2,4-Dichlorophenoxyacetic Acid Disposition after Oral Administration in Channel Catfish

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The pharmacokinetics, tissue distribution, and excretion of 2,4-dichlorophenoxyacetic acid (2,4-D) were examined in channel catfish after intravascular or oral administration (10 mg/kg of body weight). Plasma concentrations of parent 2,4-D exhibited a rapid, biphasic decline after intravascular administration with an elimination half-life of 0.76 h. After oral dosing, 2,4-D was rapidly and extensively absorbed; the absorption half-life was 1.5 h, and the bioavailability was 86%. Residue concentrations were highest in the excretory tissues, particularly in the trunk kidney; the muscle had the lowest concentrations (<1 ppm) of any tissue analyzed. Residues were not found in most tissues at 24 h. Renal excretion was the dominant route of elimination; nearly 90% of the administered dose of 2,4-D was excreted unchanged in the urine in 24 h. Less than 1% of the dose was eliminated in the bile, mostly as polar metabolites. Although 2,4-D is extensively absorbed, the limited tissue distribution and rapid renal excretion result in a low potential for accumulation in the edible portions of channel catfish.

INTRODUCTION

The chlorophenoxy compound 2,4-dichlorophenoxyacetic acid (2,4-D) is one of the most widely used herbicides in the United States. 2,4-D is currently used in aquatic environments, including aquaculture ponds, for the control of undesired vegetation. Aquatic animals may be exposed to 2,4-D via water or diet. The disposition of 2,4-D after parenteral administration has been studied in several freshwater and marine animals, including spiny lobster (James, 1982), rainbow trout (Carpenter and Eaton, 1983), winter flounder (Pritchard and James, 1979), and spiny dogfish (Guarino et al., 1977). In the channel catfish, the uptake and elimination of residues have been examined after exposure to ester and amine salt formulations of 2,4-D in water (Schultz, 1973; Rodgers and Stalling, 1972; Sikka et al., 1977). However, the fate of 2,4-D after dietary exposure has not been studied. In mammalian species, the oral absorption of phenoxy acid herbicides is rapid and extensive (Arnold and Beasley, 1989).

Knowledge of the accumulation, nature, and persistence of xenobiotics in aquatic animal tissues is useful in assessing the potential for human and wildlife exposures. Pharmacokinetic analyses also provide information on the persistence of residues. This information is then used in the design of monitoring programs. In the present study, the pharmacokinetics, tissue distribution, and excretion of 2,4-D were examined after oral administration in the channel catfish. The persistence of residues and the potential for accumulation in the edible flesh were evaluated.

EXPERIMENTAL METHODS

Chemicals and Dosing Solution. [*ring*-U-^{14}C]-2,4-D (specific activity, 13.2 μCi/μmol; radiochemical purity, >99%) and unlabeled 2,4-D were obtained from Dow Chemical Co. (Midland, MI). The dosing solution was prepared by solubilizing unlabeled 2,4-D in 1% sodium carbonate. The solution was then adjusted to pH 7.6, mixed with ^{14}C-labeled 2,4-D, and diluted to yield a final concentration of 10 mg/mL (18 μCi/mL).

Animals, Dosing, and Sampling. Channel catfish (0.5–0.9-kg body weight) were obtained from the Southeastern Fish Cultural Laboratory, U.S. Fish and Wildlife Service (Marion, AL). Fish were individually distributed and acclimated in 90-L aquaria equipped with activated carbon filters. The water temperature was 22 °C, and the pH was 8.0.

Fish were anesthetized with MS222 (ethyl *m*-aminobenzoate, Sigma Chemical Co., St. Louis, MO) during all surgical procedures. Before dosing, several fish were cannulated in the dorsal aorta and allowed to acclimate overnight, as previously described (Plakas et al., 1992). We dosed four fish (10 mg/kg; 18 μCi/kg) intravascularly via the cannulae or orally with gelatin capsules. The intravascular doses were immediately followed by 1 mL/kg saline with subsequent rinsing of the cannulae with blood. After intravascular or oral dosing, blood was serially collected via the cannulae and the plasma was obtained by centrifugation. Plasma was analyzed for parent 2,4-D and its metabolites by reversed-phase high-performance liquid chromatography (HPLC).

Additional fish were catheterized for the collection of urine (Plakas et al., 1992) before oral dosing. Urine was collected over a 24-h period and analyzed for total radioactive residues by liquid scintillation counting (LSC). Four or five animals were euthanized at 2, 4, 8, and 24 h, and the following tissues and fluids were analyzed for total residues by LSC: bile, plasma, liver, trunk (posterior) kidney, head (anterior) kidney, spleen, and muscle. We determined the distribution of radioactive residues in the urine and bile as parent 2,4-D and its metabolites by reversed-phase HPLC and LSC.

Liquid Scintillation Analysis. Total radioactivity in the tissues (0.1–0.25-g sample size) was determined in duplicate by tissue solubilization and LSC (Plakas and James, 1990). Plasma (0.1 mL), urine (0.2 mL), and bile (0.025 mL) specimens were analyzed with an Ultima Gold LSC solution (Packard Instruments Co., Downers Grove, IL). Samples were counted for 4 min. The limit of determination in the tissues was approximately 0.2 ppm (3× background counts).

HPLC Analysis. Plasma specimens (0.2 mL) were vortex mixed three times with 1 mL of acetone containing 1% H_3PO_4, followed by centrifugation. Recovery of total radioactivity in the combined supernatant was >99%. The combined supernatant was evaporated under vacuum, and the residue was solubilized in 0.5 mL of HPLC mobile phase before analysis. Urine specimens were directly analyzed by HPLC, whereas bile specimens were first diluted 1:4 with deionized water.

Parent 2,4-D and metabolites in plasma and fluid specimens were separated by HPLC with a Waters RCM 100 (C_{18}) column (Milford, MA). The mobile phase was 55% CH_3OH/45% H_2O

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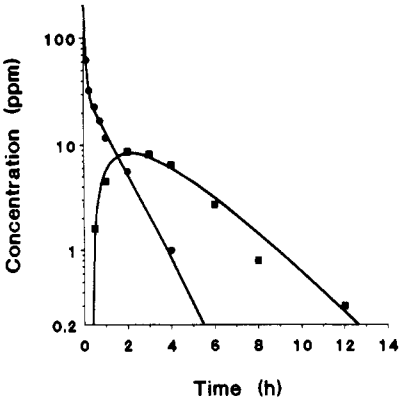

Figure 1. Plasma concentrations of parent compound after intravascular (●) or oral (■) administration (10 mg/kg) of ^{14}C-labeled 2,4-dichlorophenoxyacetic acid in channel catfish.

with H_3PO_4 added at 1 mL/L. The flow rate was 2 mL/min, and the injection loop volume was 0.2 mL. The ultraviolet detector was set at 215 nm, and we used a Berthold radioactivity monitor (Nashua, NH) with solid scintillant. In some instances, the HPLC effluent was diverted to a fraction collector and the fractions (taken at 20-s intervals) were analyzed by LSC.

To determine the presence of glucuronic acid conjugates, we incubated bile specimens at 22 °C with β-glucuronidase (type VII, Sigma) at pH 6.8 in 4 mM phosphate buffer. Enzyme-treated (24-h incubation) and untreated bile specimens were analyzed by HPLC.

Plasma Protein Binding. Plasma protein binding of 2,4-D was determined by ultrafiltration with Centrifree micropartition system ultrafilters (Amicon Corp., Danvers, MA). Control plasma was spiked with ^{14}C-labeled 2,4-D at concentrations ranging from 2.5 to 800 ppm. The spiked plasma specimens (0.2 mL) were applied to the ultrafilters and centrifuged for 30 min at 1000*g*. The entire ultrafiltrate was analyzed by LSC. Binding values were corrected for nonspecific absorption of 2,4-D to the ultrafiltration apparatus; absorption was determined with spiked plasma ultrafiltrate.

Pharmacokinetics. Plasma concentrations of 2,4-D after intravascular and oral dosing were modeled with the nonlinear, least-squares program PCNONLIN (Statistical Consultants, Inc., Lexington, KY). Plasma data after intravascular dosing were well described by a two-compartment pharmacokinetic model. Concepts of compartmental modeling and calculation of pharmacokinetic values have been previously described (Barron et al., 1990). Bioavailability was calculated by comparison of the areas under the plasma concentration–time curves (AUC) for intravascular and oral routes of adminstration.

RESULTS AND DISCUSSION

Pharmacokinetics. After intravascular dosing, plasma concentrations of parent 2,4-D exhibited a rapid, biphasic decline (Figure 1). No metabolites of 2,4-D were found in the plasma of treated animals. The plasma concentrations of parent 2,4-D (C_p) over time (t) were well described by the following biexponential equation:

$$C_p = 169e^{-13.1t} + 33.3e^{-0.917t}$$

Pharmacokinetic values derived from the above model are presented in Table I. The apparent volumes of distribution of the central (49 mL/kg) and peripheral (118 mL/kg) compartments indicated limited extravascular distribution of 2,4-D. Plasma and extracellular fluid volumes of 28 and 183 mL/kg, respectively, have been estimated in channel catfish (Kitzman et al., 1990). On the basis of the pharmacokinetics (i.e., volumes of distribution) and tissue residue data, distribution of 2,4-D appeared to have been largely confined to the extracellular fluids and excretory tissues.

Table I. Pharmacokinetic Values for 2,4-Dichlorophenoxyacetic Acid (2,4-D) after Intravascular Dosing in Channel Catfish

parameter[a]	value	parameter[a]	value
$t_{\alpha 1/2}$, h	0.05	V_{ss}, mL/kg	167
$t_{\beta 1/2}$, h	0.76	Cl_b, mL h^{-1} kg^{-1}	203
V_1, mL/kg	49	Cl_t, mL h^{-1} kg^{-1}	344
V_2, mL/kg	118		

[a] Abbreviations are as follows: $t_{\alpha 1/2}$ and $t_{\beta 1/2}$ are the half-lives for the distribution and elimination phases, respectively; V_1 and V_2 are the apparent volumes of distribution of the central and peripheral compartments, respectively; V_{ss} is the apparent volume of distribution at steady state; Cl_b and Cl_t are the total body and intercompartmental clearances, respectively.

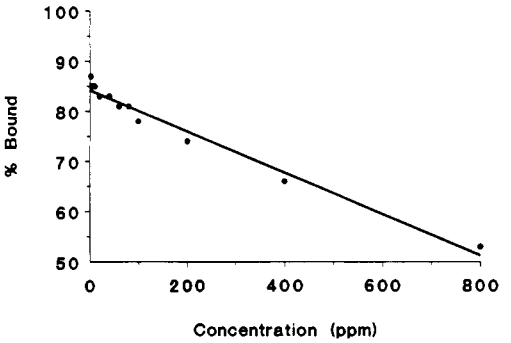

Figure 2. Plasma protein binding (% bound) of 2,4-dichlorophenoxyacetic acid at various plasma concentrations in channel catfish.

After oral administration, 2,4-D was rapidly and extensively absorbed in catfish (Figure 1). On the basis of the pharmacokinetic model, peak plasma concentration (8.5 ppm) occurred at 2.2 h with an absorption half-life of 1.5 h. The oral bioavailability of 2,4-D was 86%. Phenoxy acids are readily absorbed from the gastrointestinal tract in a variety of mammalian species (Arnold and Beasley, 1989). In humans, peak plasma concentrations (ca. 25 ppm) of 2,4-D were observed 4 h after oral dosing (5 mg/kg); on the basis of the renal excretion data, absorption of the oral dose was nearly complete (Sauerhoff et al., 1977). Ester formulations of 2,4-D are not as well absorbed as amine or alkali salt formulations (Erne, 1966).

In contrast with its extensive oral absorption, 2,4-D is poorly absorbed by other routes of exposure. After exposure of bluegills and channel catfish to 2,4-D (1 mg/L) in water for 120 h, residues were not detectable (<0.01 ppm) in the whole body or individual tissues (Rodgers and Stalling, 1972). Bioconcentration also was low during exposure of fish to the dimethylamine salt of 2,4-D (Schultz, 1973; Sikka et al., 1977). The nonpolar butoxyethanol ester of 2,4-D is much more highly absorbed (Rodgers and Stalling, 1972). In mammals, dermal absorption of 2,4-D is quite low; approximately 10% of a dermal dose of 2,4-D dimethylamine salt became systemically available in rats in 72 h (Pelletier et al., 1989).

Plasma Protein Binding. Plasma protein binding of 2,4-D was high, but exhibited saturability (Figure 2). Binding values (percent bound) declined from 87% to 54% as the spiked plasma concentration was increased from 2.5 to 800 ppm; however, the latter concentration was 10-fold greater than the highest concentration measured in the plasma of experimental animals. At 80 ppm, 2,4-D was 80% bound.

In spiny dogfish (Guarino et al., 1977) and winter flounder (Pritchard and James, 1979), plasma protein binding values of 58% and 70%, respectively, have been reported. In mammals (e.g., humans), chlorinated phen-

Table II. Residue Concentrations[a] [in 2,4-Dichlorophenoxyacetic Acid (2,4-D) Equivalents] after Oral Administration of ^{14}C-Labeled 2,4-D (10 mg/kg) in Channel Catfish

	residue concentrations, ppm, at			
tissue or fluid	2 h	4 h	8 h	24 h
bile	4.0 ± 3.2	5.6 ± 2.2	5.4 ± 1.7	15.8 ± 2.2
head kidney	4.7 ± 2.2	4.4 ± 2.9	–[b]	–[b]
liver	11.7 ± 3.9	9.5 ± 5.6	0.7 ± 0.7	0.2 ± 0.1
muscle	0.6 ± 0.2	0.6 ± 0.3	–[b]	–[b]
plasma	8.7 ± 2.4	6.5 ± 2.7	0.8 ± 0.3	–[b]
spleen	4.1 ± 3.2	6.0 ± 3.9	–[b]	–[b]
trunk kidney	15.7 ± 4.8	12.4 ± 6.9	1.3 ± 2.0	0.2 ± 0.1

[a] Mean ± SD for four animals per sampling time. [b] Below the limit of determination (0.2 ppm).

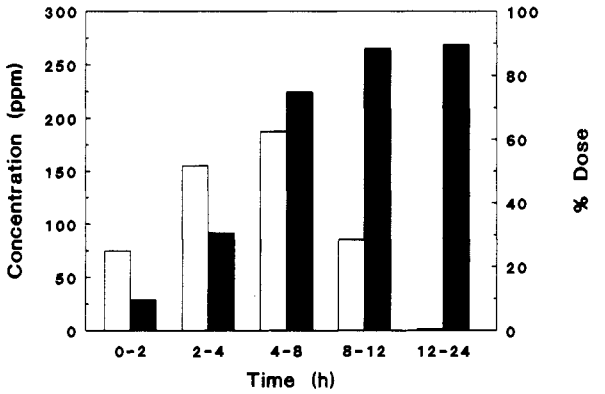

Figure 3. Concentration of 2,4-dichlorophenoxyacetic acid (open bars) and cumulative percentage of the dose excreted (black bars) in the urine after oral administration (10 mg/kg) in channel catfish.

oxy acids are extensively bound to plasma proteins (Arnold and Beasley, 1989). Saturability of plasma protein binding sites for 2,4-D has been demonstrated in the goat; binding was approximately 97% at concentrations ranging from 0.2 to 20 ppm but decreased to less than 70% at 1000 ppm (Orberg, 1980).

Tissue Distribution. After oral administration, tissue residue concentrations were highest at the 2-h sampling time (Table II). Higher concentrations of 2,4-D residues accumulated in the excretory organs (i.e., trunk kidney and liver) relative to the plasma. The trunk kidney had the highest concentrations (15.7 ppm) of any tissue analyzed and the muscle the lowest (0.6 ppm). Muscle residues were below the limit of determination (<0.2 ppm) 8 h after dosing. At 24 h, residues were found only in the excretory tissues. The residue data were consistent with the pharmacokinetic analysis that indicated limited tissue distribution of 2,4-D. Similar patterns in the tissue distribution of 2,4-D have been observed in other fish species. In both the winter flounder (Pritchard and James, 1979) and spiny dogfish (Guarino et al., 1977), only the kidney and liver had tissue:plasma concentration ratios greater than 1:1; the muscle consistently had the lowest concentrations of any tissue analyzed.

Excretion and Metabolism. Residues of 2,4-D were almost entirely eliminated by renal excretion; approximately 90% of the oral dose was excreted unchanged in the urine in 24 h (Figure 3). Trace amounts (<1% of the total radioactivity) of an unidentified polar metabolite were found in the urine at the earlier (up to 4 h) sampling times. Renal excretion of 2,4-D was nearly complete at 12 h. The mean urinary concentration of 2,4-D was highest (187.5 ppm) at the 4–8-h collection interval. The urine (4–8 h) to plasma (6 h) concentration ratio was approximately 70:1.

Table III. Comparative Metabolism of 2,4-Dichlorophenoxyacetic Acid (2,4-D) in Aquatic Species

	% urinary residues present as		
animal	free acid	taurine conjugate	other
channel catfish[a]	100		
spiny lobster[b]	100		
winter flounder[c]	90	9	1
southern flounder[d]	50	50	
spiny dogfish[e]	1	98	1

[a] Present study. Oral dosing at 10 mg/kg. [b] James (1982). Intrapericardial dosing at 10 mg/kg. [c] Pritchard and James (1979). Intramuscular dosing at 0.55 or 5.5 mg/kg. [d] James (1986). Intramuscular dosing at 10 mg/kg. [e] Guarino et al. (1977). Intravenous dosing at 1 mg/kg.

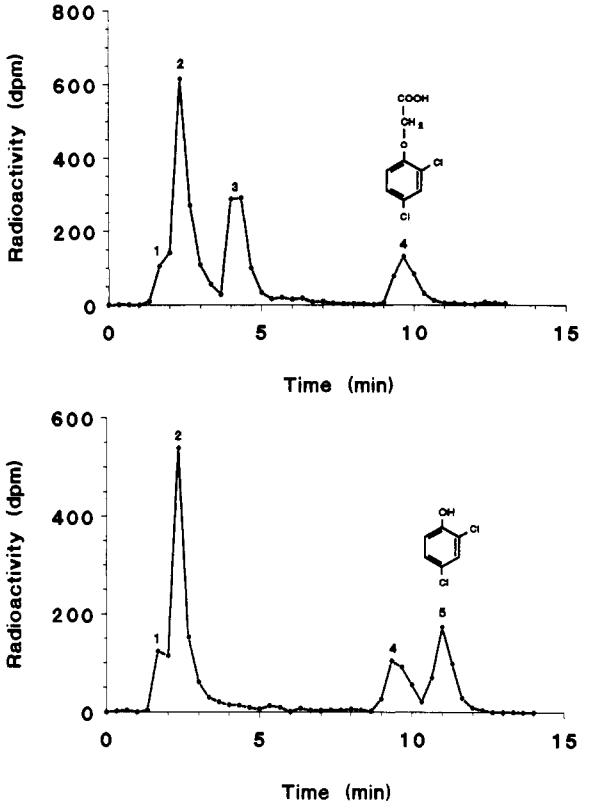

Figure 4. Representative chromatograms of the bile before (top) and after (bottom) treatment with β-glucuronidase. Peak 4 is parent 2,4-dichlorophenoxyacetic acid. Peaks 1 and 2 are unidentified polar metabolites. Polar metabolite 3 was tentatively identified as the glucuronide conjugate of dichlorophenol.

The rapid and extensive renal excretion of 2,4-D has been reported in a variety of mammalian and fish species (Arnold and Beasley, 1989; Guarino et al., 1977; Pritchard and James, 1979; Carpenter and Eaton, 1983). However, the extent of metabolism and rate of excretion may vary considerably. In most mammalian species examined, including humans, 2,4-D is primarily eliminated as the unchanged molecule (Arnold and Beasley, 1989). In contrast, some fish species (e.g., southern flounder and spiny dogfish) excrete considerable amounts of the taurine conjugate of 2,4-D; however, in studies with aquatic animals, various routes of administration have been utilized which may have influenced the extent of metabolism and rate of excretion (Table III). In general, 2,4-D is more rapidly excreted in fish than in mammals probably because of differences in renal physiology (Carpenter and Eaton, 1983; Pritchard and James, 1979).

Biliary excretion was a minor route of elimination of 2,4-D residues (<1% of the oral dose in 24 h). At 2 and 4 h, the concentrations in the bile were less than those in

the plasma (Table II); the highest concentration measured in the bile was 15.8 ppm (24 h). In contrast with urine, the bile contained mostly metabolites of 2,4-D; parent 2,4-D made up <10% of the total residues eliminated by this route (Figure 4). β-Glucuronidase treatment of bile indicated the presence of a glucuronide conjugate. The compound liberated by enzyme treatment was tentatively identified as dichlorophenol on the basis of its coelution with an analytical standard.

In summary, 2,4-D was extensively absorbed after oral dosing in catfish; however, residues were largely confined to the plasma and excretory tissues. Irrespective of the high dosage, concentrations of 2,4-D in the edible flesh never exceeded the established tolerance (1 ppm) and were rapidly eliminated. Renal excretion of the unchanged compound accounted for the majority of the administered dose. Residues of 2,4-D and metabolites were accumulated in the bile; however, the roles of biliary excretion and metabolism in the clearance of 2,4-D were insignificant.

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