[PARAQUAT] Developmental Study OPPTS 870.3700 (§83-3(a))

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DATA EVALUATION RECORD

STUDY TYPE: Prenatal Developmental Study - [rat]; OPPTS 870.3700 [§83-3 (a)]

DP BARCODE: D224841
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TEST MATERIAL (PURITY): Paraquat dichloride (38.2% paraquat ion content)

CITATION: Hodge, M. (1992) Paraquat: developmental toxicity study in the rat. ICI Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Study No. RR0593, Report No. CTL/P/3864, 30 November 1992. MRID 43964701. Unpublished.

SPONSOR: ICI Americas Inc. Agricultural Products, Wilmington, Delaware 19897

EXECUTIVE SUMMARY:

In a developmental toxicity study (MRID 43964701) paraquat dichloride (38.2% purity as paraquat ion content) was administered to 24 female Alderley Park, Wistar-derived (Alpk:APfSD) rats/dose by gavage in deionized water at dose levels of 0, 1, 3, or 8 mg paraquat ion/kg/day from days 7 through 16 of gestation.

No maternal or developmental effects were observed in the study.

The maternal NOEL is 8 mg paraquat ion/kg/day (HDT).

The developmental NOEL is 8 mg paraquat ion/kg/day (HDT).

The developmental toxicity study in the rat is classified as acceptable and satisfies the guideline requirement for a developmental toxicity study (OPPTS 870.3700; §83-3(a)) in the rat. Although there was no LEL for the study, the study is acceptable for regulatory purposes because the previous study conducted with the same strain of rat in the same laboratory in 1978 indicated that there was maternal toxicity at both 5 and 10 mg/kg/day. Because death was observed at both 5 and 10 mg/kg/day, the dose levels for this study were lowered slightly to a maximum of 8 mg/kg/day.

COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: paraquat dichloride
Description: dark brown/black aqueous liquor
Batch #: YF6219 ex. No. 9 Product Stock; Central Toxicology Laboratory reference number Y00061/160
Purity: 38.2% paraquat ion content w/v
CAS #: 1910-42-5

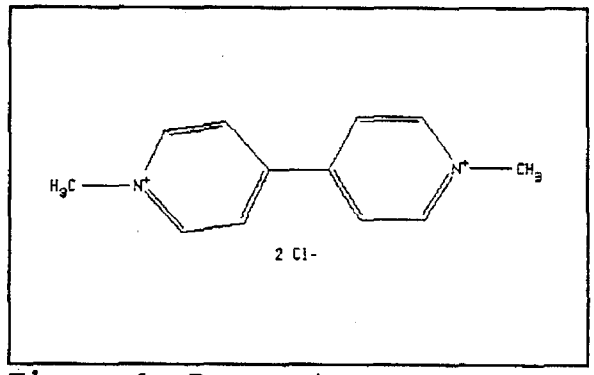

Figure 1 Paraquat

2. Vehicle: Deionized water
Description: clear liquid
Lot/Batch #: Y04517/015
Purity: 100% a.i.

3. Test animals: Species: rat
Strain: Alderley Park Wistar-derived (Alpk:SPfSD)
Age at mating: 11 wks
Weight at mating: 220 - 270g
Source: Barriered Animal Breeding Unit, ICI Pharmaceuticals, Alderley Park, Macclesfield, Cheshire, UK; the females were supplied over a 2 week period, having already been mated. Twelve females were supplied on each of 8 days.
Housing: Individually in rat racks suppled by Modular Systems and Development Company, Woolwich Industrial Estate, London; one solid stainless steel side, other side, floor, back and front constructed of stainless steel mesh.
Diet: CT1 diet suppled by Special Diets Services Limited, ad libitum
Water: tap water ad libitum

Environmental conditions:
Temperature: 19-23°C
Humidity: 40-70%
Air changes: 25-30/hr
Photoperiod: 12 hrs dark/ 12 hrs light
Acclimation period (P): Not applicable because the females were mated at the breeding unit and then sent to the laboratory.

B. PROCEDURES AND STUDY DESIGN

1. In life dates - start: May, 1992 end: June, 1992

2. Mating: Virgin females were paired overnight with unrelated males of the same strain. On the following morning, vaginal smears from the females were examined for the presence of sperm. The day when sperm were detected was designated Day 1 of gestation and on this same day successfully mated females were delivered to the experimental unit at the Central Toxicology Laboratory.

3. Animal Assignment: Animals were assigned to dose groups as indicated in Table 1. Assignment was divided into 24 replicates (computer randomized blocks). On arrival, each rat was randomly allocated to a cage (and therefore treatment group) within the replicate. Replicates were filled sequentially and 3 replicates were added to the study on each of the eight days on which rats were received.

TABLE 1 Animal Assignment

Test Group	Dose (mg/kg/day)[a]	Number of Females
Control	0	24
Low (LDT)	1	24
Mid (MDT)	3	24
High (HDT)	8	24

[a]Dosing was by gavage from days 7-16 (inclusive) with 1 ml dosing solution/100 g bodyweight.

4. Dose selection rationale: Dose levels were selected from the results of a previous developmental toxicity study in the rat (previously reviewed by the Agency).

5. Dosage preparation and analysis:

The report stated that "paraquat was formulated in deionized water and the concentration was adjusted to give a constant volume of 1 ml/100 g bodyweight for each dose level. An appropriate amount of deionized water was added to a weighed amount of paraquat to provide one dosing solution per dose level. Each dosing solution was then thoroughly mixed before being divided into aliquots....The aliquots were stored in the dark at room temperature and fresh bottles were used for each day of the study...A sample of each dosing solution was analyzed prior to the start of dosing to verify the achieved concentrations of paraquat. The chemical stability of paraquat in deionized water was determined by re-analysis of the low and high dose formulations after an interval of 32 days....The homogeneity of paraquat in water was assumed as all formulations were solutions."

Results:

Homogeneity Analysis: N/A

Stability Analysis: The stability of paraquat in aqueous solutions was shown to be satisfactory over a 32 day period. The percentages of the initial concentrations after 32 days were 101.1 and 105.1% for the 0.1 mg/ml and 0.8 mg/ml solutions, respectively.

Concentration Analysis: The concentrations of paraquat in deionized water were within 8% of nominal (range: 92.0-100.0% of nominal).

6. Dosage administration: All doses were administered once daily by gavage on gestation days 7 through 16 (inclusive) in a volume of 1 ml/100 g of body weight/day. Dosing was based on the daily body weight.

C. OBSERVATIONS

1. Maternal Observations and Evaluations: The animals were checked daily for mortality or clinical signs. Body weights were recorded on days 1 and 4 and subsequently on days 7-16 (inclusive) and on days 19 and 22 of gestation. The report stated that "the amount of food consumed by each animal over three day periods was measured by giving a weighed quantity of food contained in a glass jar on days 1, 4, 7, 10, 13, 16 and 19 and calculating the amount consumed from the residue on days 4, 7, 10, 13, 16, 19 and 22 respectively". Dams were sacrificed on day 22 of gestation. At sacrifice, each dam received a full gross post mortem examination. The intact gravid uterus (minus ovaries) was removed and weighed and the ovaries and uterus were examined. The following data were recorded: number of corpora lutea in each ovary and the number and position of implantations subdivided into live fetuses and early and late intra-uterine deaths. The intra-uterine deaths were classified as either early intra-uterine deaths showing decidual or placental tissue only or late intra-uterine deaths showing embryonic or fetal tissue in addition to placental tissue.

2. Fetal Evaluations: After weighing, the fetuses were killed with an intra-cardiac injection of pentobarbitone sodium solution. Each fetus was then examined for external abnormalities. All fetuses were examined internally for visceral abnormalities under magnification. They were sexed, eviscerated and fixed in methanol. The report stated that "the head of each fetus was cut along the fronto-parietal suture line and the brain was examined for macroscopic abnormalities. The carcasses were then returned to methanol for subsequent processing and staining with Alizarin Red S. The stained fetal skeletons were examined for abnormalities and the degree of ossification was assessed. The individual bones of the manus and pes were assessed and the result converted to a six point scale". The following points are a description of the assessment scale for skeletal ossification of the manus and pes in the rat.

"Scale

1. (good) Metacarpals/metatarsals fully ossified. 1st and 3rd rows of phalanges fully ossified.

2. Metacarpals/metatarsals fully ossified. 1st and 3rd rows of phalanges fully ossified, although an occasional phalanx (no more than 2 may be partially ossified.

3. Metacarpals/metatarsals fully ossified. Several phalanges from 1st and 3rd rows may be partially or not ossified with the remainder being fully ossified.

4. Some metacarpals/metatarsals partially ossified, the remainder being fully ossified. Several phalanges (no more than 6) from 1st and 3rd rows may be partially or not ossified.

5. Some metacarpals/metatarsals partially ossified, the remainder being fully ossified. The majority of phalanges from 1st and 3rd rows (i.e. more than 6) will be partially or not ossified, the occasional phalanx may be fully ossified.

6. (poor) Some metacarpals/metatarsals partially or not ossified the remainder being fully ossified. 1st row of phalanges usually not ossified and the 3rd row of phalanges partially or not ossified."

D. DATA ANALYSIS:

1. Statistical analyses: Maternal bodyweight during the dosing and post dosing periods was considered by analysis covariance on initial (day 7) bodyweight. Maternal food consumption during the dosing and post dosing periods, the numbers of implantations and live fetuses/dam, gravid uterus weight, mean fetal weights/litter and mean manus and pes scores were considered by analysis of variance. Maternal performance data, the proportion of fetuses with each individual manus and pes score, the proportion of fetuses with each defect and the proportion of litters with each defect were considered by Fisher's Exact Test. Pre-implantation loss, post-implantation loss, early intra-uterine deaths, late intra-uterine deaths, major external/visceral defects, minor external/visceral defects, external/visceral variants, major skeletal defects, minor skeletal defects and skeletal variants were analyzed as follows: (1) percentages were analyzed by analysis of variance following the double arcsine

transformation of Freeman and Tukey; (2) the proportion of fetuses and the proportion of litters affected were considered by Fisher's Exact Test. All statistical tests were two-sided with the exception of the analyses of each individual defect which were one-sided.

2. Indices: The following indices were calculated from cesarean section records of animals in the study:

$$\% \text{ pre-implantation loss} = \frac{\#\text{ corpora lutea} - \#\text{ implantations}}{\#\text{ corpora lutea}} \times 100$$

$$\% \text{ post-implantation loss} = \frac{\#\text{ implantations} - \#\text{ live fetuses}}{\#\text{ implantations}} \times 100$$

3. Historical control data: Historical control data were provided to allow comparison with concurrent controls. The data were for internal hydrocephaly in the brain, slightly dilated ureter and extra (14th) ribs - short. Only the brain had litter data. The rest of the data were only for the number of fetuses affected.

II. RESULTS

A. MATERNAL TOXICITY

1. Mortality and Clinical Observations: There were no mortalities during the study and no treatment-related clinical signs of toxicity were observed.

2. Body Weight: Body weight data were provided in the report, however, body weight gain data were not provided, except in graphical form. Therefore, the values in table 2 were calculated by the reviewer from the body weight data. Statistical analyses were not conducted on the body weight gain data. In the report, mean bodyweight of the high dose group (8 mg/kg/day) was statistically significantly less than the control group ($p < 0.01$ on days 8-10 and 12 and $p < 0.05$ on days 11, 13, 14 and 16). Mean bodyweights were not significantly less than controls on any of the other days. It is noted that percent decrease between the high dose group and the control ranged from 1-2%, which is not a biologically significant effect.

The study authors believed that there was a treatment-related effect at the highest dose level because there was a mean bodyweight loss between the first and second days of dosing while other groups,

including the control group showed a mean weight gain. The size of the effect varied with a maximum weight loss of 10 g for one female. There was an increased number of pregnant females affected in the high dose group (9) which lost weight compared to the control group (3) and the weight loss was generally greater in the high dose females. Eight high dose females had a weight loss of ≥ 4 g compared to a weight loss of 1-2 g in the 3 controls.

This effect is borderline at best. The maximum weight loss of 10 g in the 1 female was still only about 3-4% of her bodyweight, not a huge amount.

TABLE 2 Maternal Body Weight Gain (g)[a]

Interval	Dose in mg/kg/day (# of Dams)			
	0 (24)	1 (24)	3 (24)	8 (24)
Pretreatment: Days 1 - 7	31.6	30.9	30.7	34.6
Treatment: Days 8 - 16	39.5	38.4	41.9	38.9
Posttreatment: Days 16 - 22	65.5	66.2	71.3	61.1

a Data extracted from Table 5 of report CTL/P/3864.

3. Food Consumption: The report stated that "there was a marginal difference in food consumption [25.3 g/day versus 26.2 g/day] (which did not achieve statistical significance) for the 8 mg paraquat/kg/day group compared with the control group during Days 7-10. (The group mean bodyweight was higher than that of other groups at the start of dosing and therefore the animals might have been expected to eat more). There was no compound-related effect at 1 or 3 mg paraquat/kg/day." The figure and the table in the report support this statement.

4. Gross Pathology: No treatment-related macroscopic findings were observed in any dose group. One type of finding that was observed in all groups, including the controls was pelvic dilatation in the kidney. All other findings occurred in single animals.

5. Cesarean Section Data: No clear treatment-related effects were observed. The following table summarizes the results.

TABLE 3 Cesarean Section Observations[a]

Observation	Dose (mg/kg/day)			
	0	LDT	MDT	HDT
# Animals Assigned (Mated)	24	24	24	24
# Animals Pregnant	24	21	24	22
Pregnancy Rate (%)	(100)	(88)	(100)	(92)
# Nonpregnant	0	3	0	2
Maternal Wastage				
# Died	0	0	0	0
# Died Pregnant	0	0	0	0
# Died Nonpregnant	0	0	0	0
# Aborted	0	0	0	0
# Premature Delivery	0	0	0	0
Total # Corpora Lutea	317	277	320	290
Corpora Lutea/Dam	13.2	13.2	13.3	13.2
Total # Implantations	266	214	281	221
Implantations/Dam	11.1	10.2	11.7	10.0
Total # Litters	24	21	24	22
Total # Live Fetuses	256	206	273	206
Live Fetuses/Dam	10.7	9.8	11.4	9.4
Total # Dead Fetuses	0	0	0	0
Dead Fetuses/Dam	0	0	0	0
Total # Resorptions	10	8	8	15
Early	10	8	7	15
Late	0	0	1	0
Resorptions/Dam	0.4	0.4	0.3	0.7
Early	0.4	0.4	0.3	0.7
Late	0.0	0.0	0.04	0.0
Litters with Total Resorptions	0	0	0	0
Mean Gravid Uterus Weight (g)	72.6	65.7	75.7	64.2
Mean Litter Weight	50.1	45.3	52.4	44.0
Mean Fetal Weight (g)	4.77	4.75	4.62	4.61
Sex Ratio (% Male)	48.4	46.7	48.7	45.1
Preimplantation Loss (%)	17.2	23.7	12.4	26.0
Postimplantation Loss (%)	5.0	3.1	2.9	8.3

a Data extracted from Table 8 and Appendix 5.

B. DEVELOPMENTAL TOXICITY

No treatment-related differences between the treated and control groups were observed for either the external/visceral examinations or the skeletal examination. There were statistically significant differences for specific observations, however, there was no dose-response. The manus and pes table does not consistently show anything either. The following tables summarize findings for which a statistically significant response was observed.

TABLE 4 Intergroup Comparison of Fetal Defects and Variants[a]

Developmental Parameter	Dose Level of Paraquat (mg/kg/day)			
	0	1	3	8
Major External/Visceral Defects				
Prop. of fetuses affected	1/256	4/206	1/273	0/206
Percentage	0.4	1.5	0.4	0.0
Prop. of litters affected	1/24	3/21	1/24	0/22
Minor External/Visceral Defects Only				
Prop. of fetuses affected	2/256	6/206	1/273	6/206
Percentage	4.6	3.5	0.3	6.4
Prop. of litters affected	2/24	6/21	1/24	4/22
External/Visceral Variants				
Prop. of fetuses affected	16/256	19/206	14/273	12/206
Percentage	13.3	14.0	4.9	14.5
Prop. of litters affected	9/24	10/21	10/24	8/22
Major Skeletal Defects				
Prop. of fetuses affected	0/256	0/206	0/273	0/206
Percentage	0.0	0.0	0.0	0.0
Prop. of litters affected	0/24	0/21	0/24	0/22
Minor Skeletal Defects Only				
Prop. of fetuses affected	71/256	86/206[b]	80/273	67/206
Percentage	26.9	39.1	29.6	37/5
Prop. of litters affected	21/24	18/21	23/24	21/22
Skeletal Variants				
Prop. of fetuses affected	215/256	184/206	238/273	180/206
Percentage	85.6	86.2	87.1	87.9
Prop. of litters affected	24/24	20/21	24/24	22/22

[a]Extracted from Table 9.
[b]Statistically significant $p < 0.01$

TABLE 5 Summary of the Type and Incidence of Major Defects				
	Dose Level of Paraquat (mg/kg/day)			
	0	1	3	8
Microphthalmia (bilateral)	1			
Internal hydrocephaly		3		
Abdominal situs inversus			1	
Malrotated hindlimb (bilateral)		1		

Extracted from Table 10.

TABLE 6 Fetal External and Visceral Examinations: Group Mean Summary Data

Dose Levels (mg/kg/day)		0		1		3		8	
Findings	Type	Fetuses	Litters	Fetuses	Litters	Fetuses	Litters	Fetuses	Litters
Ureter									
Dilated - slightly	Minor	1	1	4	4	0	0	6*	4
Kinked	Variation	16	9	19	10	14	10	12	8

* = Significantly different from control fetal incidence: $p < 0.05$
** = Significantly different from control fetal incidence: $p < 0.01$
Extracted from Table 11.

TABLE 7 Fetal Skeletal Examinations: Group Mean Summary Data

Dose Levels (mg/kg/day)		0		1		3		8	
Findings	Type	Fetuses	Litters	Fetuses	Litters	Fetuses	Litters	Fetuses	Litters
Skull									
Parietals - partially ossified	Minor	0	0	5*	4*	5*	3	0	0
Fontanelle: anterior - widened slightly	Minor	2	2	2	1	0	0	2	2
Odontoid									
Not ossified	Var[a]	52	16	58*	11	71	17	42	14
Cervical vertebrae									
Arch partially ossified, 6th	Minor	0	0	4*	4*	1	1	1	1
Centrum not ossified, 2nd	Var	96	22	100*	18	94	21	66	21
Centrum not ossified, 4th	Minor	13	6	20*	9	10	7	4	4
Centrum not ossified, 3rd	Var	22	7	31*	9	27	13	5	5
Sternebrae									
Bipartite, 5th	Minor	29	15	44**	16	37	16	44**	14
Partially ossified, 5th	Var	112	23	110*	19	118	23	91	18
Extra Ribs									
14th - short length	Var	6	5	15*	8	22**	11	15*	8

* = Significantly different $p < 0.05$; ** = Significantly different $p < 0.01$
[a]Var = Variation; Extracted from Table 11.

Intergroup Comparison of Manus/Pes Assessment				
	Dose Level of Paraquat (mg/kg/day)			
	0	1	3	8
Manus Scores				
Prop. with score 3	4/256	1/206	2/273	1/206
Prop. with score 4	168/256	104/206**	166/273	116/206
Prop. with score 5	77/256	99/206	104/273	88/206**
Prop. with score 6	7/256	2/206	1/273	1/206
Mean manus score/litter	4.36	4.45	4.38	4.54
Pes Scores				
Prop. with score 3	1/256	0/206	0/273	0/206
Prop. with score 4	16/256	9/206	14/273	10/206
Prop. with score 5	229/256	179/206	245/273	190/206
Prop. with score 6	10/256	18/206*	14/273	6/206
Mean pes score/litter	4.98	4.99	4.99	5.03

*Statistically significant $p < 0.05$
**Statistically significant $p < 0.01$

III. DISCUSSION

A. INVESTIGATORS' CONCLUSIONS: The investigators believed that there was a slight maternally toxic effect on bodyweight and bodyweight gain at the highest dose level. Therefore, they set the NOEL for maternal toxicity at 3 mg paraquat ion/kg/day. The NOEL for developmental toxicity was set at 8 mg paraquat ion/kg/day (HDT) because no effects were observed at any dose level.

B. REVIEWER'S DISCUSSION

1. MATERNAL TOXICITY: The decrease in bodyweight and bodyweight gain was so slight that it did not appear to be biologically significant. Therefore, the NOEL for maternal toxicity is closer to 8 mg paraquat ion/kg/day (HDT).

2. DEVELOPMENTAL TOXICITY: There were no indications of developmental toxicity in the study. Therefore, the NOEL for developmental toxicity is 8 mg paraquat ion/kg/day (HDT).

C. STUDY DEFICIENCIES: There were no major study deficiencies. Although there was no LEL for the study, the study is acceptable for regulatory purposes because the previous study conducted with the same strain of rat in the same laboratory in 1978 indicated that there was maternal toxicity at both 5 and 10 mg/kg/day. The

dose levels for this study (1992) were based on the results of the previous study. Because death was observed at both 5 and 10 mg/kg/day, the dose levels for this study were lowered slightly to a maximum of 8 mg/kg/day.