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DISTRIBUTION AND ELIMINATION OF CHLORINATED PHENOXYACETIC ACIDS IN ANIMALS *)

By

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The distribution, elimination and metabolism in plant tissues of chlorinated phenoxyaliphatic acids have been the subject of numerous investigations (see e. g. *Audus* 1964). The fate of phenoxy acids in the animal organism, however, has not been so thoroughly investigated.

According to early studies unsubstituted phenoxyacetic acid is excreted unchanged in urine by man, dogs, rats and rabbits (*Williams* 1959). The o- and p-chloroderivatives also were shown to be excreted renally, without conjugation, in the rabbit (*Levey & Lewis* 1947). On feeding ω-phenoxybutyric and -caproic acids to rabbits, some phenoxyacetic acid was detected in the urine, along with the unchanged acids, indicating partial β-oxidation.

Recently a group of workers at Cornell University, N.Y., in a series of feeding experiments with cattle and using a gas chromatographic method of analysis, found that chlorinated phenoxyacetic acids (2,4-D, MCPA and 2,4,5-T) were completely eliminated with the urine in intact form, as was a chlorinated 2-phenoxypropionic acid (2,4,5-TP) (*Gutenmann et al.* 1963 a, b; *Lisk et al.* 1963; *Bache et al.* 1964 a, b; *St. John et al.* 1964). Chlorinated 4-phenoxybutyric acids (2,4-DB and MCPB) were largely degraded in the rumen of cattle, but small amounts of the β-oxidation products, the corresponding phenoxyacetic acids, were also detected in the urine. A 2,4,5-TP ester was largely eliminated in urine as the parent acid. None of the phenoxy herbicides given

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to cattle could be detected in the milk. *Clark et al.* (1964), applying a tracer technique, reported the rapid renal elimination of orally administered 2,4-dichlorophenoxyacetic acid in a sheep. 96 % of the activity was excreted with the urine in 72 hours, and blood levels dropped to below 0.05 p.p.m. in 24 hours.

Experiments with other animal species do not seem to have been reported.

The present paper presents results from studies of the distribution and elimination of some chlorophenoxyacetic acids in rats, pigs, calves and chickens. The experiments were performed in connection with toxicity studies which will be reported separately (*Björklund & Erne* 1966).

EXPERIMENTAL

Materials

2,4-D amine. Aqueous solution containing 20 mg of 2,4-D per ml, as the triethanolamine salt, prepared by diluting a commercial formulation.

2,4-D K-Na salt. Aqueous solution containing 20 mg of 2,4-D per ml, prepared by dissolving the pure acid (m.p. 138—142°C) in a slight excess of aqueous KOH-NaOH (1:1) and adjusting pH to about 7.

2,4,5-T amine. Aqueous solution containing 20 mg of 2,4,5-T per ml, prepared by dissolving the pure acid (m.p. 155—157°C) in a slight excess of aqueous triethanolamine and adjusting pH to about 7.

2,4-D ester. Emulsion containing 20 mg of 2,4-D per ml, as the butyl ester, prepared by homogenizing a commercial formulation, in a petroleum solvent, with water.

Animals

Data given for weight and age refer to conditions at start of experiment.

Albino rats, Anticimex strain, 180—220 g, both sexes.

Pigs, Swedish "Lantras" breed, 20—25 kg, 10—12 weeks, castrated males, and females.

Calves, SRB breed, 55—65 kg, 6—8 weeks, both sexes.

Chicks, broiler, one week, male.

Chickens, White Leghorn breed, 0.8—1.0 kg, 8—10 weeks, both sexes, and New Hampshire breed, 2.2—2.9 kg, adult hens.

Methods

Administration. Before dosing animals were starved overnight. Doses were given orally, in short term experiments by stomach tube, in long term experiments in feed or drinking water.

Sampling. At selected time intervals before and after dosing, blood samples were withdrawn in heparinized tubes, in rats at

exsanguination from a neck artery, in pigs from vena cava cranialis, in calves from a jugular vein and in chickens from a wing-vein. Plasma was separated by centrifugation.

A number of the experimental animals were sacrificed at selected time intervals, by exsanguination after anesthetizing with either chloroform (rats and poultry) or mebumal sodium (pigs). Tissues were examined for gross pathological and histological changes (*Björklund & Erne* 1966) and samples collected for analysis. Samples were stored at —20°C until analyzed.

Analytical method. The chlorinated phenoxyacetic acids were isolated from body fluids and tissues by solvent extraction, separated from extractives by thin-layer chromatography and quantitatively determined photometrically according to a technique described previously (*Erne* 1966 a). When required, the procedure was scaled down to handle samples as small as 1 g. The method gives the total concentration of chlorophenoxyacetic acids. The limit of determination of the method is 0.1—0.2 µg of 2,4-D per g of sample, with the reduced sample amounts, however, about 0.5—1 µg/g.

RESULTS

A. *Plasma levels*

1. *Single dose*

a. *2,4-D amine.* Groups of rats (male and female), pigs, calves and chickens (Leghorn and New Hampshire) were given 2,4-D amine salt, in the form of a technical formulation, as single oral doses equivalent to 50—200 mg of 2,4-D per kg body weight. On each dosage level 25 male and 10 female rats, 5—10 pigs and chickens and 2 calves were used. Blood samples were taken (in rats after killing) at 2—3-hour intervals during the first 12 hours and thereafter less frequently, and plasma analyzed for 2,4-D. Plasma concentration-time curves are shown in Figs. 1—4. (In addition, a number of the animals were killed at certain intervals in order to study tissue distribution, see section C, below.)

The peak plasma concentration of 2,4-D was usually attained within 2 hours after dosing in chickens and within 4—7 hours in the mammalian species. In the species examined a dose of 100 mg/kg gave a peak level of about 100—200 µg/ml. The plasma levels for female rats were lower than those for the males. The half-life values of 2,4-D in plasma, calculated from the curves after plotting on a semi-logarithmic scale, are given in Table 1.

b. *2,4-D K-Na salt.* The experiments were repeated with the potassium-sodium salt of 2,4-D. Ten male rats and 2 calves were given single doses of 100 mg/kg.
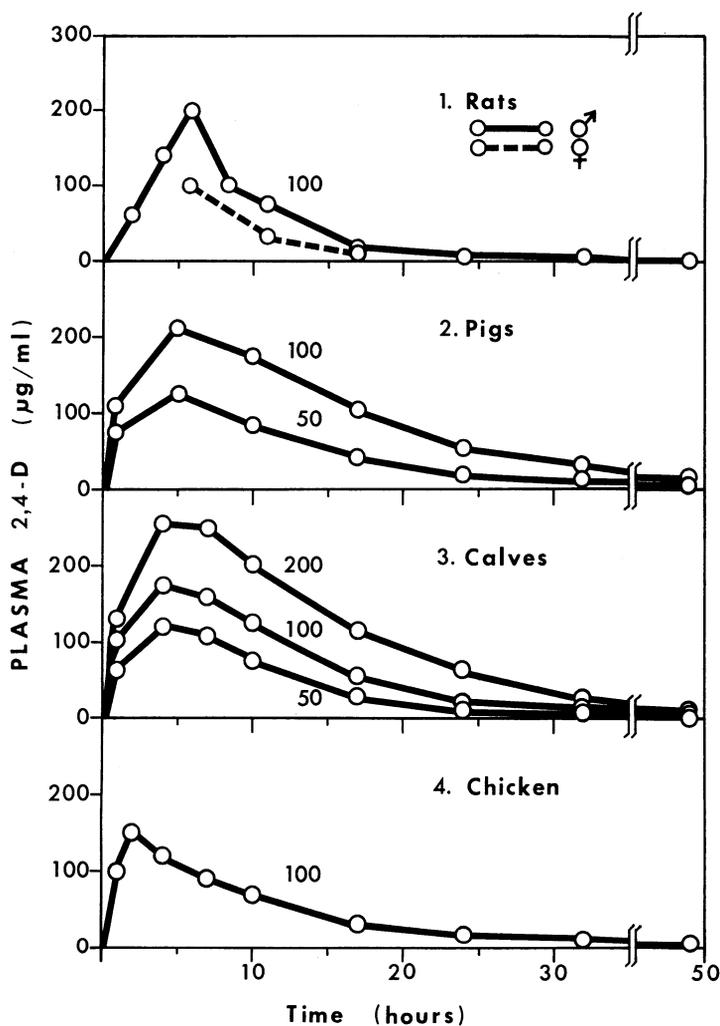
Figures 1—4. Plasma levels of 2,4-D in different species after a *single* oral dose of *2,4-D amine* (50, 100 or 200 mg 2,4-D/kg). The curves for pigs, calves and chickens were obtained with 3—5 animals on each dosage level and the curves for rats with 25 males and 10 females, 2—4 animals on each point of the curve.

The curves obtained and the half-life values found (Table 1) were similar to those obtained with 2,4-D amine.

c. *2,4,5-T amine.* Ten male rats and 2 pigs were given single oral doses of a 2,4,5-T amine salt (100 mg/kg). The plasma concentration-time curves, as well as the plasma half-life values found, were similar to those obtained with the 2,4-D salts.

d. *2,4-D ester.* A technical formulation containing the butyl ester of 2,4-D was given as single oral doses of 100 mg/kg to 14 male rats, 4 pigs and 2 calves. Plasma samples were analyzed for total 2,4-D as in the preceding experiments. Typical curves are shown in Fig. 5. Curves for pigs were intermediate between those for rats and calves. The plasma half-life values are given in Table 1.
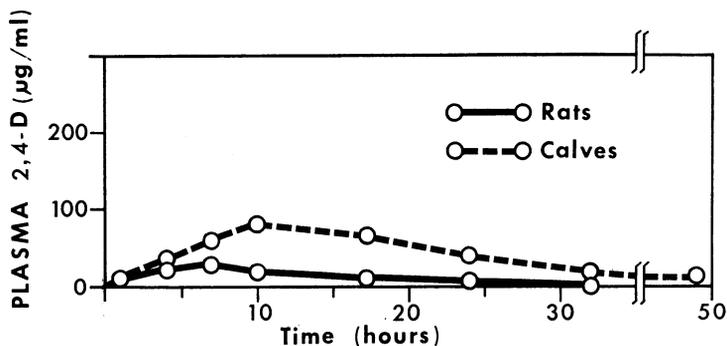
F i g u r e 5. Plasma levels of 2,4-D in rats and calves after a *single* oral dose of *2,4-D butyl ester* (100 mg 2,4-D/kg). The curves were obtained with 14 rats (2 on each point of the curve) and 2 calves.

T a b l e 1. Plasma half-life values, in different species, of phenoxy-acetic derivatives given as a single oral dose of 100 mg/kg.

Compound			Species	Plasma half-life hours
2,4-D amine salt			Rat, male	2.9 ± 0.4
,,	,,	,,	Rat, female	3.3 ± 0.5
,,	,,	,,	Pig	12 ± 2
,,	,,	,,	Calf	7.5 ± 0.8
,,	,,	,,	Chicken	7.7 ± 0.7
,,	K-Na	,,	Rat, male	3.5 ± 0.5
,,	,,	,,	Calf	8 ± 0.6
,,	ester		Rat, male	6 ± 1
,,	,,		Pig	10 ± 0.8
,,	,,		Calf	10 ± 1
2,4,5-T amine salt			Rat, male	3 ± 0.6

2. *Repeated administration*

2,4-D amine and 2,4-D ester were given as repeated oral doses (50 mg 2,4-D/kg/day) to two groups of 5 and 4 pigs, respectively.

In both groups the plasma level of 2,4-D was seen to decline — and the urinary excretion to increase — as exemplified in

Table 2. Plasma levels of 2,4-D in pigs on *repeated* oral admini-
stration of either 2,4-D amine or 2,4-D butyl ester (in each case 50 mg
2,4-D/kg/day). Pigs no. 4 A and 9 A remained unaffected clinically;
pigs no. 8 A and 15 A developed signs of poisoning. Values in brackets
denote the corresponding urinary concentrations of 2,4-D.

| Number of doses given | Plasma 2,4-D, μg/ml (at 24 hrs. after last dose) | | | |
| | Amine | | Ester | |
	Pig no. 4 A	Pig no. 8 A	Pig no. 9 A	Pig no. 15 A
3	35 (200)	230	190	155
4	—	315	240	—
7	—	335	24	295
8	8	520 (90)	25	—
23	6 (550)	—	7 (250)	—

Table 2 by pigs no. 4 A and 9 A. One animal in each group, how-
ever, did not tolerate the repeated administration; the plasma
levels continued rising during the experimental period, and signs
of poisoning gradually developed (pigs no. 8 A and 15 A, Table 2).

In an experiment with two hens, 2,4-D amine (300 mg/kg/
day) was given orally for 12 and 24 days, respectively, and during
the experimental period the plasma level (at 3 hours after dosing)
dropped from 150 to 20 μg/ml, on the average.

B. *Blood cells*

In *in vitro* experiments 2,4-D was added, at two concentration
levels, to horse blood and the samples agitated at room tem-
perature for 5 hours and 24 hours, respectively. Then the blood
cells were separated by centrifuging for 30 minutes at 2500 r.p.m.,
washed by suspending in two volumes of either fresh plasma or
physiological saline for 5 minutes and centrifuging for another
5 minutes and then haemolyzed by diluting with two volumes of
water. For comparison some samples were washed in physio-
logical saline three times. The 2,4-D levels found in plasma and
blood cells are presented in Table 3.

A small proportion of the 2,4-D, about 10 % of the plasma
level, was found in blood cells washed once. The proportion did
not change on prolonged contact between plasma and cells.
Washing with plasma removed more of the 2,4-D from the blood
cells than did washing with saline. Repeated washing with saline
removed 2,4-D almost completely from the cells.

T a b l e 3. *In vitro* distribution of 2,4-D between horse plasma and blood cells at two concentration levels. Before analysis the blood cells were washed either once (5 min.) with 2 volumes of fresh plasma (A) or of physiological saline (B) or three times (15 min. each time) with 2 volumes of physiological saline (C). Values given are means of results from duplicate experiments.

Time of contact hours	2,4-D, μg/ml							
	10 μg 2,4-D added per ml whole blood				100 μg 2,4-D added per ml whole blood			
	Plasma	Blood cells			Plasma	Blood cells		
		A	B	C		A	B	C
5	13	1.5	2.1	<0.1	135	11	17	<0.1
24	12	1.5	2.0		122	10	18	

The results reported in Table 4 were obtained under physiological conditions with rats receiving single oral doses of 2,4-D amine and 2,4-D ester.

T a b l e 4. *In vivo* distribution of 2,4-D between rat plasma and blood cells after a single oral dose of either 2,4-D amine or 2,4-D butyl ester (100 mg 2,4-D/kg in each case). Before analysis the blood cells were washed for 5 min. with 2 volumes of physiological saline. Values given are the means of results from two animals.

Time after dosing hours	2,4-D, μg/ml			
	Amine		Ester	
	Plasma	Blood cells	Plasma	Blood cells
5	170	20	25	3
24	2.5	0.8	8.5	1.2

C. *Tissues*

Groups of rats (male and female), pigs and chickens (Leghorn and New Hampshire) were given *single* oral doses of 2,4-D amine (100—200 mg 2,4-D/kg), and at different time intervals 3—4 animals from each group were sacrificed and tissues and body fluids analyzed for 2,4-D. The experiments were performed in connection with the plasma level studies described above. The results are summarized in Tables 5—7.

The effect on tissue distribution of *repeated administration* of 2,4-D was studied in feeding experiments with rats (male and

T a b l e 5. Tissue levels of 2,4-D in male and female *rats* at different intervals after a *single* oral dose of 2,4-D amine (100 mg 2,4-D/kg). Each value is the mean of results from 3—4 animals, with the range given in brackets.

| Tissue | 2,4-D, µg/g (fresh weight) | | | |
| | 6 hours | | 24 hours | |
	Males	Females	Males	Females
Plasma	150 (130—174)	70 (55— 87)	2 (1.2— 3.0)	1.5 (0.8— 2.1)
Liver	90 (65—120)	35 (23— 42)	5 (3 — 9)	3 (2.9— 5.2)
Kidney	250 (182—289)	145 (90—185)	27 (19 —40)	15 (10 —23)
Lung	140 (104—176)	60 (45— 75)	8 (6 —11)	6 (4 — 8)
Spleen	80[a])	—	6	—
Skeletal muscle	23 (12— 29)	14 (8— 21)	2 (1.3— 3.1)	0.6 (0.2— 1.3)
Feces	70 (55— 90)	—	18 (11 —27)	—

[a]) One animal.

T a b l e 6. Tissue levels of 2,4-D in *pigs* at different intervals after a *single* oral dose of 2,4-D amine (100 mg 2,4-D/kg). Each value is the mean of results from 3—4 animals, with the range given in brackets.

| Tissue | 2,4-D, µg/g (fresh weight) | | | |
	6 hours	24 hours	48 hours	72 hours
Plasma	210 (185 —230)	55 (43 — 64)	10 (5 — 16)	3 (1.5— 4.5)
Liver	115 (89 —125)	27 (18 — 35)	6 (3 — 9)	4 (2.5— 7)
Kidney	190 (165 —225)	36 (23 — 48)	10 (3 — 18)	5 (2 — 8)
Lung	90 (74 —107)	20 (14 — 32)	4 (3 — 6)	3 (2 — 4)
Spleen	65 (51 — 78)	17 (9 — 26)	3 (2 — 4)	—
Heart muscle	45 (38 — 53)	—	3[a])	—
Skeletal muscle	21 (15 — 28)	3 (2.5— 4.5)	2 (1.6— 2.7)	1.6 (1.0— 2.2)
Brain	12 (8 — 18)	3 (2 — 3.5)	1.5 (1.2— 1.8)	—
Fat	3 (1.2— 3.3)	2 (1.0— 2.1)	—	—
Skin	45 (30 — 55)	—	—	—
Bile	26 (17 — 36)	55 (40 — 71)	30 (15 — 38)	3 (1.5— 4.5)
Urine	85 (55 —125)	180 (110 —300)	95 (60 —135)	25 (10 —50)
Feces	150 (75 —205)	50 (40 — 65)	15 (10 — 25)	—
Thyroid	55 (42 — 63)	—	—	—
Adrenal	95 (80 —105)	—	—	—
Ovary	105[a])	—	—	—
Pancreas	60 (50 — 71)	—	—	—
Salivary gland	60 (48 — 69)	—	—	—
Lymph gland (mesenteric)	75 (64 — 83)	—	8[a])	—

[a]) One animal.

T a b l e 7. Tissue levels of 2,4-D in *chickens* at different intervals after a *single* oral dose of 2,4-D amine (200 mg 2,4-D/kg). Each value is the mean of results from 3—4 animals, with the range given in brackets.

Tissue	2,4-D, μg/g (fresh weight)		
	6 hours	24 hours	48 hours
Plasma	100 (72 —131)	15 (10 — 20)	5 (2 —7)
Liver	80 (50 —105)	25 (20 — 32)	3 (2 —4)
Kidney	120 (80 —170)	80 (53 —120)	7 (4 —8)
Lung	60 (40 — 82)	40 (28 — 49)	4 (2 —5)
Spleen	80 (50 — 90)	20 (10 — 28)	—
Skeletal muscle	3.5 (1.5— 4.2)	1.5 (0.8— 1.9)	1.2 (0.5—1.6)
Brain	1.5 (1.2— 2.1)	—	—
Fat	1.1 (0.9— 1.5)	—	—
Egg, yolk	<0.1	0.2 (0.1— 0.3)	0.2 (0.1—0.4)
„ white	<0.1	<0.1	<0.1

T a b l e 8. Tissue levels of 2,4-D in rats, pigs, chicks and chickens after *repeated* oral administration for 2 months. Pigs were given 500 p.p.m. of 2,4-D amine in standard feed twice daily and the other species 1000 p.p.m. of 2,4-D amine in their drinking water. The pigs were killed 24 hours after the last meal; for the other species the interval between last dose and killing was irregular. Each value given is the mean of results from 3—4 animals, with the range given in brackets.

Tissue	2,4-D, μg/g (fresh weight)				
	Rats		Pigs	Chicks	Chickens
	Males	Females			
Plasma	10 (4 —20)	5 (2—16)	22 (8 —35)	12 (3 —25)	10 (3 —31)
Liver	25 (10 —39)	3 (3—18)	6 (3 —14)	8 (5 —16)	15 (5 —20)
Kidney	45 (22 —60)	15 (8—26)	12 (10 —16)	30 (7 —71)	20 (8 —56)
Lung	30 (19 —42)	6 (3—11)	4 (2 — 6)	15 (6 —40)	12 (5 —48)
Skeletal muscle	7 (3 —10)	3 (2— 5)	2 (1.5— 3)	1.3 (0.3— 1.9)	3 (2 — 3.5)
Skin	4[a]	—	3[a]	—	—
Brain	—	—	2 (1.4— 2.6)	—	1.8[a]
Fat	1.5 (0.5— 2)	—	1.3 (0.6— 2.2)	—	1.0 (0.5— 1.3)
Egg, yolk	—	—	—	—	1.5 (0.5— 2.1)
„ white	—	—	—	—	0.2 (0.1— 0.4)

[a]) One animal.

female), pigs, chicks (broiler) and chickens (Leghorn). The pigs were fed a standard diet, with 500 p.p.m. of 2,4-D amine added, twice a day, and the other animals were given 2,4-D amine, at a level of 1000 p.p.m., in their drinking water. Animals were sacrificed after feeding periods of 2 months to 2 years.

The major clinical signs, observed in all groups were anorexia and a reduced weight gain. Further clinical and morphological observations of the experimental animals will be reported elsewhere (*Björklund & Erne* 1966). Table 8 indicates the tissue levels of 2,4-D found after a feeding period of at least 2 months.

Table 9. Tissue levels of 2,4-D in pigs showing signs of poisoning on repeated oral administration of 2,4-D amine.

Tissue	2,4-D, μg/g (fresh weight)		
	Pig no. 3 A [a]	Pig no. 8 A [b]	Pig no. 12 A [c]
Plasma	300	580	530
Liver	230	300	225
Kidney	260	370	185
Lung	220	340	190
Spleen	130	225	150
Skeletal muscle	105	—	120
Brain	40	—	65
Urine	100	90	—

[a] 10 daily doses of 50 mg/kg. Killed 48 hours after last dose.
[b] 8 ,, ,, ,, ,, ,, ,, 96 ,, ,, ,, ,,
[c] 3 ,, ,, ,, 100 ,, ,, 6 ,, ,, ,, ,,

In another experiment, repeated oral doses of 2,4-D amine (50 and 100 mg 2,4-D/kg/day) were given by stomach tube to 5 and 2 pigs, respectively. Two animals of the first and one of the second group developed signs of poisoning (vomiting, locomotory disturbances, depression) and were killed. On analysis of tissues from these animals, the results given in Table 9 were obtained.

Further, four pigs were given repeated oral doses of 2,4-D *ester* (50 mg 2,4-D/kg/day) for up to one month. In three of these animals no untoward effects were seen, and in their tissues only low levels of 2,4-D were found (Table 10). (Intact ester could not be detected in tissues from any of the animals). One animal, however, exhibited signs of poisoning and was killed after 10 days (pig no. 15 A, Table 2). On analysis comparatively

T a b l e 1 0. Tissue levels of 2,4-D in pigs after repeated oral admini-stration of 2,4-D *butyl ester* (equivalent to 50 mg 2,4-D/kg/day) for 1 month. The animals were killed 24 hours after last dose. Each value given is the mean of results from 3 animals, with the range given in brackets.

Tissue	2,4-D, μg/g (fresh weight)
Plasma	7 (5 —12)
Liver	10 (5 —17)
Kidney	17 (12 —25)
Lung	4 (2 — 7)
Skeletal muscle	1.5 (0.5— 3)
Fat	0.5 (0.2— 1.1)

high tissue levels of 2,4-D were found (230, 190, 205 and 80 μg/g in liver, kidney, lung and skeletal muscle, respectively).

Finally, with the main purpose of studying the possible effects of phenoxy acids on reproduction, a pregnant sow was given 2,4-D amine (500 p.p.m.) in the feed during the entire pregnancy (see *Björklund & Erne*). Of fifteen piglets delivered on parturition one was born dead and nine died within 24 hours. On analysis of tissues from the dead piglets considerable amounts of 2,4-D were found; for liver, kidneys and lungs the mean values, and the range, were 35 (14—77), 27 (13—58) and 30 (13—64) μg/g, respectively. The placenta contained 46 μg/g of 2,4-D and the plasma of the sow at parturition 28 μg/ml.

DISCUSSION

Absorption. 2,4-D, when administered orally as a technical formulation of an amine salt, was readily absorbed in all the animal species studied, as indicated by the peak plasma concen-trations after a single dose being attained in a few hours (about 2 hours for chickens and 4—7 hours for rats, pigs and calves, Figs. 1—4). Similar results were obtained with an alkali salt of pure 2,4-D, thus indicating the absorption rate of 2,4-D to be essentially unaffected by the amine or other ingredient of the technical formulation. 2,4,5-T amine behaved similarly.

Oral administration of 2,4-D ester, on the other hand, resulted in low plasma and tissue levels of 2,4-D as compared with the soluble salts, probably as the result of incomplete absorption (Fig. 5, Table 10). Considering the poor solubility in water of the ester, a low absorption rate is also to be expected. Not at any

time after dosing could intact ester be detected in plasma or urine of rats, pigs and calves. Only the acid was detectable, indicating the ester to undergo hydrolysis during absorption (*Erne* 1966 b).

Distribution. The absorbed phenoxy acids were distributed rapidly throughout the body (Tables 5—7). In all species the highest tissue levels were found in the excretory organs, liver and kidneys, and in the lungs and spleen. The levels in liver, kidney and lung at 6 hours approached, and at later times often exceeded, the plasma level. This was particularly true for rats and hens. A comparison between species may be based on the kidney levels. If expressed relative to the liver levels, these values at 6 hours and 24 hours, respectively, were 1.6 and 1.3 for pigs, 2.8 and 5.4 for male rats, 4.1 and 5.0 for female rats and 1.5 and 3.2 for chickens. Thus, rats exhibited the highest values throughout, and in both rats and chickens the values increased during the elimination phase. The difference in 6-hour values for male and female rats probably is not significant. Likewise, there seems to be no sex-difference in distribution pattern in rats, although the tissue levels found in the females consistently were inferior to those found in the males.

From the more detailed study performed with pigs (Table 6) is seen that comparatively high levels can be attained also in endocrine and other secretory organs. Concentrations of 2,4-D comparable to that in liver, or about 50 % of the plasma level, were found in ovaries and adrenals. In skin and heart muscle the levels were approximately 25 % and in skeletal muscle about 10 %, relative to plasma.

The brain level was usually low, about 5 % relative to plasma, although after toxic doses higher concentrations could be attained (see Table 9). In adipose tissue only traces of 2,4-D were found, or 1—2 % relative to plasma. The low brain level is consistent with current concepts of drug transfer across body membranes. For most drugs and foreign organic compounds the transfer can be explained in terms of a simple diffusion, of the unionized compound, across a lipoid membrane, the rate-determining factors being the ionization of the compound and the lipid-solubility of the unionized form (see for instance *Binns* 1964). Now, phenoxyacetic acids, as being relatively strong acids (pK_a values about 3), will be largely ionized and therefore not readily diffusible at plasma pH. Accordingly, they would not be expected to traverse the blood-brain barrier, as well as the placental barrier, readily.

Against this background, the results obtained with the pregnant
sow, suggesting a ready placental transfer of 2,4-D, seem inter-
esting. The results may be attributable to different factors, such
as change of placental permeability during pregnancy, lowering
of placental pH as compared to plasma, with a consequent lower-
ing of the degree of ionization of the phenoxy acid, and extra-
placental transfer. However, the present limited evidence does
not permit an assessment of the relative importance of the various
factors.

The distribution pattern of 2,4-D, administered as the ester,
did not differ appreciably from that observed with 2,4-D amine,
although tissue levels were consistently lower.

The apparent distribution volume of the compounds under
study can be estimated by plotting the dose-elimination curves
(Figs. 1—4) on a semi-logarithmic scale (log. conc. v. time) and
extrapolating the terminal, linear part of the plot back to zero
time. By relating the dose given to the "zero time plasma levels"
thus obtained, apparent distribution volumes of 25—50 % of
the body weight were found for the salts of 2,4-D and 2,4,5-T,
when administered orally as a single dose. These values are
intermediate between the extracellular body volume (about 20 %
of the body weight) and total body water (about 70 % of the
body weight), thus suggesting some penetration of the phenoxy
acids into the cells. Direct evidence for the permeability of blood
cells to phenoxy acids was obtained in the experiments sum-
marized in Tables 3 and 4. In the blood cells 2,4-D regularly could
be detected in concentrations ranging between 10 and 20 % of
the corresponding plasma levels. Moreover, the 2,4-D content
of the cells could be easily removed by repeated washing, which
indicates a ready passage of phenoxy acids across the erythrocyte
wall. The distribution equilibrium between plasma and blood
cells is obviously displaced towards plasma, a fact which is also
reflected in plasma being superior to saline in washing efficiency.
One reason for the equilibrium being displaced could conceivably
be a partial binding of the phenoxy acids to plasma proteins,
thus reducing the free (diffusible) fraction of phenoxy acids in
plasma. Indications of such a protein binding have been obtained
(*Erne* 1966 b).

Elimination. Elimination of the phenoxy acids from plasma,
after being administered as amine or alkali salts, was rapid in
all species studied. The semi-logarithmic dose-elimination curves

were linear in their terminal courses, indicating a first order elimination rate. The plasma half-life values calculated from these curves were about 3 hours for rats, 8 hours for calves and chickens and 12 hours for pigs (Table 1). No sex difference in respect to elimination rate was observed in rats, although the plasma levels were lower in the females than in the males.

The elimination rate from plasma of 2,4-D, given as the ester, was slightly reduced as compared with the soluble salts.

The rate of elimination of 2,4-D from tissues was lower than that from plasma (Tables 5—7). From the tabulated data, half-life values of 2,4-D ranging between 5 and 10 hours can be calculated for rats and between 10 and 30 hours for the other species. Generally, in 72 hours or less, tissue levels had dropped to a few µg/g. No retention of phenoxy acid was noted in the tissues examined. The results are in agreement with those obtained in sheep using C^{14}-labelled 2,4-D (*Clark et al.* 1964). The distribution pattern of 2,4-D on repeated administration was essentially similar to that obtained after a single dose, although individual variations were considerable, presumably owing to variations in feeding habit of the animals and in time between last water or food intake and time of killing (Tables 8 and 10). The results seem to support the view that halogenated phenoxyacetic acids are not likely to accumulate in tissues of clinically healthy animals.

Excretion. Judging from the analytical results the major excretory route is via the kidneys, at least in the mammalian species studied. Histopathological examination suggests that this is true for chickens as well (*Björklund & Erne* 1966). Only low levels of 2,4-D were found in feces from rats and pigs and in bile from pigs. That phenoxyacetic acids are predominantly excreted renally in cattle and sheep, was reported by *Lisk et al.* (1963), *Bache et al.* (1964 a), *St. John et al.* (1964) and *Clark et al.*

Interestingly, hens were found to be able to excrete part of ingested 2,4-D with the eggs (Table 6), a finding not previously reported. Most of the 2,4-D content of the egg was found in the yolk.

Adaptation. On prolonged administration of 2,4-D, given as amine or as ester in doses below the acutely toxic level, some experimental animals developed signs of an adaptation. In pigs, plasma levels of 2,4-D usually started to decline after about a

254

week, the urinary excretion concurrently increasing (Table 2, pigs no. 4 A and 9 A). A similar response was seen in chickens.

It should be remarked, however, that pigs seem to be less tolerant to prolonged exposure to phenoxy herbicides than are the other species studied. In several instances, 2,4-D given as daily doses of 50 mg/kg/day eventually produced signs of poisoning in pigs with rising plasma and tissue levels of 2,4-D (*Björklund & Erne*).

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SUMMARY

The distribution and elimination of two phenoxyacetic acids, 2,4-D and 2,4,5-T, were studied with a chemical method in rats, pigs, calves and chickens.

When administered orally as amine or alkali salts, the compounds were readily absorbed and distributed over the organism in all species studied. The absorption of 2,4-D in the form of an ester was incomplete, however, the ensuing plasma and tissue levels of 2,4-D being only low. (Intact ester could not be detected in plasma).

The highest tissue levels of 2,4-D and 2,4,5-T were found in liver, kidney, lung and spleen, the levels sometimes exceeding the plasma level. In blood cells 10—20 % of the plasma level was found. Penetration of 2,4-D into adipose tissue and into the central nervous system was restricted, whereas a ready placental transfer was demonstrated in swine. The distribution pattern did not show any significant species or — in rats — sex differences.

Elimination of the compounds was rapid, the plasma half-life being about 3 hours in rats, about 8 hours in calves and chickens and about 12 hours in pigs. The tissue half-life values ranged between 5 and 30 hours, the lower values being found in rats. No retention in tissues was noted, nor was accumulation seen on repeated administration.

In pigs and chickens an increased elimination rate was observed after repeated administration.

The major excretory route seemed to be via the kidneys in all species studied. Hens excreted small amounts of 2,4-D with the eggs.

ZUSAMMENFASSUNG

Verteilung und Elimination von chlorierten Phenoxyessigsäuren im Tierorganismus.

Die Verteilung und Elimination von zwei chlorierten Phenoxyessigsäuren, 2,4-D und 2,4,5-T, wurden an Ratten, Schweinen, Kälbern und Hühnern untersucht.

Nach oraler Eingabe, in Form der Amin- oder Alkalisalze, wurden die Verbindungen bei allen untersuchten Tierarten schnell resorbiert, und über den Organismus verteilt.

Orale Zufuhr von 2,4-D in Form eines Esters ergab jedoch nur niedrige Plasma- und Gewebsgehalte von 2,4-D, anscheinend infolge geringer Resorptionsgeschwindigkeit.

Nach einziger oraler Zufuhr von Phenoxyessigsäureaminsalzen wurden die höchsten Gewebskonzentrationen, bisweilen den Plasmaspiegel übersteigend, in Leber, Niere, Lunge und Milz gefunden. In

den roten Blutkörperchen wurden 10—20 % des Plasmagehalts nach-
gewiesen. 2,4-D gelangte nur in niedriger Konzentration ins Fettgewebe
und ins Zentralnervensystem. Dagegen wurde ein leichtes Durchdrin-
gen der Placentarbarriere beim Schwein nachgewiesen. Im Vertei-
lungsmuster waren keine geschlechtlichen (bei Ratten) und nur mäs-
sige tierartsgebundene Variationen wahrzunehmen.

Die Verbindungen wurden schnell eliminiert; die Halbwertzeit in
Plasma, nach einziger oraler Eingabe, betrug ca. 3 Stunden bei Ratten,
ca. 8 Stunden bei Kälbern und Hühnern und ca. 12 Stunden bei
Schweinen. Die Halbwertzeiten in den Geweben waren bei Ratten
5—10 Stunden und bei den anderen Tierarten 10—30 Stunden. Keine
Gewebsretention von Phenoxysäuren wurde festgestellt, ebenso keine
Ackumulation nach wiederholter Zufuhr.

Bei Schweinen und Hühnern wurde in einigen Fällen nach
wiederholter Zufuhr eine gesteigerte Ausscheidung von Phenoxysäuren
nachgewiesen.

Die Phenoxysäuren wurden bei allen untersuchten Arten haupt-
sächlich durch die Nieren ausgeschieden. Bei Hennen wurde eine
Ausscheidung von kleinen Mengen 2,4-D mit den Eiern nachgewiesen.

SAMMANFATTNING

Distribution och eliminering av klorerade fenoxiättiksyror hos djur.

Två fenoxiättiksyrederivats, 2,4-D och 2,4,5-T, distribution och
eliminering hos råttor, grisar, kalvar och höns har undersökts.

Tillförda oralt som amin- eller alkalisalter resorberades förenin-
gara lätt och fördelades snabbt över organismen hos alla undersökta
djurarter.

Efter tillförsel av 2,4-D ester påvisades endast låga plasmahalter,
synbarligen beroende på ofullständig resorption.

Lever, njure, lunga och mjälte visade de högsta vävnadshalterna,
vilka ibland översteg plasmanivån. I erytrocyter återfanns 10—20 %
av plasmahalten. Fettväv och centrala nervsystemet penetrerades en-
dast i ringa grad av 2,4-D, medan placentabarriären till synes obehin-
drat passerades hos svin. Distributionsmönstret visade ej något signi-
fikant art- eller, för råttor, könsberoende. Honråttor visade dock något
lägre plasma- och vävnadshalter än hanråttor.

Föreningarna eliminerades snabbt; halveringstiden i plasma var
c:a 3 timmar för råttor av båda können, c:a 8 timmar för kalvar och
höns och c:a 12 timmar för grisar. Halveringstiden i vävnaderna var
av storleksordningen 5—30 timmar; råttorna hade de lägre värdena.
Ingen retention i vävnaderna observerades och ej heller någon acku-
mulation efter upprepad tillförsel.

Hos grisar och höns iakttogs en upptränad utsöndring efter
upprepad tillförsel av 2,4-D.

Utsöndringen skedde huvudsakligen genom njurarna hos under-
sökta arter. Höns utsöndrade små mängder 2,4-D med äggen.