Metabolism and Fate of [^{14}C]Ethametsulfuron-methyl in Rutabaga (*Brassica napobrassica* Mill.)

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The metabolism and fate of ethametsulfuron-methyl {methyl 2-[[[[[4-ethoxy-6-(methylamino)-1,3,5-triazin-2-yl]amino]carbonyl]amino]sulfonyl]benzoate} in rutabaga were investigated. After 72 h, absorption and translocation of [^{14}C]ethametsulfuron-methyl in rutabaga did not change for the duration of the study (50 days). Less than 4% of recovered radioactivity was present in the rutabaga root. Ethametsulfuron-methyl was metabolized through a proposed unstable α-hydroxy ethoxy intermediate that dissipated 3 days after treatment to two major metabolites, *O*-desethylethametsulfuron-methyl and *N*-desmethyl-*O*-desethylethametsulfuron-methyl, as determined by liquid chromatography−mass spectrometry. It was estimated that at a spray dose of 30 g of active ingredient ha^{-1} and a harvest weight of 0.5 kg, the edible portion of the rutabaga root would contain no ethametsulfuron-methyl and ∼1.3 ppb total of both identified metabolites. Residue analysis and toxicological assessment show that ethametsulfuron-methyl and its metabolites should pose little or no risk to consumers of rutabagas.

Keywords: *Herbicide; wild mustard (Brassica kaber); dose response; toxicological assessment*

INTRODUCTION

The sulfonylurea herbicide ethametsulfuron-methyl, formerly known as DPX-A7881, inhibits the enzyme acetolactate synthase (ALS; EC 4.1.3.18), also known as acetohydroxyacid synthase (AHAS). ALS is the first enzyme of the branched-chain amino acid biosynthetic pathway responsible for the biosynthesis of valine, leucine, and isoleucine. Ethametsulfuron-methyl is a highly selective postemergent herbicide that controls Brassicaceae weeds, such as wild mustard (*Brassica kaber*) in Brassicaceae crops such as oilseed rape (*Brassica napus* and *Brassica campestris*) (Lichtner et al., 1995) and commercial brown mustard (*Brassica juncea*) (Buchanan et al., 1990a,b; Hall et al., 1992). It has been shown that the selectivity between tolerant crop and sensitive weed species to ethametsulfuron-methyl is based on its metabolism to nontoxic products in tolerant species (Lichtner et al., 1995; Hall et al., 1992).

Rutabaga is tolerant to ethametsulfuron-methyl. This herbicide can be used to selectively control Brassicaceae weeds in rutabaga crops (Pitblado et al., 1997). Wild mustard competition in canola reduced yields by 36% (McMullen et al., 1994) and has a similar effect on rutabaga yields. Currently, there is no herbicide registered for the control of wild mustard in rutabaga crops. The rutabaga growers of Ontario, the Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA), and the Pest Management Regulatory Agency (PMRA) of Health Canada, as well as Agriculture, Agri-Food Canada (AAFC), are jointly seeking information on the metabolism of ethametsulfuron-methyl in rutabaga for its registration under the User Requested Minor Use Label Expansion (URMULE) program.

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The goal of this research project was to determine the fate and persistence of ethametsulfuron-methyl and its metabolites in rutabaga. The objectives of the research were to determine (i) the dose−response of rutabaga and wild mustard to ethametsulfuron-methyl, (ii) the pattern of absorption and translocation of ethametsulfuron-methyl at various time intervals to establish the quantity of herbicide that accumulates in the edible portion of the root, (iii) the metabolism (catabolism) of ethametsulfuron-methyl in leaves of rutabaga and the edible portion of the rutabaga root at various times after herbicide application, (iv) the identity of the metabolites of ethametsulfuron-methyl, and (v) the human health safety via a toxicological assessment of harvested rutabagas after application of ethametsulfuron-methyl. On the basis of these results, the PMRA can determine whether the crop is safe for human consumption after being sprayed with the herbicide and, consequently, whether ethametsulfuron-methyl can be registered for use in rutabaga crops.

EXPERIMENTAL PROCEDURES

Chemicals. Radiolabeled ethametsulfuron-methyl {methyl 2-[[[[[4-ethoxy-6-(methylamino)-1,3,5-triazin-2-yl]amino]carbonyl]amino]sulfonyl]benzoate} was obtained from E. I. du Pont de Nemours and Co., Inc. (Wilmington, DE). The specific activities of triazine(U)-^{14}C- and phenyl(U)-^{14}C-ethametsulfuron-methyl were 752.1 and 605.1 MBq mmol^{-1}, respectively, with both labeled forms having a radiochemical purity of >94%. Muster 75 DF, the formulated commercial product of ethametsulfuron-methyl, was obtained from Dupont Canada Inc. (Missisauga, ON, Canada). All chemicals and reagents used were of reagent quality or better.

Growth of Plants. For the absorption, translocation, and metabolism experiments, three to six seeds of rutabaga (cv. Laurentian) were placed in 350 mL Styrofoam cups containing Turface, a montmorillonite clay potting medium (International Minerals and Chemical Corp., Mundelein, IL). For all other experiments, plants were grown in Premier Promix, a peat

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moss based potting medium (Premier Horticulture Inc., Red Hill, PA). All plants were grown under the same conditions, and seedlings were subsequently thinned to one plant per cup. The plants were irrigated with water and fertilized, as required, three to four times a week with 20−20−20 (N:P:K) fertilizer (20 g L^{-1}) containing micronutrients. Plants were grown in a controlled environment growth room maintained at 24/16 ± 1 °C day/night temperature with a 16 h photoperiod and an average relative humidity of 65%. The irradiance level was constant at 450 μeinstein m^{-2} s^{-1}. In all experiments, rutabaga plants were treated at the three-leaf stage.

Dose—Response Experiments. Wild mustard plants, collected in southwestern Alberta, Canada, were sprayed at the three−five-leaf stage of development, whereas rutabaga plants were treated at the three-leaf stage of development. The commercial formulation of ethametsulfuron-methyl, Muster 75 DF, plus 0.1% v/v Agral 90, a nonionic surfactant, was applied at doses ranging from 1 to 240 g of active ingredient (ai) ha^{-1} with a motorized hood sprayer equipped with a flat-fan nozzle (TeeJet 8002E) calibrated to deliver 200 L ha^{-1} of spray solution at 250 kPa. The potting medium was shielded with vermiculite during herbicide application and removed after the plants were dry. Plants were harvested 14 days after treatment (DAT) by severing the shoots from the rutabaga root. The roots were removed from the potting medium by rinsing the roots in water. Shoot and root fresh and dry weights were determined.

Absorption, Translocation, and Metabolism Experiments. *Treatment of Plants.* Experiments were conducted using either triazine(U)-^{14}C- or phenyl(U)-^{14}C-ethametsulfuron-methyl dissolved in an application solution of 0.1 M phosphate-buffered saline (PBS; pH 7.5) containing 10% ethanol and 0.5% Tween 20 (oxysorbic 20-polyoxyethylene sorbitan monolaurate). The radiolabeled herbicide solution was applied to the adaxial midsection (perpendicular to the midvein) of the third leaf of rutabaga plants. Ten 1 μL drops of ~5.1 kBq (~300000 DPM; 30 g of ai ha^{-1} $plant^{-1}$) of either the phenyl- or triazine-labeled [^{14}C]ethametsulfuron-methyl solution were applied with a Hamilton syringe equipped with an adapter calibrated to deliver 1-μL drops.

Harvest of Plants. Plants were harvested 3, 10, 15, 30, and 50 DAT and dissected into treated leaf, nontreated foliage, rutabaga root (edible portion of the root), and fibrous roots (remainder of roots). The nontreated foliage, rutabaga root, and fibrous roots were dried at 80 °C and weighed, and the level of radioactivity in these portions was determined by oxidative combustion of the samples as described later. The amount of [^{14}C]ethametsulfuron-methyl present on the treated leaf surface was determined by foliar rinse treatment (Devine et al., 1984) during which 10 mL of aqueous 10% ethanol (v/v) containing 0.5% Tween 20 was directed over the treated leaf surface. The rinse solution was collected in two 22 mL scintillation vials containing Ecolite (+) (ICN Biomedicals Inc., Irvine, CA) scintillation cocktail and quantified by liquid scintillation spectrometry (LSS) using a Beckman LS6K-SC scintillation counter (Beckman Instruments Inc., Fullerton, CA). The treated leaf samples were stored frozen at −20 °C until extraction of [^{14}C]ethametsulfuron-methyl and its metabolites. Appropriate controls were included to ensure that herbicide degradation did not occur as a result of storage or the experimental procedures.

Extraction of [^{14}C]Ethametsulfuron-methyl and Its Metabolites. The treated leaf was minced and homogenized in 3−5 mL of acetonitrile/water (7:3, v/v) per gram of tissue using a Polytron probe (Kinematica GmbH, Switzerland) and centrifuged (10000*g* for 10 min) as described by Hall et al. (1992). The supernatant was decanted and the pellet resuspended in acetonitrile/water (7:3, v/v) and extracted twice more. After the final extraction, the resulting pellet was combusted to determine the amount of unextractable radioactivity. The supernatants were pooled and concentrated under a stream of air at 35 °C. An aliquot (100 μL) was removed to determine by LSS the percent recovery of radioactivity. The supernatant was further purified, prior to high-performance liquid chromatography (HPLC) analysis, by passing an aliquot (3−5 mL) of the radioactive plant solution through a preparative C_{18} cartridge (C_{18} Sep-Pak Plus; Waters Associates, Milford, MA). The radioactivity adsorbed to the matrix was eluted with 2 mL of acetonitrile/water (7:3, v/v). Samples were stored at −20 °C until HPLC analysis.

HPLC Analysis. The partially purified plant extract (100 μL) was analyzed by HPLC on a Shimadzu model LC-6A chromatograph equipped with an ODS Spherex column (C_{18} 5 μm particle size, 250 × 10 mm; Phenomenex, Torrance, CA) as described by Hall et al. (1992). The solvents used for HPLC were acetonitrile (B) and Nanopure water with 0.1% phosphoric acid (A) or 1 M acetic acid (A). The chromatographic conditions consisted of a nonlinear concave number 2 gradient of 5−100% B over 30 min for elution, followed by 100% B for 5 min and 5% B for 5 min at a flow rate of 2 mL min^{-1}. The column temperature was maintained at ambient room temperature (20 ± 3 °C). [^{14}C]Ethametsulfuron-methyl and its radiolabeled metabolites were detected and quantified using a Radiomatic Flo-One\Beta A-250 radioactivity flow detector (Radiomatic Instruments and Chemical Co. Inc., Tampa, FL).

Primary metabolism experiments were conducted in which plants were harvested 6 and 24 h after treatment (HAT) with [^{14}C]ethametsulfuron-methyl. Plants were processed as described above. However, translocation of [^{14}C]ethametsulfuron-methyl and its metabolites was not determined in these experiments.

Oxidative Combustion of Samples. Plants were harvested 3, 10, 15, 30, and 50 DAT and dissected into treated leaf, nontreated foliage, rutabaga root (edible portion of the root), and fibrous roots (remainder of roots) as described above. The nontreated foliage, rutabaga root, and fibrous roots were dried at 80 °C and weighed. The quantity of radioactivity in the nontreated foliage, rutabaga root, fibrous roots, and pellets was determined by oxidative combustion of tissue samples to $^{14}CO_2$ using a biological oxidizer (model OX-300, R. J. Harvey Instrument Corp., Hillsdale, NJ) as described by Hall et al. (1992). The resulting $^{14}CO_2$ was trapped in carbon-14 scintillation cocktail (R. J. Harvey Instrument Corp.). $^{14}CO_2$ recovery was >96% as determined by combusting known quantities of ^{14}C[methyl] methacrylate (Spec-Chec-^{14}C, Packard Instrument Co., Downers Grove, IL). Because only 300 μg of plant tissue could be combusted at a time, large plant parts were weighed, pulverized, and homogenized using different methods depending on the tissue type, and 300 μg aliquots were combusted. Ashless filter paper was used to form aliquot pellets of 100 ± 0.5 mg of the homogenized plant material. At least three aliquots of each sample were combusted, and the amount of $^{14}CO_2$ present in each sample was determined by LSS.

Herbicide Residue Analysis in Rutabaga Root. Approximately 16.8 kBq (1000000 DPM; 100 g of ai ha^{-1} $plant^{-1}$) of phenyl-labeled [^{14}C]ethametsulfuron-methyl was applied as 1 μL droplets to all three leaves of each rutabaga plant with a Wiretrol II micropipet (Drummond Scientific Co., Broomall, PA). Plants were harvested 3 DAT and frozen at −20 °C until extraction. The rutabaga root was minced and homogenized in ~3 mL of acetonitrile/water (7:3, v/v) with a tissue grinder (Pyrex no. 7727-7, Fisher Scientific Inc.). The resulting solution was centrifuged, the supernatant collected, and the pellet resuspended in acetonitrile/water (7:3, v/v). Each sample was extracted twice in this fashion. Supernatants were pooled and concentrated in volume as described previously. An aliquot of the rutabaga root sample was filtered through a 3 mm diameter, 0.22 μm nylon syringe filter (MSI Cameo, MSI Micron Separations Inc., Honeoye Falls, NY) and the extract analyzed by HPLC for [^{14}C] metabolites, as described earlier.

Mass Spectrometry (MS) Experiments. Approximately 5.1 kBq (300000 DPM; 30 g of ai ha^{-1} $plant^{-1}$) of phenyl-labeled [^{14}C]ethametsulfuron-methyl was applied to seven rutabaga plants at the three-leaf stage. These plants were also sprayed with 300 g of ai ha^{-1} of Muster 75 DF with 0.1% (v/v) Agral 90 as described earlier. Plants were harvested 6 DAT, and the third leaf of seven plants was excised and frozen at −20 °C until extraction of ethametsulfuron-methyl and its metabolites, as described earlier. Supernatants from all seven leaves were pooled and concentrated to 2−3 mL. The solution was centri-

fuged (10000*g* for 10 min) to pellet suspended debris. The supernatant was applied to a preparative C_{18} cartridge (C_{18} Sep-Pak Plus; Waters Associates). The bound radioactivity was eluted in 3 mL of acetonitrile/water (1:1 v/v). The eluent was concentrated to ~1.5 mL and was further purified by HPLC prior to LC/MS analysis.

Chromatographic Preparation of Metabolites prior to LC-MS. Purification of the extracted aqueous sample was on an HPLC system equipped with an ODS Spherex column (C_{18} 5 μm particle size 250 × 10 mm) using the same chromatographic conditions as described before. The two metabolites (retention times of 20−21 min) were collected together in one vial, and HPLC was repeated three times. The fractions were pooled and concentrated to <1 mL. Further purification was by HPLC on an ODS Spherex column (C_{18} 5 μm particle size, 250 × 10 mm) as described by Lichtner et al. (1995). The solvents used were 0.1 M acetic acid (A) and methanol (B). An isocratic gradient of 30% B was run for 20 min followed by 100% B for 10 min at a flow rate of 2 mL min^{-1}. A diode array and radioactive flow detector (Radiomatic Flo-One\Beta A-200) were connected in series to detect the metabolites of [^{14}C]-ethametsulfuron-methyl. Under these conditions, the two metabolites (metabolites A and B) were separated and collected between 10.5 and 11.5 min and between 13.5 and 14.5 min, respectively (ethametsulfuron-methyl eluted at 28.5 min). Each eluting solution containing a separate metabolite (metabolite A or B) was concentrated to <1 mL and further purified by HPLC using an isocratic gradient of 25% methanol. Metabolite A eluted between 34 and 36 min and metabolite B between 24.5 and 25.5 min. The aqueous methanol solution containing each metabolite was reduced to near dryness under low heat (<25 °C). The residue was dissolved in 200 μL of acetonitrile/water (1:1, v/v) prior to LC-MS analysis.

LC-MS Analysis. The LC was performed using a Prodigy 5 ODS-2 column (150 × 3.2 mm i.d. with a 5 μm particle size). Mobile phase, consisting of 50% water and 50% acetonitrile, was filtered using 0.22 μm membrane filters. The mobile phase was delivered by binary LC pumps (Hewlett-Packard 1090 series II//L, Palo Alto, CA). The eluent was passed through the sample injection valve (a Rheodyne model 7010) to an electrospray ion source at a flow rate of 0.1 mL min^{-1}. A sample volume of 20 μL was injected by an autosampler (Hewlett-Packard 1090 series II//L).

MS detection was achieved using a VG Quattro II (Fisons, U.K., Ltd., Altricham, U.K.) triple-quadropole mass spectrometer equipped with an electrospray (ES) ion source. MassLynx software package was used for instrument control, data acquisition, and data processing.

Liquid N_2 was used as both the drying and ES nebulizing gas. The drying and ES nebulizing gas flow rates were 500 and 20 L h^{-1}, respectively. The instrument was operated in +ve or −ve ion mode with an ion source temperature of 95 °C, a capillary needle potential of +3.75 kV, and a cone voltage of 15 V.

The total ion chromatogram (TIC) of LC-MS was obtained by scanning the first quadrupole from *m*/*z* 125 to 700 at a rate of 400 amu s^{-1} in full scan mode with an interscan delay of 0.10 s. Data were acquired in continuum mode.

Product-ion scan was performed by tandem mass spectrometry (MS/MS) by transmitting the protonated molecular ion ($[M + H]^+$) or the deprotonated molecular ion ($[M + H]^-$) through the first quadrupole into the second quadrupole containing ultrapure argon gas at a pressure of 2.0×10^{-4} mbar. The product-ion chromatogram was recorded by scanning the third quadrupole from *m*/*z* 50 to 450 in 1.0 s. The collision energy was varied between 20 and 50 eV to optimize fragmentation of the selected protonated molecular ion. Data were acquired in continuum mode.

Statistical Analysis. All experiments were conducted at least twice, with at least three replications per treatment. All data analysis was performed using SigmaStat 2.0 software. Experimental data were subjected to analysis of variance (ANOVA) and were pooled when possible. Differences between treatment means were analyzed using Tukey's multiple comparison procedure. Statistical tests were conducted at the 95% confidence level.

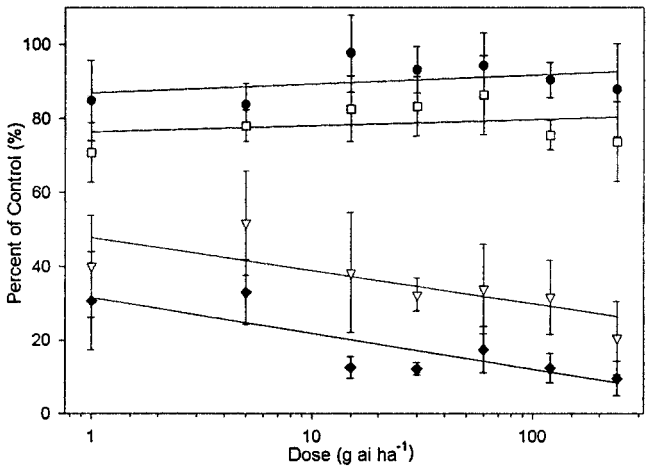

Figure 1. Effect of ethametsulfuron-methyl on wild mustard shoot (◆) and root (▽) and on rutabaga shoot (●) and root (□) dry weight 14 DAT with Muster 75 DF, the formulated product of ethametsulfuron-methyl, plus 0.1% v/v Agral 90, a nonionic surfactant. Data are expressed as percent of control. Bars represent standard errors of the treatment means. The equations for the lines for wild mustard shoot and root dry weight are $y = -9.76(\log x) + 31.64$ ($r^2 = 0.71$) and $y = -8.98(\log x) + 47.80$ ($r^2 = 0.61$), respectively.

RESULTS AND DISCUSSION

Dose—Response. At doses of 1 g of ai ha^{-1} and higher there was significant reduction in wild mustard shoot and root dry weight (Figure 1). Conversely, growth of rutabaga was not affected at any of the doses tested. The nonionic surfactant Agral 90, which is added to Muster 75 DF in field applications to improve uptake, did not affect dry weight or cause any visible injury to the plants. Rutabaga was very tolerant of ethametsulfuron-methyl; ED_{50} values could not be calculated within the dose range used (1−240 g of ai ha^{-1}). In contrast, wild mustard was extremely sensitive to ethametsulfuron-methyl; the ED_{50} values for shoot and root were <1.0 g of ai ha^{-1} (Figure 1). These results are in agreement with those of Hall et al. (1992), who obtained ED_{50} values for tolerant commercial brown mustard and susceptible wild mustard in response to foliar-applied ethametsulfuron-methyl of 148 and 0.85 g of ai ha^{-1}, respectively.

The margin of crop safety between rutabaga and wild mustard with respect to phytotoxicity of ethametsulfuron-methyl was very large. Other researchers have reported a high degree of selectivity between sensitive weeds and tolerant crop species (Hutchison et al., 1987; Buchanan et al., 1990a; McMullan et al., 1994).

Absorption and Translocation. [^{14}C]Ethametsulfuron-methyl was applied to the foliage at a dose equivalent to 30 g of ai ha^{-1}, which is twice the proposed field dose of 15 g of ai ha^{-1} for rutabaga crops. Data for phenyl and triazine labels were combined at each harvest time because there were no differences in absorption, translocation, or metabolism of the two labels. Of the applied [^{14}C]ethametsulfuron-methyl, 92.8 ± 2.9, 89.9 ± 1.3, 84.2 ± 2.4, 87.3 ± 2.4, and 83.0 ± 3.1% of ^{14}C was recovered from rutabaga plants 3, 10, 15, 30, and 50 DAT, respectively. Radioactivity levels in the leaf rinse were 29.0 ± 4.7 and 22.4 ± 2.0% of applied dose 6 and 24 HAT, respectively. Approximately

Table 1. Distribution of Radioactivity in Various Plant Parts of Rutabaga, Expressed as a Percentage of Recovered ^{14}C and ppm Values (ng/mg)[a]

plant part	time of harvest (DAT)	no. of samples	distribution of radioactivity[a] (% of recovered)	wt[b] of plant part[a] (g)	ppm[d] (ng/mg)
leaf rinse	3	18	4.5 (0.49)	10 mL[c]	0.1
	10	4	1.4 (0.29)	10 mL	0.04
	15	4	1.0 (0.20)	10 mL	0.03
	30	15	1.4 (0.52)	10 mL	0.04
	50	15	2.7 (1.82)	10 mL	0.1
treated leaf	3	18	86.6 (0.49)	1.2 (0.10)	23.0
	10	4	87.3 (0.68)	5.1 (0.62)	5.3
	15	4	88.3 (1.20)	5.8 (0.34)	4.8
	30	15	88.6 (0.65)	4.0 (0.47)	6.9
	50	15	85.2 (1.32)	1.8 (0.26)	15.2
unextractable	3	18	1.1 (0.06)	0.1 (0.10)	3.0
	10	4	1.7 (0.10)	0.5 (0.62)	1.1
	15	4	1.9 (0.16)	0.6 (0.34)	1.0
	30	15	2.6 (0.24)	0.4 (0.47)	2.0
	50	15	4.4 (0.90)	0.2 (0.26)	7.9
foliage	3	18	6.0 (0.33)	0.4 (0.03)	4.2
	10	4	7.7 (0.53)	1.8 (0.30)	1.3
	15	4	6.8 (1.14)	4.3 (0.50)	0.5
	30	15	4.9 (0.51)	15.9 (1.3)	0.1
	50	15	4.9 (0.39)	30.1 (0.88)	0.05
fibrous roots	3	18	0.4 (0.03)	0.1 (0.01)	0. 9
	10	4	0.4 (0.05)	0.4 (0.08)	0.2
	15	4	0.4 (0.05)	0.9 (0.11)	0.1
	30	15	0.5 (0.04)	2.5 (0.14)	0.06
	50	15	1.0 (0.10)	5.0 (0.36)	0.06
rutabaga	3	18	1.4 (0.12)	0.02 (0.002)	20.3
	10	4	1.5 (0.26)	0.1 (0.02)	3.7
	15	4	1.2 (0.20)	0.3 (0.05)	1.1
	30	15	2.3 (0.16)	3.7 (0.39)	0.2
	50	15	3.1 (0.33)	14.8 (2.87)	0.07

[a] Plants were treated at the three-leaf stage of development and harvested 3, 10, 15, 30, and 50 DAT of the third leaf with [^{14}C]ethametsulfuron-methyl. Results are shown as means followed by SEM in parentheses. [b] Weights are expressed as dry weights for all plant parts except the treated leaf, which is fresh weight. [c] 10 mL indicates the volume of solution used to rinse the treated leaf. [d] ppm (ng/mg) values were calculated from the average radioactivity recovered (column 4) and the plant part weight (column 5); therefore, SEM will be the same as in column 4.

95% of the recovered dose was absorbed by the foliage of rutabaga 3, 10, 15, 30, and 50 DAT (Table 1). Regardless of the harvest times, <10% of the recovered radioactivity translocated out of the treated leaf (Table 1). More than 5% of the total recovered radioactivity moved acropetally to the nontreated leaves, whereas <5% of the total recovered radioactivity moved basipetally into the roots of the rutabaga plants (Table 1). Regardless of the time of harvest, <4% of the total recovered radioactivity was found in the storage root of the rutabaga, whereas ~1% or less of the total recovered radioactivity was found in the fibrous roots (Table 1). Hall et al. (1992) found similar uptake and translocation in both commercial brown mustard and wild mustard. They found >95% of [^{14}C]ethametsulfuron-methyl was absorbed in both species 12 HAT and 10% of the recovered radioactivity translocated out of the treated leaf 3 DAT (Hall et al., 1992).

Metabolism. There were no quantitative or qualitative differences between the metabolism of either phenyl- or triazine-labeled [^{14}C]ethametsulfuron-methyl (data not shown); therefore, data were pooled. At 6 HAT, >30% of the herbicide was metabolized (Table 2) to an "intermediate" metabolite and metabolite A, having retention times of 29 and 22 min, respectively (Figure 2B). At 24 HAT, there was an increase in the relative concentration of metabolite A and the appearance of metabolite B (retention time of 21 min). At and beyond 3 days, approximately 55−70 and 30−45% of the recovered radioactivity remained as metabolites A and B, respectively (Figure 2C). The half-life of ethametsulfuron-methyl in rutabaga was ~24 h. The presence of the intermediate metabolite (29 min) decreased with time and was not detected beyond 3 DAT (Figure 2D). Using an isocratic gradient of 30% methanol and 70% 0.1 M acetic acid, metabolites A and B were separated and had retention times of 15 and 11 min, respectively (Figure 3).

Our results on the metabolism of [^{14}C]ethametsulfuron-methyl in rutabaga are similar to those of Lichtner et al. (1995) and Hall et al. (1992). Hall et al. (1992) reported two hydrophilic metabolites in wild mustard and commercial brown mustard eluting at approximately 21 and 29 min, which directly corresponds to the metabolites observed in rutabaga. Lichtner et al. (1995) observed two metabolites in oilseed rape with hydrophilicities similar to those for metabolites A and B. Furthermore, Lichtner et al. (1995) and Hall et al. (1992) observed that ethametsulfuron-methyl was rapidly metabolized in tolerant species, commercial brown mustard and oilseed rape, compared to susceptible wild

Table 2. Metabolism of [^{14}C]Ethametsulfuron-methyl, Harvested 6 and 24 HAT and 3, 10, 15, 30, and 50 DAT with [^{14}C]Ethametsulfuron-methyl as Determined by HPLC Analysis[a]

			metabolism (% of recovered ^{14}C)[a]									
			metabolite A[b]		metabolite B[b]		intermediate metabolite[b]		ethametsulfuron-methyl[b]		all other metabolites[b]	
time of harvest	no. of samples	plant wt (g)	%	ppm[c]	%	ppm[c]	%	ppm[c]	%	ppm[c]	%	ppm[c]
6 HAT	13	1.91[d]	20.1 (2.8)	**3.31**	0.3 (0.3)	**0.04**	15.4 (1.2)	**2.53**	63.4 (3.0)	**10.41**	1.0 (0.6)	**0.16**
24 HAT	5	1.91[d]	27.8 (4.8)	**4.57**	7.9 (3.7)	**1.39**	12.4 (1.7)	**2.05**	51.1 (5.7)	**8.40**	0.5 (0.3)	**0.09**
3 DAT	15	1.91	63.4 (2.4)	**10.41**	29.0 (1.6)	**4.77**	1.6 (0.4)	**0.25**	5.0 (1.4)	**0.83**	1.3 (0.5)	**0.21**
10 DAT	4	8.06	64.4 (2.0)	**2.51**	32.5 (2.7)	**1.27**	ND[e]	ND	1.2 (1.2)	**0.05**	1.9 (0.5)	**0.07**
15 DAT	4	11.87	69.6 (2.7)	**1.84**	29.1 (2.4)	**0.77**	ND	ND	ND	ND	1.4 (0.5)	**0.04**
30 DAT	16	26.61	55.0 (2.9)	**0.65**	39.4 (2.8)	**0.46**	ND	ND	2.0 (0.8)	**0.02**	3.6 (0.6)	**0.04**
50 DAT	15	51.84	58.6 (1.8)	**0.35**	35.6 (1.7)	**0.22**	ND	ND	1.1 (0.7)	**0.01**	2.3 (0.7)	**0.01**

[a] Rutabaga plants were treated at the three-leaf stage of development. Data are expressed as percent of recovered radioactivity and ppm (ng/mg). Results are shown as means followed by SEM in parentheses. [b] Retention times of each metabolite were as follows: Metabolite A, 21 min; metabolite B, 20 min; intermediate metabolite, 29 min; and ethametsulfuron-methyl, 31 min. [c] ppm (ng/mg) values were calculated using the average percent of radioactivity recovered and the plant weight (column 3); therefore, SEM will be the same as percent of radioactivity recovered. [d] Plant weights were not determined 6 and 24 HAT; therefore, the average plant weight at 3 DAT was used in the calculations. [e] ND indicates that peaks were not detected on the radiochromatogram and therefore cannot be quantified.

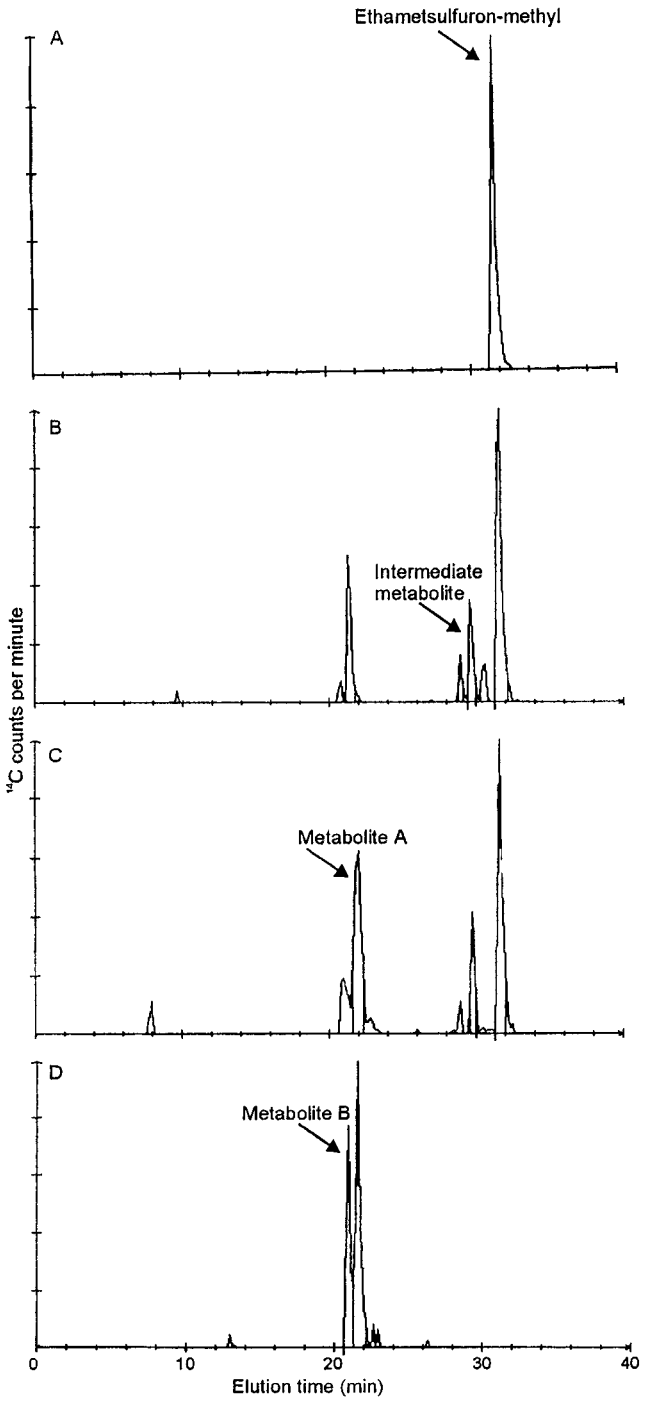

Figure 2. Typical HPLC chromatograph of extracts obtained from rutabaga plants treated at the three-leaf stage of development with [^{14}C]ethametsulfuron-methyl. Plants were harvested 0.1 (A), 6 (B), 24 (C), and 72 HAT (D). Retention times for metabolite A, metabolite B, intermediate metabolite, and ethametsulfuron-methyl were 22, 21, 29, and 31 min, respectively. Chromatograph conditions consisted of a a nonlinear concave 2 gradient of 5−100% B over 30 min, followed by 100% B for 5 min (0.1% phosphoric acid or 1.0 M acetic acid for solvent A and acetonitrile for solvent B).

mustard. These two research groups attributed the tolerance of both species to rapid metabolic degradation of the herbicide to the two major hydrophilic metabolites (Lichtner et al., 1995; Hall et al., 1992). The higher rate of metabolism of ethametsulfuron-methyl in rutabaga compared to wild mustard is the likely explanation for the different responses between the two species to ethametsulfuron-methyl.

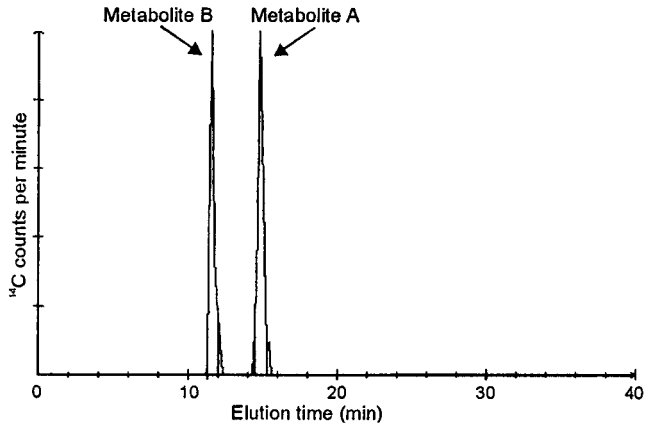

Figure 3. Typical HPLC chromatograph of plant extracts prepared for HPLC purification prior to MS. Retention times were 11 and 15 min for metabolites B and A, respectively. Chromatograph conditions consisted of an isocratic gradient of 30% methanol and 70% 0.1 M acetic acid.

MS. A characteristic ion for an authentic standard of ethametsulfuron-methyl (*m*/*z* 411.2) was obtained using positive ion electrospray ionization mass spectrometry. The spectrum of ions obtained following fragmentation of ethametsulfuron-methyl (411 ES+) included the acidic sulfonamide portion of the herbicide (*m*/*z* 195.9 and 199) and the triazine portion of the parent molecule (*m*/*z* 168.4, 155, and 141.7; Figure 4A). The negative ion electrospray ionization mass spectrum of the parent herbicide has a characteristic parent ion (*m*/*z* 409.2) and expected daughter ions of ethametsulfuron-methyl (*m*/*z* 181.7, 168.0, and 140; Figure 4B).

Metabolite A had an *m*/*z* ratio of 383 that corresponds to the loss of an ethyl group from ethametsulfuron-methyl; thus, metabolite A (Figure 5) was hypothesized to be *O*-desethylethametsulfuron-methyl. The mass spectrum of the daughter ions of metabolite A (383 ES+) showed many characteristic ions of *O*-desethylethametsulfuron-methyl, including the acidic sulfonamide portion (*m*/*z* 198.9) and the triazine portion of *O*-desethylethametsulfuron-methyl (*m*/*z* 168.2 and 141.8) (Figure 5), further supporting our proposal that metabolite A is *O*-desethylethametsulfuron-methyl.

The positive ion electrospray ionization mass spectrum of metabolite B showed a molecular ion with an *m*/*z* ratio of 369.2, which corresponds to the loss of methyl and ethyl constituents from ethametsulfuron-methyl. Therefore, metabolite B is proposed to be *N*-desmethyl-*O*-desethylethametsulfuron-methyl. Further examination of the daughter ions of metabolite B (369.2 ES+) reveals characteristic ions of *N*-desmethyl-*O*-desethylethametsulfuron-methyl including the acidic sulfonamide portion (*m*/*z* 199.0 and 135.3) and the triazine portion of the compound (*m*/*z* 154 and 127.9; Figure 6A). Negative ion electrospray ionization mass spectra of metabolite B provide further evidence that the metabolite is *N*-desmethyl-*O*-desethylethametsulfuron-methyl, because it has a characteristic parent ion (*m*/*z* 367.2) and two daughter ions (*m*/*z* 152.0 and 125.8; Figure 6B).

Lichtner et al. (1995) isolated two metabolites of ethametsulfuron-methyl from oilseed rape seeds and analyzed them using thermospray LC-MS, packed capillary LC-MS, and cochromatography with authentic standards. They identified the two major metabolites as *O*-desethylethametsulfuron-methyl and *N*-desmethyl-*O*-desethylethametsulfuron-methyl, which is consistent

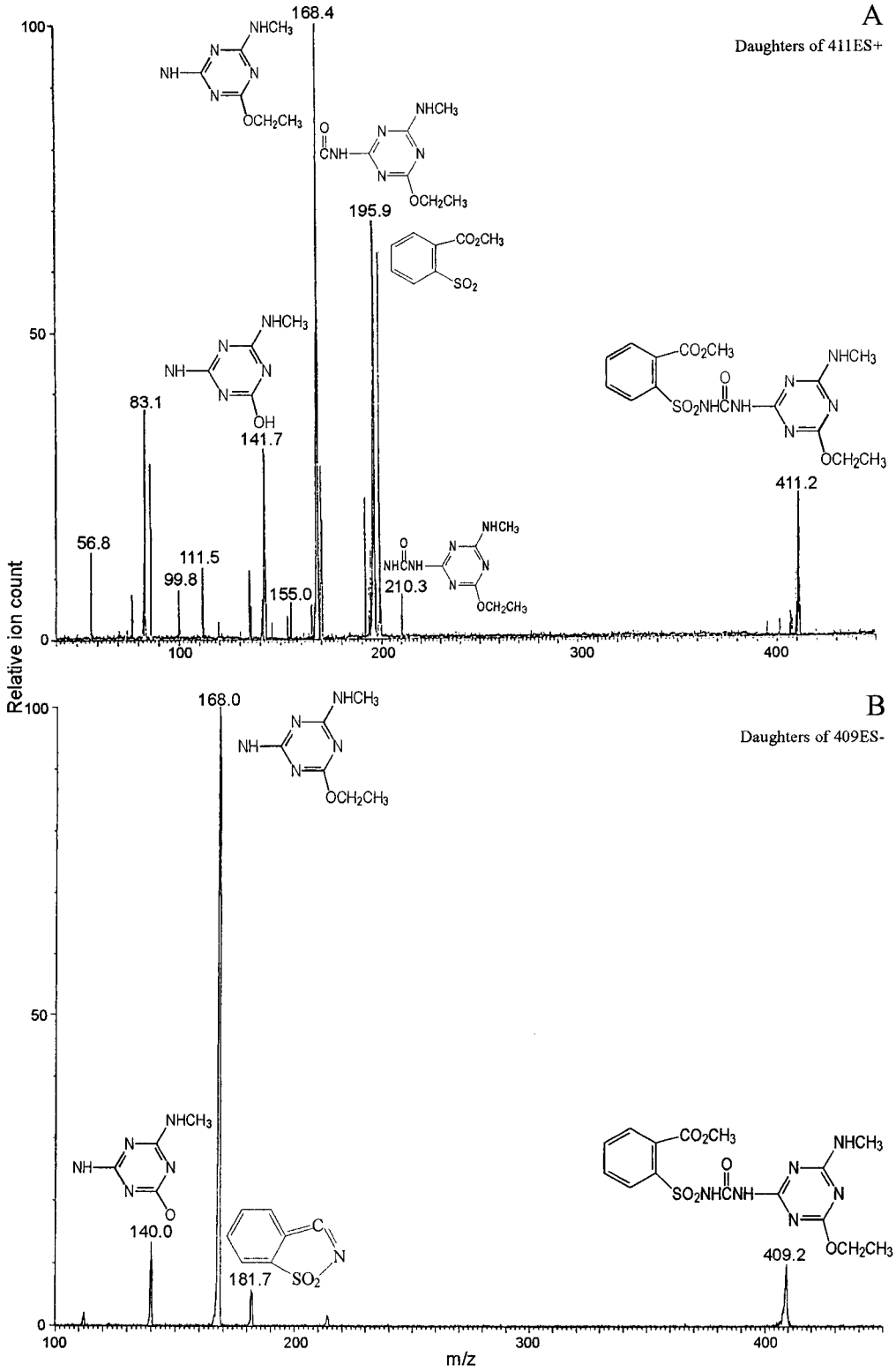

Figure 4. Mass spectra of the daughter ions of ethametsulfuron-methyl (MW 410.2) following positive ion (top spectrum) (*m*/*z* 411.2) and negative ion (bottom spectrum) (*m*/*z* 409) electrospray ionization of an authentic standard of ethametsulfuron-methyl.

with our LC-MS results in rutabaga. Furthermore, in whole plant studies, Litchner et al. (1995) determined that *O*-desethylethametsulfuron-methyl was herbicidally inactive.

The proposed pathway of metabolism of ethametsulfuron-methyl in rutabaga is illustrated in Figure 7. The identity of the intermediary metabolite with a retention time of 29 min remains unknown; however, this metabolite has been observed before in commercial brown and wild mustard (Hall et al., 1992). This intermediate metabolite is believed to be a product of hydroxylation of the ethoxy group on the triazine ring of ethametsulfuron-methyl (Figure 7). A similar intermediate of bensulfuron-methyl has been observed in rice (Takeda et al., 1986; Beyer et al., 1988), and this reaction is thought to proceed through hydroxylation of

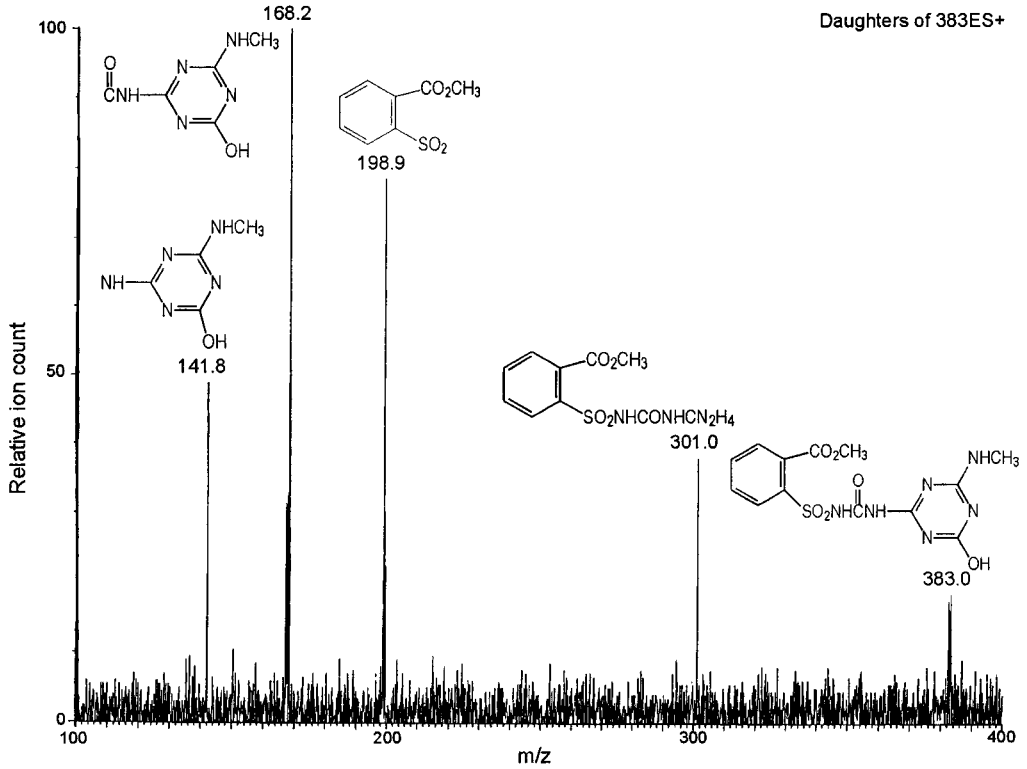

Figure 5. Mass spectrum of the daughter ions of metabolite A (MW 382.2) following positive ion (*m*/*z* 383.0) electrospray ionization.

the methoxy substituent of bensulfuron-methyl to form the unstable formyl adduct, which decomposes to the hydroxylated metabolite (Brown, 1990; Brown and Kearney, 1991). On the basis of our results, we propose that similar metabolism of ethametsulfuron-methyl occurs in rutabaga, where an unstable α-hydroxy ethoxy intermediate is metabolized to *O*-desethylethametsulfuron-methyl and subsequently to *N*-desmethyl-*O*-desethylethametsulfuron-methyl (Figure 7).

Herbicide Residue Analysis in Rutabaga Root. The extraction of radioactivity from the rutabaga root indicated that, of the applied radioactivity, 1.24 ± 0.06% was recovered in the rutabaga root 3 DAT. HLPC analysis revealed that there was no parent herbicide present in the edible portion of the rutabaga root (data not shown). Metabolites A (68.80 ± 2.75%) and B (26.76 ± 2.71%) were present in relative percentages similar to those found in the treated leaf. Similar to the treated leaf, trace quantities of other metabolites (<5%) were detected with retention times of 7 and 29 min.

In these experiments, [^{14}C]ethametsulfuron-methyl was applied to one leaf of rutabaga at a dose equivalent to 30 g of ai ha^{-1}. At this application rate, we estimated that a total of 130 ng of metabolite A and B will translocate from one treated leaf to the edible portion of the rutabaga root. Therefore, the combined concentration of *O*-desethylethametsulfuron-methyl and *N*-desmethyl-*O*-desethylethametsulfuron-methyl (no ethametsulfuron-methyl was found in root) would be 650 ng root^{-1} in plants treated at the five-leaf stage of development (130 ng per treated leaf × 5 treated leaves). Assuming a conservative harvest weight of 500 g for the edible portion of the rutabaga root, the combined, potential concentration of *O*-desethylethametsulfuron-methyl and *N*-desmethyl-*O*-desethylethametsulfuron-methyl will be 1.3 ng g^{-1} of edible root (1.3 ppb). Lichtner et al. (1995) observed comparable amounts (5 ppb) of ethametsulfuron-methyl and its metabolites in the seeds of oilseed rape.

Toxicological Assessment. Results from this study and available toxicological data were used to determine the safety of ethametsulfuron-methyl-treated rutabaga for human consumption based on acceptable pesticide residue levels with regard to risk parameters in the edible portion of the rutabaga root. Steps have been outlined (Ritter, 1998) to ensure safe pesticide residue levels in food and are as follows: (1) select a no observable (adverse) effect level [NO(A)EL], (2) calculate an acceptable daily intake (ADI), (3) propose a maximum residue level (MRL), (4) determine the theoretical daily intake (TDI), and (5) validate the MRL. The ADI is equal to the NO(A)EL divided by a safety factor. The NO(A)EL selected was 87 mg/kg of body weight (bw)/day, which was the lowest NO(A)EL cited in the literature by Agriculture Canada (1992) and used for registration of canola. The safety factor was 100, a 10× factor each for animal to human variation and for human to human variation, which is a typical factor used for other sulfonylurea herbicides. Therefore, the ADI for ethametsulfuron-methyl was calculated as 0.87 mg/kg of bw/day. The TDI is derived by multiplying the MRL by the average daily consumption of each food. Typically, when there is no parent herbicide present in the edible portion of the vegetable, the MRL is set at 0.1 ppm. As the average daily consumption of rutabaga was not available, for these calculations an excessive average daily consumption of rutabaga was proposed to be 0.25 kg/day. Therefore, the TDI for ethametsulfuron-methyl was estimated to be 0.0251 mg/kg of bw/day, the TDI summation of 0.0001 and 0.025 mg/kg of bw/day for canola (Agriculture Canada, 1992) and rutabaga, respectively. Validation of the MRL involves comparing the TDI to the ADI. Because the TDI is less, by more than an order of magnitude, than the ADI, we

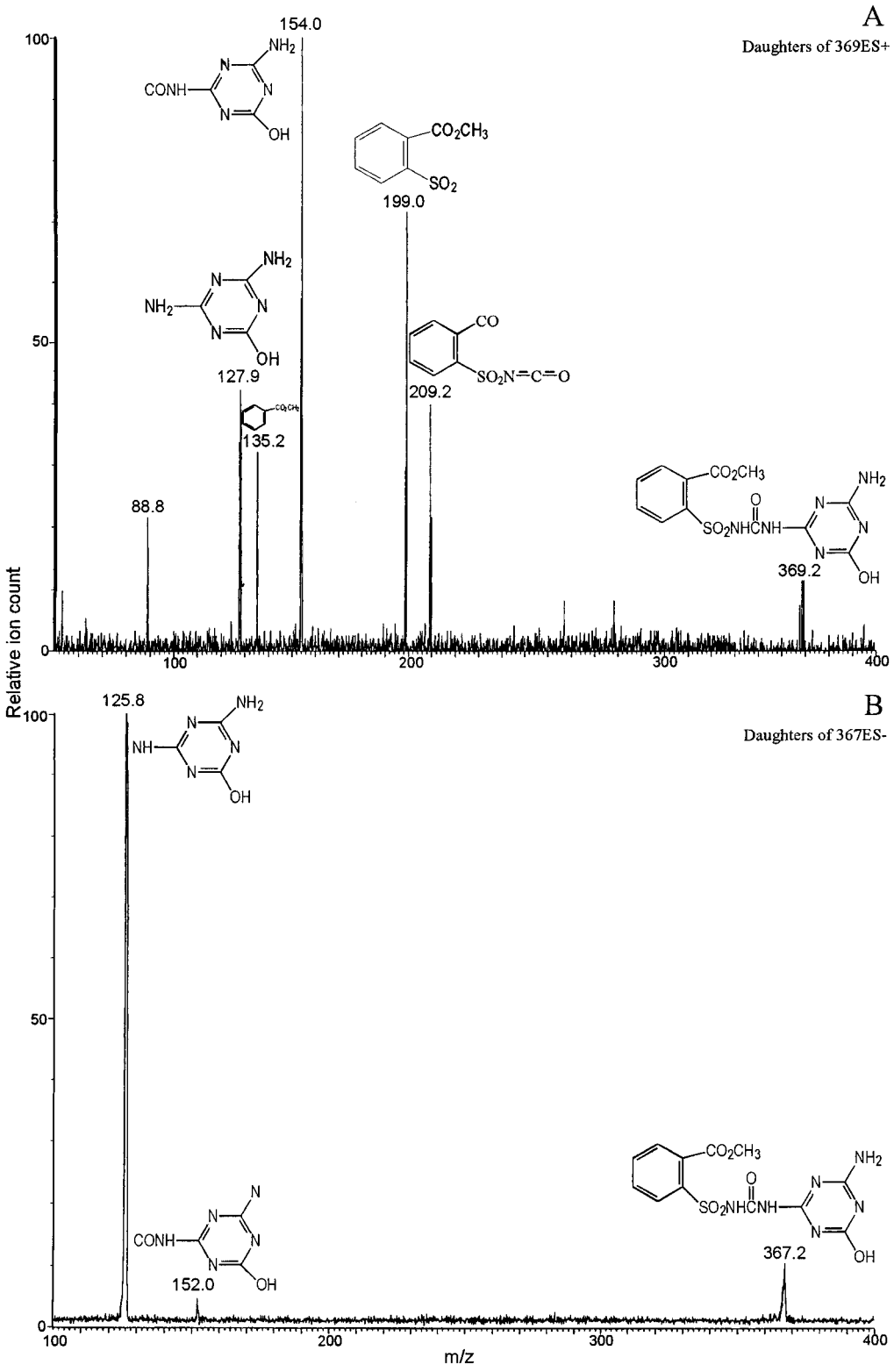

Figure 6. Mass spectra of the daughter ions of metabolite B (MW 368.2) following positive ion (*m/z* 369.2; top spectrum) and negative ion (*m/z* 367.2; bottom spectrum) electrospray ionization.

conclude that ethametsulfuron-methyl is safe for use in rutabaga crops that will be consumed.

Conclusions. Ethametsulfuron-methyl effectively inhibits wild mustard growth while remaining nonphytotoxic to rutabaga. The metabolism of ethametsulfuron-methyl in rutabaga is proposed to be through an unstable α-hydroxy ethoxy intermediate metabolite that dissipates within 3 days to *O*-desethylethametsulfuron-methyl, which is further metabolized to *N*-desmethyl-*O*-desethylethametsulfuron-methyl. No parent herbicide was present in the rutabaga root, but totals of 1.3 ng g^{-1} of *O*-desethylethametsulfuron-methyl and *N*-desmethyl-*O*-desethylethametsulfuron-methyl were estimated to be present in the edible portion of a 500 g rutabaga root at harvest. On the basis of our results and those of Lichtner et al. (1995) in oilseed rape, it is reasonable to expect that following application of ethametsulfuron-methyl for weed control in rutabaga

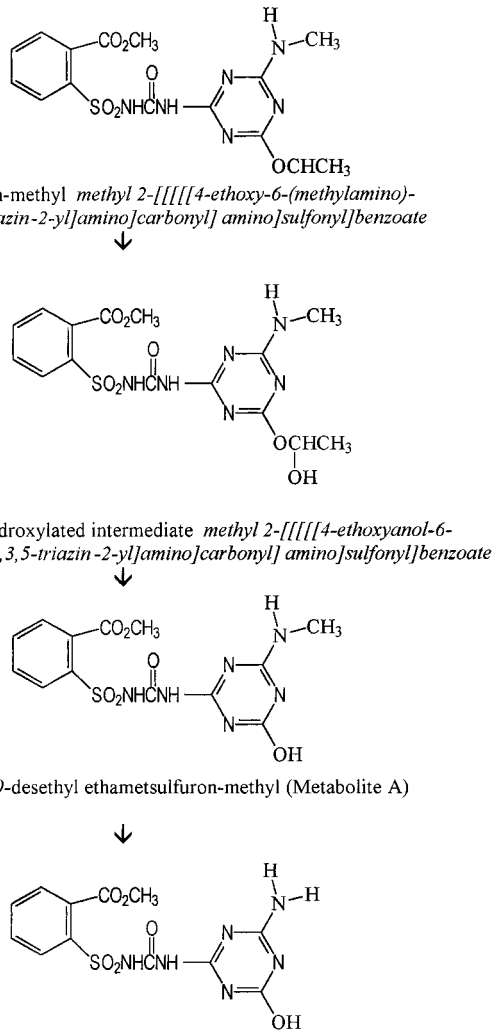

Figure 7. Proposed pathway for metabolism of ethametsulfuron-methyl in rutabaga.

crops, it will be safe to consume the edible portion of the rutabaga root.

ABBREVIATIONS USED

AAFC, Agriculture, Agri-Food Canada; AHAS, acetohydroxyacid synthase; ADI, acceptable daily intake; ALS, acetolactate synthase; ANOVA, analysis of variance; BW, body weight; DAT, days after treatment; DPM, dissipations per minute; ethametsulfuron-methyl or DPX-A7881, methyl 2-[[[[[4-ethoxy-6-(methylamino)-1,3,5-triazin-2-yl]amino]carbonyl] amino]sulfonyl]benzoate; ED_{50}, effective dose at which 50% of population is affected; ES, electrospray; HAT, hours after treatment; HLPC, high-performance liquid chromatography; LC-MS, liquid chromatography−mass spectrometry; LSS, liquid scintillation spectrometry; MRL, maximum residue level; MS, mass spectrometry; MS/MS, tandem mass spectrometry; NO(A)EL, no observable (adverse) effect level; OMAFRA, Ontario Ministry of Agriculture, Food and Rural Affairs; PBS, phosphate-buffered saline; PMRA, Pest Management Regulatory Agency; TDI, theoretical daily intake; TIC, total ion chromatogram; URMULE, User Requested Minor Use Label Expansion.

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LITERATURE CITED

Agriculture Canada. Ethametsulfuron methyl herbicide: decision document. Food Production and Inspection Branch: 1992; pp 7−16.

Beyer, E. M.; Duffy, M. J.; Hay, J. V.; Schlueter, D. D. Sulfonylureas. In *Herbicides: Chemistry, Degradation, and Mode of Action*; Kearney, P. C., Kaufman, D. D., Eds.; Dekker: New York, 1988; Chapter 3, pp 117−189.

Brown, H. M. Mode of action, crop selectivity, and soil relations of the sulfonylurea herbicides. *Pestic. Sci.* **1990**, *29*, 263−281.

Brown, H. M.; Kearney, P. C. Plant biochemistry, environmental properties and global impact of the sulfonylurea herbicides. In *Synthesis and Chemistry of Agrochemicals II*; Baker, D. R., Fenges, J. G., Moberg, W. K., Eds.; ACS Symposium Series 443; American Chemical Society: Washington, DC, 1991; pp 32−49.

Buchanan, F. S.; Gillespie, T. J.; Swanton, C. J. Environmental factors affecting the herbicidal activity of DPX-A7881. *Weed Sci.* **1990a**, *30*, 271−278.

Buchanan, F. S.; Swanton, C. J.; Gillespie, T. J. Differential response of selected species of *Brassicaceae* to DPX-A7881. *Can. J. Plant Sci.* **1990b**, *70*, 873−877.

Devine, M. D.; Bestman, H. D.; Hall, J. C.; Vanden Born, W. H. Leaf wash techniques for estimation of foliar absorption of herbicides. *Weed Sci.* **1984**, *32*, 418−425.

Hall, J. C.; Swanton, C. J.; Devine M. D. Physiological and biochemical investigation of the selectivity of ethametsulfuron in commercial brown mustard and wild mustard. *Pestic. Biochem. Physiol.* **1992**, *42*, 188−192.

Hutchison, J. M.; Peter, C. J.; Amuti, K. S.; Roy, G. S.; Stichbury, R. DPX A7881: A new herbicide for oilseed rape. *Proc. Br. Crop Prot. Conf.−Weeds* **1987**, *2*, 63−67.

Lichtner, F. T.; Dietrich, R. F.; Brown, H. M. Ethametsulfuron methyl metabolism and crop selectivity in spring oilseed rape (*Brassica napus* L.). *Pestic. Biochem. Physiol.* **1995**, *52*, 12−24.

McMullan, P. M.; Daun, J. K.; DeClercq, D. R. Effect of wild mustard (*Brassica kaber*) competition on yield and quality of triazine-tolerant and triazine-susceptible canola (*Brassica napus* and *Brassica rapa*). *Can. J. Plant Sci.* **1994**, *74*, 369−374.

Pitblado, R.; Sikkema, P.; Zandstra J. Rutabaga Research Results. *Ridgetown College* **1997**, 18−25.

Ritter, L. Toxicological Principles (Mammals). Chapter 2. In *Advanced Principles of Toxicology*; Solomon, K. R., Ritter, L., Eds.; Canadian Network of Toxicology Centres: Guelph, ON, 1998; pp 3−12.

Takeda, S.; Erbes, D. L.; Sweetser, P. B.; Hay, J. V.; Yuyama, T. Mode of herbicidal and selective action of DXP−F5384 between rice and weeds. *Weed Res. (Jpn.)* **1986**, *31*, 157−163.

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