Table VII. Root Yield, Plant Population, and Sugar Yield of Sugar Beet Crops Treated at Rates of 120, 150, 180, and 360 g of a.i./ha

| treatment, g of a.i./ha | root yield, kg/ha | plant population, n/ha | sugar % | sugar kg/ha | sugar purity |
|---|---|---|---|---|---|
| 120 | 73 350 | 71 479 | 14.40 | 10 562 | 87.8 |
| 150 | 79 016 | 77 405 | 15.12 | 11 947 | 87.8 |
| 180 | 75 646 | 79 627 | 15.48 | 11 710 | 88.9 |
| 360 | 73 016 | 73 331 | 14.62 | 10 675 | 86.6 |
| untreated | 76 572 | 77 035 | 15.00 | 11 486 | 88.3 |

after 15 days as it seems to in Tables I–IV but continues with the plant growth during more or less 2 months. This absorption does not increase proportionally with the beet weight; that is the reason of the ppm profiles decrease after 15 days.

During this experimentation, as it appears in Table VII, it was proved that a 3,6-DCP treatment, even at 3 times the usual dose, does not influence the root yield or the plant population and the sugar percentage in comparison with that of the control.

In conclusion, 3,6-DCP can ensure the protection of the sugar beet crop with a minimum residue level at the harvesting (0.2–0.6 ppm total for treatment rates of 120–360 g of a.i./ha) and has no effect on sugar production. The persistence of the herbicide in soil will be also taken into account in a further study.

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**Registry No.** 3,6-DCP, 1702-17-6.

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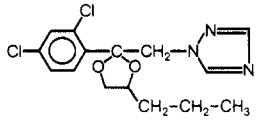
# Gas Chromatographic Determination of Propiconazole and Etaconazole in Plant Material, Soil, and Water

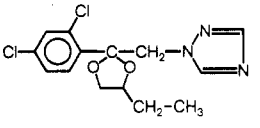
Bruno Büttler

Propiconazole and etaconazole are two representatives of a new type of broad spectrum systemic fungicides. They are the active ingredients of Tilt and Sonax or Vangard (trademark used in the United States only), respectively. A method for the determination of each of the two fungicides in various crops, soil, and water is reported. The procedure involves the extraction of the samples with methanol, dilution of the extract with water, partition into dichloromethane, and cleanup on an alumina column. An additional cleanup by gel chromatography is described for straw. The analysis of water samples starts with the partition into dichloromethane. Residues are quantitatively determined by gas chromatography using an alkali flame ionizaton detector operating in the nitrogen-sensitive mode. Recoveries in the range of 76–100% indicate that this procedure is suitable for the residue analysis of these fungicides with detection limits of 0.02 mg/kg in fruit, grain, and soil, 0.05 mg/kg in other plant materials, and 0.001 mg/kg in water. Extraction efficiency of weathered residues was investigated, and results were presented to prove that the proposed procedure is adequate. The specificity of the method was tested with a series of important nitrogen- and/or phosphorus-containing fungicides, herbicides, and insecticides. No interferences were observed.

Propiconazole [1-[[2-(2,4-dichlorophenyl)-4-propyl-1,3-dioxolan-2-yl]methyl]-1*H*-1,2,4-triazole, I] and etaconazole



I



II

[1-[[2-(2,4-dichlorophenyl)-4-ethyl-1,3-dioxolan-2-yl]-methyl]-1*H*-1,2,4-triazole, II], developed under the code numbers CGA 64 250 and CGA 64 251, respectively, are two important representatives of a new class of fungitoxic chemicals. Both are broad-spectrum systemic fungicides with activity against powdery mildew, rust, scab, and leaf spot diseases on different crops. Propiconazole is currently used on cereals and in grapes while etaconazole is mainly used in deciduous fruits. The combined or alternative application of these two fungicides is not recommended so that they should not be found simultaneously on the same crop.

The almost identical chemical structure of the two compounds suggested nevertheless the development of a common method which could be used for the residue determination of either of these fungicides in cereals and deciduous fruits. The proposed procedure involves com-

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mon extraction and cleanup steps, the final resolution of propiconazole and etaconazole by gas chromatography, and their detection by an alkali flame ionization detector operating in the nitrogen–phosphorus-specific mode.

As during spray treatment of crops part of the pesticide may also reach the soil and from there might get into underground water, procedures for analysis of soil and water were also developed.

## EXPERIMENTAL SECTION

**Sample Preparation.** A representative 800–1000-g subsample of green plant material (green cereal plants), straw, or fruit (apples, pears, grape berries) was chopped in a food cutter. Dry plant materials (chopped straw, grain) were entirely ground together with dry ice in a cross-beater mill. The dry ice was allowed to evaporate. Stones were removed from soil and the soil was passed through a 2-mm sieve. The sample was then thoroughly homogenized in a planetary blender. A subsample was air-dried to correct residue results on dry soil weight.

**Extraction.** A 25-g subsample of grain or fruit was placed into a 500-$cm^3$ wide-mouth jar. Methanol (200 $cm^3$) was added. Then 10 g of green plant material or 20 of straw was placed in a 250-$cm^3$ wide-mouth jar and methanol (200 $cm^3$) was added. A total of 25 g of soil was weighed into a 500-$cm^3$ wide-mouth jar and 200 $cm^3$ of aqueous methanol (20% water) was added. Cold extraction of soil was proven to be as efficient as hot extraction. For the ease of operation the cold extraction was preferred. The tightly closed wide-mouth jars were shaken for 2 h on a mechanical shaker. The slurry was filtered through a Büchner funnel by using suction. The filter cake was washed twice with methanol (20 $cm^3$). Filtrate and washings were combined and collected.

**Cleanup and Partition.** The methanolic plant, fruit, or soil extract was transferred to a 1000-$cm^3$ separatory funnel and diluted with 500 $cm^3$ of deionized water and 20 $cm^3$ of saturated sodium chloride solution. The aqueous methanol solution was shaken 2 times with 75 $cm^3$ of dichloromethane each. The dichloromethane extracts were collected, filtered through a plug of cotton, and evaporated to dryness by using a rotary evaporator, water bath temperature 30 °C, and slight suction. The aqueous phases were discarded.

The water sample (500 $cm^3$) was transferred to a 1000-$cm^3$ separatory funnel. The sample vessel was rinsed with 75 $cm^3$ of dichloromethane. The washing was transferred to the separatory funnel. The water sample was extracted 2 times with 75 $cm^3$ of dichloromethane each. The dichloromethane extracts were collected, filtered through a plug of cotton, and evaporated to dryness. The water phases were discarded. The water extracts could generally be analyzed by gas chromatography without any additional cleanup.

**Gel Column (for Straw Only).** An AutoPrep 1001 gel permeation chromatograph (GPC) (Analytical Biochemistry Laboratories, Inc., Columbia, MO) equipped with a 60.0 cm × 2.5 cm i.d. column, packed with 50 g of Bio-Beads SX3 resin, 37–75 μm (Bio-Rad Laboratories, Richmond, CA), compressed to a bed length of approximately 30 cm was used. The eluting solvent was cyclohexane–ethyl acetate (1:1) pumped at a constant flow rate of 2.5 $cm^3$/min, with an operating pressure of approximately 20 kPa. Before the samples could be processed with the gel permeation system, it was necessary to determine the elution volume of the two fungicides. The AutoPrep 1001 GPC autofractionates a sample into 23 10-$cm^3$ fractions for elution profile determination. This is accomplished by collecting the gel column eluant for 4 min from each of the 23 sample collection tubes at a constant flow rate of 2.5 $cm^3$/min. Under these conditions propiconazole and etaconazole eluted in the 110–175-$cm^3$ fraction. The fractions were analyzed by injection into the gas chromatograph equipped with an alkali flame ionization detector.

Table I. Typical Recoveries of Etaconazole

| sample | fortification level, mg/kg | % recovery ± SD (no. of analyses) |
|---|---|---|
| apples, pears | 0.04 | 88 ± 7.6 ($n$ = 8) |
| | 0.4 | 90 ± 3 ($n$ = 9) |
| grain | 0.04 | 82 ± 5.9 ($n$ = 3) |
| | 0.4 | 83 ± 3.7 ($n$ = 3) |
| soil | 0.04 | 87 ± 8.3 ($n$ = 3) |
| | 0.4 | 100 ± 6.4 ($n$ = 3) |
| water | 0.002 | 106 ± 6 ($n$ = 3) |
| | 0.02 | 99 ± 4 ($n$ = 3) |

The residue of the straw sample from the partition step was dissolved in 10 $cm^3$ of cyclohexane–ethyl acetate (1:1). A 5-$cm^3$ aliquot was injected onto the GPC and processed according to the conditions indicated above. The 110–175-$cm^3$ fraction was collected and evaporated to dryness by using a rotary evaporator, a water bath temperature of 40 °C, and slight suction. If no automatic gel chromatograph is available, manual execution of the above steps is possible, although time consuming.

**Alumina Column.** A total of 30 $cm^3$ of alumina basic activity grade V (Alumina Woelm B, Super I, Woelm Pharma GmbH & Co., D-3440 Eschwege, FRG) was poured into a chromatographic tube of 23-mm i.d. and 30-cm length to yield a column of 70 ± 5 mm height. The tube was filled before with 10 $cm^3$ of $n$-hexane. The residue from the partition step or from the gel chromatographic cleanup was dissolved in 2 $cm^3$ of toluene by swirling the flask and transferred to the column. The solvent in the column was drained to the top of the alumina. The flask was rinsed twice with further 2 $cm^3$ $n$-hexane which were also transferred to the column. The column was washed with 50 $cm^3$ of a mixture of $n$-hexane (analytical grade)–dichloromethane (analytical grade) (6:4). This eluate was discarded as a fore cut. The active ingredients were eluted with 75 $cm^3$ of $n$-hexane–dichloromethane (4:6). The eluate was collected and evaporated to dryness by using a rotary evaporator, a water bath temperature of 40 °C, and slight suction.

**Gas Chromatography.** A Hewlett-Packard Model 5710 A/30A gas chromatograph equipped with a nitrogen–phosphorus flame ionization detector, Model 18789 A, was operated as follows: temperature, column 225 °C, detector 250 °C, and injector 250 °C. The flow rate of the carrier gas nitrogen was 36 $cm^3$/min. The flow rates of the detector gases were 3 $cm^3$/min hydrogen and 50 $cm^3$/min air. A glass column (150 × 0.2 cm i.d.) packed with 3% Carbowax 40 M on Gas-Chrom Q (size 0.15–0.18 mm) was used. The retention time was 3.0 min and 20 s for etaconazole and 4 min for propiconazole.

Standard solutions of 0.25–10 μg/$cm^3$ were prepared from the two fungicides in $n$-hexane–ethanol (1:1). A total of 2 $mm^3$ each of at least four standard solutions was injected into the gas chromatograph. Linear plots of the fungicide concentration vs. the recorder responses (peak heights) yielded straight lines ($r^2 \geq 0.989$).

The dried residues of the eluate from the alumina column or the dried water sample extracts were dissolved in 2 $cm^3$ of $n$-hexane–ethanol (1:1). From these solutions 2 $mm^3$ was injected into the gas chromatograph corresponding to 25 mg of fruit, grain, or soil extract or to 10 mg of green plant material or straw extract or to 500 mg of water extract.

Table II. Typical Recoveries of Propiconazole

| sample | fortification level, mg/kg | % recovery ± SD (no. of analyses) |
|---|---|---|
| apples, pears | 0.04 | 96 ± 9.8 ($n$ = 13) |
| | 0.4 | 89 ± 5.0 ($n$ = 13) |
| grain | 0.04 | 101 ± 12.9 ($n$ = 5) |
| | 0.4 | 87 ± 4.7 ($n$ = 6) |
| straw | 0.1 | 98 ± 10.6 ($n$ = 10) |
| | 0.5 | 90 ± 6.0 ($n$ = 6) |
| soil | 0.04 | 85 ± 7.6 ($n$ = 3) |
| | 0.4 | 95 ± 6.5 ($n$ = 3) |
| water | 0.002 | 111 ± 6 ($n$ = 3) |
| | 0.02 | 101 ± 4 ($n$ = 3) |

Table III. Recoveries of Etaconazole and Propiconazole: Simultaneous Fortification and Determination

| substance | sample | fortification level, mg/kg | % recoveries |
|---|---|---|---|
| propiconazole | grain | 0.04 | 100/95 |
| | | 0.4 | 88/89/100 |
| | soil | 0.04 | 85 |
| | | 0.4 | 95 |
| | apples | 0.04 | 80 |
| | | 0.4 | 85 |
| etaconazole | grain | 0.04 | 87/100 |
| | | 0.4 | 82/90/112 |
| | soil | 0.04 | 87 |
| | | 0.4 | 100 |
| | apples | 0.04 | 78 |
| | | 0.4 | 87 |

## RESULTS AND DISCUSSION

The extraction of residues, especially of systematic pesticides, cannot be checked with fortified samples. For this purpose some residue-containing field samples were chosen and subjected to different extraction procedures.

| crop | solvent | extraction procedure | mg/kg[a] found |
|---|---|---|---|
| grapes | acetone | blending, twice | 0.086 |
| | methanol | shaker, 2 h | 0.090 |
| soil | acetone | hot extraction, 2 h | 0.023 |
| | methanol–water (8:2) | hot extraction, 2 h | 0.022 |
| | methanol–water (8:2) | shaker, 2 h | 0.022 |

[a] Mean value of two analyses.

As the standard deviation of the analytical procedure is at least 7%, the obtained results must be considered to be identical. For the ease of operation we therefore chose the cold extraction procedure without blending, which consists of shaking the samples for 2 h on a mechanical shaker. Methanol was used for plant materials and methanol–water for soil.

Our sample preparation procedures are especially chosen to give as finely as possible chopped and milled materials. In cases where the samples are only poorly crushed, blending before shaking is strongly recommended.

The suitability of the proposed analytical method was checked by analyzing a series of fortified samples. Recovery experiments were made by dropwise adding 0.5–1 $cm^3$ of a standard solution of these fungicides to the chopped or ground material. After evaporation of the solvent the extraction solvent was added and samples were analyzed according to the described procedure. The average recoveries of propiconazole and etaconazole at two fortification levels are given in Tables I and II. Fortification was made with each fungicide separately.

In Table III recovery values obtained by adding both fungicides simultaneously to the sample material were presented. Evaluation was with a standard curve containing also both pesticides simultaneously. Recovery values were identical with those found when each chemical was added and determined separately.

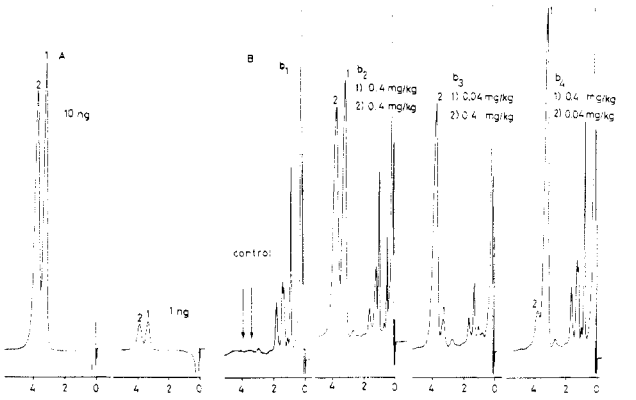


**Figure 1.** (A) Chromatograms of propiconzaole (2) and etaconazole (1). Column, 3% Carbowax 40 M on Gas-Chrom Q; oven temperature, 225 °C; sensitivity 1 × 16. (B) Chromatograms of grain sample; equivalent of 25 mg of sample injected; propiconazole (2); etaconazole (1). ($b_1$) Control. ($b_2$) Chromatogram of sample fortified with 0.4 mg/kg etaconazole and propiconazole. Recoveries: 90% and 89%, respectively. ($b_3$) Chromatogram of sample fortified with 0.04 mg/kg etaconazole and 0.4 mg/kg propiconazole. Recoveries: 100% and 95%, respectively. ($b_4$) Chromatogram of sample fortified with 0.4 mg/kg etaconazole and 0.04 mg/kg propiconazole. Recoveries: 112% and 100%, respectively.

Table IV. Series of Pesticides Tested To Show the Selectivity of the Proposed Method

| pesticide | | clean-up[b] | GC/PN detector[c] |
|---|---|---|---|
| acrolein | H[a] | – | – |
| amitrole | H | – | – |
| atrazine | H | + | ⊕, – |
| benomyl | F | – | – |
| bromofenoxim | H | – | – |
| captafol | F | – | – |
| carbendazim | F | – | – |
| chlordimeform | I | – | ⊕, – |
| chlorfenvinfos | I | + | ⊕, – |
| chlortoluron | I | – | – |
| desmetryn | H | + | ⊕, – |
| diazinon | I | + | ⊕, – |
| dicrotophos | I | – | ⊕, – |
| ditalimphos | F | – | ⊕, – |
| ethirimol | F | – | – |
| fluometuron | H | – | – |
| mancozeb | F | – | – |
| maneb | F | – | – |
| methidathion | I | * | ⊕, – |
| metolachlor | H | + | ⊕, – |
| monocrotophos | I | * | ⊕, – |
| phosphamidon | I | – | ⊕, – |
| profenofos | I | – | ⊕, – |
| propineb | F | – | – |
| simazine | H | + | ⊕, – |
| terbumeton | H | + | ⊕, – |
| thiabendazol | F | – | – |
| triadimefon | F | – | ⊕, – |
| lineb | F | – | – |

[a] F, H, and I = fungicide, herbicide, and insecticide. [b] Cleanup: (–) eliminated through proposed column cleanup; (+) elutes partially or totally from the column under the indicated conditions; (*) not tested. [c] GC/PN D: (–) cannot be chromatographed; (⊕) can be chromatographed; does not interfere with propiconazole/etaconazole resolution >1.5.

Chromatograms showing the simultaneous detection of propiconazole and etaconazole are presented in Figure 1.

The resolution of the two fungicides is generally satisfactory. Only when small amounts of propiconazole were present with large concentrations of etaconazole may evaluation give some problems. Good results were obtained by using tangent skimming and peak height for evaluation. No further attention has been paid to this fact as in practice simultaneous or alternating application of these fungicides is not recommended. As much more important we considered the selectivity of our method against other pesticides with recommended application on the same crops as propiconazole and etaconazole.

A series of important pesticides which theoretically can be detected by a nitrogen–phosphorus-selective detector were investigated. All compounds mentioned in Table IV were mixed with the two fungicides and injected into the gas chromatograph. None interfered with propiconazole and etaconazole. The mixture was also chromatographed on the proposed alumina column. Most of the compounds were either retained or eluted completely in the fore cut.

The analysis procedure described was applied to the routine analysis of these fungicides in different crops: apples, pears, grapes, prunes, peaches, oranges, wheat and barley (grain and straw), soil, and water were analyzed. The samples were from supervised trials. The analyses were performed with no difficulties and no interferences were observed. Only straw samples needed an additional gel permeation cleanup in order to be clean enough for the final determination. This cleanup is also recommended for oily or fatty samples. Recoveries obtained were in the range of 76–100%. We therefore consider this method to be suitable for the determination either of propiconazole or of etaconazole. The simultaneous determination of the two fungicides is also possible, although they should only exceptionally be found on the same crop.

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# Effects of Phosphorus, Potassium, Dolomite, and Nitrogen Fertilization on the Quality of Soybean. Yields, Proteins, and Lipids

Emile M. Gaydou*[1] and Jacques Arrivets

Effects of phosphorus, potassium, dolomite, and nitrogen fertilization upon yield, protein, and lipid contents of soybean var. "Davis" were investigated. Ground rock phosphate (0, 150, and 300 kg/ha), potassium chloride (0, 50, 100, and 150 kg/ha), and dolomite (0, 250, and 500 kg/ha) were applied, showing a significant ($p < 0.01$) positive response with yield. Urea was applied at rates ranging from 0 to 133 kg/ha but no change in yield was observed. Maximum yield (3300 kg/ha) was obtained with 150 kg/ha phosphate, 100 kg/ha potassium chloride, and 500 kg/ha dolomite. When the fertilizer amount was raised, an increase with phosphorus and a decrease with potassium were observed for protein content while an increase with phosphorus, potassium, and dolomite was observed for oil content. An interrelationship exists between the four main fatty acids for some fertilizer treatments. A positive correlation between linoleic and linolenic and a negative correlation between linolenic and oleic acids were observed.

Research has played in the last 20 years an important role in making soybean the premier of oilseed protein and the dominant vegetable oil in the world. Leng (1973) pointed out the potential of tropical countries to become soybean producers. Yields over 3000 kg/ha could be obtained in these countries, but some limiting factors existed such as the need for suitable varieties and the use of adequate cultural production practices (Lam-Sanchez, 1981). The problem of adapted varieties is almost solved in some countries but the research to improve the quality of soybeans has been limited. The ideal soybean would be (a) high in protein and (b) high in oil. The oil should contain low levels of fatty acids, which cause stability problems. It is generally accepted that the linolenic acid content of the oil is responsible for flavor problems that limits its acceptance for use in cooking and frying (Dutton et al., 1951; Evans et al., 1971). It would be (c) low in indigestible carbohydrates and (d) low in antinutritional factors (Smith, 1981). The responses to fertilizer have often been inconsistent. In the past, in some areas, soybeans have had the reputation of not responding to fertilizer (Nelson, 1971). Nitrogen can be supplied by a proper symbiosis with the nitrogen fixed by *Rhizobium japonicum*, and in Mississippi, atmospheric nitrogen was fixed to levels of 180 kg/ha (Hinson and Hartwig, 1977). The effects of inoculation and N fertilizer on soybean var. "Clark" were studied (Haque et al., 1980), and the influence of N nutrition on total N, nitrate, and carbohydrate levels was studied by Brevedan et al. (1977). The use of lime in soybean production is a common practice which, besides raising soil pH to proper levels for an adequate symbiosis (pH 5.8–7.0), provides the ions $Ca^{2+}$ and $Mg^{2+}$ and removes $Al^{3+}$ and $Mn^{2+}$ into less toxic forms (Lam-Sanchez, 1981). The response of soybean to potassium fertilizer (Chevalier, 1977) has been undertaken, and attention must be given

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