Residues of Diphenamid and Its Phytotoxic Metabolite in Flue-Cured Tobacco

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Diphenamid (*N,N*-dimethyl-2,2-diphenylacetamide) has been registered and used to control weeds in flue-cured tobacco (*Nicotiana tabacum* L.) since 1975. Surveys of tobacco leaf being offered for sale on the Ontario auction floors in 1976 and 1977 revealed residues of less than 0.5 mg/kg where the phytotoxic metabolite *N*-methyl-2,2-diphenylacetamide predominated. At the same time only trace residues of the less phytotoxic metabolite 2,2-diphenylacetamide were detected. Diphenamid and its metabolites were extracted with benzene and quantitated on an electroconductivity (Coulson) detection system with a detection limit of 0.01 mg/kg. The obtained data indicated that diphenamid was converted into *N*-methyl-2,2-diphenylacetamide and 2,2-diphenylacetamide as the major and minor metabolites, respectively. Flue-cured tobacco crops were treated at the prescribed rate of 6.75 kg of active ingredient/hectare (a.i./ha) on a 25-cm band or 1.6 kg of a.i./ha on a whole-field basis, and residues ranged from 9 mg/kg in the sand leaves to 0.2 mg/kg in the tip leaves. It is suggested that rapid translocation and stepwise demethylation are the protective degradation mechanism.

The selective preemergence herbicide diphenamid was registered in Canada in 1964 for tomatoes (*Lycopersicon esculentum* L. Mill) and for tobacco (*Nicotiana tabacum* L.) 10 years later in 1974. In Ontario diphenamid was recommended for the control of annual grasses and broadleaf weeds in tobacco fields (Ontario Ministry of Agriculture and Food, 1975). The phytotoxicity of diphenamid metabolites was investigated by Gentner (1969). He reported that with a progressive demethylation the phytotoxicity of diphenamid decreased and the activity of diphenylacetic acid was negligible. Since diphenamid and its metabolites may persist in the soil and injure fall-seeded crops, it became imperative that the herbicide be applied in a 10–25-cm band and that the soil be worked at right angles to the rows after the tobacco harvest to disperse the residues (Ontario Ministry of Agriculture and Food, 1980).

Surveys on pesticide use in Ontario conducted by Roller (1975,1979) confirmed that no diphenamid was used in 1973, but by 1978 11% of the tobacco hectarage (43 000) was being treated (Table I).

In 1976 and 1977 the crop offered for sale was monitored for diphenamid residues. The residues levels found warranted further studies on farms where diphenamid was used and in experimental tobacco plots at the Delhi Research Station.

The purpose of our investigation was to determine the persistence of diphenamid and its metabolites in flue-cured tobacco.

MATERIALS AND METHODS

Sample Collection. (*1*) *Warehouse Survey.* Flue-cured tobacco samples were collected in 1976 and 1977 at three tobacco auction exchange of the Ontario Flue-cured Tobacco Growers Marketing Board located at Aylmer, Delhi, and Tillsonburg. Approximately 1500 flats (usually 25–30 bales/flat) from both the fall and the winter deliveries were randomly sampled with three whole leaves taken from one bale in every fifth flat delivered. Leaves were separated according to stalk position and then composited on a weekly basis so that each sample represented 1 week's delivery at each exchange.

Table I.[a] Use Pattern of Herbicides in Flue-Cured Tobacco Grown in Ontario: 1973 and 1978 (Roller, 1975, 1979)

	year of survey							
	1973				1978			
	treated		use		treated		use	
herbicides	ha	%	kg	kg/ha	ha	%	kg	kg/ha
diphenamid	0				4680	11	9050	1.9
pebulate	2820	6.6	14 200	5.1	1290	3.0	6080	4.7
trifluralin	0				20	0.05	10	0.5

[a] Reprinted with permission from Roller (1975, 1979). Copyright 1975 and 1979 O.M.A.F. Economics Branch.

Sample collections were carried out by inspectors of the Farm Products Quality Branch, Ontario Ministry of Agriculture and Food (O.M.A.F.). The leaf samples were separated by stalk positions, dried, and ground in a Wiley mill at the Tobacco Research Station, Agriculture Canada. Herbicide residue analyses were performed at the Provincial Pesticide Residue Testing Laboratory, O.M.A.F.

(*2*) *Farm Survey.* Flue-cured tobacco samples were collected from 34 farms in 1976, 1977, and 1978 in the course of several related studies. On farms where diphenamid had been used, an extra sample was collected for analysis of diphenamid and its metabolites. In all cases diphenamid had been used at 6.75 kg of a.i./ha, the recommended rate, as a 25-cm band over the row following the transplanting of tobacco seedlings. On a total area basis, this represented an application of 1.7 kg of a.i./ha. After collection, cured leaves were separated according to stalk position, and grades were identified. The cured leaves were dried, ground in a Wiley mill, and stored for analysis at ambient temperatures. Pebulate was used on three of the selected farms in 1978, and trifluralin on one farm in 1979. Samples from these farms were also collected and are included in this investigation.

(*3*) *Field Experiments.* Field experiments were conducted at the Delhi Research Station during 1978 and 1979 to measure the uptake of diphenamid into tobacco tissues. The experimental plots were located on a Fox loamy sand, typical of the tobacco belt soils. A randomized block design of four replicates was employed, and the tobacco, variety Delhi 76, was transplanted into plots around June

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1st in each of the 2 years. Plots were 2.1 × 11.6 m and contained three rows of tobacco, the center row being harvested while the two side rows were planted for protection against herbicide drift.

Diphenamid (Enide WP 50) was applied in a band 25 cm wide over the transplants immediately following planting. The diphenamid was applied in 450 L of water/ha at 276 kPa of nozzle pressure. Mature tobacco leaves were harvested in five primings, cured, dried, and ground in a Wiley mill to pass through a 2-mm sieve. Before analyses the ground tobacco samples were homogenized in a Waring blender.

Residue Analysis. The selected benzene extraction was based on that of Long and Thompson (1974) with some modifications. This technique has been tested by using carbonyl-labeled [^{14}C] diphenamid on soybean plants (Krzeminski et al., 1972), on tomato plants (Bingham and Shaver, 1971), and on tobacco leaves (Long et al., 1974). The recoveries were 40–60%, 95%, and 94–97%, respectively, and indicated that the conjugation of diphenamid is not dominating metabolism path in all plants and largely absent in tomatoes and tobacco. The moderate polarity of benzene [$E^0|Al_2O_3$) = 0.32] reduced the amount of coextractives while the high solubility of diphenamid and its metabolites in benzene facilitated the partition.

(1) *Chemicals and Equipment.* The solvents used were nanograde quality supplied by Caledon Laboratories, Georgetown, Ontario. The standards (1) *N*,*N*-dimethyl-2,2-diphenylacetamide, (2) *N*-methyl-2,2-diphenylacetamide, and (3) 2,2-diphenylacetamide were obtained from Elanco Product Co., Division of Eli Lilly and Co.

A Tracor 550 gas–liquid chromatography apparatus with an electron-conductivity detection system (Coulson) operated in the nitrogen mode was used for identification and quantitation of diphenamid and its metabolites. The compounds were separated on a 183 cm × 6 mm o.d. borosilicate glass column packed with 5% Carbowax 20M on Varaport 30, 80–100 mesh, operated at 210 °C. Other temperatures were as follows: injector, 240 °C; transfer line and outlet, 220 °C; pyrolyser, 880 °C. Bridge potential was set at 30 V. The water was purified by percolating through an ion-exchange resin column, 2.5 × 25 cm, consisting of Bio-Rad AG x8, OH form (23 cm), topped with Amberlite MB-1, monobed (2 cm), both 20–50 mesh. The water flow rate through the electrolytic cell was well below 3 mL/min, reduced by insertion of a stainless steel wire into the supply channel.

(2) *Procedure.* Ten grams of ground, homogenized tobacco sample was extracted in a 500-mL boiling flask with 150 mL of benzene by shaking on a mechanical shaker for 60 min. The extract was filtered through a Whatman glass filter paper on a Büchner funnel using suction. The filter and the glassware were washed with 100 mL of benzene. The extract was returned to the original boiling flask and evaporated almost to dryness on a rotary evaporator equipped with a 50 °C water bath. The residue was transferred by using 100 mL of acetonitrile into a 250-mL separatory funnel and washed 4 times with 100 mL of hexane saturated with CH_3CN. The hexane washes were discarded and the CH_3CN fraction was evaporated to dryness. The residue was dissolved in 10 mL of dichloromethane.

The residue extract was purified by passing it through a Florisil cleanup column. The chromatography column, 400 × 25 mm o.d. with a Teflon stopcock, was packed by inserting a piece of glass wool at the bottom of the tube and then adding 10 g of anhydrous sodium sulfate, followed by 40–50 g of activated Florisil. The column was topped with 10 g of anhydrous Na_2SO_4. The dichloromethane solution of residues was transferred onto the column. After the sample solution had penetrated into the column, 200 mL of 1% methanol in dichloromethane was percolated through the column. The eluate was discarded. The diphenamid and its metabolites were eluted with 200 mL of 3% methanol in dichloromethane. The dichloromethane–methanol eluate was evaporated to dryness on a rotary evaporator (40 °C water bath). The final traces of CH_2Cl_2 were removed by adding 10 mL of methanol and evaporating. The residue was transferred into a graduate test tube by using methanol and the final volume ajusted to 5 mL or 2 g/mL.

Standard recovery curves were prepared from 10 g of tobacco samples fortified to 0.25, 0.50, and 1.00 μg/mL levels with methanolic solutions of standards 1, 2, and 3 24 h before extraction (Table II; Figure 1). The recoveries of diphenamid and *N*-methyl-2,2-diphenylacetamide were acceptable, but those of 2,2-diphenylacetamide were low.

Table II. Recovery of Diphenamid, *N*-Methyl-2,2-diphenylacetamide, and 2,2-Diphenylacetamide from Fortified Tobacco Samples

		recovery, %[a]		
	fortified, μg/g	diphenamid	*N*-methyl-2,2-diphenylacetamide	2,2-diphenylacetamide
flue-cured tobacco	0.25	83.5	92.5	53.8
	0.50	79.1	89.3	46.1
	1.0	80.2	87.5	46.2

[a] Average values of three replicates.

Table III. Residues of Diphenamid and Its Metabolite[a] (*N*-Methyl-2,2-diphenylacetamide) in Cured Leaf Collected from Three Auction Warehouses in 1976 and 1977

	residues in cured tobacco leaf, mg/kg					
	1976[b]			1977[b]		
tobacco leaf	diphenamid	metabolite	total	diphenamid	metabolite	total
sands	<0.01	0.35	0.36	<0.01	0.48	0.48
cutters	0.01	0.14	0.15	0.06	0.41	0.47
leaf	0.02	0.05	0.07	<0.01	0.27	0.27
tips	0.02	0.03	0.05	<0.01	0.15	0.15
mean	0.01	0.14	0.15	0.02	0.27	0.29
SD	0.04	0.24	0.25		0.18	0.16

[a] The metabolite 2,2-diphenylacetamide was sought but was not detected at residue levels above 0.01 mg/kg. [b] In 1976 and 1977, 48 samples were analyzed.

RESULTS

(a) Warehouse Survey. In this investigation data were gathered on the residues of diphenamid and its main phytotoxic metabolite, *N*-methyl-2,2-diphenylacetamide. The metabolites of diphenamid were not detected in the formulations available in Canada. The analytical results indicate that residues of diphenamid and *N*-methyl-2,2-diphenylacetamide were present in Ontario flue-cured tobacco being offered for sale on the auction floor (Table III). Residues of the second metabolite, 2,2-diphenylacetamide, were close to or below the detection level in both years of the survey. The major residues were those of the partly demethylated metabolite and were highest in the same leaves. Residues ranged from a high of 0.48 mg/kg in sand leaves to a low of 0.03 mg/kg in tip leaves.

(b) Farm Survey. In the Ontario farm survey, 5 and 11 of the 33 and 34 farms surveyed used diphenamid in

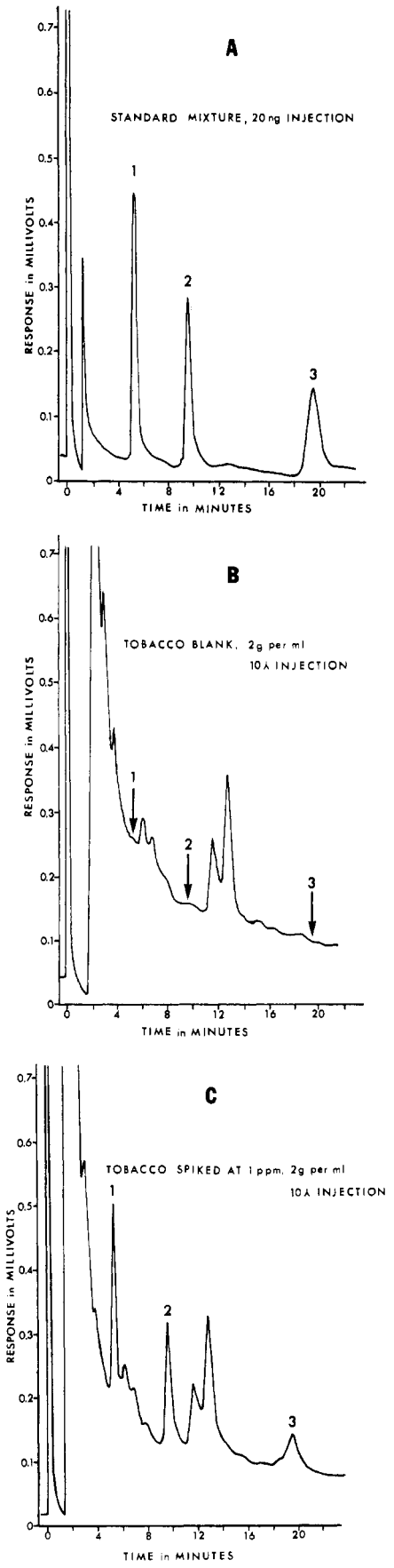

Figure 1. GLC chromatographs from analysis of fortified tobacco samples. (A) Standard mixture; injection of 20 ng each of (1) *N,N*-dimethyl-2,2-diphenylacetamide, (2) *N*-methyl-2,2-diphenylacetamide, and (3) 2,2-diphenylacetamide. (B) Untreated tobacco. (C) Tobacco fortified at 1 μg/g, 2 g/mL; 10-μL (20-ng) injection.

Table IV. Cured Tobacco Samples Collected for Residue Determination between 1976 and 1979 on farms Using the Recommended Rate of Diphenamid: The Rate of Application was 6.75 kg/ha on a Band 25 cm Wide on Rows 105-110 cm Apart[a]

		residue in cured dried tobacco leaf, mg/kg, for year of farm survey			
item		1976[b]	1977	1978	1979
no. of farms analyzed		10	6	11	5
no. of farms analyzed		33	33	34	34
P-1, sands	diphenamid		0.16	0.18	0.25
	metabolite		3.52	0.14	2.26
	total	3.01	3.68	0.32	2.51
P-2, cutters	diphenamid		0.01	0.19	0.09
	metabolite		2.17	0.18	1.21
	total	1.25	2.18	0.37	1.30
P-3 & 4, leaf	diphenamid		0.01	0.15	0.03
	metabolite		0.59	0.10	0.32
	total	0.44	0.60	0.25	0.35
P-5, tips	diphenamid		<0.01	0.11	0.02
	metabolite		0.24	0.07	0.16
	total	0.38	0.25	0.18	0.18
mean total residue:		1.27	1.68	0.28	1.09
SD:		1.05	2.56	0.36	1.57

[a] Actual rate per hectare of land was 1.6 kg. [b] In 1976 the residue levels of the metabolite and diphenamid are not reported.

the years 1976–1979. This represents 15–32% of these farms (Table IV). In 1976 the analysis involved a determination of both ingredients, together, while in the later work in 1977 the diphenamid and *N*-methyl-2,2-diphenylacetamide were separated. Other than 1978, residues of diphenamid and its metabolite were consistently higher in the sands and cutters than in the leaf and tips. In 1978, residues of diphenamid and its main metabolite were similar for all five leaf positions; however, in other years there was a substantial difference, and residues of the phytotoxic metabolite exceeded 10–20-fold that of the parent herbicide. An explanation for these differences has not been found.

In 1978 three farmers used pebulate on their tobacco crops; however, analysis of a few primings did not reveal any residues. Similarly, residues were not found when trifluralin was used.

DISCUSSION

In the late sixties Lemin (1966), using tomato (*L. esculentum* L. Mill), and Golab et al. (1966), using strawberry (*Fragaria* sp. L), reported that ^{14}C-labeled diphenamid entered the plants through the roots and was translocated into a leaves and, to a lesser extent, into other parts of the plant. Deli and Warren (1970), working with oat (*Avena sativa* L.) seedlings, and Bingham and Shaver (1971), using tomato, bermuda grass (*Cynodon dactylon* L.), and winged euonymus [*Euonymus alatus* (Thunb.)], confirmed and extended Lemin's findings by describing the demethylation and translocation processes as a protective mechanism used by tolerant species. Krzeminski et al. (1972), in following [^{14}C]diphenamid uptake studies with soybeans (*Glycine max* L.), further elucidated the pattern of distribution in resistant plant tissues. They observed lower diphenamid residue levels in the upper leaves of soybeans and concluded that the herbicide, while being taken up by the plant, was demethylated and partially absorbed through conjugation. Long et al. (1974) found similar uptake into tobacco seedlings with much lower residue levels in the roots than in the leaves. In this study

Table V. Residues of Diphenamid and Its Metabolite (*N*-Methyl-2,2-diphenylacetamide) in Cured Tobacco Leaf following Its Use on Soil at Planting Time (1978)

priming	residue	residues in cured dried leaf, mg/kg, at a rate of diphenamid on band, kg/ha, of 0[a]	2.25[b]	4.50	6.75
P-1, sands	diphenamid	0.02	0.14	0.35	0.67
	metabolite	<0.01	0.25	1.14	1.72
	total	0.02	0.39	1.49	2.39
P-2, cutters	diphenamid	0.04	0.15	0.30	0.32
	metabolite	0.06	0.24	0.68	0.78
	total	0.10	0.39	0.98	1.10
P-3, leaf 1	diphenamid	0.01	0.13	0.30	0.32
	metabolite	0.01	0.13	0.46	0.46
	total	0.02	0.26	0.76	0.78
P-4, leaf 2	diphenamid	<0.01	0.05	0.14	0.19
	metabolite	<0.01	0.06	0.21	0.23
	total	<0.01	0.11	0.35	0.42
P-5, tips	diphenamid	0.03	0.07	0.13	0.05
	metabolite	<0.01	0.08	0.14	0.17
	total	0.03	0.15	0.27	0.22

[a] Residues from drift from an adjacent area. [b] Application rates were to a 25-cm band with rows 107 cm apart; therefore, the rates on a whole-field basis were 0.53, 1.05, and 1.58 kg/ha.

Table VI. Residues of Diphenamid and Its Metabolite (*N*-Methyl-2,2-diphenylacetamide) in Cured Tobacco Leaf following Its Use on Soil at Planting Time (1979)

priming	residue	residues in cured dried leaf, mg/kg, at a rate of diphenamid on band, kg/ha, of 0	2.25[a]	4.50	6.75
P-1, sands	diphenamid	0	0.42	0.88	1.71
	metabolite	0	2.60	4.92	7.48
	total	0	3.02	5.80	9.19
P-2, cutters	diphenamid	0	0.19	0.55	0.70
	metabolite	0	0.70	2.48	2.51
	total	0	0.89	3.03	3.21
P-3, leaf 1	diphenamid	0	0.13	0.38	0.56
	metabolite	0	0.43	0.96	1.21
	total	0	0.56	1.34	1.77
P-4, leaf 2	diphenamid	0	0.09	0.21	0.14
	metabolite	0	0.22	0.44	0.33
	total	0	0.31	0.65	0.47
P-5, tips	diphenamid	0	0.04	0.05	0.06
	metabolite	0	0.07	0.19	0.16
	total	0	0.11	0.24	0.22

[a] Rates on a 25-cm band with rows 107 cm apart; therefore, rates on a whole-field basis were 0.53, 1.05, and 1.58 kg/ha.

translocation of diphenamid throughout the plant occurred; however, residue levels were significantly higher in the lower leaves and declined in concentration in ascending leaf order (Table III). Thus, our findings confirmed that the location of diphenamid residues in tobacco exhibited a similar pattern to that in soybeans reported by Krzeminski and co-workers.

According to Lemin (1966), after 7 days 59% of the diphenamid in tomato plants was present as the parent compound and the remainder as its demethylated metabolites. Bingham and Shaver (1971) reported that in tomatoes 60% of the absorbed diphenamid was demethylated and 39% was present as the parent herbicide. In tobacco seedlings Long et al. (1974) found only a trace of diphenamid and almost all of the residue consisted of *N*-methyl-2,2-diphenylacetamide. In the flue-cured tobacco leaves of our study, residues of diphenamid and *N*-methyl-2,2-diphenylacetamide determined in 1977 were in agreement with the findings of Long et al. (1974).

It is interesting to note that in 1978 the weather flecking or ozone damage in the field was greater than in any other year. The work by Hodgson et al. (1973, 1974) suggests modification of the diphenamid breakdown due to excessive ozone presence in the atmosphere. His findings in experiments with tomatoes indicated a more rapid breakdown of diphenamid and accompanying decreased levels of *N*-methyl-2,2-diphenylacetamide. Our study (Table V) supports the finding of Hodgson et al. (1973, 1974) that in 1978 the entire demethylation process was faster and the accumulation of the metabolites was lower than in normal years.

Long and Thompson (1974) analyzed air-cured Burley tobacco leaves at three stalk positions and found residues of diphenamid, *N*-methyl-2,2-diphenylacetamide, and 2,2-diphenylacetamide. The ratio of diphenamid to its *N*-methyl metabolite was in order of 1:10. This study (Table VI) indicated that except for 1978 a similar ratio prevailed in flue-cured tobacco leaves; however, the total residues were higher and 2,2-diphenylacetamide residues were lower than those reported by Long and Thompson (1974).

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