Drozdowska, 1981). The HPLC procedure used in the present study has the advantage of being both more sensitive and more quantitative than those older methods (Sang and Truscott, 1984). A possible explanation for the inability of nontransferred regenerant shoots to accumulate glucosinolates is that they were nutrient deficient. There were no apparent differences in root development between transferred and nontransferred shoots up to 50 days in culture. Further studies are in progress to determine optimal culture conditions for glucosinolate synthesis at the earliest distinct stage of regenerant shoot development and to determine whether it could be feasible to routinely screen such tissues for glucosinolates as part of a tissue culture breeding program.

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Registry No. 2-Hydroxybutenyl glucosinolate, 585-95-5; allyl glucosinolate, 3952-98-5; butenyl glucosinolate, 19041-09-9; (4-hydroxyindolyl)methyl glucosinolate, 83327-20-2; pentenyl glucosinolate, 19041-10-2; indolylmethyl glucosinolate, 4356-52-9; phenylethyl glucosinolate, 499-30-9; (4-methoxyindolyl)methyl glucosinolate, 83327-21-3.

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Methylation of Chlorophenoxy Acid Herbicides and Pentachlorophenol Residues in Foods Using Ion-Pair Alkylation

Marvin L. Hopper

An ion-pair alkylation (IPA) procedure was modified so that chlorophenoxy acid herbicides and pentachlorophenol residues could be methylated in the analysis of fatty and nonfatty foods. An intralaboratory evaluation and comparison between the IPA procedure and diazomethane methylation procedure currently being used showed that both were equivalent and gave high yields and good reproducibility. The IPA procedure is better than the diazomethane procedure because it gives cleaner reagent blanks and uses less toxic reagents.

The food items of the Food and Drug Administration's Total Diet Study, as described by Pennington (1983), are routinely monitored for chlorophenoxy acid (CPA) herbicides and pentachlorophenol (PCP) because they are widely used and are toxic. The analysis of 234 individual, table-ready food items requires that the CPA herbicides and PCP be converted to the methyl esters and ether, respectively, with diazomethane (Khan, 1975; Bache and Lisk, 1966; Howard and Yip, 1971). The methylation of these compounds facilitates the quantitation of them with gas–liquid chromatography (GLC). The inherent toxicity of both the starting material and the prepared diazomethane are of concern. The starting material is carcinogenic, and the diazomethane itself is an insidious poison and a potential explosive hazard, as listed in Sax (1984). For these reasons, less toxic reagents were sought to accomplish the methylation of these materials.

Cotterill (1982) described a procedure for ethylating chlorophenoxy acid and hydroxybenzonitrile herbicide residues in soil. Lianzhong et al. (1982) described an ion-pair alkylation (IPA) procedure for methylating low levels of 2,4-D and 2,4,5-T extracted from water samples. Both of these procedures use ion-pair alkylation to achieve the derivatization. The alkylation procedure of Lianzhong et al. (1982) gave high yields and good reproducibility with mild reaction conditions. This procedure is an application of the Williamson synthesis, as described in Condon and Meislich (1960). Because of their acidity, the chlorophenoxy acid herbicides and pentachlorophenol can be esterified and etherified, respectively, with an alkylating agent in the presence of an alkali such as tetrabutylammonium hydroxide (TBAH). TBAH serves to ionize the compounds, and methyl iodide is employed as the alkylating agent under conditions that form the methyl esters and ether, respectively. Methyl iodide and TBAH, as listed in Sax (1984), are less toxic than the reagents used in the diazomethane procedure.

The alkylation procedure of Lianzhong et al. (1982) appeared to be best suited for the derivatization of CPA's and PCP in foods of the Total Diet Study. This paper describes the work conducted to adapt the above alkylation procedure for the methylation of CPA's and PCP residues found in fatty and nonfatty food items. Results of an intralaboratory evaluation of the proposed method are

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presented, along with a comparison between the IPA and diazomethane procedures.

EXPERIMENTAL SECTION

Equipment. The concentrating system included a Snyder concentration column, a Kuderna-Danish (K-D) 250- or 500-mL concentrator, a 10-mL (No. K-570050-1025) or a 25-mL concentrator tube (No. K-570050-2525; Kontes, Vineland, NJ), and boiling chips of carborundum No. 12 granules (Catalog No. 133-B; Hengar Co., Philadelphia, PA).

The miniature concentrating system contained a distilling trap adapter (No. 5226) and a microevaporative concentrator (No. 6709; ACE Glass, Inc., Vineland, NJ). The filter consisted of a Millipore Swinny stainless adapter (Catalog No. IEAXX3001200) with Millipore 5.0-μm LS-type filter material (Catalog No. LSW01300; Millipore Corp., Bedford, MA).

Reagents were added with a 25-, 50-, or 100-μL Hamilton syringe (Hamilton Co., Reno, NV).

The gel permeation chromatograph (GPC) was an Auto-Prep 1001 (Analytical Biochemistry Laboratories, Inc., Columbia, MO) equipped with a 30-cm length × 2.5-cm i.d. column (Kontes), slurry-packed with 35 g of Bio-Beads SX-3 resin (200–400 mesh; Bio-Rad Laboratories, Richmond, CA) and compressed to a bed length of approximately 20 cm. The eluting solvent was methylene chloride–hexane (50:50, v/v) pumped at a flow rate of 5.0 mL/min, with an operating pressure range of 8–11 psi. The GPC system was set up with an 11-min dump, a 20-min collect, and a 0-min wash cycle.

A 300-mm length × 10-mm i.d. column (No. K-420540-0213; Kontes) was used for Florisil cleanup.

A Tracor 560 gas–liquid chromatograph, equipped with a nickel-63 electron-capture detector (ECD) and a Model 700A Hall electrolytic conductivity detector (HECD) in the halogen mode, was used for quantitation. The ECD was linked to a 1.8-m length × 4-mm i.d. glass column packed with 3% OV-101 on 100–120-mesh Chromosorb WHP. Column flow rate was 60 mL/min of helium with a purge gas flow rate of 10% methane in argon through the ECD at 70 mL/min. The HECD was linked to a 1.8-m length × 2-mm i.d. glass column packed with 5% OV-101 or 100–120 mesh Chromosorb WHP with a flow rate of 30 mL/min of hydrogen. Reaction gas flow rate through the furnace was 40 mL/min of hydrogen. The electrolytic conductivity cell had a flow rate of 0.5 mL/min of 1-propanol. Temperature parameters: inlet, 220 °C; oven, 180 °C; gas chromatography (GC)/ECD, 350 °C; GC/HECD furnace base, 250 °C; furnace, 950 °C.

Reagents. Tetrabutylammonium hydroxide (TBAH) titrant, 1.0 M in methanol, and methyl iodide, certified grade, were obtained from Fisher Scientific Co., Springfield, NJ.

Standards. Pentachlorophenol (PCP), pentachloroanisole, 2,3,6-trichlorobenzoic acid (2,3,6-TBA), (2,4-dichlorophenoxy)acetic acid (2,4-D), (2,4,5-trichlorophenoxy)acetic acid (2,4,5-T), 4-(2,4-dichlorophenoxy)butyric acid (2,4-DB), 4-(2,4,5-trichlorophenoxy)butyric acid (2,4,5-TB), and methyl esters of each were obtained from the Pesticide and Industrial Chemicals Repository, Environmental Protection Agency. All mixed standards were diluted from 1 mg/mL stock solutions of the individual standards.

Standard solutions: 1A, 2,3,6-TBA (1.0 μg/mL) and PCP (0.5 μg/mL) in acetone; 1B, 2,3,6-TBA (0.1 μg/mL) and pentachloroanisole (0.05 μg/mL) in isooctane; 2A, chlorophenoxy acids 2,4-D (2.5 μg/mL), 2,4,5-T (1.0 μg/mL), 2,4-DB (5.0 μg/mL), and 2,4,5-TB (2.0 μg/mL) in acetone; 2B, chlorophenoxy methyl esters of 2,4-D (0.25 μg/mL), 2,4,5-T (0.1 μg/mL), 2,4-DB (0.5 μg/mL), and 2,4,5-TB (0.2 μg/mL) in isooctane. A 1-mL volume of standards 1A, 2A, or both was used in all tests and fortifications. Standards 1B and 2B were used in quantitating all recoveries.

Solvents. Methylene chloride, acetone, hexane, isooctane, methanol, acetonitrile, ethyl ether, and petroleum ether were all pesticide-grade reagents (Burdick & Jackson, Muskegon, MI.).

Test Materials. A cross section of 23 items from the Total Diet Study was used in evaluating the ion-pair and diazomethane methylation procedures. The extraction and cleanup procedures used for each item are listed in Table I and are found in McMahon et al. (1968).

Ion-Pair Alkylation Procedure. The collected fraction was transferred from the GPC to a 250-mL K-D concentrator fitted with a 10-mL graduated tube. Boiling chips were then added, and the fraction was evaporated to approximately 5 mL with the aid of steam and a Snyder concentrator column. Following addition of 50 mL of acetone, the sample was reevaporated to approximately 1 mL and allowed to cool. The tube was detached and the fraction was evaporated to approximately 1 mL with a microconcentration apparatus and steam. After dilution to 3 mL with acetone, 80 μL of 1.0 M TBAH in methanol and 40 μL of methyl iodide were added, and each tube was immediately stoppered. (A well-ventilated hood and protective gloves should be used when adding reagents for methylation.) The stoppered tubes were mixed and placed in a 40 °C water bath for 1.5 h, with the water level of the bath above the fluid level in each reaction vessel. The tubes were removed from the water bath and attached to a 250-mL K-D concentrator. Boiling chips and 50 mL of hexane were added to each K-D, and the fractions were evaporated to a volume of approximately 1 mL (avoiding dryness) with the aid of steam and a Snyder concentrator column. All eluates were diluted to an appropriate volume with hexane and shaken with 2 mL of distilled water. The water was discarded and the fraction was quantitated by GLC, if further cleanup such as with Florisil was not performed.

Diazomethane Procedure. See Section 221.12c in McMahon et al. (1968) for the preparation of diazomethane. This involves the generation of diazomethane from a basic solution containing *N*-methyl-*N*-nitro-*N*-nitrosoguanidine into chilled ethyl ether. Methylation of a sample or standard contained in 4 mL of ethyl ether was accomplished by adding 5 mL of the diazomethane–ether solution and allowing the mixture to sit for 30 min after being mixed.

Florisil Cleanup. See Section 221.16 in McMahon et al. (1968) for the preparation of the Florisil column. Pentachloroanisole was eluted from the column with 35 mL of 20% methylene chloride in hexane. The remaining methylated herbicides of interest were eluted from the column with 60 mL of 50% methylene chloride/0.35% acetonitrile/49.65% hexane.

RESULTS AND DISCUSSION

Optimization of the IPA Procedure. The water bath temperature, reaction time, amount of methyl iodide, volume of the reaction solution, and the amount of TBAH needed for complete methylation of CPA herbicides and PCP were systematically tested.

Complete methylation of PCP and 2,3,6-TBA was achieved after being reacted for 30 min at 40 °C with 40 μL of methyl iodide and 20 μL of 1.0 M TBAH in methanol. A 40 °C reaction temperature ensured complete

Table I. Items Extracted and Cleaned Up[a]

item	% fat	extraction procedure[b]	extraction solvent	sample wt, g	samples cleaned up on GPC, g equiv wt
evaporated milk	7.5	221.13a	mixed ethers	100	5.0
cheddar cheese	31.4	221.13a	mixed ethers	50	6.0
pork sausage	37.6	221.13b	mixed ethers	50	10.0
roasted chicken	18.7	211.13b	mixed ethers	50	5.0
frankfurter	29.1	221.13b	mixed ethers	50	5.0
shrimp	9.6	221.13b	mixed ethers	50	5.0
soft-boiled egg	10.3	221.13b	mixed ethers	50	5.0
peas	<2	221.13c	methanol	100	16.2
corn	<2	221.13c	methanol	100	14.6
cantaloupe	<2	221.13e	methylene chloride	100	22.4
orange juice	<2	221.13e	methylene chloride	100	23.2
spinach	<2	221.13e	methylene chloride	100	18.3
sweet pepper	<2	221.13e	methylene chloride	100	21.8
brown gravy	<2	221.13e	methylene chloride	50	11.1
beer	<2	221.13e	methylene chloride	100	23.4
mixed vegetables	<2	221.13e	methylene chloride	100	20.5
peanut butter	57.1	221.13h	mixed ethers	50	5.0
granola	10.0	221.13h	mixed ethers	100	10.0
scalloped potatoes	4.0	221.13h	mixed ethers	25	5.0
pot pie	10.3	221.13h	mixed ethers	50	10.0
mayonnaise	80.0	221.13h	mixed ethers	25	5.0
chocolate chip cookies	22.5	221.13h	mixed ethers	100	6.6
high meat and vegetables (baby food)	4.5	221.13h	mixed ethers	50	10.0

[a] All extractions, GPC cleanup, mini-Florisil, and diazomethane methylation procedures used in this paper are found in McMahon et al. (1968). [b] Methods: GPC, 221.14; diazomethane, 221.15; Florisil, 221.16.

Table II. Comparison between Ion-Pair and Diazomethane Methylation Procedures for Samples Fortified after GPC Cleanup

compd	proced std ion	proced std dia	mayonnaise ion	mayonnaise dia	pork sausage ion	pork sausage dia	frankfurter ion	frankfurter dia	spinach ion	spinach dia	sweet pepper ion	sweet pepper dia
2,3,6-TBA	109	92 (1)[a]	105	102	100	89	114	98	104	96	111	96
PCP	100	95 (0.5)	101	100	89	95	104	100	102	93	102	100
2,4-D	107	96 (2.5)	107	96	97	92	106	88	94	86	107	98
2,4,5-T	107	92 (1)	100	101	108	102	103	96	100	92	110	93
2,4-DB	108	95 (5)	100	102	108	95	88	92	102	100	111	97
2,4,5-TB	100	97 (2)	94	101	103	97	104	97	102	94	108	101

[a] Numbers in parentheses represent micrograms of herbicides methylated.

reaction in a reasonable time and did not cause the other reagents used in the reaction solution to boil. A 40-μL amount of methyl iodide was found to be an adequate excess for methylating standards 1A and 2A (see Standards in the Experimental Section).

The efficiency of the methylation was further tested on standard 1A by using various amounts of TBAH, reaction times, and final reaction volumes. The results showed that more reaction time was needed to achieve complete methylation of standard 1A when the concentration of the TBAH and the final reaction volume were increased. A reaction time of 1.5 h was needed for complete reaction of 2,3,6-TBA and PCP, when reacted at 40 °C with 80 μL of 1.0 M TBAH in methanol and 40 μL of methyl iodide in 3 mL of acetone. Final reaction volumes ranging from 2 to 5 mL were investigated, and 3 mL was found to be optimum.

Four separate aliquots of a frankfurter extract (each equivalent to 5 g of frankfurter) were cleaned up on GPC, and each was fortified with standards 1A and 2A. All eluates were methylated in 3 mL of acetone with 20–80 μL of 1.0 M TBAH in 20-μL increments with 40 μL of methyl iodide and reacted at 40 °C for 1.5 h. The eluates were quantitated by GLC after Florisil cleanup. The results showed that 80 μL of 1.0 M TBAH in methanol is essential for good conversion of PCP and five CPA herbicides in a sample matrix. The ion-pair alkylation procedure in the Experimental Section was developed from the above test results.

Evaluation of the Ion-Pair Alkylation Procedure. A comparison between the IPA and diazomethane methylation procedures was made on extracted residues from pork sausage, frankfurter, mayonnaise, sweet pepper, and spinach. Four separate aliquots of each sample extract were cleaned up on GPC, and two of these eluates were each fortified with six herbicides. A set of fortified and nonfortified sample extracts along with a procedural standard were methylated by the IPA procedure and the diazomethane methylation procedure. The samples were cleaned up on Florisil and quantitated on GC/ECD and GC/HECD. The results in Table II show that the IPA and diazomethane methylation procedures are equivalent for methylating PCP and five CPA herbicides in sample matrices fortified after GPC cleanup.

The IPA and diazomethane methylation procedures were compared on 23 food items. These items were chosen because they represent a cross section of the 234 samples analyzed for PCP and CPA herbicides. The comparison was conducted on duplicate sample weights of each fatty or nonfatty item, fortified before extraction and cleanup. One sample weight for each item was fortified with six herbicides. All fortified and nonfortified samples were extracted, and duplicate eluates of each were cleaned up on GPC. All samples were extracted, cleaned up, methylated, and quantitated as described above. Table III shows that the IPA procedure gives results comparable to the diazomethane procedure for the methylation of PCP and five CPA herbicides in a wide variety of sample matrices. The low recoveries for CPA herbicides in boiled eggs by both procedures and PCP in frankfurters methylated

Table III. Comparison between Ion-Pair and Diazomethane Methylation Procedures for Samples Fortified before Extraction and GPC Cleanup

		percent recoveries											
		shrimp		boiled eggs		pork sausage		frankfurter		hi meat & turkey		roast chicken	
compd	spiking level,[a] ppm	ion	dia	ion	dia	ion	dia	ion	dia	ion	dia	ion	dia
2,3,6-TBA	0.02	86	67	tr[b]	tr	79	84	92	60	92	70	43	49
PCP	0.01	96	81	97	82	76	80	82	14	93	82	76	78
2,4-D	0.05	60	62	tr	tr	84	79	78	66	90	73	74	61
2,4,5-T	0.02	71	66	tr	tr	100	90	100	83	86	75	70	64
2,4-DB	0.10	79	67	56	54	88	86	85	80	92	70	82	74
2,4,5-TB	0.04	78	63	46	46	86	88	94	102	80	63	67	69

		percent recoveries							
		pot pie		brown gravy		peanut butter		cheddar cheese	
compd	spiking level,[a] ppm	ion	dia	ion	dia	ion	dia	ion	dia
2,3,6-TBA	0.02	80	83	74	72	86	90	90	67
PCP	0.01	85	86	86	86	93	81	76	83
2,4-D	0.05	70	70	81	80	76	64	76	77
2,4,5-T	0.02	85	72	94	88	73	76	83	91
2,4-DB	0.10	77	68	101	97	74	72	76	67
2,4,5-TB	0.04	77	66	98	95	89	84	99	83

		percent recoveries									
		spinach		sweet pepper		evap milk		mixed veg		beer	
compd	spiking level,[c] ppm	ion	dia	ion	dia	ion	dia	ion	dia	ion	dia
2,3,6-TBA	0.01	82	82	100	88	80	66	84	101	81	92
PCP	0.005	73	68	98	80	98	96	94	92	92	95
2,4-D	0.025	85	76	100	89	49	38	93	88	94	84
2,4,5-T	0.01	92	86	87	81	57	53	96	83	96	88
2,4-DB	0.05	93	84	110	92	64	62	104	84	88	86
2,4,5-TB	0.02	80	74	90	92	74	74	96	93	99	100

		percent recoveries									
		corn		granola		peas		orange juice		cantaloupe	
compd	spiking level,[c] ppm	ion	dia	ion	dia	ion	dia	ion	dia	ion	dia
2,3,6-TBA	0.01	91	89	64	60	60	59	94	89	105	87
PCP	0.005	92	96	92	98	86	90	94	95	92	88
2,4-D	0.025	98	84	90	83	99	94	99	85	103	101
2,4,5-T	0.01	93	84	100	89	103	90	100	92	92	101
2,4-DB	0.05	92	96	78	82	108	96	99	92	92	105
2,4,5-TB	0.02	108	110	98	100	103	110	95	98	98	105

		percent recovery, cookies			percent recoveries			
					mayonnaise		scalloped potato	
compd	spiking level,[c] ppm	ion	dia	spiking level,[d] ppm	ion	dia	ion	dia
2,3,6-TBA	0.01	75	87	0.04	48	60	78	80
PCP	0.005	90	89	0.02	89	86	80	89
2,4-D	0.025	76	79	0.10	73	60	65	71
2,4,5-T	0.01	82	87	0.04	80	63	82	89
2,4-DB	0.05	78	71	0.20	90	74	87	95
2,4,5-TB	0.02	95	100	0.08	98	94	94	98

[a] For a 50-g sample. [b] Less than 10%. [c] For a 100-g sample. [d] For a 25-g sample.

with diazomethane is unexplainable.

Intralaboratory Evaluation. An intralaboratory evaluation using the IPA procedure was conducted on the five food items used in the IPA and diazomethane comparison, in which the extracts were cleaned up on GPC and then fortified. One set of fortified and nonfortified extracts of each sample along with a procedural standard were methylated by the IPA procedure. All samples were cleaned up on Florisil, and the herbicides were quantitated. The results presented in Table IV demonstrate that the IPA procedure can be duplicated.

The reagent blanks of the IPA procedure and the diazomethane procedure were compared on a GC/ECD before being cleanedup on Florisil. Both reagent blanks were prepared by the different methylation procedures and diluted to final volumes of 10 mL in hexane. The diazomethane reagent blank, as shown in Figure 1, required additional cleanup to obtain a usable chromatogram. The IPA reagent blank could be used in an ECD analysis

Table IV. Percent Recoveries for Intralaboratory Evaluation

compd	proced std	mayonnaise	pork sausage	frankfurter	spinach	sweet pepper
2,3,6-TBA	114 (1)[a]	105	110	89	103	98
PCP	111 (0.5)	107	106	94	86	94
2,4-D	98 (2.5)	97	94	107	97	97
2,4,5-T	111 (1)	84	104	103	109	102
2,4-DB	108 (5)	106	111	98	107	104
2,4,5-TB	97 (2)	104	105	95	100	82

[a] Numbers in parentheses represent micrograms of herbicides methylated.

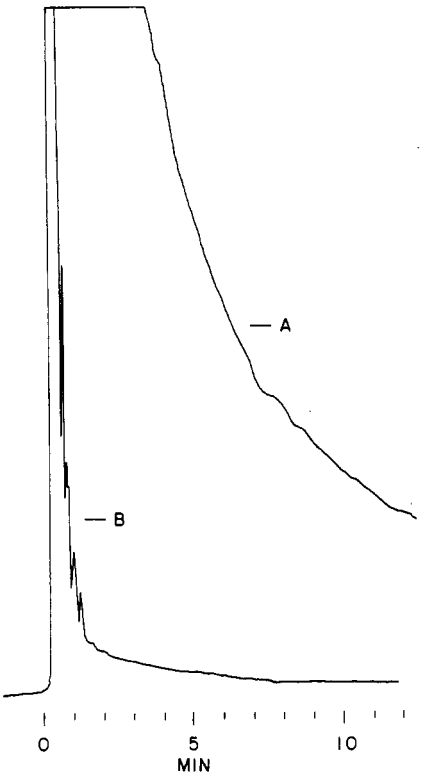

Figure 1. Chromatograms of ion-pair alkylation and diazomethane reagent blanks using GC/ECD: A, diazomethane reagent blank; B, ion-pair alkylation reagent blank.

without further cleanup.

CONCLUSION

These experiments have shown that the ion-pair alkylation procedure is equivalent to the diazomethane procedure for giving high yields and good reproducibility for methylating CPA's and PCP in food residues. The alkylation procedure is better than the diazomethane procedure, because it gives cleaner reagent blanks and involves the handling of less toxic more stable reagents. The ion-pair alkylation procedure can be used as an alternate methylation procedure to the diazomethane procedure. Further methylation studies on other compounds using this alkylation procedure are in progress and will be reported in the future.

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Gas Chromatographic Analysis of Fluazifop-butyl (Fusilade) in Potatoes, Soybeans, and Soil

B. Stephen Clegg

Fluazifop-butyl, (+)-butyl 2-[4-[[5-(trifluoromethyl)-2-pyridyl]oxy]phenoxy]propionate, is the active ingredient in Fusilade, a new highly selective systemic postemergence grass herbicide introduced by ICI for use in broadleaf crops. A specific gas chromatographic procedure is described for the determination of residues of fluazifop-butyl in potatoes, soybeans, and soil samples. Following an initial alkaline hydrolysis, residues are extracted with dichloromethane, methylated with diazomethane, subjected to Florisil column cleanup, and determined by capillary gas–liquid chromatography with a nitrogen-specific detector. The method is suitable for the determination of fluazifop, fluazifop-butyl, and its conjugates in various crops and soil samples. Recoveries of fluazifop-butyl, determined as its methyl ester derivative, were greater than 70% following fortification levels of 0.05–1.0 $\mu g\ g^{-1}$. Residues in potatoes and soybeans treated with Fusilade at recommended rates were below 0.05 $\mu g\ g^{-1}$ when harvested 90 days after herbicide application.

Fluazifop-butyl, (+)-butyl 2-[4-[[5-(trifluoromethyl)-2-pyridyl]oxy]phenoxy]propionate, is the active ingredient of the herbicide Fusilade, and the structure is presented in Figure 1.

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Fusilade is a highly selective systemic postemergence grass herbicide for use in broadleaf crops (Bates et al., 1982; Wagner, 1983). Currently, fluazifop-butyl under the trade name Fusilade is temporarily registered in Canada for use on field crops such as flax, sugar beets, and sunflowers. Legume forage crops include alfalfa, red clover, and birdsfoot trefoil, while potato is the only vegetable listed (OMAF, 1985).

Fluazifop-butyl, a (pyridyloxy)phenoxy compound is related structurally to a new series of herbicides often

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