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DATA EVALUATION REPORT

STUDY TYPE: Metabolism Study (rat): Single Oral Dose

MRID NO.: 116493 TOX. CHEM. No.: 320

SPONSOR: The Boots Company Ltd., Nottingham
(Phenoxyacid Herbicide Consortium)

TESTING FACILITY: Report prepared by Hazleton Laboratories Europe Ltd., England

CITATION: Gilbert, C.; Hopkins, R.; Bibby, M.; et al. (1978) The Metabolic Fate of (^{14}C)-dichlorprop (dl-2-(2,4-dichloro (Ring-u-14C) Phenoxy)propionic Acid) in the Rat: Report No. 1313R2-277/1. (Unpublished study received Mar 28, 1979 under 264-222; prepared by Hazleton Laboratories Europe Ltd., Eng., submitted by Union Carbide Agricultural Products Co., Inc., Research Triangle Park, NC; CDL:237982-A)

CONCLUSION:

Groups of rats (3/sex) were administered labelled and unlabelled 2,4-DP acid by gavage at a single dose of 117 mg/kg. The results indicated that radiolabelled 2,4-DP acid was absorbed rapidly and subsequently underwent enterohepatic circulation in both males and females. Majority of the administered dose in both sexes was excreted in urine within 96 hr of dosing. No radioactivity was found in the expired air. No signifiaccumulation of radioactivity was found in any particular tissue.

This study has many deficiencies which include insufficient number of animals used (at least 5 animals/sex) and metabolites have not been identified in either unrine or feces. The study is unacceptable.

MATERIALS:

a) Male and female Sprague Dawley rats were obtained from Charles River Ltd., Kent, England. They weighed between 140 and 250 g.

b) Ring labelled ^{14}C-2,4-DP was used in the studies. The chemical analysis data indicated that specific activity was 4.7 mCi/mmol (20 uCi/mg), and radiochemical purity was 97%.

c) Unlabelled 2,4-DP was a white crystalline powder, and the chemical purity was assessed to be 99.7%.

METHODS:

1). Test agent: 25 mg of ^{14}C-2,4-DP and 1033 mg of unlabelled 2,4-DP were ground together in 45 ml of saline containing methyl cellulose (1%, w/v). The specific radioactivity of the suspension was determined before and after dosing; for the excretion study, the mean specific radioactivity was 11.6 uCi/ml (0.495 uCi/mg); for tissue distribution, 11.9 uCi/ml (0.509 uCi/mg); for bile cannulation, 12.7 uCi/ml (0.543 uCi/mg); for whole body autoradiography, 48.3 uCi/ml (2.06 uCi/mg).

2). Excretion: Group of rats (3/sex) were administered by gavage the mixture at approximately 1 ml/200 g of body weight (117 mg/kg). The animals were fasted for 17 hrs before dosing. Each dosed animal was placed in a metabolism cage for 96 hrs. Fecal samples were collected at 24 hr intervals; urine samples at 6, 12, 24, 48, 72, and 96 hr. For the first 24 hrs the expired air was monitored in a dosed male and female to determine release of ^{14}C-CO_2. At the end of the study, the cage washings were retained and assayed for any radioactivity present.

3). Tissue Distribution: Groups of fasted rats (14/sex) were administered (gavage) the mixture of labelled and unlabelled 2,4-DP in a volume of 1 ml 2,4-DP which was approximately equivalent to 11.9 uCi/200 gm body weight. At 1.5, 3, 6, 12, 24, 48, and 96 hr after dosing 2 animals/sex were sacrificed. Samples of blood and certain tissues were removed for determining tissue distribution of radiolabelled 2,4-DP acid.

4). Bile Duct Cannulation: The common bile duct of 3 males was cannulated for determining the amount of ^{14}C-2,4-DP acid in the bile. These rats were administered ^{14}C-2,4-DP acid by gavage at a dose of 117 mg/kg body, and the samples of bile were collected at intervals of 1, 2, 4.5, 8, and 24 hr after dosing.

5). Whole Body Autoradiography: Groups of rats (3/sex) were

administered by gavage 50 uCi of ^{14}C-2,4-DP acid. Two rats (one male and one female) were sacrificed at each interval after dosing (6, 24, and 48 hr). Longitudinal slices of the whole body were prepared for radiography.

RESULTS:

1). Excretion: Table 1 shows the mean percent of the administered radioactive dose in urine, feces, and expired air. Majority of the administered dose of 2,4-DP acid was excreted via urine for both male and female rats. No radioactivity was found in the expired air. The data indicated that majority of the administered dose was excreted after 96 hours of dosing (male, 84.8%; female, 90.1%).

2). Tissue Distribution: Figure 1 shows at 1.5 hr after dosing the plasma radioactivity concentration reached the highest level in both males and females. Subsequently the concentration dropped sharply at 3 hr and followed by a marked increase at 6 hr. The secondary increase in the plasma radioactivity concentration indicated reabsorption was occurring. Similar patterns were also observed in liver, kidney,and fat.

At 96 hr after dosing, more radioactivity was found in colon, skin, and fat relative to other tissues (Table 2), but the major portion of the radioactivity was excreted.

3). Bile Duct Cannulation: Between 40 and 52% of the administered radioactive 2,4-DP acid or its metabolites was found in the bile within 24 hr of dosing in the 3 males tested (Table 3).

4). Whole Body Autoradiography: The report stated that much of the radioactivity was located in the bladder, kidney, and peritoneal cavity of male rats sacrificed 24 hr after dosing whereas in females the radioactivity was detected in kidney, lower intestine, peritoneal cavity, and the skin.

It should be noted that the report available to this reviewer was reproduced from the microfiche, and the clearity of the autoradiograms was compromised.

DISCUSSION:

When radioactive 2,4-DP acid was administered by gavage to male and female rats with a single dose, the majority of the radioactivity was excreted within 96 hr (male, 84.8%; female, 90.1%). The major route of excretion was via urine (male, 73.5%; female, 82.1%). The remaining radioactivity appeared to accumulate in fat, skin, and colon of both male and female rats.

The absorption of 2,4-DP acid from the intestinal tract appeared to be rather rapid as evidenced by the high levels of radioactivity detected in plasma and other organs of both males and females at 1.5 hr after dosing (Table 2). In addition, 2,4-DP acid also underwent substantial enterohepatic circulation as indicated by the results of the bile duct cannulation experiment.

Another study, which applied two-dimensional chromatography to identify the possible metabolites in the urine samples collected in this study, found that approximately 75% of the administered dose was excreted unchanged in urine. It provides useful information to the understanding of the metabolic fate of 2,4-DP acid.

This study had many deficiencies which include insufficient number of test animals was used (at least 5 animals/sex) and not all metabolites were identified in either urine or feces samples.

TABLE 1

Mean percent recovery of the radioactive dose in urine, faeces and expired air following a single oral administration of (^{14}C)-DICHLORPROP to the rat at a dose level of 117 mg/kg body weight.

(Data taken from submission; MRID No. 116493)

Sample type	Collection period (hr)	Percent of radioactive dose		Cumulative percent of radioactive dose	
		Male	Female	Male	Female
Urine	0- 6	10.1	9.3	10.1	9.3
	6-12	20.5	29.9	30.6	39.2
	12-24	20.3	27.7	50.9	66.9
	24-48	18.3	13.0	69.2	79.9
	48-72	2.6	1.5	71.8	81.4
	72-96	1.7	0.7	73.5	82.1
	Total	73.5	82.1	73.5	82.1
Faeces	0-24	6.6	5.4	6.6	5.4
	24-48	2.0	1.1	8.6	6.5
	48-72	0.6	0.4	9.2	6.9
	72-96	0.9	0.4	10.1	7.3
	Total	10.1	7.3	10.1	7.3
Expired air	0-24	0	0	0	0
Cage washings	0-96	1.0	0.7	1.0	0.7
	Mean total recovery *	84.8	90.1		

Values are the means from three animals of each sex

* Values calculated from the total radioactivity recovered (Appendix 10.8)

Figure 1

(Data taken from submission; MRID No. 116493)

Mean concentration of radioactivity in plasma and tissues of the rat following a single oral administration of (^{14}C)-DICHLORPROP at a dose level of 117 mg/Kg body weight.

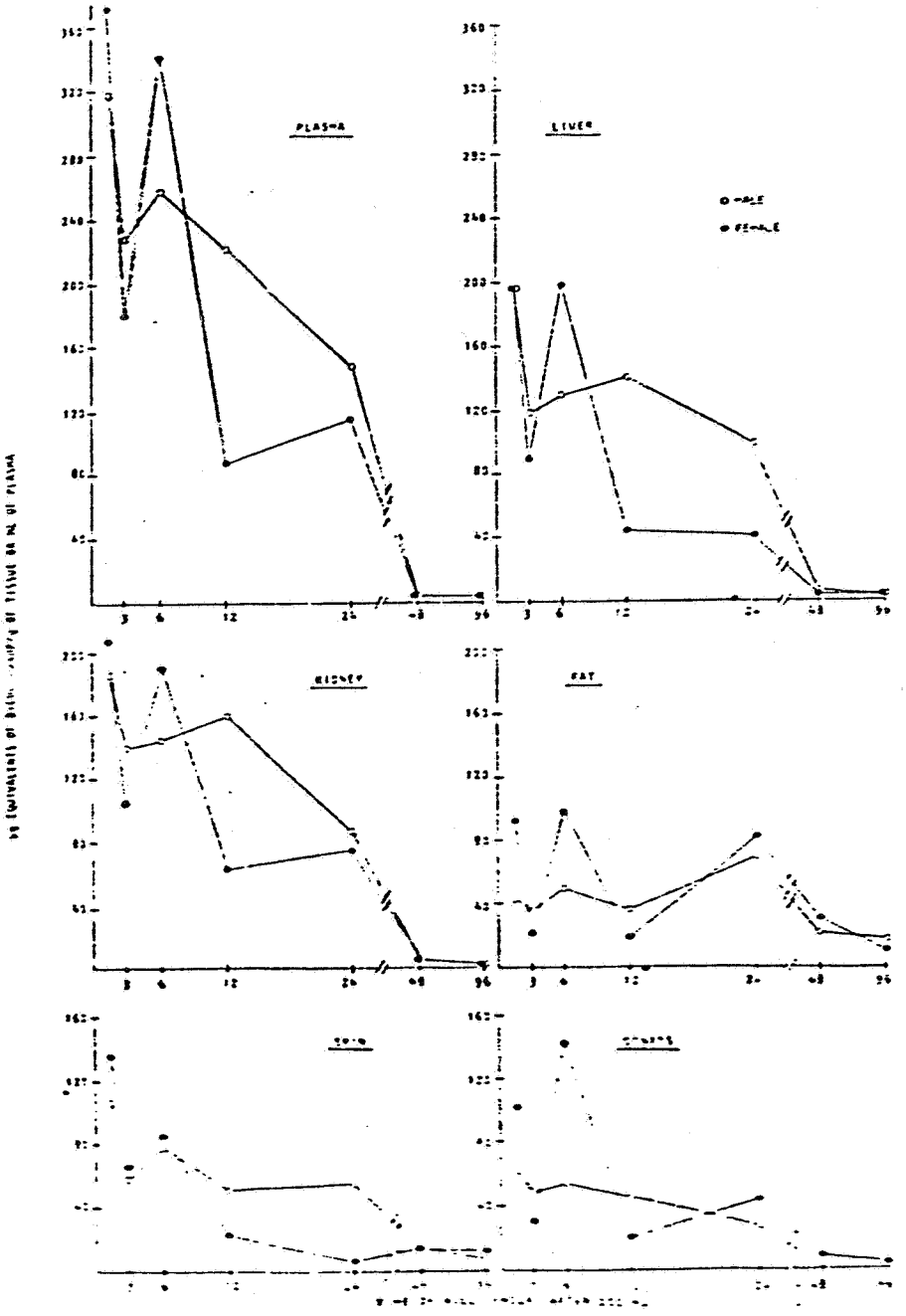

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TABLE 2

Mean tissue distribution of radioactivity following a single oral administration of (^{14}C)-bromacil to the rat at a dose level of 110 mg/kg body weight

(Data taken from submission; MRID No. 116493)

Tissue	Sex	Time of kill (in hours after dosing)						
		1.5 hr	3 hr	6 hr	12 hr	24 hr	48 hr	96 hr
Liver	♂	196.00	118.00	130.00	141.00	99.40	5.69	2.06
	♀	196.00	89.50	199.00	44.00	41.90	3.04	1.62
Kidney	♂	185.00	139.00	145.00	160.00	87.00	5.59	1.84
	♀	207.00	106.00	189.00	64.10	74.60	4.29	3.08
Heart	♂	127.00	90.00	77.80	74.10	53.90	1.97	1.15
	♀	162.00	70.30	126.00	26.70	3.06	1.50	0.64
Lung	♂	154.00	128.00	110.00	124.00	58.90	3.09	1.26
	♀	203.00	100.00	158.00	35.30	35.10	3.63	1.83
Spleen	♂	70.90	46.70	49.20	36.60	28.70	1.39	1.11
	♀	89.70	38.70	81.80	8.23	25.40	1.37	0.78
Adrenals	♂	56.90	135.00	106.00	56.40	64.60	21.30	2.65*
	♀	132.00	53.40	99.20	26.40	55.70	20.50	0.81
Thyroid	♂	188.00	129.00	58.00	58.00	45.80	2.85	0.5o*
	♀	169.00	68.20	93.90	23.70	46.20	3.18	1.27
Gonads	♂	59.00	49.70	53.90	45.30	27.30	0.62	0.67
	♀	102.00	30.30	142.00	20.10	45.50	6.50	3.10
Stomach	♂	716.00	555.00	261.00	313.00	51.80	5.13	3.35
	♀	869.00	125.00	136.00	129.00	30.40	5.90	2.28
Ileum	♂	119.00	70.90	81.30	69.20	48.00	6.24	1.98
	♀	116.00	73.20	96.30	19.60	27.90	5.31	2.00
Colon	♂	67.40	60.80	79.00	54.90	45.40	6.72	5.22
	♀	65.00	33.20	91.10	25.00	38.20	9.04	8.9o
Brain	♂	13.00	12.40	9.09	5.91	3.09	0.41	0.31
	♀	23.40	6.28	13.00	2.01	2.89	0.43	0.40
Eyes	♂	23.60	19.40	22.00	9.70	14.00	0.7o	0.10
	♀	28.60	16.10	28.20	3.39	3.22	0.39	0.21
Skin	♂	107.00	57.60	78.10	51.00	55.30	13.90	7.95
	♀	134.00	66.30	84.60	21.70	4.27	11.70	11.30
Muscle	♂	68.50	48.60	52.70	37.30	28.00	2.7o	1.o3
	♀	91.90	30.70	68.90	12.50	3.55	2.32	1.73
Fat	♂	42.90	3o.90	49.20	3o.10	70.00	21.50	1o.90
	♀	91.70	21.9o	97.40	18.10	40.3	24.40	9.59
Carcass	♂	101.00	34.00	70.30	37.30	4o.50	9.04	5.65
	♀	o3.50	29.10	74.90	21.10	41.50	9.09	7.33
Plasma	♂	319.00	228.00	258.00	221.00	14o.00	3.1o	1.31
	♀	372.00	180.00	341.00	o7.50	11o.00	2.26	1.2o

Radioactivity expressed in terms of [illegible] equivalents per [illegible] tissue, except for tissues where it is [illegible] equivalents ml

These values are the mean of two animals.

* Individual values.

TABLE 3

Percent recovery of radioactive dose in the bile of bile duct cannulated rats following a single oral administration of (^{14}C)-DICHLORPROP at a dose level of 117 mg/kg body weight

(Data taken from submission; MRID No. 116493)

Sample	Percent of radioactive dose in each sample			Cumulative percent recovery		
	1♂	2♂	3♂	1♀	2♀	3♀
Bile 0-1 hr	0.8	1.4	0.3	0.8	1.4	0.3
Bile 1-2 hr	1.2	1.6	0.6	2.0	3.0	0.9
Bile 2-4.5 hr	2.6	5.9	2.2	4.6	8.9	3.1
Bile 4.5-8 hr	8.9	10.8	10.0	13.5	19.7	13.1
Bile 8-24 hr	26.9	21.3	38.6	40.4	41.0	51.7
Total	40.4	41.0	51.7	40.4	41.0	51.7