

s is increasing,
ing such studies
lities, they will

Toxicology, 8 (1977) 3-11
© Elsevier/North-Holland Scientific Publishers, Ltd.

THE FATE OF 2,4-DICHLOROPHENOXYACETIC ACID (2,4-D) FOLLOWING ORAL ADMINISTRATION TO MAN

M.W. SAUERHOFF *, W.H. BRAUN *, G.E. BLAU ** and P.J. GEHRING *

Health and Environmental Research and Computation Laboratory**, The Dow
Chemical Co., Midland, Michigan 48640 (U.S.A.)*

(Received November 9th, 1976)

(Accepted January 4th, 1977)

SUMMARY

The pharmacokinetic profile of 2,4-D is defined in man. Five male human volunteers ingested a single dose of 5 mg/kg 2,4-D without detectable clinical effects. Concentration of 2,4-D were determined in plasma in 3 of 5 subjects and in urine in all subjects at intervals after ingestion. The elimination of 2,4-D from plasma in all subjects occurred by an apparent first-order rate process with an average half-life ($t_{1/2}$) of 11.6 h. All subjects excreted 2,4-D in the urine with an average $t_{1/2}$ of 17.7 h. Excretion occurred mainly as 2,4-D' (82.3%) with smaller amounts excreted as a 2,4-D conjugate (12.8%). Essentially all of the 2,4-D was absorbed from the gastrointestinal tract in man. Clearance of 2,4-D from the plasma and excretion from the body are first-order rate processes. There was no evidence that 2,4-D would accumulate following repeated administration.

INTRODUCTION

2,4-Dichlorophenoxyacetic acid (2,4-D) is a chlorophenoxy acid herbicide. Following oral administration to rats, pigs, calves and chickens, 2,4-D is readily absorbed, distributed and eliminated in the urine [1]. The half-life values for the apparent first-order clearance of 2,4-D from plasma were reported to be 3 h for rats, 8 h for calves and chickens and 12 h for pigs. The fate of 2,4-D following ingestion by man has not been reported. A

All correspondence to: M.W. Sauerhoff, Toxicology Research Laboratory, Health and Environmental Research, The Dow Chemical Company, 1803 Building, Midland, Michigan 48640 (U.S.A.).

Abbreviation: 2,4-D, 2,4-dichlorophenoxyacetic acid.

pharmacokinetic study of 2,4-D in man would provide critical information relative to the potential for 2,4-D to accumulate in the body. This report defines the pharmacokinetic profile and metabolism of 2,4-D following oral administration to 5 human male volunteers.

MATERIALS AND METHODS

Subjects

Five healthy male volunteers aged 29–40, weighing 70–90 kg, participated in the study. The medical history of each individual was reviewed and a physical examination conducted prior to the study. Included in the examination were blood pressure, pulse rate, pulmonary function, electrocardiogram, chest X-ray, hemoglobin, packed cell volume, red and white blood cell counts including differential, and sedimentation rate, serum concentration of total calcium, cholesterol, triglycerides, glucose, inorganic phosphate, albumin, total protein, uric acid, bilirubin, glutamicoxaloacetic transaminase, lactic dehydrogenase, alkaline phosphatase activities, as well as urinary protein and sugar. All values were in the normal range.

Dosage and sample collection for analysis

Five subjects ingested 5 mg/kg analytical grade 2,4-D (sample AGR 30653C, Dow Chemical Co.). Two subjects ingested the free acid of 2,4-D as a slurry in milk and 3 ingested it directly from a weighing paper followed by a few swallows of water. All subjects ingested 2,4-D between 8:00 and 8:05 a.m. Subjects were not fasted prior to ingestion of the dose.

Blood samples, 10 ml, were collected from 3 subjects in tubes containing 4 mg of potassium oxalate at 1, 4, 8, 12, 24, 36, 48, 72, 96, 120 and 144 h after ingestion. Following collection, the whole blood samples were centrifuged, the plasma separated and placed in tared vials and frozen for subsequent analysis of 2,4-D.

All the urine voided by each subject during successive 12-h intervals post-dosing was collected and the volume recorded. The urine collected during each 12-h interval was thoroughly mixed and aliquots were frozen until subsequently analyzed for 2,4-D.

Samples of plasma and urine were analyzed for 2,4-D by gas chromatography-mass spectrometry. Urine and plasma samples (5 ml) were acidified with 1 ml of 1.0 N HCl and extracted twice with 5 ml of diethyl ether. To determine whether hydrolyzable conjugates of 2,4-D may be present, additional samples of urine (5 ml) from the 0–12-, 12–24- and 24–36 h collection intervals were acidified with 1 ml of concentrated hydrochloric acid and heated at 80°C for 1 h. Subsequently, these samples were extracted twice with diethyl ether and analyzed for 2,4-D together with non-hydrolyzed urine samples. The ether extracts were methylated with diazomethane, evaporated to dryness and redissolved in 0.5 ml of hexane. A 2 μ l aliquot of

the l
eter
with
peak
metr
quar
St
expe
stanc

Phar

N.
cons
adeq

RESI

N
dete
T
tion
plasi
can
ject

2,4-D plasma levels (μ g/g)
30
10
10

0.10

0.01

0.001

Fig.

the hexane was injected into a LKB 9000 gas chromatograph-mass spectrometer (GC-MS). The glass GC column was packed (6 ft x 2.0 mm i.d.) with 10% OV-1 on Chromosorb W 80/100 mesh. The m/e 234, 236 and 238 peaks were monitored for sample quantitation. Recorded peaks were symmetrical; therefore, peak heights, not peak areas, were used for sample quantitation.

Standards of 2,4-D prepared in plasma and urine were stored with the experimental samples. No decomposition of 2,4-D was observed in any of the standards during the storage period.

Pharmacokinetic parameters and statistics

Nonlinear parameter estimation was used to calculate the optimal rate constants and half-life values [2]. The lack of fit test [3] was used to test the adequacy of the models described.

RESULTS

No untoward effects associated with ingestion of 5 mg/kg 2,4-D were detected in any of the subjects.

The concentration of 2,4-D in plasma, $\mu\text{g/g}$, is shown in Fig. 1 as a function of time following administration. The concentrations found in the plasma of each subject are presented. The data suggest that subjects 2 and 3 can be characterized by a 1-compartment pharmacokinetic model while subject 1 may be characterized by 1- or 2-compartment model.

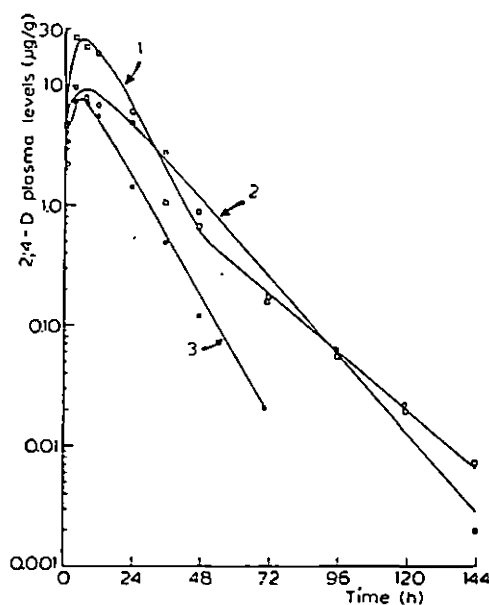


Fig. 1. Plasma levels of 2,4-D in man following a single oral dose of 5 mg/kg 2,4-D.

TABLE I

RATE CONSTANTS AND HALF-LIFE VALUES FOR ABSORPTION AND CLEARANCE OF 2,4-D FROM PLASMA IN 3 MALE SUBJECTS GIVEN A SINGLE ORAL DOSE 5 mg/kg 2,4-D

Subject	Model	Absorption		Elimination		Distribution V_d (ml/kg)
		Rate constant	Half-life	Rate constant	Half-life	
		k_a (h^{-1})	$t_{1/2}$ (h)	k_e (h^{-1})	$t_{1/2}$ (h)	
1	2 Compartment	0.165 ± 0.055	4.20 ± 1.40	$k_{12}^a = 0.013 ± 0.009$		$V_1^d = 83.6 ± 28.4$
				$k_{21}^a = 0.048 ± 0.007$		$V_2^e = 218 ± 15.7$
				$k^b = 0.145 ± 0.049$		
				$\alpha^c = 0.163 ± 0.048$	4.25 ± 1.25	
2	1 Compartment	0.202 ± 0.047	3.43 ± 0.80	$\beta^c = 0.043 ± 0.012$	16.2 ± 4.31	
				0.062 ± 0.002	11.2 ± 0.36	283 ± 42
3	1 Compartment	0.415 ± 0.082	1.67 ± 0.33	0.095 ± 0.003	7.29 ± 0.23	294 ± 39

^a k_{12} , k_{21} , First-order distribution constants between the plasma and slow exchange compartments.

^b k_e , First-order rate constant for overall elimination from the central compartment.

^c α , β , hybrid rate constants for α and β portions of plasma clearance curve.

^d V_1 , Volume of central compartment.

^e V_2 , Volume of slow exchange compartment.

^a V_1 , Volume of central compartment.
^c V_2 , Volume of slow exchange compartment.

TABLE II

PERCENTAGES OF 2,4-D (FREE AND CONJUGATE) EXCRETED IN URINE DURING SUCCESSIVE TIME INTERVALS FOLLOWING A SINGLE ORAL DOSE OF 5 mg/kg 2,4-D

Subject	1		2		3		4		5	
	Free	Conjugate	Free	Conjugate	Free	Conjugate	Free	Conjugate	Free	Conjugate
0-12	14.3	2.50	29.9	—	40.6	10.5	21.0	—	24.7	5.70
12-24	13.9	8.80	33.9	—	17.3	11.8	17.1	3.10	13.8	3.00
24-36	31.6	10.8	9.18	—	8.64	4.76	14.1	1.70	10.7	1.10
36-48	15.9		8.78		2.33		11.0		10.0	
48-60	5.07		3.91		0.68		9.98		8.99	
60-72	1.10		1.10		0.31		7.15		7.90	
72-84	0.86		0.49		0.16		4.04		6.03	
84-96	0.93		0.22		0.12		2.97		1.25	
96-108	0.27		0.06		0.02					
108-120	0.11		0.05		0.04					
120-132	0.08		0.03		0.01					
132-144	0.07		0.01		0.01					
Total	84.2	22.1	87.6	0.00	70.1	27.1	86.3	4.8	83.4	9.8
Total ^a	106.3		87.6		97.2		91.1		93.2	

^a Free + conjugate.

parameters for 3 subjects were calculated using a nonlinear parameter estimation program [2]. Parameters for a 2-compartment model were calculated for subject 1. They are reported in Table I with their linearized standard deviations. Comparing the parameter estimates reveals the importance of characterizing each subject individually. For example, the absorption constant (k_a) for subjects 2 and 3 are significantly different at the 95% confidence levels as are the elimination rate constants (k_e).

A lack of fit test was performed for all compartmental models based on the known analytical error [3]. Each model was shown to be inadequate to describe the data at the 95% confidence level.

The volume of distribution (V_d) was calculated for all subjects (refer to Table I). The V_d for subjects 2 and 3 were 238 and 294 ml/kg, respectively. The V_d for subject 1, V_1 and V_2 , were 83 and 218 ml/kg. This indicates 2,4-D is not extensively distributed to tissues.

The urinary excretion of 2,4-D expressed as a percentage of the dose excreted during each collection interval is presented in Table II and represented graphically in Fig. 2. Subjects were modeled individually using a 1-compartment linear pharmacokinetic model. The pharmacokinetic parameters determined using a nonlinear parameter estimation program are presented in Table III [2]. The half-life of elimination ranged from 10.2 to

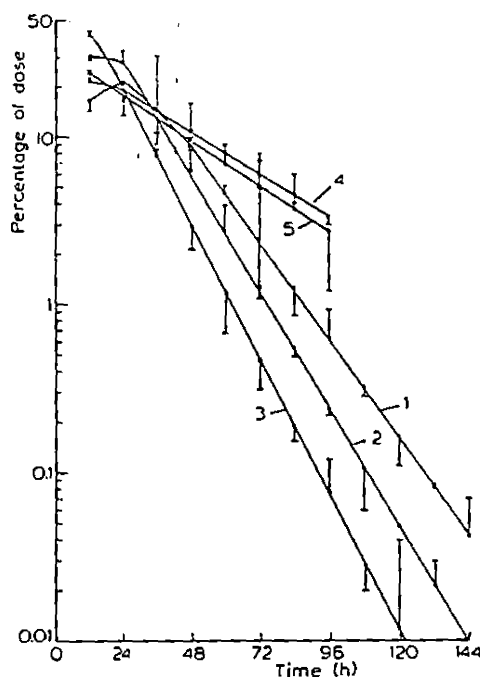


Fig. 2. Excretion of 2,4-D in urine expressed as percentage of dose excreted during successive time intervals following a single oral dose of 5 mg/kg 2,4-D.

dose excreted during

RATE CONSTANTS AND HALF-LIFE VALUES FOR THE ELIMINATION OF 2,4-D IN THE URINE OF INDIVIDUALS FOLLOWING A SINGLE ORAL DOSE OF 5 mg/kg

Subject	Estimated fraction of dose eliminated ^a	Absorption constant k_a (h^{-1})	$t_{1/2}$ (h) absorption	Elimination constant k_e (h^{-1})	$t_{1/2}$ (h) elimination
1	71.4 ± 18.9	0.108 ± 0.052	6.42 ± 3.09	0.0566 ± 0.0060	12.2 ± 1.3
2	84.3 ± 14.5	0.192 ± 0.098	3.61 ± 1.84	0.0678 ± 0.0023	10.2 ± 0.44
3	47.8 ± 15.5	—	—	0.0638 ± 0.0029	10.9 ± 0.50
4	96.5 ± 4.67	0.522 ± 4.67	1.33 ± 1.44	0.0244 ± 0.0020	28.4 ± 2.38
5	88.4 ± 18.5	—	—	0.0290 ± 0.0065	23.9 ± 5.42

^b Meaningful estimate of absorption constant could not be obtained from these two subjects.

28.5 h for subjects 2 and 4, respectively. Recovery of 2,4-D ranged from 87.6 to 106.3% of the administered dose.

DISCUSSION

These data demonstrated that orally administered 2,4-D was well absorbed in man and excreted in urine, at the dose provided, mainly as 2,4-D. A fraction of administered 2,4-D was also excreted in the urine as a conjugate. The average recovery of 2,4-D and conjugate was 95.1%. Recovery of greater than 90% in human studies reflects quantitative excretion of the administered chemical.

After ingestion of 5 mg/kg 2,4-D, plasma concentrations increased and reached a peak of approximately 25 $\mu\text{g/ml}$ at 4 h. Clearance of 2,4-D from the plasma of subjects 2 and 3, as well as the excretion in urine from all 5 subjects, was monophasic. The plasma data for subject 1 exhibited a biphasic clearance phenomenon. The explanation for this is not available. It is interesting to note that this biphasic clearance in subject 1 is accompanied by the delayed peak in the urine excretion data. The slow clearance phase parameter for the plasma data of subject 1 is only slightly slower than the monophasic clearance for subjects 2 and 3. This suggests that the overall clearance characteristics of subjects 1, 2 and 3 will not be markedly different.

Statistical tests [3] performed to determine the suitability of the compartmental models to characterize the 2,4-D plasma data based on a combined analytical and sampling error of 10.2%, indicates that there was a significant lack of fit between the models and the experimental data. A significant lack of fit implies either that a more complex model (i.e., one having more parameters such as a 2-compartment model) is required or that the data are sufficiently precise to reveal deviations from the linear approximations inherent to compartment models. In this case, the latter situation is occurring. If, for example, the experimental error had been 15% rather than 10.2%, no significant lack of fit would have been detected. This means that the differences between data points and the line drawn through the plasma data in Fig. 1 are real differences and not random scatter. Moreover, these differences represent real departures from the assumed first-order rate processes.

Excretion data are presented for 5 subjects ingesting 5 mg/kg 2,4-D. It is not reasonable to pool the individual subject data because of the difference in the pattern of excretion. One of the 5 subjects did not form the conjugate of 2,4-D while other subjects excreted between 4.8 to 27.1% of the administered dose as 2,4-D conjugate. Modeling of the data was based on the excretion of 2,4-D and not the conjugate.

The data presented in Table II and Fig. 2 show significance between subject variation in the excretion of 2,4-D from the body. The slower excretion of 2,4-D in subjects 4 and 5 may be attributable to such factors as increased protein binding, decreased capability of the kidney to excrete 2,4-D or differences in reabsorption from the tubular filtrate. (In addition,

-D ranged from

the urine samples from subjects 4 and 5 were stored refrigerated for 18 months before they were analyzed.) Data presented in this report indicated first-order excretion of 2,4-D

The pooled data half-life values for clearance of 2,4-D from plasma was 11.6 h. It may be useful to compare this data with pooled data from human 2,4,5-T pharmacokinetic studies. Gehring et al. [4] reported that the plasma clearance $t_{1/2}$ of 2,4,5-T was 23.1 h. Decreasing the number of chlorines from 3 to 2 on the phenoxy ring apparently increased the rate of clearance of these chlorophenoxy acids from the body.

A practical question is: What will be the concentration of a chemical in the plasma or other tissues after repeated administration? Assuming the first-order elimination process following a single dose does not change, is not induced or saturated, the cumulative concentration in plasma may be determined by addition of the concentrations attributable to each exposure. In practical terms, the time required to attain 90% of the steady-state value is about 3.5 times the biological half-life; for 99% of steady-state value it requires about 7 times the biological half-life. Steady-state concentrations of 2,4-D would be reached by approximately 3 days. No additional accumulation would occur after this time. Based on our analysis of the data collected in this study, 2,4-D would not accumulate in the body with repeated administration.

In conclusion, 2,4-D is well absorbed following oral administration to man and eliminated as unchanged 2,4-D (82.3%) and 2,4-D conjugate (12.8%) in urine. Clearance of 2,4-D from plasma and elimination from the body occur by apparent first-order rate processes. No evidence of nonlinear kinetics was observed following the 5 mg/kg oral dose of 2,4-D.

REFERENCES

- 1 K. Erne, *Acta Vet. Scand.*, 7 (1966) 240.
- 2 Y.J. Bard, *Nonlinear Parameter Estimation*, Academic Press, New York, 1974
- 3 P.M. Riley and G.E. Blau, *Can. J. Chem. Eng.*, 52 (1974) 289.
- 4 P.J. Gehring, C.G. Kramer, B.A. Schwetz, J.Q. Rose and V.K. Rowe, *Toxicol. Appl. Pharmacol.*, 26 (1973) 352.

mg/kg 2,4-D. It is of the difference from the conjugate is 27.1% of the was based on the

ificance between The slower excretion such factors as kidney to excrete rate. (In addition,