Study Type: Reproduction

Study Title: Paraquat: Multigeneration Reproduction Study in Rats - Three Generations. Report No. CTL/P/719

Accession No.: 249911 and 249912

Record No.: 16071

Sponsor: Chevron Chemical Company, Richmond, California

Testing Lab.: Imperial Chemical Industries PLC, Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, England.

Date of Final Report: December 22, 1982

Test Material: Paraquat (1,1'-dimethyl-4,4'-bipyridylium) dichloride; technical grade - a brown, aqueous solution containing 32.7% w/w of paraquat ion.

PROTOCOL

I. Experimental Design: 21-Day old Wistar-derived rats of the Alderley Park strain, 15 males and 30 females/dose level (F_0 parents), were first acclimated for 8 days and then were fed diets containing 0, 25, 75 or 150 ppm of paraquat ion for 12 weeks (premating period) before they were mated to produce the F_{1A} litters. The F_{1A} litters were weaned at 21 days of age, but were not bred; the F_0 parents were remated 7 days later to produce the F_{1B} litters. The F_{1B} pups were weaned at 28 days of age, but remained housed by litter until 35 days of age, at which time 15 males and 30 females/dose level were selected for treatment with paraquat and for production of the F_{2A} and F_{2B} litters. The process of selection, feeding paraquat and mating was then repeated with the F_{2B} animals in order to obtain the F_{3A} and F_{3B} litters. The study was terminated after weaning of the F_{3B} litters, at 28 days of age. The F_0, F_1 and F_2 mothers were fed paraquat continuously throughout the two mating, gestation and lactation periods. The F_0, F_1 and F_2 fathers were fed paraquat continuously throughout the two mating periods. All surviving female parents from each generation were killed after weaning of the B litters. All surviving male parents from each generation were killed after completion of mating to produce the B litters. The sequence of events in this 3-generation reproduction study is illustrated in Figure 1.

The same batch of paraquat (S 358) was used throughout the study. The animals were assigned to different groups by a shuffle card method and were numbered by ear punching. The rats (F_0 generation) were obtained from the Animal Breeding Unit of Imperial Chemical Industries (ICI). The Alderley Park strain was used because background information was available on the reproductive performance of this strain from previous studies conducted by ICI. During the acclimation period, the rats were housed by sex in litters. During the premating period, males were housed individually and females were housed in groups of 2/cage. During mating, 2 females were housed with 1 male for periods ranging from 1 to 10 days. If there was no evidence of mating after 10 days, the first male was removed and after 3 days was replaced by another male. During the gestation and lactation periods, male and female rats were housed individually, the males being kept in a separate room. The temperature in the animal rooms was 22-25°C and the humidity 38-60%.

Food and water were supplied without restrictions. The basic diet fed was Porton Combined Diet (composition attached) which was purchased as a meal. Appropriate volumes of diluted paraquat were added to this diet, the mixtures were pelletized and the pellets dried and stored at room temperature. Dates of preparation of batches of diets and periods for which each batch was fed were reported for the entire study. Diets were analyzed frequently for the concentration of paraquat, but the homogeneity of paraquat in diets, using the standard mixing procedure, was determined before the initiation of the study. Chemical stability of paraquat in pellets was also studied.

II. Parameters Examined for the F_0, F_1 and F_2 Parents

1. Observations for abnormalities. Animals were observed daily for toxic signs and mortality. Each rat was also examined weekly, in detail, for clinical abnormalities.

2. Body weights were determined weekly for male and female rats throughout the premating period. After the premating period, male rats were weighed every 4 weeks until they were sacrificed and female rats were weighed on days 0, 7, 14 and 21 of gestation period.

3. Food consumption and utilization were determined for each cage of rats throughout the premating period. Food utilization was calculated as the total food consumed divided by the total weight gained by the animals.

4. Fertility of male and female rats.

5. Length of gestation.

6. Urinary paraquat. Paraquat was determined in the urine of 3 male and 3 female rats/dose level during the premating period. Individual urine samples were collected over a 3- to 4-hour period during week 8 for the F_0 parents and during weeks 7-10 for the F_1 and F_2 parents. Samples were pooled by sex and dose level for analysis. The sensitivity of the procedure used (detailed in the submission) was 0.05 ug of paraquat ion/ml.

7. Gross necropsy was performed on all F_0, F_1 and F_2 parents at the termination of a generation and on animals which died or had to be sacrificed (moribund animals) during the course of the study.

8. Histopathology was performed on the following tissues:

   a. Grossly abnormal tissues from all male and female rats used in this study.

   b. Reproductive tracts of infertile males and females. These included: prostate, epididymides, seminal vesicles, testes, ovaries, uterus and cervix.

   c. Uterus, cervix and ovaries from pregnant females which failed to litter.

   d. Tests from all F_0, F_1 and F_2 male parents.

   e. Lungs from F_0 and F_2 parents (8 males and 8 females/dose level).

   f. Trachea, brain, spinal cord, sciatic nerves, lungs, liver, spleen, kidneys, stomach, heart, pituitary, prostate, epididymides, seminal vesicles, testes, ovaries, uterus, cervix and mammary glands - from F_1 parents (25 females and 10 males/dose level).

   g. Tissues listed above (from F_1 parents) and eyes, thyroid, salivary glands, cervical lymph node, thymus, pancreas, esophagus, duodenum, jejunum, ileum, cecum, colon, mesenteric lymph node, adrenals, bladder, voluntary muscle, ribs and sciatic nerves - from moribund animals.

III. Parameters Examined for the Litters

1. Pups were checked daily for gross abnormalities and mortality.

2. Live and stillborn (or dead) pups were counted and sexed within 24 hours of parturition and on days 4, 10 and 21 post partum.

3. All pups were weighed individually within 24 hours of birth (day 0) and thereafter on days 4, 10, 21, 28 and 35 (only those selected for breeding).

4. Teratological (soft tissue) examination was conducted on all grossly abnormal pups and on those found dead, aged 18 days and younger. The following procedure was used: Wilson, J.G. (1965). Methods for Administering Agents and Detecting Malformations in Experimental Animals in 'Teratology: Principles and Techniques" eds. Wilson, J.G. and Warkany, J. Univ. of Chicago Press.

5. Necropsy was performed on pups over 18 days of age. The following pups were used:

   a. Pups found dead or moribundt (A and B litters).

   b. About 50% of the F_{1A}, F_{2A} and F_{3A} litters, at weaning time. (Remaining pups were discarded).

   c. All A and B pups with clinical abnormalities.

   d. About 50% of the F_{1B}, F_{2B} and F_{3B} pups which were not selected for breeding or histopathology examination. (Remainding pups were discarded).

6. Histopathology was performed on pups over 18 days of age. The following tissues were examined:

   a. Tissues listed under II.8.f. and g., but not the reproductive tracts and the mammary glands - from pups found dead or moribundt. If pups were dead or moribundt due to maternal death, at least one pup/litter was examined.

   b. All abnormal tissues from the A and B litters.

c. Tissues listed under II.8.f. and g., but not the mammary glands, from the following pups: 5 males and 5 females/dose level from the F_{1B} and F_{2B} litters; and 10 males and 10 females/dose level from the F_{3B} litters. Brain and spinal cord of the F_{3B} litters were not examined.

IV. Statistical Analysis

Body weight gain during the premating and gestation periods, food consumption and utilization, duration of the gestation period, proportion of pups born alive (Live Born Index), proportion of viable pups which survived to day 21 (Survival Index), total litter size on days 0, 10, 21 and 28, initial litter weight and litter weight gain on days 4, 10, 21 and 28 were analyzed statistically by comparing the treatment group means with the control group means, using a two-sided Student's t-test. One-sided Fisher's exact test was used in comparing the treatment group means with the control group means for the fertility of male and female rats and the proportion of viable litters in which all pups died by day 10 (Maternal Neglect Index).

V. Deviations from the Original Design of the Study

1. Reduction in the high-dose level of paraquat from 150 ppm to 145 ppm and then to 140 ppm. This study was started on 12/19/79 and was completed on 11/28/81. Because 3 high-dose female rats died from lung injury caused by paraquat during the second week of study, the 150 ppm level was decreased to 145 ppm. Because paraquat lung injury was also responsible for the deaths of several lactating F_1 females, the 145 ppm level was further decreased to 140 ppm. The actual feeding schedule for the high-dose animals was as follows:

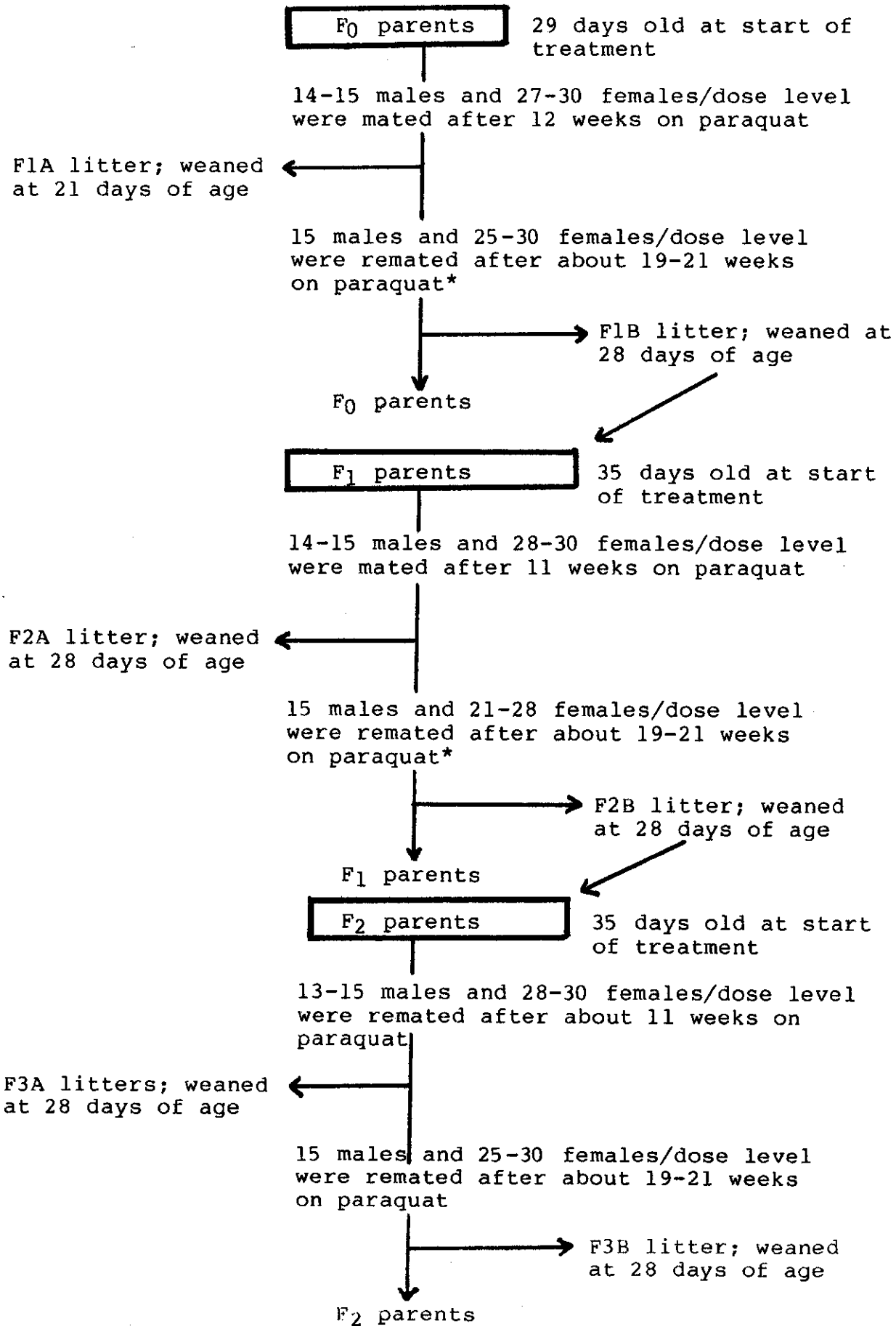
Paraquat ion (ppm)	Feeding Interval*
150	12/19/79 - 1/4/80; about 2 weeks.
145	1/4/80 - 12/10/80; about 10.5 months.
140	12/21/80 - 12/28/81; about 11 month and 1 week.

*Based on APPENDIX 6 of the submission.

2. Increased ingestion of paraquat during 8/12/80 - 8/28/80. Due to an error in the preparation and analysis of the 1% solution of paraquat used to prepare the test diets, animals in the 25 ppm and 75 ppm (nominal concentrations of paraquat ion) groups received diets containing, respectively, 52 ppm and 151 ppm of paraquat ion (analytical concentrations) for 15 days. The high-dose animals (nominal concentration of paraquat ion at that time: 145 ppm) received a diet containing actually 281 ppm of paraquat ion, for one day. (APPENDIX 3 and 6)

3. Remating of females infertile in the first mating. Because of deaths and suspected infertility in both the F_0 and F_1 parents, the number of females available for second mating was low in these groups. In an attempt to obtain sufficient B litters, females which did not produce litters were, therefore, remated.

Figure 1. Sequence of events in the 3-generation rat reproduction study (No. CTL/P/719; 12/22/82).



*Too few females were available for the second mating in these groups and the females which did not become pregnant during the first mating were, therefore, remated in order to produce sufficient B litters.

Composition of Porton Combined Diet*

	% w/v	
Barley	5.5 7.5	(up to 10.3.80) (after 10.3.80)
Wheat	20.0	
Maize	10.0	
Oats	17.5	
Wheat Feed	20.0	
Extracted Soya Bean Meal	10.0 8.0	(up to 10.3.80) (after 10.3.80)
Unextracted Yeast	2.5	
Denatured Skim Milk	10.5	
White Fish Meal	2.0	
Vitamin/mineral premix	2.0	

*As reported in APPENDIX 2 of the submission. The contaminants found in the diet (not specified) were not considered to be present in sufficient concentration to have influenced the outcome of the study.

RESULTS OBTAINED FOR PARENTS

1. Mortality and toxic signs

Mortality was reported for the male and female rats as numbers of animals found dead or killed moribundt and as percent of total (15 males and 30 females) in each group. Causes of mortality were also reported.

Total deaths in the control, low-dose (25 ppm) and mid-dose (75 ppm) male and female groups, and in the high-dose (150 ppm) male group of each generation ranged from 0 to 1, or 0-6.7% for the males and 0-3.3% for the females. Only in the F_2 male control group 2 moribundt rats had to be sacrificed. The highest incidence of mortality, attributed mostly to lung damage by paraquat, occurred among the high dose females. These data are summarized in Table 1.

Table 1. Mortality among the high-dose female rats*

Females rats	Found dead or sacrificed	Percent of total females	Deaths due to:	
			Lung damage[a] by paraquat	Other causes
F_0	8	26.7	7	1[b]
F_1	13	43.3	13	0
F_2	8	26.7	6[d]	2[c]

*This table is based on TABLES 6, 45, 50 and 55 of the submission.

a: Classified as severe acute, subacute or chronic.

b: Urinary tract disease.

c: Animals killed because of imperforate vaginas.

d: According to TABLE 6, paraquat was responsible for 5 deaths; according to TABLE 55, 6 deaths were associated with paraquat.

According to the testing laboratory, 3 females in the high-dose F_0 group died from paraquat toxicity during the first two weeks of the study, whereas the remaining females in that group and those in the high-dose F_1 and F_2 groups had litters of weaning age at the time of their deaths. Most of the F_0 and F_1 females died after producing, respectively, B and A littres, but no trend was apparent in the F_2 group. There were no deaths among the high-dose F_0, F_1 and F_2 male rats.

Other than hair loss (incidence not reported), considered by the testing laboratory to be unrelated to treatment, toxic signs were not observed in the parental groups.

2. Body weight gain by male and female rats during the premating period.

These data were reported as initial mean body weights for each test group and as weekly mean body weight gains for the premating peirods of 12 weeks (F_0 parents) and 11 weeks (F_1 and F_2 parents). Data included also statistical evaluation (two-sided t-test and approximate 95% confidence limits).

Paraquat, at all levels tested, had no effect on the body weight gains of most animals, both males and females when compared with the controls. Some animals gained less weight (mid-dose F_0 males) or more weight (mid-dose F_2 males and females, and high-dose F_2 males) than did the controls. These decreases or increases in weight gain occurred either during most of a premating period or during a part of it, were statistically significant at the 1% or 5% level (two-sided t-test), but were small (5-9%).

3. Food consumption

These data were reported as group mean weekly food consumption (g/rat) and as total food consumption (g/rat) during the premating weeks 1-12 (F_0 parents) and 1-11 (F_1 and F_2 parents). Data included also statistical evaluation (two-sided t-test and approximate 95% confidence limits). Most means were based on 15 cages of rats per dose level, except for the F_1 control groups where means were based on 12-14 cages of rats. In the case of the mid-dose F_1 parents, means were based on only 4 cages of rats during the premating week 5. In the case of the high-dose F_1 parents, means were based on only 2, 3 or 8 cages of rats during the premating weeks 4 and 5.

Paraquat, at all levels tested, had no effect on the food consumption of the male and female rats. In the case of the F_0 parents, statistically significant increases (13-20%) or decreases (7-9%) in the food intake occurred only occasionally and were dose-unrelated. In the case of the F_1 parents, dose-unrelated and mostly statistically insignificant decreases (3-14%) in the food intake occurred primarily during the second half of the premating period. In the case of the F_2 parents, the dose-unrelated and statistically insignificant decreases in the food consumption were 5-13% for the females and 2-7% for the males. The corresponding values for the increases in the food consumption were 9-10% and 2-18%.

4. Food utilization

These data were reported as group mean food utilization (grams of food consumed/grams of weight gained) for the premating weeks 1-4, 5-8, 9-11(12) and 1-11(12) for the F_0 and F_2 parents, and for the premating weeks 1-3, 6-11 and 1-11 for the F_1 parents. Data included also statistical evaluation (two-sided t-test and approximate 95% confidence limits. Incomplete food consumption data were available for the mid-dose and high-dose F_1 parents during the premating weeks 4 and 5, and food utilization was therefore not calculated during that period.

Paraquat, at all levels tested, had no effect on the food utilization of male and female rats. In the case of the F_0 and F_2 parents, both the controls and the paraquat-treated groups, food utilization was most effective during the first 3-4 weeks of the premating period and least effective during the last 3-4 weeks of the premating period. Similar finding were reported for the F_1 group.

The F_0 paraquat-treated male and female rats (all three levels) utilized food somewhat less efficiently when compared with the controls, but the differences were small (6% for the males and 1-8% for the females), statistically insignificant and dose-unrelated.

The F_1 and F_2 paraquat-treated male and female rats generally utilized food more efficiently than did the controls, but the differences were dose-unrelated and, in most instances, small and statistically insignificant. In the case of the F_2 parents, the increases in food utilization ranged from 2% to 10% for the males and from 1% to 6% for the females (group means for 11 premating weeks). In the case of the F_1 parents, the group mean increases in food utilization for the low-dose and mid-dose male rats were 1% and 9%, respectively. The corresponding values for the female rats were 5% and 11%.

In the case of the high-dose F_1 rats, there was a decrease in food utilization during the premating weeks 1-3 (8% for the males and 5% for the females) and an increase in food utilization during the premating weeks 6-11 (17% for the males and 7% for the females). None of these decreases or increases was statistically significant. As was already indicated, food utilization could not be calculated for the high-dose F_1 group during the premating weeks 4 and 5.

5. Urinary paraquat

Urinary levels of paraquat increased with doses, but varied greatly within each dose for the F_0, F_1 and F_2 parents. These data are summarized in Table 2. Paraquat was not detected in the urine of control rats.

Table 2. Concentration of paraquat in urine of the F_0, F_1 and F_2 parents*

Paraquat ion in diet (ppm)	25	75	150
Parents	Paraquat ion in urine (ug/rat)		
Males	1.6-2.1	4.5-9.6	9.9-10.6
Females	0.6-2.4	2.0-4.9	7.8-12.9
	Paraquat ion in urine (ug/ml)		
Males	0.7-1.8	2.1-4.5	3.6-5.3
Females	0.3-1.6	1.0-5.6	2.6-4.6

*This table is based on TABLE 24 of the submission. The sensitivity of the procedure used was 0.05 ug of paraquat ion/ml.

6. Necropsy and histopathology

The numbers of rats sacrificed and necropsied at the termination of each generation appear in Table 3.

Table 3. Rats examined grossly at terminal sacrifice

Paraquat ion (ppm)	0	25	75	150
Parents	FEMALES			
F_0	29	28	29	21
F_1	27	28	28	17
F_2	30	30	29	24
	MALES			
F_0	14	15	14	15
F_1	14	15	14	15
F_2	13	15	14	15

*This table is based on TABLES 46, 48, 51, 53, 56 and 58 of the submission. Gross necropsy findings were not reported.

The predominant microscopic lesions observed in the F_0, F_1 and F_2 parents at sacrifice were lung injury and focal alveolar histiocytosis, also in the lungs. Lung injury, attributed to paraquat, occurred only in the female rats and in the high-dose group. These data are summarized in Table 4.

Table 4. Incidence of lung injury in high-dose female rats due to paraquat*

High-dose female parents	F_0	F_1	F_2
Number examined	20	17	15
Number affected	6	6	4
Incidence (%)	30	35	27

*This table is based on TABLES 46, 51 and 56 of the submission.

Of the total number of lung injuries reported, 2 were classified as severe subacute and 14 as chronic (6 mild, 5 moderate and 3 severe).

Focal alveolar histiocytosis (FAH), described by the testing laboratory as an accumulation of large, foamy macrophages in subpleural and peribronchial tissue, was observed at all dose levels, including the controls. Within each sex, the incidence and severity of FAH were, in most instances, about the same in the control and the 25 ppm groups, but the incidence and severity of FAH were increased with dose in the 75 ppm and the 150 ppm groups. In the control and low-dose groups, FAH occurred generally more frequently in the female than in the male parents. In the mid-dose and high-dose groups, with one exception (F_2 males), the incidence of FAH was about the same in the male and female parents. Data concerned with incidence and severity of FAH are summarized in Tables 5 and 6.

Table 5. Incidence of focal alveolar histiocytosis in the lungs of male and female rats*

Paraquat ion (ppm)	0	25	75	150
	Female parents			
Number examined				
F_0	10	13	19	20
F_1	25	25	26	17
F_2	10	13	15	15
Number affected				
F_0	3	7	15	20
F_1	7	7	16	14
F_2	4	6	12	12
Incidence (%)				
F_0	30	54	79	100
F_1	28	28	62	82
F_2	40	46	80	80
	Male parents			
Number examined				
F_0	9	8	7**	10
F_1	10	10	11	14
F_2	9	8	10	14
Number affected				
F_0	1	1	5	8
F_1	3	1	5	12
F_2	1	0	1	5
Incidence (%)				
F_0	11	13	71	80
F_1	30	10	46	86
F_2	11	0	10	50

*This table is based on TABLES 46, 48, 51, 53, 56 and 58 of the submission.

**Lung tissue from one animal was accidentally omitted in this group. (Lung tissue from at least 8 rats should have been examined).

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Table 6. Severity of focal alveolar histiocytosis in the lungs of male and female rats*

Paraquat ion (ppm)	0	25	75	150
	F_0, F_1 and F_2 female parents			
Number examined	45	51	60	52
Severity of FAH**				
Minimal	13	17	27	24
Mild	1	3	10	19
Moderate	0	0	5	3
Marked	0	0	1	0
TOTAL	14	20	43	46
Incidence (%)	31	39	72	89
	F_0, F_1 and F_2 male parents			
Number examined	28	26	28	34
Severity of FAH**				
Minimal	3	2	8	14
Mild	1	0	2	10
Moderate	1	0	1	2
TOTAL	5	2	11	26
Incidence (%)	18	8	39	77

*This table is based on TABLES 46, 48, 51, 53, 56 and 58 of the submission.

**Focal alveolar histiocytosis.

Other lesions observed mostly in the F_1 male and female parents at termination were nephrocalcinosis, nephropathy, focal accumulation of lymphocytes or mixed inflammatory cells and focal hepatic necrosis. These lesions, classified as minimal, mild or moderate, occurred in the controls and the paraquat-treated rats, and were dose unrelated.

Adenomas or carcinomas were detected in the following animals: lung adenoma, in one high-dose F_0 female; pituitary adenoma, in one mid-dose F_1 female; and salivary gland carcinoma, in one control F_2 male.

7. Effect of paraquat on fertility of male and female F_0, F_1 and F_2 rats.

These data were reported as follows: (1) number of male and female rats mated at each dose level to produce A and B litters (TABLES 47, 49, 52, 54 and 57 of the submission); (2) total number of infertile F_0, F_1 and F_2 females/dose level (TABLES 47, 52 and 57); (3) total number of infertile F_0 and F_1 males/dose level (TABLES 49 and 54; F_2 infertile males were not reported); and (4) proportion of fertile animals (TABLE 25).

TABLE 25 of the submission, entitled INTERGROUP COMPARISON OF FERTILITY (proportion of fertile animals), consists of entries such as 23/30 77%, but neither numerators nor denominators or the term "fertility" are defined. Considering statements on pages 13 and 14 of the submission -

-"The fertility or otherwise of each male and female was established by the success (as measured by the production of a viable litter) of each mating. Where it was not possible to establish whether individual animals were fertile or infertile, these animals were excluded from consideration in the fertility indices" - it is confusing what was meant by "fertility"? Was it difficult to establish if a litter was viable?

Based on the reported data, paraquat had no effect on the fertility of the male and female F_0, F_1 and F_2 rats. The fertility rates (proportion of fertile animals) for the F_0, F_1 and F_2 parents were 86-100% for the males and 77-93% for the females. The dose-unrelated fertility rates for the paraquat-treated males and females were 85-100% and 73-96%, respectively. The lowest fertility rate (73%) occurred in the 25 ppm F_1 female group during the production of the A litter and was statistically insignificant when compared with the controls. Other than mating failure in some instances, no reasons could be found for infertility during necropsy and histopathological examinations.

If it is assumed that the denominators in TABLE 25 represent the numbers of animals which were mated, then some discrepancies exist between TABLE 25 and TABLES 47, 52 or 57. These discrepancies are summarized in TABLE 7.

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Table 7. Discrepancies in the number of female rats mated

Females mated	Paraquat ion (ppm)	Number of females mated according to:	
		TABLE 25	TABLES 47, 52 or 57
F_0, to produce F_{1A} litter	25	29	30
F_0, to produce F_{1A} litter	150	26	27
F_1, to produce F_{2A} litter	75	28	29
F_1, to produce F_{2A} litter	150	29	30
F_1, to produce F_{2B} litter	150	20	21
F_2, to produce F_{3B} litter	0	29	30
F_2, to produce F_{3B} litter	75	29	30

The number of infertile F_0, F_1 and F_2 rats, obtained from TABLES 47, 49, 52, 54 and 57, appear in Table 8, but it is difficult to relate these data to those reported in TABLE 25.

Table 8. Infertile rats in the production of the A and B litters

Paraquat ion (ppm)	0	25	75	150
Generation	Females			
F_0	3	1	1	2
F_1	2	4	3	1
F_2	2	2	1	1
	Males*			
F_0	2	2	1	1
F_1	1	2	2	2
F_2	Not reported			

*Rats suspected of infertility.

8. Length of gestation

These data were reported as group mean gestation lengths, with standard deviations (sd) and number of observations also included. Gestation lengths for individual animals were not reported. According to the MATERIALS AND METHODS section, the mean gestation period was measured in days, from date of positive smear to date of birth.

According to the reported data (TABLE 29 of the submission), paraquat, at all levels tested, had no effect on the length of gestation. In the case of the F_0, F_1 and F_2 controls (A and B litters), the gestation periods ranged from 21.95 to 22.50 days (sd: 0.21-0.60 days). In the case of the paraquat-treated F_0, F_1 and F_2 rats (A and B litters), the gestation periods lasted 21.90-22.32 days (sd: 0.20-1.32). These data were reported for 22-25, 20-26, 20-27 and 17-23 rats in the control, low-dose, mid-dose and high-dose groups, respectively. In three instances (2 in the low-dose and 1 in the mid-dose groups), there were statistically significant but very small (1 or 2%) increases or decreases in the gestation periods when the paraquat-treated rats were compared with the controls.

The absence of individual data and the inability, therefore, to clarify confusing points makes the evaluation of data summarized (by the testing laboratory) in TABLE 29 somewhat difficult. Illustrations appear below:

1. Acording to TABLE 29, 25 F_0 low-dose females had A litters and 22 F_2 control females had B litters. According to TABLE 26, there were 25 pregnant (with A litters) low-dose females on the gestation day 0; 24 on the gestation day 7; 25 on the gestation day 14; and 24 on the gestation day 21. The corresponding values for the F_2 control females were 22, 21, 22 and 21 (TABLE 28).

2. According to TABLE 27 (Litter F_{2B}), 23 rats were pregnant in the control group on the gestation day 21. However, the mean gestation length is reported for only 22 rats in that group, in TABLE 29.

3. According to TABLE 28 (Litter F^3_B), 18 high-dose rats were pregnant on the gestation days 0, 7, 14 and 21. However, the mean gestation length is reported for 20 rats in that group, in TABLE 29.

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RESULTS OBTAINED FOR LITTERS

1. Pups born alive

These data were reported as group mean percentages of pups born alive (Live Born Index) in the F_1, F_2 and F_3 A and B litters (TABLE 30 of the submission). TABLE 30 contains also number of litters for each generation and dose level, but it is unclear what these values represent. The term "number of litters" is not defined and the numbers reported in TABLE 30 are higher in all instances but three than the numbers of pregnant rats reported in TABLES 26, 27, 28 and 29. The total number of pups born at each dose level or the number of pups born to each animal within that level was not reported.

Based on the data summarized in TABLE 30 by the testing laboratory, paraquat, at all levels tested, had no effect on the Live Born Index. The percentages of pups born alive in the F_0, F_1 and F_2 control groups (F_1, F_2 and F_3 A and B litters) ranged from 97.3 to 99.7. The percentages of pup born alive in the low-dose, mid-dose and high-dose groups were 95.2-99.3, 95.7-99.0 and 97.6-99.7, respectively. The small differences between the control and the low-dose and mid-dose groups were not statistically significant.

According to the testing laboratory, the proportion of pups born alive was "considered by analysis of variance following transformation using the double arcsine transformation (Freeman and Tukey, 1950)"*. According to Mr. Bertram Litt, Biostatistician, Toxicology Branch/HED, this transformation is inappropriate for the data reported in TABLE 30.

2. Pups dying by day 10

These data were reported as numbers and percentages of viable F_1, F_2 and F_3 A and B litters in which all pups died by day 10 after their birth (Maternal Neglect Index; TABLE 31). The numbers of viable litters were reported as 1/23, 2/26 etc. The denominators were not defined.

*Freeman, M.F. and Tukey, J.W. (1950). Transformations Related to the Angular and the Square Root. Ann. Math. Statist. 21 607.

According to TABLE 31, the highest death rate occurred in the F_{1B} litters, was dose-unrelated and was attributed to environmental disturbance. According to APPENDIX 6 of the submission, maintenance work involving an electric drill was carried out in adjacent rooms during or shortly after the birth of the F_{1B} litters. The animals were bothered by noise and several temporarily stopped lactating. These data are summarized in Table 9.

Table 9. Maternal Neglect Indexes*

Paraquat ion (ppm)	0	25	75	150
Generation	Percent of litters in which all pups died by 10 days of age			
F_{1A}	4	8	7	9
F_{1B}	19	11	11	5
F_{2A}	4	9	5	0
F_{2B}	0	4	0	0
F_{3A}	4	4	0	0
F_{3B}	0	4	0	5

*This table is based on TABLE 31 of the submission.

There were no statistically significant differences in the Maternal Neglect Indexes between the paraquat-treated and the control groups. The actual numbers of pups dying in each litter, at each dose level, by the time they reached 10 days of age were not reported.

3. Pups surviving to day 21

These data were reported as group mean percentages of pups surviving to day 21 (Survival Index; TABLE 32 of the submission). TABLE 32 contains also number of litters for each generation and dose level. The term "number of litters" is not defined.

Based on the data summarized in TABLE 32 by the testing laboratory, paraquat, at all levels tested, had no effect on the Survival Index. The percentages of pups surviving to day 21 in the F_1, F_2 and F_3 control groups (A and B litters) ranged from 75.3 to 96.5. The percentages of pups surviving to day 21 in the low-dose, mid-dose and high-dose groups were 79.6-93.6, 79.4-95.0 and 83.5-93.4, respectively. The small, dose-unrelated decreases in the Survival Index in the F_{1A} group (3.4-6.0%) and the F_{3B} group (2.7-7.2%) were statistically insignificant when compared with the control values.

4. Group mean litter size

These data were reported as group mean litter size for the F_1, F_2 and F_3 A and B litters, on the post partum days 0 (within 24 hours of birth), 4, 21 and 28 (TABLES 33-38 of the submission). TABLES 33-38 contain also "number of litters", but this term is not defined. For all litters and treatment groups, the number of litters remains the same at each time interval when counting was made. This is confusing because, according to TABLE 31, certain numbers of whole litters were lost by day 10. Individual data (number of pups born to each female) were not reported. The numbers of male and female pups born at each dose level, in each generation, were also not reported.

Based on the data summarized in TABLES 33-38 by the testing laboratory, paraquat, at all levels tested, had no effect on the group mean litter size. On days 0 and 28, there were, respectively, 11-13 and 9-11 F_1, F_2 and F_3 pups/litter in the control groups. On day 0, the low-dose, mid-dose and high-dose groups had 11-13, 10-14 and 11-12 pups/litter, respectively. On day 28, the same groups had 10-11, 9-11 and 10-11 F_1, F_2 or F_3 pups/litter, respectively. The decreases at all dose levels in the number of pups/litter, when days 0 and 28 are compared, must apparently reflect death of pups during the post partum period.

5. Group mean litter weight gain

These data were reported as group mean initial weight and as group mean weight gain (in grams) on days 4, 10, 21 and 28, for the F_1, F_2 and F_3 A and B litters (TABLES 39-44 of the submission). Each TABLE contains also "number of litters" for each dose level and weighing day, but this term is not defined. If this term denotes the numbers of litters used to determine weight gain of the pups, it is then unclear why these numbers are sometimes different for male and female pups, unless there were litters with only male or only female pups which appears unlikely. Furthermore, the numbers of litters reported in TABLES 39-44 do not always correspond to those reported in TABLES 33-38 (in which the group mean litter size was reported). Verification was not possible because individual data (number of male and female pups born to each rat) were not reported. It was not even reported how many male and female pups were born at each dose level, in each generation.

Based on the data summarized in TABLES 39-44 by the testing laboratory, paraquat had no effect on the weight gain of the male and female pups. Although the F_{1A}, F_{1B} and F_{3B} male and female pups and the F_{3A} male pups, born to the paraquat-treated rats, gained less weight than did pups born to the controls, these weight gain decreases were in most instances small (2-11%), dose-unrelated and statistically insignificant. The statistically significant (all at the 5% level; two-sided t-test) group mean lower weight gains were reported in only 10 instances, 8 for the F_1 A and B litters and 2 for the F_{3A} litter. Of the 10 instances of the lower weight gains, 4 occurred in the low-dose groups, 5 in the mid-dose group and 1 in the high-dose (F_{1A}) group. These 10 statistically significant decreases in the group mean litter weight gains were mostly dose-unrelated and ranged from 11 to 28% (8 of them were 11-15%) when compared with the controls.

In the case of the remaining litters (F_2 A and B males and females, and F_{3A} females), the group mean litter weight gains were either the same or higher than those of the controls. The higher weight gains were statistically insignificant.

6. Soft tissue examination

The results of this examination were briefly discussed by the testing laboratory, but the incidence of pelvic dilatation of the kidney, the most frequent finding, was tabulated. A total of 266 (18 days old or younger) pups were examined, 35 from the control groups (3-7 F_1, F_2, or F_3 A or B pups/dose level) and 78, 100 and 53 from the low-dose, mid-dose and high-dose groups, respectively (3-31 pups/dose level).

The incidence of pelvic dilatation was reported as the number of the A and B litters affected/dose level/generation, but the number of pups affected was not reported. According to TABLES 59-61 of the submission, pelvic dilatation was observed in 2 litters born to the control groups and in 5, 9 and 3 litters born to the low-dose, mid-dose and high-dose paraquat-treated groups, respectively. The following abnormalities were also observed:

Abnormality	Number of pups affected and generation	Paraquat ion (ppm)
Large cavity within the cranium and brain situated to one side	1 F_{1A}	75
Internal bleeding and subarachnoid hemorrhage	2 F_{2A}	25
Cleft palate and enlarged lateral ventricles of the brain	1 F_{2B}	25
One toe missing from each of the front legs	1 F_{2B}	150
Slightly reduced jaw	1 F_{3A}	25
Extra testis and seminal vesicle	1 F_{3A}	75

A comment was made that "The incidence of these and other abnormalities was low and findings were of a type naturally occurring in the strain of rats used."

7. Gross necropsy and histopathology of the offsprings

Gross necropsy and histopathology were performed on pups over 18 days of age. These pups either died or had to be sacrificed during the study, or were sacrificed at the termination of the study. According to TABLES 62, 64, 66 and 70 of the submission, a total of 10 male and 21 female pups died or had to be sacrificed during the study. According to TABLES 63, 65, 67, 68, 69 and 71 of the submission, 35-94 F_1, F_2 or F_3 A or B pups per dose level, per sex, were grossly examined at the termination of the study. Gross necropsy findings were not reported.

The unscheduled deaths occurred as follows: 1 in the female F_{1B} control group; 2 in the female F_{1A} mid-dose group; and 28 in the male and female F_{1A}, F_{1B}, F_{2A}, F_{2B} or F_{3B} high-dose groups. Twenty-five deaths (10 male and 15 female pups) in the high-dose group were caused by the deaths of mothers, due to paraquat toxicity, before the pups were weaned. These pups belonged to two litters. The remaining pups in this and other groups died because of kidney disease and/or urinary tract disease.

The most frequent findings at the termination of the study were hydronephrosis, nephrocalcinosis (in females only), lung congestion and/or alveolar hemorrhage, perivascular inflammatory cell infiltration in the lungs (in high-dose pups only), focal accumulation of lymphocytes in the liver, and hypoplasia, atrophy and/or necrosis of the testes. The incidence of these findings is summarized by the reviewer in Tables 10 and 11, and is based on the numbers of pups examined. However, nothing can be said, in most instances, about the incidence of abnormalities in the whole litter for the following reasons:

°In the case of the A litters, only grossly abnormal pups were examined histologically and "grossly abnormal" was not defined.

°In the case of the F_1 and F_2 B litters, histopathological findings were reported for 5 pups and all grossly abnormal pups/dose level/sex, without separating abnormal pups from the randomly selected pups. Because these findings, as reported, were not based on random samples, it is impossible to relate the observed incidence to that in the whole litter.

°In the case of the F_{3B} litters, histopathological findings were reported for 10 pups and all grossly abnormal pups/dose level/sex, without separating abnormal pups from the randomly selected pups.

Table 10. Most frequent histological observations in males offsprings at termination*

Paraquat ion (ppm)	0	25	75	150
Tissue and test group	Number of pups examined			
Kidney: F_{1A}	6	1	2	1
F_{1B}	8	6	5	6
F_{2B}	10	7	5	5
F_{3B}	11	12	12	14
Lung: F_{1A}	0	2	5	0
F_{1B}	6	5	5	5
F_{3B}	12	11	11	11
Liver: F_{3B}	10	10	10	10
Testes: F_{1B}	5	5	5	6
F_{2B}	5	5	6	5
F_{3B}	10	10	10	10
Observation and test group	Incidence (%)			
Kidney: hydronephrosis; F_{1A}	100	100	100	100
F_{1B}	38	17	0	17
F_{2B}	50	29	0	20
F_{3B}	9	25	42	29
Lung: congestion and/or focal alveolar hemorrhage; F_{1A}	0	50	80	0
F_{1B}	17	0	5	20
F_{3B}	25	9	18	9
Lung: perivascular inflammatory cell infiltration; F_{1B}	0	0	0	80
Liver: focal accumulation of lymphocytes; F_{3B}	20	30	30	20
Testes: hypoplasia; F_{2B}	0	0	0	20
F_{3B}	0	0	0	10
Testes: atrophy; F_{2B}	0	20	17	40
F_{3B}	10	10	0	20
Testes: necrosis; F_{1B}	0	0	0	17

*This table is based on TABLES 63, 65, 67, 68, 69 and 71 of the submission.

Table 10. Most frequent histological observations in females offsprings at termination*

Paraquat ion (ppm)	0	25	75	150
Tissue and test group	Number of pups examined histologically			
Kidney: F_{1A}	0	4	3	3
F_{1B}	5	6	6	6
F_{2B}	7	5	5	7
F_{3B}	13	10	10	11
Lung: F_{1A}	1	2	5	4
F_{1B}	6	5	6	6
F_{3B}	10	11	10	10
Liver: F_{1B}	6	6	8	5
F_{3B}	10	10	10	10
Observation and test group	Incidence (%)			
Kidney: hydronephrosis; F_{1A}	0	100	100	100
F_{1B}	0	17	33	17
F_{2B}	57	0	0	29
F_{3B}	15	0	0	9
Kidney: nephrocalcinosis; F_{1B}	60	67	50	67
F_{2B}	14	0	20	29
F_{3B}	0	10	30	18
Lung: congestion and/or focal alveolar hemorrhage; F_{1A}	0	100	60	75
F_{1B}	17	0	33	0
F_{3B}	20	9	10	10
Lung: perivascular inflammatory cell infiltration; F_{1B}	0	0	0	33
Liver: focal accumulation of lymphocytes; F_{1B}	17	0	25	40
F_{3B}	50	40	40	40

*This table is based on TABLES 63, 65, 67, 68, 69 and 71 of the submission.

SUMMARY

Wistar-derived Alderley Park strain of rats, 15 males and 30 females/dose level, were fed technical grade paraquat (cation content: 32.7% w/w) for 11-12 weeks before they were mated to produce F_1, F_2 and F_3 A litters, and subsequently F_1, F_2 and F_3 B litters. The following levels of paraquat cation were fed in the diet: 0, 25, 75 and 150 ppm. The following parameters were examined for the F_0, F_1 and F_2 parents:

1. Toxic signs and mortality.
2. Body weight gain during the premating and gestation periods.
3. Food consumption and utilization during the premating period.
4. Fertility of male and female rats.
5. Length of gestation.
6. Gross necropsy of all animals.
7. Histopathology of all grossly abnormal tissues and of designated number of grossly normal tissues.
8. Paraquat levels in urine during the premating weeks 7, 8 or 10.

The following parameters were examined for the F_1, F_2 and F_3 A and B litters.

1. Group mean litter size.
2. Group mean litter weight gain.
3. Life Born Index (group mean percentage of pups born alive).
4. Maternal Neglect Index (number and percentage of viable litters in which all pups died by day 10).
5. Survival Index (group mean percentage of pups surviving to day 21).

6. Soft tissue (teratogenic) examination, conduted on 18-day old or younger pups (all grossly abnormal and those found dead).

7. Gross necropsy and histopathology, conducted on pups over 18 days of age (all pups with clinical abnormalities and designated number of pups/dose level/sex).

Statistical analyses (two-sided Student's t-test or one-sided Fisher's exact test) were applied to parameters 2-5 in the case of parents and 1-5 in the case of litters.

Paraquat, at all levels tested, had no effect on parameters 2-5, in the case of the F_0, F_1 and F_2 parents. However, there was a high incidence of mortality (27-43%) in the high-dose F_1, F_2 and F_3 female rats, due mostly to severe lung damage caused by paraquat. The incidence of lung injury (red or purple discoloration, congestion, edema, fibrosis, hyaline membrane formation, inflammatory cell infiltration and/or hyperplasia) ranged from 27 to 35%. There was also an increased incidence of alveolar histiocytosis in the lungs of the mid-dose and high-dose male and female parents. In the case of the F_0, F_1 and F_2 females, the incidence was 28-40% (control groups), 28-54% (low-dose groups), 62-80% (mid-dose groups), 80-100% (high-dose groups). The corresponding incidence for the males was 11-30%, 0-13%, 10-71% and 50-86%, respectively. The urinary levels of paraquat ion increased with dose, but varied greatly within each dose.

Paraquat, at all levels tested, had no effecct on parameters 1-5, in the case of the F_1, F_2 and F_3 A and B litters. The most frequent finding during the soft tissue examination was pelvic dilatation of the kidney. The numbers of litters affected were 2, 5, 9 and 3 in the control, low-dose, mid-dose and high-dose groups, respectively. However, nothing can be said about the incidence because the number of litters examined or the number of pups affected in each group were not reported.

The most frequent histological findings at the termination of the study were hydronephrosis, nephrocalcinosis (in female pups only), lung congestion and/or alveolar hemorrhage, perivascular inflammatory cell infiltration in the lungs (in high-dose F_{1B} pups only), focal accumulation of lymphocytes in the liver, and hypoplasia (in the high-dose F_2 and F_{3B} groups only), atrophy and/or necrosis (in the high-dose F_{1B} group only) of the testes. Based on the reported data, the

incidence of these findings was not dose-related. However, nothing can really be said about the incidence because either only the grossly abnormal pups (in the case of A litters) or the grossly abnormal pups and 5 or 10 grossly normal pups/dose (in the case of B litters) were examined. In the latter case, no distinction was made between the grossly abnormal pups and the randomly selected pups in the reporting of the data.

Paraquat was homogeneously distributed in the diets and was stable in the diets "up to at least 7 weeks".

COMMENTS

This study is poorly reported. With the exception of the histopathology data, which were tabulated without identifying any animals, most of the remaining data were reported as group means only, making it impossible to perform an independent evaluation and/or to clarify ambiguous statements. Although approximate 95% confidence limits were included in the body weight gain and food consumption and utilization data, it was stated that the data included also adjustments for missing values. However, without individual data, the reader has no idea how many or when adjustments were made.

In several instances, tables carry notation "number of litters", but the term is never defined. This leads to ambiguities, as in TABLES 33-38 of the submission, where the group mean litter size does not change during the 28-day post partum period, although 1-5 whole litters died in same groups during the first 10 days of that period. Other ambiguities are concerned with numbers of pregnant females, numbers of animals mated, definition of "fertility" and absence of footnotes and/or descriptions for some of the tabulated data, and are detailed in the review.

Although the term "grossly abnormal" (tissue or organ) is frequently used, the abnormalities were never described and gross necropsy findings were not reported for either parents or the pups. In the instances where histological findings for the grossly abnormal tissues were not separated from those for the randomly selected grossly normal tissues in the reporting of the data, nothing can be said about the incidence of these findings. Details appear in the review.

NOELs and CORE CLASSIFICATION OF STUDY

Provisional reproductive NOEL: 150 ppm (highest tested)
Reproductive LEL effects: Not determined.

Provisional systemic NOEL: 25 ppm
Provisional systemic LEL: 75 ppm (alveolar histiocytosis in male and female parents).

Core Classification: Supplementary

Toxicology Branch accepted the NOELs as provisional because of absence of individual data and because of deficiencies and/or ambiguities which were detailed in the review. The provisional NOELs and core classification will be updated upon the receipt and evaluation of the following data:

1. Summary table showing number of males mated and siring live young; number of females mated, killed or died, non-pregnant and those rearing young to weaning; and number of females with total resorption and total litter loss. The data should be separated for each dose level and generation.

2. Table(s) showing number of offsprings, stillbirth and live births for each female.

3. Distribution of sex between litters.

4. Individual body weight or body weight gain data showing adjustments for missing values.

5. Data showing when in which test group(s) and for how many animals adjustments were made in the food consumption data.

6. Gross necropsy findings for adult rats (and their ID numbers) and for the offsprings.

7. Clarification of data by supplying footnotes and/or explanations. Details appear in the review.