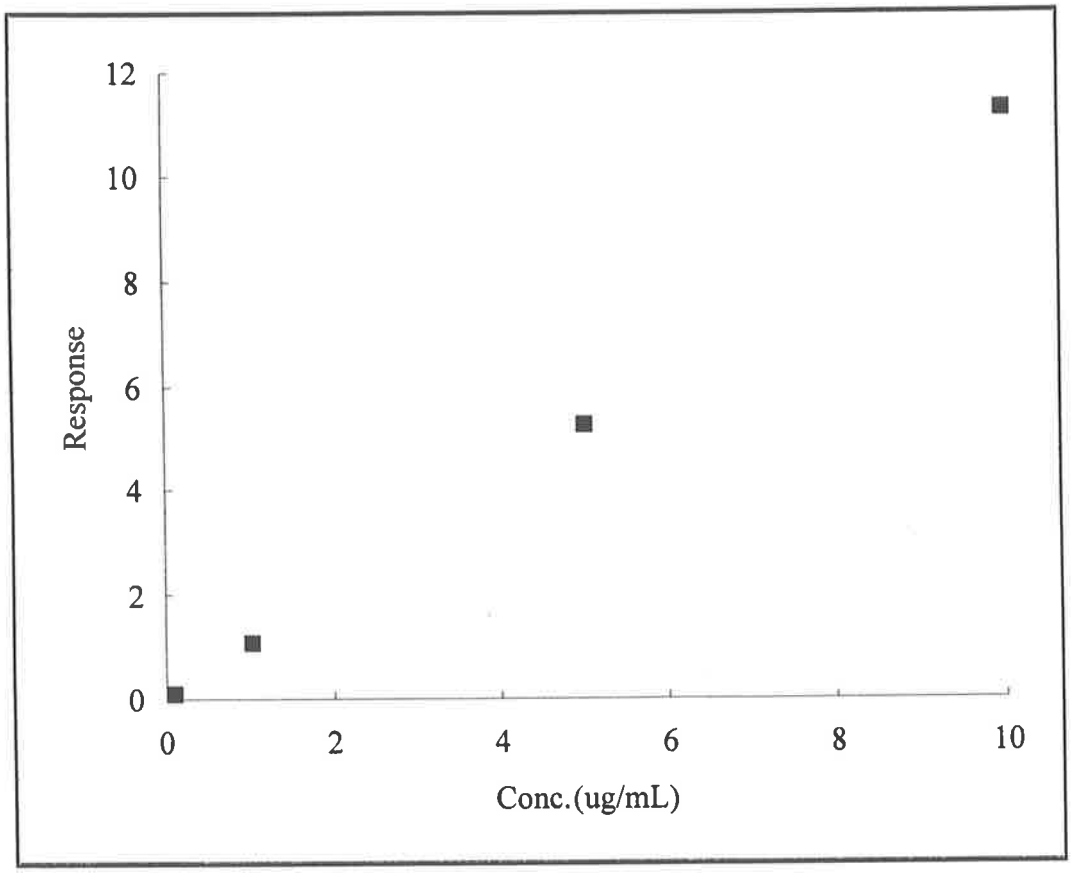


Figure 3.10 Calibration curve of p-nitroacetophenone standard (280nm)(R^2=0.9987)

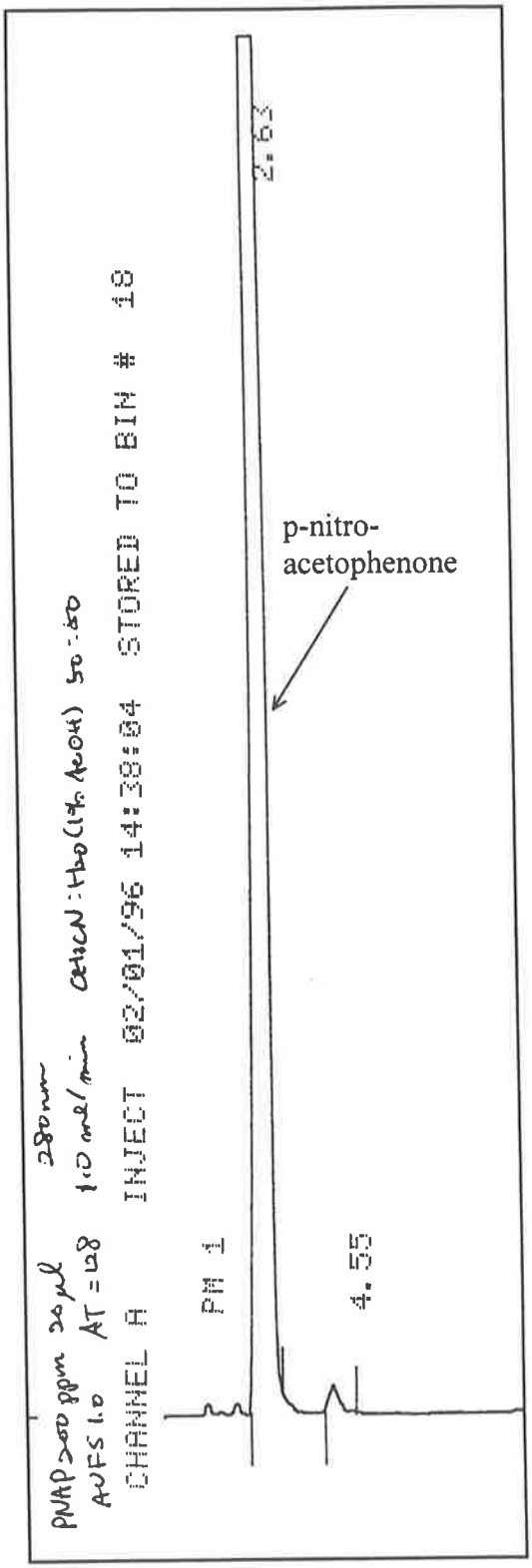


Figure 3.11 Typical chromatogram of p-nitroacetophenone standard

GC method development

Although the thermal lability of dithianon suggested that GC was never likely to be the most appropriate method for the routine determination of this compound in these studies, the behaviour of this compound was investigated to determine the optimum operating conditions for GC/MS analysis. A typical gas chromatogram of a dithianon standard solution is shown in Figure 3.12. It shows one peak, which one might expect to be due to dithianon. However, mass spectrometry determined that the compound producing this signal was not dithianon but rather suggests the sulphur-extruded derivative, XXIX (Figure 3.13) (mass spectrum analysis, m/z : 264 (M+), 236 (M-CO), 208 (M-CO,-CO)). On the other hand, direct injection MS of dithianon produced a mass spectrum showing the same fragmentation pattern as obtained from a library search (mass spectrum analysis, m/z ; 296 (M+), 268 (M-CO), 240 (M-CO,-CO)) (Figure 3.14). These results confirmed that GC was not an appropriate analytical method for dithianon determination.

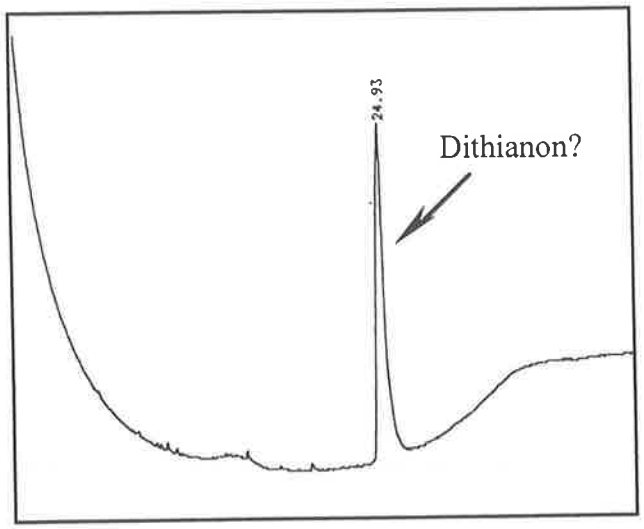


Figure 3.12 GC chromatogram of dithianon injection

Operating conditions: injector 280 °C, detector 300 °C, temperature program, column 150 °C, increase to 300 °C at 5 °C/min.

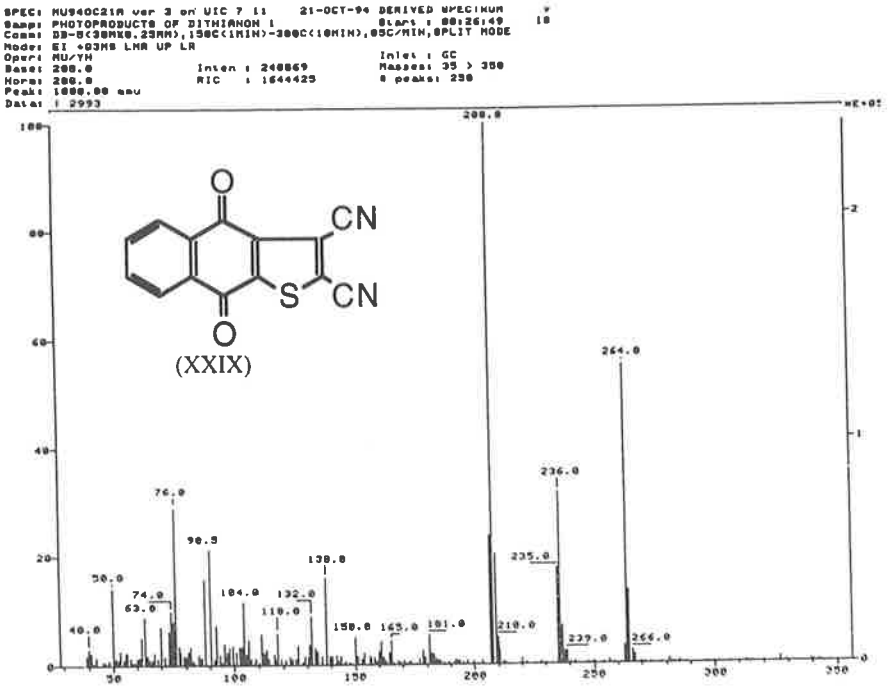


Figure 3.13 Mass spectrum of suspected sulphur extruded dithianon derivative

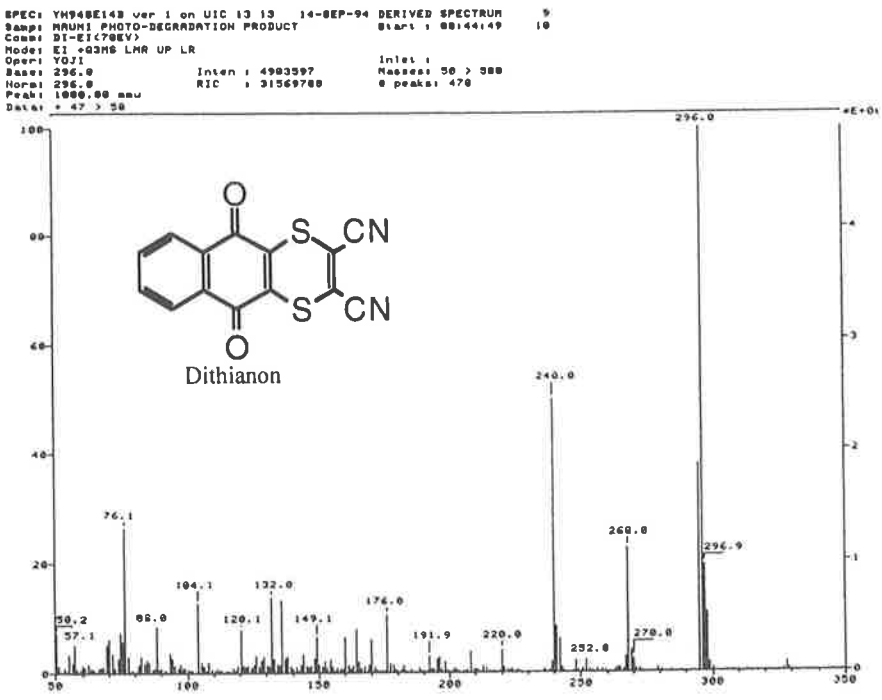


Figure 3.14 Mass spectrum of dithianon

3.4 Conclusion

Exploratory HPLC and GC experiments confirmed the impression obtained from literature surveys that HPLC was the most appropriate analytical system to use for the routine detection and determination of dithianon, vinclozolin, their metabolites and associated actinometer chemicals during this study. Ultimately, it was determined that the most appropriate operating conditions were :

dithianon

- standard solutions prepared in 99 : 1 acetonitrile : acetic acid.
- column : 150 x 3.9 mm i.d. stainless steel column packed with reversed-phase dimethylphenylpropylsilyl bonded amorphous silica (Nova-Pak® Phenyl 60Å 4µm Millipore Corporation, MA, USA)
- mobile phase : 50:50:1 acetonitrile : water : acetic acid
- flow rate : 1.0 mL/min
- UV detection - detector set at 254 nm

vinclozolin and *3,5-dichloroaniline*

- standard solutions prepared in acetonitrile
- column : 150 x 3.9 mm i.d. stainless steel column packed with reversed-phase dimethylphenylpropylsilyl bonded amorphous silica (Nova-Pak® Phenyl 60Å 4µm Millipore Corporation, MA, USA)
- mobile phase : 50:50 acetonitrile : water
- flow rate : 1.0 mL/min

- UV detection - detector set at 210 nm

p-nitroacetophenone

- standard solutions prepared in acetonitrile
- column : 150 x 3.9 mm i.d. stainless steel column packed with reversed-phase dimethylphenylpropylsilyl bonded amorphous silica (Nova-Pak® Phenyl 60Å 4µm Millipore Corporation, MA, USA)
- mobile phase : 50:50 acetonitrile : water
- flow rate : 1.0 mL/min
- UV detection - detector set at 280 nm

4. Sample Preparation

4.1 Introduction

This project investigated the fate of the fungicides dithianon and vinclozolin in a number of different matrices e.g. water, grape juice, wines, and soil. These matrices contain potential interferences which must be separated from the active ingredients and their breakdown products prior to analysis. Traditionally, liquid-liquid and liquid-solid phase extraction methods have commonly been used, but increasingly solid phase extraction (SPE) methods are preferred, especially as the whole system can be automated. SPE is based on the principle that the components of interest are retained on a special sorbent contained in a disposable cartridge. By using SPE one can remove matrix interferences (these either pass through the cartridge or are subsequently washed off) and then isolate with selective enrichment one's target compounds. Multiple extractions can be done simultaneously, rapidly and relatively inexpensively. The final extract from SPE is well suited to chromatographic analysis. SPE methods have been reported for the isolation of vinclozolin from water samples (Schlett, 1991; de la Vigne et al. 1991; Balinova, 1995; Just et al., 1995; Butz and Stan, 1995; Eisert et al., 1995), broccoli, carrot, celery, oranges (Hsu, R. C. et al., 1991), and grapes (Newsome and Collins, 1989). However, there have been no published reports of the use of SPE for the isolation of dithianon residues from water or biological matrices. This section presents rapid and simple SPE methods for the isolation of dithianon and vinclozolin residues from water, grapes, wine and soil based on modified versions of the aforementioned procedures for vinclozolin using Sep-Pak® C18 cartridges and analyte elution by acetonitrile.

4.2 Materials and Methods

(a) Chemicals, fungicide standards, solvent degassing and the liquid chromatographic system (HPLC column, solvent system and UV detector) are as described in Chapter 3.

(b) *Extraction cartridges* - Sep-Pak® C18 solid phase extraction cartridges (adsorbent 500mg; hold-up volume 0.8mL; Waters Australian Pty. Ltd.).

(d) *Extraction manifold* - demountable vacuum manifold with 12 screw type vacuum bleed connections for SPE columns coupled to water aspirator pump; sample loaded through Visiprep large volume samplers (Sigma-Aldrich Pty. Ltd., Sydney, Australia).

(e) *Grapes and wines* - grapes were harvested from vines at the Nuriootpa Research Station, Primary Industry of South Australia, and Roseworthy Campus, University of Adelaide. Grape varieties : Nuriootpa, pinot noir (red) and semillon (white); Roseworthy, bastardo, cinsaut (red) and rkaziteli and goyura (white). Wines were made from pinot noir and semillon grapes by personnel of Wine and Grape Research Unit at Roseworthy, University of Adelaide, while wines were made from bastardo, cinsaut, rkaziteli and goyura grapes by the author in the laboratory.

(f) *Soil samples* - soil samples were extracted from sites near Rutherglen in north-east Victoria, and near Overland Corner in the South Australian Riverland. Soil characteristics are detailed in Chapter 7.

General method for solid phase extraction

Figure 4.1 illustrates the general method used for the solid phase extraction of dithianon and vinclozolin from water, grape juice, wine and soil extracts. The SPE cartridges were first conditioned by sequential washing with acetonitrile (6mL), then water (6mL). Liquid samples were filtered through glassfibre filter paper and then loaded onto the cartridge at room temperature at a flow rate of ca. 5-10 mL/min. The cartridge was then sequentially washed with water (6mL) then 30% ethanol : water (6mL), then vacuum dried. Both dithianon and vinclozolin were eluted from the cartridges with acetonitrile (2mL). Fungicide concentrations were determined directly from the acetonitrile solutions by HPLC. Analyte determination was done by the external standard method. All analyses were undertaken in at least triplicate.

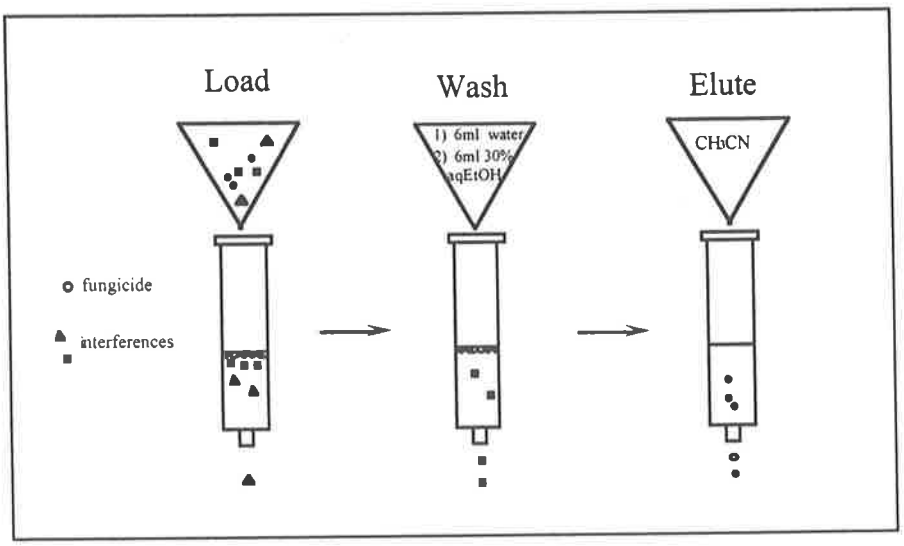


Figure 4.1 Solid phase extraction procedure

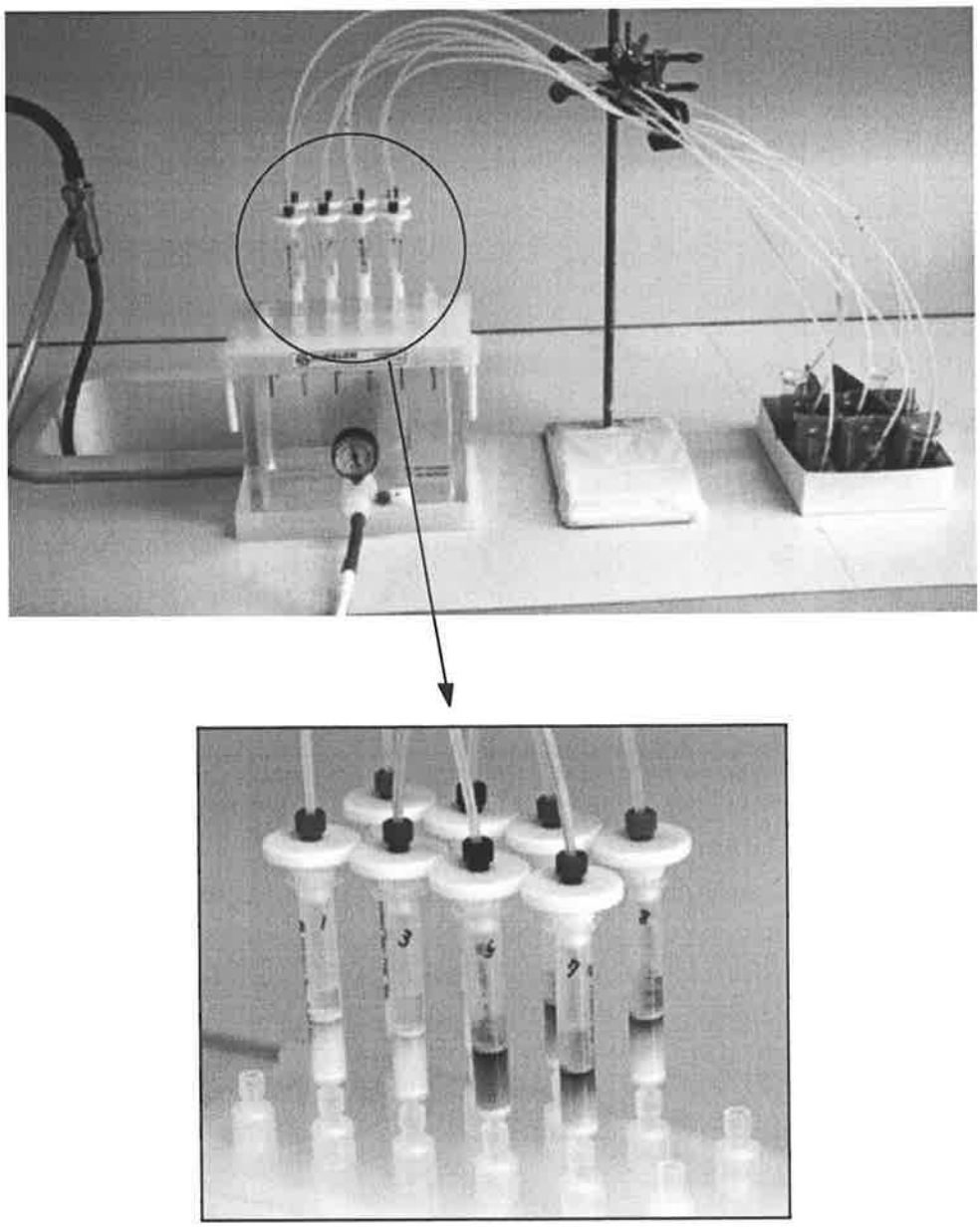


Figure 4.2 Solid phase extraction equipment

4.3 Results and Discussion

Dithianon and vinclozolin recoveries from water

Dithianon and vinclozolin were spiked into a blend of leachate (100 mL) collected prior to application of fungicide to the soil columns extracted from a paddock near Rutherglen, Victoria (see Chapter 7). The fungicides were spiked into this semi-natural water at two levels - 10 and 100 µg. Recoveries were determined in triplicate at each level of spike. In general, the extraction method proved to be rapid, simple, and robust. Both dithianon and vinclozolin could be quantitatively recovered with excellent reproducibility from the leachate. There was no statistical difference in the recovery between the 10 µg and 100 µg spikes; combined mean recovery for dithianon 95.6 %, coefficient of variation (c.v.) 4.1 %; combined mean recovery for vinclozolin 99.3 %, c.v. 4.1 % (Table 4.1).

Table 4.1 Summary of dithianon and vinclozolin recoveries from water

Fungicide	Matrix	Sample	Spike	Recovery		Mean Recovery	
			(µg)	(µg)	(%)	(%)	c.v. (%)
Dithianon	Leachate	R-D1	10.1	9.9	97.7	98.7	1.0
		R-D2		10.1	99.7		
		R-D3		10	98.6		
		R-D4	60.6	55.6	91.8	91.8	3.0
		R-D5		57.4	94.6		
		R-D6		53.9	89		
Vinclozolin	Leachate	R-V1	10.6	10.2	96.1	95.9	0.5
		R-V2		10.2	96.3		
		R-V3		10.1	95.3		
		R-V4	106.0	105.6	99.6	103.0	3.0
		R-V5		109.8	103.6		
		R-V6		111.0	104.7		

c.v. = coefficient of variation

Dithianon and vinclozolin recoveries from grape berries

Further sample preparation steps are required prior to extraction of both vinclozolin and dithianon from grape berries. The grape berry sample is first frozen. Thereafter, frozen berries (100 g) are mixed with powdered solid carbon dioxide and ground to a fine powder in a stainless steel blender. A portion of the crushed grape berries (50 g) is stirred with methanol (30 mL). This mixing with methanol effects fungicide extraction. After stirring for 10 min, the mixture was filtered through a bed of celite, the residue washed with methanol (10 mL), and then water (10 mL). The filtrate is diluted made up to 200 mL with deionised water. This has the effect of making the resultant solution approximately 20 % v/v methanol. An aliquot (40 mL) of this solution was loaded onto the SPE cartridges in the manner previously described. Each analysis was repeated three times.

Dithianon was found to degrade very quickly in grape juice. This is discussed in more detail in Chapter 5. The speed of this degradation, $t_{1/2} \sim 30$ min, precluded the

Table 4.2 Vinclozolin recovery from grapes

Fungicide	Matrix	Sample	Spike (µg)	Recovery (µg)	Recovery (%)	Mean Recovery (%)	Mean Recovery c.v. (%)
Vinclozolin	red grapes (cinsaut)	rg-V1	10.0	9.08	90.8	91	3.9
		rg-V2		8.76	87.6		
		rg-V3		9.46	94.6		
	white grapes (goyura)	wg-V4	10.0	9.08	90.8	96.2	8.7
		wg-V5		9.20	92.0		
		wg-V6		10.58	105.8		

c.v. = coefficient of variation

gathering of accurate recovery data. Vinclozolin, on the other hand, is stable in grape juice, and can be quantitatively recovered from grapes (recovery 91 - 96.2 %) with good reproducibility (c.v. 3.5 - 8.3 %) by this method. Vinclozolin recovery data are shown in Table 4.2. Typical chromatograms are shown in Figures 4.3 - 4.6.

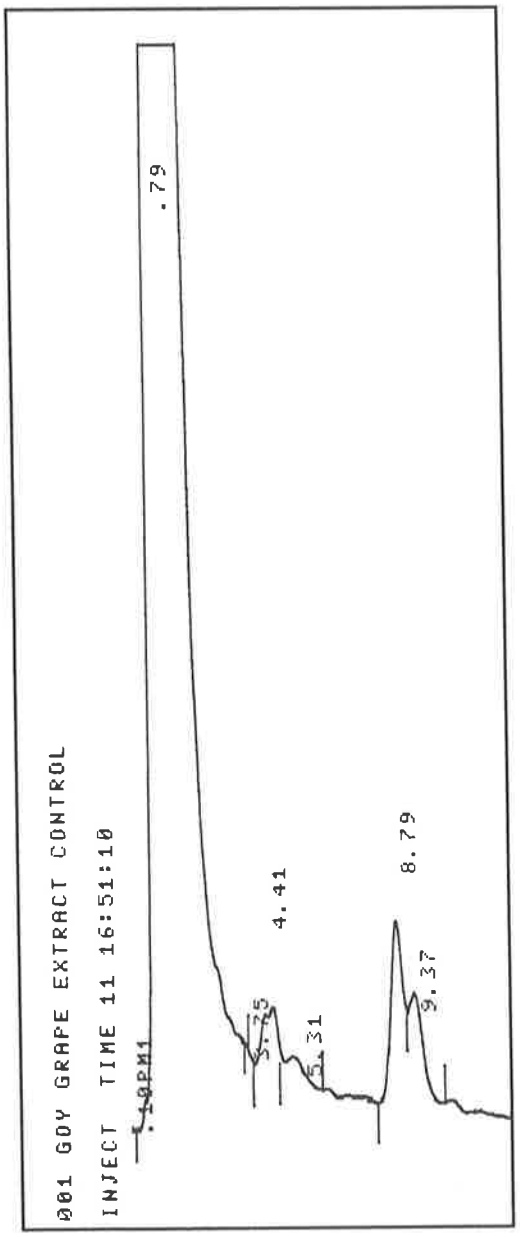


Figure 4.3

White grape juice control (goyura)

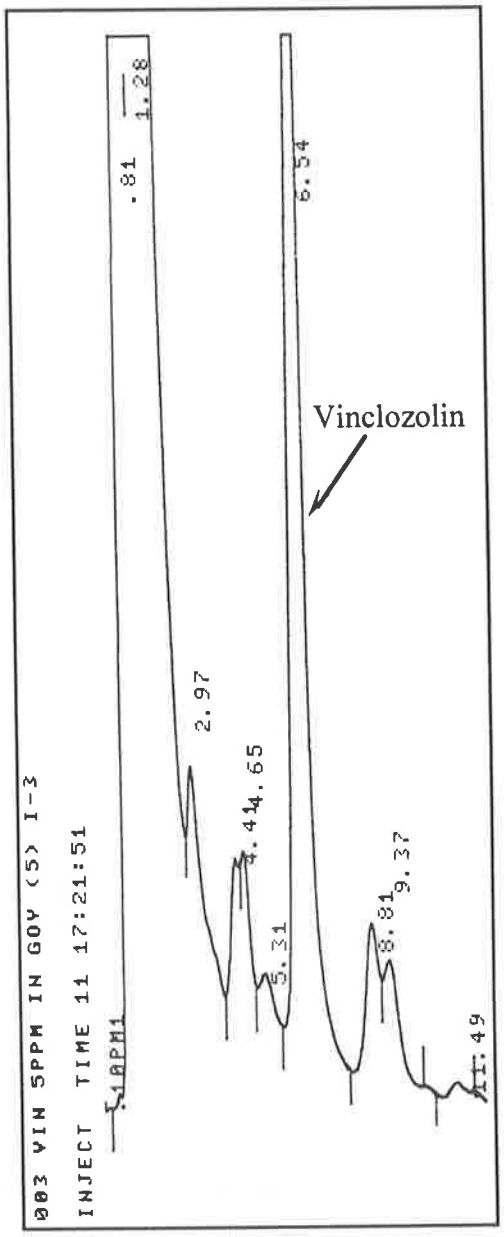


Figure 4.4

Vinclozolin in white grape juice (goyura)

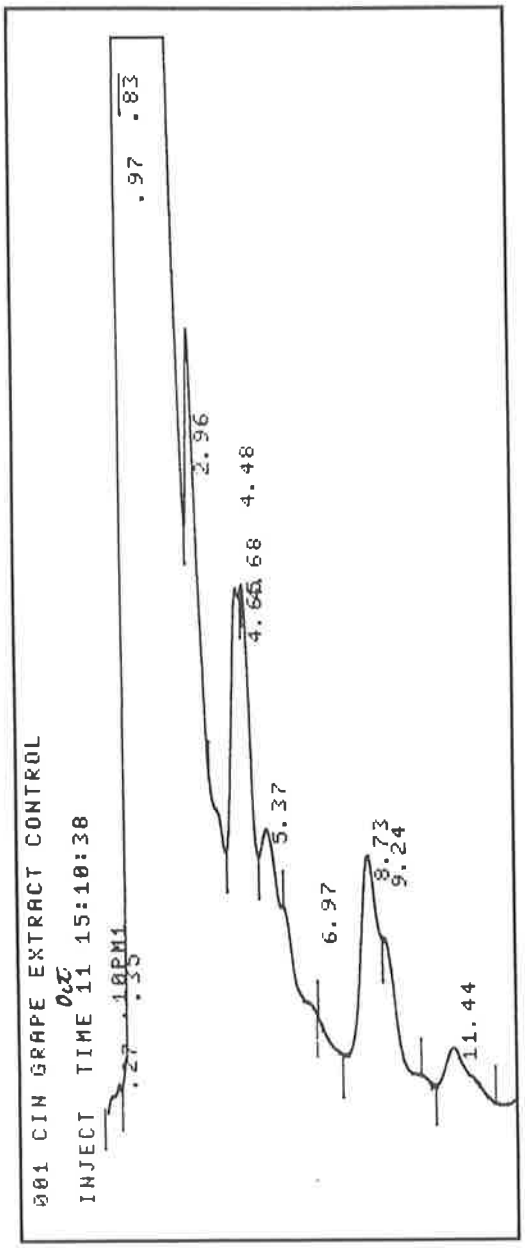


Figure 4.5

Red grape juice control (cinsaut)

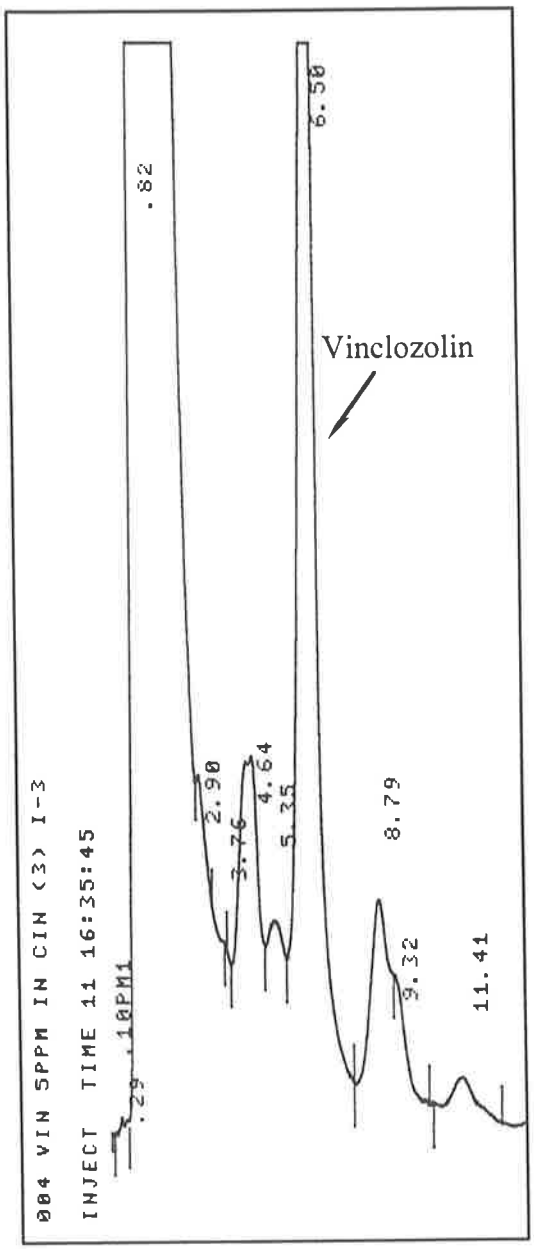


Figure 4.6

Vinclozolin in red grape juice (cinsaut)

Dithianon and vinclozolin recoveries from wine

White and red wines were made in the laboratory in the manner described in Chapter 6. A quantity of both dithianon and vinclozolin standard (10 µg) was added to white wine (10 mL) made from goyura grapes, and red wine (5 mL) made from cinsaut grapes. The wine was loaded onto the preconditioned cartridges in the manner described above for water samples. Each analysis was repeated 5 times.

Dithianon was found to degrade very quickly in both red and white wine. This is discussed in more detail in Chapter 5. The speed of this degradation, $t_{1/2}$ ~ 30 min, precluded the gathering of accurate recovery data. Vinclozolin was found to be stable in both red and white wine, and can be quantitatively recovered (recovery 114 - 116 %) with excellent reproducibility (c.v. 1.9 - 2.5 %) by this method. Vinclozolin recovery data are shown in Table 4.3. Typical chromatograms are shown in Figures 4.7 and 4.8.

Table 4.3 Vinclozolin recovery from wines

Fungicide	Matrix	Sample	Spike	Recovery		Mean Recovery	
			(µg)	(µg)	(%)	(%)	c.v. (%)
Vinclozolin	red wine	rw-V1	10.0	11.48	114.8	114.3	1.7
	(cinsaut)	rw-V2		11.22	112.2		
		rw-V3		11.26	112.6		
		rw-V4		11.52	115.2		
		rw-V5		11.68	116.8		
	white wine	ww-V6	10.0	11.38	113.8	116.4	2.1
	(goyura)	ww-V7		12.00	120.0		
		ww-V8		11.64	116.4		
		ww-V9		11.44	114.4		
		ww-V10		11.74	117.4		

c.v. = coefficient of variation

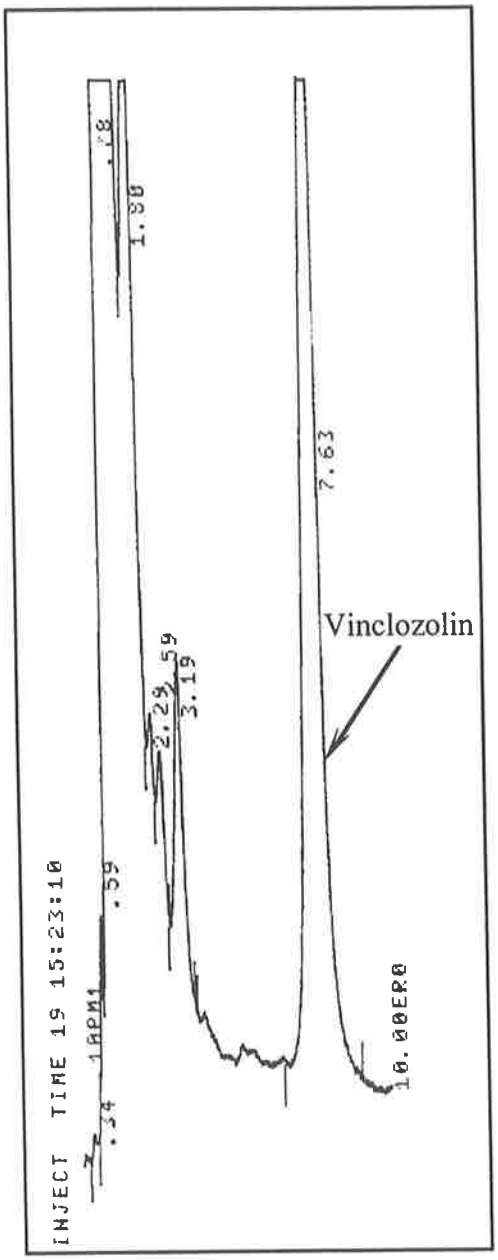


Figure 4.7 Vinclozolin in white wine

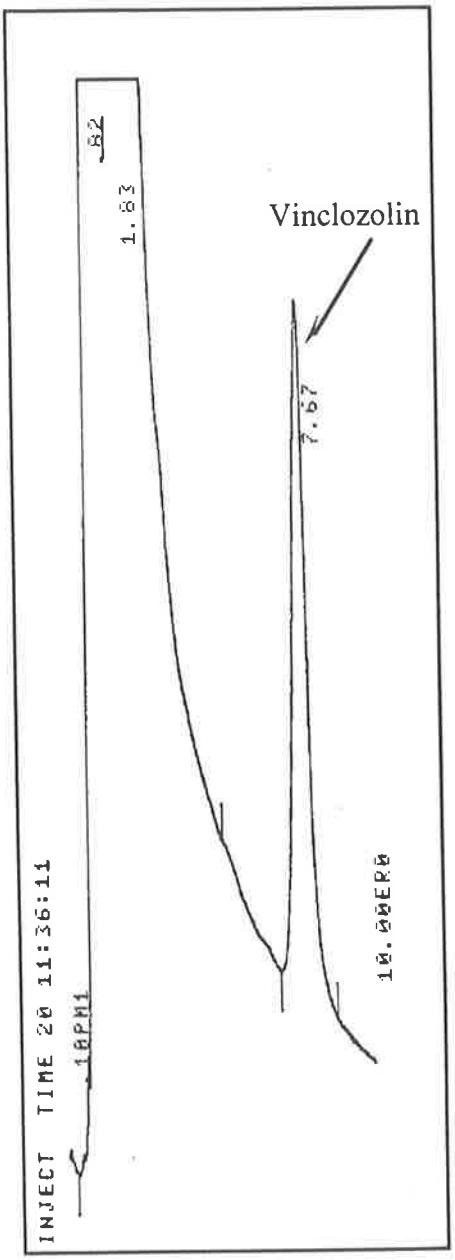


Figure 4.8 Vinclozolin in red wine

In order to determine whether it was the alcohol in the wine that was degrading the dithianon, an artificial wine was made by dissolving ethanol (100 mL) and tartaric acid (5 g) in one litre of water. A quantity of dithianon (10 μg) was added to the artificial wine (10 mL) and deionised water controls (10 mL). The samples were loaded onto the preconditioned cartridges in the manner described above for water samples. Each analysis was repeated 3 times. Dithianon was found to be stable in both the artificial wine and the deionised water control, suggesting that it is not the alcohol that causes dithianon breakdown in wine, and can be quantitatively recovered (recovery 111 - 118 %) with excellent reproducibility (c.v. 2.8 - 3.1 %) by this method. Dithianon recovery data is shown in Table 4.4.

Table 4.4 Dithianon recovery from deionised water and artificial wine

Fungicide	Matrix	Sample	Spike (μg)	Recovery (μg)	(%)	Mean Recovery (%)	c.v. (%)
Dithianon	deionised water	di-D1	10	11.5	115	117.7	2.6
		di-D2		11.7	117		
		di-D3		12.1	121		
	artificial wine	aw-D4	10	10.8	108	111.3	2.6
		aw-D5		11.3	113		
		aw-D6		11.3	113		

c.v. = coefficient of variation

Dithianon and vinclozolin recoveries from Rutherglen topsoil and subsoil

Further sample preparation steps are required prior to extraction of both vinclozolin and dithianon from soil.

Table 4.5 Summary of results from Rutherglen soil recovery experiments

Fungicide	Matrix	Sample	Spike (µg)	Recovery (µg)	(%)	Mean Recovery (%)	c.v. (%)
Dithianon	Topsoil	R-D7	10.1	7.7	76.2	79.1	14
		R-D8		9.2	91.0		
		R-D9		7.1	70.1		
		R-D10	101	79	78.2	92.5	13.5
		R-D11		102.2	101.1		
		R-D12		99.2	98.2		
	Subsoil	R-D13	10.1	9.1	89.6	88.9	7
		R-D14		9.6	94.7		
		R-D15		8.3	82.3		
		R-D16	101	89.8	89.0	96.4	6.8
		R-D17		99.5	98.5		
		R-D18		102.7	101.7		
Vinclozolin	Topsoil	R-V7	10.6	10.5	99.1	100.9	14
		R-V8		12.3	115.8		
		R-V9		9.3	87.8		
		R-V10	106	105.8	107.9	105.4	3.4
		R-V11		98.2	106.2		
		R-V12		99.8	107.2		
		R-V19		106.1	100.1		
	Subsoil	R-V13	10.6	10.0	94.0	102.8	8.2
		R-V14		11.0	103.6		
		R-V15		11.8	110.8		
		R-V16	106	104.5	98.6	96.9	3
		R-V17		103.8	97.9		
		R-V18		99.2	93.6		

c.v. = coefficient of variation

Both dithianon and vinclozolin were added to samples of topsoil and subsoil (5 g) collected from the core extraction site. The fungicides were spiked at two levels - 10 and 100 µg. The samples were shaken with methanol : water (80 : 20) for one hour. Thereafter, the mixture was filtered, the residue washed with water, and the combined filtrate and washings diluted to 200 mL with deionised water. Thereafter, the aqueous solution was loaded onto an SPE cartridge and the analyte isolated in the manner described for aqueous solutions. Recoveries were determined in triplicate at each level of spike. Again, the extraction procedure proved to be rapid, simple, and robust. The fungicides were found to be stable in both topsoil and subsoil. Recoveries of both dithianon and vinclozolin were again good, with good reproducibility. There was no statistical difference in the recovery from topsoil or subsoil: combined mean recovery for dithianon 89.2 %, c.v. 11.7 %; combined mean recovery for vinclozolin 101.7 %, c.v. 7.6 % (Table 4.5).

Dithianon was found to be stable in all topsoil and subsoil and can be quantitatively recovered (recovery 79.1 - 99.2 %) with good reproducibility (c.v. 2.8 - 12.5 %) by this method. Vinclozolin was also found to be stable in all topsoil, subsoil, and sandy soil and can be quantitatively recovered (recovery 96.7 - 107.1 %) with excellent reproducibility (c.v. 0.9 - 14.1 %) by this method.

Fungicide recovery from Overland Corner topsoil

Both dithianon and vinclozolin were added to samples of topsoil collected from the extraction site. Vinclozolin was added at one of two levels - 10.6 µg and 106 µg, dithianon at 10.1 µg or 101 µg. The samples were shaken with methanol : water (80 :

20) for one hour. Thereafter, the mixture was filtered, the residue washed with water, and the combined filtrate and washings diluted to 200 mL with deionised water. Thereafter, the aqueous solution was loaded onto an SPE cartridge and the analyte isolated in the manner described for aqueous solutions. Recoveries were determined for each fungicide in triplicate at each level of spike. Again, the extraction procedure proved to be simple, rapid and robust. The fungicides were found to be stable in both topsoil and subsoil over the time period of this experiment. Both dithianon and vinclozolin were quantitatively recovered with good reproducibility (Table 4.6). There was no significant difference in the rate of recovery of either high or low level spikes : combined mean recovery, 100.7% ; c.v. = 5 %.

Table 4.6 Summary of results from Overland Corner topsoil recovery experiments

Fungicide	Matrix	Sample	Spike	Recovery		Mean Recovery	
			(µg)	(µg)	(%)	(%)	c.v. (%)
Dithianon	Topsoil	OC-D1	10.1	9.8	96.9	95.1	3
		OC-D2		9.8	96.5		
		OC-D3		9.3	91.8		
		OC-D4	101	100.7	99.7	99.2	4.2
		OC-D5		95.7	94.7		
		OC-D6		104.1	103.1		
Vinclozolin	Topsoil	OC-V1	10.6	10.7	100.6	101.4	1.4
		OC-V2		10.7	100.6		
		OC-V3		10.9	103.1		
		OC-V4	106	114.4	107.9	107.1	0.8
		OC-V5		112.6	106.2		
		OC-V6		113.7	107.2		

c.v.= coefficient of variance

4.4 Conclusion

The matrices inherently required to be investigated in any study of the environmental fate of viticultural pesticides i.e. water, grape juice, wines, and soil, all contain organic compounds which are potential analytical interferences. The solid-phase extraction (SPE) methods developed heretofore were able to separate the analytes dithianon and vinclozolin from those naturally occurring organic species prior to analysis. The methods were rapid and robust, yet capable of providing extract solutions from which sub-ppm concentrations of the analytes could be determined with excellent reproducibility. Multiple extractions could be done simultaneously, rapidly and relatively inexpensively. The final extracts from the SPE methods were well suited to the chromatographic analysis described in Chapter 3. As such, in this case SPE was preferred to, and had considerable advantages over, more traditional approaches such as liquid-liquid and liquid-solid phase extraction methods.

5. Stability of Dithianon and Vinclozolin

5.1 Introduction

Australia's grape growing and wine making industries comprises over 5,000 independent grape growers and more than 800 wineries spread across all States and Territories. Members of the industry range in size from the very small, hobby grape-grower to the large multinational. Vines are grown across an extensive range of soils and climatic conditions, with viticulturists using a wide range of vine, pest, and vineyard water and soil management practices (GWRDC, 1996). Like other Australian agricultural industries, agrochemicals are used extensively by the industry. Once sprayed onto vines, there are a number of possible outcomes for pesticides such as dithianon and vinclozolin. They may persist on the surface of grapes and be transported into the wine making process, the fungicides may chemically degrade, or they may be washed off the vines onto the soil, and from there reach surface waters by means of surface run-off, or leach through the soil to groundwater.

A number of basic experiments help to predict the behaviour of pesticides in the natural environment : the first of these involves kinetic studies investigating hydrolysis patterns in water at different pHs and temperatures. Dithianon is known to be decomposed by alkaline media, by concentrated acids, and by prolonged heating (RSC, 1993). The rates of hydrolysis of dithianon in buffered solution at different pH has been reported (FAO/WHO, 1992) and the reported half-life values are summarised in Table 2.1. Dithianon stability decreases dramatically with increasing temperature. At room temperature (22 ^{o}C), dithianon half-lives range from 0.15 h. at pH 9, to 295 h. at pH 5. However, no data was presented for this temperature at the

lower pHs typical of wine i.e. pH 3 - 4. In addition, it is not unusual for Australian watercourses to reach temperatures over 30 °C during the summer months, while groundwater may be heated to higher temperatures with increasing depth. High temperature kinetic data has only been reported for dithianon for buffered solutions at pH 4. At 50 °C, the half-life was reported to be 278 h. Vinclozolin is also known to be more susceptible to hydrolysis in basic solutions than in acidic ones (Melkebeke et al., 1986; FAO/WHO, 1986). The kinetics of hydrolysis of vinclozolin in aqueous buffers of pH 4.5 - 8.3 at 13 - 35 °C has been studied (Szeto et al., 1989a). The reaction was reported as being base-catalysed and the rate proportional to pH i.e. the higher the pH, the faster the degradation. This data is summarised in Table 2.6.

The second type of basic experiment used to predict the behaviour of pesticides, kinetic studies investigating hydrolysis patterns in grape juice and wine, has been neglected for dithianon. There have been no published reports detailing investigations on the stability of dithianon in wine and grape juice. And, while other aspects of the oenological and environmental chemistry of vinclozolin have been extensively studied, the kinetics of the persistence, or otherwise, of vinclozolin in grape juice and wine has been little studied. Only one major study has been reported (Cabras et al., 1984). That study reported that the pseudo-first order rate constants defining the disappearance of vinclozolin in wines of pH 3.0 and 4.0 were elevated at the higher pH.

The third of the simple chemical experiments that may be used to help predict the behaviour of pesticides in the natural environment are photochemical studies of the

stability of such chemicals in both the aqueous and solid phase. Degradation of pesticides in aqueous solutions may occur chemically, particularly in the case of grape juice and wine, or be promoted by the interaction of the pesticide with light. Such photodegradation in natural waters by natural sunlight is of significant environmental concern because the degradation products may have different environmental effects to their parent compounds, i.e. the degradation products may be more toxic, or more persistent than the original active ingredients of the pesticide formulations. Organic pollutants in the aquatic environment may be exposed to sunlight in the surface layers of a natural water body in a subtly different manner to exposure in the solid phase from direct sunlight. However no studies have been reported detailing either dithianon or vinclozolin photolysis in aqueous solutions, nor is data available regarding the stability of these compounds in the solid phase to direct sunlight.

The primary aim of these studies being to compare and contrast the environmental behaviour of dithianon and vinclozolin, this chapter describes investigations into the stability of these fungicides in water, grape juice and wine, and towards solar radiation, both in aqueous solution and in the solid-phase.

5.2 Materials

(a) Chemicals, fungicide standards, solvent degassing and the liquid chromatographic system (HPLC column, solvent system and UV detector) as described in Chapter 3.

(b) Extraction cartridges, manifold, and methods as described in Chapter 4.

(c) *pH meter* : Hannah Instruments Model HI 8519.

(d) *Water bath* : 60 L constant temperature bath with variable temperature control.

(e) *Grape juice and wines* : grapes of known chemical treatment history were harvested from both Nuriootpa Research Station, Primary Industries of South Australia and vineyard at Roseworthy Campus, University of Adelaide. Grape varieties were pinot noir (red) and semillon (white) from Nuriootpa, and bastardo, cinsaut (red) and rkaziteli and goyura (white) from Roseworthy. Wines made from pinot noir and semillon grapes were prepared by personnel in the winery of Wine and Grape Research Unit at Roseworthy, University of Adelaide. Wines made from bastardo, cinsaut, rkaziteli and goyura grapes were prepared by the author in the laboratory.

5.3 Stability of Dithianon and Vinclozolin in Water

The primary purpose of this aspect of this study was to fill in some of the blanks apparent in the tables of dithianon and vinclozolin half-life values (Tables 2.1 and 2.6). The information so provided was also vital if these studies were going to be able to compare rates of hydrolysis in water with rates in other aqueous media such, as

grape juice and wine, and also with photodegradation. This section describes hydrolysis of dithianon and vinclozolin at 20 and 35 °C, and pH 4, 5.4, 7 and 9.

5.3.1 Methods

Buffer solutions

Acetate buffers (100 mL) were made in the manner of Dawson et al. (1986) :

- pH 4 : 18 mL of 0.2 M sodium acetate plus 82 mL of 0.2 M acetic acid
- pH 5.4 : 86 mL of 0.2 M sodium acetate plus 14 mL of 0.2 M acetic acid

Phosphate buffer (100 mL) was made in the manner of Dawson et al. (1986) :

- pH 7 : 30.5 mL of 0.2 M di-sodium hydrogen orthophosphate plus 19.5 mL of 0.2 M sodium dihydrogen orthophosphate plus 50 mL deionised water.

Borate buffer (100 mL) was made in the manner of Dawson et al. (1986) :

- pH 9 : 50 mL of 0.025 M sodium tetraborate decahydrate plus 4.6 mL of hydrochloric acid plus 45.4 mL deionised water.

Hydrolysis experiments

Buffer solutions (50 mL; pH 4, 5.4, 7, and 9) were spiked with fungicide standard solutions at two levels : 2 µg/mL with the water bath set at 20 °C, and 3 - 12 µg/mL with the bath set at 35 °C. Essentially, the reason for the use of higher concentrations in the latter experiments was concern over the expected more rapid degradation at elevated temperatures i.e. since degradation was fast at 20 °C one would expect degradation to be significantly faster at higher temperatures. The solutions were incubated in light-tight ground-glass jointed, stoppered boiling tubes

(50 mL) for several weeks in the constant temperature water bath. Aliquots of the solution (20 µL) were taken regularly and injected directly into HPLC system. The LC mobile phases were 50 : 50 acetonitrile : water for 20 °C experiment, and 1 : 50 : 50 acetic acid : acetonitrile : water for 35 °C experiment. At the end of the experiments, the solutions were extracted by SPE using C18 cartridges.

5.3.2 Results and Discussion

Disappearance of vinclozolin

The disappearance of vinclozolin at 20 °C was very fast at basic pH (Figure 5.1(g,h)), but considerably slower at neutral (Figure 5.1(e,f)) and acidic pHs (Figure 5.1(a-d)). This trend is consistent with those reported by Melkebeke et al. (1986) and Szeto et al. (1989a). These researchers studied the kinetics of the chemical hydrolysis of vinclozolin and both reported that the disappearance of vinclozolin in aqueous buffer solutions followed pseudo first order kinetics, although they disagreed over the exact values for the rate constants and half-lives. The results obtained in this study also suggest that the disappearance of vinclozolin follows pseudo first order kinetics. The apparent deviation from simple first order kinetics observed at low concentrations (approximately 10% of initial dose) does not seem to have been observed by Szeto et al. (1989a; Figure 5.5), even though the experimental protocols and buffer solutions used were identical. However, the initial concentrations reported by Szeto et al. (40 µM; 1989a; Figure 5.5)) is approximately 4.5 times that used in this experiment (8.7 mM). The data does not support second, third, fourth or even fifth order kinetic interpretation. It is assumed that the data is unreliable at low concentrations, since the vinclozolin observed at low concentrations cannot be due to

contamination of the syringe or injector port between standard and sample injections because blank solvent injections undertaken immediately after injection of the analytical standard did not give latent vinclozolin peaks (Pers. Comm., Dr. F.Stagnitti, Deakin University). Although the half-life at pH 7 calculated from the data obtained in this experiment (Table 5.1) is twice that observed by Szeto (Table 2.6), this may simply be a result of the unreliability of the data at vinclozolin concentrations close to the minimum determinable limit.

In order to study the influence of temperature on the rate constant, the experiments were repeated at 35°C (also the same temperature used by Szeto et al., 1989). The disappearance of vinclozolin at 35°C was very fast at basic pH (Figure 5.2 (g,h)), but considerably slower at neutral (Figure 5.2 (e,f)) and acidic pH's (Figure 5.2 (a-d)). In this case, the half-life at pH 7 calculated from the data obtained in this experiment (Table 5.1) is consistent with that observed by Szeto (Table 2.6). Again, these results suggest that the disappearance of vinclozolin follows pseudo first order kinetics.

Table 5.1 Summary of kinetic data of hydrolysis of vinclozolin in aqueous (buffered) solutions of pH 4 - 9

Temp °C	pH	correlation coefficient	k h-1	t1/2 h
20	4	0.965	0.004	1724
	5.4	0.937	0.0048	144
	7	0.975	0.0118	59
	9	0.99	0.7745	0.89
35	4	0.9	0.0017	409
	5.4	0.954	0.0211	33
	7	0.99	0.0793	8.7
	9	0.981	0.64535	0.19

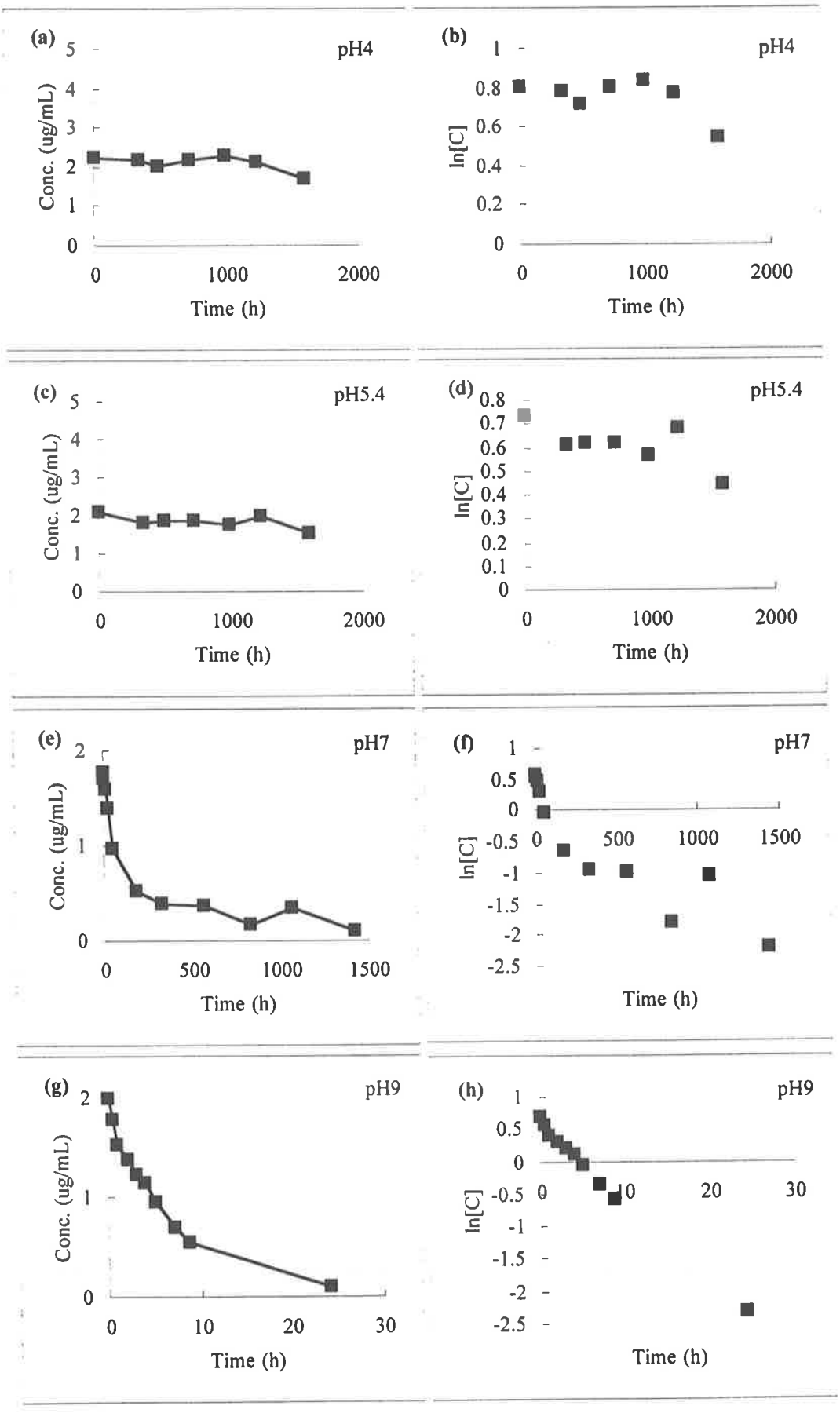


Figure 5.1 Persistence of vinclozolin in aqueous (buffered) solution at 20 °C

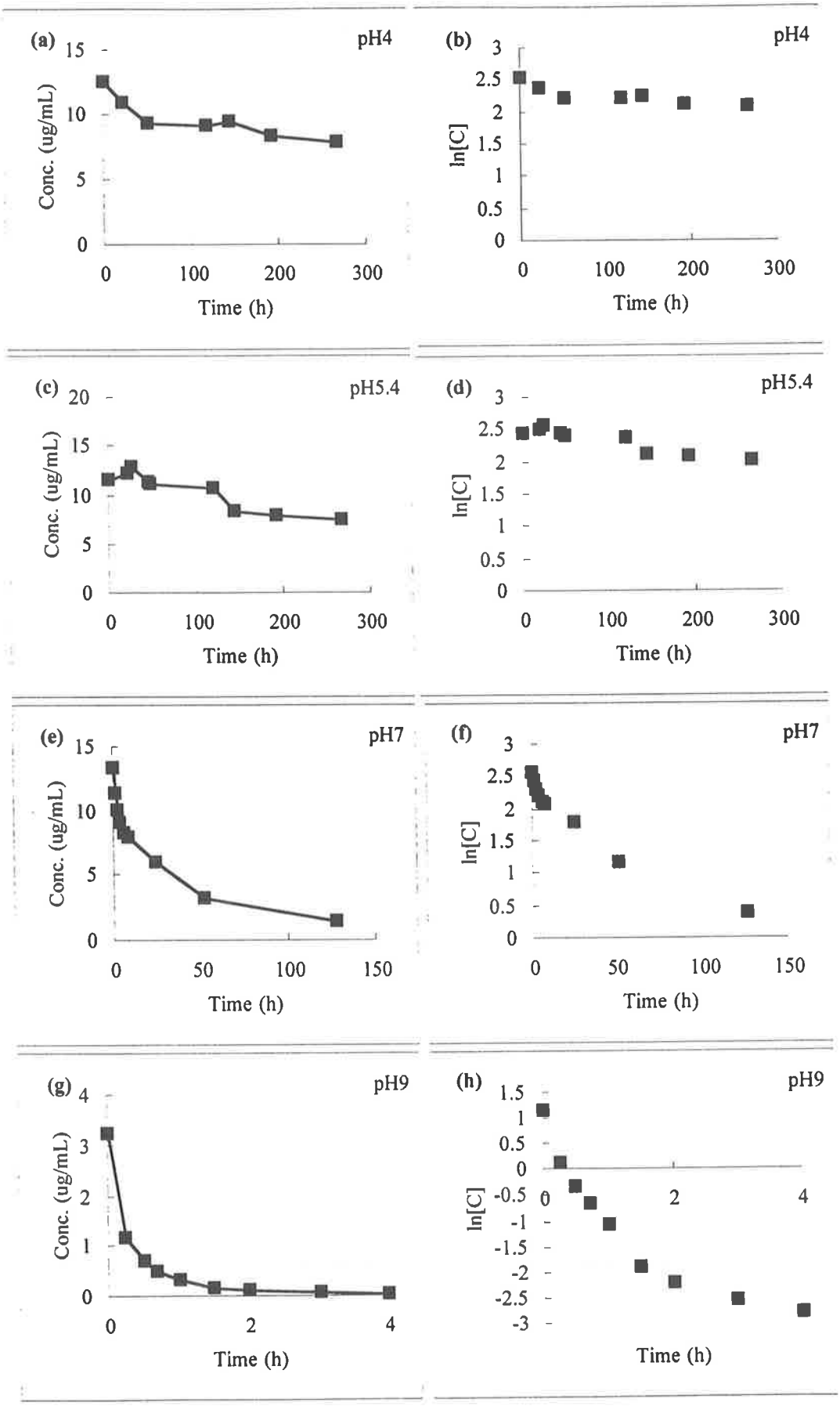
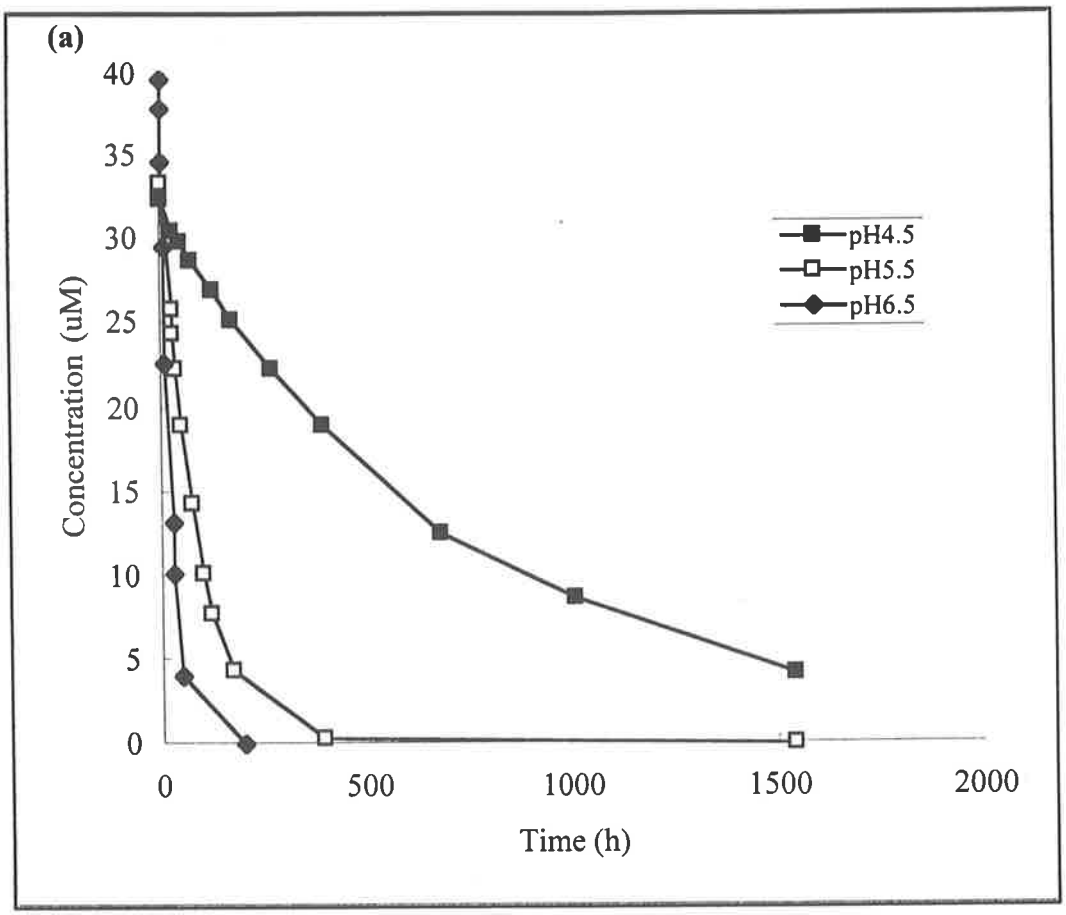


Figure 5.2 Persistence of vinclozolin in aqueous (buffered) solution at 35 °C



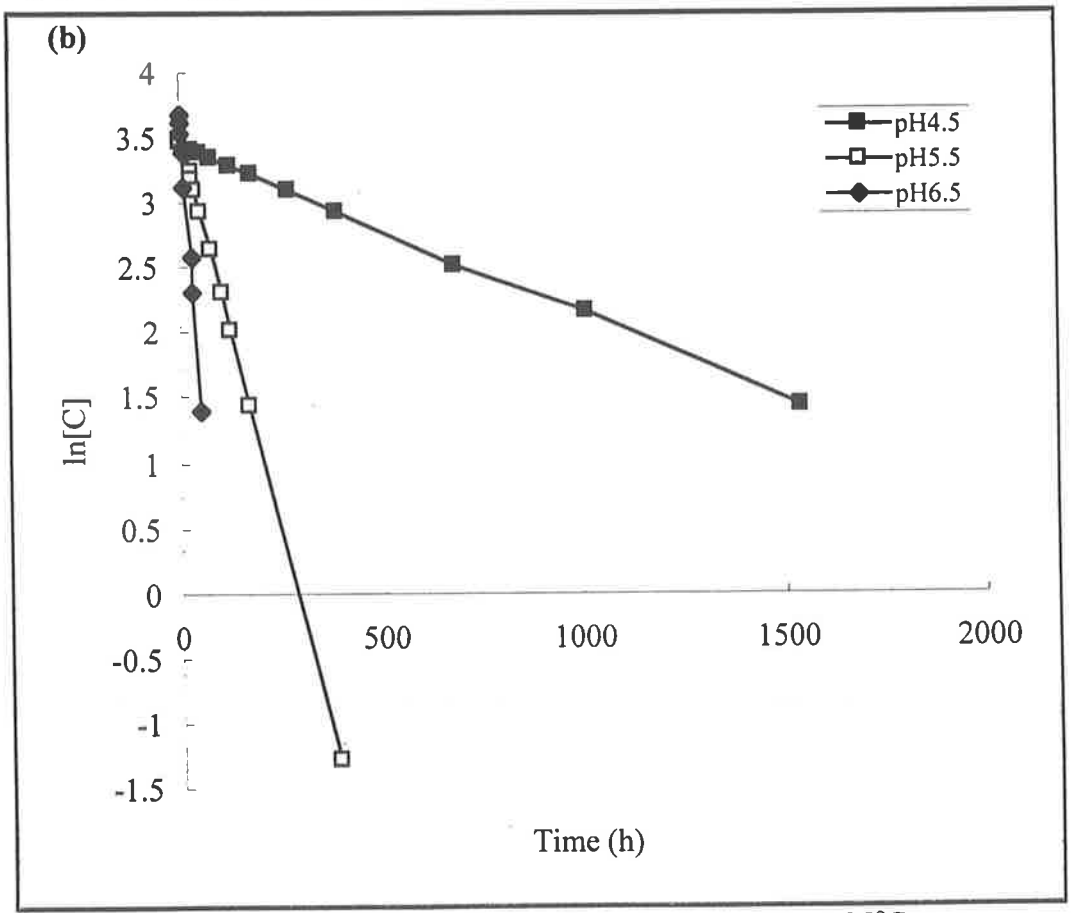


Figure 5.3 Summary of reported vinclozolin hydrolysis data at 35°C (Szeto et al., 1989a)

Disappearance of dithianon

With the aqueous degradation experimental protocols validated by the replication of Szeto's vinclozolin results, attention turned to the behaviour of dithianon in aqueous solution. The disappearance of dithianon at 20 °C was very fast at basic pH (Figure 5.4(g,h)), but considerably slower at neutral pH (Figure 5.4(e,f)), and somewhat slower still at acidic pH's (Figure 5.4(a-d)). The general trends observed - rapid disappearance of dithianon in alkaline solutions, slow in acidic - are consistent with those reported by the FAO/WHO (1992). That report suggested that the disappearance of dithianon in aqueous buffer solutions followed pseudo first order kinetics. The results reported herein also suggest that the disappearance of dithianon follows pseudo first order kinetics, although again the apparent deviation from simple first order kinetics at low concentrations (approximately 10% of initial dose) does not seem to have been observed by researchers involved in the FAO/WHO report (1992). The deviation from simple first order kinetics observed during is not due to contamination of the syringe or injector port between standard and sample injections since blank solvent injections undertaken immediately after injection of the analytical standard did not give latent dithianon peaks. The data does not support second, third, fourth or even fifth order kinetic interpretation. It is assumed that the deviation from simple first order kinetics seen at low concentrations results from the data being unreliable at concentrations close to the minimum determinable limit (Pers. Comm., Dr. F.Stagnitti, Deakin University).

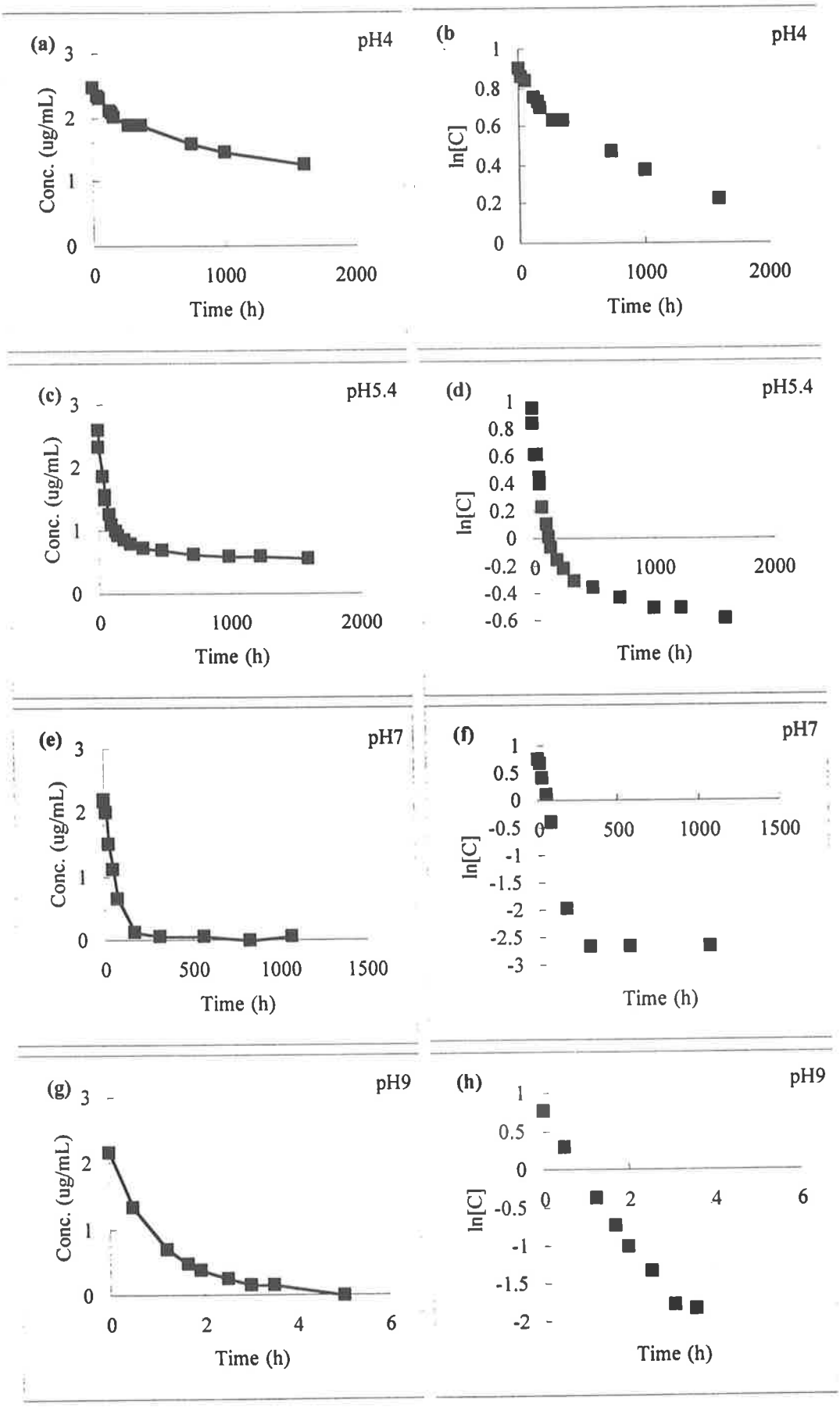


Figure 5.4 Persistence of dithianon in aqueous (buffered) solution at 20 °C

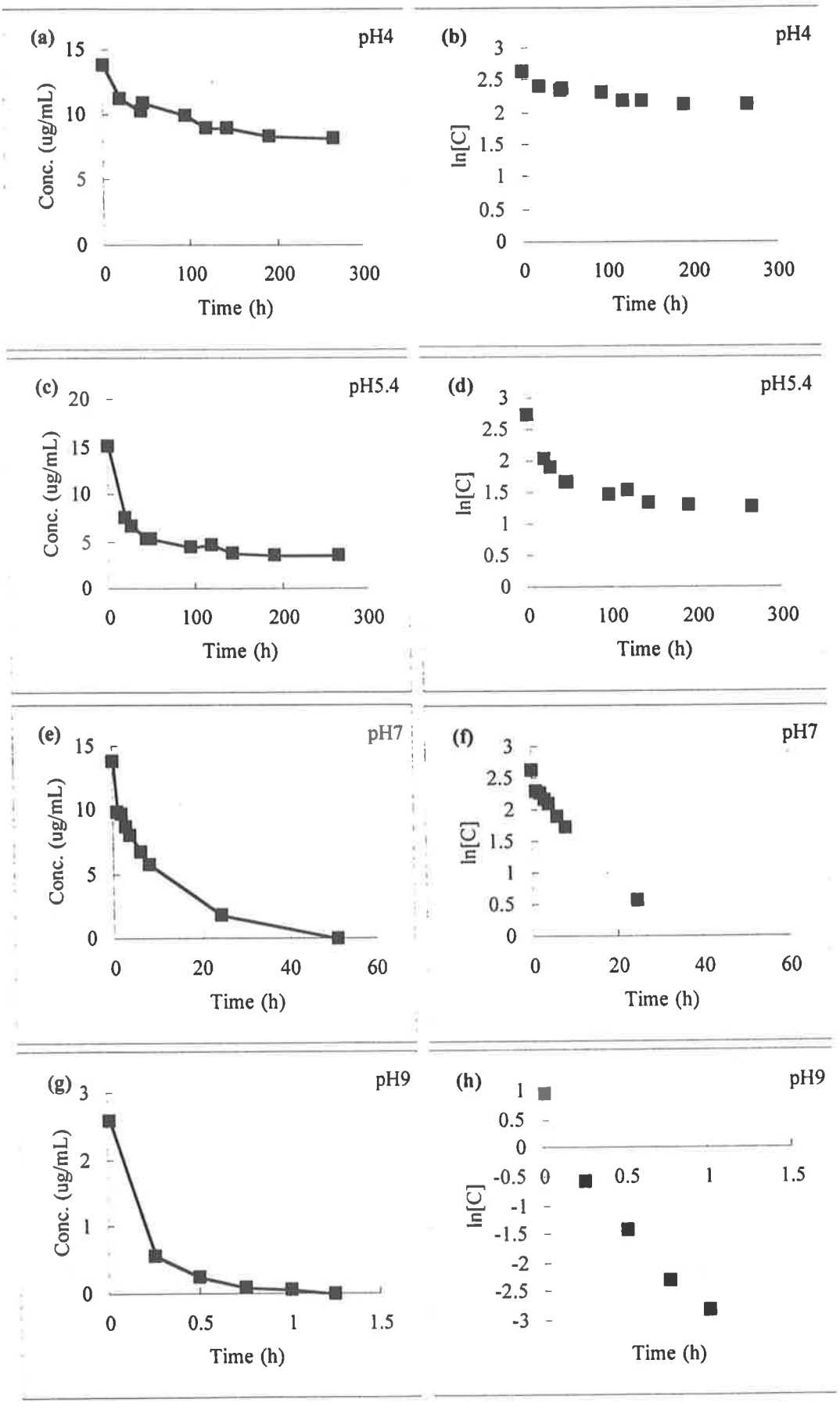


Figure 5.5 Persistence of dithianon in aqueous (buffered) solution at 35 °C

Table 5.2 Summary of kinetic data of hydrolysis of dithianon in aqueous (buffered) solutions of pH 4 - 9

Temp °C	pH	correlation coefficient	k h-1	t1/2 h
	4	0.601	0.0001	6443
20	5.4	0.717	0.0001	5720
	7	0.905	0.0018	392
	9	0.997	0.1228	5.6
	4	0.889	0.0014	485
35	5.4	0.941	0.0021	329
	7	0.97	0.0164	42
	9	0.977	1.9197	0.36

In order to study the influence of temperature on the rate constant, the experiments were repeated at 35 °C. The disappearance of dithianon at 35 °C was very fast at basic pH (Figure 5.5 (g,h)), but considerably slower at neutral (Figure 5.5 (e,f)) and acidic pH's (Figure 5.5 (a-d)). Increasing the temperature has a significant effect on the rate constants and associated half-lives. The half-lives were significantly reduced at 35 °C i.e. pH 4, 35 °C $t_{1/2}$ = 409 h., cf. 20 °C $t_{1/2}$ = 1724 h.

5.3.3 Conclusion

By comparison with other crop protection agents, vinclozolin and dithianon appear to be more resistant to hydrolysis. For instance, the pseudo first order rate constants of hydrolysis at 28 °C and about pH 7 are ~ 0.23 h^{-1} for captan, ~ 0.28 h^{-1} for captafol and ~ 0.5 h^{-1} for folpet (Wolfe et al., 1976). By comparison, the value for vinclozolin at 35 °C and about pH 7 is estimated to be 0.0793 h^{-1}, while that for dithianon is estimated to be 0.0164 h^{-1}.

For both dithianon and vinclozolin the rate constants were both pH and temperature dependent. When compared with each other, the disappearance of dithianon at both 20 °C and 35 °C was slower than vinclozolin at basic pH, a little slower at neutral pH, and a little slower still at acidic pH's (Tables 5.1 and 5.2). In the range pH 5.4 - 9.0, which covers the range typical for natural Australian waters, both dithianon and vinclozolin underwent hydrolysis readily. At 20 °C, a not atypical groundwater temperature, the half-live of dithianon at pH 7.0 was 392 h., while that of vinclozolin was 59 h. These findings suggest that both Delan and Ronilan formulations may be unstable in water, especially slightly basic waters, and that to ensure the efficacy of both these fungicides it is important to prepare the spray mix fresh with neutral or slightly acidic water. On the other hand, these results also suggest that should either dithianon or vinclozolin be accidentally released into Australian waters then their lability to hydrolysis would suggest that they are likely to be rapidly hydrolysed, particularly in slightly basic waters, and therefore these chemicals should pose little direct long-term environmental threat.

5.4 Stability in Grape Juice and Wine

There have been no published reports detailing investigations on the stability of dithianon in wine and grape juice. This section describes experiments investigating the persistence of dithianon and vinclozolin in grape juice and wine.

5.4.1 Methods

Methods for the extraction and determination of dithianon and vinclozolin in grape and wine samples are detailed in Chapter 3. Other methods specific to this aspect of these studies are explained hereafter.

Persistence of dithianon and vinclozolin in wine

The bulk wine sample (red wine 50 mL; white wine 100 mL) was spiked with the fungicide at such a level as to produce concentrations between 1 - 5 μg/mL in the bulk wine sample. Immediately after spiking the samples, an aliquot of the wine (red wine, 5 mL; white wine, 10mL) was taken and the fungicide re-isolated using the SPE method described in Chapter 3. Thereafter, further aliquots of wine were taken from the bulk sample at regular intervals and the fungicide isolated from the sample in the same manner. Each recovery run was repeated a minimum of three times.

Dithianon recovery from polyvinylpolypyrrolidone (PVPP) treated wines

Polyvinylpolypyrrolidone (PVPP, Sigma Chemical Co., 10 g) was added to a bulk wine sample (100mL). The mixture shaken, and then filtered through glassfibre filter paper. The filtrate was then spiked with the fungicide at such a level as to produce a

1 μg/mL concentration in the bulk wine sample. Immediately after spiking the samples, an aliquot of the wine (red wine, 5 mL; white wine, 10mL) was taken and the fungicide re-isolated using the SPE method described in Chapter 3. Thereafter, further aliquots of wine were taken from the bulk sample at regular intervals and the fungicide isolated from the sample in the same manner. Each recovery run was repeated a minimum of three times.

Dithianon recovery from inactivated wine

Bulk samples of wine (500mL) were sterilised by heating in an autoclave (120 °C / 30 min). Thereafter, the wine was allowed to cool to room temperature overnight. Samples of red and white wines (100mL) were spiked with fungicide to give a 1 μg/mL concentration in the bulk wine sample. Immediately after spiking the samples, an aliquot of the wine (red wine, 5 mL; white wine, 10mL) was taken and extracted using the SPE (C18 cartridge) method described in Chapter 3. Thereafter, further aliquots of wine were taken from the bulk sample at regular intervals and the fungicide isolated from the sample in the same manner. Each recovery run was repeated a minimum of three times.

5.4.2 Results and Discussion

Vinclozolin was recovered quantitatively from both wine and grape juice (Table 4.2 and 4.3). Since it was known that vinclozolin persisted through the winemaking process (Chapter 6) it was thought that this chemical might be stable in wine. Therefore, once more, the stability experiments were performed first on vinclozolin in order to use the behaviour of this well-studied compound as a model for the little

studied dithianon. A bulk white wine sample (semillon) was spiked with vinclozolin such that the concentration in the wine was 1.0 μg / mL. Regular sampling of the bulk wine suggested that this compound is indeed relatively stable in wine, with a half-life, $t_{1/2}$ of about 800 hours (33 days) (Figure 5.6). Similar results were observed for red wine (pinot noir).

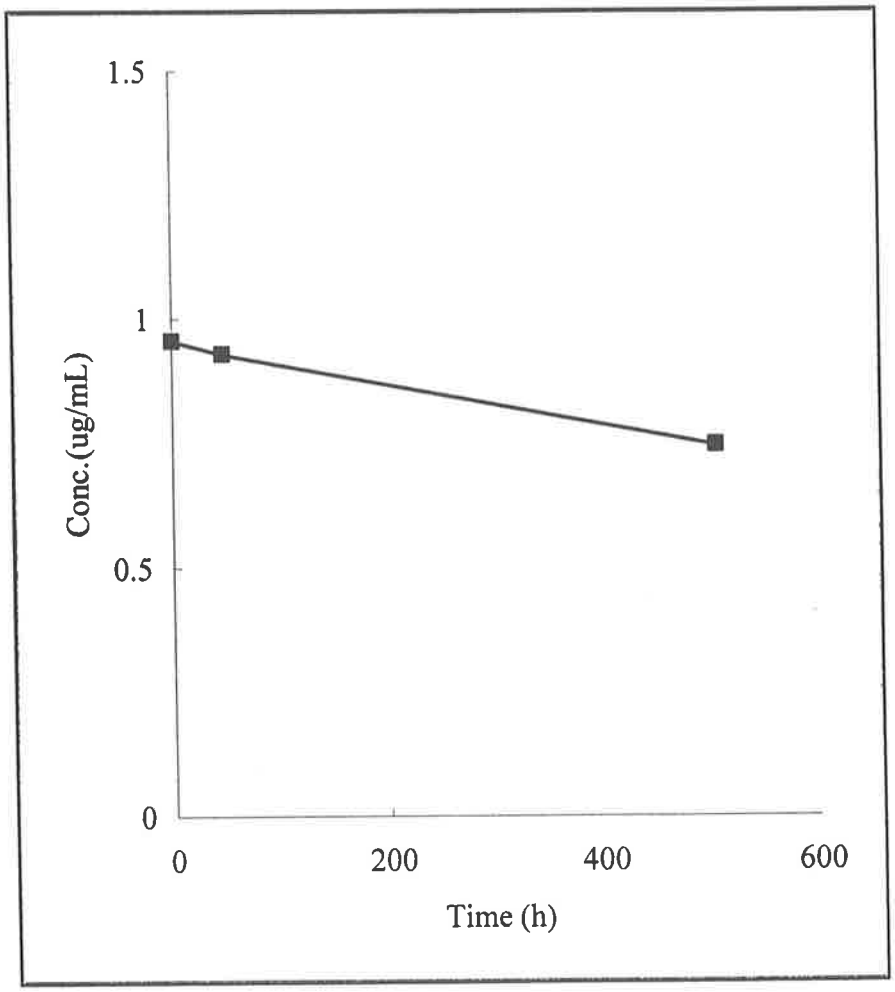


Figure 5.6 Persistence of vinclozolin in white wine (Semillon)

Dithianon recoveries from grape juice and wine were variable, even when recovery studies were performed immediately after spiking. Since vinclozolin showed stable recoveries at around 100% from wine, the extraction method was deemed to be acceptable and, therefore, that the poor recovery of dithianon from wine was due to chemical degradation not poor chemical recovery.

To investigate the rate of degradation of dithianon in grape juice, samples of juice derived from pinot noir grapes (red) and semillon grapes (white) were spiked with dithianon. Samples of the juice were re-extracted immediately after spiking and at regular intervals thereafter. The results are shown in Figures 5.7(a,b), and suggest that dithianon is totally degraded within twenty-four hours of exposure to both red and white grape juice. Dithianon in pinot-noir grape juice was marginally more resistant to degradation than that in semillon grape juice. The half-life in red grape juice was approximately 4.5 h., that in white grape juice approximately 2.8 h. These results suggest that the chemical instability of dithianon in grape juice is common to all grape juices, not just restricted to those from any particular sub-species of grape.

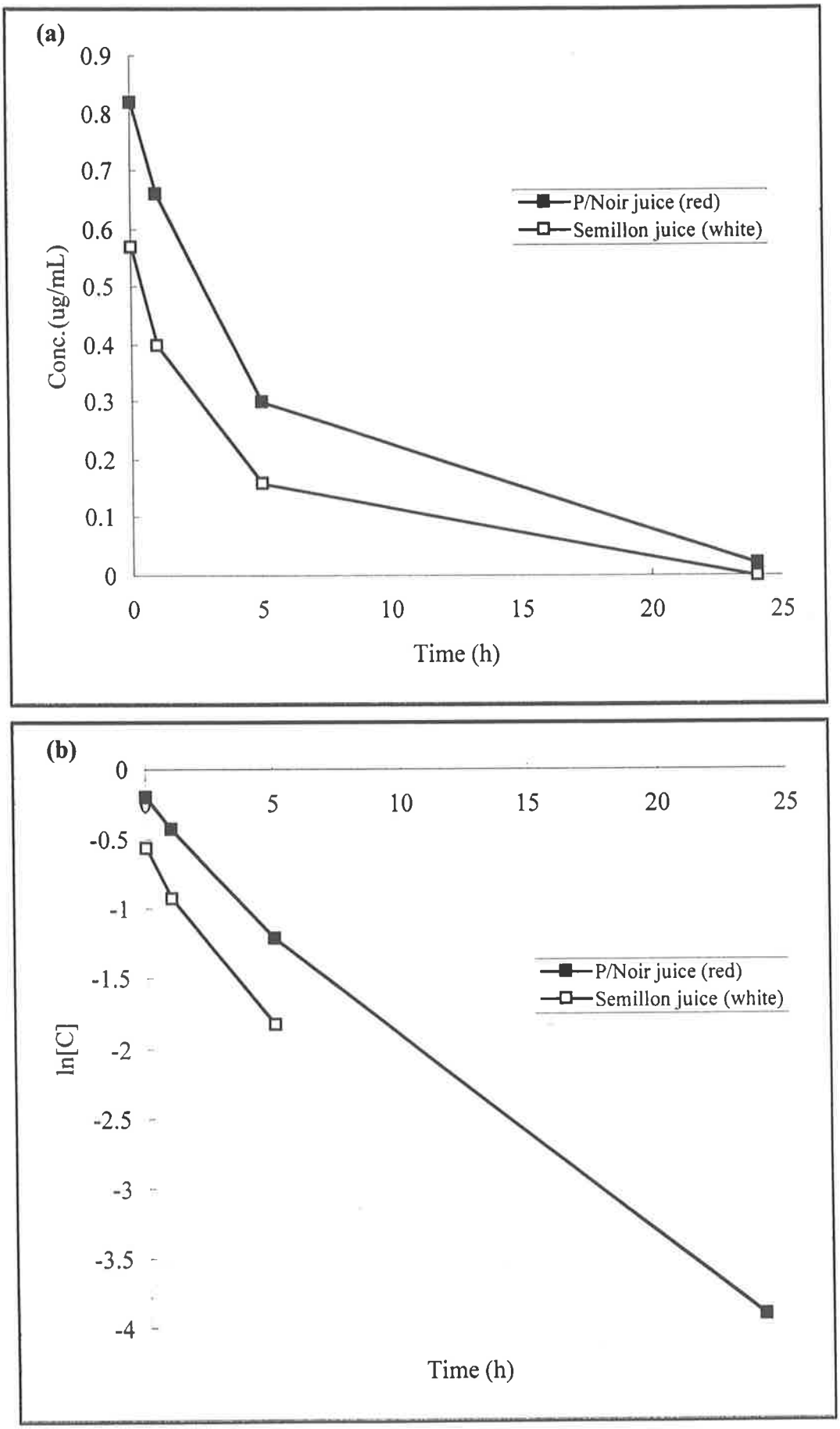


Figure 5.7 Persistence of dithianon in red and white grape juice

To determine the rate of degradation of dithianon in wine, samples of wine derived from pinot noir grapes (red) and semillon grapes (white) were spiked with dithianon. Samples of the wine were re-extracted immediately after spiking and at regular intervals thereafter. The results suggest that dithianon is totally degraded within five hours of exposure to red and white wines (Figures 5.8(a,b) and 5.9(a,b)). Dithianon in pinot-noir wine was marginally more resistant to degradation than that in semillon wine. The higher the initial concentration, the longer the half-life in red and white wines. At first glance, this suggests that the dithianon half-life is concentration dependent i.e. dithianon half-life in pinot noir wine is approximately 0.5 h. at an initial concentration (C_o) of 1 μg/mL, but approximately 13 h. at an initial concentration, C_o = 5 μg/mL (Figure 5.10). Again, dithianon half-life in semillon wine is approximately 1 h. at C_o = 1 μg/mL, but approximately 28 h. at C_o = 5 μg/mL (Figure 5.11). However, if the dithianon degradation / decomposition obeys first-order or pseudo-first order kinetic, as is suggested, then the rate of decomposition / degradation must be concentration independent. These results may, therefore, suggest that the kinetics of the chemical instability of dithianon in wine may result from mixed order reactions (ie there is more than one competing degradation / decomposition process) that are common to all wines, not just restricted to those made from any particular sub-species of grape.

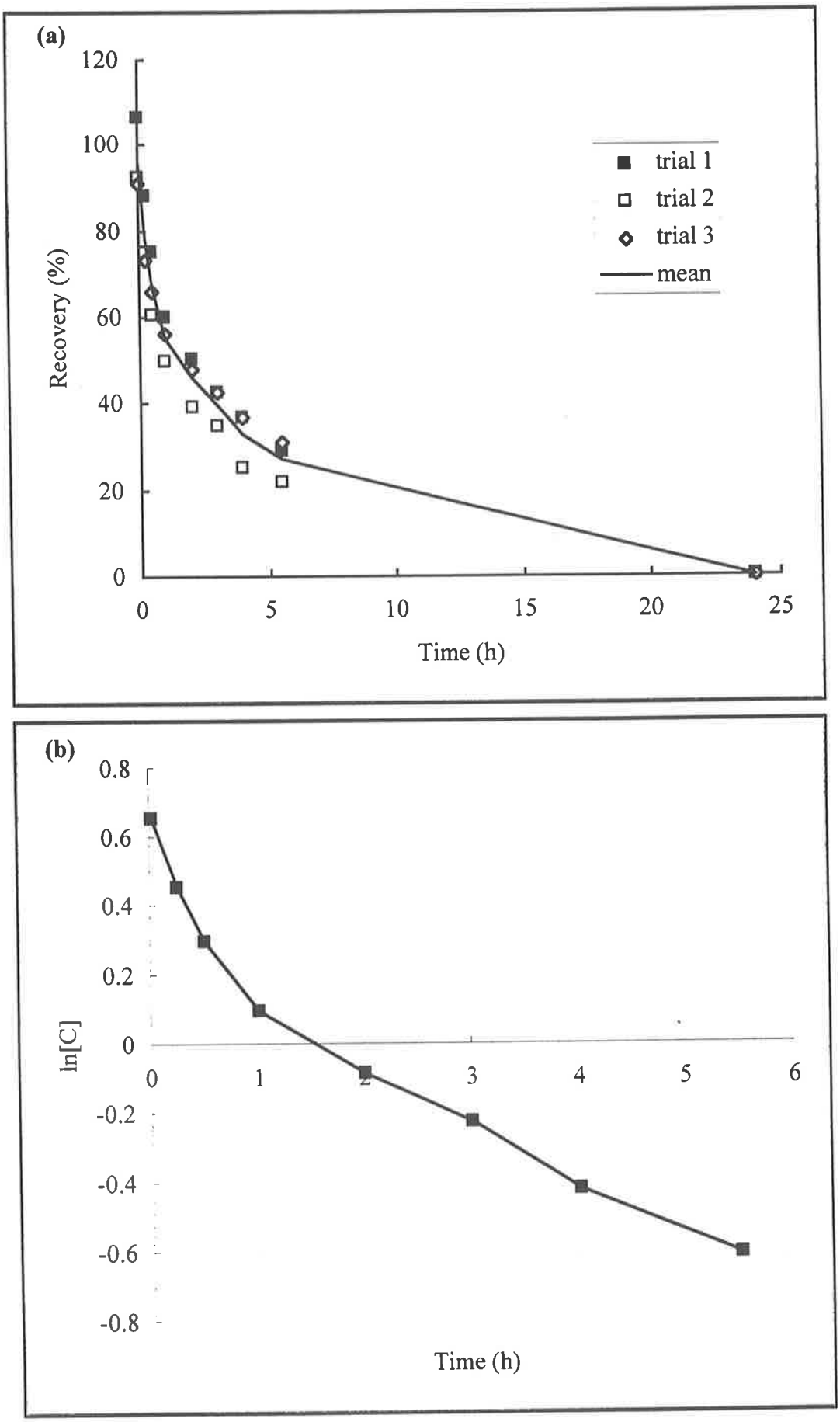


Figure 5.8 Persistence of dithianon (Co=2µg/mL) in red wine (pinot noir)

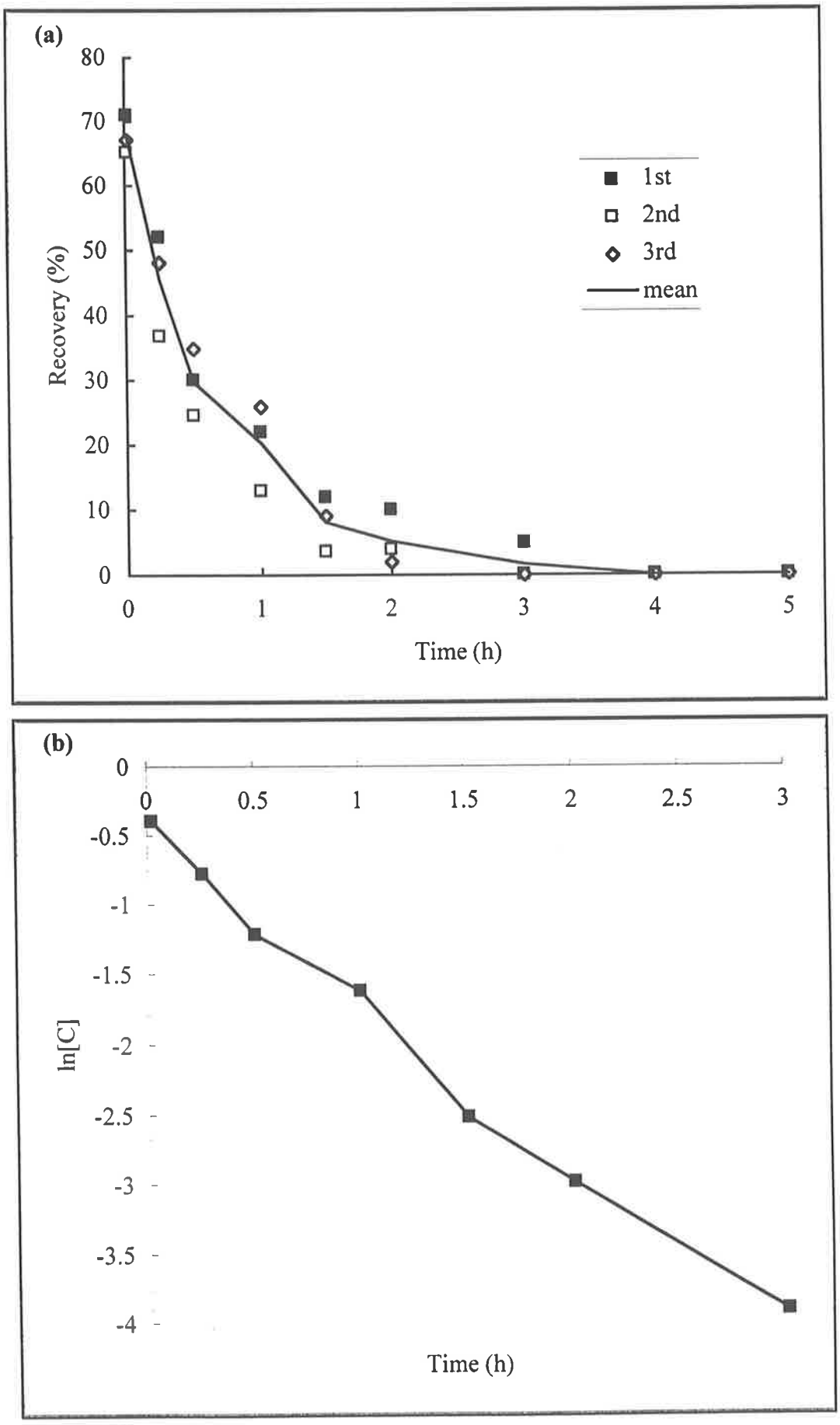


Figure 5.9 Persistence of dithianon (Co=1µg/mL) in white wine (semillon)

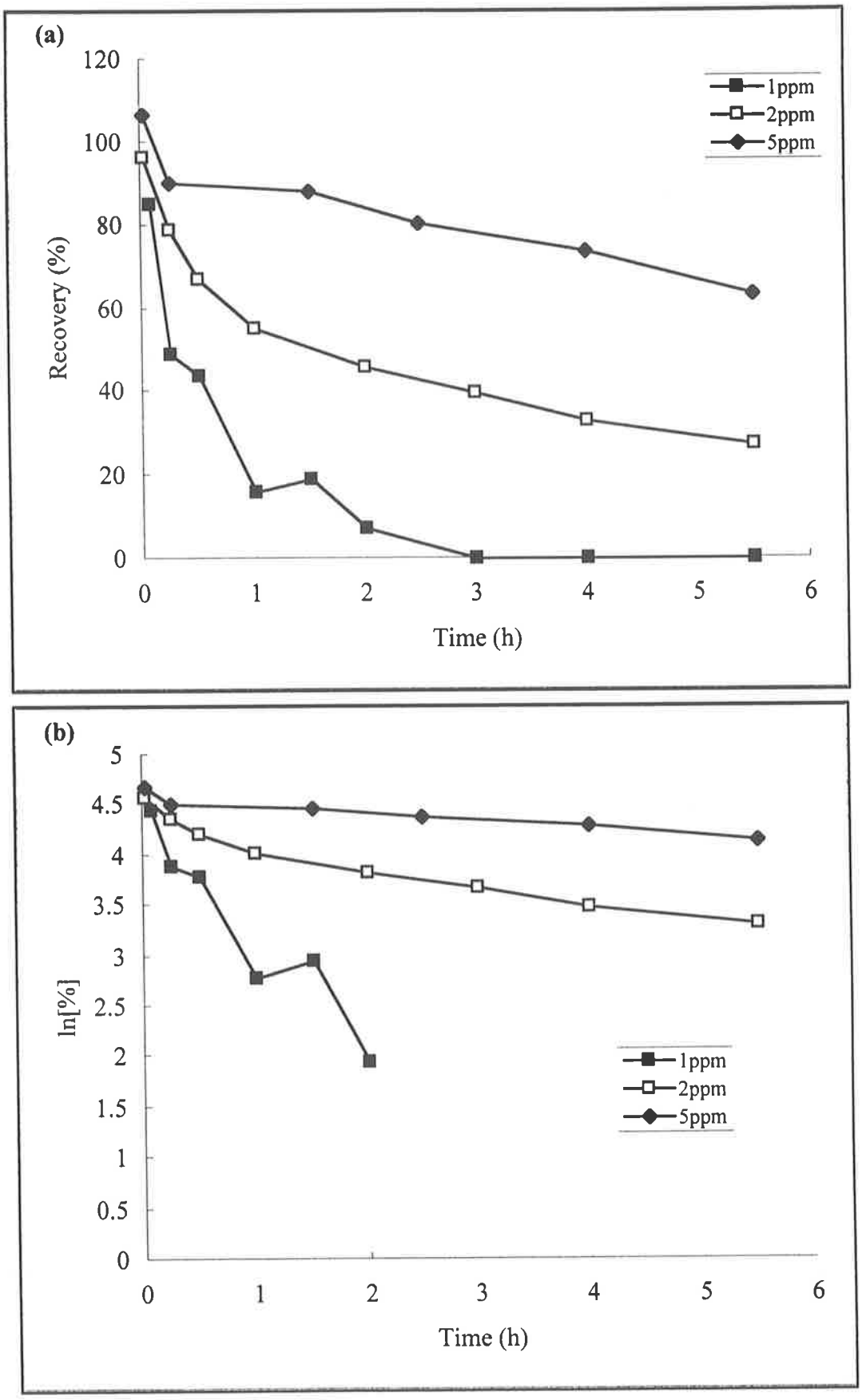


Figure 5.10 Variation in the rate of disappearance of dithianon with initial fungicide concentration in red wine (pinot noir)

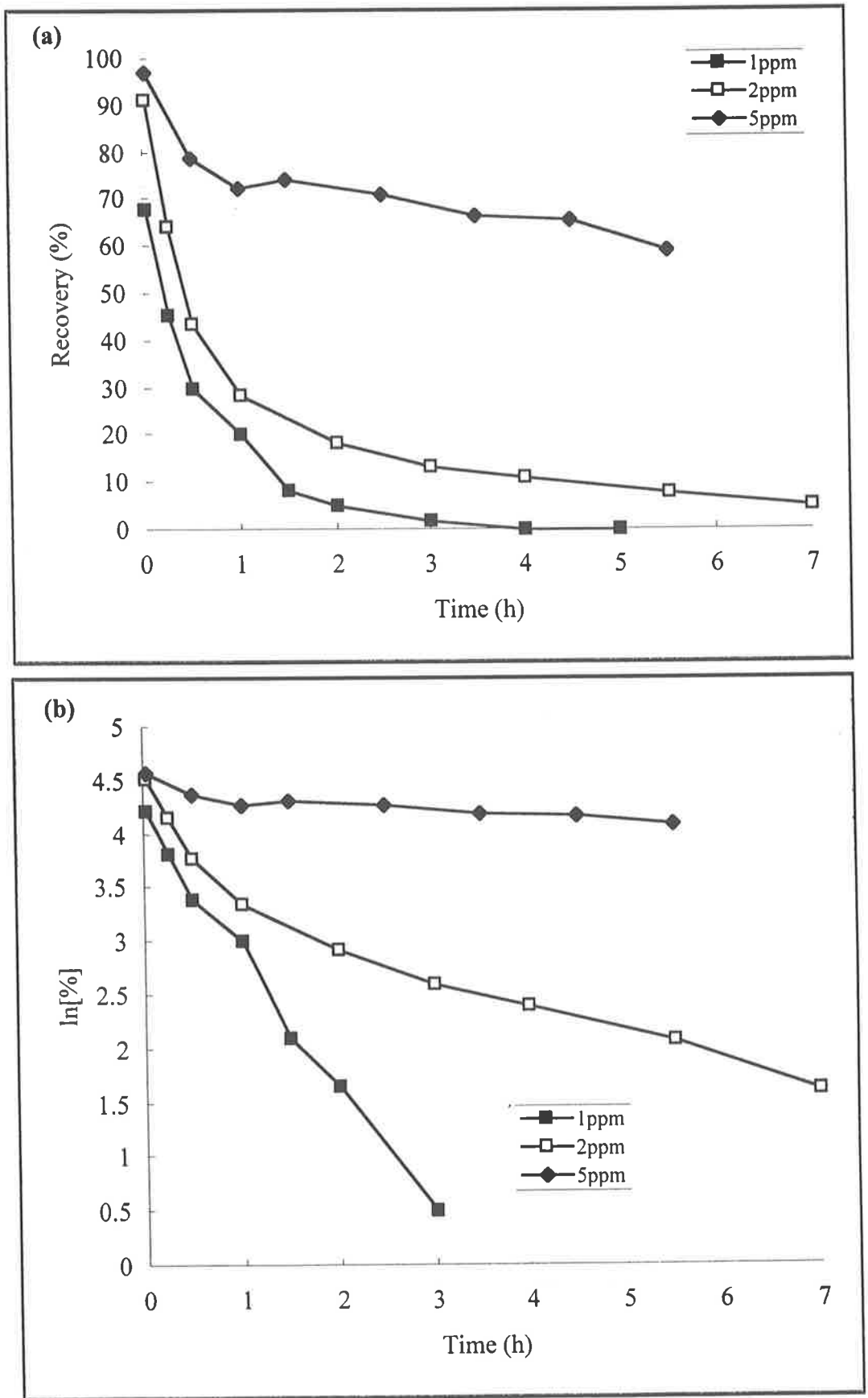


Figure 5.11 Variation in the rate of disappearance of dithianon with initial fungicide

Samples of wine made from red wine (pinot noir) and white wine (semillon) were also treated with polyvinylpolypyrrolidone (PVPP), an agent used to clarify wine. Fining agents such as PVPP have been shown to reduce phenolic levels and alter the colour and / or sensory characteristics of wine. PVPP typically binds with, and removes low molecular weight phenolics, particularly ellagic acid (Sims et al., 1995). If a phenolic compound was responsible for the degradation of dithianon in wine, treating the wine with PVPP, and hence removing the phenolic compound, would reduce the rate of degradation. Samples of the PVPP treated wine were re-extracted immediately after spiking and at regular intervals thereafter. concentration in white wine (semillon).

The results suggest that there was some slowing of the rate of degradation of dithianon in white wine but that in red wine degradation is slowed considerably (Figure 5.12 (a,b)). Dithianon in pinot-noir wine was significantly more resistant to degradation than that in semillon wine. Given that red wines are generally considered to contain significantly higher levels of phenolic compounds that white wines (Oszmianski et al., 1988), these results suggest that dithianon degradation is at least partially caused by phenolic compounds. However, given that degradation still occurs, there is still the possibility that other chemicals and reaction mechanisms may be involved in the degradation process.

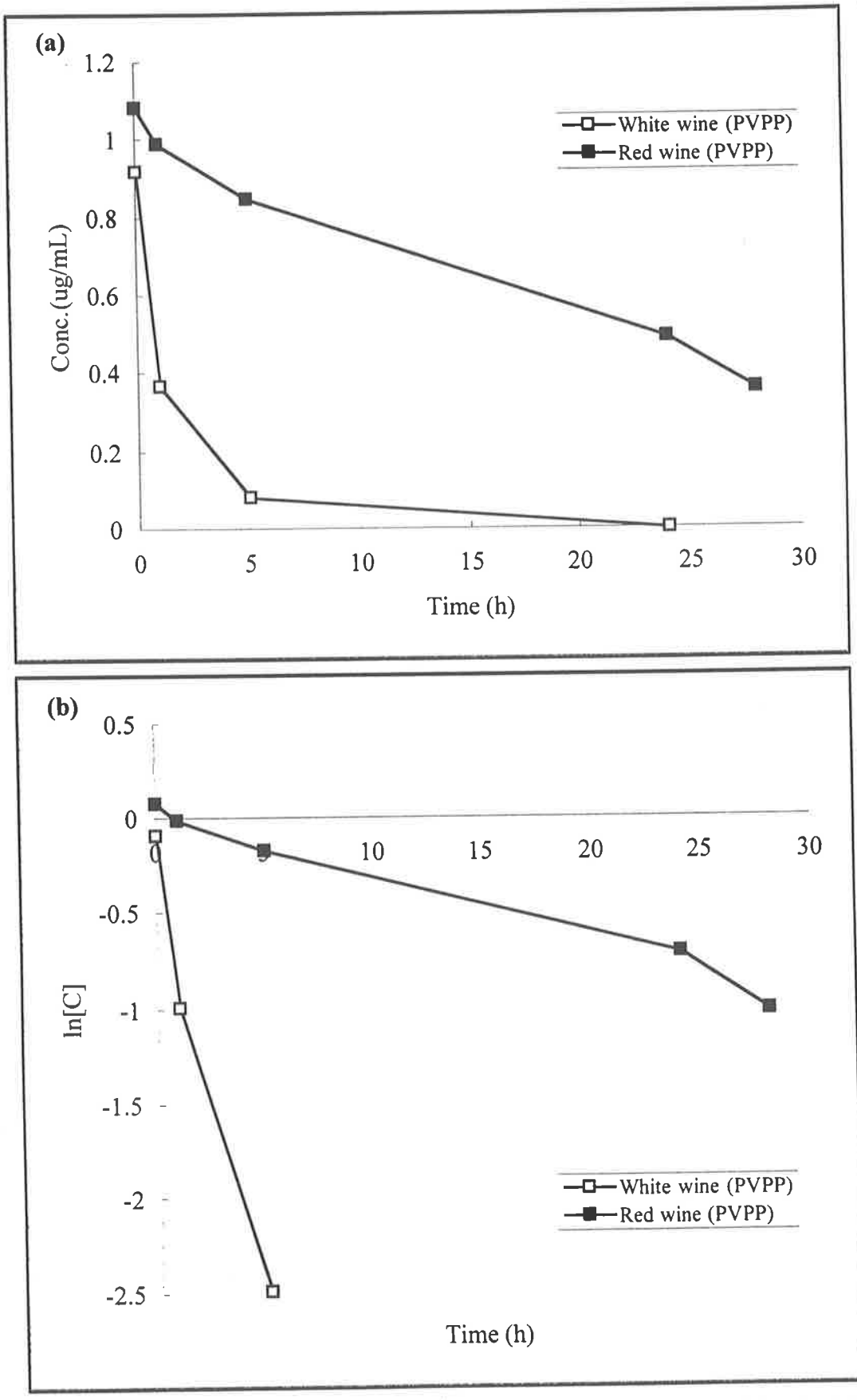


Figure 5.12 Disappearance of dithianon in PVPP treated red (pinot noir) and white wines (semillon)

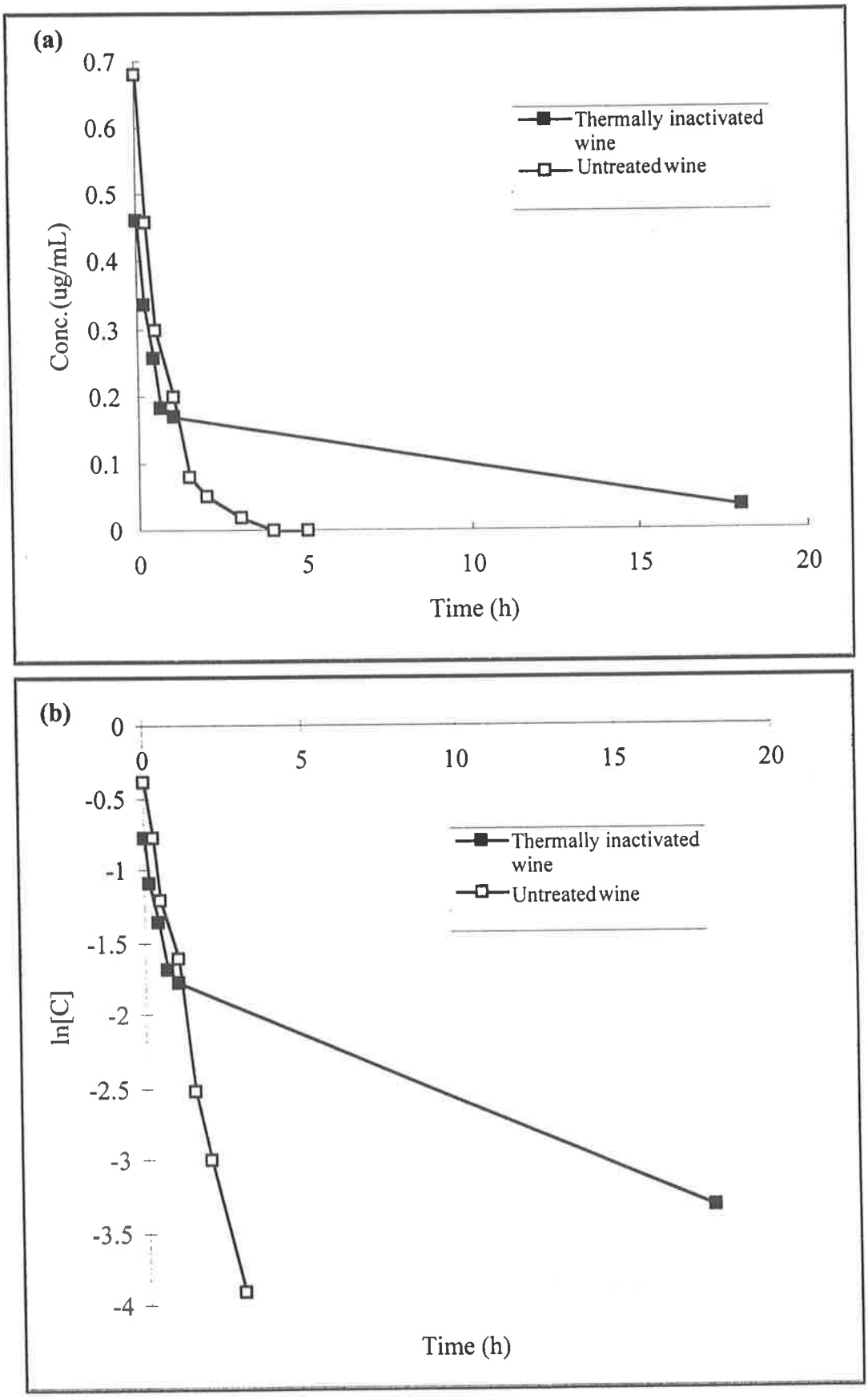


Figure 5.13 Persistence of dithianon in heat-treated wine

Samples of wine made from red wine (pinot noir) and white wine (semillon) were also sterilised. If a micro-organism or bio-active enzyme were responsible for the degradation of dithianon in wine, heat-treating the wine would either kill the micro-organism or denature the enzyme and therefore reduce the rate of degradation. Samples of the heat-treated wine were re-extracted immediately after spiking and at regular intervals thereafter. The difference between the sterilised and unsterilised wines is within experimental error (Figure 5.13(a,b)). Dithianon in pinot-noir wine degrades at a similar rate to that in semillon wine i.e. $t_{1/2}$ (red wine) 4.6 h., cf. $t_{1/2}$ (white wine) 2.8 h. These results, therefore, leave open the possibility of dithianon degradation by either micro-organisms or bio-active enzymes present in the wine.

5.4.3 Conclusion

Dithianon is totally degraded within twenty-four hours of exposure to both red and white grape juice. The half-life in red grape juice was 4.5 h., that in white grape juice 2.8 h. Dithianon is totally degraded within five hours of exposure to red and white wines. The half-life was concentration dependent e.g. dithianon half-life in pinot noir wine is 0.5 h. at an initial concentration of 1 µg/mL, but 13 h. at an initial concentration of 5 µg/mL (Table 5.3 and 5.4).

Vinclozolin is more stable in both red and white grape juice and wine, with a half-life of about 800 hours in both. Essentially, these results show that there should be little threat to human health should either dithianon or vinclozolin residues be found on

grapes destined for vinification since in both cases any chemical residue partitioning into the aqueous phase (the wine) will degrade well before the wine reaches the consumer (Figures 5.14 , 5.15 and 5.16).

Table 5.3 Summary of dithianon kinetic data in white wines and grape juices (semillon)

conc. (µg/mL)	k (h^{-1})	$t_{1/2}$ (h)	correlation coefficient
1	1.191	0.582	0.993
2	0.376	1.845	0.954
5	0.0251	27.582	0.958
1 (PVPP)	0.450	1.540	0.981
1 (juice)	0.248	2.799	0.996

Table 5.4 Summary of dithianon kinetic data in red wines and grape juices (pinot noir)

conc. (µg/mL)	k (h^{-1})	$t_{1/2}$ (h)	correlation coefficient
1	2.393	0.290	0.941
2	0.212	3.272	0.963
5	0.0532	13.030	0.998
1(PVPP)	0.0344	20.165	0.996
1(juice)	0.152	4.554	0.998

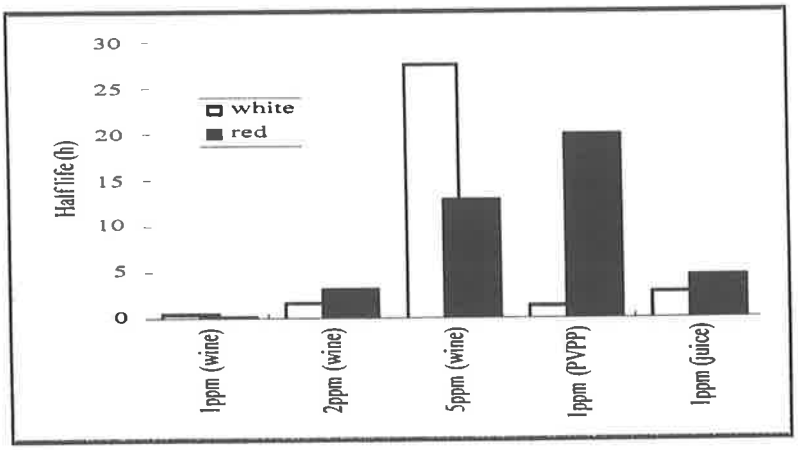


Figure 5.14 Half-life of dithianon in wine and grape juice

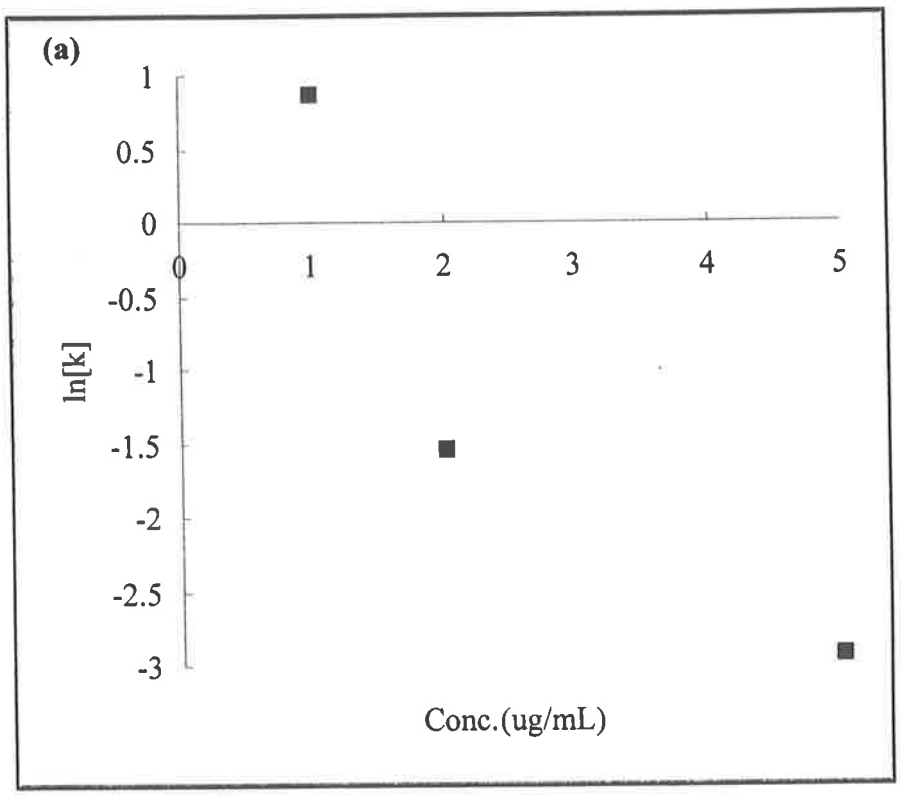


Figure 5.15 Profile of log rate constant vs concentration of dithianon in red wine

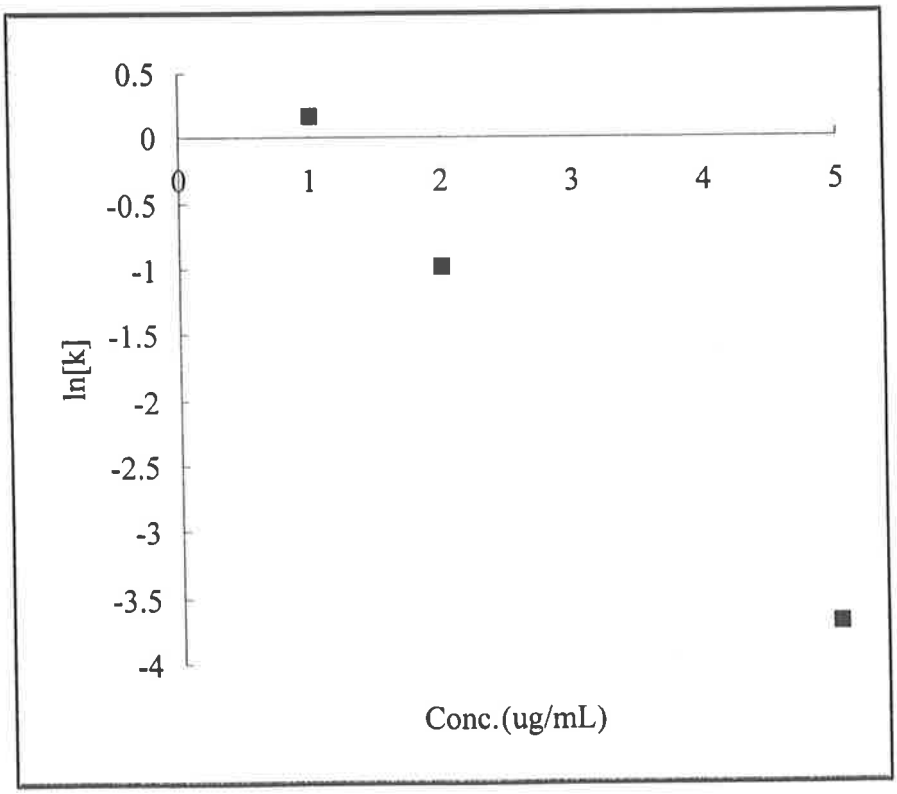


Figure 5.16 Profile of log rate constant vs concentration of dithianon in white wine

5.5 Photodegradation

5.5.1 Introduction

The aim of this aspect of these studies was to determine the rates of photolytic degradation of dithianon and vinclozolin in aqueous solution, and, if possible, identify their photolytic breakdown products. However, equipment limitations and limited access to the required external facilities, such as GC/MS and LC/MS, severely restricted the experimental design, essentially limiting experimentation to the primary aim of assessing photodegradation rates.

Pesticides may reach natural waters by a variety of means, e.g. spray drift, surface run-off or leaching into groundwater. The experimental apparatus used to mimic aqueous photodegradation was essentially a transparent container containing aqueous solutions of these chemicals. The apparatus was designed to be simple and robust, since not only did the fungicides need to be exposed to the harsh South Australian summer weather for a considerable period of time in order to determine rates of degradation, but also regular, and hence easy access sampling, was required. Since the South Australian climate can be quite extreme during the summer months, simple thermal degradation was also considered to be a distinct possibility, and an indoor, light sealed control was also used. Regular HPLC analysis provided information on solution composition. Finally, the aqueous samples were concentrated by SPE (solid phase extraction). This latter step helped in the investigation of the degradation products using GC/MS facilities available in outside institutions.

Solid phase photodegradation of dithianon and vinclozolin was also studied. Fungicide formulations are generally sprayed onto the surface of grapes and vines. These are known to dry out quickly, and thus most of the time the solar radiation is incident on fungicides in the solid phase on the grape berries or vines, rather than in solution.

The outdoor incident solar irradiation could not be directly monitored using available equipment. Rather, it was chemically monitored using a p-nitroacetophenone (PNAP) / pyridine actinometer solution (Woodburn et al., 1993).

5.5.2 Methods

Preliminary dithianon photodegradation experiments

Dithianon (5 and 10 mg) was dissolved in small amount of acetone (1 mL) and diluted with water such that the final aqueous solution concentrations were 10 µg/mL and 100 µg/mL. The solutions (100 and 500 mL) were placed in ground-glass jointed, stoppered, single-neck, pyrex round bottom flasks (100 and 500 mL). These in turn were placed on the roof of the laboratory at Roseworthy. The flasks were placed on cork rings on top of a magnetic stirrer. The solutions were stirred by way of magnetic fleas placed in the flasks (Figure 5.17). Pyrex in not transparent to solar radiation below 295 nm, so the use of pyrex flasks will have meant that the dithianon solutions were only exposed to solar radiation above 295 nm. Control solutions were kept in the dark in a refrigerator (T = 4 °C). Dithianon concentration was monitored by direct injection of an aliquot of the aqueous solution (100 µL) into the HPLC. After two months the solutions were concentrated by extracting with C18 cartridges

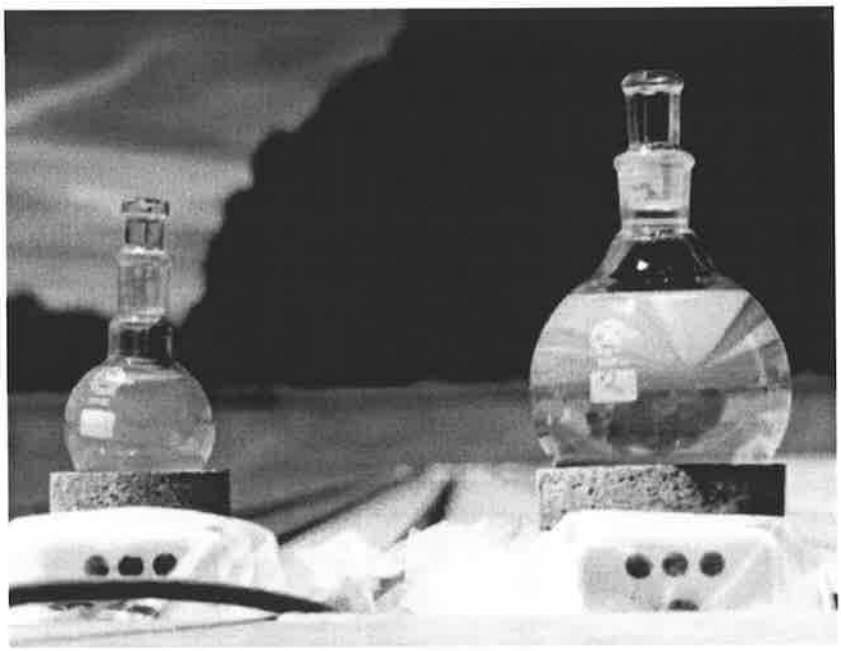


Figure 5.17 Preliminary dithianon photodegradation experiments

(Aug - Oct 1994 and Jan - Mar 1995). The composition of the final eluant was determined by HPLC-PDA, GC/MS, MS, and LC/MS analysis.

Aqueous phase photodegradation of dithianon and vinclozolin

Small volumes of dithianon and vinclozolin standard solutions were injected into small volumes of water (20 mL) in a ground-glass jointed, stoppered test tube such that the final aqueous fungicide concentration was 2 μg/mL. The test tubes were placed on the roof of the laboratory for between 20 - 50 days (Dec 1995 - Jan 1996 and Feb - Apr 1996). Fungicide concentrations were monitored by direct injection of an aliquot of the aqueous solution (100 μL) into the HPLC. After two months the solutions were concentrated by extracting with C18 cartridges. The composition of the final eluant was investigated by HPLC-PDA, GC/MS, MS, and LC/MS analysis.

Thermal degradation of aqueous dithianon and vinclozolin

In order to differentiate between photodegradation and thermal degradation fungicide solutions were heated in the absence of light. Small volumes of dithianon standard solution (12 μg/mL; 200 mL) were heated in an oven (96 °C) for 17 hours. For vinclozolin, small volumes of vinclozolin standard solutions (2 μg/mL; 20 mL) were placed in ground-glass jointed, stoppered test tubes. The test tubes were wrapped in aluminium foil to exclude light, and then placed on the roof of the laboratory for between 20 - 50 days (Dec 1995 - Jan 1996 and Feb - Apr 1996). In both cases, fungicide concentrations were monitored by direct injection of an aliquot of the aqueous solution (100 μL) into the HPLC.

Solid phase photodegradation of dithianon and vinclozolin

A small volume (100 µL of 200 µg/mL standard solution) of fungicide analytical standard solution was placed into several small vials. The organic solvent was evaporated to leave a 20 µg fungicide residue. The vials were then placed on the roof of the laboratory at Roseworthy for between 20 - 50 days (Dec 1995 - Jan 1996 and Feb - Apr 1996). Fungicide levels were monitored by grab sampling a vial at random, dissolving the contents in acetonitrile, and then injecting an aliquot of the solution (20 µL) into the HPLC.

Actinometer system

Essentially the system consisted of an 2 mM aqueous pyridine solution with a 20 µM PNAP concentration. Small volumes of the actinometer solution (20 mL) were placed in ground-glass jointed, stoppered test tubes. The test tubes were placed on the roof of the laboratory for between 20 - 50 days (Dec 1995 - Jan 1996 and Feb - Apr 1996). Fungicide and PNAP concentrations were monitored by direct injection of aliquots of the aqueous solutions.

5.5.3 Results and Discussion

Preliminary dithianon aqueous photodegradation experiments

A relatively low dithianon concentration was used in the preliminary experiment, essentially to determine if any degradation of dithianon actually occurred in dithianon solutions exposed to the natural sunlight. The first trial was run during October 1994

when a 10 μg/mL dithianon solution was exposed to natural sunlight on the roof of the laboratory for 10 days. The results are shown in Figure 5.18 and suggested that dithianon was unstable towards natural sunlight, with the concentration being reduced by approximately 50% during the test period. In addition, it was noticeable that during the lifetime of the experiment an unknown compound, U1, with a retention time 20% faster than that of dithianon was produced (Figure 5.20). However, given that the apparent concentration of U1 did not increase as the dithianon concentration fell, it was assumed that U1 was also unstable (Figure 5.18). The experiment was repeated again between January and March 1995 at a higher concentration and longer time with the aim of both confirming the instability of dithianon towards solar radiation, and also of isolating and determining the identity of U1 (Figure 5.19). In this case a dark control was added. The results are summarised in Figure 5.19. The fungicide concentration in the dithianon dark-control solution was stable over the 50 day test period. However, the results again suggested that dithianon was unstable towards natural sunlight, although this time only 25% of the initial concentration degraded during the test period. In this case the photodegradation half-life was estimated to be 100 days, an order of magnitude greater than that observed during the first trial. The reason for this discrepancy is not fully understood, although the discrepancy may be due to the limited light intensity entering the sample. Again, the appearance of U1 was observed. However, again the apparent concentration of U1 did not increase as the dithianon concentration fell, once more suggesting that U1 was also unstable. At the end of the experiment the remaining aqueous solution was concentrated by SPE. The SPE extract was investigated by GC/MS at the Australian Wine Research Institute, and by LC/MS courtesy of JEOL Ltd. in Japan.

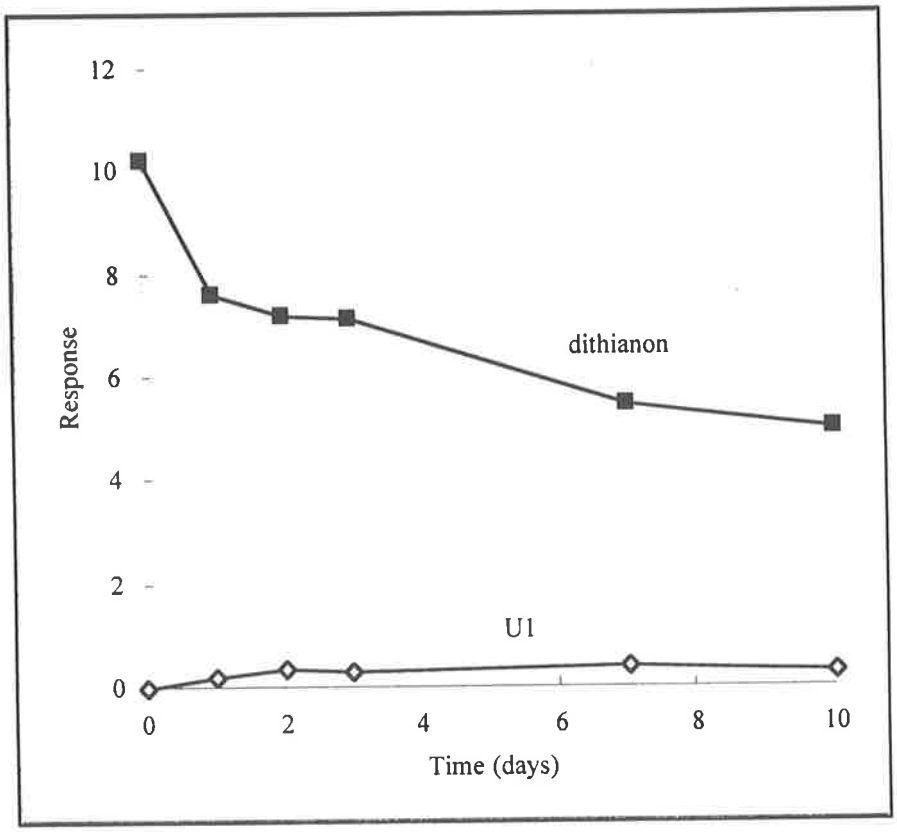


Figure 5.18 Photodegradation of dithianon (10 µg/mL) in water and appearance of putative degradation product U1

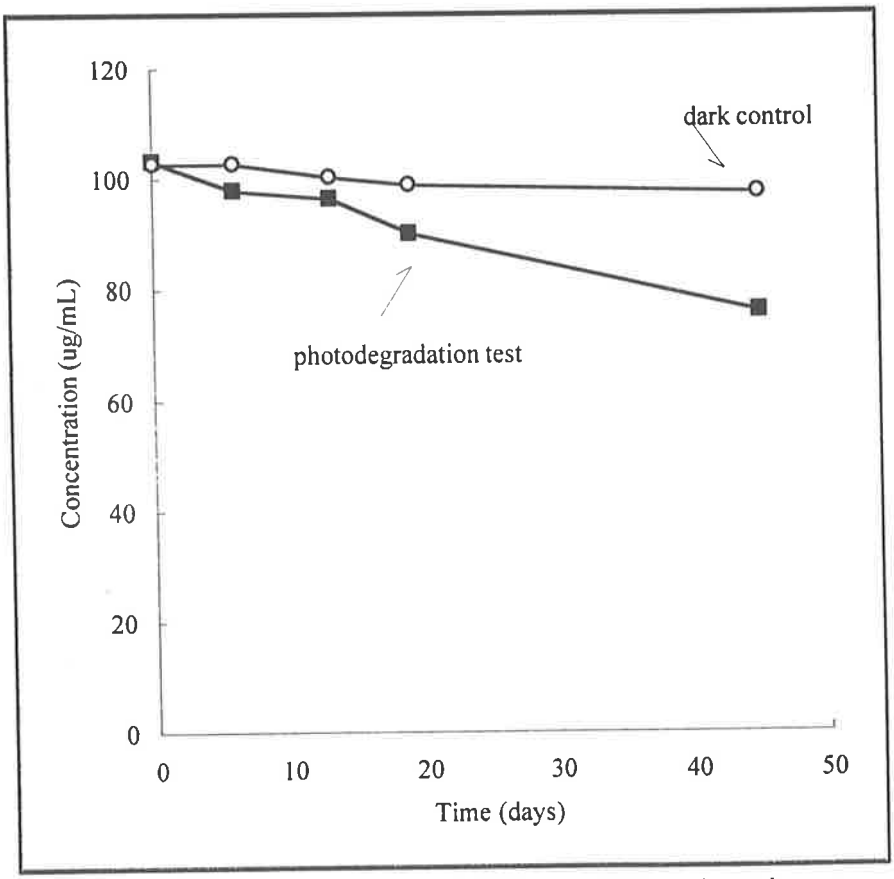


Figure 5.19 Photodegradation of dithianon (100 µg/mL) in water

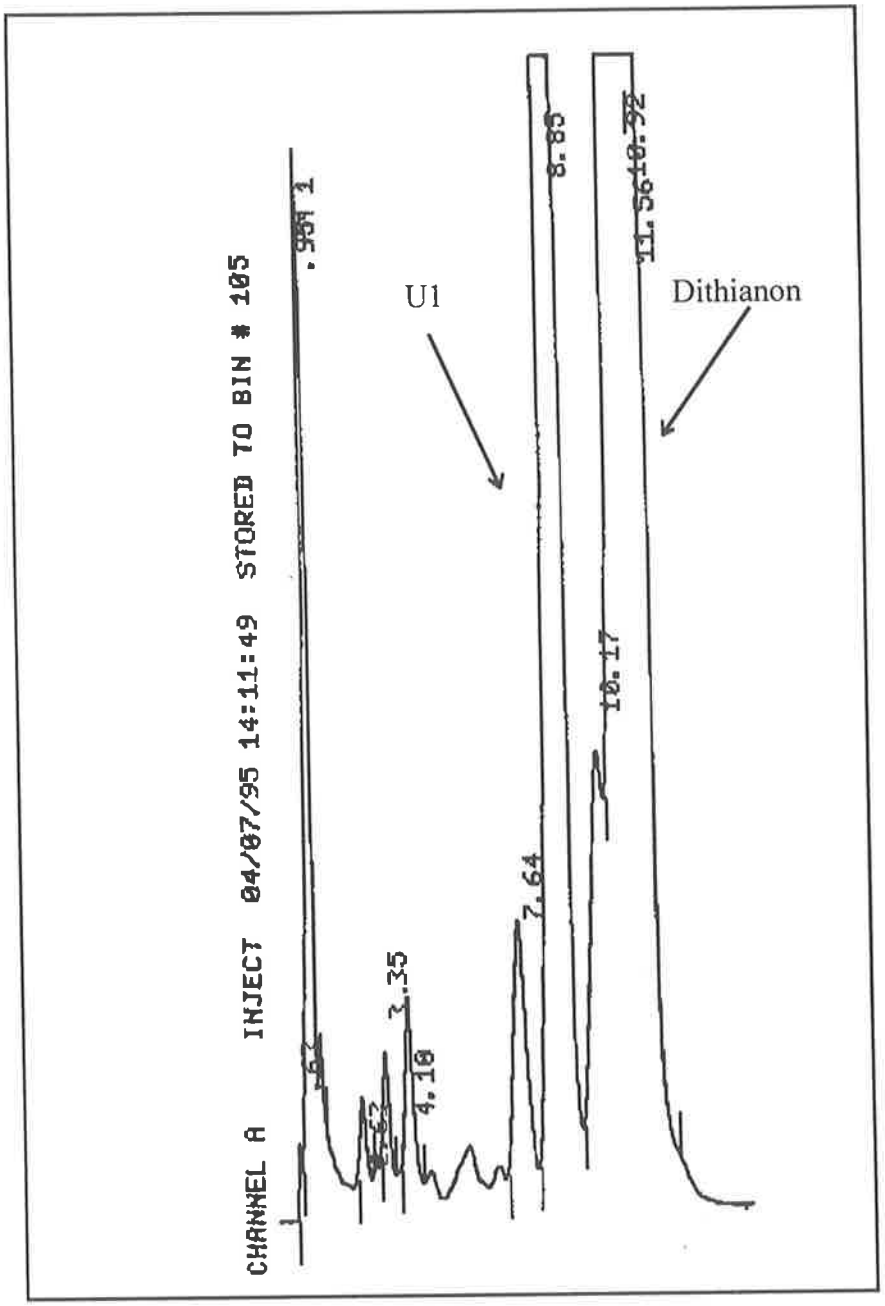


Figure 5.20 Typical chromatogram of photoirradiated aqueous dithianon solution (concentrated by C18 cartridge)

HPLC condition - 50 : 50 CH_3CN : H_2O
254nm, injection volume 20µL

GC/MS investigations showed two significant peaks : the suspected sulphur extruded dithianon derivative, and a second signal at longer retention time suspected of being U1. The latter peak can not be positively identified as U1 because of the disparity in operating conditions between the routine HPLC system, and the GC/MS. The former uses a reverse phase column in which, in general, the more polar the compound, the more quickly it is eluted from the column - hence the assumption that U1 is more polar than dithianon. The GC/MS system used a 30 m DB-5 column on which, in general, the *less polar* the compound, the more quickly the elution from the column. The appearance of a compound with longer retention time than the suspected sulphur extruded dithianon derivative strongly suggests that this is U1. However, in this case we are not monitoring dithianon but the suspected sulphur extruded dithianon derivative, and the relative difference in HPLC retention times between U1 and the suspected sulphur extruded dithianon derivative are not known. Be that as it may, the results do suggest that the second peak observed in the GC/MS may be U1. Analysis of the mass spectrum of U1 (Figure 5.22) indicates that it has the chemical structure shown in Figure 5.21, tentatively identified as 2,3-dihydro-1,4-dithia-anthraquinone.

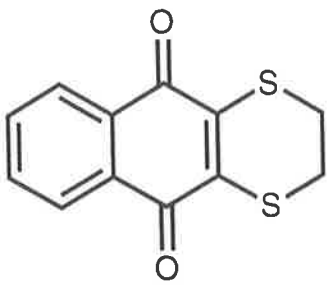


Figure 5.21 Proposed chemical structure of suspected dithianon photodegradation product U1

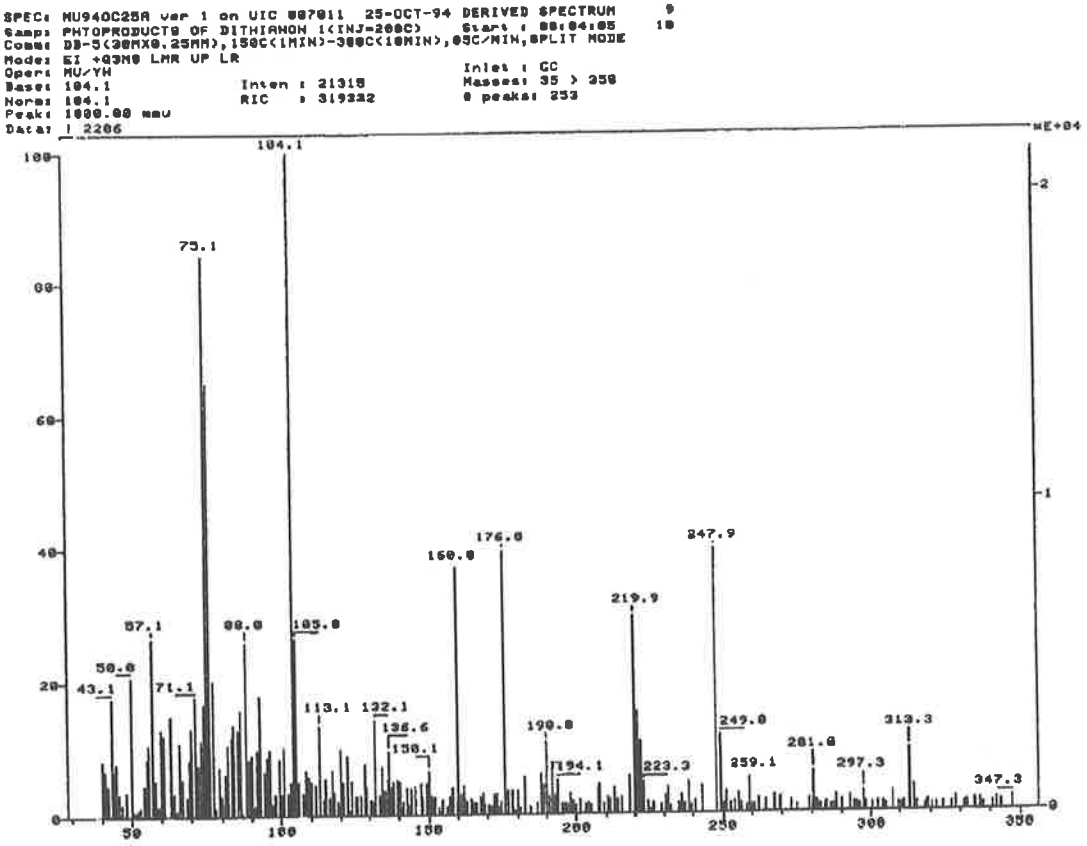


Figure 5.22 Mass spectrum of suspected dithianon photodegradation product U1 obtained by GC/MS

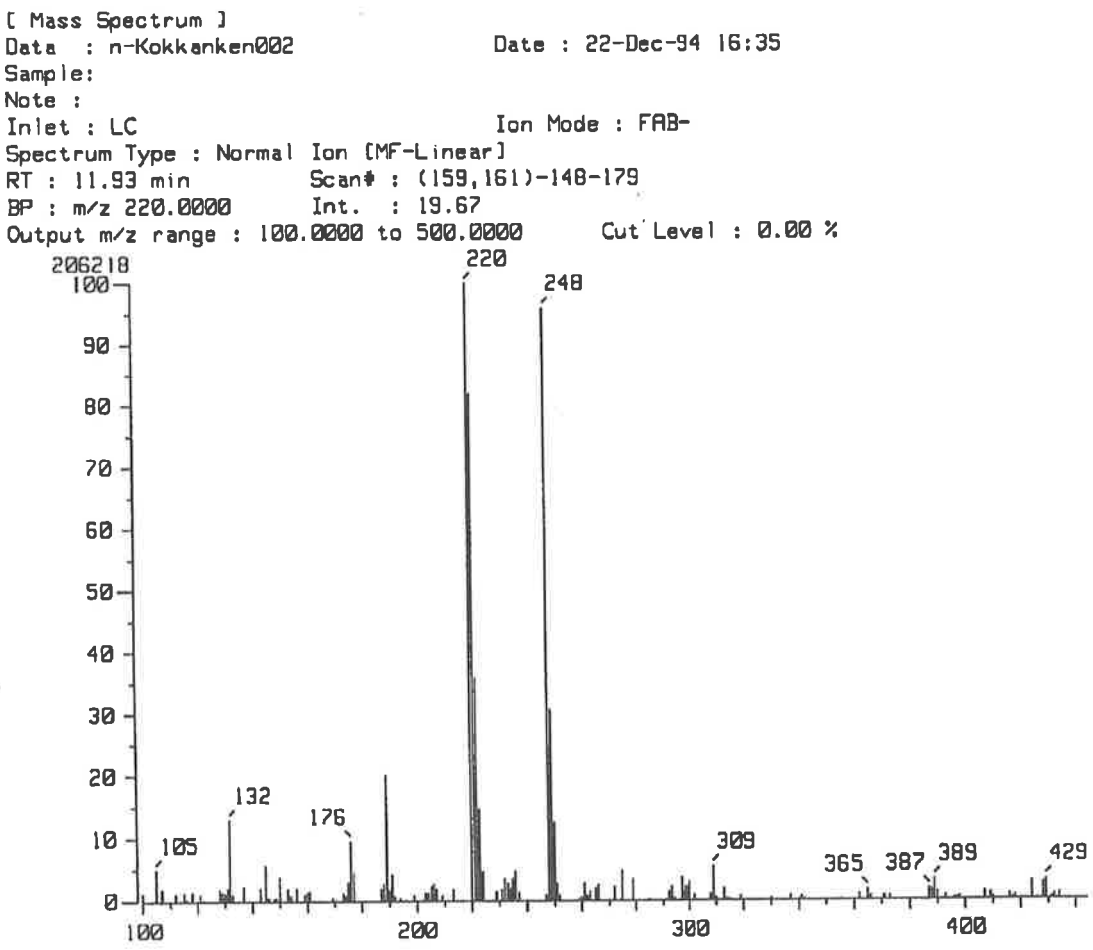


Figure 5.23 Mass spectrum of suspected dithianon photodegradation product U1 obtained by LC/MS

LC/MS investigations again showed two significant peaks. In this case dithianon and a second signal suspected of being U1. Again, the latter peak can not be positively identified as U1 because of the disparity in operating conditions between the routine HPLC system, and the LC/MS system. However, advice from the mass spectrometer operators at JEOL Ltd. in Japan was that the signals observed were strongly suggestive of being U1. Analysis of the mass spectrum of U1 (Figure 5.23) again indicates that it has the chemical structure of the compound suspected of being U1 that was identified by GC/MS (Figure 5.21).

Thermal degradation of aqueous dithianon and vinclozolin

In order to differentiate between photodegradation and thermal degradation fungicide solutions were heated in the absence of light. Initially, it was thought that degradation would be slow, so dithianon solutions were heated in an oven to try to speed up thermal degradation. The HPLC chromatograms obtained after 17 h. at 96°C showed a number of new peaks compared to the standard. However, the pattern of the peaks was significantly different from those HPLC chromatograms observed during the photodegradaton. It appeared that most of the thermal degradation products were very polar, possibly hydrolysed compounds - much more polar than their photodegraded counterparts. From these investigations it was concluded that photodegradation, not thermal degradation was really occurring in these experiments.

For vinclozolin the situation is more ambiguous. The thermal hydrolysis products obtained by Szeto et al. (1989a) are not dissimilar to those observed by Schwack et

al. (1995) after photoirradiation of vinclozolin in organic solvents. The HPLC chromatograms obtained during these thermal degradation experiments were similar to those obtained from photodegradation experiments, so no degradation pathway could be ruled out merely on the grounds of the chromatograms observed.

Actinometer

Sunlight plays an important role in the degradation of some pollutants in the aquatic environment (Wan et al., 1994). The photodegradation of chemicals may occur by both direct and indirect photochemical processes. Direct photolysis involves direct absorption of light by the chemicals followed by further chemical reaction. Indirect photolysis may be initiated via light absorption by other substances in the aquatic environment. The rate of direct photolysis of pesticides in aquatic environments depends on two factors related to the physico-chemical characteristics of the pesticides themselves : the rate of light absorption and the quantum yield for direct photolysis. This latter is a very useful parameter for assessing the relative importance of direct photolysis under a given set of environmental conditions where other competing processes, such as hydrolysis or bio-degradation, may occur. The application of kinetic data derived from photolysis under one set of sunlight conditions to a wide range of sunlight conditions requires the use of standard actinometers to monitor the variable solar irradiance experienced by both the actinometer and pesticide during the test period (Dulin and Mill, 1982).

The p-nitroacetophenone (PNAP) / pyridine actinometer system used in these studies has been quite extensively studied (Dulin and Mill, 1982; Woodburn et al.,

1992; Wan et al., 1994). It is a variable quantum yield actinometer useful for relatively short term sunlight experiments lasting several months. Estimation of the rate constant for photolysis in sunlight (κ_{pE}) for a specific pesticide such as dithianon or vinclozolin in aqueous solution requires data on the absorption spectrum of the chemical, the quantum yield, or efficiency, of the process (Φ) and the intensity of the sunlight as a function of wavelength, latitude, season, and possibly even time of day. Estimates of κ_{pE} and Φ can be made by direct exposure of aqueous pesticide solutions to sunlight provided solar irradiance is measured in some way. One way to account for fluctuations in solar irradiance induced by weather changes, the change from day to night and seasonal changes is to simultaneously expose another chemical, the actinometer, that has a known value of Φ that does not vary with wavelength (Dulin and Mill, 1982).

The upper atmosphere cuts off almost all solar irradiance below about 290 nm. Chemicals in the terrestrial environment can therefore only absorb light (photons) at longer wavelengths. In sunlight the rate constant for photolysis (κ_{pE}) is given by

$$d[C]/ dt = 2.3r\Phi \sum L_\lambda E_\lambda [C] = \kappa_{pE}[C] \qquad (5.1)$$

where

- E_λ is the molar extinction coefficient at wavelength λ
- L_λ is the solar irradiance at wavelength λ

- Φ is the wavelength independent quantum yield
- r is a reaction parameter characteristic of the system

Integration of (5.1) gives

$$\ln [C_o] / [C_t] = \kappa_{pE}\, t \qquad (5.2)$$

where $[C_o]$ and $[C_t]$ refer to concentrations at time zero and time t.

Equation (5.2) may be used to estimate κ_{pE} if the losses of the chemical (C) and actinometer (A) are followed while both are exposed to sunlight. Regression of ln $[A_o]$ / $[A_t]$ vs. ln $[C_o]$ / $[C_t]$ gives the slope S, from which the average or environmental quantum yield for photolysis of the chemical (Φ_{cE}) can be estimated using the relationship

$$\Phi_{CE} = \Phi_A \left[\frac{1\ \Sigma L_\lambda E_\lambda{}^A}{S\ \Sigma L_\lambda E_\lambda{}^C} \right] \qquad (5.3)$$

where superscripts A and C refer to actinometer and chemical, respectively. Once Φ_{CE} is known, it may be used to estimate at other latitudes and seasons in clear weather (Dulin and Mill, 1982). However, equation (5.3) assumes that the ratio of light absorbed by the chemical and actinometer are constant over changes in sky conditions, latitudes and seasons. This assumption is held to be good above 350 nm, but increasingly error prone below this wavelength. Another problem arising from the use of equation (5.3) is the need to use values of $L\lambda$ that, if available, are usually

listed for a limited number of dates in any given season. Despite this, chemical actinometry offers several advantages over instrumental methods for measuring sunlight intensity, the most important of which is that proper use of an actinometer can integrate solar flux during the time and weather conditions experienced by the test chemical (Dulin and Mill, 1982).

Wan et al. (1994) reported that the degradation quantum yield of a pesticide may be calculated if the pesticide (P) and actinometer (A) are followed while both are exposed to sunlight, and using the equation

$$\Phi_P = (slope)_P / (slope)_A \times E_A \Phi_A E_P \qquad (5.4)$$

where

- $(slope)_P$ is the gradient of the plot of ln concentration of the pesticide vs exposure time
- $(slope)_A$ is the gradient of the plot of ln concentration of the actinometer vs exposure time
- E_A is the molar extinction coefficient of the actinometer
- E_P is the molar extinction coefficient of the pesticide
- Φ_A is the quantum yield for the reaction of the actinometer
- Φ_P is the quantum yield for the reaction of the pesticide

In the experiments described hereafter, efforts were made to sample, and analyse those samples, in a manner such that the temporal change in concentration of the

pesticides and the actinometer were monitored with the aim of calculating the quantum yields of the photodegradation reactions using equation (5.4).

Aqueous phase photodegradation of dithianon and vinclozolin

Test tubes containing 2 μg/mL concentrations of dithianon were placed on the roof of the laboratory and exposed to the summer sun for between 20 - 50 days (Dec 1995 - Jan 1996 and Feb - Apr 1996). During that time, dithianon concentration was monitored by direct injection of an aliquot of the aqueous solution (100 μL) into the HPLC. Figure 5.24 shows the effect of those natural sunlight conditions on the concentration of dithianon in the tubes. Based on this information it is estimated that the half-life of dithianon in aqueous solution when exposed to natural sunlight is of the order of 42 days. A typical chromatogram is shown in Figure 5.20.

Figure 5.25 shows the effect of the same natural sunlight conditions on the actinometer. Based on this experiment it is estimated that the half-life of PNAP in the actinometer system when exposed to natural sunlight is of the order of 70 days. In their paper describing the development of the PNAP/pyr actinometer system, they predicted that the half-life of PNAP in sunlight as a function of [pyr] at 40° N would be approximately 20 days. Given that Roseworthy lies at a latitude of approximately 35° S, a half-life of the order of 70 days is not inconsistent with the predicted half-life based on the results of Dulin and Mill (1982).

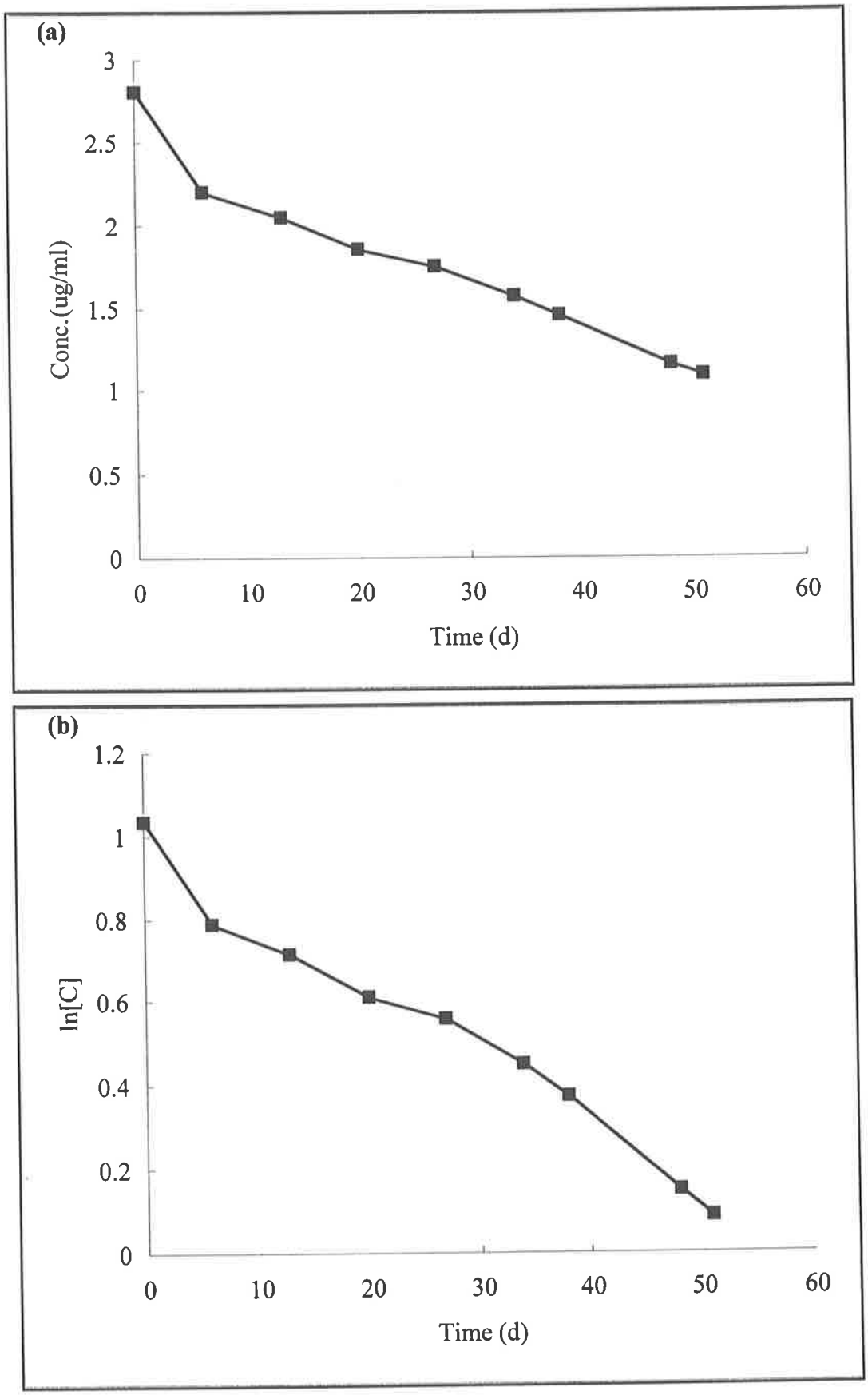
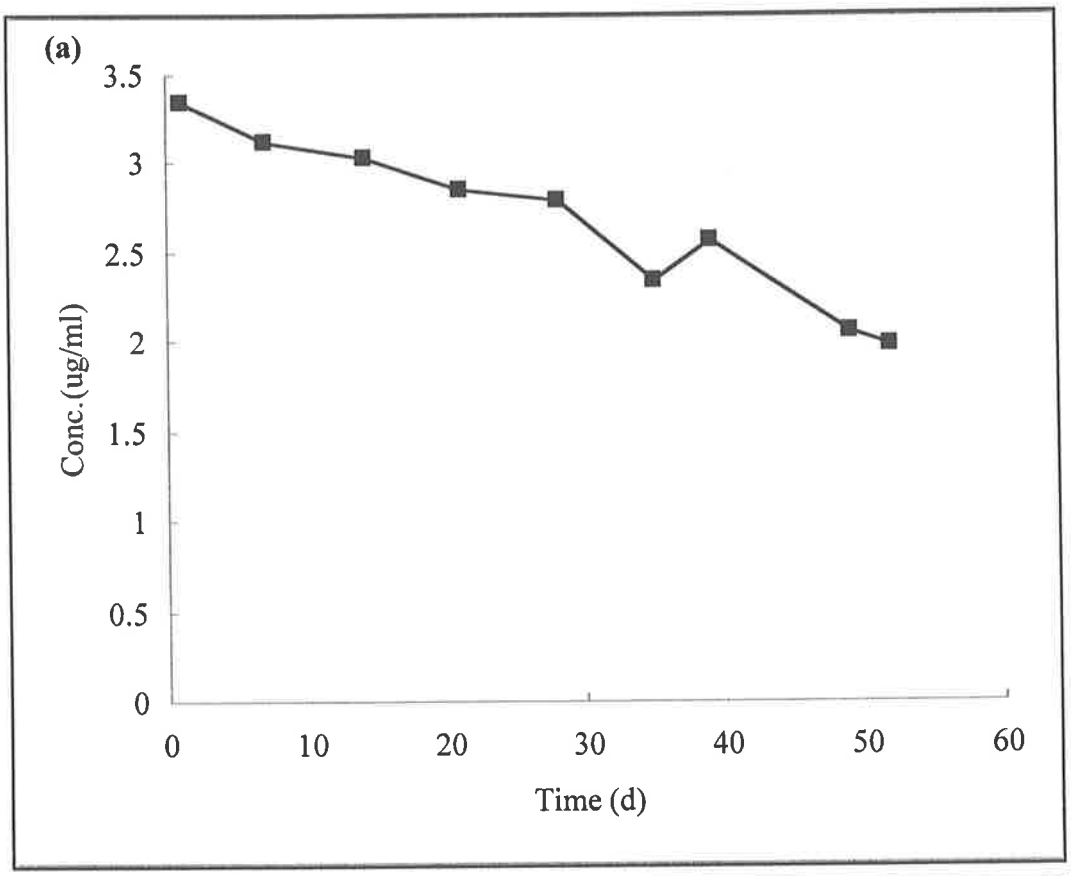


Figure 5.24 Photodegradation of dithianon in water



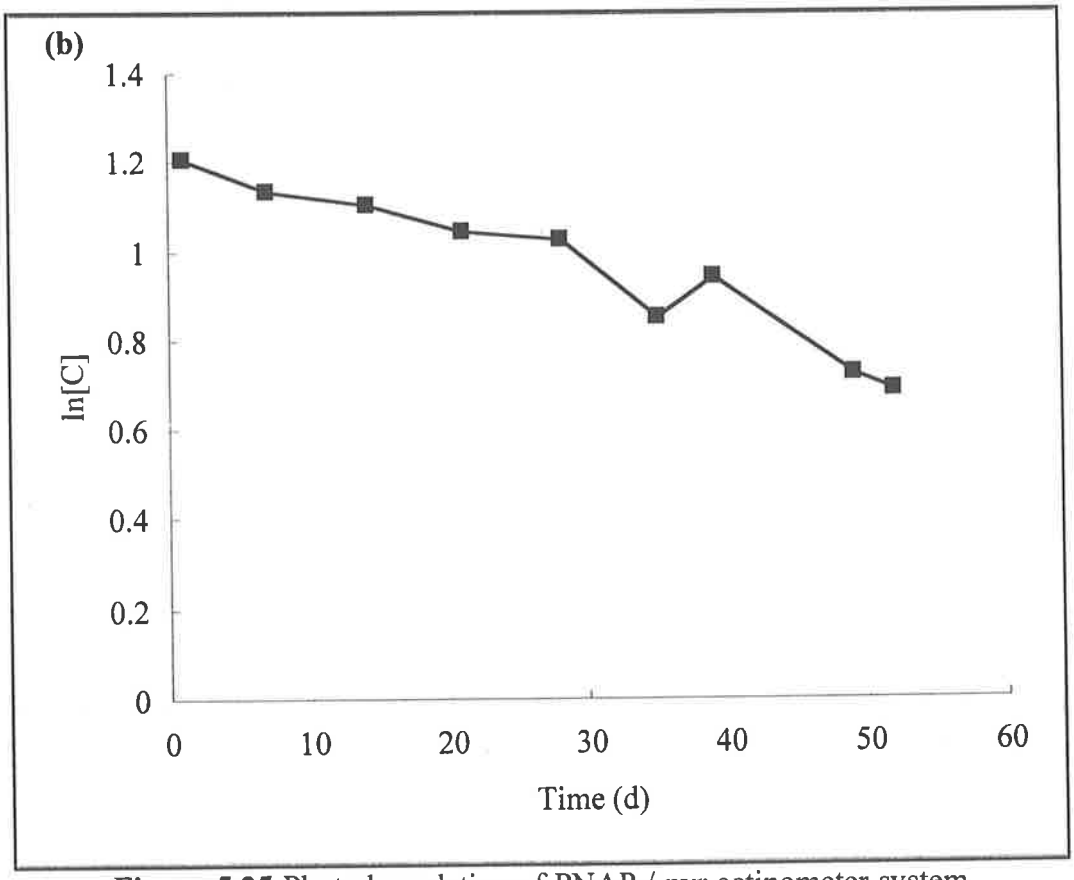


Figure 5.25 Photodegradation of PNAP / pyr actinometer system

The rate of photodegradation of dithianon was slower than that of thermal degradation discussed earlier i.e. photodegradation half-life of 70 days in deionised water, thermal degradation half-life 2 - 20 days pH 7 - 4 in buffered aqueous solution. There remains some doubt as to whether photodegradation is being observed, or aqueous degradation. One clue lies in the HPLC traces. A typical chromatogram for dithianon solutions is shown in Figure 5.20. These are different from those obtained after heating dithianon overnight in an oven. If one assumes that this overnight heating has merely increased the rate of production of the aqueous degradation products there is, therefore, some suggestion that photodegradation was being observed. If this is so, then the quantum yield for dithianon can be calculated from equation 5.4. A regression slope of k = 0.0166 day^{-1} (r = 0.987) was obtained from ln[dithianon] vs time, a regression slope of k = 0.00978 day^{-1} (r = 0.966) was obtained from ln[PNAP] vs time. These two values were used in equation 5.4, with values of molar extinction coefficients at 265 nm calculated from the UV spectrum of each chemical. Φ_A was taken as being the reported value of 3.4 x 10^{-5} for PNAP (Dulin and Mill, 1982). Under these conditions, the quantum yield for dithianon was calculated to be 5457. This value is not inconsistent with the results of Wan et al. (1994), who reported quantum yields for organophosphate esters and their phenolic counterparts ranging from 100 - 1,100,000 under similar conditions.

Test tubes containing 2 µg/mL concentrations vinclozolin were placed on the roof of the laboratory and exposed to the summer sun for between 20 - 30 days (Dec 1995 - Jan 1996 and Feb - Apr 1996). During that time, vinclozolin concentration was monitored by direct injection of an aliquot of the aqueous solution (100 µL) into the

HPLC. Figure 5.26 shows the effect of those natural sunlight conditions on the concentration of vinclozolin in both light tight and exposed tubes. Based on this information there appears to be no difference in the half-life in aqueous solution when exposed to natural sunlight and that when not exposed to light. Figure 5.26 suggests that both reactions have a half life of about 5 days. One possible explanation is that the presence of aluminium foil, while preventing photodegradation, enhanced thermal degradation by keeping the solution warmer longer in the evenings, prolonging thermal degradation. In addition, this small difference in half-lives may not be statistically significant, although there is not enough data to determine this. A typical chromatogram is shown in Figure 5.27. However, given that the rates of degradation in the light-tight and exposed solutions are so similar, and considering the UV-Vis absorption spectrum would suggest that vinclozolin will not absorb solar radiation (*cf.* the spectrum of dithianon which does suggest dithianon will absorb solar radiation,and might undergo photolysis), it is probable that degradation is by the thermal pathway, not as the result of photoirradiation.

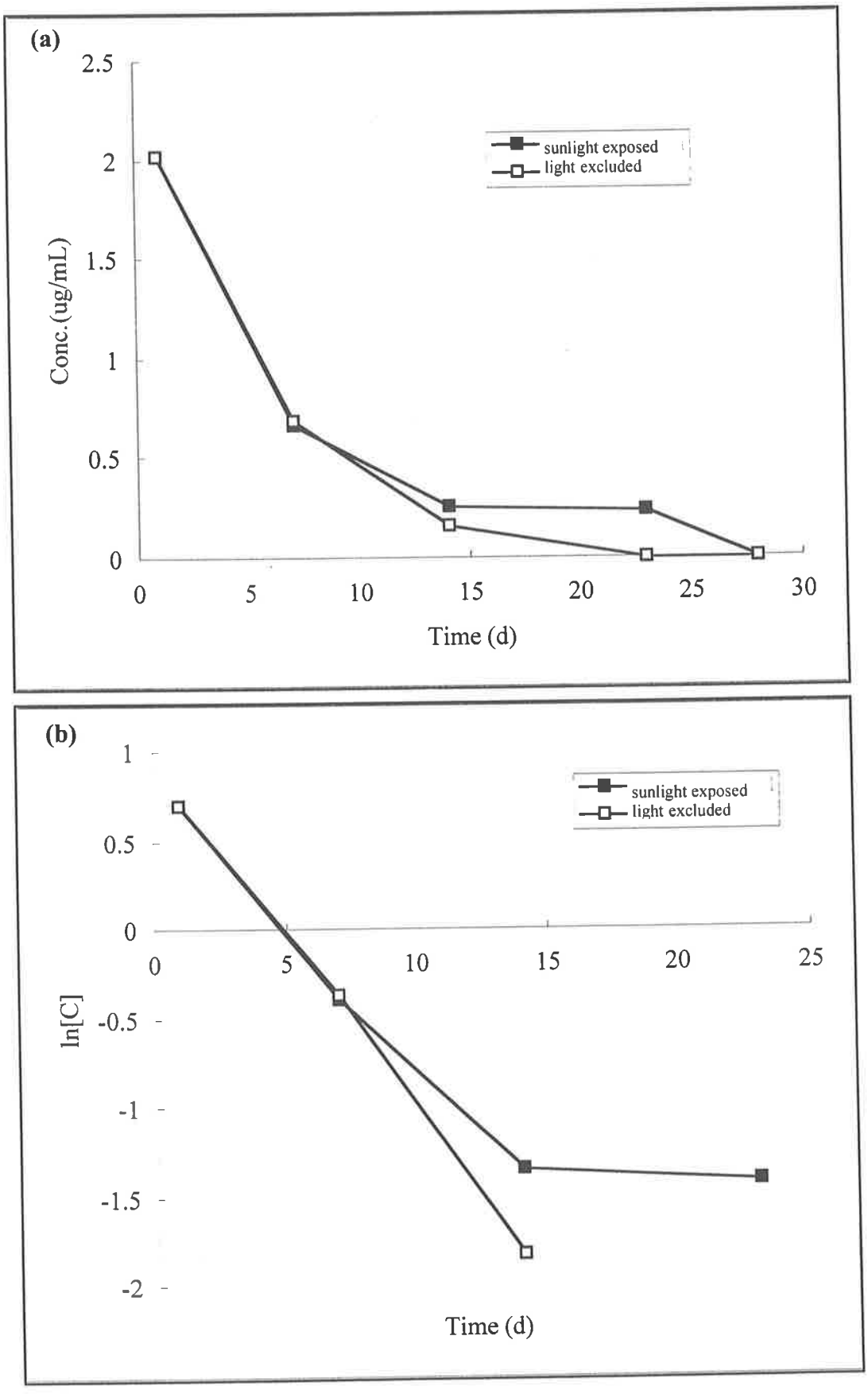


Figure 5.26 Photodegradation of vinclozolin in water

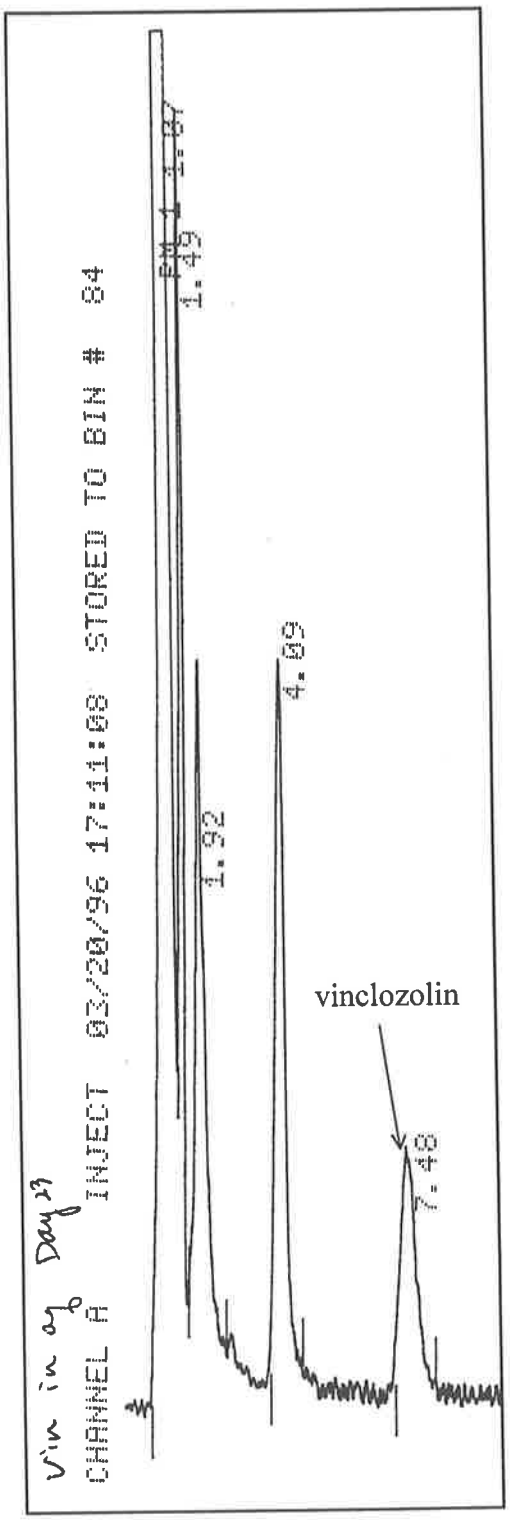


Figure 5.27 Typical chromatogram of photoirradiated aqueous vinclozolin solution

HPLC condition - 50 : 50 CH3CN : H2O
210nm, injection volume 100µL

Solid phase photodegradation of dithianon and vinclozolin

Vials containing small amount of dithianon were also placed on the roof of the laboratory at Roseworthy for between 20 - 50 days. Fungicide levels were monitored by grab sampling a vial at random, dissolving the contents in acetonitrile, and then injecting an aliquot of the solution (20 μL) into the HPLC. Figure 5.28 shows the results obtained from the dithianon solid-phase photoirradiation experiments. In this case, dithianon again is degraded, with a half-life of approximately 68 days. This is very similar to the 70 day aqueous photoirradiation half-life. A typical chromatogram is shown in Figure 5.29.

Vials containing small amount of vinclozolin were also placed on the roof of the laboratory at Roseworthy for between 20 - 50 days. However, these vials suffered a rather mysterious accident part-way through the experiment - they were part-filled with water from an unknown source - and so this data was not obtained.

5.5.4 Conclusion

Dithianon is unstable towards natural sunlight, with an aqueous photodegradative half-life of approximately 70 days. Thermal hydrolysis does not seem to be the preferred pathway even when solutions are heated by the summer sun since the HPLC traces observed after photoirradiation are somewhat different from those obtained after heating dithianon overnight in an oven. During aqueous phase photodegradation an unknown compound, U1, tentatively identified as 2,3-dihydro-1,4-dithia-anthraquinone, is produced. However, this compound may also be either

thermally or photolytically unstable since its apparent concentration remains at a constant level throughout the photolysis experiments.

For vinclozolin the situation is more ambiguous. In this case if photodegradation was being observed, the photolytic half-life is approximately 7 days. However, the thermal hydrolysis products and rates of hydrolysis are not dissimilar to those observed after photoirradiation of vinclozolin in aqueous solution. It is, therefore, probable that degradation is by the thermal pathway, not the result of photoirradiation.

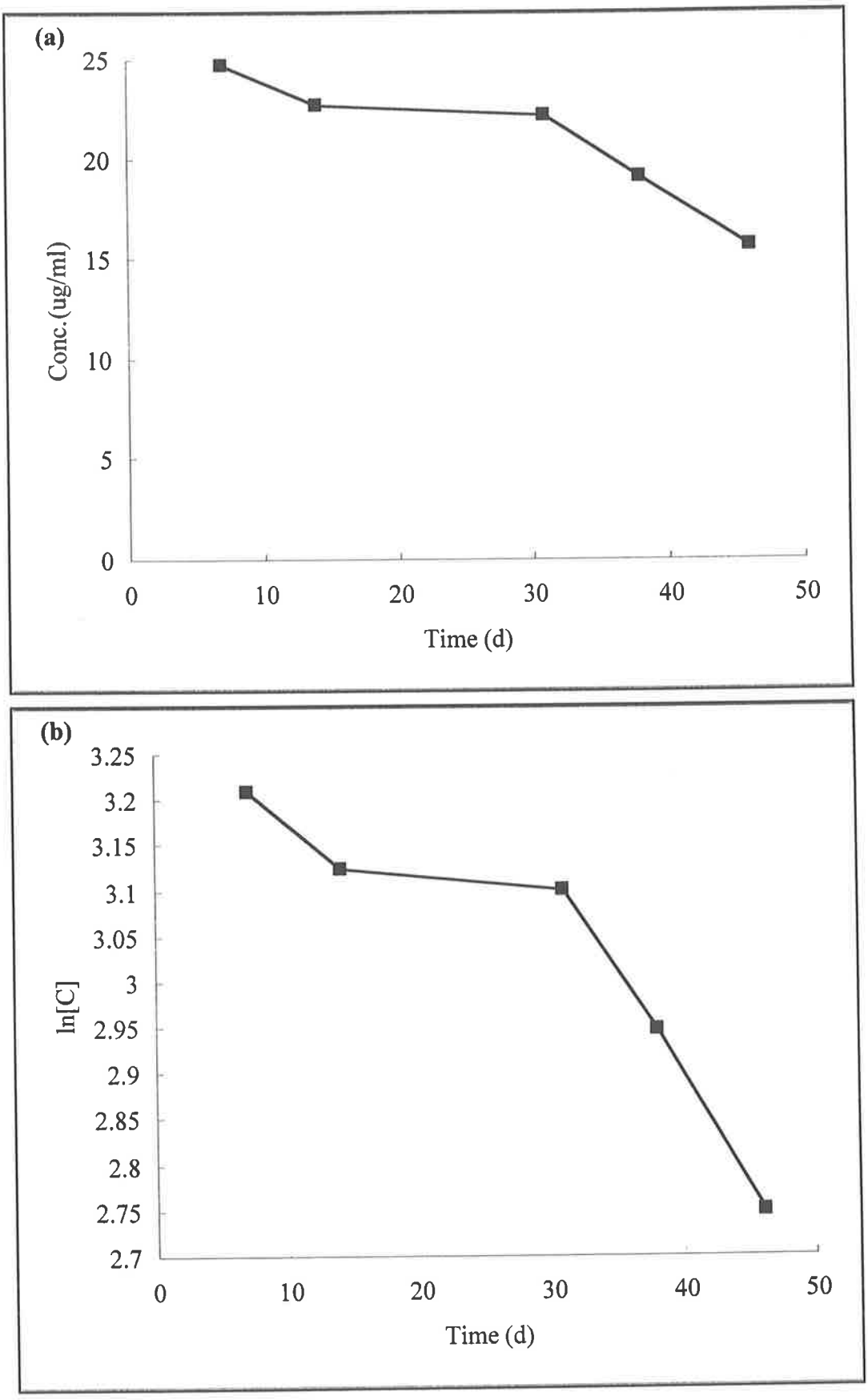


Figure 5.28 Photodegradation of dithianon (solid phase)

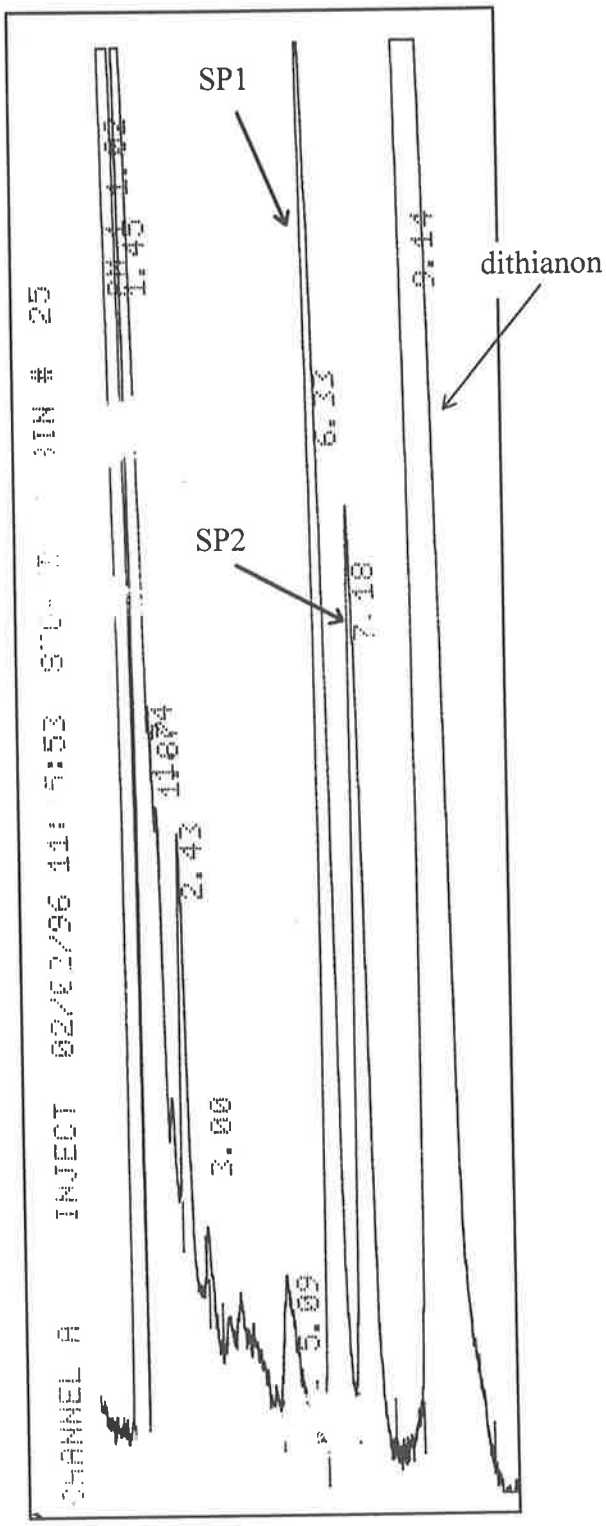


Figure 5.29 Typical chromatogram of photoirradiated dithianon (solid phase)

HPLC condition - 50 : 50 CH3CN : H2O
254nm, injection volume 20µL

6. Fate of Dithianon and Vinclozolin from Vine to Wine

6.1 Introduction

Pesticides are still used quite extensively in Australian agriculture and grape growing is no exception. Indeed, over seventy agrochemicals were registered for use in Australian viticulture in 1996 (AWRI, 1996). Dithianon is used for the control of *excoriosis* (black spot disease or dead arm disease) caused by *Phomopsis viticloa* (Brendel, 1970; Bulit and Bugaret, 1972; Arya, 1988); botrytis bunch or grey mould caused by *Botrytis cinerea* (Chkheidze, 1977); anthracnose caused by *Gloeosporium species* (Sadamatsu and Sanematsu, 1978); and downy mildew caused by *Plasmopara viticolla* (Schuck and Matos, 1985). Vinclozolin finds extensive use for the control of *Botrytis spp.* (grey moulds) on grapes, perhaps most importantly control of Botrytis bunch rot caused by *Botrytis cinerea.*

The Codex Alimentarius - an international body established by the United Nations' Food & Agriculture Organisation and World Health Organisation - sets Maximum Residue Limits (MRLs) for agrochemicals in a range of crops, including grapes. These MRLs are used as benchmarks in Australian viticulture, where MRLs are set to reflect 'good agricultural practice,' e.g. if viticulturists follow the directions provided by the manufacturer, formulate, and spray the product at the appropriate time and in the right concentrations, then their crops should have residues below the permitted Australian MRL (AWRI, 1996). However, some of the chemicals used during the growing of grapes in Australia do not have an MRL in some, or all, overseas markets. Essentially, this is often because the importing country does not grow grapes itself and therefore the chemicals are not registered for use on grapes in

that particular country. In addition, MRLs are rarely set for wine. Often MRLs in wine are simply those adopted for the raw materials i.e. grapes in this case. In the absence of an MRL manufacturers will often prudently apply the CODEX MRL, if one exists. Thus, the production of grapes for domestic consumption, export or for wine requires careful planning on the part of the viticulturist to ensure that the use and application of agricultural chemicals will not result in residue limits that will bar the product from Australia's major export markets.

Table 6.1 Some MRLs for dithianon and vinclozolin in grapes in selected countries

Active Ingredient	Registered Products	Maximum Residue Limits (µg / g)				
		Australia	Canada	Germany	N.Z.	USA
dithianon	Delan	2	7	3	2	no MRL
vinclozolin	Ronilan	5	5	5	5	6

MRLs have been set in a limited number of countries for dithianon in grapes (Table 6.1). The MRLs for vinclozolin (which is registered for use on grapes in many more countries) in those countries are similar to those set for dithianon, ~ 5 µg/g. Again these MRLs, while set for grapes, are also set for wines.

Meeting MRL standards is essentially a symbiosis of four factors - the right chemical, in the right proportions, on the right crop, at the right time. In general, provided an agriculturist prepares and applies pesticide formulations correctly, statistical analysis of national residue surveys undertaken in Australia suggest that there is little danger that the MRLs in the crop will be exceeded (Pers. Comm., G. Roberts, State Chemistry laboratory, Agriculture Victoria). Perhaps the two most

important points for viticulturists seeking to meet MRLs is not to get the chemical onto the vines / grapes in the wrong proportions or at the wrong time. Too great an applied dose, even early in the season, may lead to lingering residues at harvest, while application too close to harvest may save a crop but leave significant residues on the grapes. Manufacturers and regulatory authorities address these points by recommending crop specific application rates and chemical 'with-holding periods' - the minimum length of time *before* harvest that an agrochemical may be applied. Dithianon application rates in most countries are 0.1 - 0.4 kg a.i./ha, depending on climatic conditions, infection pressure and agricultural practices. Application - harvest withholding periods vary considerably from country to country and from crop to crop, e.g. apples: 35 days (Australia) to 14 days (South Africa); stone fruits: 14 days (Chile) to 21 days (Australia); peaches: 7 days (Japan) to 21 days (Australia) (FAO/WHO, 1992). The recommended application rate for grapes in South Australia (1992) is 50 g a.i./100L, with a pre-harvest withholding period of 21 days (Roger, 1992). Vinclozolin application rates in most countries are somewhat higher than the corresponding rates for dithianon, generally 0.5 - 1.5 kg a.i./ha, again depending on climatic conditions, infection pressure and agricultural practices. Again, application-harvest with-holding periods vary considerably with country and crop, e.g. strawberries: 1 day (Australia) to 21 days (Italy); stone fruits: 1 day (New Zealand) to 21 days (Belgium, Luxembourg); lettuce: 1 day (Australia) to 28 days (The Netherlands, USA); fruiting vegetables, outdoors and under cover (glasshouse or plastic) in most countries: 3-4 days, but 21 days for outdoor tomatoes in Italy (FAO/WHO, 1986). For Botrytis control on grapes, the recommended application rate in South Australia in 1992 is 100 g a.i./100L, with a pre-harvest withholding period of 7 days (Rogers, 1992).

The physical and chemical heterogeneity of the vine environment makes the accurate prediction of the fate of agrochemicalsused in viticulture very difficult. Regardless, authorities worldwide are under pressure to use some basic chemical information to guide agrochemical users in selecting pesticides with minimal residuals since significant pesticide toxicity is often seen at sub-mg/L concentrations and only a comparatively small amount need persist on a crop such as grapes to cause significant contamination of food (Franklin et al., 1994). A significant body of work is available detailing physico-chemical investigations into the environmental fate of pesticides under atmospheric and climatic conditions prevalent in the grape growing regions of the northern hemisphere. However, the climatic conditions in Australia, in particular the shorter summer daylight hours but hotter, drier, more intense sunlight are somewhat different from Europe. This, in turn, may influence the physico-chemical behaviour of pesticides compared with applications in the northern hemisphere, and may therefore affect such critical parameters as application rates (in terms of effectiveness and persistence) and pre-harvest with-holding days. This section details such investigations into the fate of the fungicides dithianon and vinclozolin when sprayed onto grapes on vines planted in South Australia (Barossa Valley and at Roseworthy), subjected to ambient climatic conditions for the normal withholding period, harvested, crushed, and fermented into wine.

6.2 Materials and Method

6.2.1 Materials

(a) Chemicals, fungicide standards, solvent degassing and the liquid chromatographic system (HPLC column, solvent system and UV detector) are as described in Chapter 3.

(b) Extraction cartridges, manifold, and methods as described in Chapter 4.

(c) *Grapes* - grapes were harvested from vines grown at the Nuriootpa Research Station, Primary Industries of South Australia (Figure 6.1), and at Roseworthy Campus, University of Adelaide. The grape varieties involved in this study were pinot noir, bastardo and cinsaut (red), semillon, rkaziteli and goyura (white). Pinot noir (Figure 6.2) is the principal variety of red grape used for the wines of Burgundy, and one of the principal varieties for the white sparkling wines of Champagne. Pinot noir plantings in Australia have increased more than 10-fold since 1974 from less than 10 ha to more than 670 ha in 1987 (Dry & Gregory, 1995). Half of the planted area is in South Australia, particularly the Barossa region. Bastardo (Figure 6.4) is another red wine grape. It is considered to be one of the better port varieties of Portugal. The area grown in Australia is small, less than 100 ha, with the majority of the plantings in South Australia. Considered best suitable for fortified wines (Dry & Gregory, 1995). Cinsaut (Figure 6.5) originated in the south of France where it is regarded as a good wine grape, particularly for blending. It is the principal red wine grape of South Africa. It is not of great importance in Australia and now the total area planted is less than 100 ha. Much of the plantings are in the Barossa, although there is a significant amount near Mildura, Victoria. Readily harvested mechanically, it is used for red

table wine and medium quality ports (Dry & Gregory, 1995). Semillon (Figure 6.3) is one of the world's most widespread white wine grape varieties. In the 1980's, semillon was Australia's fourth most important white wine variety, with over 3000 ha of plantings. It is generally used for dry white table wines which develop distinctive and desirable flavour characteristics with aging (Dry & Gregory, 1995). Goyura (Figure 6.6) is a white wine grape variety designed to put quality into Australia's wine casks. Developed at Merbein, South Australia, goyura is a cross between Muscat of Alexandria x sultana. Goyura has a vaguely Muscat flavour and is considered very useful for its high levels of natural acids (Robinson, 1986). Rkaziteli (Figure 6.7) was the old Soviet Union's most planted wine grape, covering almost 250,000 ha. Also grown in North and South America, this variety constitutes one of the world's most important grape varieties. However, plantings are limited in Australia. In 1988, there were less than 100 ha of this variety (Robinson, 1986; Dry & Gregory, 1995).

(d) *Wine making materials* - wine yeast (provided by Wine and Grape Research Unit at Roseworthy), potassium metabisulphite $K_2S_2O_5$, flagons, rubber bung, fermenter (air lock), tubing for syphon, specific hydrometer and baume hydrometer (S&B Hillis Pty Ltd, Australia).

6.2.2 General Method

Methods for the extraction and determination of dithianon and vinclozolin in grape and wine samples are detailed in Chapter 4. Other methods specific to this aspect of these studies are explained hereafter.

Figure 6.1 Nuriootpa Research Station, PISA

Figure 6.2 Pinot noir

Figure 6.3 Semillon

Figure 6.4 Bastardo

Figure 6.5 Cinsaut

Figure 6.6 Goyura

Figure 6.7 Rkaziteli

Wine making

Two trials investigating the persistence of dithianon and vinclozolin on grape berries and through the wine making process were undertaken in the 1994-95 and 1995-96 grape-growing seasons. The first trial was conducted on one row each of pinot noir (30 vines; clone D5V12 (2051)) and semillon grape vines (45 vines; clone LRC 147) grown at the Nuriootpa Research Centre. The vines of each variety were divided equally into three groups : pinot noir, control (10 vines), dithianon treated (10 vines) and vinclozolin treated (10 vines), semillon, control (15 vines), dithianon treated (15 vines) and vinclozolin treated (15 vines). The grape bunches on treated vines were dipped into fungicide solution at the manufacturer's recommended application rate (dithianon (Delan®) : 10 L of 0.5 g / L solution, ; vinclozolin (Ronilan®) : 10 L of 0.1 mL / L solution). The grapes were harvested by hand eight days later. The harvested grapes were then transported to the Grape and Wine Research Unit, Roseworthy where fermentation was conducted in 20 L glass bottles by Unit personnel.

The second trial (1995-1996) was conducted on three vines each of the varieties goyura, rkaziteli, cinsaut, and bastardo grown in the Grape and Wine Research Unit vineyard at Roseworthy. The varieties were divided into two groups : dithianon treated (bastardo and rkaziteli) and vinclozolin treated (cinsaut and goyura). The three vines of each variety were then further sub-divided into a control group (one vine) and treatment group (two vines). The grape bunches on the treated vines were sprayed directly with fungicide solution at the manufacturer's recommended application rate (dithianon (Delan®) : 2 L of 0.5 g / L solution,; vinclozolin (Ronilan®) : 1.3 L of 0.1 mL / L solution). All of the control grapes, and 4 - 5 kg of

the treated grapes were harvested when the grape BRIX of a variety was greater than 22 % (rkaziteli, day 6; bastardo, day 7; cinsaut, day 10; goyura, day 11). Wine was made from these grapes in accordance with the standard wine making procedure adopted by the Grape and Wine Research Unit (Ewart and Sitters, 1991; Figures 6.8 and 6.9). Fermentation was in 2 L flagons in the laboratory. Grab samples of the remaining treated grapes were taken 14 days after spraying.

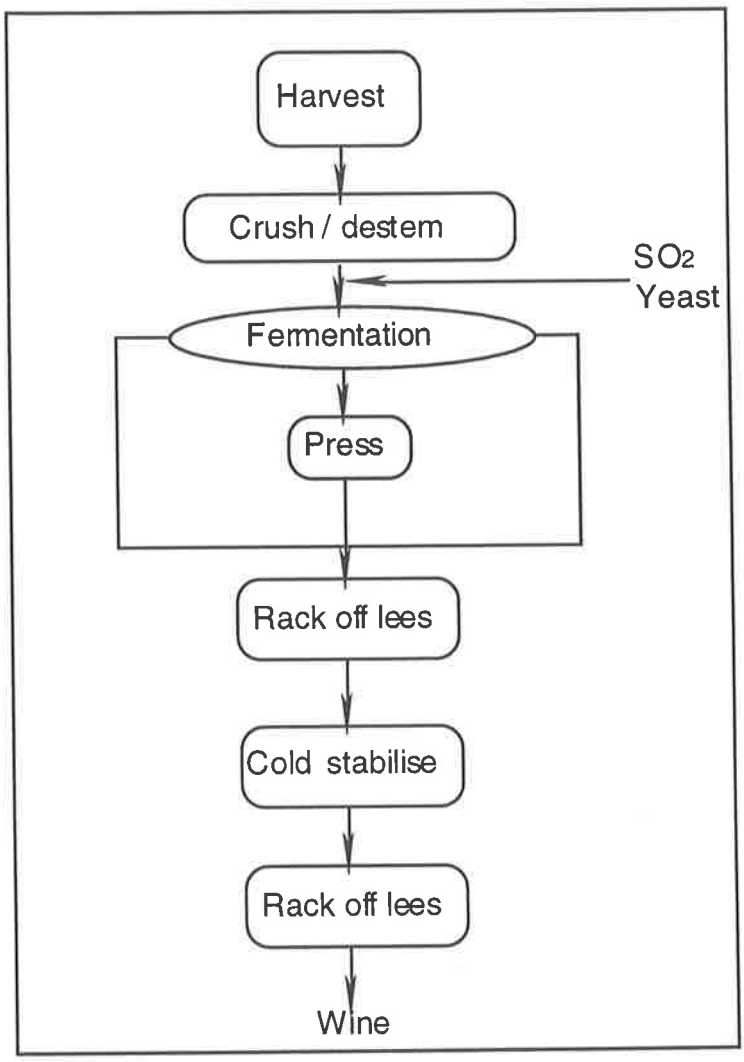


Figure 6.8 Red wine making procedure

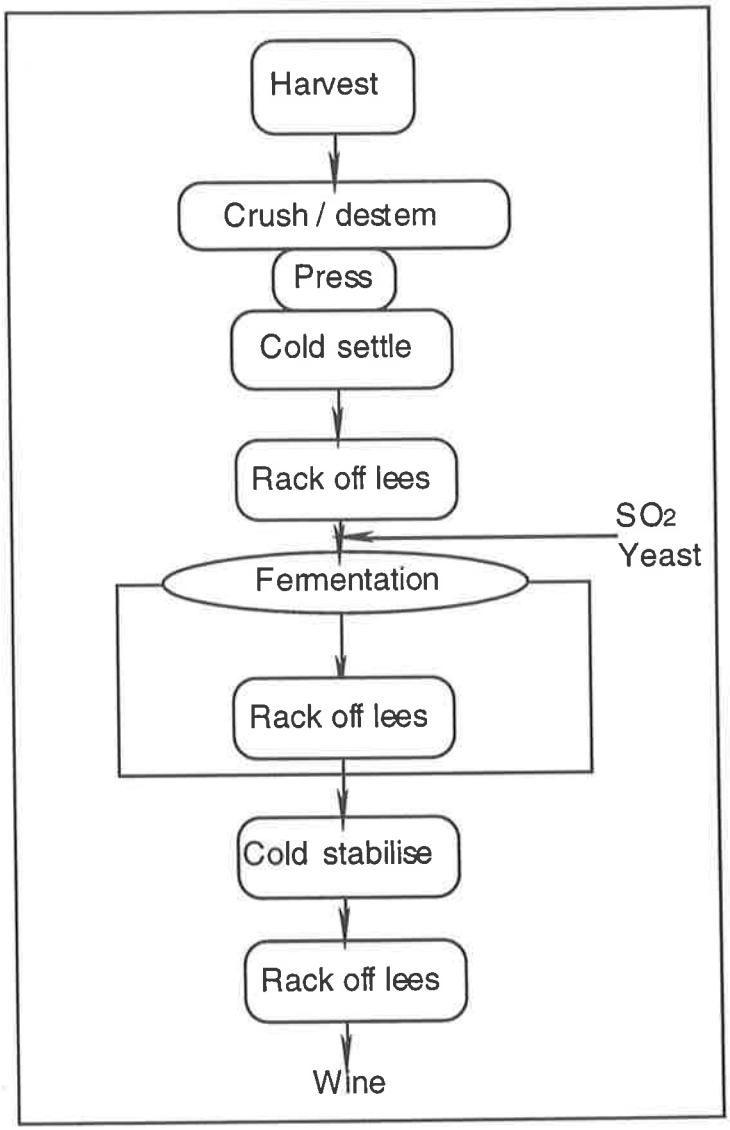


Figure 6.9 White wine making procedure

Extraction of dithianon and vinclozolin from the surface of grapes

Five bunches of grapes were picked at random from both the control and treated vines of each variety immediately after spraying (day 0), on harvest day, and on day 14 after spraying. Each bunch was weighed and placed into a 1000 mL beaker. The surface of each bunch was then washed first with methanol (40 mL; dropped onto the bunch using a Pasteur pipette), then water (160 mL). The combined aqueous solution was filtered through glass fibre filter paper and then extracted and analyte

concentrations determined in the manner described for aqueous samples in Chapter 4. The methanol-washed grape berry samples were then frozen, and residual fungicide concentrations determined in the manner described for grape berries in Chapter 4.

Extraction of dithianon and vinclozolin from grape skins

The grape skins were removed from the press and frozen. Thereafter, residual fungicide concentrations were determined in the manner described for grape berries in Chapter 4.

Extraction of dithianon and vinclozolin from lees

The bulk of the wine was first racked off from the lees. The remaining mixture was then centrifuged to separate the last of the wine from the lees. The isolated lee samples were then frozen. The lees (10 g) were mixed with methanol (20 mL), and made up to 100 mL total volume with water. Thereafter, residual fungicide concentrations were determined in the manner described for aqueous solutions in Chapter 4.

Solid phase extraction of dithianon and vinclozolin from wine

Residual fungicide concentrations in wine were determined in the manner described in Chapter 4.

6.3 Results and Discussion

Two trials investigating the fate of dithianon and vinclozolin from vine to wine were undertaken during the 1994-95 and 1995-96 grape-growing seasons. The first trial was designed to answer two questions - first, do these fungicides persist on the grape berries and, second, do they persist through the wine making process.

Trial 1 : Persistence of dithianon and vinclozolin in pinot noir and semillon grapes and wine

Ten pinot noir and fifteen semillon vines grown at the Nuriootpa Research Centre were treated with dithianon, the same numbers of each variety treated with vinclozolin, and a third cohort of the same size of each variety left untreated as controls. The grapes were harvested by hand eight days later, and made into wine by the Grape and Wine Research Unit, Roseworthy. Grab samples of berries taken at Day 0 and Day 8, and the young wine were analysed for fungicide residues.

No dithianon was detected in control grape or wine samples. In addition, no dithianon was detected in treated grape or wine samples. The non-detection of dithianon in the grape samples, particularly the Day 0 samples, was initially of great concern. However, these results were understandable once it was determined that dithianon decomposes extremely rapidly in grape juice (see Chapter 5). During this trial, the whole grapes were crushed and left at ambient temperature for several hours before extraction. It is, therefore, highly likely that the dithianon decomposed during that time.

No vinclozolin was detected in control grape or wine samples. However, significant quantities of vinclozolin were determined on treated grapes and in wine made from such grapes (Table 6.2). In both pinot noir and semillon samples it was noticeable that vinclozolin levels decreased significantly between Day 0 and Day 8. Vinclozolin is known to be thermally unstable (see Chapter 5) so this decrease may have been due to thermal degradation beneath the vine canopy. However, the fact that there was a rain event during the eight day period between spraying and harvesting meant one can not rule out the possibility that significant amounts of the fungicide had simply been washed off the grapes. Approximately 10 % of the average vinclozolin levels in the grapes were determined to have survived the wine making process. These results are consistent with previously reported data from Europe. For instance, vinclozolin residues in the range 0.5 - 4.8 µg/g on grapes, and 0.03 - 0.8 µg/g in wine in Germany and France (FAO/WHO, 1986).

Table 6.2 Summary of Trial 1 fungicide residue from vine to wine

Variety	Fungicide	Fungicide Concentration In Grape Berries				In Young Wine	
		Day 0		Day 8			
		Conc. (µg/g)	Mean (c.v. %) (µg/g)	Conc. (µg/g)	Mean (c.v. %) (µg/g)	Conc. (µg/mL)	Mean (µg/mL)
Pinot Noir	dithianon	N/D	N/D	N/D	N/D	N/D	N/D
		N/D		N/D		N/D	
		N/D		N/D			
	vinclozolin	4.77	4.13 (12)	1.32	1.12 (17)	0.61	0.58
		3.77		0.95		0.52	
		4.40		1.09			
Semillon	dithianon	N/D	N/D	N/D	N/D	N/D	N/D
		N/D		N/D			
		N/D		N/D			
	vinclozolin	9.09	9.77 (7.8)	4.03	4.48 (13)	0.55	0.52
		10.59		5.11		0.49	
		9.63		4.29			

Trial 2 : Persistence of dithianon and vinclozolin in bastardo, cinsaut, goyura and rkaziteli grapes and wine

The results of Trial 1 lead to a number of modifications being made to the experimental protocols for Trial 2 i.e. smaller number of vines, grab samples of grapes were taken at three times over a two week period, the grapes were stored at cooler temperatures, the fungicides were washed from the surface of the grapes, and the grapes were frozen as quickly as possible.

Two vines each of the grape varieties bastardo (red) and rkaziteli (white) were sprayed with dithianon. Similarly, two vines each of the grape varieties cinsaut (red) and goyura (white) were treated with vinclozolin. One vine of each variety was left untreated as part of the control cohort. 4 - 5 kg of control and treated grapes of a variety were harvested when the grape BRIX was greater than 22 % (rkaziteli, Day 6; bastardo, Day 7; cinsaut, Day 10; goyura, Day 11). Wine was made from these grapes in 2 L flagons in accordance with the standard wine making procedure adopted by the Grape and Wine Research Unit (Ewart and Sitters, 1991; Figures 6.8 and 6.9).

Grab samples of five bunches of control and treated grapes were taken on the day of spraying, at harvest and 14 days after spraying. No dithianon was detected in control grapes or wine samples. However, unlike Trial 1 where residues were determined by analysing whole grapes, during Trial 2 the surfaces of the treated grapes were washed with methanol. This methanol wash was designed to extract the fungicides from the grape surfaces without the risk of chemical degradation of the fungicide due to contact with chemicals from the grape's interior. The methanol wash was simple but very effective, removing approximately 90% of the total dithianon

residues as determined by sequential methanol washing and then analysis of the grapes. However, since dithianon degrades very quickly in grape juice, it was not possible to determine with absolute confidence residual dithianon levels on the surface of the grape berries after the methanol wash.

There was no significant difference in the levels of dithianon on the surfaces of both red or white grapes (bastardo and rkaziteli, respectively) at Day 0, at harvest, or Day 14 (bastardo, only) (Tables 6.3 and 6.4; Figure 6.10 and 6.11). This suggests that dithianon is both chemically stable on the grape surface, and thermally stable in the conditions prevalent under the vine canopy over the time period studied. Such a finding would disturbingly suggest that dithianon residues surviving until harvest might be transported through the wine making process to the wine if it were not for the fact that dithianon degrades very quickly in both red and white wine (Chapter 5). Analysis of the wines made from red (pinot noir and bastardo) and white (semillon and rkaziteli) grapes sprayed with dithianon indicated that none contained detectable dithianon residues. However, although the wines did not contain determinable levels of dithianon, the author did not attempt to isolate and / or identify breakdown products. There still exists the possibility, therefore, that significant residues of dithianon breakdown products may have been present in the wines and which, depending on their relative toxicities, may equally be a human health threat.

Table 6.3 Summary of dithianon residues on the surface of red grapes (bastardo)

Bunch	Day 0			Day 7			Day 14		
	Bunch size (g)	Residue (µg)	Conc. (µg/g)	Bunch size (g)	Residue (µg)	Conc. (µg/g)	Bunch size (g)	Residue (µg)	Conc. (µg/g)
1	98	229	2.3	136	691	5.1	174	162	0.9
2	222	165	0.7	150	358	2.4	153	136	0.9
3	330	301	0.9	194	285	1.5	127	246	1.8
4	220	165	0.6	154	349	2.3	147	427	1.8
5	185	238	1.6	135	193	1.4	124	231	1.9
mean	211	219.6	1.22	153.8	375.2	2.54	145	240.4	1.46
c.v. (%)	40	26	59	16	50	59	14	47	35

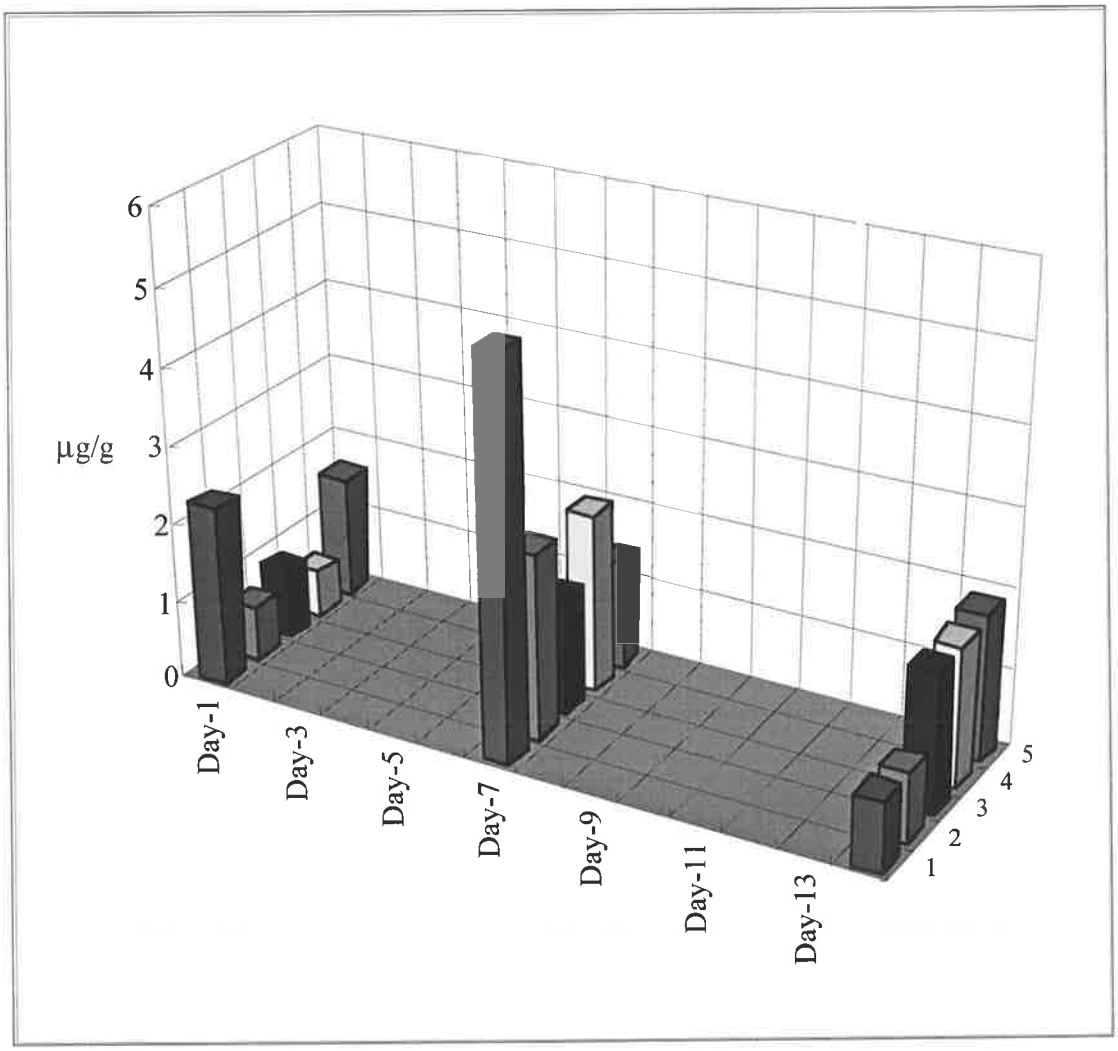


Figure 6.10 Dithianon residues on the surface of red grapes (bastardo)

Table 6.4 Summary of dithianon residues on the surface of white grapes (rkaziteli)

Bunch	Day 0			Day 6		
	Bunch size (g)	Residue (µg)	Conc. (µg/g)	Bunch size (g)	Residue (µg)	Conc. (µg/g)
1	267	254	1.0	189	192	1.0
2	146	96	0.7	165	394	2.4
3	168	64	0.4	183	85	0.5
4	110	50	0.5	143	75	0.5
5	213	29	0.1	185	115	0.6
mean	180.8	98.6	0.54	173	172.2	1.0
c.v. (%)	34	92	62	11	77	84

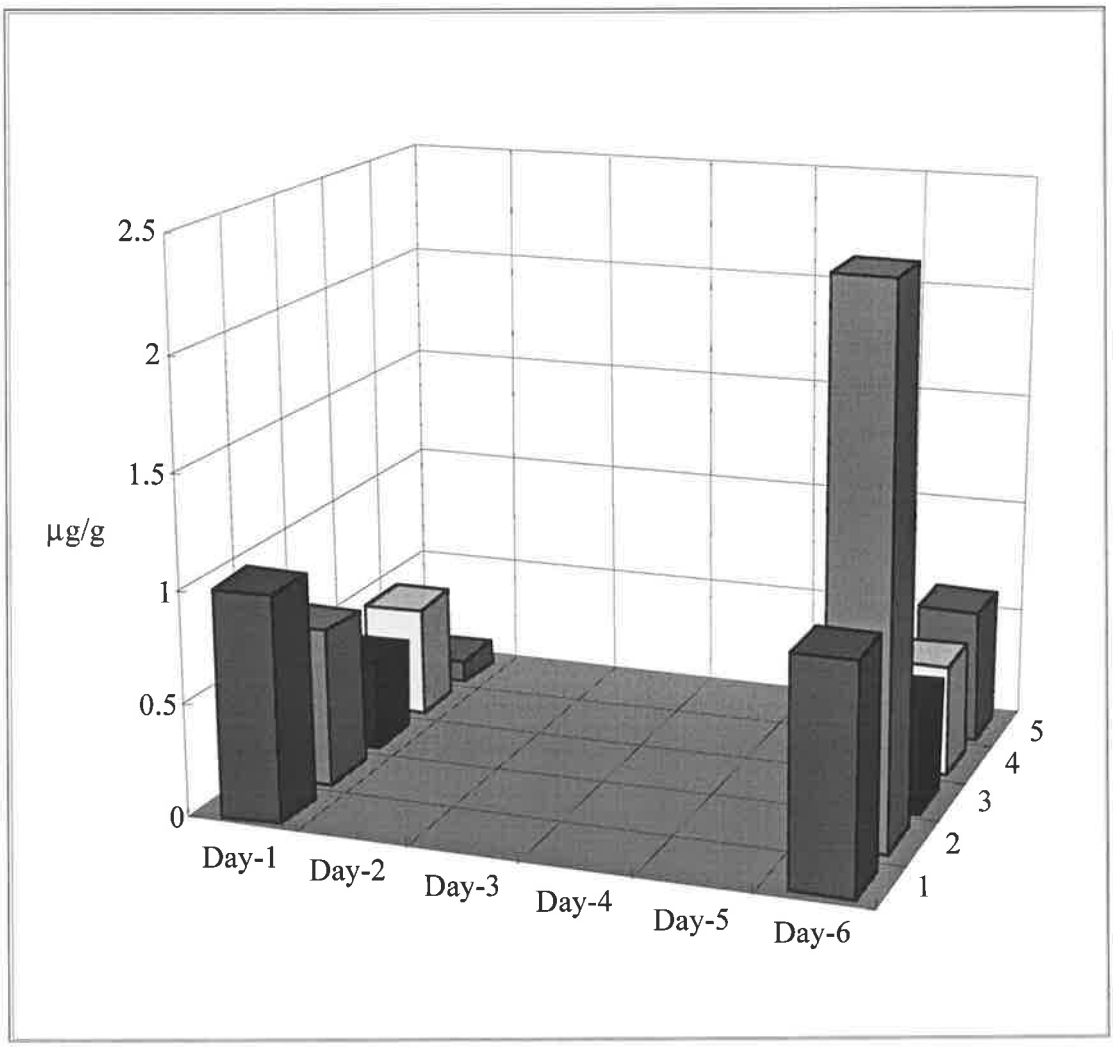


Figure 6.11 Dithianon residues on the surface of white grapes (rkaziteli)

Vinclozolin, on the other hand, showed a significant drop in the levels found on the surface of both red and white grapes (cinsaut and goyura) one week after spraying (Tables 6.5 and 6.6; Figures 6.12 and 6.13). These results are similar to those found in Trial 1. Once more, the most likely cause of this disappearance is thermal degradation since in this case wash out is unlikely because there were no rain events during the trial, nor could irrigation be responsible since the vines were watered by drip irrigation.

Table 6.5 Summary of vinclozolin residues in red grapes (cinsaut)

Sample code	Bunch mass	Mass of vinclozolin					
		From surface		From berries after wash		Total mass from grape	
	(g)	(µg)	(µg/g)	(µg)	(µg/g)	(µg)	(µg/g)
Cin-1 D-0	143	519	3.6	11	0.08	530	3.71
Cin-2 D-0	205	608	3.0	ND	0	608	2.97
Cin-3 D-0	318	975	3.1	51	0.16	1026	3.23
Cin-4 D-0	285	881	3.1	34	0.12	915	3.21
Cin-5 D-0	298	499	1.7	72	0.24	571	1.92
mean	249.8	696.4	2.9	33.6	0.12	730	3.01
c.v. (%)	29	31	25	87	75	31	22
Cin-1 D-10	222	125	0.6	42	0.19	167	0.75
Cin-2 D-10	137	143	1.0	53	0.39	196	1.43
Cin-3 D-10	235	80	0.3	54	0.23	134	0.57
Cin-4 D-10	190	132	0.7	29	0.15	161	0.85
Cin-5 D-10	188	23	0.1	13	0.07	36	0.19
mean	194.4	100.6	0.54	38.2	0.21	138.8	0.76
c.v.	20	49	65	45	51	44	60
Cin-1 D-14	258	45	0.2	-	-	45	0.17
Cin-2 D-14	196	107	0.6	43	0.22	150	0.77
Cin-3 D-14	194	126	0.7	19	0.1	145	0.75
Cin-4 D-14	214	59	0.3	9	0.04	68	0.32
Cin-5 D-14	230	62	0.3	21	0.09	83	0.36
mean	218.4	79.8	0.42	22.9	0.11	98.2	0.5
c.v.	12	44	52	62	68	48	57

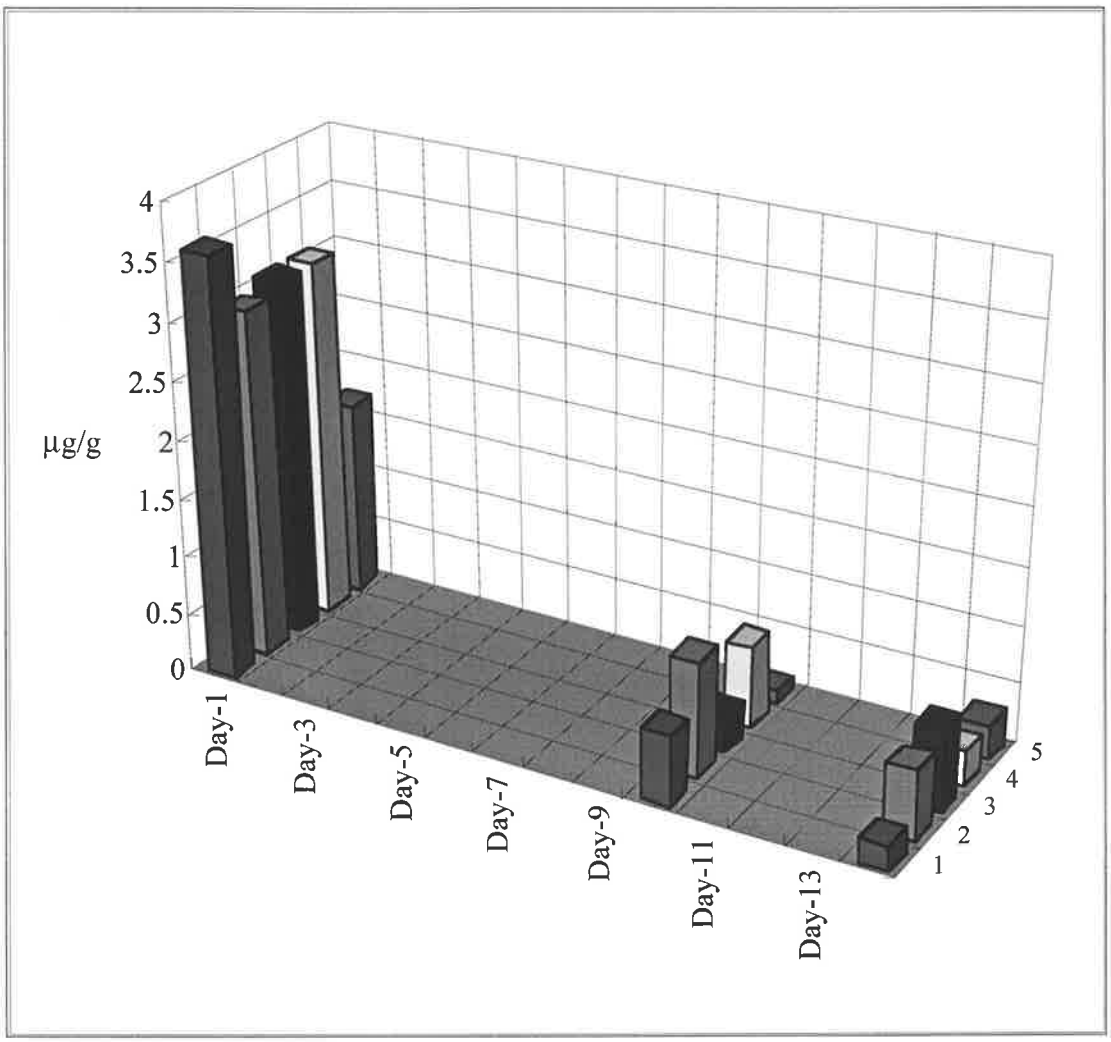


Figure 6.12 Vinclozolin residues on the surface of red grapes (cincaut)

Table 6.6 Summary of vinclozolin residues from white grapes (goyura)

Sample code	Bunch mass	Mass of vinclozolin					
		From surface		From berries after wash		Total mass from grape	
	(g)	(μg)	(μg/g)	(μg)	(μg/g)	(μg)	(μg/g)
Goy-1 D-0	196	385	2.0	11.76	0.06	396.76	2.02
Goy-2 D-0	154	759	5.0	6.16	0.04	608	3.95
Goy-3 D-0	138	762	5.5	12.42	0.09	774.42	5.61
Goy-4 D-0	126	839	6.7	6.3	0.05	845.3	6.71
Goy-5 D-0	257	1363	5.3	33.41	0.13	1396.41	5.43
mean	174.2	821.6	4.9	14.01	0.074	804.178	4.75
c.v. (%)	31	43	36	80	49	46	38
Goy-1 D-11	344	121	0.4	48.16	0.14	169.16	0.49
Goy-2 D-11	215	6	0.03	15.05	0.07	21.05	0.10
Goy-3 D-11	332	19	0.06	19.92	0.06	38.92	0.12
Goy-4 D-11	345	71	0.2	41.4	0.12	112.4	0.33
Goy-5 D-11	182	35	0.2	30.94	0.17	65.94	0.36
mean	283.6	50.4	0.178	31.094	0.11	81.494	0.28
c.v.	28	92	82	45	42	73	59
Goy-1 D-14	234	8	0.03	16.38	0.07	24	0.10
Goy-2 D-14	246	58	0.2	42	0.17	100	0.41
Goy-3 D-14	278	142	0.5	67	0.24	209	0.75
Goy-4 D-14	278	305	1.1	192	0.69	497	1.79
Goy-5 D-14	314	131	0.4	72	0.23	203	0.65
mean	270	128.8	0.446	77.8	0.28	206.6	0.7
c.v.	12	88	92	87	85	87	86

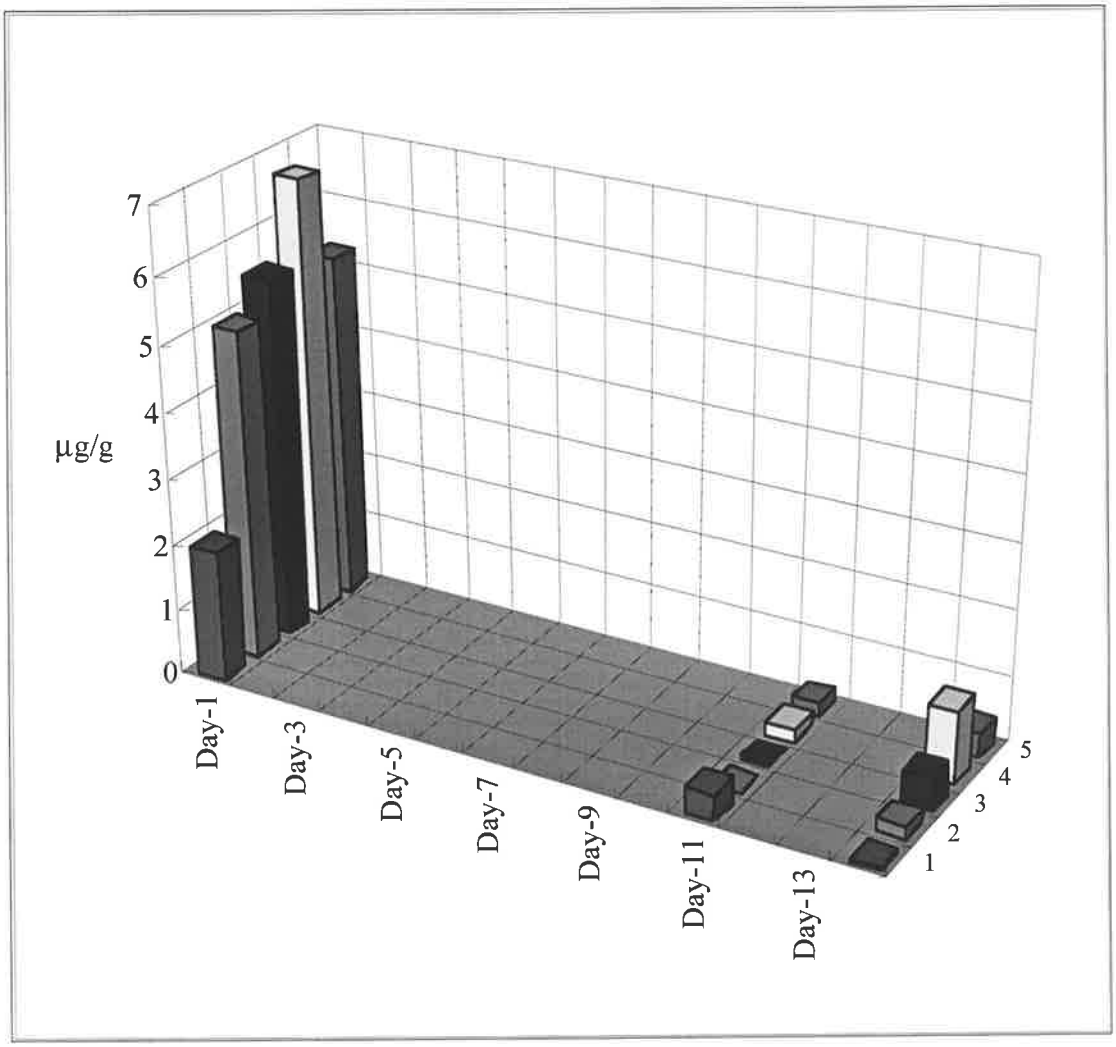


Figure 6.13 Vinclozolin residues on the surface of white grapes (goyura)

To check that the total mass of vinclozolin determined by sequential methanol washing and whole grape analysis was not introducing any systematic inaccuracies, a further series of grab samples of red (cinsaut) and white (goyura) grape bunches were taken from those collected at harvest, frozen immediately, and subsequently analysed using the method detailed in Chapter 4 (Table 6.7). The results of these cross-checks are shown in Tables 6.5, 6.6 and 6.7. They confirm the average levels of vinclozolin at harvest were 0.76 - 1.42 μg/g on red grapes, and 0.28 - 0.43 μg/g on white grapes. This apparent difference in residue levels on red and white grapes is probably a function of the physical differences between the grapes themselves, e.g. cinsaut grapes are smaller, more tightly bunched and lighter than the larger, heavier goyura grapes.

Table 6.7 Summary of vinclozolin residues in whole red and white grapes (cinsaut and goyura) at harvest

Sample code	Sample mass (g)	Harvest day (μg)	(μg/g)
Cin-1	50	56.6	1.13
Cin-2	50	87.6	1.75
Cin-3	50	69.3	1.39
mean		71.2	1.42
c.v.		22	22
Goy-1	50	33.5	0.67
Goy-2	50	15.0	0.30
Goy-3	50	15.3	0.31
mean		21.3	0.43
c.v.		50	49

Vinclozolin is stable in grape juice and wine (Chapter 5), and can be quantitatively recovered with good reproducibility. This suggests that any vinclozolin residues

remaining on the grape surface at harvest may persist through the wine making process and may ultimately be found in the final product, the wine. To assess this, wine was made from both the treated and control grapes. Samples were taken throughout the wine making process e.g. the grape skins, the lees and the final young wine. Grape skins were separated from the grape juice prior to fermentation of white wine grapes (goyura), and from the fermented must of red wine (cinsaut). The grape skins were quickly frozen and analysed. A significant proportion of the vinclozolin residues appeared to remain on the grape skins and not be transferred to grape juice or must (Table 6.8). A significant proportion of the vinclozolin residues that were transferred to grape juice or must remained in the discarded lees (Table 6.9).

Table 6.8 Summary of vinclozolin residues on grape skins taken from wine making process

Grape	Sample mass (g)	Mass of vinclozolin (µg)	(µg/g)
red (Cin)	50	62.6	1.25
white(Goy)	50	22.8	0.46

Table 6.9 Summary of vinclozolin residue in lees

Grape	Lees type	Sample mass (g)	Mass of vinclozolin (µg)	(µg/g)
red (Cin)	last lees	10	44.2	4.42
white(Goy)	1st lees	10	31.0	3.10
	2nd lees	10	30.6	0.37
	last lees	10	8.8	0.88

Finally, the young wines made from grapes treated with vinclozolin were analysed for vinclozolin using the method detailed in Chapter 4. Low levels of vinclozolin were found in all these young wines (Table 6.10). Having determined that vinclozolin

was relatively stable in wine (Chapter 5) the wines were stored in a cold store with the intention of long term monitoring of the vinclozolin levels. However, it was found that the wines 'went off' within a few weeks, presumably a function of their low alcohol contents (generally < 10%). It was therefore decided to abandon long term monitoring since the rapid change in the chemical constitutions of the wines made any further conclusions on the stability of this compound in wine somewhat difficult to justify.

Table 6.10 Vinclozolin residues from wines

Wine	Sample	Volume (mL)	Mass of vinclozolin (µg)	(µg/mL wine)
red	cin-1	5	0.54	0.11
white	goy-must	10	0.18	0.018
	goy-1	10	0.48	0.048
	goy-2	10	0.86	0.086

By following vinclozolin residues through the wine making process, it was possible to be determine that the greatest proportion of this chemical resident on the grape berries was removed from the grape juice / wine when the skins were discarded (Table 6.11). Thereafter, further small quantities of vinclozolin are removed with the lees. A small, but significant amount reaches the bottled wine. A total mass balance was difficult to achieve becuase there was significant variance (60 %) in the level of vinclozolin estimated to be on the surface of the 1.7 - 2.5 kg of grapes used to make the wine, and that vinclozolin will have been slowly degrading during the wine making process. Despite this, one can assume all of the vinclozolin has been accounted for.

Table 6.11 Summary of vinclozolin residues determined in wine making processes

Variety	Total mass of vinclozolin						
	On crushed berries	On grape skins		In lees		In young wine (2 L)	
	(µg)	(µg)	(%)	(µg)	(%)	(µg)	(%)
red	2480	1330	53.6	90	3.6	220	8.9
white	1100	500	45.5	60	5.5	134	12.2

6.4 Conclusion

Dithianon is stable on the surface of grapes under the ambient conditions prevalent under the grape canopy for at least two weeks. This result suggests that spraying with dithianon two weeks before harvest of table grapes would result in exposure to essentially the full dose of active ingredient sprayed, and increase the risk to human health through ingestion of this compound. However, the direct threat to human health from ingestion of dithianon is limited since any contact with grape juice or wine leads to rapid decomposition by some as yet undetermined process. However, there remains the possibility that dithianon breakdown products in the wine or grape juice may pose a threat to hman health. Vinclozolin appears to be unstable on the surface of grapes and that a fourteen day withholding period would see decomposition of 80 - 90 % of the applied dose. Again, however, spraying table grapes would lead to some, albeit reduced, risk of human exposure to vinclozolin through ingestion of the grapes. The bulk of that portion of the vinclozolin that enters the wine making process is discarded with the grapeskins and lees. Only approximately 10% of the residues entering the vinification process are mobilised into the wine. In addition, the slow decomposition of this compound in wine makes it unlikely to pose any threat to human health through ingestion of wine.

7. Transport and Behaviour of Dithianon and Vinclozolin in Soil

7.1 Introduction

Within the 5,000 or so independent grape growers that make up Australia's viticultural industry, there is a range of management systems, particularly in relation to the use of pesticides (insecticides, herbicides, fungicides), all arguably 'best practice' for the particular combination of soil and climatic conditions under which a vineyard operates (Pers. comm., V. Patrick, Mildara Wines, Coonawarra, S.A.). Even within a region growing conditions can change markedly. For instance, it is estimated that in the Barossa there are *at least* twenty seven different soils used for viticulture (Northcote, 1995). In addition, a short drive through the vineyards would reveal a range of vine management practices : many vineyards using traditional viticultural practices maintain a clean soil surface by means of ploughing, although this can lead to soil compaction and other associated problems. Some viticulturists use herbicide regimes to achieve the same effect. More recently, the use of cover crops grown between and beneath the vines e.g. grass sod, barley, or natural herbage, has been popular, although concern at the spread of Australian grapevine yellows may yet see a return to clearing of groundcover (Pers. comm., V. Patrick, Mildara Wines, Coonawarra, S.A.). When using cover crops some viticulturists adopt total ground cover regimes, some grow inter-row cover crops, but have no groundcover beneath the vines, and yet others have herbage beneath the vines but not between rows. As a result of these different management practices, there may be significant underestimation of the environmental risks to water supplies from pesticide use across the range of soils and climates experienced by the industry.

The physical and chemical heterogeneity of the natural environment makes the accurate prediction of the fate of agrochemicals very difficult. Regardless, authorities worldwide are under pressure to use soils information and chemical characteristics to guide agrochemical users in selecting pesticides with minimal residuals and least prone to leaching into groundwater (Franklin et al., 1994). Since significant pesticide toxicity is often seen at sub-mg/L concentrations only a comparatively small amount need leach through the soil to cause significant contamination of groundwater. In screening and registration programs, it is still common practice to estimate pesticide mobility by simply determining physical and chemical properties of the pesticides e.g., adsorption constants, water solubilities and degradation rates, and predict leachability based on such information. It is even more common to conduct short-term leaching tests on sterile, homogeneous soils. However, the environmental conditions in these tests are quite different from natural soils and field conditions. Predicting pesticide transport is further complicated by networks of interconnected pathways within the soil which transmit water and substances dissolved in it. These pathways result from geological activity, such as sub-surface erosion, faults and fractures, shrink-swell cracks, and biological forces such as animal burrows, worm holes, decaying roots, etc. They may transmit water and dissolved compounds at very much higher rates than anticipated by many current theories (Stagnitti et al., 1995; Bergstrom & Jarvis, 1993; Glotfelty et al., 1984; Frank et al., 1979). Despite these difficulties, the fact that some pesticides can be transported long distances by soil water and be detected far from the application site makes the prediction of their environmental fate through experimentally validated models of utmost importance.

Undisturbed soil columns offer a better means of studying preferential flow of pesticides under field conditions than the aforementioned standard leaching tests, because they preserve the natural structure of the soil (Steenhuis et al., 1991). In this section the behaviour and transport of dithianon and vinclozolin through small, undisturbed soil cores extracted from sites near Rutherglen in north-east Victoria, and near Overland Corner in the South Australian Riverland are discussed.

7.2 Materials and Methods

(a) Chemicals, fungicide standards, solvent degassing and the liquid chromatographic system (HPLC column, solvent system and UV detector) were as described in Chapter 3.

(b) Extraction cartridges, manifold and methods were as descried in Chapter 4.

(c) *Irrigation system* - Model CPP 30 Peristaltic Pump (ChemLab, England); Flow Measured Pump tubes (i.d. 0.38 mm, A.I., Queensland, Australia).

(d) *pH meter* - Hannah Instruments Model HI 8519.

Leaching experiments

The site was thoroughly soaked with water prior to excavation. A 1.5 m deep, 3 m long trench was carefully excavated, first using a back-hoe, then by shovels and spades. Soil columns were carefully carved out of the soil structure and encased in treated marine-ply boxes of dimensions 15 x 15 x 15 cm. A plywood lid was fitted to the top of the core and the core carefully removed from the site by excavating around the base and slicing and tipping the core (Figure 7.3). The excess soil on the base of the soil column was carefully trimmed flush with the base of the box, and a plywood lid screwed to the base.

Figure 7.1 The back-hoe at work at the Rutherglen site

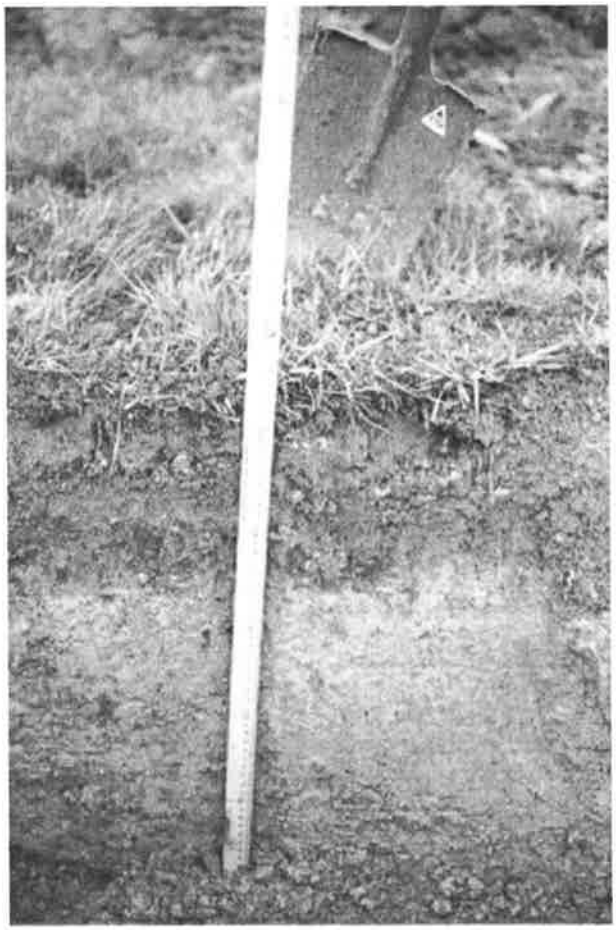


Figure 7.2 Rutherglen site soil profile
Note the sharp delineation between the topsoil and subsoil, and the spatial variation in the clay / buckshot density.

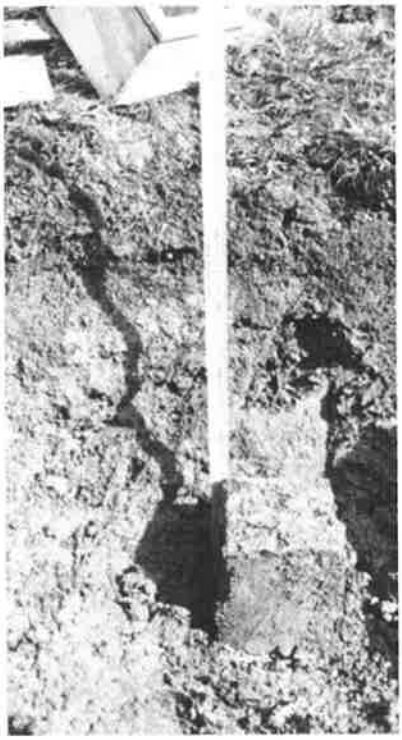


Figure 7.3 Soil core immediately before extraction from site

Figure 7.4 Leaching experiment : soil cores mounted on lysimeters

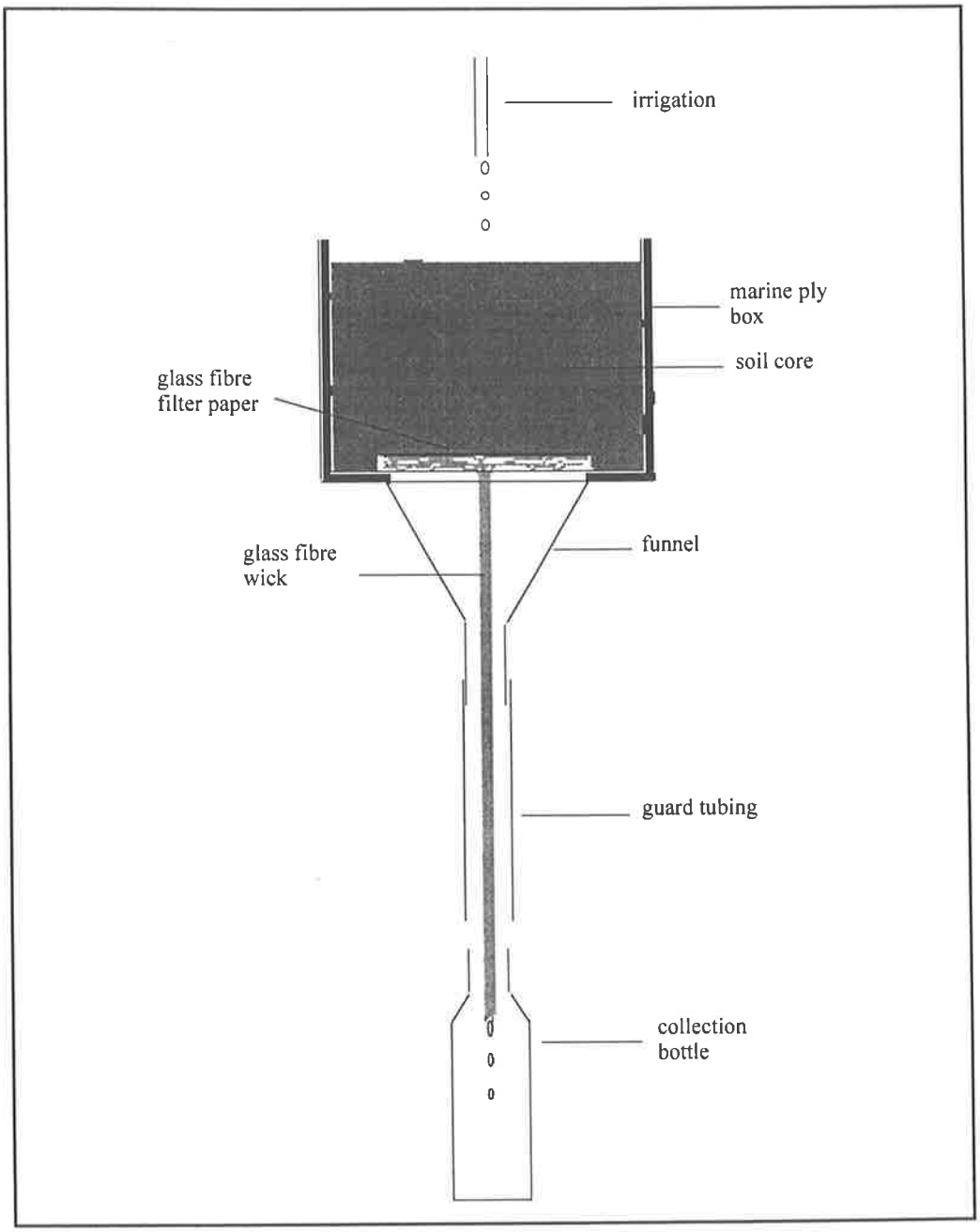


Figure 7.5 Diagram of single-wick lysimeter

The cores were transported back to Deakin University, Warrnambool and placed in a controlled environment laboratory (20 ± 2 °C) (Figure 7.4). The bases were removed and the exposed soil gently brushed and lightly vacuumed. The core was then fitted onto a single-wick lysimeter consisting of a 12.5 cm diameter glass fibre filter paper pressed onto a 40 cm glass fibre wick, the top 15 cm of which was spread out evenly underneath the filter paper (Figure 7.5). The wick lysimeter system was designed to provide a capillary suction comparable to natural field conditions. Such lysimeters have been tested previously and have been found to provide minimal field-flow distortions at the soil-apparatus interface (Stagnitti et al., 1995; Steenhuis et al., 1995). The cores were irrigated at 5-10 mL/h for several weeks. Water leaching from the cores was collected every two days, and the volume and pH determined.

Thereafter, dithianon and vinclozolin were applied to the cores over the centre of the irrigation area. The cores were irrigated further at the same rate for several more weeks. The leachate was collected thereafter at regular intervals. At the end of the experiment, a 2.5 cm diameter column of soil was taken through the full depth of the cores at the centre of the irrigation area. Where possible, this column was extracted from the core whole and then divided into 2 cm lengths for core pesticide residue profiling. Leachate and soil were extracted and any fungicide residues therein determined by the methods described in Chapter 4.

Soil pH

Triplicate samples of soil (5 g) were mixed with deionised water (50 mL) and shaken for 1 hour. After standing for a further 30 min., the pH of the supernatant aqueous phase was determined (Rayments and Higginson, 1992).

7.3 Results and Discussion

7.3.1 Behaviour of Dithianon and Vinclozolin in the Acidic Soils of the Rutherglen Region of Victoria

The 'North-East' grape growing region of Victoria in part comprises plantings at Rutherglen adjacent to the River Murray. The growing season is hot (mean January temperature 21 - 22.9°C), with low humidity and moderate rainfall. Water stress is common during the summer, although some vineyards obviate this by irrigating with water extracted from the River Murray (Northcote, 1995). The soils of this area are very important viticulturally, the region has a good reputation in Australia for the quality of its fortified wines. Small undisturbed soil cores were extracted from the topsoil and underlying subsoil of a paddock owned by the Rutherglen Research Institute, Agriculture Victoria approximately 10 km S.E. of Rutherglen, Victoria. The soil above the bedrock consisted of a thin (20 cm) layer of red-brown, acidic surface soil with a moderate organic content (1.2 - 4.1 %; Table 7.1), below which was a yellow-brown bleached clay containing substantial amounts of buckshot (iron rich nodules 3-8 mm in diameter) (Figure 7.2). The paddock was known never to have been sprayed with either dithianon or vinclozolin. Although vines are rarely grown in such clayey acidic soils (Northcote, 1995), both dithianon and vinclozolin are registered for use on other crops such as strawberries, fruit and ornamentals, which may be grown in similar soils. In addition, vinclozolin is registered for use in the control of *Scleortina*, *Helminthosporium*, and *Corticum* spp. in turf (BCPC/RSC, 1994). Surface fluxes of fungicide were applied at the maximum recommended application rates to the cores, which were then irrigated and residue levels in the leachate determined.

Table 7.1 Rutherglen soil characteristics

Core	Soil Depth (cm)	Munsell Soil Colour	Initial Average Moisture Content (%)	Total Organic Carbon (%)	Phosporous Adsorption Maxima PO_4^{3-}/P (mg/kg)	Nitrogen Adsorption Maxima NO_3^-/N (mg/kg)	Soil pH	Leachate pH	Particle Size Distribution (µm; %)				
									< 1200	< 300	< 150	< 65	> 65
R1, R2	0 - 5	10 YR 3/2 greyish brown	23	4.1	159	56	5.3	6.2 , 6.4	12.7	10.4	5.5	0.4	71.0
	5 - 15	5 YR 3/4 reddish brown	17	1.2	650	90	5.8		18.1	14.6	12.1	26.3	28.9
R3, R4	30 - 40	10 YR 5/3 brown	21	1.7	938	1470	6.2	6.6 , 6.7	4.0	13.0	6.2	2.4	74.4
R5, R6	45 - 55	10 YR 4/6 dark yellow brown	29	1.6	1294	2200	6.6	N/A , 6.4	5.7	2.8	3.4	29.9	58.2

N/A = Not Available

Leaching experiment

The Rutherglen soil cores were extracted from the Rutherglen Research Institute paddock in August 1996 before the spring rains and floods made the site too difficult to work in. Two cores were extracted from the surface soil layer (R1 and R2), two from the subsoil at a depth of 20-35 cm (R3 and R4) and a further two cores from a depth of 40-55cm (R5 and R6). The cores were irrigated at 5-10 mL/h for several weeks, a rate comparable to heavy winter rainfall in the area. Water leaching from the cores was collected every two days, and the volume and pH determined. Thereafter, dithianon and vinclozolin were applied to the cores over the centre of the irrigation area: dithianon 3.85 mg (10 mL of 0.5 g/L aqueous solution of Delan®; cores R1, R3, R5); vinclozolin 5.00 mg (10 mL of 1 mL/L aqueous solution of Ronilan®; cores R2, R4, R6). The cores were irrigated further at the same rate for a several more weeks. The leachate was collected at 40.5, 64.5, 115.5, 160.5, 184, 329, 548, 729, 832, and 884 hours after fungicide application. At the end of this time, a 2.5 cm diameter column of soil was taken through the full depth of the cores at the centre of the irrigationarea.

Cores R1 and R2 were extracted exclusively from the topsoil / turf root zone. As a result there was significant quantities of live roots in these cores and, indeed, grass growing during the experiment. Cores R3 and R4 were extracted from just below the A^2 horizon. However, there was still some evidence that turf roots extended into the top few cm of these clayey cores. Cores R5 and R6 were extracted from much deeper in the subsoil. Although the subsoil below the A^2 horizon appeared to have a relatively uniform clayey appearance, on closer inspection core R4 was found to

contain substantially greater quantities of buckshot than the other cores. Core R5 contained substantially more clay than the other subsoils. This clay appeared to be responsible for the core swelling after only a few days of irrigation, ultimately sealing the core which resulted in irrigated water pounding on the surface. No water was observed to leach from this core. Comparisons between the rate of irrigation and collected leachings indicated that up to 50% of the applied water did not leach into the lysimeter. Much of this was presumably lost from the core surface by evaporation. In general, the average soil pH was lower than that of the respective leachate (Table 7.1).

No dithianon was detected in the leachate from any of cores R1, R3 or R5. However, approximately 5% of the applied vinclozolin dose leached through the topsoil (R2), and 8 - 10% through the subsoil cores (R4, R6) (Table 7.2). The temporal distribution of vinclozolin residues is shown in Figure 7.6. Pesticide breakthrough occurred after approximately 40 hours in R2 (topsoil) and R4 (subsoil taken from immediately below the A^2 horizon), but was considerably slower in R6 (the subsoil taken from deeper in the B horizon). That substantially greater quantities of fungicide leached through R4 and R6 compared to R2 suggests that the higher organic content of the latter core (topsoil) may have been promoting fungicide immobilisation. In addition it is possible that the non-sterile topsoil contained a much greater quantity and variety of microflora than the subsoil R6, and that with greater immobilisation there was enhanced biodegradation. This latter suggestion agrees with reports that the rate of vinclozolin degradation increases with repeated application of this fungicide to the soil, suggesting that the soil microflora actively degrade this compound (Walker et al., 1986; Walker, 1987a,b).

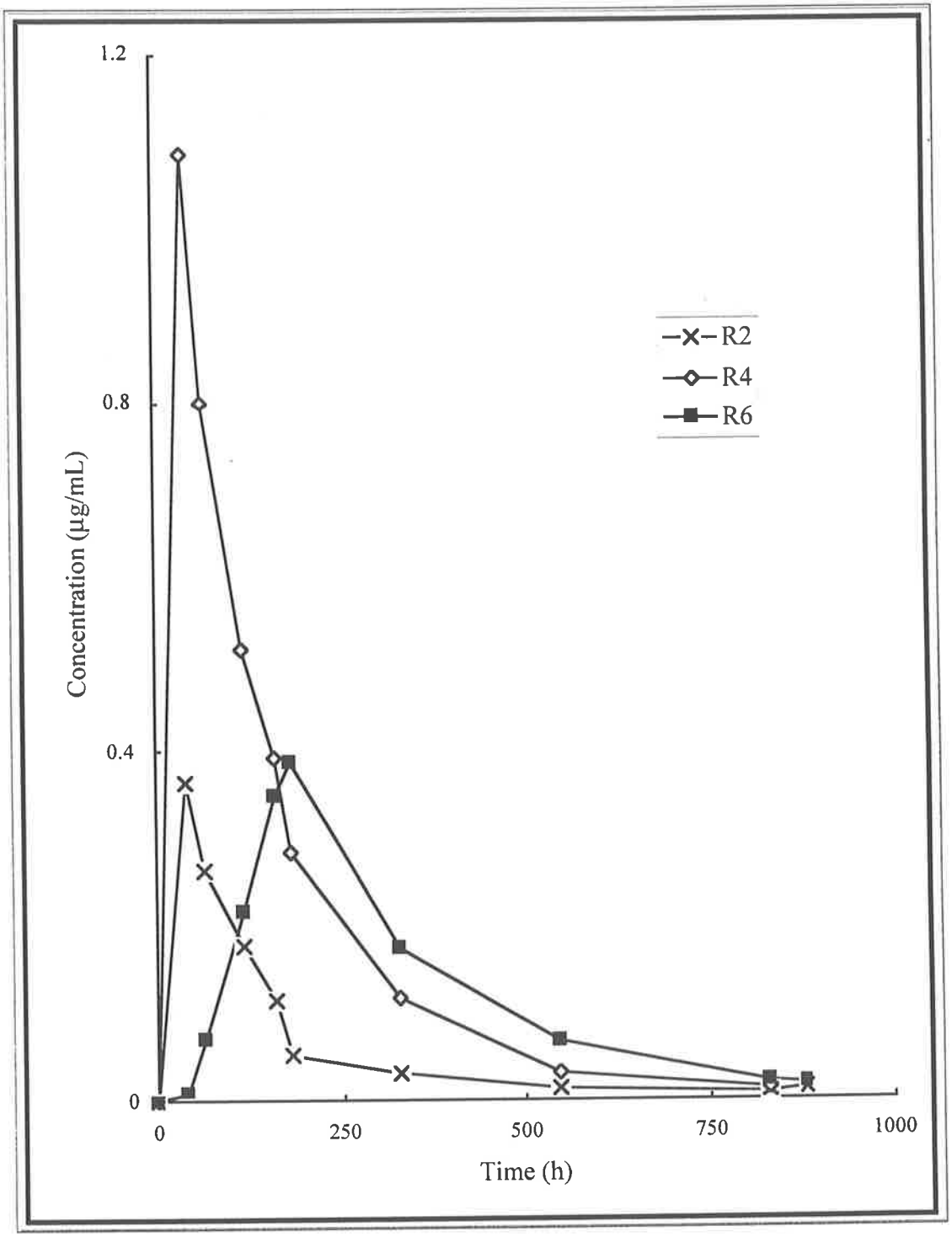


Figure 7.6 Temporal distribution of vinclozolin residues in the leachate

Table 7.2 Summary of fungicide residues in Rutherglen soil cores and leachate

Core (fungicide applied)	Depth of Core in Soil Profile (cm)	Total Mass of Fungicide Detected in Leachate (µg)	Fraction of Applied Dose (%)	Core Section	Depth of Section in Core Profile (cm)	Mass of Fungicide (µg)	Fraction of Applied Dose (%)
R1 (dithianon)	0 - 15	ND	-	1a	0 - 2	284.5	7.4
				1b	2 - 4	3.7	0.1
				1c	4 - 6	2.1	0.05
				1d	6 - 8	ND	-
				1e	8 - 15	ND	-
R3 (dithianon)	25 - 40	ND	-	3a	0 -2	1462.1	38.0
				3b	2 - 4	186.3	4.8
				3c	4 - 6	59.3	1.5
				3d	6 - 10	ND	-
				3e	10 - 14	ND	-
R2 (vinclozolin)	0 - 15	256	5.1	2a	0 - 2	14.5	0.3
				2b	2 - 4	9.9	0.2
				2c	4 - 6	12.2	0.25
				2d	6 - 10	6.6	0.1
				2e	10 - 14.5	4.0	0.08
R4 (vinclozolin)	25 - 40	544	10.9	4a	0 - 7	ND	-
				4b	7 - 14	2.2	0.02
R6 (vinclozolin)	45 - 60	404	8.1	6a	0 - 1.5	1.2	0.02
				6b	1.5 - 10	2.6	0.05
				6c	10 - 15	3.4	0.07

ND = not detected

That the two cores comprised predominantly of clays, R4 and R6, had such radically different breakthrough rates may be explained if the high clay content of R6 delayed pesticide breakthrough through swelling of the core and a concomitant reduction in the leaching rate; and that the substantial quantities of buckshot in R4 may have provided preferential flow pathways within the core, promoting leaching and hastening pesticide breakthrough.

At the end of the irrigation phase of these studies, a 2.5 cm diameter column of soil was taken through the full depth of the cores at the centre of the irrigation zone. The columns taken from cores R1, R2 and R3 were extracted from the core whole and then divided into 2 cm lengths for core pesticide residue profiling. The predominantly clay columns R4 and R6 compacted during extraction and were divided along natural breaks. That dithianon was found only in the top 2 cm of both topsoil (R1, 7.4% applied dose) and subsoil (R3, 38% applied dose) (Table 7.2) suggests that this compound is relatively immobile in these mildly acidic clayey soils. The relatively low levels of dithianon found in the top 2 cm of these cores suggests this compound is also relative unstable in such in mildly acidic conditions. This observation agrees with the reported dithianon half-life, $t_{1/2}$, at 22 ^{o}C and similar pHs i.e. pH 5, $t_{1/2}$ 295 h., pH 7, $t_{1/2}$ 15.7 h. (BCPC/RSC, 1994; FAO/WHO, 1993). Although 5-10% of the applied vinclozolin dose leached from cores R2, R4 and R6, vinclozolin was found only at extremely low levels throughout the core profile. This suggests vinclozolin is degraded relatively rapidly in non-sterile mildly acidic soils. This agrees with published reports that over 90% degradation of applied vinclozolin 40 days after application to a sand loam soil previously untreated with this fungicide; over 50% lost after 7 days after application to a clay loam soil; and that over 50% of

the applied dose degraded within 30 days of application to a silty clay loam soil of pH 6.5 (Walker et al., 1986; Walker, 1987a,b).

7.3.2 Behaviour of Dithianon and Vinclozolin in the Soils of the South Australian Riverland

The soils of the South Australian Riverland are very important viticulturally, producing more than one-third of the grapes used in Australian winemaking. The growing season is hot (mean January temperature 23 - 24.9 ^{o}C), with very low humidity and scant rainfall. Water stress is common during the summer, although vineyards obviate this by irrigating with water extracted from the River Murray (Northcote, 1995). Small, undisturbed cores were extracted from a vineyard approximately 10 km south-west of Overland Corner. The vineyard is located on a gentle south-facing incline (north-south slope, approximately 10 %) overlooking the Murray River valley. The vineyard soil, a highly alkaline reddish brown sand loam of variable depth (1 - 4 m) and moderate organic carbon content (1 - 2 %), was typical of the soils of the mallee highlands near the River Murray used for viticulture (Northcote, 1995). This vineyard had never applied dithianon or vinclozolin to its grapes or vines. The cores were extracted from the top soil (surface 25 cm) between the rows of vines near the lower edge of the vineyard. Surface fluxes of pesticide were applied at the maximum recommended application rates to the cores, which were then irrigated and residue levels in the leachate determined.

Fungicide recovery from leachate

Both dithianon and vinclozolin were known to be unstable in alkaline aqueous media (Chapter 5). Therefore, it was assumed that any dithianon or vinclozolin still present in any aqueous sample could be quantitatively recovered with excellent reproducibility by the method described earlier in Chapter 4 (Section 4.3 Recovery from leachate; Table 4.1).

Leaching experiments

The soil cores were extracted in December 1996, approximately half-way through the grape growing season. The cores were extracted from between the rows of vines near the lower edge of the vineyard. The site was thoroughly soaked with water prior to excavation. All work was undertaken in the first few hours after dawn to avoid the extreme heat of the day, and to avoid the cores drying out with concomitant loss of soil pore structure. Dithianon and vinclozolin were applied to the cores over the centre of the irrigation area: dithianon 3.85 mg (10 mL of 0.5 g/L aqueous solution of Delan®; cores OC3, OC4); vinclozolin 5.00 mg (10 mL of 1 mL/L aqueous solution of Ronilan®; cores OC1, OC2). The cores were irrigated further at the same rate for several more weeks. The leachate was collected at 26, 42, 66, 91, 121, 144, 168, 211, and 240 hours after fungicide application.

The cores were taken exclusively from the topsoil / turf root zone of the vineyard. The inter-row management practice operating in the vineyard involved the annual sowing of barley followed by regular slashing of the cover crop and weeds. As a

Figure 7.7 Backhoe at work between the vines at the Overland Corner site

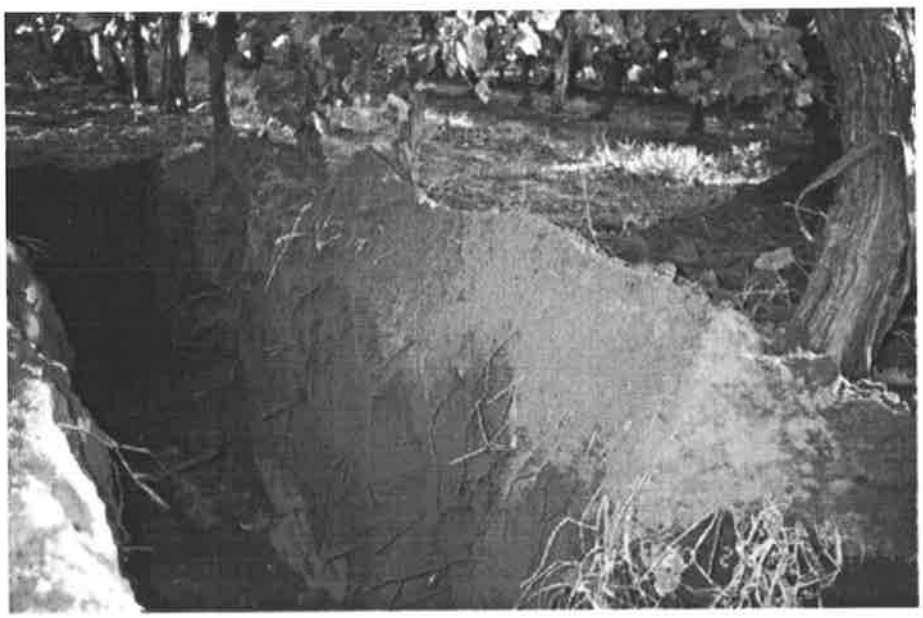


Figure 7.8 Excavated trench showing almost uniform soil structure

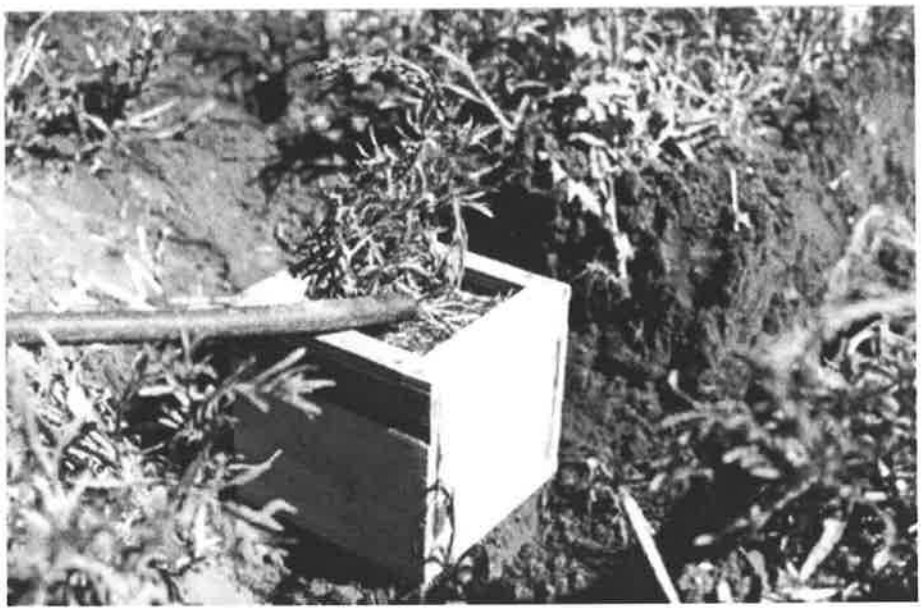


Figure 7.9 Soil core immediately after encasing

Note core is being soaked with water.

This prevents core break-up during the extraction process.

result there was significant quantities of live roots in these cores and, indeed, with grasses growing during the experiment, a real possibility of preferential solute transport.

The soil was alkaline, and the average soil pH was significantly higher than that of the respective leachate (Table 7.3). Comparisons between the rate of irrigation and collected leachings indicated that up to 50% of the applied water did not leach into the lysimeter. This loss was presumably from the soil surface by evaporation.

Table 7.3 Summary of soil and leachate pH

pH	Core			
	OC1	OC2	OC3	OC4
Soil	8.9	9.2	9.1	9.0
Leachate	8.1	8.4	8.2	8.6

Analysis of leachate prior to fungicide application showed a number of small peaks due to unknown organic compounds, none of which interfered with the determination of either vinclozolin or dithianon. Leachate was collected approximately every twenty-four hours over a two week period. No dithianon or vinclozolin was detected in any leachate.

At the end of the irrigation phase of these studies, a 2.5 cm diameter column of soil was taken through the full depth of the cores at the centre of the irrigation zone. The columns taken from cores OC1 and OC3 were extracted from the core whole and then divided into 2 cm lengths for core pesticide residue profiling. The column extracted from OC4 compacted somewhat during extraction and was divided into two along a

natural break. Core OC2 was considerably drier than the others. The column could not be extracted intact. The soil from this column was blended and analysed as a single sample.

No dithianon was detected in extracts of soil taken from OC4. Dithianon was found only in the top 2 cm of OC3 (37.2% of applied dose, Table 7.4). These results suggest that this compound is both relatively immobile in alkaline soils and also relative unstable under alkaline conditions. These observations agree with the reported dithianon half-life $t_{1/2}$ at 22 °C and similar pHs i.e. pH 7, $t_{1/2}$ 15.7 h., pH9, 0.15 h. (FAO/WHO, 1992).

Table 7.4 Summary of total dithianon and vinclozolin (μg) found in soil core profiles

Fungicide	Core			
	OC 1	OC 2	OC 3	OC 4
Dithianon	N/A	N/A	1431.2	N/D
Vinclozolin	6.8	69.4	N/A	N/A

N/A = not applied; N/D = not detected

Although none of the applied dose was detected in the leachate, vinclozolin was found at low levels throughout cores OC1 and OC2 (< 1.2% applied dose, Table 7.4). This suggests that vinclozolin is both somewhat mobile in these soils, but also degraded rapidly in non-sterile alkaline soils. Vinclozolin is known to be unstable under alkaline conditions at 25 °C: pH 7, $t_{1/2}$ 19.7 h. (Szeto et al., 1989a), pH9, 0.26 h. (FAO/WHO, 1992), and our observations agree with those reported by Walker who stated that over 90% of applied vinclozolin dose was lost after 40 days when

applied to a sand loam soil previously untreated with this fungicide (Walker, 1987a,b; Walker et al., 1986).

7.4 Conclusion

The studies suggest that the mildly acidic topsoils and subsoils of the Rutherglen region may be prone to pesticide leaching, particularly those soils with preferential flow pathways. However, pesticide transport potential will primarily be dependent on the physico-chemical characteristics of the pesticide. In these studies we determined that vinclozolin is relatively mobile in both topsoil and subsoil (breakthrough occurring 5 - 10 days after application), but also relatively unstable, with approximately 90 - 95% of the applied dose degrading in six weeks. Dithianon, on the other hand, was immobilised in the top 2 cm of the soil cores, but also relatively unstable. As a result of this chemical instability, it appears unlikely that either vinclozolin or dithianon would pose any significant threat to groundwater supplies in the Rutherglen area.

These studies also determined that dithianon was immobile in the red calcareous earth soils of the South Australian Riverland. Dithianon was also unstable in such alkaline conditions. Vinclozolin, on the other hand was somewhat mobile as evidenced by its detection throughout the soil profiles, but also unstable under the alkaline conditions. These results suggest that the red calcareous earth soils of the South Australian Riverland are unlikely to be prone to leaching of viticulturally derived dithianon or vinclozolin, nor, as a result of their chemical instability, is it likely that either fungicide would pose any significant threat to groundwater supplies in the area.

8. Conclusion - Environmental Fate of Dithianon and Vinclozolin

The physical and chemical heterogeneity of the natural environment makes the accurate prediction of the fate of chemicals used in agriculture or other industrial practices very difficult. Australian vineyards and the chemicals used therein are no different. The climatic conditions in Australian vineyards, in particular the shorter, hotter, drier, more intense, summer daylight hours, are different from those in Europe where much of the research on such critical parameters as application rates (in terms of effectiveness and persistence) and pre-harvest with-holding days has been undertaken. The main objective of this study was to compare and contrast the environmental fate and behaviour of two fungicides registered for use in Australian viticulture, namely dithianon and vinclozolin, by :

1. Developing an accurate and precise analytical method for their detection and determination in extracts from aqueous, grapes, grape juice, wine and soil matrices.
2. Developing rapid, robust solid phase extraction methods for their extraction from aqueous, grapes, grape juice, wine and soil matrices.
3. Examining their persistence in aqueous solutions of different temperature and pH.
4. Examining their thermal stability.
5. Examining their persistence in grape juice and wine.
6. Examiningtheir stability towards photoradiation in both the solid and aqueous phases.
7. Examining their persistence on the surface of grapes under the vine canopy.
8. Determining their fate during the vinification process.

9. Examining their transport potential and mobility in soils typically used in Australian viticulture.

That this study was successful in achieving its aims and objectives is witnessed by the fact that :

1. Development of an accurate and precise analytical method for their detection and determination of dithianon and vinclozolin was relatively straightforward once the appropriate column, mobile phase and flow rates were determined.

2. The HPLC method developed greatly facilitated the development of rapid, yet robust, solid phase extraction methods for the extraction of these chemicals from aqueous, grapes, grape juice, wine and soil matrices once the appropriate volumes / quantities of matrix i.e. the maximum cartridge loading before analyte breakthrough was observed, and the appropriate cartridge conditioning, washing and elution parameters had been determined.

3. A linear relationship was observed between compound stability and decreasing pH i.e. lower the pH, the greater the persistence of dithianon and vinclozolin in aqueous solution. In the range pH 5.4 - 9.0, which covers the range typical for natural Australian waters, both dithianon and vinclozolin underwent hydrolysis readily. At 20 ^{o}C, the half-live of dithianon at pH 7.0 was 392 h., while that of vinclozolin was 59 h. A relationship was observed between compound stability and temperature i.e. lower the temperature, the greater the persistence of dithianon and vinclozolin in aqueous solution.

4. Both dithianon and vinclozolin are thermally unstable.

5. Both compounds are unstable in grape juice and wine. Dithianon is totally degraded within twenty-four hours of exposure to both red and white grape juice. The half-life in red grape juice was 4.5 h., that in white grape juice 2.8 h. Dithianon is totally degraded within five hours of exposure to red and white wines. The half-life was concentration dependent e.g. dithianon half-life in pinot noir (red) wine is 0.5 h. at an initial concentration of 1 mg/mL, but 13 h. at an initial concentration of 5 mg/mL. Vinclozolin is more stable in both red and white grape juice and wine, with a half-life of about 800 hours in both.

6. When exposed to South Australian summer sunlight, both dithianon and vinclozolin underwent aqueous phase degradation readily, although with the equipment available it was difficult to distinguish between thermal and photolytic degradation. The half-life of dithianon in aqueous solution was 1008 h., while that of vinclozolin was 17.5 h. The half-life of dithianon in the solid phase was 1632 h.

7. One week after spraying, there was no significant difference in the levels of dithianon on the surfaces of either red or white grapes, suggesting that dithianon persists in the conditions prevalent under the vine canopy. One week after spraying, there was a significant drop in the levels of vinclozolin found on the surface of both red and white grapes, suggesting that vinclozolin does not persist in the conditions prevalent under the vine canopy.

8. Analysis of wines made from grapes sprayed with dithianon found no detectable dithianon residues, suggesting that none of the residual dithianon found on the grape surfaces persists through the vinification process to the young wine. Analysis of wine made from grapes sprayed with vinclozolin indicated that about 10% of the residual vinclozolin found on grape surfaces persisted through the vinification process to the young wine.

9. Dithianon was immobilised in the top 2 cm of both clayey soil cores extracted from the Rutherglen region of Victoria and alkaline sandy loam soil cores extracted from the South Australian Riverland. However, dithianon was also relatively unstable, over 90% degrading in six weeks. Vinclozolin was mobile in acidic topsoil and subsoil clayey soil cores extracted from the Rutherglen region of Victoria (10 % of the applied dose leached through the cores in the first ten days), and the alkaline sandy loam soil cores extracted from the South Australian Riverland. However, vinclozolin was also unstable in the latter soil cores, with over 95% of the applied dose degrading in six weeks..

These findings suggest that both dithianon and vinclozolin containing fungicide formulations may be unstable in water, especially slightly basic waters, and that to ensure the efficacy of both these fungicides it is important to prepare the spray mix fresh with neutral or slightly acidic water. On the other hand, these results also suggest that should either dithianon or vinclozolin be accidentally released into Australian waters then their susceptibility to hydrolysis would suggest that they are likely to be rapidly hydrolysed, particularly in slightly basic waters, and therefore should pose little long term environmental threat. In addition, these results suggest

that dithianon and vinclozolin residues in wine are unlikely, again due to their proneness to hydrolysis, and that they should therefore pose little threat to human health through consumption of contaminated wines. However, significant residues do persist on grape skins and therefore care should be taken when using either compound on table grapes.

The main recommendations for further-research coming out of this study would be :

1. That the on-grape stability of dithianon be re-investigated under Australian conditions with a view to critically examiningthe current withholding period for dithianon use on table grapes.

2. That the stability of both dithianon and vinclozolin in aqueous buffered solutions be re-examined using alternate buffers, deionised and natural waters to determine their persistence in aqueous solutions of different temperature and pH, and to investigate the reaction kinetics further.

3. That the photolytic stability of both dithianon and vinclozolin in aqueous solutions and the solid phase be re-examined using sealed, natural-sunlight transparent, constant temperature experimental apparatus to determine whether the degradation observed during these studies was truly photolytic degradation, thermal degradation or a mixed pathway.

4. The above studies would be greatly facilitated if access was available to more sophisticated analytical equipment, in particular LC/MS instrumentation, on a

routine basis. This would greatly facilitate the identification of degradation pathways and products.

5. Although these chemicals degrade rapidly in water, there is still the possibility that large spills may have an adverse effect on local aquatic ecosystems in much the same way that endosulfan spills have embarrassed the cotton industry in New South Wales. A further recommended study would be to examine the effect of acute exposure to elevated levels of dithianon and vinclozolin on the hatchability and survivability of the eggs and larvae of native Australian aquatic invertebrates, fish and amphibians, their effect on growth of juveniles, fecundity of adults, their gross toxicities to native Australian aquatic organisms and any synergistic effects of temperature, pH, salinity etc.

6. Finally, examine the effect of these chemicals on the flora and fauna found in soils typically used in Australian viticulture, and the consequences of spills, spray drift on soil fertility and ultimately crop production.

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