AN ABSTRACT OF THE THESIS OF

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Title: MESUROL (METHIOCARB) RESIDUES IN GRAPES AND WINE AND THEIR POSSIBLE EFFECTS ON FERMENTATION AND WINE QUALITY

Abstract approved: D. A. Heatherbell

Wines were made from Pinot Noir and White Riesling grapes which had received field application rates of 2 and 4 lb./acre of the experimental bird repellent Mesurol (methiocarb or 3, 5 dimethyl-4(methylthio)phenylmethylcarbamate). In addition, Pinot Noir must and press juice and White Riesling press juice were fortified with 15 ppm Mesurol (active ingredient) and vinified.

Mesurol and its sulfoxide and sulfone metabolites were determined by gas chromstographic analysis as mesylate derivatives following fractionation on Florisil. Residues were also oxidized to and expressed as total sulfone.

Mesurol residues, methiocarb and total sulfone, in field treated grapes sprayed with 4 lb./acre Mesurol exceeded the 15 ppm temporary tolerance established by the Environmental Protection Agency.

Methiocarb residues in pomace, calculated on a dry weight basis, obtained from grapes harvested one day after the final application of Mesurol were in excess of the 75 ppm EPA temporary tolerance.

Approximately 40 to 51% of the methiocarb residues measured in field treated grapes remained associated with the pomace after pressing. Finished Pinot Noir and White Riesling wine, prior to bottling, contained in the order of 3-8 ppm representing 34 to 49% and 13%, respectively, of the methiocarb residues originally present in grapes.

The fate of Mesurol sulfide, sulfoxide and sulfone residues during the processing of Mesurol fortified White Reisling press juice was investigated. Settling of press juice was found to reduce methiocarb residues. Oxidation during vinification was minimal.

Mesurol sulfide, sulfoxide, and sulfone residues in finished wine remained unchanged after 12 months of storage at cellar temperatures. Trace amounts of Mesurol sulfide phenol were detected with Mass Spectrometry.

Volclay bentonite (KWK) fining at 4 and 8 lb./1000 gal. and membrane filtration (0.45 μ) were not effective in reducing Mesurol residues in wine.

Reference-difference tests were used to evaluate the effect of Mesurol on finished wine sensory qualities. The sensory properties

of wines made from grapes harvested seven days after the last application of Mesurol and Mesurol Fortified must and juices were not affected. Also, the chemical composition of these wines was not altered.

Lag time and fermentation rate of Mesurol Fortified Pinot Noir must and juice processed into wine were not affected. The course of fermentation in the presence of Mesurol was also monitored by recording mass loss in model studies. A slight inhibition in fermentation rate was observed at levels below 25 ppm while a stimulation was evident at concentrations of up to 500 ppm.

Mesurol (Methiocarb) Residues in Grapes and Wine and Their Possible Effects on Fermentation and Wine Quality

by

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INTRODUCTION

Grape growers contend with much adversity in order to harvest a successful crop. Bird damage to ripening fruit is undoubtedly one of the most frustrating problems confronting viticulturists today. A nationwide survey in 1972 revealed most producers believed that bird control measures available for grapes were either too expensive or ineffective. A minimum of 4.4 million dollars were reported lost as a result of avian mediated destruction (Zabadal and Hothem 1979).

Several techniques have been utilized to reduce bird damage. Birds are often frightened with propane exploders, shotguns, alarm devices, recorded distress calls or pyrotechnics. Netting, live trapping and poisoning are common alternatives. However, none of the measures currently applicable can be considered as absolute solutions.

A novel approach to bird control was introduced in field trials in California, New York and Oregon vineyards during 1978. The U.S. Fisheries and Wildlife Service (FWS) evaluated the potential use of the carbamate insecticide Mesurol or methiocarb as a chemical bird repellent on wine grapes. Bird damage was significantly reduced in

treated plots. Modifications in current application methods are expected to make Mesurol usage cost effective (Zabadal and Hothem 1979).

Prior to full registration, however, an enological evaluation of methiocarb was warranted. The stability of methiocarb residues in grapes, juices and wines; possible effects on alcoholic fermentation, wine chemical composition and sensory properties; and methods for residue reduction were not considered by governmental researchers. Therefore, this study was initiated to investigate these areas of concern.

LITERATURE REVIEW

Mesurol is a proprietary formulation of Chemagro Agricultural Division, Mobay Chemical Corporation, marketed as a 75% wettable powder. The active ingredient, methiocarb (3, 5 dimethyl-4(methylthio)phenyl-methylcarbamate) is effective in the control of numerous insects on fruit, field, and vegetable crops and ornamental flowers (Chemagro 1964).

Methiocarb is unstable in highly alkaline conditions and is susceptible to oxidation and hydrolysis (Starr and Cunnigham 1974). Methiocarb and its five known metabolites are presented in Figure 1.

Methiocarb has recently been recommended for combating several viticultural pests. Mesurol vineyard application is efficient in controlling the larvae of the variegated grape moth (Lobesia botrana) (Dirimanov and Kharizanov 1972), severe infestations of mole crickets (Gryllopta gryllopta) in young vineyards (Werth 1976) and overwintering grape leaf hopper [Erythroneura comes (Say)] adults (Taschenberg 1973). Van den Berg (1971) found methiocarb to be a reliable substitute for DDT (dichlorodiphenyltrichloroethane) in controlling various weevils capable of attacking South African vines.

The possible use of methiocarb as a chemical bird repellent was reported by Schafer and Brunton (1971). Field studies have

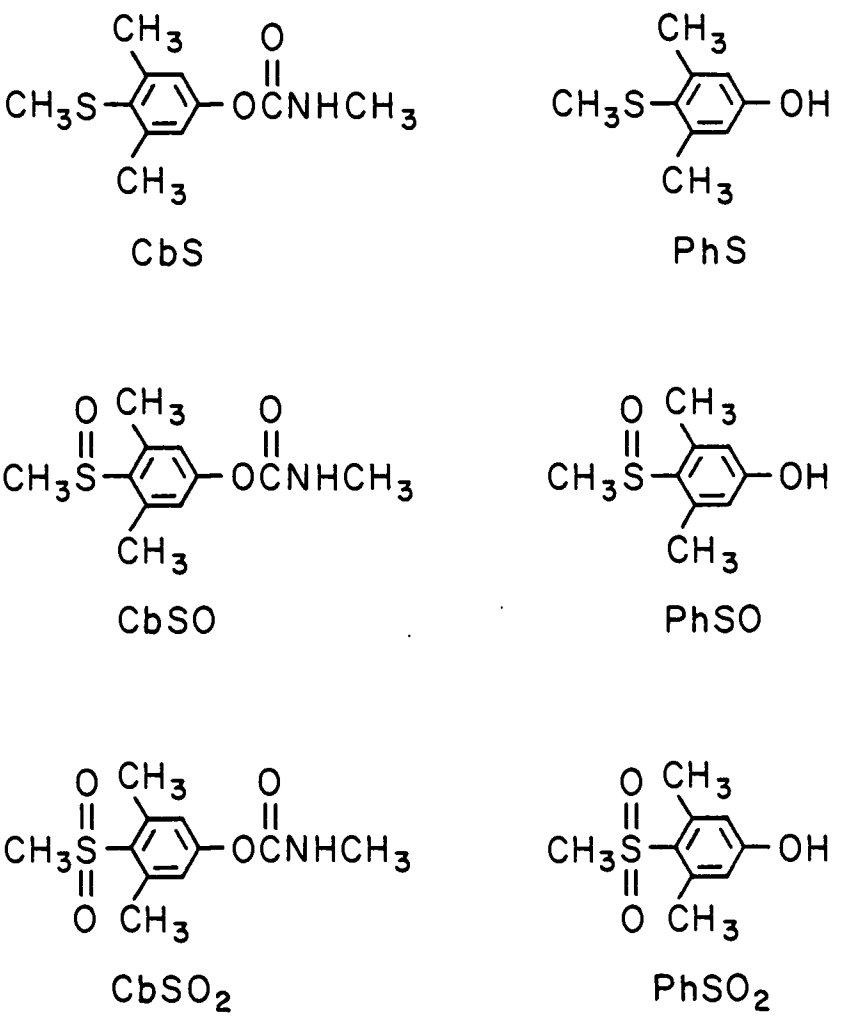

Figure 1. Methiocarb and its five metabolites. Cb = carbamate, Ph = phenol, S = sulfide, SO = sulfoxide, and SO_2 = sulfone.

shown Mesurol to be effective in reducing bird damage in: sprouting corn in South Dakota (West and Dunks 1969), Texas (Guarino and Forbes 1970) and South Carolina (Stickley and Guarino 1972); ripening rice in California De haven et al. 1971); ripening cherries in Michigan (Guarino et al. 1974); and ripening blueberries in Canada (Wood and Pierce 1977), Maine (Schmnitz 1976) and Michigan (Stone et al. 1974).

Bollengier et al. (1971) evaluated Mesurol vineyard application and resulting avian protection in New Hampshire. Random grape clusters sampled from the two most susceptible varieties weighed six times more than those taken from untreated vines. Mesurol sprayed on Gordo (Muscat of Alexandria) and Doradillo grapes was shown by Bailey and Smith (1979) to increase yields two fold (e.g. treated versus untreated) and to significantly reduce damage caused by blackbirds (Turdus merula) and grey breasted silvereyes (Zosterups lateralis). Methiocarb is an acetylcholinesterase inhibitor, however, its bird repellent properties are related to conditioned aversion (Rogers 1973). The compound is a potent emetic and recovery is complete (Anon. 1976). A variety of avian species plague American vineyards (Zabadal and Hothem 1979); robins (Turdus migratorius) predominate in Oregon (Mott et al. 1978).

Mesurol Residue Analysis

The development of reliable methodology for the analysis of carbamate pesticide residues in food stuffs and crops has been gradual. Numerous approaches have been attempted by researchers. Until recently, Mesurol residues were often determined by colorimetry, thin-layer chromatography or infrared spectroscopy (Broderick et al. 1966; Henkel 1966; Wales et al. 1968; Belliveau et al. 1970; El-Dib 1970; Mendoza and Shields 1970; Mendoza and Shields 1971; Mendoza and Shields 1973; Ernst et al. 1975).

Many carbamates, particularly N-methylcarbamates, are thermally labile and decompose to the corresponding phenol under normal gas chromatographic (GC) conditions (Zielinski and Fishbein 1965; Strother 1968). The development of heat stable derivatives that react with intact carbamates or their hydrolysis products have virtually eliminated this problem (Drozd 1975). Several derivatives permit the use of element specific detectors and have helped to improve sensitivity and selectivity (Drozd 1975).

Various GC analytical procedures for Mesurol residues have been reported in the literature. Van Middelem et al. (1965) determined Mesurol residues in plant tissues with GC/Electron Capture (EC) detection by brominating the phenol obtained from hydrolysis. Bache and Lisk (1968) used a microwave emission detector to

quantitate Mesurol as the phenol in fortified extracts. Bowman and Beroza (1969) were able to partition Mesurol residues in fortified apples, pears and corn into sulfide, sulfoxide and sulfone fractions with liquid-solid chromatography (LSC) on silica-gel; the carbamate and phenol present in each fraction were separated with LSC on alumina. The carbamates were subsequently hydrolyzed. Mesurol and its metabolites were then determined as the respective phenol with GC/Flame Photometric detection (FPD). Thornton and Drager (1973) oxidized the Mesurol sulfide and sulfoxide to the sulfone which was then silylated for GC/FPD total residue analysis. Mesurol analysis of plant materials was accomplished by Holden (1973) and Ernst et al. (1975) by producing the 2,4-dinitrophenyl or 2,6-dinitro-4 trifluoromethyl ethers of the sulfide phenol for GC/EC analysis. Lorah and Hemphill (1973) directly chromatographed Mesurol (methiocarb) using Chromosorb W support, surface modified with Carbowax 20 M, for Alkaline Flame Ionization detection.

The GC characteristics of Mesurol can be improved by derivatization of the NH group (Sieber 1978). Greenhalgh et al. (1976) was successful in derivatizing Mesurol and its metabolites, oxidative and hydrolytic, with trifluoroacetic anhydride (TFA). The analysis of Mesurol sulfide, sulfoxide, and sulfone in lowbush blueberries was reported by Greenhalgh et al. (1977) using TFA and GC/FPD; Silica gel was utilized to separate the Mesurol carbamates into

distinct fractions prior to derivatization. Mobay Corporation, Strankowski and Stanley (1975), recommends sulfide and sulfoxide oxidation to the sulfone, hydrolysis and silylation for GC/FPD analysis of Mesurol and its metabolites.

High Pressure Liquid Chromatography (HPLC) has been suggested for Mesurol quantitation. Mesurol was reacted with dansyl chloride for HPLC/fluorometric detection in soil and water samples (Frei et al. 1974). Direct quantitation of Mesurol in food stuffs was reported by Lawrence (1977) using HPLC/Ultra-Violet detection (UV). Reverse-phase HPLC/UV was applied by Aten and Bourke (1977) for Mesurol analysis in Brussel Sprouts. A post column flowogenic labeling reaction between O-pthaldehyde (OPA) and methylamine, a hydrolysis product of N-methylcarbamates, is described by Moye et al. (1973) for Mesurol residue analysis with a modular fluorogenic HPLC system.

The successful production of Mesurol mesylate derivatives and application to GC/FPD analysis was reported by Maitlen and Mc Donough (1980). The carbamate was hydrolyzed to the phenol in methanolic KOH and reacted with methane sulfonylchloride to produce the mesylate derivative. The structure of the Mesurol sulfide mesylate is shown in Figure 2.

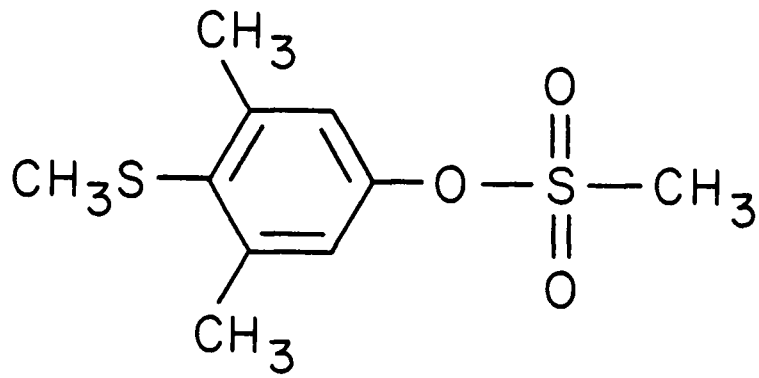

Figure 2. Structure of Mesurol sulfide mesylate.

Effects of Biocides on Alcoholic Fermentation

Many of the biocides available for use by viticulturists are important adjuncts in the control of vine diseases and pests. Fungicides, in particular, have been evaluated for potential wine yeast inhibitory properties. Wine yeast, Saccharomyces cerevisiae, are members of the fungi class Ascomycetes (Phaff et al. 1966).

Castor et al. (1957) reported a one day delay in the onset of fermentation of California grapes containing one ppm Captan residues. Captan, N-(trichloromethylthio)-4-cyclohexene-1,2-dicarboximide, is active against bunch rot caused by Botrytis cinera. Adams (1960, 1968) demonstrated a delay in cherry and grape juice fermentations with levels of Captan and Folpet as low as 1-2 ng/ml. Minarik and Ragala (1966), and Van Zyl and Du Plessis (1961) described a similar inhibitory effect of Captan residues during grape juice fermentation.

Canterelli et al. (1964) studied the effects of Zineb preparations

(zinc ethylenebisdithiocarbamate) on winemaking. Dithiocarbamates are useful in the control of grape mildews. Wine yeast were able to grow and ferment in concentrations of up to 500 ppm Zineb. Some stimulation in the rate of fermentation at high levels was evident. Minarik and Ragala (1966) concluded that Zineb formulations had no significant influence on wine yeast. Lemperle (1973) found that the dithiocarbamates, Methylmetriam and Mancozeb, did not affect fermentation when used in recommended quantities.

Adams (1968) tested ten fungicides and six insecticides commonly used in wine production for the ability to inhibit yeast growth and fermentation. Twelve wine yeast strains were screened; differences in sensitivity to these compounds were demonstrated. Six of the biocides failed to show any effect at concentrations as high as 1,000 ppm during fermentation.

Eschenbruch (1972) showed that Benomyl (a semi-systemic fungicide used to control Botrytis cinera) delayed grape juice fermentation at 200 ppm. It was concluded that Benomyl did not influence wine yeast at concentrations prescribed commercially. Inhibitory levels would be difficult to attain from normal field application. However, Beuchat (1973) reported that Benomyl inhibited peach pulp fermentations at levels as low as 5 ng/ml. Studies conducted by Marais and Kruger (1976) demonstrated a delay in the fermentation

of grape juice containing 50 mg/L Benomyl. The fermentation of grapes sprayed at suggested application rates was not affected.

MATERIALS AND METHODS

Field Treatment and Sampling

Wine grapes, in 70-100 lb. lots, were obtained from field trials conducted by Mobay Chem. Corp. and FWS in Oregon commercial vineyards in the fall of 1978. Pinot Noir and White Riesling grapes were sprayed with 4.0 lb. (active ingredient) of Mesurol (75% wettable powder, Mobay Chem. Corp., Kansas City, Mo.) in 30 gal. of water with a Foliar High Concentrate Ground Sprayer. Each variety received four applications. Treated and control fruit were harvested at one and seven days following the last Mesurol treatment. Grapes were transported the same day to Corvallis and processed into wine at the OSU winery.

In addition, treated and control Pinot Noir grapes were obtained from an efficacy trial administered by FWS. Efficacy sites were sprayed twice with 2.0 lb. (active ingredient) of Mesurol in 50 gal. of water. These grapes were processed into wine by a commercial winery.

Randomly sampled 3-5 lb. lots of fruit were stored frozen at -10°F until analyzed.

Vinification

Yeast Starter

A two percent inoculum of rehydrated dry wine yeast, champagne strain (Vi-A-Dry, Scott Labs., San Rafael, Cal.) was prepared in 1:1 grape juice-water at 105°F. The rehydrated culture was incubated at room temperature for three hours.

White Riesling Processing

Field treated fruit and controls were crushed and pressed immediately at the OSU winery. A portion of the one day control[1] was subdivided and fortified to 15 ppm (mg active ingredient of methiocarb/kg juice) with Mesurol 75% wettable powder. A 1% solution of $K_2S_2O_5$ was used to adjust SO_2 levels to 50 ppm (mg/L); juices were allowed to settle overnight. The settled juice was racked into five gallon glass carboys and inoculated with 2% yeast starter. The containers were sealed with fermentation locks. All wines were fermented to dryness at 58°F, racked twice and held at 38°F until bottled.

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[1] Abridged description of Mesurol treatments utilized are presented in Table 21, appendix.

Pinot Noir Processing

Field treated fruit and controls were crushed; 50 ppm SO_2 was added. A 15 ppm (active ingredient) 75% wettable powder fortification was prepared from the seven day control. One half of the fortified must was fermented on the skins while the remainder was pressed and fermented without the skins. Appropriate controls were prepared by dividing the seven day control. All samples were inoculated with a 2% yeast starter. Musts were fermented until 2-3 °Brix, pressed and fermented to dryness at 68 °F in ten gal. plastic tubs. The seven day press juice spike and control were taken to dryness at 68 °F. Wines were racked three times and held at 38 °F until bottled.

Bottling

Wines were adjusted to 30 ppm free SO_2 and bottled without filtration approximately 11 months after crushing.

Wine and Must Analysis

The recommended procedures of Amerine and Ough (1974) were used for the determination of pH, titratable acidity, reducing sugar (Lane and Eynon), volatile acidity, alcohol (micro distillation, dichromate procedure), soluble solids (refractive index), total

phenol, malic acid (paper chromatography), and color in wine and or must/juice samples.

Mesurol Residue Analysis

Samples were stored frozen at -10°F and warmed to room temperature prior to extraction.

Total Residue Analysis as the Mesurol Sulfone

Extraction

Grapes. Fifty grams of fruit (randomly sampled), 50 ml. acetonitrile (Baker 'Resi Grade'), 15 ml. precipitating solution (25 ml. of concentrated H_3PO_4 and 1.25 g reagent grade NH_4 Cl per liter) and five grams Hyflo Supercell (Johns Mansville) were blended in a Sorvall Omnimixer for one minute at high speed. The extract was filtered under vacuum through an 11 cm Buchner funnel with Reeve Angel 202 filter paper. The filter cake was remacerated with 50 ml. acetonitrile and 15 ml. precipitating solution. The extract was recovered with vacuum filtration. The mixer cup and filter cake were washed with 25 ml. acetonitrile. The combined extracts were transferred to a 250 ml. separatory funnel. The upper layer was collected. The remaining aqueous phase was extracted with 50 and 25 ml portions of acetonitrile. The combined acetonitrile extracts were stored at 4°C for one hour. The acetonitrile extract was returned to the funnel; any water that might have separated during cooling was removed. The acetonitrile was taken to near dryness (< 5 ml.) in

a Buchii rotary evaporator (34 °C). Ten ml. of 10% NaOH (w/v) was added to the flask; the contents were transferred to a 250 ml. separatory funnel and backwashed with three 10 ml. portions of chloroform. The chloroform was collected and discarded. The aqueous phase in the separatory funnel was acidified with 15 ml. of 5% sulfuric acid (v/v) and extracted with three 25 ml. portions of chloroform. The round bottom flask was rinsed with the preceding chloroform fractions prior to extraction of the aqueous layer. All final chloroform extracts were combined in an additional 500 ml. round bottom flask; the chloroform was removed with a rotary evaporator (34 °C).

Juice and Wine. Fifty ml. of wine or juice samples and five grams Hyflo Supercell were filtered through an 11 cm. Buchner funnel, with Reeve Angel 202 filter paper, into a collection flask using vacuum. The filtered sample was transferred to a 250 ml. separatory funnel. The filter cake was rinsed with 50 ml. acetonitrile. The acetonitrile was transferred to the separatory funnel; an additional 50 ml. of solvent was added. The funnel contents were extracted for 30 sec. The remaining extractions were identical to those utilized in fruit analysis. Ten grams of reagent grade NaCl was added to wine samples prior to the initial acetonitrile extraction. Ten percent ethyl ether/acetonitrile was used in the extraction of several problem samples to reduce emulsions.

Oxidation

Mesurol residues (fruit, juice and wine) were oxidized to the sulfone with 4 ml. of 1% m-chloroperbenzoic acid (w/v) at room temperature for 30 min. Twenty-five ml. of a saturated solution of Na_2SO_3 was added to the round bottom flask; the contents were transferred to a 250 ml. separatory funnel. Twenty-five ml. of saturated $NaHCO_3$ were placed into the funnel; the aqueous mixture was gently agitated. The aqueous layer was then extracted with three 25 ml. aliquots of chloroform. The combined organic extract was transferred to a 500 ml. round bottom flask. Solvent was removed with a rotary evaporator (34 °C).

Derivatization

The Mesurol sulfone phenol, prepared by alkaline hydrolysis, was converted to the mesylate derivative using the procedure described by Maitlen and McDonough (1980). One ml. of 0.25 M KOH in methanol was added to the round bottom flask used for oxidation. The flask was stoppered and allowed to stand at room temperature for 15 min. Two ml. of 5% pyridine (v/v) in benzene (redistilled) and 1 ml. of 1% acetophenone (v/v, reagent grade) in benzene (keeper solution) were placed into the flask. The solvents were removed under a dry air jet at room temperature. Two ml. of 1% methane

sulfonylchloride (v/v, Aldrich Co.) in benzene were added; the flask was sealed and stored at ambient temperature for 30 min. The flask contents were taken to dryness with an air jet at room temperature. The residues were diluted with benzene (redistilled), as needed, for gas chromatographic analysis.

Gas Chromatographic Equipment and Conditions

A Varian 3700 equipped with a dual flame photometric detector operated in the sulfur mode was used. The column, 18 in. by 2 mm. i.d. glass, was packed with 5% OV-101 on 120/140 mesh Chromosorb HPW. GC operating conditions were: column temperature 195°C, detector temperature 230°C, carrier gas flow 30 ml. N_2/min., gas flows to the detector 80, 170 and 140 ml./min. for air one, air two and hydrogen, respectively. The peak heights of a Mesurol sulfone standard and sample were matched in triplicate. The square root of the peak height (cm) for each standard and sample were derived and compared to determine the total residues as the sulfone present in the sample.

Mesurol Sulfide, Sulfoxide and Sulfone Analysis

Parent compound analysis was performed on grape, pomace, grape juice and wine samples from all trials. Cellar and bottle-aged

wine produced from one day Pinot Noir grapes and samples obtained during the processing of the White Riesling press juice fortification series were analyzed for Mesurol sulfide, sulfoxide and sulfone.

Extraction

The initial extraction of fruit juice and wine samples with acetonitrile was identical to that used for "Total Residue" analysis. However, 100, 50 and 50 ml. portions of solvent were used. The combined acetonitrile extracts were transferred to a two L. separatory funnel. Fifty ml. of chloroform (redistilled) was added; the contents were gently mixed for 30 sec. and 800 ml. of distilled water added. The aqueous phase generated was extracted with the chloroform previously added. The organic layer was drained through anhydrous Na_2SO_4 into a 500 ml. round bottom flask. The aqueous phase was re-extracted with 50 ml. chloroform. The chloroform extracts were combined in the flask and taken just to dryness with a rotary evaporator (34 °C).

Derivatization

Mesurol residues were derivatized with methane sulfonylchloride using the procedure previously described for "Total Residue" analysis.

Partitioning and Clean-up

A Florisil chromatographic column was prepared as follows: a plug of glass wool, 1/2 in. anhydrous Na_2SO_4, 10 grams Florisil (Floridin Company, 100/200 mesh, activated at 450°C for 12 hours, stored at 130°C), 1/2 in. Na_2SO_4 were added successively to an 18 mm i.d. x 22 mm. o.d. glass column equipped with a teflon stopcock. The column was packed in 50 ml. hexane (redistilled). Residues in the derivatization flask were solubilized with 2.5 ml methylene chloride (Baker 'Res.' Grade). The precipitate present in the flask was gently scrapped into the solvent with a capillary pipette. The methylene chloride was transferred to the Florisil column quantitatively with the same pipette. The solvent rinse and transfer were repeated. The combined methylene chloride washes (5 ml) were allowed to drain to the top of the salt bed. Three additional residue rinses and transfers (5 ml.) were performed and placed successively on the column.

The Mesurol sulfide and sulfone mesylate were eluted with 80 ml. 10% acetone/methylene chloride (v/v, Baker 'Resi-Grade'). The eluant was collected in a 250 ml. round bottom flask. The Mesurol sulfoxide mesylate was eluted with 50 ml. 50% acetone/methylene chloride and received in an additional 250 ml. round bottom flask. Solvents were removed with a rotary evaporator

(34 °C). Residues were solubilized in benzene and diluted as necessary for GC analysis.

Gas Chromatographic Equipment and Conditions

GC equipment, conditions and data evaluation were identical to those used in "Total Residue" analysis. Appropriate Mesurol sulfide, sulfoxide and sulfone mesylate standards were prepared. In addition, column temperatures of 170° and 175 °C were utilized in parent compound analysis of fruit samples.

Modifications in Fruit Extraction

Three hundred grams of fruit were ground with an equal weight of dry ice in a commercial Waring Blender. The samples were placed in a -10°F freezer to permit the sublimation of the dry ice. Ten percent ethyl ether (redistilled) was added to the acetonitrile used in the initial extraction and 16 grams NaCl (reagent grade) to the water necessary for the chloroform extraction to reduce emulsion problems. A 1:1 hexane (acetonitrile saturated)-acetonitirle backwash was used to remove non-polar oils. Clean-up was accomplished with Florisil LSC described elsewhere. Only parent compound (methiocarb) analysis was performed on fruit samples.

Modifications in Pomace Extraction

Frozen pomace samples were ground in a Hobart food chopper. A 50 g. subsample was placed in a Sorvall Omnimixer with 150 ml. acetonitrile, 25 ml. precipitating solution, 5 g. Hyflo Supercell and blended at high speed for 1 minute. The extract was recovered by filtering the contents through a 11 cm. Buchner funnel, with Reeve Angel 202 filter paper, into a filter flask under vacuum. The filter cake was remacerated with 100 ml. acetonitrile and 25 ml. precipitating solution. The second extract was separated from pomace solids with vacuum filtration. The filter cake was rinsed with 50 ml. acetonitrile. Twenty-five ml. portions of treated samples and a 50 ml. aliquot of control were sampled from the combined acetonitrile extracts and transferred to 250 ml. separatory funnels. A 4x acetonitrile volume (present in funnel) of distilled water was added to each sample. The aqueous phase produced was extracted with 25 ml. chloroform for one minute; the chloroform was filtered through anhydrous Na_2SO_4 into a 250 ml. round bottom flask. The aqueous layer was re-extracted with 25 ml. of chloroform. The combined chloroform extracts were taken just to dryness with a rotary evaporator (34°C). The Mesurol sulfide was measured in pomace samples; clean-up was not essential. Total methiocarb residues were calculated using the appropriate dilution factor.

Simultaneous with Mesurol analysis, moisture determinations were run on each pomace sample (A.O.A.C. 7.003).

Mass Spectral Confirmation

The presence of Mesurol sulfide, sulfoxide and sulfone residues in wine was confirmed by gas chromatograph-mass spectrometry (GC-MS). A Finnigan Gas Chromatography/EI-CI Mass Spectrometer System was used. The ionizing voltage was 10 eV; the source temperature 300°C; separator over, temperature 27°C; injection port temperature 275°C and the column temperature 240°C. The gas chromatograph was equipped with a 6 in. x 2 mm. i.d. glass column packed with 3% OV-17 on 100/200 Chromosorb HPW.

Determination of Mesurol Sulfide Phenol

Wines were extracted with acetonitrile and chloroform (see Extraction. Mesurol sulfide, sulfoxide and sulfone analysis). The combined chloroform extracts were removed with a rotary evaporator (34°C). Mesurol residues were quantitatively transferred in benzene to a graduated 10.0 ml. concentration tube with a capillary pipette. The solvent volume was reduced to 0.25 ml. under an air jet. Approximately 40% of extract was injected into a Waters Associates Liquid Chromatograph equipped with a differential UV

detector (254 nm); a 25 cm. x 4.6 mm. i.d. stainless steel column, packed with a 5 μ Lichosorb silica was used. The instrument was operated under isocratic conditions with 5% isopropanol/trimethylpentane (Baker 'HPLC Grade'). Mesurol sulfide phenol and carbamates standards in benzene or methanol were used to determine retention volumes. The Mesurol sulfide phenol observed was collected as it eluted in successive injections totaling 100 μl in 20 x 150 mm test tubes. The trapped solvent was quantitatively transferred to a 250 ml. round bottom flask and removed with a rotary evaporator (34 °C). The Mesurol residues were transferred in benzene (capillary pipette) to a graduate 10.0 ml. screw cap tube. The benzene was concentrated to 0.5 ml under an air jet. Twenty-five μl of Pierce BSA N-o-5.3-(trimethylsilyl)acetamide were added. The tube was capped.

The Mesurol sulfide phenol was determined as the trimethylsilyl derivative by comparing the intensity of the 240 m/e ion obtained from Mass Spectrometry with that of a standard. A Finnigan Gas Chromatography EI-CI Mass Spectrometer System was employed for the analysis. A 6 ft. x 2 mm i.d. glass column packed with 3% OV-101 on 100/200 mesh Chromosorb HPW was used. Operating conditions were: source voltage 70 eV, source temperature 300°C, separator over 275 °C, injector port 275 °C, and column temperature 220°C.

Bentonite Fining of Mesurol Treated Wine

A 30 g/L suspension of Volclay (KWK) bentonite was prepared in distilled water and heated to ambient boiling three times prior to use. One ml. of the suspension in 250 ml. of wine is equivalent to 1 lb/1000 gal. Mesurol treated wines were fined at the equivalent rate of 4 and 8 lb./1000 gal. in 250 ml. screw cap bottles. After thoroughly mixing samples were settled for 2 days, racked, rough filtered under vacuum (Whatman number 1) and filtered through a 0.65 μ Millipore membrane (Millipore cartridge syringe) into 60 ml. bottles. A 50 ml. aliquot of the control was filtered through a 0.45 μ Millipore membrane prior to analysis. The control and three treatments were analyzed for Mesurol sulfide, sulfoxide and sulfone using GC/FPD as previously described.

Sensory Evaluation

Three 750 ml. bottles of wine, two control and one treated, were used for each trial. Twenty ml. serving portions were measured into 6 oz. wine glasses. The glasses were placed on trays and served to judges sitting in individual testing booths. Each test tray consisted of three glasses. One glass labeled "Ref." contained the control sample. The remaining two glasses were coded with three digit random numbers and contained a duplicate control sample and the treated sample.

The judges were asked to score the coded samples in direct relation to the "Ref." sample on a scale from one to seven defining desirability and overall difference. Refer to example score card, Figure 3. Trials with common controls were combined.

The 20 judges performed two replicate tests on each lot of samples during a one hour test period. The judges were experienced in reference-difference methodology. They were not, however, selected for wine tasting ability. The data was analyzed with three factor analysis of variance and evaluated by mean flavor scores and treatment least significant differences (LSD) at the 5% level.

Fermentation Studies

Model System

One hundred ml. of grape juice (composite sample, Vitis vinifera) were placed into 250 ml. Erlenmeyer flasks. Aqueous suspensions of Mesurol 75% wettable powder were prepared in solutions containing 5 g/L potassium bitartrate, 0.2 g/L citric acid and 3 g/L malic acid. The concentration of methiocarb in the suspensions was such that a 5 ml. aliquot would yield the desired level of active ingredient in 105 ml. Triplicate Flasks were prepared with 500, 250, 100, 50, 25, 15, 5 and 0 ppm (mg/L) methiocarb.

Five grams of Vi-A-Dry Yeast, Champagne strain, were added

Dept. of Food Science & Technology
Oregon State University

Product: _____ Name _____

Date _____

1. Please SMELL - TASTE - EXPECTORATE the samples.
2. Compare the flavor of the coded samples in direct comparison to the reference sample.
3. Score the desirability of the coded samples.

Reference-Difference		Over-All Desirability	
_____	Same as Reference	_____	Very Desirable
_____	Slight	_____	Moderately Desirable
_____	Moderate	_____	Slightly Desirable
_____	Pronounced	_____	Neutral
_____	Strong	_____	Slightly Undesirable
_____	Very Strong	_____	Moderately Undesirable
_____	Extremely Different	_____	Very Undesirable

If different, please describe the difference:

Figure 3. Sample scorecard used in sensory analysis.

to 300 ml. of 1:1 juice-distilled water at 105°F. The yeast starter was incubated at room temperature for six hours. Flasks were inoculated at a rate of 2% and sealed with fermentation locks. The course of fermentation was determined by recording mass loss with a Mettler P 1210 balance at regular intervals after inoculation. Mass loss values were calculated by averaging the results obtained in triplate for each concentration.

Pinot Noir Press Juice and Must Mesurol Fortification Trials

Samples were secured from Pinot Noir press juice and must fortification (15 mg methiocarb/kg must) trials in approximate 12 hour intervals. (See Vinification, Pinot Noir Processing.) Fermentation was monitored in both series by measuring the percent alcohol (v/v) in the samples (see Wine and Must Analysis).

RESULTS AND DISCUSSION

Methiocarb and Total Mesurol Residue Analysis

The precision of gas chromatographic measurements was estimated by determining the relative standard deviations of detector response factors (K) for Mesurol sulfide, sulfoxide and sulfone mesylate standards injected during a single analysis period. These data are presented in Table 1. A greater variation with the Mesurol sulfoxide mesylate is observed. The increased sensitivity (i.e.- lower minimum detectable level) for the Mesurol sulfide mesylate, relative to Mesurol sulfoxide and sulfone mesylates, was expected because of the lesser polarity of the compound.

Typical GC chromatograms of Mesurol sulfide, sulfoxide and sulfone mesylates and controls are shown in Figure 4. The retention times of Mesurol sulfide, sulfoxide and sulfone mesylates at 195 °C are 1.0 min., 2.5 min., and 2.6 min., respectively. Mesurol sulfide and sulfone mesylates elute off the Florisil column in the primary cut (see Materials and Methods. Mesurol sulfide, sulfoxide and sulfone analysis. Partitioning and clean-up). The Mesurol sulfoxide eluted last during Florisil clean-up in a separate fraction.

The results of replicate methiocarb analysis are presented in Table 2. Wine A averaged 3.4± 0.8 ppm while the mean

Table 1. Detector response factors (K)[1] and minimum detectable levels of MS, MSO and MSO_2 mesylate derivatives.

Compound	K[2]	std. dev.	Minimum Detectable Level (ppm)
Mesurol sulfide	0.289	0.014	0.01
Mesurol sulfoxide	0.108	0.007	0.03
Mesurol sulfone	0.121	0.006	0.04

[1] $K = (\text{Peak height, cm})^{1/2}/\text{ng injected.}$

[2] Calculated from 6 determinations during one G.C. analysis period.

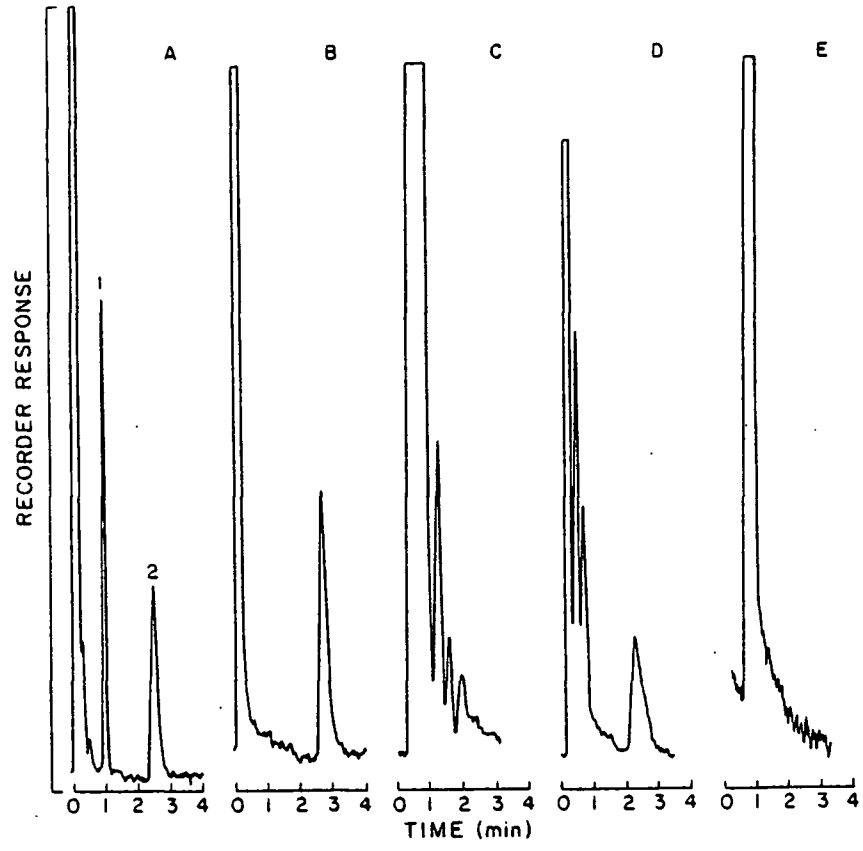

Figure 4. Representative GC chromatograms of Mesurol mesylate derivatives and blanks. Column temperature - 195°C. Attenuation - 8X. (A) Separation of Mesurol sulfide (peak 1) and sulfone (peak 2) Mesylate derivatives (B) Mesurol sulfoxide mesylate. (C) White Riesling juice control, primary. Florisil cut (sulfide and sulfone mesylates). (D) White Riesling juice control, final Florisil cut (sulfoxide mesylate) (E) Pinot Noir juice control, Mesurol total sulfone residue analysis (oxidation).

Table 2. Methiocarb analysis of Pinot Noir wines.[1]

Wine A: methiocarb ppm[2]	3.7	3.6	4.0	2.3
Wine B: methiocarb ppm[3]	6.9	7.6	7.6	7.3[4]

[1] Replicate analysis performed over five month period. Samples stored at -10°F.

[2] Commercial Trial wine.

[3] Wine processed from fruit harvested one day after the last application of Mesurol.

[4] Florisil clean-up.

for the B series was 7.4±0.3 ppm. The preceding represents an acceptable degree of precision for methiocarb analysis. Analysis of these wines was performed on frozen samples over a five month period. Clearly, the data also suggests minimal degradation during frozen storage at -10°F. Repeated analysis of frozen samples over an eleven month period also indicated minimal degradation during frozen storage.

Mass Spectral analysis following mesylate derivatization confirmed the presence of methiocarb in a control wine. Background responses were substracted in all recovery studies.

Methiocarb and Total Mesurol Residues in Fruit

Table 3 contains the residues determined on field treated fruit using parent compound (methiocarb) and total sulfone (oxidation) analysis techniques. In no instance are residues in grapes sprayed at 4 lbs./acre, detected by either method, less than the 15 ppm temporary tolerance established by the Environmental Protection Agency.[2] Total sulfone levels ranged from 19 to 36 ppm. The

[2]Mobay Corporation obtained a temporary residue tolerance of 15 ppm as the total sulfone (MSO_2) on wine grapes and an Emergency Use Permit (No. 3125-EUP-140) from the EPA to allow expert mental vineyard application and testing in the fall of 1978. The 15 ppm limit also encompassed products derived from fruit during the winemaking process (e.g.-juice and wine). A 75 ppm tolerance was established for grape pomace, wet or dry.

Pinot Noir total sulfone residues increased in the seven day sample from 22 to 36 ppm. The increase in Mesurol residues in Pinot Noir grapes may be related to sampling technique; exposed berries were removed from whole clusters. Bailey and Smith (1979) observed a gradual reduction of Mesurol residues in Gordo grapes; Mesurol residues, as the sulfone, between 28 and 30 ppm declined to 1.3 ppm in 28 days. It should be noted, however, that methiocarb residues decreased in the seven day samples of Pinot Noir and White Riesling grapes. Greenhalgh et al. (1977) reported a similar loss in methiocarb residues in lowbush blueberries and indicated field oxidation to the sulfoxide was a contributing factor.

Recovery data for methiocarb and total sulfone analysis in Pinot Noir grapes are summarized in Tables 4 and 5, respectively. Methiocarb recoveries averaged 46±12% fortified at 1.0 and 4.0 ppm while the mean total residue recovery with 0.5 ppm Mesurol sulfoxide or sulfone spikes was 96±7.9%. Greenhalgh et al. (1977) found that methiocarb was oxidized to the sulfoxide when solutions were taken to dryness with a rotary evaporator during extraction and clean-up steps. A rotary evaporator is utilized in this manner in the mesylate procedure and could account for low recoveries in fruit. Despite low recoveries, Mesurol residues determined with both techniques, parent compound and total sulfone, are similar. Oxidation converts most

Table 3. Methiocarb (MS) and total[1] Mesurol (MSO_2) residues in field treated grapes.

Fruit	Application rate	Days after last application			
		1 day		7-days	
		MS ppm	MSO_2 ppm	MS ppm	MSO_2 ppm
White Riesling	4 lb./acre	38	25	25	19
Pinot Noir	4 lb./acre	22	22	16	36
Pinot Noir	2 lb./acre	12	--	--	--

[1] Residues oxidized to and expressed as total sulfone.

Table 4. Recovery of methiocarb from fortified Pinot Noir grape samples.

Added ppm	Replicate A % Recovery	Replicate B % Recovery	Replicate D % Recovery
1.0	44	40	34
4.0	68	46	46

Table 5. Recovery of Mesurol sulfoxide (MSO) and sulfone (MSO_2) as the total sulfone[1] from 0.5 ppm fortified Pinot Noir grape samples.

Compound	Replicate A % Recovery	Replicate B % Recovery
Mesurol sulfoxide	98	84
Mesurol sulfone	98	102

[1] Residues oxidized to and expressed as total sulfone.

tissue extractives and pigments to a water soluble form, thus facilitating their removal (Thornton and Droger 1973). Better recoveries might be expected with the total residue oxidation technique with the elimination of these potential interferences. In addition, it is possible for extracted plant material to occlue pesticides (Bowman and Beroza 1969). In this study there was no evidence of methiocarb loss during residue partitioning with solvents or on Florisil columns. Radiotracer studies would be necessary to effectively resolve recovery losses.

Methiocarb Residues in Pomace

Methiocarb residues in pomace ranged from 16 to 40 ppm on a wet weight basis in pomace produced during the vinification of Mesurol field treated grapes and fortified Pinot Noir must (15 mg methiocarb/kg juice), Table 6. The pomace from the one day Pinot Noir and White Riesling series contained 101 and 142 ppm methiocarb on a dry weight basis, respectively, in excess of 75 ppm EPA tolerance. Sixty-five, 62 and 52 ppm on a dry weight basis were found in commercial, must fortification, and seven day Pinot Noir trial pomace, respectively. A 71% recovery of a 6.0 ppm methiocarb spike was obtained (Table 6). Calculations based on observed press yields indicate that 40-51% of the methiocarb residues remain associated with pomace after pressing grapes which had received Mesurol application

Table 6. Methiocarb residues in pomace from field treated grapes and fortified[1] must.

Sample	Percent Moisture	Methiocarb ppm wet weight	Methiocarb ppm dry weight
Pinot Noir commercial trial	60	26	65
Pinot Noir 1-day field trial	72	28	101
Pinot Noir 7-day field trial	70	16	52
Pinot Noir must fortification trial	74	16	62
White Riesling 1-day field trial	72	40	142
Pinot Noir 300 μg[2] fortification 7-day control	74	4.2[3]	16[3]

[1] 15 mg methiocarb/kg must.

[2] 50 grams sample, 6.0 ppm at blender.

[3] 71% recovery.

rates of 4 lb/acre.

The commercial trial Pinot Noir pomace consisted of approximately 60% moisture compared to the 70% or greater values measured in OSU processed samples. This might be related to the differing press efficiencies observed in the two wineries. Thus, calculation of methiocarb pomace residues on a dry weight basis provides a more accurate comparison of samples produced using unstandardized practices.

Methiocarb and Total Mesurol Residues in Juice and Wine

Methiocarb and total Mesurol residues were reduced in White Riesling wine when compared to levels detected in the respective musts (Table 7). Mesurol is insoluble in aqueous solutions (Chemagro 1964). Therefore, residual losses could be accounted for with juice settling or successive rackings. However, residues in Pinot Noir wine did not greatly differ from must values. The higher residues observed in Pinot Noir wines might be the result of ethanolic extraction (e.g.-fermentation) prior to pressing. A comparison with Table 3 indicates finished Pinot Noir wine, prior to bottling, contained 34 to 49% of the methiocarb residues originally present in the fruit while White Riesling wine retained 13%. Results detected by both techniques in common samples are similar.

Table 7. Methiocarb (MS) and total[1] Mesurol (MSO_2) residues in must and wine[2] produced from field treated grapes.[3]

Sample	Days after last application			
	1-day		7-days	
	MS ppm	MSO_2 ppm	MS ppm	MSO_2 ppm
White Riesling Juice	15	12	5.1	8.8
White Riesling wine	4.8	8.0	3.3	4.9
Pinot Noir must[4]	10	9.9	7.1	5.7
Pinot Noir wine	7.4	9.7	7.9	4.6

[1] Residues oxidized to and expressed as total sulfone.

[2] 2 months at 55°F, 6 months at 38°F.

[3] 4 lb. active ingredient, 4 applications, 30 gal./acre.

[4] Fermented on skins juice.

Total Mesurol residues should be higher than parent compound levels if oxidation occurred in the field or during processing.

Methiocarb recoveries as the total sulfone in Pinot Noir and White Riesling juice and wine (0.5 ppm) averaged 88±5.4% (Table 8). The mean recovery for methiocarb fortifications (0.5 ppm) in White Riesling juice and wine were 82±6.9% and 75±18%, respectively (Table 9).

Fate of Methiocarb During the Vinification of Artificially Fortified White Riesling Press Juice and Pinot Noir Must

White Riesling press juice which was fortified to 15 ppm methiocarb contained 10.4 ppm Mesurol sulfide, 0.6 ppm Mesurol sulfoxide and 0.1 ppm Mesurol sulfone (Table 10). Natural sedimentation (overnight) reduced methiocarb juice levels to 6.2 ppm; 29.3 ppm methiocarb were detected in the sediment after racking. Less than one ppm Mesurol sulfoxide or sulfone were detected in the settled juice. Adsorption of methiocarb to sedimented particulate matter or co-precipitation, therefore, provides an important route for residue reduction in the juice stage of white table wine production. However, the data suggests that fermentation lees would not contain significant quantities of Mesurol residues; 1/2 fermented juice and cellared wine had identical residues for Mesurol sulfide,

Table 8. Recovery of methiocarb (MS) as the total sulfone (MSO_2) from 0.5 ppm fortified grape juice and wine.

Variety	Grape Juice % Recovery	Wine % Recovery
Pinot Noir	83	94
White Riesling	90	83

[1] Residues oxidized to and expressed as total sulfone.

Table 9. Recovery of methiocarb (MS) from 0.5 ppm fortified White Riesling juice and wine.

Sample	Replicate A % Recovery	Replicate B % Recovery	Replicate C % Recovery	Replicate D % Recovery
Juice[1]	84	72	84	88
Wine	56	64	92	88

[1] Florisil clean-up.

Table 10. Mesurol sulfide, sulfoxide and sulfone residue partitioning observed during the processing of fortified[1] White Riesling press juice.

Sample	Mesurol Sulfide (p.p.m.)	Mesurol Sulfoxide (p.p.m.)	Mesurol Sulfone (p.p.m.)
Press Juice	10.4	0.6	0.1
Sediment	29.3[2]	0.7[2]	0.5[2]
Settled Juice	6.2	0.2	0.9
1/2 Fermented Juice	2.7	0.2	0.1
Wine[3]	2.7	0.2	0.1

[1] 15 mg methiocarb/Kg juice.

[2] w/w

[3] Sampled from cellar, 38°F, approximately 8 months following fermentation.

sulfoxide and sulfone: 2.7 ppm, 0.2 ppm and 0.1 ppm, respectively. Thus, sedimentation following alcoholic fermentation may not be a viable mechanism for reducing Mesurol residues in wine.

Oxidation of methiocarb to the sulfoxide or the sulfone was minimal during processing; sulfoxide and sulfone residues account for 10% of the total Mesurol levels detected in 1/2 fermented juice and cellared wine. This may be related to the reducing action or antioxident properties of the sulfur dioxide used during production. However, oxidation of methiocarb might have occurred during analysis (See Results and Discussion. Methiocarb Residues in Pomace). Mesurol sulfide and sulfoxide recoveries in White Riesling juice and wine, Tables 11 and 12, respectively, improved with increased fortification. Mesurol sulfone recoveries were better than 81% at all concentrations utilized.

Methiocarb residues in must, juice and in wine at first racking and after 6 months storage at 38°F (Table 13) were unchanged in the Pinot Noir commercial, press juice fortification and must fortification trials.

In comparison, Kawar et al. (1977) found 8.8 ppm parathion in finished wine derived from reconstituted Semillion grape juice concentrate fortified to 25 ppm (w/w); fermentation lees contained 156 ppm parathion. Hydrolysis of parathion to p-nitrophenol was an important consideration in accounting for lower pesticide levels in

Table 11. Recovery of Mesurol sulfide, sulfoxide and sulfone from White Riesling juice.

Mesurol sulfide			Mesurol sulfone			Mesurol sulfoxide		
µg added	µg recovered	% recovery	µg added	µg recovered	% recovery	µg added	µg recovered	% recovery
21	9	43	21	25	119	20	21	105
42	26	62	42	51	121	40	34	85
63	54	86	63	64	102	60	46	77
105	85	81	105	110	105	100	78	78
105[a]	54	51	105[a]	102	97	100[a]	53	53

[a]Press juice sediment control obtained from White Riesling fortification trial; w/w.

Table 12. Recovery of Mesurol sulfide, sulfoxide, and sulfone from White Riesling wine.

Mesurol sulfide			Mesurol sulfone			Mesurol sulfoxide		
µg added	µg recoveral	% recovery	µg added	µg recovered	% recovery	µg added	µg recovered	% recovery
21	12	57	21	22	105	20	12	60
42	25	60	42	34	81	40	22	55
63	48	76	63	61	97	60	54	90

Table 13. Methiocarb residues in Pinot Noir must juice and wine obtained from press juice fortification, must fortification and commercial trials.

Trial	Juice		Wine	
	Press MS ppm	Fermented on[a] skins MS ppm	First Racking MS ppm	Cold[b] stabilized MS ppm
Press Juice Fortification	6.0	4.5[c]	3.8	5.1
Must Fortification	d	3.3	5.2	4.0
Commercial	4.2	4.9	3.3	3.6

[a]Prior to pressing.

[b]Wine stored at 38°F, 6 months.

[c]Settled juice.

[d]Not sampled.

wine. Oxidation, however, was not. Similar evaluations were conducted by Kawar and associates (1979) using diluted Zinfandel concentrates spiked at 25 ppm with Diaflor, Dimethioate and Methidathon. Residues of Dimethioate remained constant going from must to finished wine. Wine prior to bottling contained 46% of the Methidathon and 10% of the Diaflor added to the grape juice. In contrast, 26% of the methiocarb detected in the fortified White Riesling press juice was found in the cellared wine. Methiocarb residues determined during the vinification of Mesurol field treated grapes are summarized in Tables 21-23, appendix.

Effect of Bentonite Fining and Membrane Filtration on Mesurol Sulfide, Sulfoxide and Sulfone Residues in White Riesling Wine Produced from Field Treated Grapes

Bentonite fining at 4 and 8 lb. per 1000 gal. equivalents was not active in reducing Mesurol residues in White Riesling wine produced from field treated fruit, Table 14. Filtration through a 0.45μ membrane also did not effect Mesurol levels. Volclay bentonite (KWK) addition and membrane filtration are, therefore, not effective practices for decreasing Mesurol residues in wine. However, fining of juice prior to fermentation and various types of bentonite (e.g.- sodium, calcium, and calcium/sodium) should be evaluated before

Table 14. Effect of bentonite fining and membrane filtration on Mesurol sulfide, sulfoxide and sulfone residues in White Riesling wine produced from field treated fruit.[1]

Treatment	Mesurol Sulfide	Mesurol Sulfoxide	Mesurol Sulfone
Control	5.9	1.1	0.1
Membrane Filtration[2]	5.4	1.0	0.1
Bentonite - 4 lb/10^3 gal	6.7	1.1	0.1
Bentonite - 8 lb/10^3 gal	5.2	0.8	0.1

[1]Harvested 7 days after the last application of Mesurol.

[2]0.45 μ Millipore membrane.

dismissing clay treatments as a vehicle for Mesurol residue reduction.

Gnaegi and Lipka (1974), however, reported bentonite fining to be a reliable method for reducing Benzidazole Methyl Carbamate (BMC), a systemic fungicide to counter grey rot, residues in finished wine. Forty to 90% removal of BMC was achieved with different bentonites. Greater residue losses were observed with increasing doses of bentonite, but was independent of BMC concentration at 1 to 5 ppm.

Mesurol Residues in Cellar and Bottle Aged Pinot Noir Wine

The hydrolytic and oxidative stability of Mesurol in finished wine is an important consideration in evaluating the safety of the biocide. The sulfoxide is known to be a more potent acetylcholinesterase inhibitor than the parent sulfide (Oonithon and Casida 1966).

A control wine, Pinot Noir, was fortified with 0.5 ppm Mesurol sulfide, sulfoxide and sulfone phenol. The wine was extracted and the phenols silylated (See Materials and Methods. Determination of Mesurol Sulfide Phenol). Recoveries were calculated by comparing the intensity of selected ions produced by Mass Spectrometry with those of standards. Seventy-two percent of the Mesurol sulfide phenol and sulfone phenol were recovered; 24% of the Mesurol sulfoxide added

Table 15. Mesurol sulfide (MS) phenol, sulfoxide (MSO) phenol and sulfone (MSO_2) phenol recoveries from 0.5 ppm fortified Pinot Noir wine.[1]

Compound	MS Phenol	MSO Phenol	MSO_2 Phenol
% Recovery	72	24	72

[1]Determined by Mass Spectrometry as the trimethylsilyl derivative.

was detected (Table 15). Thus, the extraction procedure utilized for carbamate analysis also removes phenols. The carbamates are hydrolyzed prior to derivatization with methane sulfonylchloride. It is apparent that any carbamate residues determined as the mesylate include the corresponding phenols.

Pinot Noir wines from "one day fruit" which had been cold stabilized (2 months at 55°F, 6 months at 38°F) and had two months bottle age (2 months at 55°F, 8 months at 38°F, 2 months at 55°F in the bottle) were chosen for analysis. Both samples were estimated to contain 0.05 ppm Mesurol sulfide phenol (Table 16). HPLC collection and Mass Spectrometry detection were used for the determination. Figure 5 contains representative HPLC chromatograms for a Mesurol sulfide phenol standarded and treated Pinot Noir wine, respectively. Mesurol sulfide, sulfoxide and sulfone carbamate residues in both samples were nearly identical (Table 16). The residues in both wines, exclusive of the sulfide phenol, total between 10 and 11 ppm. This is in agreement with the 9.9 ppm residues as the sulfone detected in the one day Pinot Noir wine (Table 7).

The mass spectra of Mesurol sulfide, sulfoxide and sulfone mesylates obtained from combining the final extracts of the two wines and of corresponding standards are shown in Figures 6-11. The presence of 247 and 262 m/e ions in the wine sulfone mesylate spectra

Table 16. Mesurol sulfide, sulfide phenol,[1] sulfoxide and sulfone residues in cellar[2] and bottle aged wine[3] produced from field treated Pinot Noir grapes.[4]

Sample	Mesurol sulfide ppm	Mesurol sulfide phenol ppm	Mesurol sulfoxide ppm	Mesurol sulfone ppm
Cellar	7.3	0.05	2.1	0.8
Bottle	8.0	0.05	2.1	0.8

[1]Determined by HPLC collection and Mass Spectrometry detection as the trimethylsilyl derivative.

[2]2 months at 55°F, 6 months at 38°F.

[3]2 months at 55°F, 8 months at 38°F, 2 months in bottle at 55°F.

[4]Harvested one day after the last application of Mesurol.

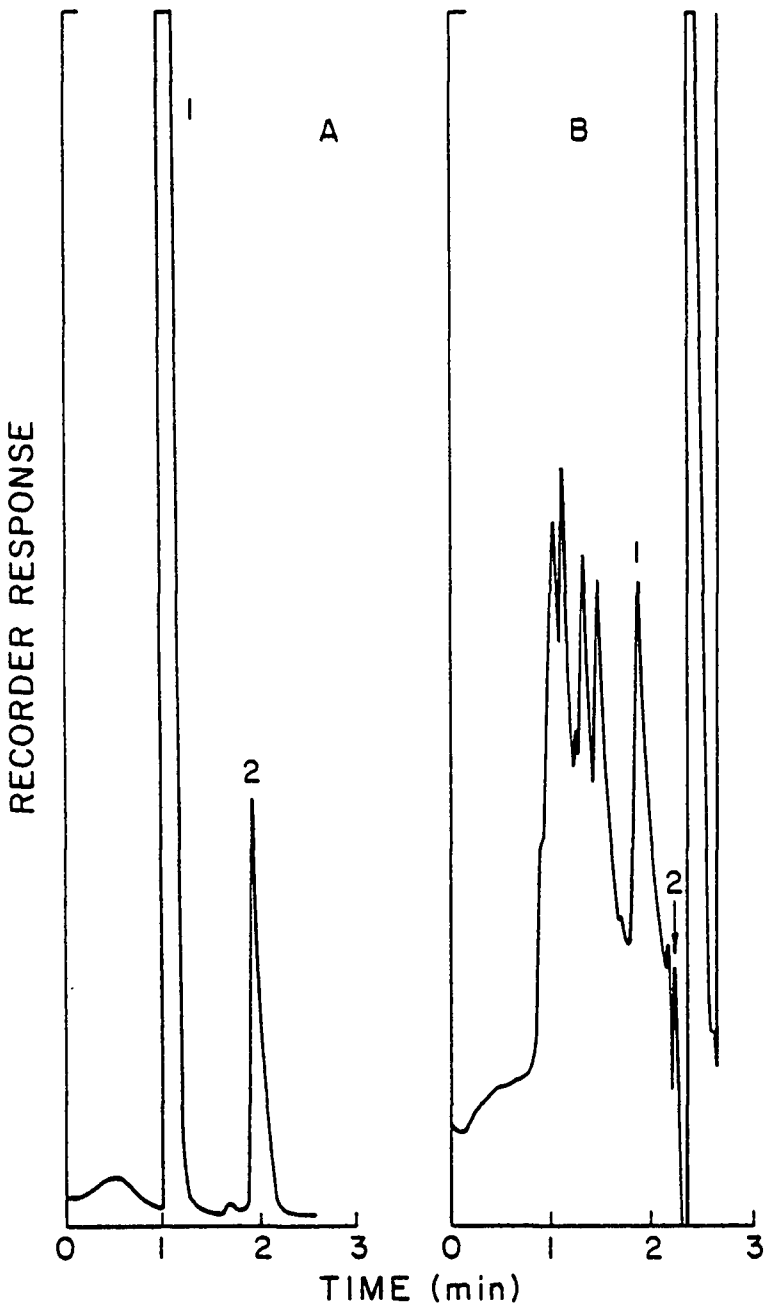

Figure 5. Representative HPLC chromatograms. Attenuation - 4X. Flow rate - 2 ml/min. (A) Mesurol sulfide phenol (peak 2) in benzene (peak 1). (B) Mesurol treated Pinot Noir wine. Peak 1 was collected. Flow rate was increased to 3 ml/min after peak 2 had eluted.

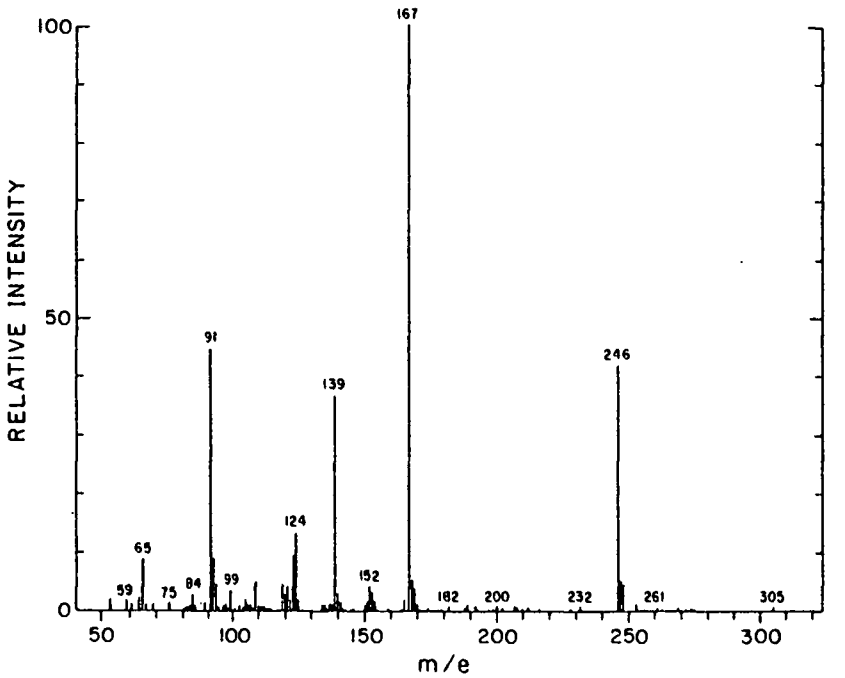

Figure 6. Mass Spectra of Mesurol sulfide mesylate standard.

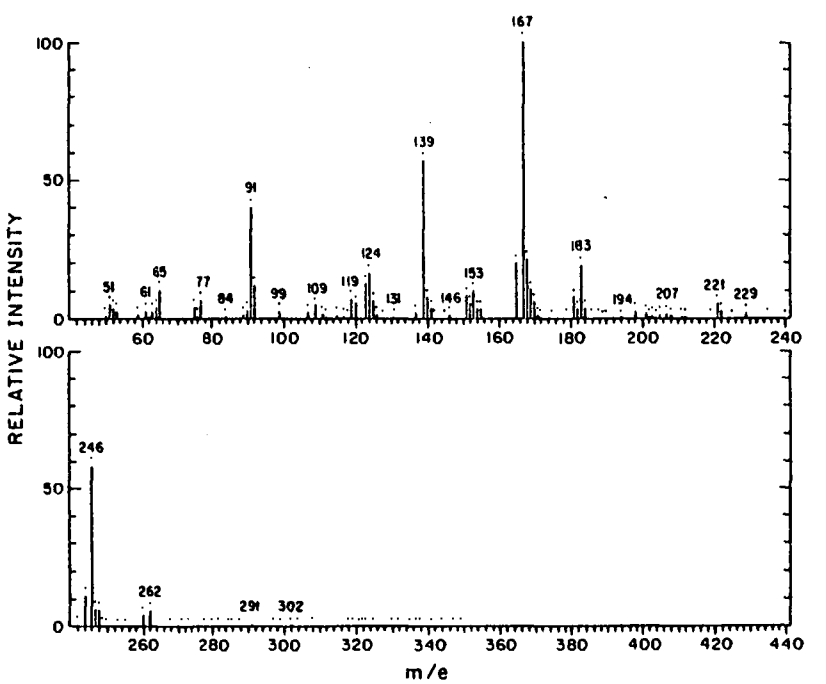

Figure 7. Mass Spectra of Mesurol sulfide mesylate detected in Pinot Noir wine produced from field treated grapes.

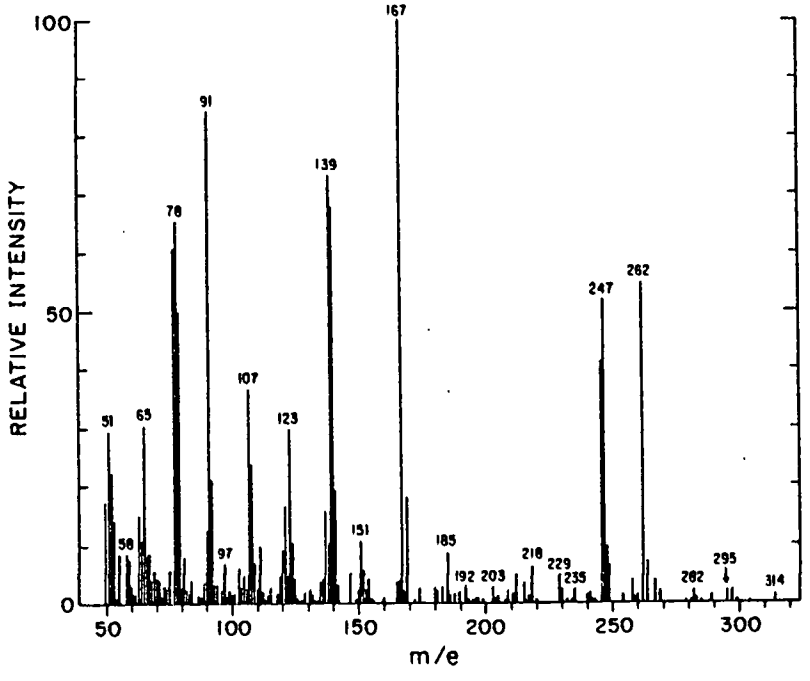

Figure 8. Mass Spectra of Mesurol sulfoxide standard.

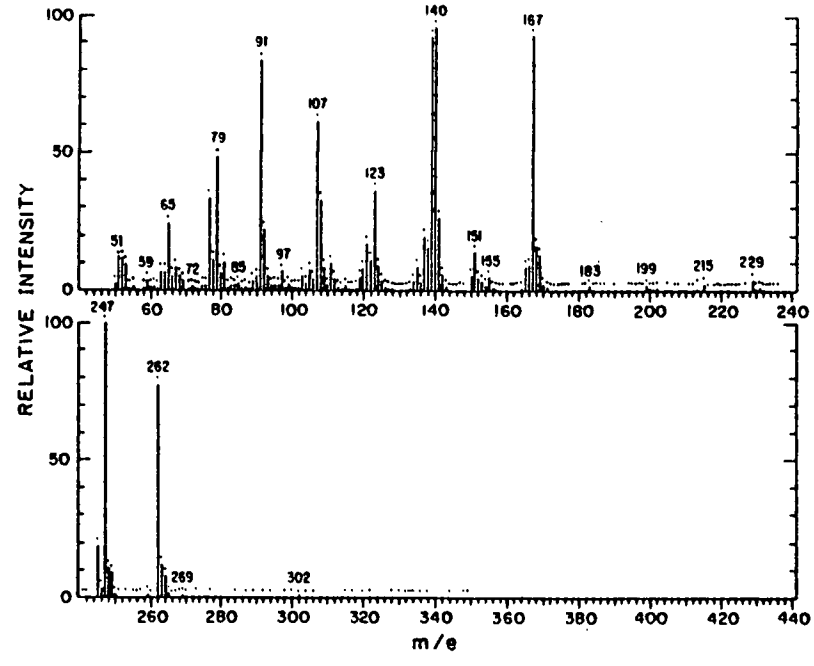

Figure 9. Mass Spectra of Mesurol sulfoxide mesylate detected in Pinot Noir wine produced from field treated grapes.

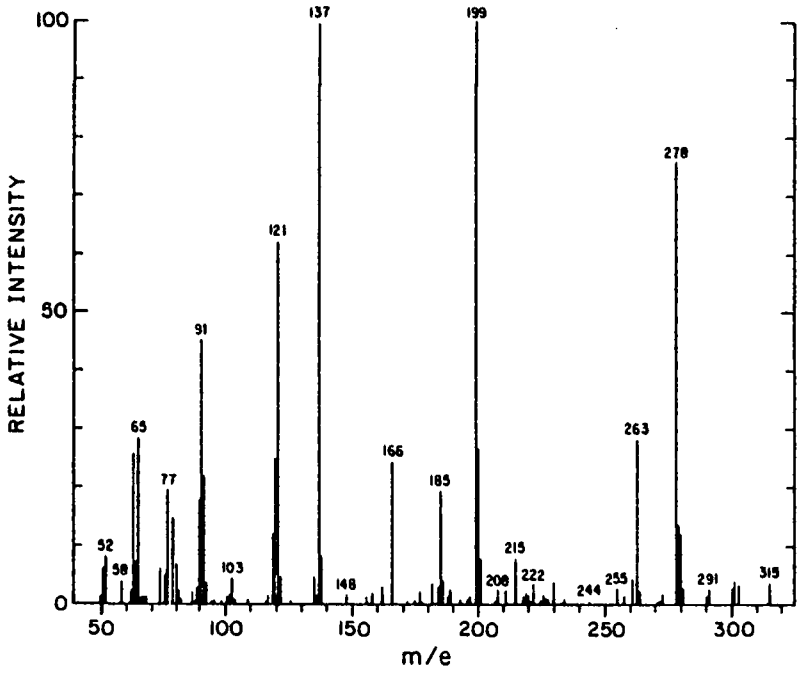

Figure 10. Mass Spectra of Mesurol sulfone mesylate standard.

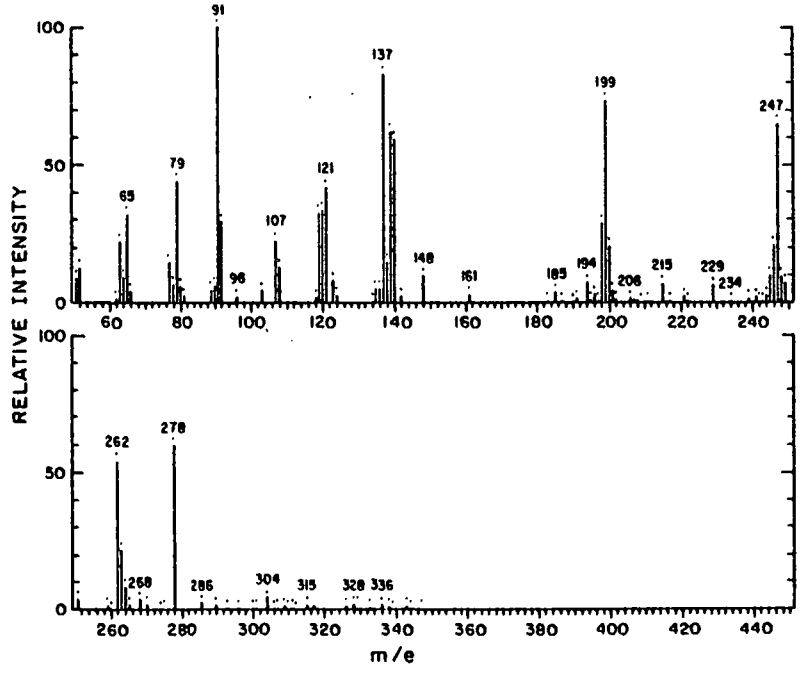

Figure 11. Mass Spectra of Mesurol sulfone detected in Pinot Noir wine produced from field treated grapes.

Table 17. Mesurol sulfide (MS), sulfoxide (MSO) and sulfone (MSO_2) recoveries from Pinot Noir wine.

Compound	µg added	µg recovered	% recovery
Mesurol	100[a]	62	62
Mesurol sulfoxide	25[b]	34	136
Mesurol sulfone	25	23	92

[a] 2.0 ppm, 50 g sample.

[b] 0.5 ppm, 50 g sample.

(Figure 11) indicates partial elution or tailing of the sulfoxide in the sulfide and sulfone cut during Florisil clean-up.

A control wine, Pinot Noir, was spiked with 2.0 ppm Mesurol sulfide and 0.5 ppm Mesurol sulfoxide and sulfone. Ninety-two percent of the sulfone, 62% of the sulfide and 136% of the sulfoxide were recovered (Table 17). Oxidation during the analytical procedure in this experiment is suggested and may also contribute to sulfone and sulfoxide residues in treated samples. Oxidation was not apparent in White Riesling juice and wine Mesurol sulfide, sulfoxide and sulfone fortification studies, Tables 11 and 12, respectively. Cellar and bottle aged Pinor wines each contained 2.1 ppm sulfoxide and 0.8 ppm sulfone (Table 16). These residues could also be the result of methiocarb field oxidation rather than degradation during processing or analysis. However, total Mesurol levels did not change in 12 months. Mesurol residues are, therefore, stabilized by vinification, there being little evidence of degradation to phenolic or oxidation metabolites during cellar and bottle storage.

Effect of Mesurol on Wine Composition and Sensory Properties

The chemical composition of White Riesling and Pinot Noir Mesurol treated and control musts, Table 18, used in this study were nearly identical. The only major disparity in composition between

Table 18. Chemical characterization of Mesurol treated and control musts.

Sample	pH	T.A.[1]	°Brix[2]
Pinot Noir			
Control - 1 day	3.30	0.99	22.8
Treated - 1 day	3.30	1.00	22.8
Control - 7 days	3.40	1.04	23.9
Treated - 7 day	3.30	1.04	23.4
White Riesling			
Control - 1 day	3.20	0.97	22.0
Treated - 1 day	3.25	0.96	22.6

[1]Titratable activity expressed as g. tartaric acid/100 ml.

[2]Soluble solids expressed as g. sucrose/100 g.

Table 19. Chemical composition of Mesurol treated and control wines.

Sample	pH	T. A.[3]	V. A.[4]	R. S.[5]	Ethanol (% v/v)	Phenols (p.p.m. G.A.E.)[6]	Color (O. D. 420 nm)	Color (O. D. 520nm)
White Riesling								
Control - 1 day	3. 07	0. 800	0. o73	0. 24	12. 92	276	0. 089	--
Treated - 1 day	3. 26	0. 811	0. 077	0. 33	13. 23	271	0. 118	--
Fortification - Press Juice[1]	3. 13	0. 800	0. 069	0. 29	12. 64	268	0. 093	--
Control - 7 day	3. 12	0. 709	0. 062	0. 44	13. 00	268	0. 097	--
Treated - 7 day	3. 18	0. 753	0. 070	0. 46	13. 58	279	0. 099	--
Pinot Noir								
Control - 1 day	3. 49	0. 687	0. 012	0. 17	11. 91	838	0. 083	0. 122
Treated - 1 day	3. 55	0. 664	0. 012	0. 18	12. 19	827	0. 094	0. 128
Control - 7 day	3. 77	0. 630	0. 040	0. 19	13. 35	1006	0. 109	0. 112
Treated - 7 day	3. 76	0. 641	0. 043	0. 19	13. 43	1000	0. 119	0. 121
Fortification - Must[2]	3. 75	0. 663	0. 045	0. 18	13. 32	994	0. 112	0. 118
Control - Press Juice	3. 74	0. 658	0. 060	0. 20	14. 32	531	0. 041	0. 041
Fortification - Press Juice	3. 75	0. 688	0. 059	0. 17	14. 02	609	0. 062	0. 060
Control - Commercial	3. 94	0. 524	0. 070	0. 21	13. 97	1732	0. 211	0. 269
Treated - Commercial	3. 85	0. 550	0. 093	0. 22	14. 03	1531	0. 170	0. 220

[1] Control 1 -day control, White Riesling.

[2] Control 7 -day control, Pinot Noir.

[3] Titratable acidity expressed as g tartaric acid/ 100 ml.

[4] Volatile acidity expressed as g acetic acid/ 100 ml.

[5] Reducing sugar expressed as g/ 100 ml.

[6] G. A. E. = Gallic acid equivalent.

Table 20. Mean difference and desirability scores and treatment least significant differences at the 5% level for Mesurol treated and control wines.

Sample	Mean Difference Scores	Mean Difference Scores
White Riesling		
Control - 1 day	6.18	5.02
Treated - 1 day	5.95	5.15
Fortification - Press Juice	6.00	5.25
L.S.D. (.05)	0.39	0.36
Control - 7 day	6.02	5.08
Treated - 7 day	5.97	4.70[a]
L.S.D. (.05)	0.49	0.33
Pinot Noir		
Control - 1 day	6.25	4.68
Treated - 7 day	5.58[a]	4.00[a]
L.S.D. (.05)	0.51	0.44
Control - 7 day	5.95	4.60
Treated - 7 day	5.81	4.67
Fortification - Must	5.95	4.74
L.S.D. (.05)	0.39	0.34
Control - Press Juice	6.45	4.50
Fortification - Press Juice	6.05[a]	4.32
L.S.D. (.05)	6.34	0.30
Control - Commercial	6.42	4.98
Treated - Commercial	5.77[a]	4.82
L.S.D. (.05)	0.40	0.37

[a]Significantly different from the control sample at the 5% level.

treated and control wines was found in the phenol levels of the commercial trial Pinot Noir, Table 19. A difference of 201 ppm is evident. Singelton et al. (1975) reported that increases of 85-100 mg/L in total phenols can produce a threshold difference in astringency.

The Pinot Noir wine produced from grapes harvested one day after the last application of Mesurol was scored significantly different in flavor and less desirable, Table 20, than wine made from control fruit (e.g.-difference in mean scores is greater than LSD at the 5% level). The wine resulting from Mesurol fortified Pinot Noir press juice was found to be significantly different from the respective control as was the commercial trial Pinot Noir wine produced from field treated fruit. The 7 day White Riesling treated wine was preferred over the control when no overall difference was recorded.

The significant difference registered for the Pinot Noir press juice wines is probably related to the partial malo-lactic fermentation detected in the control. The malo-lectic transformation is a bacterial mediated enzymatic decarboxylation of malic acid. Increased levels of diacetyl and acetoin have been reported in such wines (Amerine and Joslyn 1970). The statistical difference scored in the commercial trial wines is due in part to the contrasting phenol concentrations. The slight bias for the 7 day White Riesling control wine demonstrated by the judges, while not scoring any difference,

can be attributed to a lack of familiarity with wine and wine evaluation. The significant difference between desirability scores should be considered a 5 in 100 statistic when the difference scores are not significantly different. Differences recorded in the one day Pinot Noir wines could be the result of lot to lot variation.

The effect of Mesurol on the sensory properties and chemical composition of wines prepared from field treated Gomay Beaujolais and Pinot Blanc grapes was recently examined by Noble (1980). Wines were screened for undefined differences with Duo Trio tests. It was concluded that Mesurol did not influence the sensory qualities or chemical composition of wines produced from grapes harvested one week after the final application of the pesticide. Significant differences observed ($P < 0.05$) between treated and control samples and among controls from different sites in Gamay Beaujolais wines were ascribed to variations between lots and sites. Mesurol residues in finished wines were below 7 ppm. Total dosages, application methods and rates were similar to those used in Oregon trials; Gamay Beaujolais received three treatments and Pinot blanc four.

It can be concluded, in agreement with Noble (1980), that the chemical composition and organoleptic properties of wines made from grapes picked 7 days following the last Mesurol application were not affected. In addition, Mesurol fortification of musts and juices did not influence finished wine quality.

Effect of Methiocarb Residues on the Fermentation of Grape Juice and Must

The incorporation of methiocarb into grape juice in model system studies did not alter fermentation lag time at any concentration tested (Figures 12 and 13). A slight inhibition in fermentation rate is demonstrated at levels below 25 ppm methiocarb, while a stimulation is observed at higher concentrations of up to 500 ppm.

The effect of Mesurol fortification (15 mg methiocarb/kg must) on Pinot Noir press juice and must bulk trial fermentations is presented in Figures 14 and 15, respectively. In both series, lag time and fermentation rate are similar for control and treated lots. The chemical composition of both treated and control wines (Table 19) are analogous. In addition, sulfur containing volatiles, such as H_2S, which has been correlated to fungicides (Eschenbruch 1978), were not detected (sensory evaluation). Therefore, Mesurol does not significantly influence the fermentation of grape juice or must.

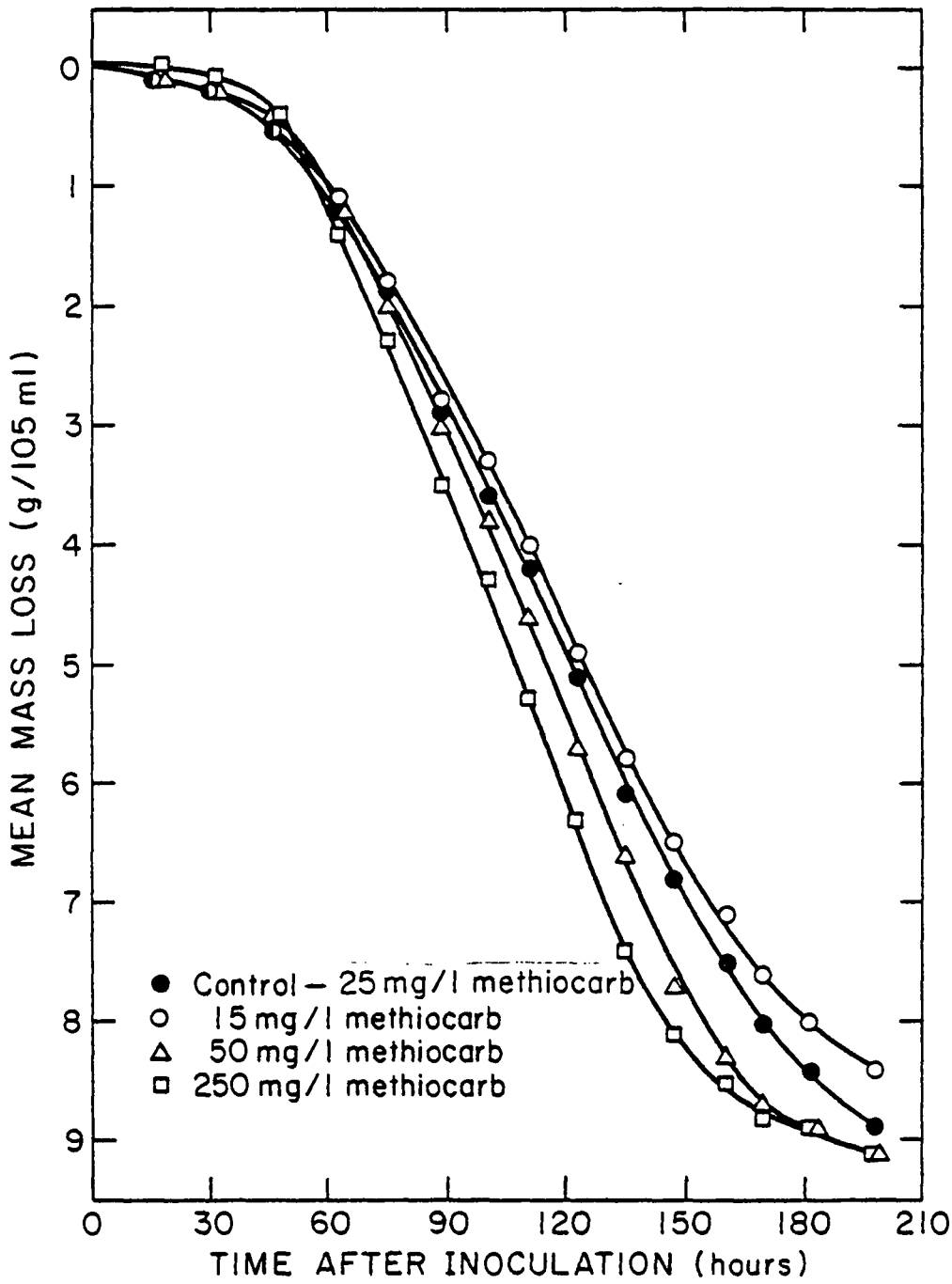

Figure 12. Mass loss fermentation curves of grape juice samples with different concentrations of Mesurol. Saccharomyces cerevisiae, Champagne strain.

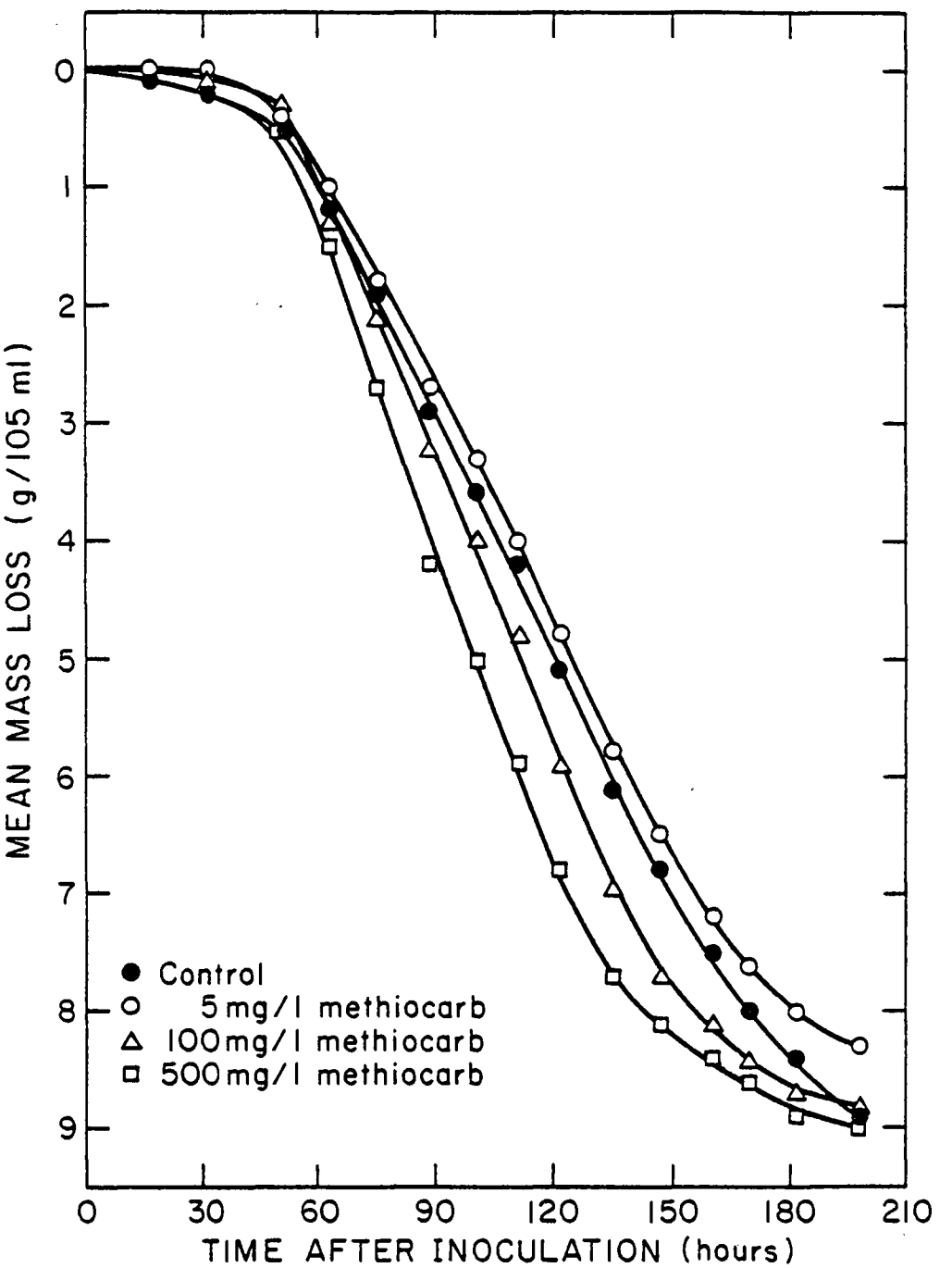

Figure 13. Mass loss fermentation curves of grape juice samples with different concentrations of Mesurol. Saccharomyces cerevisiae, Champagne strain.

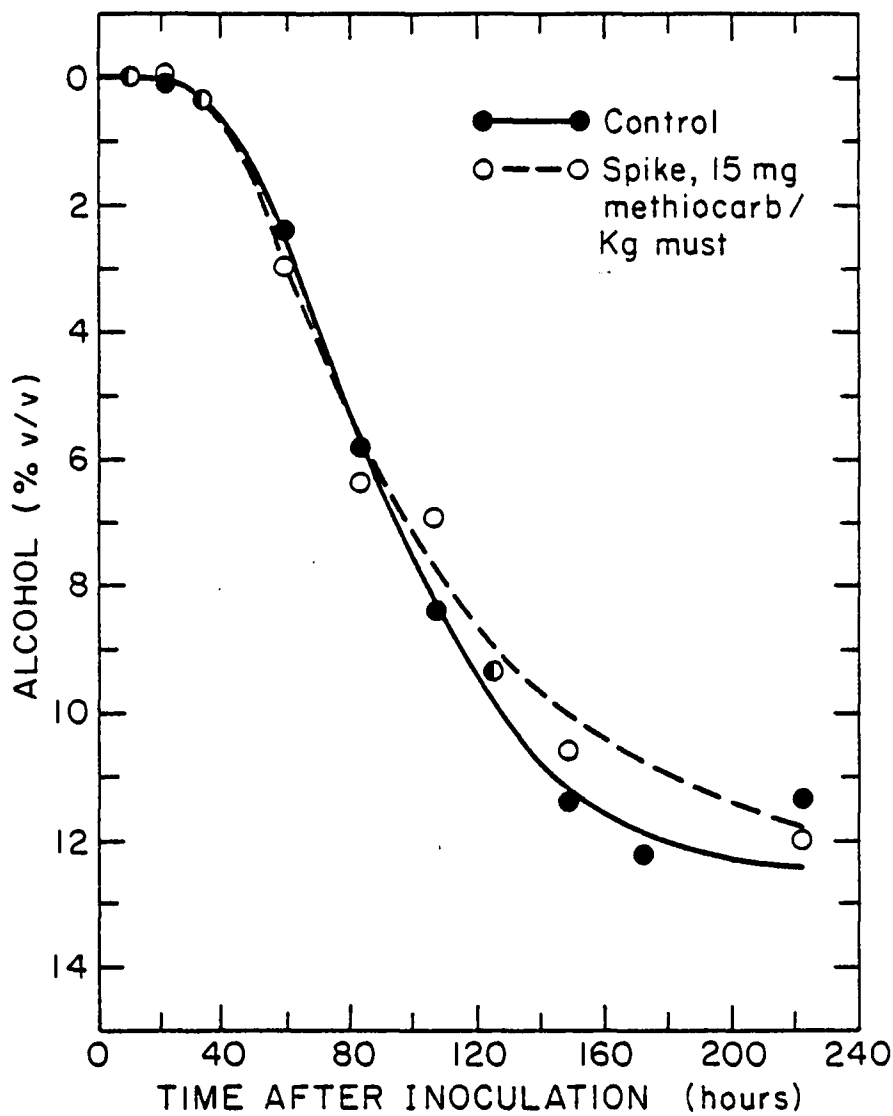

Figure 14. Effect of Mesurol fortification on Pinot Noir press juice fermentation. Saccharomyces cerevisiae, Champagne strain.

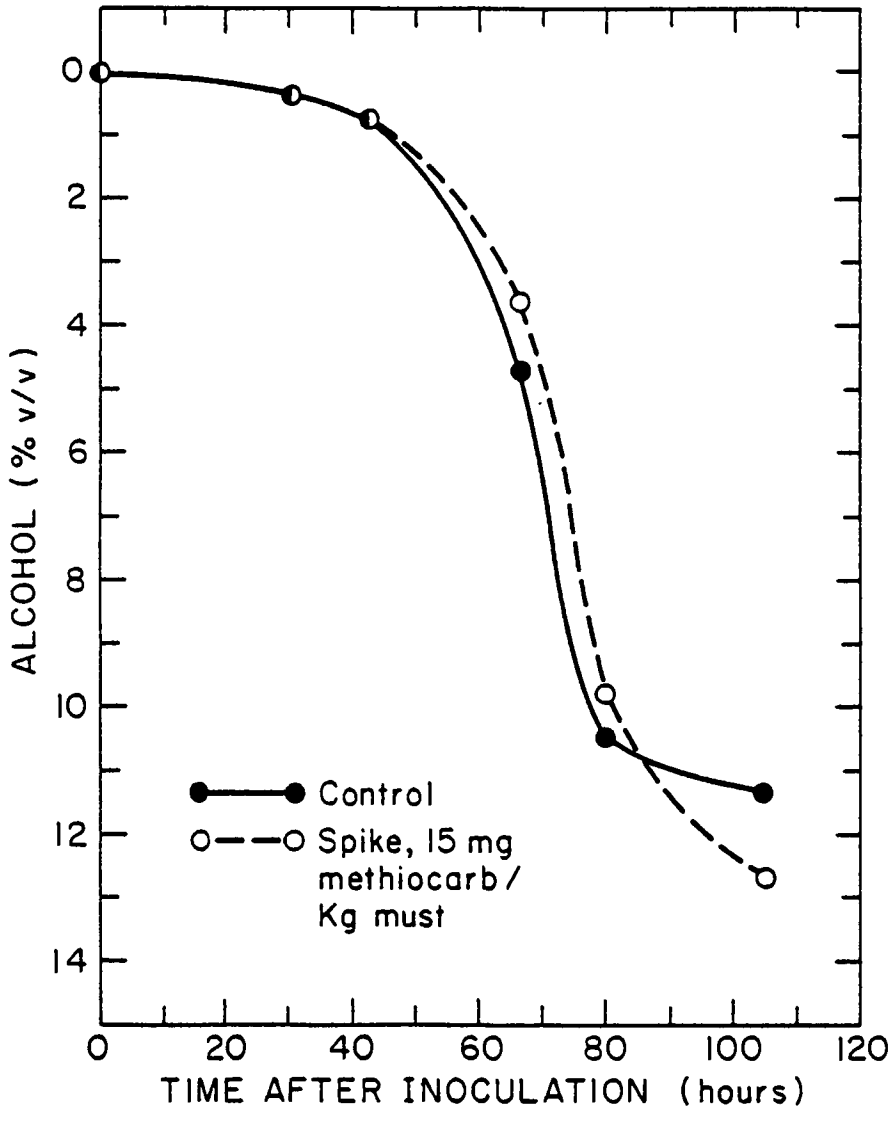

Figure 15. Effect of Mesurol fortification on Pinot Noir must (plus skins) fermentation. Saccharomyces cerevisiae, Champagne strain.

SUMMARY AND CONCLUSIONS

Wines were prepared from Mesurol field treated grapes and Mesurol fortified musts and juices. Mesurol residues were determined in grapes and various products derived from vinification. The fate of Mesurol and its metabolites during the processing of fortified White Riesling juice and the stability of Mesurol residues in finished wine were also studied. The influence of membrane filtration (0.45 μ) and bentonite fining (KWK) on Mesurol residues in wine was investigated. The effect of Mesurol on wine sensory properties, wine chemical composition, and alcoholic fermentation was evaluated. The following results were obtained:

1. Methiocarb (MS) and total sulfone (MSO_2-oxidation) residues in field treated grapes sprayed with 4 lb./acre methiocarb in 4 applications exceeded the 15 ppm temporary tolerance established by the EPA.

2. Methiocarb (MS) residues in pomace, calculated on a dry weight basis, derived from Pinot Noir and White Riesling grapes harvested one day after the last application of Mesurol exceeded the 75 ppm temporary established by the EPA.

3. Methiocarb (MS) residues representing 40 to 51% of the residues determined in whole grapes remained associated with pomace after pressing.

4. Finished Pinot Noir and White Riesling wines, prior to bottling, contained in the order of 3-8 ppm Methiocarb (MS) representing 34 to 49% and 13%, respectively, of the residues originally present in grapes.

5. Methiocarb (MS) residues in Mesurol fortified Whie Riesling press juice (15 mg/kg) were reduced by settling. Oxidation during processing was minimal.

6. Volclay bentonite (KWK) fining at 4 and 8 lb./1000 gal. and membrane filtration (0.45 μ) did not reduce Mesurol (MS, MSO, MSO_2) residues in finished wine.

7. Mesurol (MS, MSO, MSO_2) residues in Pinot Noir wine were not affected by 12 months after storage at cellar temperatures. Trace amounts of Mesurol sulfide phenol were detected.

8. The chemical composition and sensory properties of wines made from grapes harvested seven days after the last Mesurol treatment and Mesurol fortified must and juices were not affected.

9. The lag time and fermentation rate of Mesurol fortified Pinot Noir press juice and must (15 mg methiocarb/Kg) processed into wine were not significantly altered. Model studies demonstrated a slight inhibition in fermentation rate at methiocarb (MS) levels below 25 ppm while a stimulation was indicated at residues of up to 500 ppm.

BIBLIOGRAPHY

Adams, A. M. 1968. Effect of biocidal residues on wine making. Proc. 1st Internl. Biodeterioration Symp., Southampton, England. pp. 685-692.

Adams, A. M. 1960. Functional inhibition of Saccharomyces species by Captan and Phaltan. Rept. Hort. Expt. Sta. Prod. hab., Vineland, Ontario, Can. pp. 72-78.

Amerine, M. A. and Joslyn, M. A. 1970. Table Wines. The technology of their production. p. 471. University of California Press, Berkeley, CA.

Amerine, M. A. and Ough, C. S. 1972. Recent advances in enology. In "Critical Reviews in Food Technology." T. A. Furia (ed.) Chemical Rubber Co. Cleveland. 2:407-515.

Amerine, M. A. and Ough, C. S. 1974. Wine and Must Analysis. John Wiley and Sons. N. Y.

Anonymous. 1976. Methiocarb seen by USDI as an acceptable bird repellent on certain crops. Pest. Chem. News. September 29:16.

A.O.A.C. 1975a. Official Methods of Analysis. 12th ed. p. 129, 7.0003. Association of Official Analytical Chemists, Washington, D.C.

Aten, C. F. and Bourke, J. B. 1977. Reverse-phase liquid chromatographic behaviour of some carbamate and urea pesticides. J. Agr. Food Chem. 25(6):1428-1430.

Bailey, P. T. and Smith, G. 1979. Methiocarb as a bird repellent on wine grapes. Aust. J. Agr. Anim. Husbandry 19(4):247-250.

Basche, C. A. and Disk, D. J. 1968. Microwave emission residue analysis of carbamate and triazine pesticides. J. Gas. Chromatogr. 48(3):478-483.

Belliveau, P. E., Mellet, V. and Frei, R. W. 1970. A spray method for fluorescent detection of sulfur containing compounds. J. Chromatogr. 48(3):478-483.

Berg, H. C. van den. 1971. The biology and control of vine snout beetles. Deciduous Fruit Grower. 22:83-85.

Beuchat, L. R. 1973. Inhibitory effects of Benomyl on Saccharomyces coevisiae during peach fermentation. Am. J. Enol. Viticult. 24:110-115.

Bollingier, R. M., Stickley, A. R., Jr. and Besser, J. F. 1971. The effectiveness of methiocarb for protecting ripening grapes in New Hampshire. Report No. 33, Denver Wildlife Research Center. 5 pp. (typed).

Bowman, M. C. and Beroza, M. 1969. Determination of Mesurol and five of its metabolites in apples, pears and corn. J. Assoc. Off. Anal. Chem. 982-985.

Broderick, E. J., Bourke, J. B., Mettick, L. R., Taschenberg, E. F. and Aten, A. W. 1966. Determination of methylcarbamate pesticide in the presence of methyl anthranilate. J. Assn. Off. Anal. Chem. 49(5):982-985.

Cantarelli, C., Tafuri, F. and Martini, A. 1964. Chemical and microbiological surveys on the effects of dithiocarbamate fungicides on wine making. J. Agr. Food Chem. 15(5):186-196.

Castor, J. G., Nelson, K. E. and Harvey, J. M. 1957. Effects of captan residues on fermentation of grapes. Amer. J. Enol. Viticult. 8:50-57.

Chemagro Corp., Kansas City, Mo., Technical Data Sheet on Bayer 37344. Jan. 1964.

Dehaven, R. W., Guarino, J. L., Crase, F. T. and Schaffer, E. W., Jr. 1971. Methiocarb for repelling blackbirds from ripening rice. Int. Rice Comm. Newsletter. 20(4):25-30.

Dirimenov, M. and Kharizonov, A. 1972. Test of preparations for the control of the variegated grape moth. Rastitelna Zashchita. 20(10):23-26.

Drozd, J. 1975. Chemical derivatization in gas chromatography. J. Chromatogr. Chromatogr. Rev. 113(3):303-356.

El-Dib, M. A. 1970. Thin-layer chromatography detection of carbamate and phenylurea pesticide residues in natural waters. J. Assn. Off. Anal. Chem. 53(4):756-760.

Ernst, G. F., Roder, S. J., Tjan, G. H. and Jansen, J. T. A. 1975. Thin-layer chromatographic detection and indirect gas chromatographic determination of three carbamate pesticides. J. Assn. Off. Anal. Chem. 58(5):1015-1019.

Eschenbruch, R. 1971. The influence of fungicides on the formation of hydrogen sulfide (H_2S) on the fermentation of grape juice. Die Wynboer. 8(64):22-23.

Eschenbruch, R. 1972. Benlate, a semi systemic fungicide against Botrytis cinera. Die Wynboer. 490:21-22.

Frei, R. W., Lawrence, J. F., Hope, J. and Cassidy, R. M. 1974. Analysis of carbamate insecticides by flourogenic labeling and High Speed Liquid Chromatography. J. Chromatogr. Sci. 12(1):40-44.

Gnaegi, F. and Lipka, G. 1974. Residues of systematic fungicides in wines. Influence of wine production method and the possibility of elimination by bentonite. Revue Suisse de Viticulture, Aboriculture, Horticulture. 6(4):117-120.

Greenhalgh, K., Marshall, W. D. and King, P. R. 1976. Trifluoroacetylation of Mesurol(4-methylthio-3, 5-xylyl-N-methylcarbamate), its sulfoxide, sulfone and phenol analogs for analysis by gas chromatography. J. Agr. Food Chem. 28(2):266-270.

Greenhalgh, R., Wood, G. W. and Pearce, P. A. 1977. A rapid gas chromatography method of monitoring Mesurol(4-(methylthio)-3, 5-xylyl-N-methylcarbamate) and its sulfoxide and sulfone metabolites and their persistence in lowbush blueberries. J. Environ. Sci. Health B12(4):229-244.

Guarino, J. L. and Forbes, J. E. 1970. Preventing blackbird damage to sprouting corn with a carbamete repellent. N. Y. Fish and Game J. 17(2):117-120.

Guarino, J. L., Shake, W. F. and Schafer, E. W., Jr. 1974. Reducing bird damage to ripening cherries with methiocarb. J. Wildl. Manage. 38(2):338-342.

Henkel, H. G. 1966. Dünnschichtchromatographische Trennung und Nachweis insektizidwirksamer carbamate. J. Chromatogr. 21(2):396-398.

Holden, E. R. 1973. Gas chromatographic determination of residues of methylcarbamate insecticides in crops as their 2,4-dinitrophenyl ether derivatives. J. Assoc. Off. Anal. Chem. 56(3):713-717.

Kawar, N. S., Grunther, F. A. and Iwata, Y. 1978. Fate of Parathion in artificially fortified grape juice processed into wine. J. Environ. Sci. Heal. B13(1);1-9.

Kawar, N. S., Iwata, Y., Düsch, M. E. andGunther, F. A. 1979. Behaviour of Dialifor, Dimethoate, and Methidathon in artificially fortified grape juice processed into wine. J. Environ. Sci. Heal. B14(5):505-513.

Lawrence, J. F. 1977. Direct analysis of some carbamate pesticides in foods by High Pressure Liquid Chromatography. J. Agr. Food Chem. 25(1)211-212.

Lemperle, E., Kerner, E., Strecker, H. and Waibel, A. 1973. Active ingredient residues and effects on fermentation after application of fungicides in grape growing. Deutshe Lebensmitted-Rundschau. 69(9):313-322.

Loran, E. J. and Hemphill, D. D. 1974. Direct chromatography of some N-methylcarbamate pesticides. J. Assoc. Off. Anal. Chem. 87(3):870-875.

Maitlen, J. C. and McDonough, L. M. 1980. Derivatization of several carbamate pesticides with methane sulfonylchloride and detection by gas-liquid chromatography with flame photometric detection. Application of carbaryl residues in Lentil straw. J. Agr. Food Chem. 28(1):78-82.

Marais, P. G. and Kruger, M. M. 1976. Effect of Benomyl on fermentation and hydrogen sulfide formation during winemaking, Agrochemophysica 8:61-64.

Mendoza, C. D. and Shields, J. B. 1970. Sensitivity of pig liver esterase in determining twelve carbamate pesticides on thin-layer chromatograms. J. Chromatogr. 50(1):92-102.

Mendoza, C. E. and Shields, J. B. 1971. Esterase specificity and sensitivity to organophosphorous and carbamate pesticides: Factors affecting determination by thin-layer chromatography. J. Assoc. Off. Anal. Chem. 54(3):507-512.

Mendoza, C. E. and Shields, J. B. 1973. Determination of some carbamate pesticides by enzyme inhibition techniques using thin-layer chromatography and colorimetry. J. Agr. Food Chem. 21(2):178-184.

Minarik, E. and Ragala, P. 1969. Actions of fungicides on yeasts during the fermentation of grape musts. Yeasts Proc. 2nd. Symp. Yeasts. House Slovak Acad. Sci. Bratislava. p. 109.

Mott, D. F., Guarino, J. L., Royall, W. C., Jr. and Twedt, D. J. 1978. An evaluation of Mesurol for preventing bird damage to ripening wine grapes in Oregon. Bird Damage Research Report No. 99, Denver Wildl. Res. Cen. 7 pp. (typed).

Moye, H. A., Scheir, S. J. and St. John, P. A. 1977. Dynamic flourogenic labeling of pesticides for High Pressure Liquid Chromatography: Detection of N-methylcarbamates with o-pthaldehyde. Anal. Lett. 10(13):1049-1073.

Noble, A. C. 1980. Research Note. Effect of use of the bird repellent Mesurol on wine composition and flavor. Am. J. Enol. Viticult. 31(1):98-100.

Oomithan, E. S. and Casida, J. E. 1966. Metabolites of methyl- and dimethylcarbamate insecticide chemicals as formed by rat liver microsomes. Bull. Expt. Cont. Tox. 1(2):59-69.

Phoff, H. J., Miller, M. W. and Mrak, E. M. 1966. The Life of Yeasts. Their nature, activity, ecology and relation to mankind. p. 12. Harvard University Press, Cambridge, Mass.

Rogers, J. G., Jr. 1974. Responses of caged red-winged blackbirds to two types of repellents. J. Wildl. Manage. 38(3): 418-23.

Schafer, E. W., Jr. and Brunton, R. B. 1971. Chemicals as bird repellents: two promising agents. J. Wildl. Manage. 35(3): 569-572.

Schemnitz, S. D., Ismail, A. A. and Gramlich, F. J. 1976. Effectiveness of methiocarb in repelling birds in central Maine lowbush blueberry fields. Maine, Life Sci. Agr. Expt. Stat. Res. Life Sci. 23(12):6 pp.

Seiber, J. N. 1972. N-perflouroacyl derivatives for methylcarbamate analysis by gas-chromatography. J. Agr. Food Chem. 20(2):443-446.

Singleton, V. L., Sieberhagen, H. A., deWet, P. and vanWyk, C. J. 1975. Composition and sensory qualities of wines prepared from white grapes by fermentation with and without grape solids. Am. J. Enol. Vitic. 26:62-69.

Starr, R. I. and Cunningham, D. J. 1974. Degradation of ^{14}C-labeled Mesurol in soil and water. Am. Chem. Soc. Abst. No. 59.

Stickley, A. R., Jr. and Guarino, J. L. 1972. A repellent for protecting corn seed from blackbirds and crows. J. Wildl. Manage. 36(1):150-152.

Stone, C. P., Shake, W. F. and Langowski, D. J. 1974. Reducing bird damage to highbush blueberries with carbamate repellent. Wildl. Soc. Bull. 2(3):135-139.

Strankowski, K. J. and Stanley, C. W. 1975. Determination of residues of Mesurol and its toxic metabolites in crops. Chemagro Agricultural Division of Mobay Chemical Corporation. Personal communication.

Strother, A. 1968. Gas-chromatography of various pheny N-methylcarbamates. J. Gas. Chromatogr. 6(2):110-113.

Taschenberg, E. F 1973. Economic status and control of the grape leaf hopper in western New York. Search Agriculture (Entomology). 3. 4. V. 9 pp.

APPENDIX

Thornton, J. S. and Drager, G. 1973. Determination of residues of Mesurol and its toxic metabolites in plant and animal tissues. Int. J. Environ. Anal. Chem. 2:229-239.

Van Middlem, T. I., Norwood, T. L. and Weites, R. E. 1965. Electron affinity for GLC residue determination of Sevin and other carbamates following hydrolysis and bromination. J. Gas Chromatogr. 3(9):310.

Van Zyl, J. A. and Du Plessis, L. de W. 1962. The microbiology of South African wine making. Part 1. The yeasts occurring in vineyards, musts and wines. S. A. J. Agr. Sci. 4:393-403.

Wales, P. J., McLeod, H. A. and McKinley, W. P. 1968. T.L.C.-enxyme inhibition procedure to detect some carbamete standards and Carb yl in food extracts. J. Assoc. Off. Anal. Chem. 52(5):1054-1063.

Werth, K. 1976. Die Maulwurfsgrillen oder Werren. Obstbau Weinbau. 13(6):196.

West, R. R. and Dunks, J. M. 1969. Repelling boattailed grackles from sprouting corn with a carbamate compound. Texas J. Sci. 21(2):231-233.

Wood, G. W. and Pierce, P. A. 1977. Reducing bird damage to lowbush blueberries with a carbamate repellent. Acta Horticult. No. 61:201-204.

Zabadal, T. J. and Hothem, R. L. 1979. Mesurol - it's for the birds. Wines and Vines. 60(11):38-41).

Zielinski, W. L. and Fishbein, L. 1965. Gas chromatography of carbamate derivatives. 2. N-substituted carbamates. J. Gas Chromatogr. 3(10):333-335.

Table 21. Abridged descriptions of Mesurol treatments utilized.

Variety	Treatment	Abridged Description
Pinot Noir	Fruit harvested one day after the last application of Mesurol[1]	Pinot Noir 1 day trial
	Fruit harvested seven days after the last application of Mesurol[1]	Pinot Noir 7 day trial
	Commercially processed Mesurol treated fruit[2]	Pinot Noir commercial trial
	Mesurol fortified must[3]	Pinot Noir must fortification trial
	Mesurol fortified press juice[4]	Pinot Noir press juice fortification trial
White Riesling	Fruit harvested one day after the last application of Mesurol[1]	White Riesling 1 day trial
	Fruit harvested seven days after the last application of Mesurol[1]	White Riesling 7 day trial
	Mesurol fortified press juice[4]	White Riesling press juice fortification trial

[1] 4 lb./acre methiocarb, 4 applications.

[2] 2 lb./acre methiocarb, 2 applications.

[3] 15 mg methiocarb/Kg must; must-juice plus skins prior to pressing. Fermented on skins.

[4] 15 mg methiocarb/Kg must. Pressed. Fermented without skins.

Table 22. Methiocarb (MS) residues (ppm) determined during the vinification of Mesurol field treated Pinot Noir grapes.[1]

Grapes	Juice[2]	Pomace	Wine (First Rack)	Wine (Finished)
22	10	28	7.6	7.4

[1] 4 lb./acre, 4 applications. Harvested one day after the last application of Mesurol.

[2] Fermented on skins.

Table 23. Methiocarb (MS) residues (ppm) determined during the vinification of Mesurol field treated White Riesling Grapes.[1]

Grapes	Press Juice	Pomace	Wine (First Rack)	Wine (Finished)
38	15.3	40	6.3	4.8

[1] 4 lb./acre, 4 applications. Harvested one day after the last application of Mesurol.

Table 24. Methiocarb (MS) residues (ppm) determined during the vinification of Mesurol field treated Pinot Noir grapes.[1]

Grapes	Juice[2]	Pomace	Wine (First Rack)	Wine (Finished)
12	4.9	20	3.3	3.6

[1] 2 lb./acre, 2 applications. Commercial trial.

[2] Fermented on skins.