Evaluation of Renal Function in Rhesus Monkeys and Comparison to Beagle Dogs Following Oral Administration of the Organic Acid Triclopyr (3,5,6-Trichloro-2-pyridinyloxyacetic Acid)

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The current study evaluated the effects of triclopyr (3,5,6-trichloro-2-pyridinyloxyacetic acid) on renal function following oral administration in the beagle dog and rhesus monkey. Male rhesus monkeys were orally administered triclopyr by gavage at a dose of 5 mg/kg/day, 7 days/week for 28 days, after which the dosage was increased to 20 mg/kg/day for 102 consecutive days. Groups of male dogs were administered either a single oral dose of 5 mg/kg triclopyr or were fed a diet spiked with triclopyr at a dose of 5 mg/kg/day for 47 consecutive days. The following functional and clinical chemistry parameters were evaluated: exogenous phenolsulfonphthalein (PSP) excretion, inulin and para-aminomaleicure (PAH) clearance (monkeys only), endogenous serum creatinine, and blood urea nitrogen (BUN) at multiple time points during the study. Creatinine, BUN, and inulin clearance were within the normal range from both species following triclopyr administration which indicates that repeated administration of triclopyr in the beagle dog and rhesus monkey had no effect on glomerular filtration rate (GFR). Therefore, the no-observed-adverse-effect level (NOAEL) was considered to be 5 mg/kg/day, which was 10 fold greater than the observed NOEL in this same study. In comparison, female rhesus monkeys demonstrated no treatment-related changes were interpreted to be a reversible physiological adaptation associated with excretion of the test material. Therefore, the no-observed-adverse-effect level (NOAEL) was considered to be 5 mg/kg/day, which was 10 fold greater than the observed NOEL in this same study. In comparison, female rhesus monkeys demonstrated no treatment-related effects when administered triclopyr acid by gavage at doses of 0, 0.5, 2.5, and 5 mg/kg/day; the kidney was identified as a target organ (1988, unpublished report of the Dow Chemical Co., Midland, MI). The NOEL for the chronic dog study was 0.5 mg/kg/day. The kidney effects observed at 2.5 and 5 mg/kg/day were characterized by a slight reduction in phenolsulfonphthalein (PSP) excretion, slight increases in BUN and creatinine, and an increase in normally occurring pigment in the proximal tubules of the kidneys. These minor changes were interpreted to be a reversible physiological adaptation associated with excretion of the test material.

Triclopyr (3,5,6-trichloro-2-pyridinyloxyacetic acid) is the active ingredient in Garlon® herbicides and is used in the control of a wide variety of broad leaf and woody plants. Triclopyr is an organic acid which has a moderate acute toxicity; oral LD50 in rats is 713 mg/kg (Worthing, 1987). Subchronic (13 week) dietary administration of 5–250 mg/kg/day of triclopyr resulted in slight degeneration of the descending proximal tubules (S3 segment) of the kidneys in rats given 20 mg/kg/day or more (Landry et al., 1985). Chronic dietary administration to rats resulted in no tumorigenic response and the no-observed-effect-level (NOEL) was 3 mg triclopyr/kg/day (Eisenbrandt et al., 1988). A 1-year chronic dietary study was conducted in the dog at doses of 0, 0.5, 2.5, and 5 mg/kg/day; the kidney was identified as a target organ (1988, unpublished report of the Dow Chemical Co., Midland, MI). The NOEL for the chronic dog study was 0.5 mg/kg/day. The kidney effects observed at 2.5 and 5 mg/kg/day were characterized by a slight reduction in phenolsulfonphthalein (PSP) excretion, slight increases in BUN and creatinine, and an increase in normally occurring pigment in the proximal tubules of the kidneys. These minor changes were interpreted to be a reversible physiological adaptation associated with excretion of the test material. Therefore, the no-observed-adverse-effect level (NOAEL) was considered to be 5 mg/kg/day, which was 10 fold greater than the observed NOEL in this same study. In comparison, female rhesus monkeys demonstrated no treatment-related effects when administered triclopyr acid by gavage at doses of 0, 10, 20, and 30 mg/kg/day for 28 days (1976, unpublished report of the Dow Chemical Co.).

The pharmacokinetics of triclopyr in the dog were markedly different from those in monkey, rat, or humans (Timchalk et al., 1990, 1996; Carmichael et al., 1989). In humans,

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an orally administered dose of 0.1 or 0.5 mg/kg provided no indication of nonlinear pharmacokinetics and was rapidly eliminated with a terminal phase half-life in blood of 5-hr ( Carmichael et al., 1989 ). An intravenously administered dose of 30 mg triclopyr/kg of body weight in a monkey was rapidly eliminated from the plasma ( $t_\text{1/2} = 6.3$ hr ), with >95% of the urinary $^{14}C$ radioactivity excreted within 24 hr. In contrast, the kinetics of triclopyr excretion in the dog were nonlinear with virtually all the dose excreted in the urine by 72 hr postdosing; however, the fraction excreted in the urine decreased with increasing dose ( Timchalk et al., 1996 ). Overall, the slower renal excretion in the dog was a function of extensive nonlinear plasma protein binding and tubular reabsorption of triclopyr. These findings were consistent with previous dog studies with organic acids which suggested that they have a lower capacity than other species for the renal clearance of organic acids ( Gehrings and Betso, 1978 ; Piper et al., 1973 ; Hook et al., 1974, 1975 ; Cherkovsky, 1995 ).

The objective of the current study was to evaluate the effects of repeated triclopyr administration on kidney function in the monkey, and to compare this response to that observed in the dog following single or repeated exposure. These data provide additional perspective on the relevance of the renal effects observed in the dog chronic toxicity data for human risk extrapolation.

**MATERIALS AND METHODS**

**Test Material and Dosing**

Technical-grade triclopyr (chemical purity >99.4%) was obtained from DowElanco (Indianapolis, IN). Dogs were administered the single oral 5 mg triclopyr/kg body wt dose by gavage at a volume of 2 mL/kg as an aqueous solution. Rhesus monkeys were administered triclopyr dissolved in a 0.05 M phosphate buffer (pH 7.0) which was orally administered by gavage at a volume of 1 mL/kg body wt, at a dose of 5 mg/kg/day, 7 days/ week for 28 days, after which the dosage was increased to 20 mg/kg/day for an additional 102 days (130 daily doses). Repeated dietary administration in dogs was accomplished by placing a weighted amount of triclopyr into a gelatin capsule which was then mixed with a small amount of ground beef which the dogs were allowed to immediately eat. Dogs received a nominal dose of 5 mg/kg/day for 47 consecutive days. No attempt was made to determine the stability of triclopyr within the gelatin capsule/ground beef matrix. However, numerous studies within the laboratory have demonstrated that triclopyr is extremely stable in a variety of matrices, such as in the diet and as an aqueous solution (personal communication).

**Animals**

Four male Rhesus monkeys (Macaca mulatta) were selected from the colony at the International Center of Environmental Safety (Holloman Air Force Base, NM). The monkeys were housed individually in air-conditioned quarters with feed and tap water available ad libitum throughout the study. Eight male beagle dogs were purchased from HRP, Inc. (Cumberland, VA) for use in the single-dose experiment and were acclimated to the laboratory environment. These dogs were housed in outside runs except during experimentation and had feed and water available ad libitum. However, dogs were fasted overnight preceding each day that PSP and creatinine clearance were measured.

Two male beagle dogs for the repeated dietary administration were purchased from Marshall Research Animals (North Rose, NY), and were likewise acclimated to the laboratory environment. The dogs were housed in appropriate air-conditioned quarters with feed and tap water available ad libitum throughout the study. All four monkeys and all dogs were administered triclopyr with an appropriate preadministration period serving as the control period. Therefore, each animal served as its own control.

**Single-Dose Administration (Dogs Only)**

**Experimental design.** Body weight determinations and baseline values for PSP excretion and endogenous creatinine clearance were obtained on all dogs 23 hr prior to triclopyr administration. Triclopyr (5 mg/kg) was administered by gavage to six dogs with two dogs serving as untreated time controls. Untreated controls did not vary from baseline for any of the parameters evaluated (data not shown). PSP excretion was determined as described by Osborne et al. (1972). All dogs had PSP and endogenous creatinine clearance measured repeatedly at predose and 1, 2, 3, and 73 hr postdosing and all procedures were performed in an identical manner during the four test times. Dogs were placed in Pavlov slings and were given 200 mL of water by gavage to increase urine flow rates. A venous catheter was placed in the saphenous or cephalic vein for use in blood specimen collection and a urethral catheter was put in place for collection of urine. Immediately after rinsing the bladder with sterile water, a clinical test dose of PSP (6 mg/animal) was given intravenously. Urine was then collected using a catheter over the following 20 min; the bladder was flushed with sterile water to assure complete collection of PSP and endogenous creatinine. The amount of PSP excreted during the 20-min interval was expressed as a percentage of the original dose of PSP. A blood specimen was taken at the midpoint of the collection interval and centrifuged to obtain plasma which was analyzed for triclopyr and endogenous creatinine. Urine and bladder rinse were pooled and analyzed for creatinine and PSP. The analysis for triclopyr, creatinine, and PSP in specimens of plasma and urine was as previously described ( Carmichael et al., 1989 ; Fincio, 1971, 1993 ).

**Repeated Administration**

**Experimental design.** Animals were observed daily for overt signs of toxicity or changes in demeanor and for pharmacologic or other effects. Individual body weights were obtained for the rhesus monkeys prior to the initiation of daily dosing and on Days 28, 52, 66, and 115. Clinical chemistry parameters for the monkeys were evaluated predose and following 8, 59, and 115 days of dosing, whereas for dogs, these parameters were evaluated predose and on dosing Days 16 and 37.

Urine analysis and PSP clearance assays were conducted for the monkeys predose and on Days 8, 24, 52, 65, 100, and 113 days of dosing and for dogs at predose and on Days 17 and 31. Monkeys and dogs were fasted overnight and a clinical test dose of PSP (6 mg/animal) was given intravenously as previously described. In general, the PSP excretion evaluation was conducted in the morning prior to the daily administration of triclopyr. However, in one instance (Day 113, 20 mg/kg), the dose of triclopyr was infused intravenously, rather than given orally, during the 20 min period following injection of PSP in the monkey. This change in triclopyr dose administration had no impact on the results of this study.

In addition to the PSP evaluation, inulin and para-aminohippurate (PAH) clearance were evaluated, in only the monkeys, using an adaptation of the constant-infusion technique of Cole et al. (1972), at predose and on Days 26 and 66 of dosing. Priming doses of 40 mg/kg of inulin and 8 mg/kg PAH were initially given intravenously while a sustained infusion of inulin (2.7–3.4 mg/mL) and PAH (0.4–1.4 mg/mL) in physiological saline was administered by a constant-speed infusion pump (0.7–1 mL/min) to maintain steady-state blood levels. Blood inulin and
TRICLOPYR EFFECTS ON RENAL FUNCTION

FIG. 1. Endogenous creatinine clearance in male beagle dogs administered a single oral dose of 5 mg triclopyr/kg of body weight dose determined at baseline, 1, 25, and 73 hr postdosing. The values represent mean ± SD for six animals.

PAH levels for clearance calculations were determined at ~4 hr of infusion. Inulin was measured according to the method of Heyrovsky (1956). PAH was determined using the method of Bratton and Marshall (1939) as modified by Smith et al. (1945).

Statistical Analysis

Predose values (baseline) were compared with values obtained postdosing using a paired t-test (α = 0.05) (Steel and Torrie, 1980).

RESULTS

Dogs administered a single oral dose of 5 mg triclopyr/kg body wt were clinically normal at the initiation of the study and remained normal throughout the trial. The mean body weight at the study start was 8.10 ± 0.93 kg. Values for endogenous creatinine clearance (Fig. 1) following this single-dose triclopyr administration were not statistically different from baseline (control) levels. The percentage PSP excretion and triclopyr plasma concentration (µg/mL) following a single 5 mg/kg oral dose in dogs are presented in Fig. 2. PSP excretion was significantly decreased to 63 and 74% of baseline levels at 1 and 25 hr postdosing, respectively. However, by 73 hr postdosing PSP excretion had returned to near baseline levels (96%). The plasma triclopyr concentration and percentage PSP excretion were inversely related over time. Plasma triclopyr concentration showed a decreasing trend with time, ranging from ~14 µg/mL (1 hr postdosing) to ~1 µg/mL (72 hr postdosing).

The monkeys and dogs tolerated the repeated administration of triclopyr at doses of 5 and 20 mg/kg/day (monkeys only) over a 130- or 41-day dosing period, respectively. Body weight changes for the monkeys are shown in Table 1 and increased over the study duration. One dog maintained its

**TABLE 1**

<table>
<thead>
<tr>
<th>Dose group</th>
<th>Day of dosing</th>
<th>Body wt (kg)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>0</td>
<td>2.78 ± 0.34</td>
</tr>
<tr>
<td>5 mg/kg</td>
<td>28</td>
<td>2.95 ± 0.34</td>
</tr>
<tr>
<td>20 mg/kg</td>
<td>52</td>
<td>2.93 ± 0.34</td>
</tr>
<tr>
<td>20 mg/kg</td>
<td>66</td>
<td>3.43 ± 0.49</td>
</tr>
<tr>
<td>20 mg/kg</td>
<td>115</td>
<td>3.35 ± 0.54</td>
</tr>
</tbody>
</table>

*Values represent means ± SD for four animals.
variability among test days, in general the response exceeded
baseline measurements and in no case was PSP excretion
significantly reduced. On the other hand, PSP excretion in
dogs was reduced approximately 50% from baseline on Test
Days 17 and 31 following the 5 mg/kg dose.

Inulin and PAH clearance also were examined in the mon-
key as indicators of renal function following triclopyr admin-
istration (Fig. 6). Inulin clearance following oral administra-
tion of triclopyr at 5 mg/kg/day (Day 26) and 20 mg/kg/
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A.

B.

FIG. 5. Percentage PSP excretion in male rhesus monkeys (A) and beagle dogs (B). Monkeys were gavaged with triclopyr at a dose of 5 mg/kg/day for 28 days followed by an additional 20 mg/kg/day for 102 days (130 daily doses). PSP analysis was conducted on Days 8, 24, 52, 65, 100, and 113 of dosing. Dogs were fed triclopyr in their diet at a dose of 5 mg/kg/day for 47 days and PSP was determined on dose Days 16 and 37. For monkeys, the values represent means ± SD for four animals. Values identified as statistically different are identified with an asterisk (*) (α = 0.05). For dogs, the values represent mean for two animals and individual animal data are presented in parentheses.

day (Day 60) was not significantly different from baseline clearance. para-Aminohippurate clearance demonstrated a considerable degree of variability when measured on Day 26 following the 5 mg/kg/day dose, but was comparable to baseline clearance, whereas the PAH clearance following 60 days of repeated triclopyr administration at a dose of 20 mg/kg/day was significantly increased above baseline.

DISCUSSION

Several studies have suggested that, relative to other species, including humans, the dog has a reduced capacity to effectively eliminate organic acids via the kidneys (Timchalk et al., 1996; Cherkofsky, 1995; Gehring and Betso, 1978; Hook et al., 1974; Piper et al., 1973). This study was conducted to evaluate the effects of triclopyr administration on kidney function in the monkey and to compare their response to that observed in the dog.

Blood urea nitrogen and serum creatinine are routinely utilized to evaluate renal function, although BUN and creatinine have limited sensitivity since renal function must be decreased to 25% of normal or less to be detected by these measurements (Finco, 1989; Schuster and Seldin, 1992). Nonetheless, the lack of an effect on BUN and creatinine levels in monkeys and dogs following repeated triclopyr administration suggests that triclopyr had no effect on normal kidney function in both species.

Phenolsulfonphthalein is a weak organic acid that is excreted by the kidneys primarily by an active secretory process in the proximal tubules (Finco, 1980). In monkeys there was no evidence for a decrease in PSP excretion following repeated triclopyr exposure at doses of 5 or 20 mg/kg/day. In contrast, PSP excretion appeared to increase following repeated triclopyr doses of 20 mg/kg/day in the monkey. Since both triclopyr and PSP are reported to extensively bind to plasma proteins (Timchalk et al., 1996; Ochwadt and Pitt, 1962), a possible explanation for increased renal excretion of PSP may be the result of competition between triclopyr and PSP for the same plasma protein-binding sites. A shift to greater amounts of non-plasma-bound PSP would enhance its glomerular filtration increasing the percentage PSP excreted in the urine.

Phenolsulphonphthalein excretion in dogs was inversely related to plasma triclopyr concentration. At high plasma triclopyr concentrations (3–15 µg triclopyr/mL plasma) PSP excretion was clearly depressed but returned to baseline as triclopyr plasma concentration decreased (~1 µg/mL). This effect of triclopyr on PSP excretion is consistent with a physiological response due to competition of triclopyr and PSP for the same active secretory system. These findings are consistent with those of Timchalk et al. (1996), who suggested that, in the dog, the renal clearance of triclopyr involves both a high-affinity low-capacity active secretory site and glomerular filtration of non-protein-bound triclopyr. In addition, numerous studies have demonstrated decreased renal clearance of organic acids, including PSP, when coadministered with other organic acids (Russel et al., 1989,
FIG. 6. Inulin (A) and PAH (B) clearance in male rhesus monkeys repeatedly administered triclopyr at a dose of 5 mg/kg/day for 28 days followed by an additional 102 days at 20 mg/kg/day (130 daily doses). Analysis was conducted on dose Days 26 and 60. The values represent means ± SD for four animals. Values identified as statistically different are identified with an asterisk (*) (α = 0.05).

1987; Mann et al., 1991; Foreman, 1984; Weiner, 1973). Overall these data clearly indicate that triclopyr effectively competes with PSP for the active secretory site within the dog kidney proximal tubules. In contrast, the monkey appears to be insensitive to the effects of triclopyr on the active secretory process even at doses fourfold higher (20 mg/kg/day) than the effective dose in the dog (5 mg/kg/day).

Inulin and PAH studies were initiated as a means of further evaluating renal function in the monkey. The glomerular filtration rate (GFR) as determined by inulin clearance represents a very sensitive test for renal function and has been reported to detect abnormalities with decrements as small as 20% (Schuster and Seldin, 1992). The results indicate that GFR as measured by inulin clearance was not affected by triclopyr administration and was well within the reported normal range of 2.2–3.6 mL/min/kg for adult male monkeys (Altman and Dittman, 1974). These data support the conclusion that triclopyr administration at doses as high as 20 mg/kg/day did not adversely impact renal function or produce associated renal toxicity in the monkey.

*para*-Aminohippurate is a weak organic acid that is extensively plasma protein-bound and is handled by the kidneys in a similar fashion as PSP (Finco, 1989). The slightly elevated PAH clearance following the 20 mg/kg/day dose of triclopyr was consistent with PSP excretion data at this higher dose level. As noted for the effects seen with PSP, competition for plasma protein-binding between triclopyr and PAH is also a reasonable explanation for this slight increase in PAH clearance. The failure of triclopyr to decrease PAH clearance in the monkey provides additional support for the conclusion that triclopyr does not significantly impact the active secretory process in monkeys.

The findings in this study indicate that the monkey was insensitive to the effects of triclopyr and support previous data which indicated that monkeys administered doses of triclopyr as high as 30 mg/kg/day for 28 days exhibited no treatment-related effects (1976, unpublished report of the Dow Chemical Co.). In contrast, dog renal physiology appears to be more sensitive to the effects of triclopyr administration. Perspective on the sensitivity of the dog to the renal effects of triclopyr and other organic acids such as phenoxyacid herbicides (2,4-D, 2,4,5-T) and phenoxyalkanoic acids has been previously reported (Timchalk et al., 1996; Gehring and Betso, 1978). Allometric comparisons of triclopyr clearance and half-life between rat, dog, monkey, and human clearly demonstrated that the dog has a decreased capacity to clear triclopyr relative to other species including humans (Timchalk et al., 1996). Most recently, Cherkofsky (1995) compared the pharmacokinetics of the organic acid 1-amino-cyclopropanecarboxylic acid in the mouse, rat, monkey, dog, and human and likewise demonstrated that the dog has a significantly slower clearance and longer half-life for this organic acid. Collectively, these findings suggest that dogs have a reduced capacity to clear organic acids, like triclopyr, and are consistent with the effects on renal function in dogs in this study. This unique physiological response of the dog, relative to the monkey, raises into question the relevance of utilizing renal function changes in the dog as endpoints for human risk extrapolation for organic acids such as triclopyr.

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REFERENCES


