Animal Bioavailability of Defined Xenobiotic Lignin Metabolites

Heinrich Sandermann, Jr.,* Masood Arjmand,‡ Ingrid Gennity,§ and Reinhard Winkler

GSF, Institut für Biochemische Pflanzenpathologie, D-8042 Neuherberg, FRG

Craig B. Struble and Peter W. Aschbacher

Red River Valley Agricultural Research Center, U.S. Department of Agriculture, Fargo, North Dakota 58105

Lignin has been recognized as a major component of bound pesticide residues in plants and is thought to be undigestible in animals. Two defined ring-U-^{14}C-labeled chloroaniline/lignin metabolites have now been fed to rats, where a release of ~66% of the bound xenobiotic occurred in the form of simple chloroaniline derivatives. The observed high degree of bioavailability indicates that bound pesticidal residues may possess ecotoxicological significance. In parallel studies, the white-rot fungus *Phanerochaete chrysosporium* was more efficient, and a soil system was much less efficient, in the degradation of the [ring-U-^{14}C]chloroaniline/lignin metabolites.

INTRODUCTION

The plant metabolism of pesticides often leads in high yield to bound residue fractions which are difficult to characterize, although the cell wall lignin fraction has been recognized to represent a major binding site (Sandermann et al., 1983; Huber and Otto, 1983; Pillmoor and Roberts, 1985). In the case of chlorinated anilines, a chemical mechanism for lignin incorporation has been derived (v.d. Trenck et al., 1981; Still et al., 1981). This result was obtained by use of defined lignin metabolites made in vitro by the classical procedure (Freudenberg, 1968)) employing coniferyl alcohol, hydrogen peroxide, and commercial horseradish peroxidase. Certain other xenobiotics are also copolymerized into lignin under these in vitro conditions (v.d. Trenck and Sandermann, 1981; Schäfer and Sandermann, 1988). Defined chloroaniline/lignin metabolites (v.d. Trenck et al., 1981; Arjmand and Sandermann, 1985) are employed in the present study to determine bioavailability in rats. A major type of linkage of the xenobiotic to the lignin polymers used is shown in Figure 1.

Plant lignin is generally thought to be nondigestible in animals, although its molecular weight may be lowered by physical mechanisms (Zeikus, 1981). The total bound plant metabolites derived from several pesticides were likewise reported to remain undigested in animals (Pillmoor and Roberts, 1985; Harvey, 1983; Khan and Dupont, 1987; Akhtar, 1987; Edwards and Hutson, 1986). The ecotoxicological significance of "insoluble" metabolite fractions depends on the potential release of low molecular weight xenobiotic fragments (Huber and Otto, 1983; Kovacs, 1986). It will now be reported that the two defined chloroaniline/lignin copolymers employed were to a high degree solubilized in the digestive tract of the rat, presumably due to bacterial degradation. A comparison is made with the degradation of the same xenobiotic lignin polymers by the white-rot fungus *Phanerochaete chrysosporium* and by a soil system.

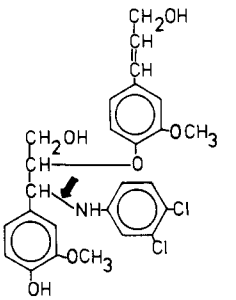

Figure 1. Linkage of a chloroaniline molecule to a segment of the lignin matrix [based on Sandermann et al. (1983)].

EXPERIMENTAL PROCEDURES

Materials. The [^{14}C]chloroaniline/lignin copolymers were prepared and characterized by previously described procedures (Arjmand and Sandermann, 1985). The [^{14}C]-4-chloroaniline/lignin copolymer had a specific activity of 1.6×10^5 dpm/mg. The [^{14}C]-3,4-dichloroaniline/lignin copolymer had a specific activity of 1.5×10^5 dpm/mg and was the same material previously employed to determine fungal bioavailability. The lignin metabolites were stored in dimethylformamide under nitrogen at −20 °C. The copolymers were characterized as macromolecules by gel permeation chromatography in dimethylformamide. Both copolymers had identical elution profiles, the elution diagram of the [^{14}C]-3,4-dichloroaniline/lignin copolymer having already been published (Arjmand and Sandermann, 1985). The previous three different methods of hydrolysis released the free [^{14}C]chloroanilines from the polymers in ≥93% yield [determined by TLC as described Arjmand and Sandermann (1985)].

Chromatographic Procedures. TLC was carried out on precoated silica gel G plates (Merck 5554) using the following solvent systems (parts by volume): A, methylene chloride/methanol, 9:1; B, *n*-hexane/benzene/acetone, 7:3:1; C, ethyl acetate/propanol-2/water, 6:2:1; D, diethyl ether/petroleum ether (30–60 °C), 4:1.

Descending paper chromatography (Whatman 3 MM paper; Shandon 500 tank; solvent system E; 47-cm migration distance of the solvent) was employed to examine for the presence of *N*-glucuronides. Solvent system E contained butanol-1/acetic acid/water, 3:1:1 (parts by volume). Phenyl- and 4-nitrophenyl *O*-β-D-glucuronides were employed as reference compounds and had $R_f \sim 0.6$. The aqueous urine and bile samples were directly applied, or their ethyl acetate extracts were studied. In the case of feces samples, the initial one-phasic organic extract was applied. The urine and bile samples were adjusted to pH ~8.0 by addition of sodium bicarbonate, followed by repeated

* Author to whom correspondence should be addressed.
‡ Present address: Pesticide Research Laboratory, The Pennsylvania State University, University Park, PA 16802.
§ Present address: Enimont America Inc., 2000 Cornwall Road, Monmouth Junction, NJ 08852.

0021-8561/90/1438-1877$02.50/0

extraction with ethyl acetate and TLC in solvent system A. In some cases, TLC spots were eluted with methanol and subjected to GC/MS on a Finnigan 4023 system. A DB-5 column (0.32 mm × 25 m) with a 0.25-μm coating was used at 100 °C with helium (~40 cm/s) as carrier gas. After 1 min at constant 80 °C, a linear temperature gradient up to 250 °C was applied with 8 °C/min.

For two-dimensional TLC, cleanup was first performed (see below). The methanolic eluate containing most of the radioactive material was evaporated at ≤30 °C to near dryness. Aliquots (5 μL) of 10 mg/mL standard solutions in methanol were added. The sample mixture was applied as a single spot to a TLC plate followed by development with solvent system C. The plate was then developed in the second dimension with solvent system D. The standards were localized under UV light. In addition, radioactivity was localized by a two-dimensional scan with the Berthold TLC scanning system.

Cleanup Procedures. Cleanup of the organic fecal extract was performed on a column (1.8 × 30 cm) of silica gel 60 (Merck 7734) packed in *n*-hexane. The organic sample solution was evaporated together with a small portion of the silica gel 60 and applied to the column as a powder. Stepwise elution was performed with 50-mL portions of neat *n*-hexane, 50% ethyl acetate in *n*-hexane, 75% ethyl acetate in *n*-hexane, neat ethyl acetate, 25% methanol in ethyl acetate, 33% methanol in ethyl acetate, 50% methanol in ethyl acetate, and, finally, neat methanol. Most of the radioactivity appeared in the fractions eluted with 33% and 50% methanol in ethyl acetate.

Cleanup could also be performed by adsorbing the sample in 50 μL of methanol to a Sep-Pak C_{18} cartridge (Waters Associates, Inc.; No. 51910), washing with water (20 mL), and eluting with 10-mL portions of 10%-wise increased methanol in water. Most of the nonradioactive impurities were eluted by the initial water and the final neat methanol eluents. The radioactive material was mainly present in the 60–90% aqueous methanol fractions.

Feeding Procedure. Each rat (250–300-g male Sprague-Dawley) was given 4 mg (~0.3 μCi) of the chloroaniline/lignin complex. The dose was placed on the feed, and the dioxane was allowed to evaporate overnight or was removed under vacuum. The rats had been previously trained to eat a 7.5-g meal relatively rapidly. The rats were dosed 24 h after cannulation of the bile duct and duodenum. Procedures for cannulation of rats were as described by Struble et al. (1983), except that the reentry cannula was placed directly in the duodenum and secured with a purse string suture. Previously collected bile was infused into the duodenum at about the rate of flow from the bile duct (0.5–1 mL/h). The rats were held in restraining cages (Bollman, 1948) from 24 h before dosing until sacrificing. Feces and urine were separated by a stainless steel screen. All rats were killed 72 h after dosing. Most of the ^{14}C material had been excreted from the large intestine before the rats were sacrificed. Respired $^{14}CO_2$ or other volatiles were not trapped since the amounts were insignificant. Samples of urine and bile were assayed directly by liquid scintillation. Feces and tissues were homogenized and freeze-dried; a sample was combusted, and the resulting CO_2 was trapped for assay by liquid scintillation (Packard Model 306 oxidizer, Packard Instrument Co., 2200 Warrenville Rd., Downers Grove, IL 60515).

Fractionation of Feces. Feces samples of about 300 mg dry weight were suspended in 3 mL of chloroform/methanol/water, 1:2:0.8 (parts by volume), and were sonicated for 3 × 1 min with a microtip of a Branson B-15 sonifier. The pellet fraction obtained by centrifugation (5 min, 3000*g*) was three times reextracted. The final pellet was dried, and a portion was combusted for determination of radioactivity. The final dry weight was in all cases within 85 ± 5% of the initial feces dry weight. Water (4 mL) was added to the pooled supernatant extracts of the centrifugation steps to induce the formation of a two-phase system. Aliquots of the upper aqueous and the lower organic phases were used for the determination of radioactivity by scintillation counting. Further *n*-hexane extraction of the concentrated organic phase dissolved only ~6% of the radioactivity present, and *n*-hexane extraction was therefore not routinely employed.

Soil and Fungal Degradation. Soil degradation data were obtained by the previously described general procedure (Haider and Kladivko, 1980). Briefly, 30 mg of the lignin metabolite with 1.2–4.5 × 10^6 dpm in 300 μL of dimethylformamide was dispersed in 150 g of Aseler Schwarzerde and 2 g of wheat straw powder, with addition of 37.5 mL of water containing 361 mg of KNO_3 and 61.3 mg of K_2HPO_4. Liberated $^{14}CO_2$ was trapped in 4% (w/v) NaOH and determined every 3–6 days. The experimental procedures for measuring fungal degradation have been described in detail (Arjmand and Sandermann, 1985).

In the case of the white-rot fungus and the soil system, control experiments were performed with a nonxenobiotic lignin carrying a [ring-U-^{14}C]coniferyl alcohol label [cf. Arjmand and Sandermann (1985)].

Table I. Distributions of ^{14}C after Orally Feeding [ring-U-^{14}C]Chloroaniline/Lignin Copolymers to Rats

	% of ^{14}C in					
rat	feces	urine	bile	GI tract	carcass	total recovery
	[^{14}C]-4-Chloroaniline/Lignin Metabolite					
1	83.0	9.0		0.2	1.7	93.9
2	77.6	7.9		0.2	1.7	87.4
3	84.0	6.8	8.4	0.1	0.7	100.0
4	80.9	6.9	8.5	0.1	0.7	97.1
	[^{14}C]-3,4-Dichloroaniline/Lignin Metabolite					
5	86.6	6.8	10.3	0.1	0	98.7
6	82.1	6.3	11.1	0	0	99.6
7	87.7	9.3		0	0	97.0
8	88.5	9.1		0	0	97.7

Table II. Distributions of ^{14}C between Fecal Fractions[a]

	% of initial ^{14}C in			
sample	aqueous phase	organic phase	final residue	total recovery
	[^{14}C]-4-Chloroaniline/Lignin Complex			
0–24 h feces	11	48	40	99
24–48 h feces	7	57	38	102
	[^{14}C]-3,4-Dichloroaniline/Lignin Complex			
0–24 h feces	6	50	39	94
24–48 h feces	9	56	30	95

[a] A total of 30 extractions were carried out with feces samples coming from the various rat experiments of Table I with or without bile cannula treatment. The exact solvent ratios and homogenization conditions were varied, but variability of radioactivity values was in all cases within a range of ≤15% of the values shown.

RESULTS

Determination of Bioavailability. Male albino rats were used for bioavailability studies. Prior to feeding, the polymeric nature of the sample was reexamined by TLC (solvent system B). Polymeric material remained at the origin, whereas degradation products were mobile. The ^{14}C percentages with $R_f \leq 0.1$ were ~92% in both cases.

The distributions of radioactivity 3 days after dosing normal or bile duct cannulated rats are shown in Table I. The urinary and fecal excretion rates were comparable to those reported in the only available similar study previously published (Sutherland, 1976). In that case, bound [ring-U-^{14}C]-3,4-dichloroaniline/propanil residues from rice plants had been fed to rats. Rat bile was not examined in the previous study, although less than 0.05% of the ^{14}C dose appeared in the bile of dogs and mice (Sutherland, 1976). In contrast, bile fluid in the present experiments contained ~10% of the applied ^{14}C dose (Table I). In the previous study (Sutherland, 1976) the feces fractions had not been further characterized. In the present study, a chloroform/methanol/water extraction was performed and led in all experiments to solubilization of ~60% of the fecal radioactivity (Table II). Bioavailability calculated as the sum of radioactivity excreted into bile, urine, and the soluble portion of feces amounted

Table III. Bioavailability of [ring-U-^{14}C]Chloroaniline/Lignin Metabolites in the Rat, in the White-Rot Fungus (*P. chrysosporium*), and in a Soil System[a]

	rat (3 days)			fungus (27 days)			soil (90 days)	
	4-CA	DCA		4-CA	DCA		4-CA	DCA
feces, soluble metabolites	50	51	volatiles	2.4	1.4	carbon dioxide	0.5	0.2
urine	6.8	6.5	soluble metabolites	38.6	29.7			
bile	8.4	10.7	carbon dioxide	57.8	64.1			
sum	65.2	68.2		98.8	95.2		0.5	0.2

[a] All presently available data are summarized and originate from the following sources: rat data from the present Tables I and II; fungal data from Arjmand and Sandermann (1985); soil data from Haider et al. (1985). Abbreviations: 4-CA, 4-chloroaniline; DCA, 3,4-dichloroaniline.

to ~66% of the applied radioactivity of the two metabolites tested.

Metabolite Characterization in Urine and Bile. The preliminary chemical characterization of the excreted materials was done as follows. Attempts to localize *N*-glucuronide conjugates by PC were negative with all excreted fractions. In each case examined, ≥95% of the radioactivity migrated near the solvent front, together with the free chloroaniline and chloroacetanilide standards. Chemical decomposition of the *N*-glucuronides in the acidic solvent system used could, however, not be excluded. The acid sensitivity of *N*-glucosides of chlorinated anilines has recently been described (Winkler and Sandermann, 1989). The known *N*-acetyl conjugates could be located in urine and bile by TLC and GC/MS. Ethyl acetate extracts of the urine and bile samples originating from the [ring-U-^{14}C]-3,4-dichloroaniline copolymer were studied by TLC in solvent systems A–D. About 60% of the radioactivity in the organic extract from urine and ~30% of the radioactivity in the organic extract from bile cochromatographed with the 3,4-dichloroacetanilide standard. The corresponding TLC spot of the bile sample was studied by GC/MS. At the retention time of the 3,4-dichloroacetanilide standard (9.20 min) a typical mass spectrum with the parent ion at m/z 203 and parent ion minus ketene at m/z 161 was obtained with the metabolite sample. The chloroacetanilides appeared to predominate in urine, but several additional TLC peaks were also present and predominated in the bile fractions. One of these components in bile cochromatographed with the predominant component in feces.

Metabolite Characterization in Feces. TLC analysis of the organic fecal extracts of Table II required prior cleanup due to the presence of high amounts of nonradioactive material. Most of the soluble fecal radioactivity appeared to be present in a single component as documented here by two-dimensional TLC (Figure 2). The major portion of the soluble fecal radioactivity was in all cases further differentiated from the free chloroanilines and the chloroacetanilides by TLC in solvent systems A–C for which R_f values of various chloroaniline reference compounds have been reported (Winkler and Sandermann, 1989). A great number of soluble aniline-derived metabolites are known as animal urinary excretion products (Parke, 1960; Boyland et al., 1963; Kao et al., 1978). Due to the extremely low absolute metabolite amounts available, chemical identification of the present rat excretion products has not been possible.

DISCUSSION

Comparison between Animal, Fungal, and Soil Bioavailability. The [ring-U-^{14}C]chloroaniline/lignin metabolites tested above in rats have also been studied in a standardized soil system and in cultures of the white-rot fungus *P. chrysosporium*. All bioavailability results obtained so far are summarized in Table III. The rat

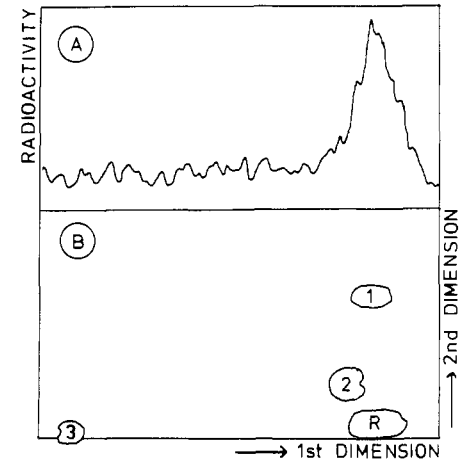

Figure 2. Two-dimensional TLC of the soluble portion of rat feces obtained after feeding the [^{14}C]-4-chloroaniline/lignin metabolite. The following reference compounds were examined: (1) free 4-chloroaniline; (2) 4-chloroacetanilide; (3) 4-nitrophenyl *O*-β-D-glucuronide. A radioactivity scan of the sample lane is shown in panel A. The positions of the reference compounds 1–3 and of radioactivity (R) are shown in panel B.

formed a total of ~66% soluble metabolites, whereas this figure was ~33% in the case of the fungus. However, the fungus also formed ~60% [^{14}C]carbon dioxide, so total fungal bioavailability of the chloroaniline/lignin metabolites amounted to 95–99%. In soil, a mere 0.5% of the initial radioactivity was liberated as [^{14}C]carbon dioxide after 90 days.

The fungus was unusual in its high capacity for complete mineralization and in making no difference between the ring-U-^{14}C label being in either the xenobiotic chloroaniline or the natural lignin monomer. In the soil system, mineralization was much higher in the latter case, and 31.2% of the initial ^{14}C label was trapped as carbon dioxide over the time interval of 90 days. A major soluble metabolite formed by the white-rot fungus has been identified as a novel succinimide conjugate (Arjmand and Sandermann, 1987). The fungal mineralization process has recently been shown to be strictly inducible and independent of the extracellular ligninase complex whose formation is also inducible (Winkler and Sandermann, 1990). Mineralization in the soil system was very low for the [ring-U-^{14}C]-4-chloroaniline/lignin metabolite, and mineralization was even lower when a second chlorine substituent was present. When the ring-U-^{14}C label was in the natural lignin monomer, mineralization was dramatically increased. In summary, Table III illustrates remarkable differences between the three systems studied.

Bioavailability in the Rat. Contrary to what one might expect from the literature (Pillmoor and Roberts, 1985; Harvey, 1983; Khan and Dupont, 1987; Akhtar, 1987; Edwards and Hutson, 1986), the two defined xenobiotic lignin metabolites tested were to a high degree solubilized in the digestive tract of the rat. One might speculate

that the chloroaniline/lignin complex was cleaved in the cecum or large intestine where bacteria are most active and that absorption may have been less efficient in that part of the intestine. Absorption of the soluble ^{14}C material possibly could have occurred in other sections of the gastrointestinal tract if coprophagy had been permitted.

It has been proposed that the bioavailability of the radiolabel in bound residues will be highly dependent on the digestibility of the natural polymer matrix (Edwards and Hutson, 1986). However, the structure shown in Figure 1 indicates that much of the xenobiotic may be bound as a simple substituent rather than an integral component of the polymer matrix. Cleavage of a single bond (arrow in Figure 1) may suffice to liberate the xenobiotic from the matrix material. Sequential approaches have been suggested to determine the significance of bound pesticide residues in plants (Huber and Otto, 1983; Kovacs, 1986). On the basis of these literature criteria, the present results indicate for the first time that bound pesticidal residues may possess ecotoxicological significance in animals. This conclusion has not been drawn in previous reviews (Pillmoor and Roberts, 1985; Harvey, 1983; Khan and Dupont, 1987; Akhtar, 1987; Edwards and Hutson, 1986).

ACKNOWLEDGMENT

We thank Dr. K. Haider, FAL Braunschweig, for contributing the soil degradation data. We gratefully acknowledge the excellent technical assistance of E. Mattes and the GC/MS determination of L. Lattmann, GSF-Institut für Ökologische Chemie. This work has been supported by the Bundesministerium für Forschung und Technology (Project 0386573), the Deutsche Forschungsgemeinschaft (Project Sa 180/15-5), and the Fonds der Chemischen Industrie.

LITERATURE CITED

Akhtar, M. H. The disposition of bound ^{14}C-agrochemical residues in plants and animal tissues when consumed by animals. In *Pesticide Science and Technology*; Greenhalgh, R., Roberts, T. R., Eds.; Blackwell Scientific Publications: Oxford, U.K., 1987; pp 509–512.

Arjmand, M.; Sandermann, H. Mineralization of chloroaniline/lignin conjugates and of free chloroanilines by the white-rot fungus Phanerochaete chrysosporium. *J. Agric. Food Chem.* **1985**, *33*, 1055–1060.

Arjmand, M.; Sandermann, H. Plant biochemistry of xenobiotics. Mineralization of chloroaniline/lignin metabolites from wheat by the white-rot fungus, Phanerochaete chrysosporium. *Z. Naturforsch.* **1986**, *41C*, 206–214.

Arjmand, M.; Sandermann, H. N-(Chlorophenyl)-succinimides: A novel metabolite class isolated from Phanerochaete chrysosporium. *Pestic. Biochem. Physiol.* **1987**, *27*, 173–181.

Bollman, J. L. A cage which limits the activity of rats. *J. Lab. Clin. Med.* **1948**, *33*, 1348.

Boyland, E.; Manson, D.; Nery, R. The biochemistry of aromatic amines. 9. Mercapturic acids as metabolites of aniline and 2-naphthylamine. *Biochem. J.* **1963**, *86*, 263–271.

Edwards, V. T.; Hutson, D. H. The disposition of plant xenobiotic conjugates in animals. In *Xenobiotic Conjugation Chemistry*; Paulson, G. D., Caldwell, J., Hutson, D. H., Menn, J. J., Eds.; American Chemical Society: Washington, DC, 1986; pp 322–340.

Freudenberg, K. In *Constitution and Biosynthesis of Lignin*; Freudenberg, K., Neish, A. C., Eds.; Springer-Verlag: Berlin, 1968; pp 45–122.

Haider, K.; Kladivko, E. J. Transformation of [^{14}C] and [^{35}S]-labeled lignosulfonates during soil incubation. *Soil Biol. Biochem.* **1980**, *12*, 275–279.

Haider, K.; Arjmand, M.; Sandermann, H., FAL Braunschweig und Universität Freiburg, 1985, unpublished studies.

Harvey, J. The fate of pesticide plant metabolites in vertebrates. In *Pesticide Chemistry. Human Welfare and the Environment*; Miyamoto, J., Kearney, P. C., Eds.; Pergamon Press: Oxford, U.K., 1983; Vol. 3, pp 369–374.

Huber, R.; Otto, S. Bound pesticide residues in plants. In *Pesticide Chemistry. Human Welfare and the Environment*; Miyamoto, J., Kearney, P. C., Eds.; Pergamon Press: Oxford, U.K., 1983; Vol. 3, pp 357–362.

Kao, J.; Faulkner, J.; Bridges, J. W. Metabolism of aniline in rats, pigs and sheep. *Drug Metab. Dispos.* **1978**, *6*, 549–555.

Khan, S. U.; Dupont, S. Bound pesticide residues and their bioavailability. In *Pesticide Science and Technology*; Greenhalgh, R., Roberts, T. R., Eds.; Blackwell Scientific Publications: Oxford, U.K., 1987; pp 417–420.

Kovacs, M. F. Regulatory aspects of bound residues (chemistry). *Residue Rev.* **1986**, *97*, 1–17.

Parke, D. V. Studies in detoxication. 84. The metabolism of [^{14}C]aniline in the rabbit and other animals. *Biochem. J.* **1960**, *77*, 493–503.

Pillmoor, J. B.; Roberts, T. R. Approaches to the study of non-extractable (bound) pesticide residues in plants. *Prog. Pestic. Biochem. Toxicol.* **1985**, *4*, 85–101.

Sandermann, H.; Scheel, D.; v.d. Trenck, K. T. Metabolism of environmental chemicals by plants-copolymerization into lignin. *J. Appl. Polym. Sci., Appl. Polym. Symp.* **1983**, *37*, 407–420.

Schäfer, W.; Sandermann, H. Metabolism of pentachlorophenol in cell suspension cultures of wheat (Triticum aestivum L.). Tetrachlorocatechol as a primary metabolite. *J. Agric. Food Chem.* **1988**, *36*, 370–377.

Still, G. G.; Balba, H. M.; Mansager, E. R. Studies on the nature and identity of bound chloroaniline residues in plants. *J. Agric. Food Chem.* **1981**, *29*, 739–746.

Struble, C. B.; Pekas, J. C.; Gerst, J. W. Enterohepatic circulation and biliary secretion of 5,6-dihydro-5,6-dihydroxy-^{14}C-carbaryl glucuronide in the rat. *Pestic. Biochem. Physiol.* **1983**, *19*, 95–103.

Sutherland, N. L. In *Bound and Conjugated Pesticide Residues*; Kaufman, D. D., Still, G. G., Paulson, G. D., Bandal, S. K., Eds.; American Chemical Society: Washington, DC, 1976; pp 153–155.

v.d. Trenck, K. T.; Hunkler, D.; Sandermann, H. Incorporation of chlorinated anilines into lignin. *Z. Naturforsch.* **1981**, *36C*, 714–720.

v.d. Trenck, K. T.; Sandermann, H. Incorporation of benzo(*a*)-pyrene quinones into lignin. *FEBS Lett.* **1981**, *125*, 72–76.

Winkler, R.; Sandermann, H. Plant metabolism of chlorinated anilines: Isolation and identification of N-glucosyl and N-malonyl conjugates. *Pestic. Biochem. Physiol.* **1989**, *33*, 239–248.

Winkler, R.; Sandermann, H. Mineralization of 3,4-dichloroaniline by the white-rot fungus, Phanerochaete chrysosporium. *Abstract Book*, IUPAC—Congress on Pesticide Chemistry, Hamburg, 1990, in press.

Zeikus, J. G. Lignin metabolism and the carbon cycle. Polymer biosynthesis, biodegradation, and environmental recalcitrance. *Adv. Microb. Ecol.* **1981**, *5*, 211–243.

Received for review December 12, 1989. Revised manuscript received March 27, 1990. Accepted April 20, 1990.

Registry No. 4-Chloroaniline/lignin copolymer, 98541-31-2; 3,4-dichloroaniline/lignin copolymer, 77716-53-1.