JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Metabolic Fate of [^{14}C] Chlorophenols in Radish (*Raphanus sativus*), Lettuce (*Lactuca sativa*), and Spinach (*Spinacia oleracea*)

SOPHIE PASCAL-LORBER,*,† SABRINA DESPOUX,† ESTELLE RATHAHAO,‡ CÉCILE CANLET,† LAURENT DEBRAUWER,† AND FRANÇOIS LAURENT†

INRA, UMR1089 Xénobiotiques, 180 ch. de Tournefeuille, BP3, F-31931 Toulouse, Cedex 9, France, and INRA/INA P-G, UMR0214 Chimie Analytique, Ingénierie Analytique pour la Qualité des Aliments - IAQA, 16 rue Claude Bernard, F-75231 Paris, Cedex 05, France

Chlorophenols are potentially harmful pollutants that are found in numerous natural and agricultural systems. Plants are a sink for xenobiotics, which occur either intentionally or not, as they are unable to eliminate them although they generally metabolize them into less toxic compounds. The metabolic fate of [^{14}C] 4-chlorophenol (4-CP), [^{14}C] 2,4-dichlorophenol (2,4-DCP), and [^{14}C] 2,4,5-trichlorophenol (2,4,5-TCP) was investigated in lettuce, spinach, and radish to locate putative toxic metabolites that could become bioavailable to food chains. Radish plants were grown on sand for four weeks before roots were dipped in a solution of radiolabeled chlorophenol. The leaves of six-week old lettuce and spinach were treated. Three weeks after treatments, metabolites from edible plant parts were extracted and analyzed by high performance liquid chromatography (HPLC) and characterized by mass spectrometry (MS), and nuclear magnetic resonance spectroscopy (NMR). Characterization of compounds highlighted the presence of complex glycosides. Upon hydrolysis in the digestive tract of animals or humans, these conjugates could return to the toxic parent compound, and this should be kept in mind for registration studies.

KEYWORDS: ***Lactuca sativa*; *Spinacia oleracea*; *Raphanus sativus*; 4-chlorophenol; 2,4-dichlorophenol; 2,4,5-trichlorophenol; metabolism; glycosides**

INTRODUCTION

Chlorophenols have been widely used as biocides and for wood preservation, and they can also be a byproduct of the bleaching process of pulp and paper mills, chlorination of drinking water, and incineration of organic waste. They are widespread in the environment and have been detected even in natural biotopes, and in both aquatic and terrestrial food chains. They are of great environmental concern because some are suspected to be persistent, mutagenic, carcinogenic, and difficult to decompose biologically because of their chlorinated nature (*1*). For these reasons, they are included in the USEPA list of priority pollutants (http://www.epa.gov/superfund/programs/clp/svtarget.htm and http://www.epa.gov/superfund/programs/clp/target.htm). In addition, chlorophenols are one of the putative precursors of highly potent toxicants, dioxins, during combustion of wastes (*2*). Among chlorophenols, 2,4,5-trichlorophenol (2,4,5-TCP) has been used as a biocide and as an industrial intermediate in the manufacture of the herbicide 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), one of the most widely used herbicides in the past century. 2,4-Dichlorophenol (2,4-DCP) is a precursor in the production of the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D). 2,4,5-TCP is the first intermediate in microbial degradation of 2,4,5-T (*3*) and 2,4-DCP the main catabolic metabolite of 2,4-D in aerated soil (*4, 5*). Moreover, 2,4-DCP is a degradation product resulting from the aqueous photolysis of triclosan, a widely used antibacterial agent (*6*). 4-Chlorophenol (4-CP) is widely used for the production of dyes, drugs, pesticides, and fungicides (*7*). Chlorophenols are also sometimes used as model pollutants representative of chlorophenol contamination (*8*). These chlorinated compounds can also cause a food safety problem when they enter the food chain by means of edible plants. Plants are unable to mineralize chlorophenols, although they can metabolize them mainly by glycosidation, a typical phase II conjugation process (*9*). Previous studies on the metabolism of phenol and chlorinated phenols have shown the formation of glucosyl conjugates in aquatic plants (*Lemna gibba*) (*10*−*12*). 2,4-DCP-(malonyl) and -(pentosyl)glucoside are also found in aquatic plants and in cell suspension cultures (*13, 14*). Recently, Day and Saunders (*15*) have characterized more complex 2,4-DCP and 2,4,5-TCP glycosides such as a glucose-apiose, a hydroxymethyl-3 tetrose conjugate in *Lemna minor*. But little information is available on chlorophenol metabolism in edible plants (*16*). 2,4-DCP has

* Corresponding author. Tel: +33 5 61 28 53 92. Fax: +33 5 61 28 52 44. E-mail: spascal@toulouse.inra.fr.
† UMR1089 Xénobiotiques.
‡ UMR0214 Chimie Analytique.

10.1021/jf8016354 CCC: $40.75
Published on Web 09/03/2008

been studied as a model pollutant in wheat and soybean; it is conjugated with glucose and malonic acid to give the malonylglucoside derivative (*17*). Nevertheless, it is important to characterize the structure of these sugar conjugates in order to evaluate their bioavailability and the risk they represent to human health. As shown with the absorption of endogenous compounds of plants such as flavonoids, the glycosidic structure affects the efficiency of their absorption and therefore their bioavailability in animals and humans (*18*). The purpose of this study was thus to elucidate the metabolic fate of [^{14}C] 4-CP, [^{14}C] 2,4-DCP, and [^{14}C] 2,4,5-TCP in three market garden plants, lettuce, spinach, and radish. The distribution of radioactivity in soluble and unextractable residues was determined, and the structure of metabolites was characterized by MS and ^{1}H NMR analyses. Our study clearly identified new sugar-conjugated metabolites of chlorophenols.

MATERIALS AND METHODS

Chemicals. 4-CP (98% pure), 2,4-DCP (99% pure), 2,4,5-TCP (99% pure), and almond β-glucosidase (G0395) were purchased from Sigma-Aldrich (Saint Quentin Fallavier, France). [U-*phenyl*-^{14}C] 4-CP (specific activity 1.92 GBq mmol^{-1}, radiochemical purity >98% as determined by radio-RP-HPLC analysis), [U-*phenyl*-^{14}C] 2,4-DCP (specific activity 2.96 GBq mmol^{-1}, radiochemical purity >99% as determined by radio-RP-HPLC analysis), and [U-*phenyl*-^{14}C] 2,4,5-TCP (specific activity 2.03 GBq mmol^{-1}, radiochemical purity >98% as determined by radio-RP-HPLC analysis) were purchased from ARC (St. Louis, MO). Solvents used for extractions and radio-HPLC analyses were obtained from Scharlau Chemie (Barcelona, Spain). Unless otherwise specified, all other chemicals were of analytical grade. 2,4-DCP-glucose was synthesized according to the procedure of Sinnot and Souchard (*19*).

Plant Materials and Growth Conditions. Seeds of lettuce (*Lactuca sativa* cv Carlane), spinach (*Spinacia oleracea* cv Space), and radish (*Raphanus sativus* cv Fluo) were current seeds obtained from *Les Espaces Verts du Languedoc* (Toulouse, France). Seeds of lettuce and spinach were sown in 50 mL plastic pots containing a humus/sand mixture (2:1, w/w) and grown for one week in a climate-controlled cabinet under a 16 h photoperiod at 22/19 °C (day/night) and 350 μEm^{-2}s^{-1} photosynthetically active radiation (PAR). To accommodate the larger development of the leaves, plants were subsequently individually transplanted in 1 L pots containing the same soil mixture and grown under field conditions. Plants were supplied with quarter strength Hoagland solution (Sigma H2395). Radish seeds were directly planted in quartz sand in 400 mL plastic pots and irrigated with the same solution.

Plant Treatment. *Lettuce and Spinach Plants.* Droplets (0.5 μL) of [^{14}C] 2,4-DCP or [^{14}C] 2,4,5-TCP, and 37000 Bq and 40 μM of unlabeled molecule dissolved in 1 mL of a Tween 80 0.1% H_2O/acetone (50:50, v/v) solution were applied with a microsyringe (Hamilton) to the higher face of fully expanded leaves of six-week-old lettuce and spinach plants. Each treatment consisted of four to six replicates of individual plants. Treated leaves of lettuce and spinach plants were harvested after three weeks and rinsed with methanol to remove residues adsorbed on the surface. Samples were freeze-dried for 48 h to remove water and stored at −80 °C before further analysis.

Radish Plants. Four-week-old radish plants were carefully removed from the sand, their roots were briefly rinsed under running water, and then dipped in 1 mL of [^{14}C] 4-CP, [^{14}C] 2,4-DCP, or [^{14}C] 2,4,5-TCP (37000 Bq and 40 μM of unlabeled molecule), dissolved in nutrient solution. Each treatment consisted of four to six replicates of individual plants. After three weeks, root peel, root core, root hairs, and leaves of radish plants were harvested separately using a razor blade and rinsed with methanol. Samples were freeze-dried for 48 h to remove water and stored at −80 °C before further analysis.

Preparation of Plant Extracts. Plant parts were cut into small pieces and then ground in a chilled mortar. An aliquot of the resulting powder was kept to measure the total radioactivity absorbed by plants. Ground tissues were extracted with water (5 mL g^{-1} FW, fresh weight) and then with methanol/dichloromethane (2:1, v/v), homogenized for 4 h in a vortex, and stored overnight at −20 °C. The homogenate was then sonicated with an ultrasonic cell disrupter (Branson sonifier 450, Fischer Bioblock, Illkirch, France) for 5 min and centrifuged at 10,000*g* for 10 min, and the pellet was washed twice in the solvent mixture. The three supernatants were combined and contained the soluble residues. The residual material, consisting of the unextractable residues, was allowed to air-dry for 24 h to remove organic solvents, and then freeze-dried for 48 h to remove water.

Determination of Radioactivity. Radioactivity of plant parts and of unextractable residues was measured after oxidative combustion of aliquots in an oxidizer (model Packard 307, PerkinElmer Life and Analytical Sciences, Courtaboeuf, France). The resulting $^{14}CO_2$ was trapped in a scintillation mixture (Permafluor and Carbosorb, PerkinElmer LAS) and counted in a Packard Tricarb 2200CA scintillation counter. Aliquots of extractable residues were directly counted with Ultimagold (PerkinElmer LAS) as scintillation cocktail. Amounts of residues were calculated from the specific activity of 4-CP, 2,4-DCP, or 2,4,5-TCP, as equivalent parent compound.

Enzymatic Metabolite Hydrolyses. Samples (about 167 Bq) under investigation were evaporated to dryness under vacuum or in a nitrogen stream before treatment.

β-D-Glucosidase Hydrolysis. Hydrolysis was performed in 200 μL of reaction medium, according to the method of Schmitt et al. (*20*). Samples were incubated at 30 °C for 2 h with almond β-glucosidase (2 units) in 0.1 M sodium acetate buffer, pH 5.0.

Malonyl Esterase Hydrolysis. Samples were incubated with 250 μL of a crude enzyme preparation made from parsley (*Petroselinum hortense*) stems (*21*) in 0.1 M potassium phosphate buffer, pH 7.5, at 30 °C for 2 h.

Chromatography. Soluble residues were analyzed by reverse-phase-HPLC with a Hewlett-Packard 1100 liquid chromatograph (Les Ulis, France) equipped with a HP1100 UV detector. Radioactivity was monitored with an online Packard Flo-One β A500 scintillation detector (cell volume, 0.5 mL; scintillation cocktail ratio, 2 mL scintillation liquid/1 mL HPLC effluent), using Flo-Scint II as scintillation counting cocktail (PerkinElmer LAS). Separation was carried out on a C 18 Bischoff reverse-phase column (Prontosil Eurobond, 250 × 4.6 mm, 5.0 μm) with a 1 cm guard cartridge. Elution was performed at 30 °C at a flow rate of 1 mL/min. The column was equilibrated with 100% of solvent A (water/acetonitrile/formic acid, 90:10:0.2, v/v/v). Elution conditions were as follows: a 30 min linear increase of solvent B (water/acetonitrile/formic acid, 60:40:0.2, v/v/v) from 0 to 100% and then 100% solvent B for 15 min. Retention times of standards were as follows: 2,4-DCP-Glu, 14.60 min; 4-CP, 25.10 min; 2,4-DCP, 27.40 min; 2,4,5-TCP, 34.90 min. Retention times of metabolites are given in **Table 1**.

Isolation of Metabolites. Metabolites and the parent compound (soluble residues) were isolated by coupling the HPLC system with a fraction collector (model FC-204, Gilson Medical Electronics, Middleton, WI) set at 4 fractions min^{-1}. An aliquot of each fraction was counted in a scintillation counter to monitor radioactivity. The fractions of each peak were combined and evaporated to dryness under vacuum and stored at −20 °C until analysis by mass spectrometry (MS) or nuclear magnetic resonance spectroscopy (^{1}H NMR).

Mass Spectrometry (Table 1). Structural characterization of the metabolites was conducted on a quadrupole ion trap mass spectrometer (Finnigan LCQ, Thermo Finnigan, Les Ulis, France) fitted with an electrospray ionization source. To obtain complementary information, both positive and negative ionization modes were used. Samples (typically 1ng μL^{-1} in MeOH/H_2O (50−50, v/v)) were introduced into the ionization source at a flow rate of 3 μL min^{-1}. For positive ESI experiments, lithium chloride was added to the MeOH/H_2O solution for cationization (final concentration 10 μM). The heated capillary was maintained at 200 °C. Operating parameters for production and transmission of electrosprayed ions into the ion trap analyzer (needle voltage, nebulizing gas flow rate, transfer capillary voltage, tube lens offset, etc.) were optimized for each compound to achieve maximum sensitivity. All other parameters for MS/MS experiments (isolation width, excitation voltage, and excitation time) were also adjusted to obtain maximum structural information about the compound of interest. All analyses were performed under automatic gain control conditions

Table 1. MS Characteristics of Chlorophenol Conjugates in Different Plants after HPLC Analysis and Identification by Mass Spectrometry[a]

plant	chlorophenol conjugates	*Rt* in HPLC min	$[M-H]^-$ or $[M+Li]^+$ *m/z*	precursor ion	main fragment ions *m/z*
radish	4-CP-(acetyl)hexose (*major*)	16.1	$[M-H]^-$	331	127
			$[M+Li]^+$	339	211, 193
	4-CP-(malonyl)hexose	16.1	$[M-H]^-$	375	331, 289, 127
			$[M+Li]^+$	383	339, 211
radish, (lettuce, spinach)	2,4-DCP-(acetyl)hexose (*major, except spinach*)	19.5	$[M-H]^-$	365	161
radish	2,4,5-TCP-hexose-sulfate	20.4	$[M-H]^-$	437	357, 275, 241, 195, 181*, 153*, 97*
	2,4,5-TCP-(malonyl)hexose-sulfate	22.2	$[M-H]^-$	523	479, 437, 327, 195
			$[M-H+2Li]^+$	537	457, 341, 261
	2,4,5-TCP-(malonyl)hexose-deoxyhexose	23.2	$[M-H]^-$	589	545, 503*, 399*, 195*
			$[M+Li]^+$	597	553, 511, 451, 401, 383, 357, 339, 237
	2,4,5-TCP-hexose-$C_5H_5O_5$ (*major*)	24.5	$[M-H]^-$	501	457, 399, 357, 305, 195
			$[M+Li]^+$	509	491, 447, 313, 169
lettuce	2,4,5-TCP-(acetyl)hexose (*major*)	24.4	$[M-H]^-$	399	195
	2,4,5-TCP-deoxypentose-(malonyl)hexose	24.4	$[M-H]^-$	559	515, 473, 413*, 311*, 277*, 253*
spinach	2,4-DCP-(malonyl)hexose-hexuronic acid (*major*)	13.6	$[M-H]^-$	585	541, 379, 161
spinach	2,4,5-TCP-hexose-hexose (*major*)	15.8	$[M-H]^-$	519	357, 195
			$[M+Li]^+$	527	365, 331, 313, 169
	2,4,5-TCP-(malonyl)hexose-hexuronic acid	17.5	$[M-H]^-$	619	575, 413, 195
			$[M+Li]^+$	627	609, 583, 451, 429, 407
	2,4,5-TCP-(malonyl)hexose-pentose	22.8	$[M-H]^-$	575	531, 399*, 195*
			$[M+Li]^+$	583	539, 497, 451, 387, 369, 343, 325, 237

[a] (*) Fragments obtained by MS3 carried out on the underlined fragment ion. $[M-H]^-$ obtained from NI-ESI ionization. $[M+Li]^+$ obtained from PI-ESI ionization.

using helium as damping as well as collision gas for MS/MS experiments. Unless specified in the text, all tandem mass spectrometric experiments were performed on the ^{35}Cl, $^{35}Cl_2$, and $^{35}Cl_3$ monoisotopic peaks of 4-CP, 2,4-DCP, and 2,4,5-TCP metabolites, respectively.

Nuclear Magnetic Resonance Spectroscopy (Table 2). ^1H NMR spectra were obtained at 300 K using a Bruker Avance DRX-600 spectrometer (Bruker, Wissembourg, France), operating at 600.13 MHz and equipped with a 5 mm H,C,N inverse triple resonance TXI cryoprobe attached to a cryoplatform (the preamplifier unit). Samples were dissolved in 600 μL of deuterated methanol (CD_3OD).

One-dimensional ^1H NMR spectra were acquired using a standard pulse sequence for ^1H NMR. 1024 free induction decays (FIDs) were collected with a spectral width of 12 ppm into 64 K data points. An exponential function equivalent to a line-broadening of 0.3 Hz was applied prior to Fourier transformation.

Two dimensional $^1H-^1H$ COSY spectra were acquired over a spectral width of 2400 Hz in both dimensions, into 1024 data points in the F1 dimension and 512 data points in the F2 dimension. 64 FIDs were collected per data point in the F2 dimension. A sine-bell apodization function was applied to both F1 and F2 dimensions before Fourier transformation. A summary of ^1H resonances of isolated metabolites is given in **Table 2**.

Data Analysis. Results, expressed as μg of equivalent parent compound g^{-1}, were always relative to DW. They were the average of at least six measurements for each experiment and were analyzed for statistical significance by two-tailed Student's *t* test ($p < 0.05$).

Table 2. Summary of ^1H NMR Resonances of Isolated Metabolites[a]

compound	aglycone moiety	glycoside
4-CP-*O*-(acetyl)-β-glucose	H-2: 7.15 (*d*, 8.6) H-3: 7.45 (*d*, 8.6) CH3: 2.12 (s)	H-1′: 5.18 (*d*, 8.6) H-2′: 3.68 (*t*, 8.6) H-3′: 3.65 (*t*, 8.6) H-4′: 3.63 (*t*, 8.6) H-5′: 3.93 (*ddd*, 2; 5.8; 9.3) H-6′: 4.55 (*dd*, 2; 12) 4.42 (*dd*, 5.8; 12)
2,4,5-TCP-*O*-β-glucose-$C_5H_5O_5$	H-3: 7.58 (*s*) H-6: 7.43 (*s*)	H-1′: 4.98 (*d*, 7.7) H-2′: 3.51 (*dd*, 8.8; 7.7) H-3′: 3.46 (*t*, 8.8) H-4′: 3.38 (*m*) H-5′: 3.72 (*ddd*, 1.8; 6.8; 9.7) H-6′: 4.51 (*dd*, 1.8; 11.9) 4.22 (*dd*, 6.8; 11.9)

[a] Chemical shifts (ppm) are relative to deuterated methanol (^1H, δ, 3.30). Multiplicity of signals are in italics: *s* = singlet; *d* = doublet; dd = doublet−doublet; ddd = doublet−doublet−doublet; *t* = triplet; and *m* = multiplet. Values in parentheses are $^1H-^1H$ splittings (Hz) in cases where these are clearly resolved.

RESULTS AND DISCUSSION

Uptake of [^{14}C] 4-CP, [^{14}C] 2,-4DCP, and [^{14}C] 2,4,5-TCP by Radish and Distribution of the Radioactivity. The uptake of [^{14}C] 4-CP, [^{14}C] 2,4-DCP, and [^{14}C] 2,4,5-TCP in radish plants is shown in **Table 3**. The amount of radioactivity taken up by plants and left in the nutrient solution accounted for total applied radioactivity. Whole radish plants took up the three [^{14}C] chlorophenols and [^{14}C] 2,4,5-TCP uptake appeared to be higher than that of the two other compounds (**Table 3**). Lipophilicity is one of the most important factors that drives the uptake of chemicals in plants and is generally assessed using the octanol−water partition coefficient, Kow and especially log Kow. The range of log Kow values favorable for optimal uptake by plants lies between 1 and 3.5. Log Kow values of chlorophenols are 2.85 for 4-CP, 3.05 for 2,4-DCP, and 3.66 for 2,4,5-TCP (*1*). Efficient uptake of these compounds was thus expected. The pK_a values of the chlorophenols are in the 7.4−9.4 range, 9.43, 8.51, and 7.40 for 4-CP, 2,4-DCP, and 2,4,5-TCP, respectively (*Handbook of Chemistry and Physics*, 87th edition, 2006−2007). At pH values of plant compartments (xylem and vacuoles, pH 5−5.5; cytoplasm and phloem, pH 7.5−8), 4-CP and 2,4-DCP should be neutral and were thus expected to enter plants in equal quantities. The higher log Kow value of 2,4,5-TCP could explain its higher uptake.

We found that the distribution of all three [^{14}C] chlorophenols in radish plant parts was similar and in decreasing order peel, core, and leaves. Nearly 60% of the total amount of residues persisted in root peel three weeks after treatment even though root peel represents only 10% of total weight. A small proportion, less than 12%, was translocated into aerial parts.

Table 3. Whole Residues, Extractable (ER), and Non-Extractable Residues (NER) of [^{14}C]-4-CP, [^{14}C]-2,4-DCP, and [^{14}C]-2,4,5-TCP in Radish[a]

chlorophenol	plant fraction	radish	root peel	root core	leaves
[^{14}C]-4-CP	total uptake	43.5 ± 14.1 (66.6 ± 9.4%)	82.2 ± 43.7	25.9 ± 6.0	2.4 ± 1.0
	ER	34.9 ± 6.7[b] (57.2 ± 12.8%)	70.5 ± 25.6[b]	17.4 ± 3.4[b]	1.7 ± 0.9
	NER	7.4 ± 4.1[b] (10.8 ± 4.8%)	18.2 ± 11.1[b]	2.3 ± 0.7[b]	0.9 ± 0.2
[^{14}C]-2,4-DCP	total uptake	33.7 ± 19.1 (44.7 ± 9.7%)	85.9 ± 17.7	15.0 ± 4.8	1.5 ± 0.8
	ER	19.0 ± 11.3 (24.9 ± 5.3%)	45.3 ± 23.5[b]	6.8 ± 1.6[b]	1.8 ± 1.1[b]
	NER	12.2 ± 5.6 (16.9 ± 3.3%)	13.7 ± 5.9[b]	2.6 ± 1.1[b]	0.6 ± 0.2[b]
[^{14}C]-2,4,5-TCP	total uptake	60.0 ± 22.8 (70.7 ± 6.3%)	65.0 ± 11.9	30.2 ± 16.5	3.8 ± 0.8
	ER	36.4 ± 14.1[b] (40.8 ± 5.7%)	41.0 ± 17.9[b]	27.8 ± 9.4[b]	1.9 ± 0.2[b]
	NER	9.6 ± 1.5[b] (11.8 ± 3.7%)	7.8 ± 2.0[b]	5.9 ± 3.0[b]	1.0 ± 0.2[b]

[a] Results are expressed as μg equivalent parent compound g^{-1} dry weight or as percentage of applied radioactivity (parentheses). Values are the means ± SD from at least six samples. [b] Statistically significant, ER as compared to NER ($p < 0.05$).

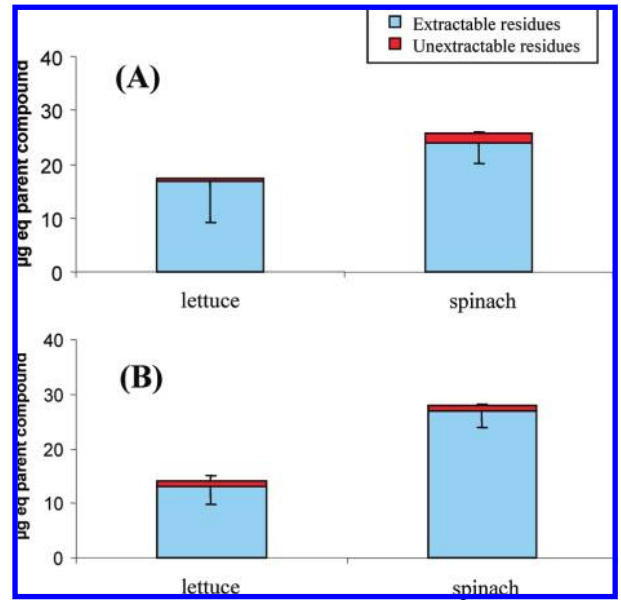

Figure 1. Quantities of residues in lettuce and spinach, expressed in μg of equivalent parent compound (**A**) [^{14}C] 2,4-DCP and (**B**) [^{14}C] 2,4,5-TCP.

Similar results were obtained in experiments where vapor phase uptake by edible parts was avoided. Autoradiography of longitudinal or cross sections of radish treated with either [^{14}C] 2,4-DCP or [^{14}C] 2,4,5-TCP showed that most radioactivity accumulated in root peel (not shown). Fujisawa et al. (*22*) have reported that the bulk of residues is sequestered in root peel when edible roots are exposed to pesticides after soil treatment. Translocation also appears to be largely determined by the lipophilicity of the compound (*23*). In spite of the fact that log Kow values of the three chlorophenols are optimal for translocation and efficient uptake, sequestration of residues in roots could be explained by a high metabolic rate (*24*).

Analysis of the distribution of radioactivity between solvent extractible and unextractable residues in radish plants showed that a large proportion of residues were soluble, whatever the chlorophenol involved (**Table 3**). It should be noted that because of the small quantities and the relative heterogeneity of aliquots, variability in the 10−20% range was observed between total uptake and the sum of extractable + unextractable residues. In the same way, the major part of radioactivity in different plant parts was found to be associated with the extractable residues at the expense of unextractable residues (**Table 3**). The low level of unextractable residues was somewhat unexpected since a large proportion of unextractable residues associated with roots has previously been reported (*25*) and could be due to differences in cell wall structures of edible roots. Actually, unlike carrots, which arise from underground plant parts, radish edible roots are tubers entirely formed from hypocotyls.

Uptake and Distribution of [^{14}C] 2,4-DCP, and [^{14}C] 2,4,5-TCP by Lettuce, and Spinach. In lettuce, the proportion of radioactivity recovered in the leaves treated with either [^{14}C] 2,4-DCP or [^{14}C] 2,4,5-TCP amounted to 13.5% of total applied radioactivity. The radioactivity taken up by spinach was 22.4 ± 3.2% for 2,4-DCP and 33.3 ± 3.1% for 2,4,5-TCP. The unrecovered radioactivity was likely due to loss by vaporization since the vapor pressure values of 2,4-DCP and 2,4,5-TCP are 8.6 and 4.2 Pa at 25 °C, respectively, and the small quantity of chlorophenol applied about 100 μg per plant. The low absorption rate could be also due to poor penetration into the plants despite the presence of a foaming agent in the formulation. Indeed, only 20% was recovered in the leaf surface washings. The radioactivity recovered in untreated or new leaves of both plants was in the 1−10% range (data not shown). The distribution of radioactivity between soluble and unextractable residues is shown in **Figure 1**. Most of the radioactivity was located in the extractable fraction. When results are expressed as μg equivalent parent compound g^{-1} DW, the proportion of unextractable residues amounted to less than 1%. A high proportion of extractable residues associated with plant aerial parts has already been reported in previous studies (*17*).

Identification of Soluble Metabolites of [^{14}C] 4-CP, [^{14}C] 2,4-DCP, and [^{14}C] 2,4,5-TCP in Radish. Typical radio-HPLC profiles obtained from radish crude extracts after [^{14}C] chlorophenol exposure are shown in **Figure 2**. Under our conditions, no parent compound was detectable in the extracts.

Most of the total extractable residues (TER), 80.5 ± 1.4%, were metabolized to one main peak (Rt = 16.1 min) in the case of [^{14}C] 4-CP (**Figure 2A**). The structure of the metabolite was further characterized by negative ESI-MS and ^{1}H NMR spectroscopy. MS/MS analysis of the [M−H]$^{-}$ ion detected at m/z 331 gave a characteristic m/z 127 fragment ion (chlorophenoxy ion) consistent with the elimination of an acetyl-glycoside moiety. This was confirmed by MS/MS data obtained on the [M+Li]$^{+}$ ion (m/z 339) generated by positive ESI (**Table 1**). In the ^{1}H NMR spectrum of this metabolite, the signals between 3 and 5.5 ppm indicated that there was a β-glucose moiety conjugated to 4-CP. The two doublets at 7.15 and 7.45 ppm observed in the aromatic region with a coupling constant of 8.6 Hz showed that the aromatic ring was para-disubstituted. The glycoside moiety was linked to the oxygen of 4-CP. Because of the high chemical shifts of the proton H-6′ of the glucose, there was certainly a substituent (like malonyl or acetyl group) linked to the carbone-6 of the glucose. The singlet at 2.12 ppm corresponded to the methyl group of acetyl-glycoside moiety. Thus, the chemical structure of the major 4-CP metabolite was attributed to its acetyl-*O*-glucoside derivative (**Tables 1** and **2**). A minor metabolite was also present and was characterized by ESI-MS. Data generated in both negative and positive ion modes (**Table 1**) showed losses of 44 and 86 amu (atomic mass unit) characteristic of a malonyl moiety, which

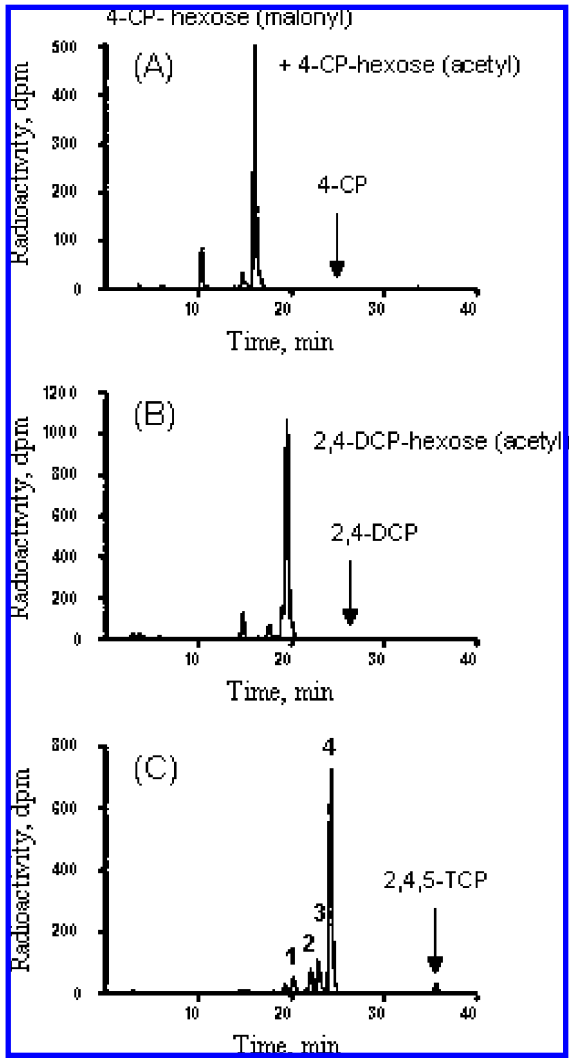

Figure 2. Typical radio-HPLC profiles of soluble residues obtained from crude extracts of radish (**A**) [^{14}C] 4-CP, (**B**) [^{14}C] 2,4-DCP, and (**C**) [^{14}C] 2,4,5-TCP. Peak 1: 2,4,5-TCP-hexose-sulfate. Peak 2: 2,4,5-TCP-(malonyl)-hexose-sulfate. Peak 3: 2,4,5-TCP-(malonyl)hexose-deoxyhexose. Peak 4: 2,4,5-TCP-hexose-$C_5H_5O_5$.

enabled identification of this metabolite as the malonyl-*O*-glucose conjugate of 4-CP. Because of the limited quantity of other metabolites, further analysis of the more polar metabolites was not undertaken.

In the case of [^{14}C] 2,4-DCP, the main peak (Rt = 19.60 min) accounted for 75.0 ± 6.6% of the TER (**Figure 2B**). The structure was further investigated by negative ESI-MS. The main fragment obtained from the MS/MS analysis of the $[M-H]^-$ ion (*m/z* 365) of this metabolite was detected at *m/z* 161. This could reflect the occurrence of either the dichlorophenoxy ion ($C_6H_3O^{35}Cl_2$) or a glycoside moiety ($C_6H_9O_5$) as part of the parent ion. A similar MS/MS experiment was carried out on the *m/z* 367 ion corresponding to the $^{35}Cl^{37}Cl$ isotopomer of the metabolite. In this case, the *m/z* 163 fragment ion was obtained, indicating that it corresponded to the 2,4-DCP moiety of the molecule, in agreement with the elimination of an acetyl glycoside from the selected $[M-H]^-$ ion (data not shown). In accordance with these MS data, the main metabolite was identified as the acetyl-*O*-glucoside conjugate of 2,4-DCP (**Table 1**). The peak at Rt = 14.80 could not be investigated by MS because of the low quantity available. Nevertheless, it coeluted in HPLC with authentic 2,4-DCP-glucose and was hydrolyzed by almond β-glucosidase to a product coeluting with the parent compound, suggesting that it corresponded to 2,4-DCP-glucose.

Concerning [^{14}C] 2,4,5-TCP, one main and three minor peaks were detected in the radio-HPLC profile and were numbered according to their elution order (**Figure 2C**). Peak 4 accounted for 70.4 ± 3.5% of the TER and was further analyzed by ESI-MS. Using both negative and positive ionization modes, the quasi-molecular species (i.e., $[M-H]^-$ at *m/z* 501 and $[M+Li]^+$ at *m/z* 509, respectively, for the $^{35}Cl_3$ isotopomer) were consistent with a structure corresponding to 2,4,5-TCP conjugated to a hexose and a $C_5H_5O_5$ moiety (**Table 1**). This metabolite was also submitted to ^{1}H NMR spectroscopy analysis, which confirmed the occurrence of a 2,4,5-TCP-*O*-β-glucose conjugate (**Table 2**). However, the high chemical shifts of the protons H-6′ of the glucose moiety compared to 2,4,5-TCP-*O*-glucose indicated that there was a substituent such as a malonyl group linked to carbon 6 of the glycoside. Unfortunately, some signals were hidden by solvent signals, and it was difficult to determine the structure of the $C_5H_5O_5$ moiety. According to the MS and NMR results, we suggest the following formula for the $C_5H_5O_5$ moiety: -CO-CH_2-CO-O-CH_2-COOH. The minor peaks, peak 1, Rt = 20.4; peak 2, Rt = 22.2; and peak 3, Rt = 23.2 min, accounted for 4.5 ± 0.8%, 7.5 ± 2.4%, and 11.4 ± 1.0% of the TER, respectively, and were further identified by both negative and positive ESI-MS. In accordance with MS data, we suggest the following structures for these metabolites: metabolite 1, 2,4,5-TCP-hexose-sulfate; metabolite 2, 2,4,5-TCP-(malonyl)hexose-sulfate; and metabolite 3, 2,4,5-TCP-(malonyl)hexose-deoxyhexose (e.g., fucose or rhamnose) (**Table 1**).

Identification of Metabolites of [^{14}C] 2,4-DCP and [^{14}C] 2,4,5-TCP in Lettuce and Spinach. After treatment with [^{14}C] 2,4-DCP or [^{14}C] 2,4,5-TCP in lettuce and spinach, crude extracts were first analyzed by radio-HPLC. Typical profiles obtained are shown in **Figure 3**.

In lettuce treated with [^{14}C] 2,4-DCP, the profile showed one main peak (Rt =19.80 min) that accounted for 85.6 ± 5.1% of the TER (**Figure 3A**). This peak was then submitted to MS analysis. The major metabolite was identified as the 2,4-DCP-(*O*-acetyl)-glucoside (**Table 1**).

In lettuce treated with [^{14}C] 2,4,5-TCP, one main peak was obtained, Rt = 24.50 min, 81.9 ± 2.5% of the TER (**Figure 3B**). The peak was submitted to MS analysis, which revealed the presence of two metabolites. The major compound displayed an $[M-H]^-$ ion at *m/z* 399 for the $^{35}Cl_3$ isotopomer, for which MS/MS analysis gave a unique fragment ion at *m/z* 195 corresponding to the 2,4,5-TCP moiety. On the same basis as for 2,4-DCP, this metabolite was identified as the 2,4,5-TCP-(*O*-acetyl)-glucoside (**Table 1**). The $[M-H]^-$ ion of the second metabolite was detected at *m/z* 559 ($^{35}Cl_3$ contribution). The MS/MS spectrum acquired from the *m/z* 559 precursor ion gave fragment ions at *m/z* 515 ($[M-H-44]^-$) and *m/z* 473 ($[M-H-86]^-$) indicating the occurrence of a malonyl moiety. Subsequent MS^3 analysis of the *m/z* 473 ion gave additional fragment ions at *m/z* 311 ($[473-162]^-$) and *m/z* 277 $[473-196]^-$), corresponding to the elimination of a hexose and of the 2,4,5-TCP moiety, respectively, from the *m/z* 473 ion (**Table 1**). All these data were consistent with the fragmentation of 2,4,5-TCP-deoxyribose-(*O*-malonyl)hexose.

The radio profile obtained from spinach treated with [^{14}C] 2,4-DCP showed a more complex pattern. Four peaks were detected at Rt = 8.40 (peak 1), 13.60 (peak 2), 14.60 (peak 3), and 19.70 min (peak 4) and accounted for 9.9 ± 0.9%, 49.7 ± 3.6%, 11.0 ± 2%, and 26.8 ± 2.8% of the TER, respectively (**Figure 3C**). Because of their high polarity and their low

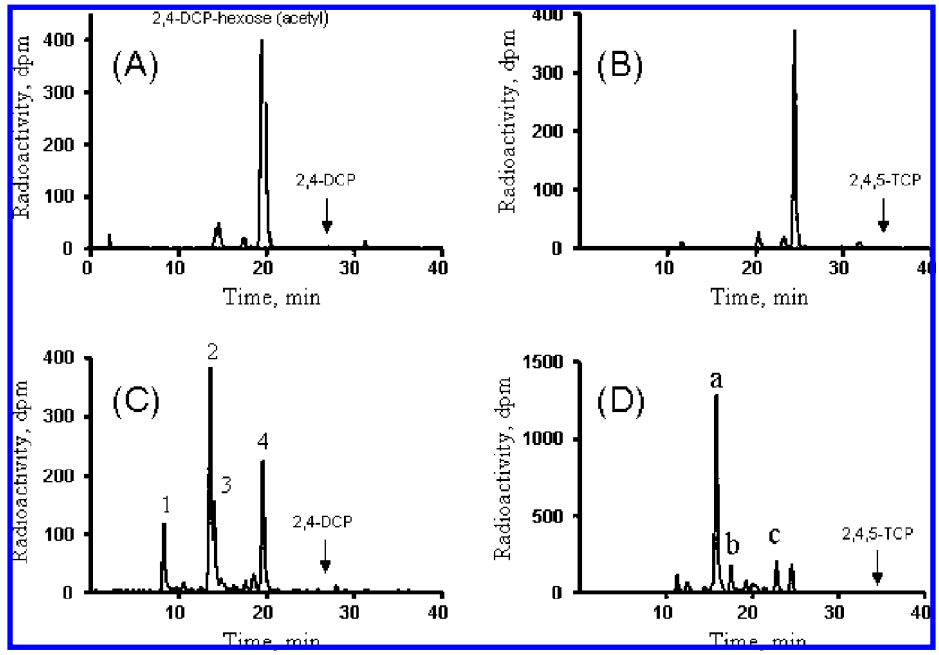

Figure 3. Typical radio-HPLC profiles of soluble residues obtained from crude extracts of lettuce (**A**) [^{14}C] 2,4-DCP, (**B**) [^{14}C] 2,4,5-TCP, and spinach (**C**) [^{14}C] 2,4-DCP. Peak 1: unknown. Peak 2: 2,4-DCP-(malonyl)hexose-hexuronic acid. Peak 3: 2,4-DCP-hexose. Peak 4: 2,4-DCP-(O-acetyl)hexose. (**D**) [^{14}C] 2,4,5-TCP. Peak a: 2,4,5-TCP-hexose-hexose. Peak b: 2,4,5-TCP-(malonyl)hexose-hexuronic acid. Peak c: 2,4,5-TCP-(malonyl)hexose-pentose.

quantity in crude extract, peaks 1 and 3 could not be characterized by MS analysis. Peak 1 was resistant to enzyme treatments such as β-glucosidase or malonyl esterase from parsley (*21*). However, peak 3 could be identified as the glucoside conjugate of 2,4-DCP, on the basis of positive almond β-glucosidase treatment. Peaks 2 and 4 were further analyzed by negative ESI-MS. On the basis of MS data, the chemical structure of metabolite 4 was attributed to the acetyl-*O*-glucoside of 2,4-DCP, which was already identified in radish and lettuce (**Table 1**). Peak 2 gave a signal at *m/z* 585 corresponding to the $[M-H]^-$ species. When submitted to resonant excitation in the ion trap device, the *m/z* 585 precursor ion decomposed into *m/z* 541 $[M-H-CO_2]^-$, *m/z* 379 $[M-H-CO_2-DCP]^-$ and *m/z* 161 (dichlorophenoxy ion) fragment ions. To clarify the fragmentation pathway, a complementary MS/MS experiment was carried out on the $^{35}Cl_2{}^{37}Cl$ isotopomer of metabolite 2. The MS/MS spectrum of the *m/z* 587 selected as the precursor ion displayed fragment ions at *m/z* 543, 379, and 163, thus confirming the above attributions (data not shown). From these data, we suggest 2,4-DCP-(malonyl)hexose-hexuronic acid as a structure for metabolite 2 (**Table 1**). It is noteworthy that peak 2 was hydrolyzed by parsley malonyl esterases to a product coeluting with peak 1 and could thus be 2,4-DCP-hexose-hexuronate.

One major and several minor peaks were detected in the radio profile obtained from the crude extract from spinach treated with [^{14}C] 2,4,5-TCP (**Figure 3D**). Three peaks (peak a, Rt = 15.8 min, 56.3 ± 0.9%; peak b, Rt = 17.5 min, 11.5 ± 3.2%; peak c, Rt = 22.8 min, 7.5 ± 1.9% of the TER) were analyzed by both negative and positive ESI-MS after lithium cationization. The analysis of peak a gave both an $[M-H]^-$ ion at *m/z* 519 and an $[M+Li]^+$ ion at *m/z* 527, in agreement with a metabolite displaying a molecular mass of 520. The MS/MS spectrum of the *m/z* 519 precursor ion gave fragment ions at *m/z* 357 and 195, corresponding to the successive elimination of one and two glycosyl moieties, respectively, (i.e., 357 = 519 − 162 and 195 = 519 − 162 − 162). These data were confirmed by MS/MS of the *m/z* 527 $[M+Li]^+$ ion (**Table 1**), enabling us to propose 2,4,5-TCP-hexose-hexose as a structure for peak a. Peak b gave an $[M-H]^-$ ion at *m/z* 619, which decomposed into *m/z* 575, 413, and 195 fragment ions in the MS^2 step. All these ions were shifted by 34 mass units compared to 2,4-DCP metabolite 2 from spinach. This shift corresponded to the additional chlorine atom in 2,4,5-TCP vs 2,4-DCP. On the basis of the same experiments as for 2,4-DCP metabolites, we tentatively identified peak b as 2,4,5-TCP-(malonyl)hexose-hexuronic acid. Positive ESI gave an $[M+Li]^+$ ion (*m/z* 627) and fragment ions (**Table 1**), which were also in agreement with this structure. Finally, the *m/z* 575 $[M-H]^-$ ion together with its related fragment ions produced by MS/MS (**Table 1**) allowed us to propose the (malonyl)hexose-pentose conjugate of 2,4,5-TCP for peak c. This was confirmed by the analysis of the same metabolite under positive ionization conditions after lithium cationization (i.e., $[M+Li]^+$ ion at *m/z* 583 and related MS/MS fragment ions, see **Table 1**).

Comparative metabolic pathways of 2,4,5-TCP transformation in lettuce, spinach, and radish are summarized in **Figure 4**. In this study, we characterized various chemical structures of metabolites of chlorophenols, but these contaminants were always conjugated to sugars, generally giving rise to more polar and less toxic compounds. Glycosylation of xenobiotics is the major detoxification mechanism described in higher plants (*26*) and has been well documented for hydroxyl, carboxyl, and amino groups (*27*−*29*). Chlorophenols such as pentachlorophenol, 2,4,5-TCP, 2,4-DCP and phenol in terrestrial and aquatic plants are usually metabolized by glucose conjugation (*30, 11*−*13, 15*). In addition, numerous glycosyltransferases have been shown to be involved in the detoxification of endogenous substrates with parallel activity toward xenobiotics including pesticides and pollutants such as 2,4,5-trichlorophenol and 3,4-dichloroaniline (*31*−*33*). Transformation by conjugation with low molecular mass peptides has been described in plants such as maize, pea, and English ryegrass for phenols and benzoic acid (*34, 35*), but no such compound could be identified in our study. We detected acetyl glycoside conjugates as main compounds in the metabolism of 4-CP in radish, 2,4-DCP in all three plants, and 2,4,5-TCP in lettuce (**Table 1**). Acetyl glycoside conjugates have also

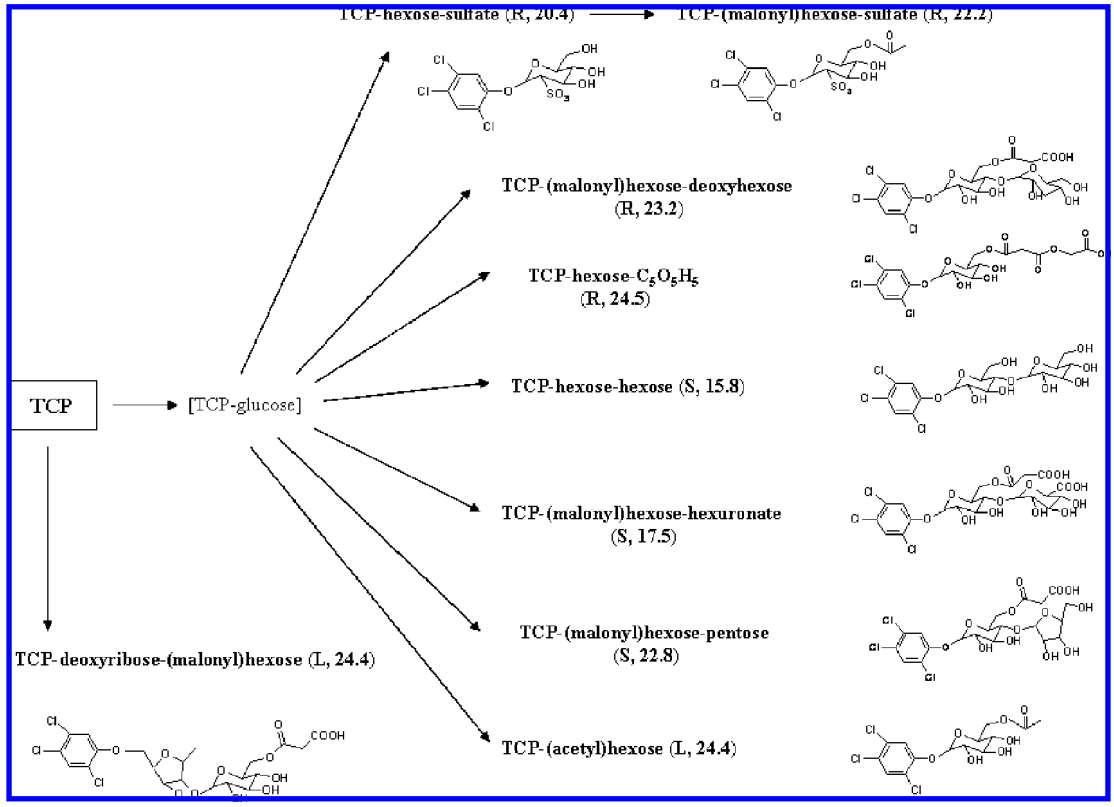

Figure 4. Proposed metabolic pathways of 2,4,5-TCP in radish (R), lettuce (L), and spinach (S). *Rt* are given in parentheses.

been found in aquatic plants (*13*) but are generally detected as minor metabolites in angiosperms (*16*). Most of the 2,4,5-TCP metabolites were disaccharide conjugates (**Figure 4**). This result is not surprising since xenobiotic glycoside conjugates are able to undergo glycosyl extension to give more complex conjugates (*9*). However, the presence of pentose residues among them has been less frequently described (*36, 37, 15*). Conjugation with pentose would prevent further saccharide chain elongation (*9*). In all three plants, we found malonyl glycoside conjugates as metabolites of 2,4,5-TCP (**Figure 4**). Acylation by malonic acid is a common process, specific to plants, and may represent a signal for sequestration of glycoside conjugates in vacuoles, the classical stores for secondary compounds and transformed xenobiotics (*38*). Nevertheless, due to the presence of various glycosidases and esterases, interconversion of these saccharide conjugates could occur and has occasionally been demonstrated (*36*). The 2,4,5-TCP metabolite 4 from radish has been suggested to be composed of a hexose conjugated to a diacidic moiety that comprised two more carbons than malonic acid. This diacid could result from the condensation of malonic acid and glycollate. The presence of such varying residues could be explained by the relatively long exposure time. Metabolites stored in the vacuoles could be further processed before exportation to the cell wall. However, very little is known about how the transformation process occurs and about the ultimate fate of sugar conjugates. To our knowledge, the characterization of deoxy-sugar conjugates (**Figure 4**) in the metabolism of xenobiotics in plants has not yet been reported. However, deoxyoses are commonly found in plant cell structures such as cell walls, and deoxyhexose derivatives of quercetin containing rhamnose are among the most abundant flavonol glycosides identified in berries (*39*). Deoxypentose conjugates have already been suspected in the metabolism of 4-*n*-nonylphenol in *Lemna minor* after six days of exposure followed by six days of depuration (*40*). Sulfation of glycosides is not common in the plant metabolism of xenobiotics. One reported case concerns the metabolism of phenmedipham in sugar beet (*41*). A second case is the identification of a glucosylsulfate conjugate of 4-bromo-2-chlorophenol, a metabolite of profenofos in cotton (*42*). Finally, 2,4-DCP-(6-*O*-sulfate) glucoside has been described as a major terminal metabolite of 2,4-D in transgenic tolerant cotton (*43*). In addition to these metabolites, glucuronide conjugates have been characterized in spinach treated with 2,4-DCP and 2,4,5-TCP (**Figure 4**). This kind of conjugate has rarely been described in plants. In contrast, many xenobiotics and endogenous compounds are known to form uronic acid, in particular glucuronic acid, conjugates in animals (*44*). However, glucuronide endogenous compounds are found in plants such as marigold (*Calendula officinalis*), which is able to conjugate oleanolic acid to glucuronic acid (*45*). In the same way, glucuronic acid residues are involved in secondary wall formation (*46*). Bockern et al. (*47*) have reported the formation of 4-*n*-nonylphenol glucuronide derivatives in wheat cell suspension cultures. A triglycoside containing a glucuronic acid has also been characterized in cell suspension cultures of tobacco (*14*).

Following oral ingestion, animals or humans can be exposed to glucuronide conjugates. In animals, acidic conjugates of xenobiotics such as glucuronides are usually excreted in urine and bile. Following hydrolysis, they may be resorbed from the intestine and enter the enterohepatic circulation (*44*). Glucuronides present in plants could also follow this pathway.

Flavonoids such as quercetin are present as glycosylated forms in food, mainly as β-glycosides. The nature and the number of sugars determine the extent of small intestine absorption of quercetin (*48*), which is enhanced by glucose conjugation (*18*). Chlorophenols are exclusively metabolized by saccharide conjugation. As reported for flavonoids, they could be hydrolyzed by enzymes in the colon and by microflora present in the digestive tract and then return to the toxic parent compound. This should be kept in mind for registration studies. Hollman et al. (*49*) have highlighted the predominant role of the sugar moiety in the

bioavailability and absorption of dietary quercetin in humans. It is consequently important to determine the exact structure of chlorophenol glycosides to evaluate their bioavailability and the risk they represent to humans.

ABBREVIATIONS USED

4-CP, 4-chlorophenol; 2,4-DCP, 2,4-dichlorophenol; 2,4,5-TCP, 2,4,5-trichlorophenol; 2,4-DCP-glucose, O-β-D-glucopyranosyl-2,4-dichlorophenol; DW, dry weight; eq, equivalent; ESI-MS, electrospray ionization-mass spectrometry; Kow, octanol−water partition coefficient; LC-MS, liquid chromatography−mass spectrometry; NMR, nuclear magnetic resonance; RP-HPLC, reverse-phase-high performance liquid chromatography; *Rt*, retention time; TER, total extractable residues.

LITERATURE CITED

(1) Jensen, J. Chlorophenols in the terrestrial environment. *Rev. Environ. Contam. Toxicol.* **1996**, *146*, 25–51.

(2) Tuppurainen, K. A.; Ruokojärvi, P. H.; Asikainen, A. H.; Aatamila, M.; Ruuskanen, J. Chlorophenols as precursors of PCDD/Fs in incineration processes: correlations, PLS modeling, and reaction mechanisms. *Environ. Sci. Technol.* **2000**, *34*, 4958–4962.

(3) Marsolek, M. D.; Kirisits, M. J.; Rittmann, B. E. Biodegradation of 2,4,5-trichlorophenol by aerobic microbial communities: biorecalcitrance, inhibition, and adaptation. *Biodegradation* **2007**, *18*, 351–358.

(4) Crespin, M. A.; Gallego, M.; Valcarcel, M. Study of the degradation of the herbicides 2,4-D and MCPA at different depths in contaminated agricultural soil. *Environ. Sci. Toxicol.* **2001**, *35*, 4265–4270.

(5) Boucard, T. K.; Bardgett, R. D.; Jones, K. C.; Semple, K. T. Influence of plants on the chemical extractability and biodegradability of 2,4-dichlorophenol in soil. *Environ. Pollut.* **2005**, *133*, 53–62.

(6) Latch, D. E.; Packer, J. L.; Stender, B. L.; VanOverbeke, J.; Arnold, W. A.; McNeill, K. Aqueous photochemistry of triclosan: formation of 2,4-dichlorophenol, 2,8-dichlorodibenzo-*p*-dioxin, and oligomerization products. *Environ. Toxicol. Chem.* **2005**, *24*, 517–525.

(7) Czaplicka, M. Sources and transformations of chlorophenols in the natural environment. *Sci. Total Environ.* **2004**, *322*, 21–39.

(8) Sinclair, G. M.; Paton, G. I.; Meharg, A. A.; Killham, K. *Lux*-biosensor assessment of pH effects on microbial sorption and toxicity of chlorophenols. *FEMS Microbiol. Lett.* **1999**, *174*, 273–278.

(9) Cole, D. J.; Edwards, R. Secondary Metabolism of Agrochemicals in Plants. In *Metabolism of Agrochemicals in Plants*; Roberts, T., Ed; John Wiley & Sons Ltd: Chichester, U.K., 2000; pp 107−154.

(10) Ensley, H. E.; Barber, J. T.; Polito, M. A.; Oliver, A. I. Toxicity and metabolism of 2,4-dichlorophenol by the aquatic angiosperm *Lemna gibba*. *Environ. Toxicol. Chem.* **1994**, *13*, 325–331.

(11) Barber, J. T.; Sharma, H. A.; Ensley, H. E.; Polito, M. A.; Thomas, D. A. Detoxification of phenol by the aquatic angiosperm *Lemna gibba*. *Chemosphere* **1995**, *31*, 3567–3574.

(12) Sharma, H. A.; Barber, J. T.; Ensley, H. E.; Polito, M. A. A comparison of the toxicity and metabolism of phenol and chlorinated phenols by *Lemna gibba*, with special reference to 2,4,5-trichlorophenol. *Environ. Toxicol. Chem.* **1997**, *16*, 346–350.

(13) Pascal-Lorber, S.; Rathahao, E.; Cravedi, J.-P.; Laurent, F. Metabolic fate of [^{14}C]-2,4-dichlorophenol in macrophytes. *Chemosphere* **2004**, *56*, 275–284.

(14) Laurent, F.; Canlet, C.; Laurent, L.; Pascal-Lorber, S. Metabolic fate of [^{14}C]-2,4-dichlorophenol in tobacco cell suspension cultures. *Environ. Toxicol. Chem.* **2007**, *26*, 22992307.

(15) Day, J. A.; Saunders, F. M. Glycosidation of chlorophenols by *Lemna minor*. *Environ. Toxicol. Chem.* **2004**, *23*, 613–620.

(16) Laurent, F.; Debrauwer, L.; Pascal-Lorber, S. Metabolism of [^{14}C]-2,4-dichlorophenol in edible plants. *Pest. Manag. Sci.* **2006**, *62*, 558–564.

(17) Pascal-Lorber, S.; Rathahao, E.; Cravedi, J.-P.; Laurent, F. Uptake and metabolic fate of [^{14}C]-2,4-dichlorophenol and [^{14}C]-2,4-dichloroaniline in wheat (*Triticum aestivum*) and soybean (*Glycine max*). *J. Agric. Food Chem.* **2003**, *51*, 4712–4718.

(18) Karakaya, S. Bioavailability of phenolic compounds. *Crit. Rev. Food Sci. Nutr.* **2004**, *44*, 453–464.

(19) Sinnot, M. L.; Souchard, I. J. The mechanism of action of β-galactosidase. *Biochem. J.* **1973**, *133*, 89–98.

(20) Schmitt, R.; Kaul, J.; van der Trenk, T.; Schaller, E.; Sandermann, H., Jr. β-D-glucosyl and O-malonyl-β-D-glucosyl conjugates of pentachlorophenol in soybean and wheat: identification and enzymatic synthesis. *Pestic. Biochem. Physiol.* **1985**, *24*, 77–85.

(21) Matern, U. 1983. Acylhydrolases from parsley (*Petroselinum hortense*). Relative contribution and properties of four esterases hydrolyzing malonic acid hemiesters of flavonoid glucosides. *Arch. Biochem. Biophys.* **1983**, *224*, 261–271.

(22) Fujisawa, T.; Ichise, K.; Fukushima, M.; Katagi, T.; Takimoto, Y. Mathematical model of the uptake of non-ionized pesticides by edible root of root crops. *J. Pestic. Sci.* **2002**, *27*, 242–248.

(23) Briggs, G. G.; Bromilow, R. H.; Evans, A. A. Relationship between lipophilicity and root uptake and translocation of non-ionised chemicals by barley. *Pestic. Sci.* **1982**, *13*, 495–504.

(24) Vila, M.; Lorber-Pascal, S.; Laurent, F. Fate of RDX and TNT in agronomic plants. *Environ. Pollut.* **2007**, *148*, 148–154.

(25) Schmidt, B. Non-extractable residues of pesticides and xenobiotics in plants−A review. *Recent Res. Devel. Agric. Food Chem.* **1999**, *3*, 329–354.

(26) Korte, F.; Kvesitadze, G.; Ugrekhelidze, D.; Gordeziani, M.; Khatisashvili, G.; Buadze, O.; Zaalishvili, G.; Coulston, F. Organic toxicants and plants. *Ecotoxicol. Environ. Saf.* **2000**, *47*, 1–26.

(27) Schmidt, B.; Schuphan, I. Metabolism of the environmental estrogen bisphenol A by plant cell suspension cultures. *Chemosphere* **2002**, *49*, 51–59.

(28) Nakajima, N.; Oshima, Y.; Edmonds, J. S.; Morita, M. Glycosylation of bisphenol A by tobacco BY-2 cells. *Phytochemistry* **2004**, *65*, 1383–1387.

(29) Vila, M.; Pascal-Lorber, S.; Rathahao, E.; Debrauwer, L.; Canlet, C.; Laurent, F. Metabolism of [^{14}C]-2,4,6-trinitrotoluene in tobacco cell suspension cultures. *Environ. Sci. Technol.* **2005**, *39*, 663–672.

(30) Roy, S.; Hänninen, O. Pentachlorophenol: uptake/elimination kinetics and metabolism in an aquatic plant *Eichhornia crassipes*. *Environ. Toxicol. Chem.* **1994**, *13*, 763–773.

(31) Meβner, B.; Thulke, O.; Schäffner, A. R. Arabidopsis glucosyltransferases with activities toward both endogenous and xenobiotic substrates. *Planta* **2003**, *217*, 138–146.

(32) Brazier-Hicks, M.; Edwards, R. Functional importance of the family 1 glucosyltransferase UGT72B1 in the metabolism of xenobiotics in *Arabidopsis thaliana*. *Plant J.* **2005**, *42*, 556–566.

(33) Loutre, C.; Dixon, D. P.; Brazier, M.; Slater, M.; Cole, D. J.; Edwards, R. Isolation of a glucosyltransferase from *Arabidopsis thaliana* active in the metabolism of the persistent pollutant 3,4-dichloroaniline. *Plant J.* **2003**, *34*, 485–493.

(34) Chrikishvili, D.; Sadunishvili, T.; Zaalishvili, G. Benzoic acid transformation via conjugation with peptides and final fate of conjugates in higher plants. *Ecotoxicol. Environ. Saf.* **2006**, *64*, 390–399.

(35) Chrikishvili, D. I.; Zaalishvili, G.; Mitaishvili, T. I.; Lomidze, E. P. Peptide conjugates of benzene and toluene metabolites in English ryegrass. *Russian J. Plant Physiol.* **2006**, *53*, 456–462.

(36) Wright, A. N.; Roberts, T. R.; Dutton, A. J.; Doig, M. V. The metabolism of cypermethrin in plants: the conjugation of the cyclopropyl moiety. *Pestic. Biochem. Physiol.* **1980**, *13*, 71–80.

(37) Lamoureux, G. L.; Rusness, D. G. Quinclorac absorption, translocation, metabolism, and toxicity in leafy spurge (*Euphorbia esula*). *Pestic. Biochem. Physiol.* **1995**, *53*, 210–226.

(38) Martinoia, E.; Maeshima, M.; Neuhaus, H. E. Vacuolar transporter and their essential role in plant metabolism. *J. Exp. Bot.* **2007**, *58*, 83–102.

(39) Häkkinen, S.; Auriola, S. High-performance liquid chromatography with electrospray ionization mass spectrometry and diode array ultraviolet detection in the identification of flavonol aglycones and glycosides in berries. *J. Chromatogr., A* **1998**, *829*, 91–100.

(40) Thibaut, R.; Jumel, A.; Debrauwer, L.; Rathahao, E.; Lagadic, L.; Cravedi, J.-P. Identification of 4-*n*-nonylphenol metabolic pathways and residues in aquatic organisms by HPLC and LC-MS analyses. *Analusis* **2000**, *28*, 793–801.

(41) Lamoureux, G. L. Plant Metabolism of Herbicides in Relation to Detoxification, Selectivity, Antidoting, and Synergism. In *Xenobiotic Metabolism and Disposition*; Kato, R., Eastabrook, R. W., Gayen, M. N., Eds; Taylor and Francis: Bristol, PA, 1989; pp 267−274.

(42) Capps, T. M.; Barringer, V. M.; Eberle, W. J.; Brown, D. R.; Sanson, D. R. Identification of a unique glucosylsulfate conjugate metabolite of profenofos in cotton. *J. Agric. Food Chem.* **1996**, *44*, 2408–2411.

(43) Laurent, F.; Debrauwer, L.; Rathahao, E.; Scalla, R. 2,4-Dichlorophenoxyacetic acid metabolism in transgenic tolerant cotton (*Gossypium hirsutum*). *J. Agric. Food Chem.* **2000**, *48*, 5307–5311.

(44) Bounds, S. V. J.; Hutson, R. The Comparative Metabolism of Agrochemicals in Plants and Mammals. In *Metabolism of Agrochemicals in Plants*; Roberts, T., Ed; John Wiley & Sons Ltd.: Chichester, U.K., 2000; pp 179−209.

(45) Ruszkowski, D.; Szakiel, A.; Janiszowska, W. Metabolism of [3-^{3}H]oleanic acid in *Calendula officinalis* L. roots. *Acta Physiol. Plant* **2003**, *25*, 311–317.

(46) Zhong, R.; Pena, M. J.; Zhou, G.-K.; Nairn, J.; Wood-Jones, A.; Richardson, E. A.; Morrison, W. H.; Darvill, A. G.; York, W. S.; Ye, Z.-H. *Arabidopsis fragile* fiber8, which encodes a putative glucuronyltransferase, is essential for normal secondary wall synthesis. *Plant Cell* **2005**, *17*, 3390–3408.

(47) Bockern, M.; Nimtz, M.; Harms, H. Metabolites of 4-n-nonylphenol in wheat cell suspension cultures. *J. Agric. Food Chem.* **1996**, *44*, 1123–1127.

(48) Manach, C.; Donovan, J. L. Pharmacokinetics and metabolism of dietary flavonoids in humans. *Free Radical Res.* **2004**, *38*, 771–785.

(49) Hollman, P. C. H.; van Trijp, J. M. P.; Buysman, M. N. C. P.; v. d. Gaag, M. S.; Mengelers, M. J. B.; de Vries, J. H. M.; Katan, M. B. Relative bioavailability of the antioxidant flavonoid quercetin from various foods in man. *FEBS Lett.* **1997**, *418*, 152–156.

Received for review May 27, 2008. Revised manuscript received July 8, 2008. Accepted July 8, 2008.

JF8016354