Table I. Herbicides Suitable for Bis Ester Chemistry[a]

acifluorfen	3,6-dichloropicolinic acid	mecoprop
benazolin	dichlorprop	naptalam
benzadox	endothall	picloram
bifenox	fenac	silvex
chloramben	flurenol	2,4,5-T
2,4-D	glyphosate	2,3,6-TBA
dalapon	glyphosine	Cl_3CCO_2H
2,4-DB	MCPA	triclopyr
dicamba	MCPB	

[a] Common name as determined by the Weed Science Society of America ("Herbicide Handbook of the Weed Science Society of America", 1979).

penetration, translocation, and detoxification characteristics of the esters vary between species. These selectivity differences, when coupled with the efficient penetration of the bis ester compound, may transform a carboxylic acid herbicide with moderate efficacy into a useful chemical for weed control.

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Effects of Repeated Application of Dichlobenil in a Commercial Apple Orchard

A commercial apple orchard of Idared on Malling 7 rootstock was treated with dichlobenil (2,6-dichlorobenzonitrile) at the maximum recommended rate (8.97 kg/ha a.i.) every other year from 1974 to 1978. Residues of the herbicide and the metabolite 2,6-dichlorobenzamide were determined in leaves, fruits, and soil. 2,6-Dichlorobenzamide was present in considerably greater amounts in the leaves and soil samples than the parent compound. Small amounts of 2,6-dichlorobenzamide was also detected in fruit. The residues levels were higher, and foliar phytotoxicity symptoms, characterized by leaf margin yellowing and leaf tip burn, were more marked in fruiting trees. However, removing the fruit to encourage growth resulted in a reduction in the leaf symptoms as well as the levels of dichlobenil and 2,6-dichlorobenzamide.

Dichlobenil (2,6-dichlorobenzonitrile) is recommended for the selective control of a wide range of annual and perennial weeds including both grasses and broadleaf species (Verloop, 1972; Beynon and Wright, 1972). Apple trees have been considered very tolerant to dichlobenil even at excessive rates (Sanford, 1962). However, Lord et al. (1973) and Lord and Damon (1974) observed that the annual applications of dichlobenil at the recommended rates caused foliar phytotoxic symptoms on young apple and peach trees. A comparison between control and dichlobenil-treated "McIntosh" apple trees showed no differences in tree growth and yield, although some relationship between phytotoxicity severity and tree growth and yield was noticed (Lord et al., 1973). In a later study, Lord and Green (1975) observed that the registered rates of dichlobenil usage in orchards, 4.47–8.97 kg/ha, had no adverse effect on tree growth even though foliar phytotoxic symptoms were present.

The occurrence of leaf margin chlorosis due to the use of dichlobenil as a soil-applied selective herbicide varies in accordance with geographical location and climatic conditions (Verloop, 1972). The leaf chlorosis varies from year to year (Verloop, 1972), probably due to soil conditions when dichlobenil is metabolized to 2,6-dichlorobenzamide, the causative agent of the leaf yellowing (Leach et al., 1971).

The herbicide dichlobenil has been used in eastern Ontario to effectively control weeds under mature apple trees. The present study was designed to determine the residue levels of dichlobenil and its metabolite, 2,6-dichlorobenzamide, in soil, leaf, and fruit after repeated applications of the herbicide in a commercial apple orchard. It was also of interest to determine whether any phytotoxicity symptoms occurred following the dichlobenil usage.

EXPERIMENTAL SECTION

Samples. Samples were collected on Aug 28, 1980, from a commercial apple orchard of Idared on Malling 7 rootstock growing on Newcastle silt loam (organic matter 5%). The trees were planted in 1974. Dichlobenil was applied (broadcast) at the maximum recommended rate of 65 g of product (8.97 kg/ha a.i.) on an area 2 × 2 m under each tree in the fall of 1974, 1976, and 1978 (Heeney et al., 1981). Excellent weed control was obtained in the area under the treated trees. The trees were allowed to fruit at an early age (1978). In 1980, fruit was removed from some of the trees to encourage growth. On Aug 28, 1980, midshoot leaf samples were collected from control and treated fruiting and defruited trees having similar dichlobenil treatments. In each case the leaves were collected from four trees and mixed thoroughly, and a subsample was retained for

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Table I. Percent Recovery of Dichlobenil and 2,6-Dichlorobenzamide from Fortified Samples at the 0.05-ppm Level (Mean Values for Triplicate Samples with Standard Errors)

	recovery, %	
sample	dichlobenil	2,6-dichloro-benzamide
soil	97.2 ± 2.4	83.9 ± 2.8
leaves	81.6 ± 1.5	71.9 ± 1.4
apples	84.4 ± 3.8	98.6 ± 3.2

analysis. Part of the leaf sample was also oven-dried (~70 °C) and analyzed for N, P, K, Mg, Ca, Mn, and Zn.

Soil samples were taken from the top 15 cm under the two types of treated trees. The control soil sample was taken from untreated areas between the rows of trees.

Fruit samples were collected at the same time. All samples were frozen and stored at −20 °C until analyzed.

Residue Analysis. Residues of dichlobenil and its primary metabolite (2,6-dichlorobenzamide) were determined as described by Montgomery et al. (1972). The soil, leaf, or fruit samples were extracted in a Goldfisch extractor with acetone for 6 h. The extract was made to 100 mL with acetone and analyzed by gas chromatography. The gas chromatograph was a Microtek 220 fitted with a ^{63}Ni electron capture detector. Residues of dichlobenil were determined by using a 1.5 m × 0.4 cm i.d. glass column packed with 3% OV-17 on 80–100-mesh Chromosorb WHP. The column was operated at 300 °C and the nitrogen carrier gas flow rate was 20 mL/min. Residues of 2,6-dichlorobenzamide were determined by using a 2 m × 0.4 cm i.d. glass column packed with 3% Carbowax 20M coated on 80–100-mesh Chromosorb WHP. The column temperature was 210 °C and the nitrogen gas flow rate was 40 mL/min. The concentration of dichlobenil and 2,6-dichlorobenzamide in the extract were determined by comparing the peak height with that of known concentrations of the reference standards. The identity of the desired peaks was ascertained by comparison of their mass spectra with those of pure reference standards and by cochromatography with the latter.

Determination of Residues in Fortified Samples. Untreated samples of leaves, fruit, and soil were fortified with 0.05 ppm of dichlobenil and 2,6-dichlorobenzamide. The solvent was allowed to evaporate and the sample mixed thoroughly. Residues were then extracted with acetone by the procedure described above and analyzed by gas chromatography.

RESULTS AND DISCUSSION

Under the gas chroamtographic conditions described, dichlobenil and 2,6-dichlorobenzamide gave retention times of 5.0 (OV-17 column) and 5.2 (Carbowax 20M column) min, respectively. The response of the electron capture detector was linear over the concentration range used (0.5–15 ng) with dichlobenil and 2,6-dichlorobenzamide requiring 3.0 and 0.6 ng, respectively, for a 50% full-scale deflection. The recovery of dichlobenil added to untreated soil, leaf and apples at the 0.05-ppm level ranged from 81 to 97% (Table I). Similarly, the recovery of 2,6-dichlorobenzamide from the samples, fortified at the 0.05-ppm level, was 72–98% (Table I). Interference from the coextractives was nearly negligible. The peaks from the sample extracts at the retention times of 5 min on the 3% OV-17 column and at 5.2 min on the 3% Carbowax 20M column were shown by their mass spectra to be dichlobenil and 2,6-dichlorobenzamide, respectively. The mass spectral fragmentation patterns of the reference standards matched with those of the compounds extracted from the soil, leaf, or apple samples.

Table II. Residues of Dichlobenil and 2,6-Dichlorobenzamide in Leaves, Soil, and Fruit in a Commercial Apple Orchard (Mean Values for Triplicate Samples with Standard Errors)

		ppb	
sample	treatment	dichlobenil	2,6-dichloro-benzamide
leaf	untreated control	ND[a]	ND
	defruited tree	89 ± 4	364 ± 11
	fruiting tree	191 ± 5	734 ± 34
apple	untreated	ND	ND
	treated	ND	42 ± 3
soil	untreated	ND	ND
	soil under defruited tree	103 ± 6	679 ± 68
	soil under fruiting tree	189 ± 7	1626 ± 22

[a] ND = none detected.

Table III. Mineral Content of Leaf Samples Collected from a Commercial Apple Orchard in August 1980

	mineral content, % dry wt basis				
sample	N	P	K	Mg	Ca
untreated orchard	2.20	0.14	1.12	0.23	1.53
treated orchard	1.80	0.22	0.48	0.32	1.51

Dichlobenil was found in soils collected from under the treated trees (Table II) although the residue levels were lower than those reported by Heeney et al. (1981) in an orchard soil that received six consecutive annual applications of the herbicide at the rate of 8.97 kg/ha. The breakdown product 2,6-dichlorobenzamide was present in considerably greater amounts than the parent compound.

No dichlobenil and only low levels of 2,6-dichlorobenzamide residues were detected in the fruit. The trees have been stunted, making little terminal growth. Removal of fruit from these stunted trees increased terminal growth by 28%. Foliar phytotoxicity symptoms, characterized by leaf margin yellowing and leaf tip burn, were observed in fruiting and nonfruiting trees but were most severe in the fruiting trees. Dichlobenil and 2,6-dichlorobenzamide levels were higher in the leaves associated with the fruiting trees.

Leaf analysis indicated that the trees were deficient in potassium but the levels of N, P, Mg, and Ca were adequate. Removal of fruit did not control the potash deficiency (Table III).

The trees were under stress due to the fruit load and also as a consequence of the low potassium. Removing the fruit stress reduced the leaf symptoms as well as the levels of the dichlobenil and 2,6-dichlorobenzamide. The reduction in the levels of the residues and symptoms in the leaves of the trees where fruit was removed may be related to growth dilution as a result of the greater leaf and shoot growth that occurred in these trees.

Malling 7 rootstock is shallow rotted with the bulk of the roots present near the surface soil where the major portion of the dichlobenil and 2,6-dichlorobenzamide have been accumulated. It has been found that even after 2 years unaltered 2,6-dichlorobenzamide could be extracted from the soil in a yield of about 90% of the applied dose, thereby indicating that the metabolite is very persistent in the soil (Verloop, 1972). Thus, for Idared on Malling 7 rootstock, toxic quantities of 2,6-dichlorobenzamide remained in the soil even 2 years after the last application of the herbicide in 1978. It has been shown that this major metabolite of dichlobenil is resistant to further breakdown

and appears to be the end product of the herbicide degradation in soil (Verloop, 1972). Leach et al. (1971) reported that 2,6-dichlorobenzamide is water soluble and is moved readily by soil water. The accumulation of this major breakdown product under eastern Ontario conditions may suggest that due to the insufficient rainfall in this area the material is not leached away from the root zone.

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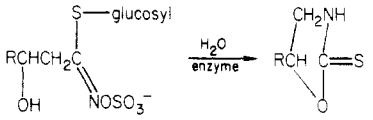
(+)-5-Allyloxazolidine-2-thione, an Enantiomer of Turnip Antithyroid Factor Isolated from *Berteroa incana* (L) D.C.

A new potential thyroid toxicant, (+)-5-allyloxazolidine-2-thione, has been isolated from *Berteroa incana* (L) D.C. seed. The new compound was characterized by comparison of its GLC, ORD, NMR, UV, and MS data to those of the previously known levo isomer from turnips and Chinese cabbage.

Oxazolidinethiones are well-known as antithyroid agents (Astwood et al., 1949; Tookey et al., 1980). They are readily formed from certain glucosinolates (GS's) in crucifer plants such as turnips, rutabagas, and Chinese cabbages. Glucosinolates that contain a β-hydroxy function (I) may hydrolyze to an intermediate isothiocyanate that



I II

cyclizes to form an oxazolidine-2-thione (OZT) (II) or may hydrolyze to form alternate aglucon products. Because glucosinolates impart flavor and can affect nutritional quality of foods and feeds, they have evoked much interest and have been extensively reviewed (Kjaer, 1960; Ettlinger and Kjaer, 1968; Tookey et al., 1980).

We wish to report a new potential toxicant, (+)-5-allyl-OZT, isolated from enzymatically hydrolyzed GS's of *Berteroa incana* (L) D.C. seed. Tapper and MacGibbon (1967) reported the corresponding levo isomer from turnips and rutabagas. The new OZT compound, and by inference a new precursor GS, was characterized by comparison with ORD and NMR data from the literature and by direct comparison of GLC, UV, and MS data with those of the authentic levorotatory isomer. We also report a convenient preparation of (+)-5-allyl-OZT that may be of use in gaining further knowledge of the toxicology of this class of thyroid poisons.

EXPERIMENTAL SECTION

Seed Preparation Analytical Procedures. Seed was collected from Wisconsin, Michigan, and Yugoslavia. Seed meal preparation, glucosinolate extraction, and subsequent analytical determinations for GS-glucose and quantitative determinations of individual aglucons were as summarized previously (Daxenbichler et al., 1980). GLC was performed with Packard 7400 series and supporting equipment as described by Daxenbichler and VanEtten (1977). GC–MS determinations were obtained as described by Spencer and Daxenbichler (1980). UV measurements were recorded with a Beckman Model DK-2A spectrophotometer. Optical rotation measurements were taken in 0.66% solution in methanol with a Perkin-Elmer No. 241 polarimeter in a 1 DM microcell and the data recorded at five wavelengths 589 (Na) and 578, 546, 436, and 365 nm (Hg). ^{1}H NMR spectra were recorded with a Varian HA-100 instrument. HPLC separations were performed with a Waters Associates instrument.

Isolation of Allyl-OZT. *Seed Preparation.* Whole seed (grown in Wisconsin) was ground in a Conco hammer mill and defatted by five separate 60-min steeps with pentane–hexane in a glass percolator. Then the meal was air-dried in a hood (yield: 77% of starting weight).

GS Extraction. Glucosinolates were extracted by inactivation of the endogenous enzyme system with boiling methanol followed by three extractions with 70% (v/v) aqueous methanol as described by Daxenbichler et al. (1980).

Thioglucosidase–Buffer Preparation. A crude thioglucosidase–buffer medium was prepared from *Sinapis alba* seed meal as follows: Whole *S. alba* seed (provided by French Co.) was flaked and defatted with pentane–hexane percolation at room temperature in a manner similar to the defatting operation of *Berteroa* seed described above.

Defatted *S. alba* seed meal (50 g) was shaken for 30 min with 500 mL, pH 7.0, phosphate buffer (0.05 M). Some solids were separated by passage through several layers of cheesecloth (excess liquid was collected from the solids in the cheesecloth by wringing and squeezing). The liquid was also filtered through Celite supported on Whatman No. 54 paper. The Celite on the filter was washed with