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TXR-2594

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

MEMORANDUM

002594

TO: Robert J. Taylor/V. K. Walters
Product Manager, Team #25
Registration Division (TS-767)

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

THRU: Edwin R. Budd, Section Head
Section II, Toxicology Branch
Hazard Evaluation Division (TS-769)

THRU: Orville E. Paynter, Chief
Toxicology Branch
Hazard Evaluation Division (TS-769)

SUBJECT: Paraquat: Miscellaneous Data - Mouse Feeding Studies.
EPA Accession No. 246502-246505. EPA Record No. 55650.
TOX Chem. No. 634

This submission consists of the following studies:

1. Mouse oncogenic study. Report No. CTL/P/556, dated 6/22/81. EPA Accession No. 246504.

2. Two 28-day preliminary mouse feeding studies. Report Nos. CTL/P/426 and CTL/P/433, both dated 7/11/79; EPA Accession Nos. 246502 and 246503, respectively.

3. Analysis of concentrated paraquat dichloride liquor (S 358) used for toxicology testing. Report No. RJ0208B, dated 3/12/81. EPA Accession No. 246505.

The purpose of the preliminary studies was to establish doses of paraquat ion for the mouse oncogenic study.

Paraquat was not oncogenic to the Alderley Park Swiss-derived mouse, male and female, under the conditions of this study. The highest level of paraquat ion fed was 100/125 ppm (100 ppm for the initial 35 weeks and then 125 ppm until the termination of the study, between 97-99 weeks).

The oncogenic study was classified as Core-Minimum. Because there are no core criteria for the remaining three studies, each one was classified as Acceptable.

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OPP:HED:TOX:K.LOCKE:sb 2/18/83 X71511 Rm 824 #m23

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PARAQUAT: 28-DAY PRELIMINARY FEEDING STUDY IN THE MOUSE

M. F. Sotheran, M. J. L. Clapp, P. R. Banham, A. Doss and K. Taylor. Imperial Chemical Industries Limited (ICI), Alderley Park, Macclesfield, Cheshire, U.K. Report No. CTL/P/426; July 11, 1979.

EPA Accession No. 246502 TOX Chemical No. 634
EPA Record No. 56650

SUMMARY

1. This preliminary study was conducted in order to determine suitable levels of paraquat ion for a lifetime feeding study (EPA Accession No. 246504). The preliminary study was initiated on May 16, 1977.

2. The test material was a crystalline paraquat (1,1'-dimethyl-4,4'-bipyridilium) dichloride of 100% purity, which was different from a technical grade paraquat dichloride that was used later in a mouse lifetime feeding study.

3. The levels of paraquat ion fed were 0, 25, 50, 75, 100, 125 or 200 ppm. However, because of errors in diet preparation, the 25 ppm group received 25 ppm of paraquat ion for 3 weeks and 38 ppm for one week. In the case of the 75 ppm group, 75 ppm of paraquat ion was fed for only one week and 45 ppm was fed for 3 weeks.

4. The diet used in this preliminary study was very similar to the diet used in the lifetime feeding study.

5. The test animals were Specific Pathogen-Free (SPF) Alderley Park Swiss-derived mice. They were used in this study because the same strain of mice was to be used in the lifetime feeding study. Twenty males and 20 females/dose were used.

6. The folowing results were obtained:

   - Paraquat at the 200 ppm level caused increased mortality in the males and the females, decreased body weight gain in the males, and increased food intake and decreased food utilization in the males and the females.

   - There was a tendency toward increased food intake and decreased food utilization with increasing levels of dietary paraquat.

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- o The lung weight and lung/body weight ratios showed dose-related increases. The weights of liver, kidneys, spleen, gonads, heart an brain were unaffected by treatment with paraquat.

- o The levels of paraquat ion in urine were dose-dependent. Paraquat was not detected in pooled samples of lung and kidney tissues, and essentially none was found in plasma.

- o Gross necropsy revealed dark red or patchy lungs in the male and female mice which died during the study, and in the male mice at the termination of the study.

- o Only lungs and kidneys were examined histopathologically from all the animals which died during the study, and from the selected animals at the termination of the study. The most common pathological changes attributed to paraquat toxicity were pulmonary congestion, edema and collapse, and kidney congestion.

7. It was concluded that the top level of paraquet ion in the lifetime feeding study should be no greater than 100 ppm. The no-effect level for the lifetime feeding study was not determined.

8. Classification of this study: Acceptable.

EXPERIMENTAL PROCEDURES

Specific Pathogen-Free (SPF) Alderley Park Swiss-derived mice, 20 males and 20 females per dose, were fed diets containing 0, 25, 50, 75, 100 or 200 ppm or paraquat ion for 28 days. The test material was a crystalline paraquat dichloride of 100% purity (batch ADY M 76/G). The diet fed in unrestricted amounts was a Porton Mouse Diet (PMD), supplied as pellets and then ground at the testing laboratory. Paraquat was added to the ground pellets as an aqueous solution, the mixture was pelletized and the pellets dried. The concentration of paraquat ion in the diet was determined in a random sample of each batch and the stability was assessed over a period of five weeks. The mice were 26-30 days old at the start of the study. They were housed 5/cage/sex, in a room maintained at the temperature of 20-24°C and the relative humidity of 51%. The acclimatization period was 7 days.

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The following parameters were examined:

1. Daily observations for symptoms of toxicity.

2. Individual body weights were recorded at the start of the study and then weekly throughout the study.

3. Food intake per cage of mice was recorded daily during the first week of the study and then weekly until the termination of the study.

4. Paraquat levels in urine, plasma, lungs and kidneys were determined as follows:

   - In urine, twice during the study, using 5 mice of each sex from the groups receiving 50, 100 or 200 ppm of paraquat ion.
   - In plasma, at the termination of the study, using 5 mice of each sex from the groups receiving 0, 50, 100 or 200 ppm of paraquat ion.
   - In lungs and kidneys, at the termination of the study, using 5 mice of each sex from the groups receiving 50, 100, or 200 ppm of paraquat ion.

5. The following organs were weighed from 7-10 mice of each sex, from groups receiving 0, 50, 100 or 200 ppm of paraquat ion: liver, kidneys, spleen, testes, lungs, heart and brain. Paired organs were weighed together.

6. All animals were autopsied. Histological evaluation was performed on samples of kidneys and lungs from all the animals that died during the study, and from 7-12 mice of each sex in the groups receiving 0, 50, 100, 125 or 200 ppm of paraquat ion.

7. Body weights, body weight gain, food consumption, food utilization and organ weights were analyzed separately for males and females by comparing the control group against each of the treatment groups using Student's t-test.

Mice were selected at random by shuffle cards for the determination of paraquet in urine, plasma and tissues; for organ weights; and for histopathological evaluations.

Detailed procedures for the assignment of animals to various test groups and for the determination of paraquat ion in animal diets, urine, plasma and tissues were submitted. The composition of the Porton Mouse Diet (attached to this evaluation) was also submitted.

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RESULTS

1. Problems encountered during the study

The following problems occurred during this study:

1. Errors were made in the preparation of some diets.
2. The automatic watering system failed (not stated for how long).
3. Five females were incorrectly sexed at the initiation of the study.

Errors in diet preparation

All batches of diet used in this study, except the control diets, were analyzed for paraquat ion concentration. With two exceptions, the 25 ppm and the 75 ppm levels, the mean results were within the experimental limits of ± 10% of the theoretical concentrations. In the case of the 75 ppm level, 75 ppm was mistakenly read as 45 ppm during the diet preparation on May 13, 1977, or 3 days before the study was started. Consequently, a diet containing 45 ppm of paraquat ion was fed to male and female mice for the three initial test weeks (out of total 4) before it was replaced with the correct (75 ppm) diet.

An error was also made in the preparation of the diet containing 25 ppm of paraquat ion. The analytical concentration of paraquat ion in the diet prepared on June 8, 1977 was 38 ppm, and not 25 ppm. This diet was fed to mice during the last test week of the study.

Failure in the automatic watering system

Shortly after the start of the study, there was a failure in the automatic watering system on rack 2, housing the following mice:

	Paraquet ion (ppm)						
Animals	0	25	50	25	100	125	200
Males	5	5	5	5	10	0	5
Females	5	0	5	5	5	5	5

It was not reported for how long the animals were left without water.

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Incorrect sexing of five female mice

Two, one and two female mice were incorrectly assigned to the male groups treated with 0, 25 or 100 ppm of paraquat ion, respectively. The error was discovered at the termination of the study. These animals were excluded from the body weight, organ weight, pathology and mortalities data, but were included in the data concerned with food consumption and urine analysis.

2. Observation for toxic signs

Toxic signs were not observed in this study. The mice which died due to administration of paraquat showed no clinical symptoms prior to death. Only the mice which were dehydrated, due to a failure in the automatic watering system, had a thin, hunched appearance. Most animals recovered within a few days after they received water again.

3. Mortalities

One male receiving 125 ppm of paraquat ion and six males and 14 females receiving 200 ppm of paraquat ion died during the test weeks 2-4, and their deaths were attributed to paraquat toxicity. The cause of death was not established for one control male and one female from the 50 ppm group; both animals died during the first test week. There were no other deaths.

4. Body weights

Male mice fed 200 ppm of paraquat ion and female mice receiving 25 ppm of paraquat ion had significantly lower body weight gains when compared with their controls. In the case of the 25 ppm group, the reduction in weight gain was insignificant when results from those animals affected by water deprivation were omitted. Mice in other groups, both males and females, were unaffected by treatment with paraquat. These data are summarized below.

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Paraquat ion (ppm)	0	25[a]	50	75[b]	100	125	200
Test animals	Mean body weight gains (g/mouse)						
Males: all animals	23.7	22.3	23.1	22.5	22.8	22.4	17.9[d]
Without those on rack 2[c]	22.8	22.2	21.3	21.5	21.4	22.4	17.7[e]
Females: all animals	14.9	11.8[d]	14.3	13.4	16.0	14.0	15.5*
Without those or rack 2[c]	13.1	11.8	12.5	12.2	12.2	12.5	14.0**

a. Due to an error in diet preparation, mice in this group were fed 25 ppm of paraquat ion for 3 weeks and 38 ppm for one (last) week.

b. Due to an error in diet preparation, mice in this group were fed 45 ppm for 3 weeks and 75 ppm for one (last) week.

c. These mice were affected by water deprivation.

d. Statistically significant at 1% level (Student's t-test).

e. Statistically significant at 5% level (Student's t-test).

* and **: Results based on only 6 and 4 animals, respectively.

5. Food Consumption

There was a tendency toward increased food intake and decreased food utilization with increasing levels of dietary paraquat. In the males, the increased food consumption occured mostly during test weeks 3 and 4 and in the females, during test weeks 2 and 3. With regard to the food consumption, the differences were most pronounced in males and females receiving 125 ppm and 200 ppm of paraquat ion, when these groups were compared with their controls. With regard to the food utilization, females did not generally utilize food as well as males. These data are summarized below.

Paraquat ion (ppm)	0	25[a]	50	75[b]	100	125	200
Test animals	Mean food consumption (g)/mouse/4 weeks						
Males[g]: all animals	168.2	188.7	185.0	174.9	174.8	206.7[d]	237.3
Without those on rack 2[c]	174.2	193.8	181.8	176.6	180.9	206.7[d]	263.5[e]
Females: all animals	141.3	148.1	167.6[e]	170.6[e]	151.4	169.3[e]	190.1[d]*
Without those or rack 2[c]	138.3	148.1	169.0	174.6	164.6	173.2[e]	186.1[e]**

	Food Utilization[f]						
Males[g]: all animals	7.4	8.6	8.1	7.9	7.7	9.2[e]	11.9
Without those on rack 2[c]	7.9	8.7	8.5	9.1	8.7	9.2[e]	13.1
Females: all animals	9.9	12.6[e]	12.3	13.7	9.8	12.6	9.5*
Without those or rack 2[c]	10.7	12.6	13.6	15.1	13.5	13.9[e]	9.5**

a. Due to an error in diet preparation, mice in this group were fed 25 ppm of paraquat ion for 3 weeks and 38 ppm for one (last) week.

b. Due to an error in diet preparation mice in this group were fed 45 ppm for 3 weeks and 75 ppm for one (last) week.

c. These mice were affected by water depravation.

d. Statistically significant at 1% level (Students t-test).

e. Statistically significant at 5% level (Student's t-test).

f. Expressed as mean food intake/g of body weight gained.

g. Five incorrectly sexed mice were included in the food consumption and utilization data for the males.

* Results based on only 6 animals.
** Results based on only 4 animals.

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6. Levels of paraquat ion in urine

These determinations were performed for groups of mice receiving 50, 100 or 200 ppm of paraquat ion in their diets, during the test days 14-17 and 22-23 for the males, and 11-15 and 21-22 for the females. The levels of paraquat ion in urine were dose-dependent. These data are summarized below.

Paraquat ion in diet (ppm)		50	100	200
Period (Day)		Paraquat ion in urine ug/ml*		
Males	14-17	5.1 3.4	14.2[a] 6.7[a]	16.0[b] 20.1[b]
Females	11-15	4.6	14.1	17.2[c]
	21-22	9.3	12.9	21.9

* Unless indicated otherwise, each value represents an average of 2 determinations, using pooled samples from 5 mice, from the same cage, per determination. The numbers of animals used in other determinations were as follows:

(a) 5 (one determination only);

(b) 2 and 4;

(c) 1 and 3; and

(d) 3 (one determination only). The limit of detection of the analytical method was not reported.

7. Levels of paraquat ion in plasma, lungs and kidneys

These determinations were performed at the termination of the study, using 5 mice of each sex from the groups receiving 50, 100 or 200 ppm of paraquat ion.

Paraquat ion was detected in plasma samples from 3 male mice only: 0.04 ppm was detected in one mouse fed 50 ppm of paraquat ion in the diet and 0.02 ppm was detected in 2 mice from the 200 ppm paraquat ion group. The limit of detection of the analytical method was 0.01 ppm.

Paraquat was not detected in any of the pooled lungs or kidneys analyzed.

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8. Organ weights and organ/body weight ratios

The weights of liver, kidneys, spleen, gonads, heart and brain were unaffected by treatment with paraquat. Lung weight and lung to body weight ratio showed a dose-related increase, as is evident from the data tabulated below.

Paraquat ion (ppm)	0	50	100	200
	Mean lung weights (g)			
Males	0.224	0.242	0.256	0.331*
Females	0.230	0.224	0.276	-
	Mean lung/body wgt ratios (g/100g)			
Males	0.571	0.635	0.631	1.092**
Females	0.705	0.730	0.888	-

*Statistically significant at 1% level (Student's t-test).
**Statistically significant at 5% level (Student's t-test).

The numbers of male mice used were 10, 9, 9 and 7 from dosages of 0, 50, 100 and 200 ppm of paraquat ion, respectively. The corresponding numbers of females were 10, 10, 9 and 0. Because of high mortality, organs were not weighed in the 200 ppm female group.

9. Gross necropsy

All animals were autopsied in this study. The only abnormal findings were dark red or patchy lungs. This was seen in all mice which died during the study and in several males autopsied at the termination of the study. Nothing remarkable was observed in the females at the termination of the study.

10. Histopathology

Only lungs and kidneys from the following animals were examined histopathologically: all the males and females that died during the study; the males, 7-11/level, that were fed 0, 50, 100 or 200 ppm of paraquat ion; and the females, 9-12/level, that were fed 0, 50 or 100 ppm of paraquat ion. Samples of lung tissue from the males and females in the 125 ppm group were also examined histopathologically. Lungs and kidneys from the female mice treated with 200 ppm of paraquat ion and sacrificed at the termination of the study were not examined.

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The most common pathological changes, attributed to paraquat toxicity, were pulmonary congestion, edema and collapse. These changes were observed chiefly in groups of mice treated with 125 ppm or 200 ppm of paraquat ion. Kidneys from mice fed 200 ppm of paraquat ion showed congestion in 50% of samples examined. Hydromephrosis was seen in all groups (including the controls), was found most frequently in the males, and was not attributed to paraquat toxicity.

11. It was concluded that the top dose level of paraquat ion in the lifetime feeding study should be no greater than 100 ppm, but a no-effect level for the lifetime feeding study was not determined.

12. Classification of this 28-day preliminary feeding study: Acceptable.

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DCR-16701:HED-13:ShirleyBailey/K.Locke:CM#2Rm824:5571511:2/22/83:efs

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PARAQUAT: 28-DAY PRELIMINARY FEEDING STUDY IN THE MOUSE

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APPENDIX 3 Parton Mouse Diet

COMPOSITION OF PMD DIET (SUPPLIED BY OAKES LTD, CONGLETON, CHESHIRE, UK)

Batch 34

Formulation

	%
Barley	5.625
Wheat	20.0
Maize	10.0
Oats	18.125
Wheatfeed	20.0
Soya (Canadian)	10.0
Roller-dried Skimmed Milk	7.5
Unext. Dried Yeast	2.5
Eng. White Fish-meal	5.0
Salt	0.25
Labvit A	0.5
Labmin K	0.5

Paraquat: Additional 28-Day Preliminary Feeding Study in the Mouse. M. F. Sotheran, M. J. L. Clapp, P. B. Banham and B. H. Woolen. Imperial Chemical Industries Limited (ICI), Alderley Park, Macclesfield, Cheshire, U.K. Report No. CTL/P/433; July 11, 1979.

EPA Accession No. 246503 TOX Chem. No. 634
EPA Record No. 56650

Summary:

1. This preliminary study was conducted in order to establish a dose of paraquat ion which could be used as a no-effect level in the mouse lifetime feeding study (EPA Accession No. 246504). The preliminary study was initiated on September 27, 1977.

2. The test material was a crystalline paraquat (1,1'--dimethyl-4,4'-bipyridilium) dichloride of 100% purity, where the ratio of paraquat ion: dichloride salt was 1:1.381.

3. The levels of paraquat ion fed were 0, 12.5, 25 or 50 ppm (analytical value was 42.5 ppm).

4. The diet used in this preliminary study was identical in composition to one of the diets used in the mouse lifetime feeding study.

5. The test animals were Specific Pathogen-Free (SPF) Alderley Park Swiss-derived mice. They were used in this study because the same strain of mice was used in another preliminary study (EPA Accession No. 246502) and was to be used in the mouse lifetime feeding study. Twenty males and 20 females/dose were used.

6. The following results were obtained:

- Paraquat, at all levels tested, produced no abnormalities in appearance, behavior, tissues (based on macroscopic examination) and body weight gains, and caused no mortalities. Histopathology was not performed.

- There was a tendency toward increased food intake and decreased food utilization with increasing levels of dietary paraquat, in males and females.

- Paraquat, at all levels tested, had no effect on the weight of the lungs in the males and the females, and the lung/body weight ratios of the males and the low-dose (12.5 ppm) females. The lung/body weight ratios of the females treated with 25 and 50 ppm of paraquat ion were 11 and 9% lower, respectively, when compared with the controls. These decreases were statistically significant at the 5% level (Student's t-test). Tissues other than lungs were not weighed.

- The levels of paraquat ion in urine were dose-dependent. The levels of paraquat ion in plasma cannot be properly evaluated because only one mouse/dose/sex was used in these determinations.

7. It was concluded that a level no greater than 12.5 ppm of paraquat ion should be used for a presumptive no-effect level in a mouse lifetime feeding study.

8. Classification of this study: Acceptable.

Experimental Procedures:

The design and execution of this study were the same as those reported for another 28-day preliminary study with mice (Report No. CTL/P/426, dated 7/11/79; EPA Accession No. 246502 and EPA Record No. 55650), except for the following:

1. The objective of this study was to establish a level of paraquat ion which could be used as a no-effect level in the mouse lifetime feeding study (EPA Accession No. 246504).

2. The levels of paraquat ion tested in this study were 0, 12.5, 25 or 50 ppm.

3. Porton Rat Diet with Vitamin E was used. This diet is very similar in composition to a Porton Mouse Diet (PMD), used in the earlier preliminary study (Report No. CTL/P/426).

4. Paraquat was not determined in tissues, tissues were not examined histopathologically, and only lungs were weighed in this study.

Results:

1. Observation for Toxic Signs:

No abnormalities in appearance or behavior were noted.

2. Mortalities:

There were no deaths in this study.

3. Body Weights:

Paraquat had no effect on body weights, when the treated mice are compared with the controls. These data are shown below.

Paraquat ion (ppm)	0	12.5	25	50
Test Animals	Mean Body Weight Gain (g/mouse)			
Males	13.1	12.0	14.3	12.4
Females	7.7	8.2	8.2	7.6

4. Food Consumption and Utilization:

There was a tendency toward increased food intake and decreased food utilization with increasing levels of dietary paraquat, in males and females. These data are summarized below.

Paraquat ion (ppm)	0	12.5	25	50 [a]
Test Animals	Mean Body Weight Gain (g/mouse/4 wks)			
Males	146.1	151.5	160.8[b]	161.3[b]
Females[c]	116.6	129.4	151.0	151.9
	Percent Increase in Food Intake			
Males	-	3.7	10.1	10.4
Females	-	11.0	29.5	30.3
	Food Utilization [d]			
Males	11.1	12.7	11.3	13.1[b]
Females	15.1	15.8	18.4	20.0

a According to diet analyses, this level was really 42.5 ppm of paraquat ion.

b Statistically significant at 5% level (Student's t-test).

c In the case of males, 4 cages of mice (total of 20 mice) per dose were used to calculate mean weekly food intake. In the case of females, 5, 10, 15 or 20 mice per dose were used to calculate mean weekly food intake. Some of the data had to be excluded because of problems with flooding of food from the antomatic watering system. Because of incomplete data, statistical analysis of total food intake and food utilization by the females were not performed.

d Expressed as mean food intake (g) per gram of body weight gained.

Despite incompleteness of data for the female mice, increased food intake and decreased food utilization with increased exposure to paraquat are evident.

5. Levels of Paraquat Ion in Urine:

Paraquat ion was determined in urine collected from one cage of mice (total of 5 mice), from each group, during the last week of the study. Data summarized below show that the concentration of paraquat in urine was dose-dependent.

Paraquat ion in Diet (ppm)	0	12.5	25	50
	Paraquat ion in Urine (ug/ml)*			
Males	0	0.37	1.20	1.50
Females	0	0.34	0.48	1.50

* The limit of detection of the method used was 0.005 ug of paraquat ion/ml of urine.

6. Levels of Paraquat Ion in Plasma:

These determinations were performed at the termination of the study, using one male and one female mouse from each test group.

Paraquat ion was detected only in plasma of mice which received 50 ppm of paraquat ion in their diet. The male mouse had 0.010 ug of paraquat ion/ml and the female mouse, 0.013 ug/ml of plasma. The limit of detection of the method used was 0.005 ug of paraquat ion/ml of plasma. However, the number of mice used (one mouse/sex/dose) was inadequate to evaluate paraquat ion in plasma.

7. Lung Weights:

Paraquat, at all levels tested, had no effect on the weight of lungs in male and female mice. The lung/body weight ratios were unaffected in the males, but were lower in the females which were fed 25 ppm and 50 ppm of paraquat ion. In the case of the 25 ppm group, the lung/body ratio was 11% lower than that of the controls. The lung/body weight ratio in the 50 ppm group was 9% lower than that of the controls. Since body weights and lung weights were unaffected by paraquat, these decreases in the lung/body weight ratios, although small, are difficult to explain. These data are summarized below.

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Paraquat ion (ppm)	0	12.5	25	50
	Mean Lung Weights (g)			
Males	0.247	0.237	0.251	0.238
Females	0.235	0.247	0.227	0.224
	Mean Lung/Body Weight Ratios[a]			
Males	0.557	0.549	0.544	0.549
Females	0.711	0.681	0.635[b]	0.645[b]

a Expressed as weight of lung (g)/100 g of body weight.

b Statistically significant at 5% level (Student's t-test).

8. Gross Necropsy:

No macroscopic changes were seen that could be associated with paraquat toxicity. Of the 20 animals examined in each group (that is, all animals), 18-20 males fed 0, 12.5 and 25 ppm of paraquat ion, and 18-19 females fed 0, 12.5 and 50 ppm of paraquat ion, had no abnormalities. There were 4 abnormalities in each, the male group receiving 50 ppm of paraquat ion and the female group receiving 25 ppm of paraquat ion. The few abnormalities, mostly single occurences, observed in the controls and the mice exposed to paraquat, were lungs with red patches and livers with white patches or white striations. Tissues were not examined histopathologically.

9. For the lifetime feeding study in the mouse, a level of 12.5 ppm of paraquat ion was recommended as a presumptive no-effect level.

10. Classification of this study: Acceptable.

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Paraquat: Analysis of Concentrated Paraquat Dichloride Liquor (S 358) used for Toxicology Testing. H. Swaine. Imperial Chemical Industries Limited (ICI), U. K. Report No. RJ0208B; March 12, 1981.

EPA Accession No. 246505
EPA Record No. 56650

TOX Chem. No. 634

Summary:

Technical grade paraquat (1,1'-dimethyl-4,4'-bipyridinium) dichloride, identified as Concentrated Paraquat Dichloride Liquor (batch No. S 358), was analyzed for the concentration of paraquat ion and impurities during 10/11/77-10/14/80. This material was used in long-term animal feeding studies, including a lifetime mouse feeding study (Report No. CTL/P/556; EPA Accession No. 246504).

The paraquat ion concentration remained essentially constant at 32.35 ± 0.18% w/w (corresponding to 44.64% w/w of paraquat dichloride) during that 3-year period of testing. Impurities, too, remained essentially constant. The purity of paraquat dichloride, with respect to non-aqueous constituents, was calculated as 96.1 ± 0.5%.

Classification of this study: Acceptable.

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PARAQUAT: LIFETIME FEEDING STUDY IN THE MOUSE

M. Sotheran, P. B. Banham, M. J. Godley, S. Lindsay, I. Pratt, K. Taylor and B. H. Woolen. Imperial Chemical Industries Limited (ICI), Alderley Park, Macclesfield, Cheshire, U.K.
Report No. CTL/P/556, June 22, 1981

EPA Accession No. 246504 Tox Chem. No. 634
EPA Record No. 56650

SUMMARY

1. This study was initiated on 10/25/77 and was terminated between weeks 97 and 99 (about 22.4 - 22.9 months) when 80% mortality was reached in a female control group and when mortality was approaching 80% in the study overall.

2. The study consisted of two parts: Part 1 contained 60 mice/sex/level for the oncogenic assessment of paraquat and Part 2 contained 10 mice/sex/level for the determination of paraquat in tissues and plasma. Part 2 was terminated after 52 weeks.

3. The test material was a technical grade paraquat (1,1'-dimethyl-4,4'-bipyridilium) dichloride, a brown aqueous solution containing 32.7% (w/w) of paraquat ion (an active ingredient). The same batch was used throughout the study. Paraquat was stable as such and after incorporation into the diets.

4. The animals used were male and female Specific Pathogen-Free (SPF) Swiss-derived mice, obtained from the Animal Breeding Unit, Alderley Park, Cheshire, U.K. The Alderley Part strain was selected because it was used in other oncogenic studies, conducted by ICI, and data on the incidence of tumors in this strain were, therefore, available.

5. The levels of paraquat fed (nominal concentrations, expressed as paraquat ion) were 0 (two controls), 12.5, 37.5 and 100/125 ppm. The mean analytical concentrations and the standard deviations of the means (in parenthesis) were 0, 12.8 (1.64), 37.8 (3.75) and 108.9 (10.23)/128.5 (5.73) ppm, or close to the nominal values. The selection of dosages for this study was based on the results of preliminary studies with the same strain of mice and 100 ppm was selected initially as a maximally tolerated level.

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Because the animals did not show toxic signs after eating 100 ppm of paraquat for 35 weeks, this dose was increased to 125 ppm at week 36 in order to evoke a toxic reaction. The highest level fed is, therefore, identified as the 100/125 ppm level.

6. Two dry diet mixtures (to which paraquat was added) were used in this study. Expanded Porton Rat Diet with a Vitamin E Supplement (PRDE) was fed to mice during the preexperimental period and from 10/25/77 (initiation of the study) until 8/21/78. At that time, a policy decision was made to change from PRDE to Porton Combined Diet (PCD), which was fed until the termination of the study (about 9/6-9/20/79; exact termination dates were not reported). The two diet formulations differed only in the content of sodium chloride, calcium and phosphorous.

7. The following determinations were made in the oncogenic (Part 1) study: daily observation for clinical and behavioral abnormalities, body weights, food consumption and utilization, urinary paraquat levels, gross necropsy and histopathology.

8. The following results were obtained in the oncogenic (Part 1) study:

   - Paraquat, at all levels tested, had no effect on the incidence of benign and malignant neoplasms in both male and female mice.

   - The most prevalent benign neoplasms were lung adenomas and Harderian gland adenomas in the males and the females, pituitary adenoma in the females and liver nodules (Type A) in the males.

   - The most prevalent malignant neoplasms were lymphosarcomas in the males and the females, and liver nodules (Type B) in the males.

   - The following treatment-related findings were reported for the 100/125 ppm level: non-neoplastic renal changes in the males and the females; sores and swelling in the genital area, increased mortality and weight loss, all in the females; and decreased food intake during test weeks 4-24 in the males.

- o The following findings, probably treatment-related, were reported for the 37.5 ppm group: renal tubular degeneration in the males; weight loss in the females; and decreased food intake during test weeks 6-20 and 56-84 in the females.

- o The only finding reported for the 12.5 ppm level (lowest tested) was a decreased food intake (5-16%) of the females during test weeks 6-24.

- o Paraquat produced minimal non-neoplastic changes in the lungs of mice in this study. Only the following findings in the 100/125 ppm group could probably be attributed to paraquat: a slight increase in the incidence of hypercellularity of alveolar walls in the males and the females; and a slight increase in the incidence of lung congestion and alveolar macrophages in the females.

- o Other organs and tissues examined were unaffected by paraquat under the conditions of this test.

- o Paraquat concentration in urine reflected a dose-related absorption, but the concentrations varied greatly within each dose.

9. Small amounts of paraquat were detected in plasma, kidneys and lungs of male and female mice. The levels observed appeared to be dose-related. However, due to analytical problems with the determination of paraquat ion in tissues, too few data were collected for the 12.5 and 37.5 ppm groups.

10. NOEL = 12.5 ppm (1.87 mg/kg), based on systemic findings; males and females. A small decrease in the food intake of females during weeks 6-24, observed at this level, is considered insignificant.

11. Paraquat was not oncogenic under the conditions of this study. The highest level tested was 100/125 ppm (15/18.75 mg/kg), in male and female mice.

12. Classification of this study: Core Minimum

EXPERIMENTAL PROCEDURES

This study consisted of 2 parts: Part 1 contained mice for the oncogenic assessment of paraquat and Part 2 contained

mice which were used only for the determination of paraquat in tissues and plasma.

In Part 1, Specific Pathogen-Free (SPF) Swiss-derived mice (Alderley Park strain), 60/sex/dose, were fed diets containing 0 (2 groups), 12.5, 37.5 and 100/125 ppm of paraquat cation for 97-99 weeks (about 22.4-22.9 months). The dose levels were selected from the results of 2 preliminary feeding studies with mice (EPA Accession Nos. 246502 and 246503). Based on these data, the 100 ppm level was selected initially as the maximally tolerated level for the mouse lifetime feeding study. Because no adverse effects occurred at the 100 ppm level after 35 weeks of feeding paraquat, this dose was increased to 125 ppm at week 36 in order to evoke a toxic reaction.

The mice were 19 days old when they arrived to the testing laboratory and 5 weeks old at the start of the study. Both the testing laboratory and the animal breeding unit were located in Alderley Park, Cheshire, England. During the study, the mice were housed by sex and dose levels, 5 mice/cage. The temperature in the animal room was 20-23°C and the relative humidity 35-72%. The Alderley Park strain of mice was used in this study because this strain was used in other oncogenic studies conducted by the laboratory (Central Toxicology Laboratory, ICI Limited). Historical data were therefore available for this strain of mice. The pre-experimental mean body weights of male and female mice were 29.8-30.3 g and 25.1-26.0 g, respectively.

Paraquat used in this study was supplied by ICI Limited as technical grade paraquat dichloride, a brown aqueous solution containing 32.7% w/w of paraquat ion. The same batch (S358) was used throughout the study. Appropriate volumes of an 1% w/w aqueous solution of paraquat dichloride were added to the dried diet mixture and the final mix was pelletized. The dry pellets were analyzed periodically for the concentration and stability of paraquat.

The diets were supplied by BP Nutrition (UK) Limited, Stepfield, Witham, Essex, England. During the pre-experimental period and from 10/25/77 (initiation of the study) until 8/21/78, Expanded Porton Rat Diet with a Vitamin E Supplement (PRDE) was fed to mice. At that time, a policy decision was made to change from PRDE to Porton Combined Diet (PCD), which was fed until the end of the study (about 9/6 - 9/20/79; exact termination dates were not reported).

The composition of the two diets was as follows:

FORMULATION OF EXPANDED PORTON COMBINED RAT DIET SUPPLEMENTED WITH VITAMIN E (PRDE) AND PORTON COMBINED DIET (PCD)

Constituent	% Content in:	
	PRDE	PCD
Barley	5.12	5.50
Wheat	20.00	20.00
Maize	10.00	10.00
Oats	18.12	17.50
Wheatfeed	20.00	20.00
Soya extracts	10.00	10.00
Non-denatured dried skim milk	7.50	--
Denatured dried skim milk	--	10.50
Unextracted dried yeast	2.50	2.50
White fishmeal	5.00	2.00
Salt	0.75	0.50
Vitamin supplement	0.50	--
Labmin K	0.50	--
Vitamin/Mineral premix	--	2.00

(It was stated that PCD contained 0.9-1.0% of calcium and 0.75-0.83% of phosphorus. In PRDE, the level of phosphorus is normally higher than that of calcium.)

The following determinations were made in the oncogenic (Part 1) study:

1. Animals were observed daily for clinical and behavioral abnormalities.

2. Body weights were recorded weekly for the first 12 test weeks; biweekly until week 36; weekly until week 40, following a change in the dose level from 100 ppm to 125 ppm; and then biweekly until the termination of the study.

3. Food consumption and utilization for each cage of mice was monitored weekly for the first 12 test weeks and during weeks 36-40; and weekly once every four weeks during test weeks 12-36 and from week 41 until the termination of the study.

4. Urinary paraquat levels were determined every three months, using 2 cages of mice/sex/dose for each determination.

5. All mice were autopsied as soon as possible after death.

6. Histopathology was performed on all Part 1 mice, on the following tissues: voluntary muscle, salivary glands, cervical lymph nodes, pancreas, spleen, liver with gall bladder, adrenals, kidneys, urinary bladder, heart, lungs, thyroid (with parathyroid in some animals), trachea, esophagus, thymus, mesenteric lymph node, jejunum, ileum, duodenum, colon, cecum, stomach, pituitary, brain, spinal cord, skin, aorta, eyes, Harderian glands and sciatic nerves, mammary gland, ovaries, cervix, uterus, testes, epididymes, seminal vesicle, preputial and prostate glands. Other tissues which appeared abnormal at autopsy were also examined histopathologically.

7. Statistical analyses were performed on most of the data. The most frequently observed clinical findings and the incidence of neoplastic and non-neoplastic findings were analyzed using a one-sided Fisher's exact test. In certain instances ("where the evidence for increased tumors, the level of tumor incidence and the mortality rates made it appropriate"), tumor incidence was also analyzed by the logrank test of Peto and Pike, 1973.* Weekly food consumption, food

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* Peto, R. and Pike, M. C., (1973). Conservatism of the Approximation Σ (O-E)Z/E in the Logrank Test for Survival Data or Tumor Incidence Data. Biometrics 29 579-584.

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utilization during the first 12 weeks of the study and body gain throughout the stud, were evaluated separately for males and females by analysis of variance. Group means were adjusted for missing values before comparisons were made. Each treated group mean was compared with the control group mean (combination of the two groups) using Student's t-test (two-sided). Mortality data were analyzed using the NCI program.** The analysis was performed with the two control groups separately and pooled. The analysis included pairwise comparisons of all groups and an overall test for trend with dose.

In Part 2 of the study, the SPF mice, 10 males and 10 females/dose, were fed diets containing 0, 12.5, 37.5 and 100/125 ppm of paraquat cation for 52 weeks. The following parameters were evaluated:

1. Animals were observed daily for clinical and behavioral abnormalities.

2. Paraquat levels were determined in lungs, kidney and plasma.

3. All animals were autopsied.

4. Histopathology was performed only on tissues which were considered abnormal.

** Thomas, D.G, Breslow N. and Gart, J.J., (1977). Trend and Homogeneity Analysis of Proportions and Life Table Data. Comps of Biomed Res 10 373-381.

RESULTS FOR PART 1 OF THE STUDY (ONCOGENIC ASSESSMENT OF PARAQUAT)

1. Clinical Observations

The most frequent observations in male and female mice (60/dose/sex) were as follows:

Paraquat ion (ppm)	0	0	12.5	37.5	100/125
Observation (males)	Number of mice affected				
Swelling in genital area	37	30	37	34	33
Sores in genital area	19	8	13	15	19
Incontinence	44	44	46	41	52
Distended Abdomen	13	11	11	12	13
Hair loss	21	20	19	18	5
Cataracts	4	2	2	2	3
Both eyelids swollen	17	21	17	13	17
Observation (females)					
Swelling in genital area	3	5	2	3	8
Sores in genital area	5	5	7	4	16
Incontinence	27	32	38	36	36
Distended abdomen	21	11	18	17	20
Hair loss	34	31	36	37	36
Cataracts	6	7	8	2	5
Both eyelids swollen	12	13	7	8	8

These data show that swelling and sores in the genital area, incontinence, hair loss and swollen eyelids predominated in the male mice. With regard to the female mice, incontinence, distended abdomen and hair loss were most frequently observed. In both sexes, these symptoms were not dose-related and were considered common findings in the Alderley strain mouse. The swelling around the genital area in males was attributed to the inflammation of the preputial gland or dilation of the ducts. Only in the females at the 100/125 ppm level were the swelling and sores in the genital area more prevalent than in other treatment or control groups. Females also had more cataracts than the males, but the incidence was not dose-related.

2. Mortality

This study was terminated between weeks 97 and 99 when 80% mortality was reached in one of the female control groups and was approaching 80% in the study overall.

The mortality data were reported for the test weeks 20-99 for the males and for the test weeks 20-98 for the females, but individual data were not reported. A portion of these data is shown below.

Paraquat ion (ppm)	0	0	12.5	37.5	100/125
Week	Percent mortalities (males)				
20	1.7	1.7	5.0	1.7	3.3
52	10.0	10.0	16.7	20.0	10.0
68	30.0	20.0	25.0	35.0	18.3
84	50.0	43.3	55.0	60.0	50.0
92	63.3	51.7	68.3	75.0	66.7
96	71.7	53.3	73.3	78.3	73.3
99	75.0	58.3	75.0	78.3	80.0
Week	Percent mortalities (females)				
20	1.7	0.0	1.7	3.3	1.7
52	16.7	16.7	15.0	16.7	16.7
68	28.3	28.3	21.7	23.3	36.7
84	50.0	46.7	46.7	51.7	61.7
92	68.3	55.0	58.3	68.3	80.0
96	75.0	60.0	66.7	73.3	83.3
98	80.0	65.0	68.3	78.3	86.7

According to these data, the mortality rates were low in all groups of mice during the first test year, but increased rapidly during the second half of the following year, as the animals grew older.

With two exceptions (males in the mid-dose group and females in the high-dose group), the mortality rates of the paraquat-treated mice were similar to those of the controls, for both males and females. The mortality rates for the male 37.5 ppm group and the female 100/125 ppm group were higher than the mortality rate of their controls. These groups showed increased mortality from about the test weeks 40 and 60, respectively. Because there was no increase in the mortality rate in the male 100/125 ppm group, it is difficult to attribute the increase in the mortality rate in the 37.5 ppm group to paraquat treatment.

3. Bodyweight Gain

Group mean body weights for male and female mice were reported for the test weeks 1-96, but individual data were not reported.

Paraquat, at all levels tested, had no effect on the bodyweight gain of male mice, but it caused weight loss in the female mice in the 37.5 ppm and 100/125 ppm groups. In the 37.5 ppm group, the weight loss started at about week 68 and was small (5-7%) during weeks 68-88. During weeks 92 and 96, the weight loss was 12 and 20%, respectively, when compared with the controls. In the 100/125 ppm group, the weight loss stated during week 44 and was 12-16% during weeks 44-72, 19-23% during weeks 73-88 and 35-45% during weeks 89-96. These weight losses were statistically significant at the 1% level (t-test, 2-sided).

4. Food Consumption and Utilization

Group mean food consumption (g/mouse/week) was reported for weeks 1-96. Because the mice were housed in groups of five per cage, the actual food intake by each mouse was unknown.

The control male mice consumed 31-44 g of food/mouse/week during this study, with most of the values being 38-44 g/mouse/week. During weeks 6-24, male mice in the 12.5 ppm and 37.5 ppm groups comsumed 3-5% less food than the controls. During weeks 4-24, male mice in the 100/125 ppm group consumed 7-15% less food than the controls and this was considered statistically significant at the 1% level (t-test, 2-sided). At other times the food intake of male mice was similar to that of controls.

The food consumption of the female control mice was 35-47 g/mouse/week, with most of the values ranging from 35 to 40 g/mouse/week during weeks 1-96. During weeks 6-24 and 76, the 12.5 ppm female group ate 5-16% and 19% less than the controls, respectively. At other times, their food consumption was similar to that of controls. At the 37.5 ppm level, there was a 3-15% decreased food intake during weeks 6-20 and sporadic decreases of 15-22% during weeks 53-84. At the 100/125 ppm level, the following decreases in food intake were reported: 8-14% during weeks 5-20; 21-34% during weeks 21-39; and 3-29 during weeks 40-88. During weeks 89-96, the 100/125 ppm female group consumed more food than the controls.

Group mean food utilization (g wt gain/g food consumed) was reported for weeks 1-12. As shown below, there was either no difference between the controls and the paraquat-fed mice or the utilization was better in the paraquat-fed mice.

Test	Food utilization (g wt gain/g food consumed)			
	Controls		Paraquat-fed mice	
	Males	Females	Males	Females
1-4	0.077	0.051	0.076-0.080	0.051-0.057
5-8	0.026	0.022	0.028-0.029	0.019-0.025
9-12	0.018	0.015	0.021-0.023	0.018-0.022

5. Paraquat ion Concentration in Urine

Paraquat was determined in the urine of mice during the test weeks 13-78. Although paraquat concentration in urine reflected a dose-related absorption, the concentrations varied greatly within each dose level, as is shown below.

Paraquat in diet, ppm	Paraquat ion in urine, ug/ml *	
	Males	Females
0	0	0.08**
12.5	0.36-0.70	0.27-0.76
37.5	1.04-4.10	0.49-2.60
100/125	2.90-8.95	1.15-7.70

Paraquat in diet, ppm	Paraquat ion excreted in urine, ug/mouse	
	Males	Females
0	0	0.22**
12.5	0.15-0.93	0.18-1.05
37.5	0.41-6.56	0.38-3.23
100/125	2.63-18.4	1.62-11.3

* Limit of detection: 0.05 ug of paraquat ion/ml.

** At the first urine sampling point (week 13), two control samples contained traces of paraquat. Exhaustive investigations failed to reveal the source of this contamination.

It is unknown now many mice were used per determination to obtain these data, especially during the second test year when the mortality rate began to increase. According to the footnotes on pages 61 and 62 of this submission (EPA Accession No. 246504), each figure in the table represented an average of two cages of aminals (5 mice/cage at beginning of study).

6. Pathology: Non-neoplastic Findings

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The non-neoplastic findings were reported in tabular form for animals surviving to the termination of the study (terminal kill) and for those dying before the end of the study (intercurrent deaths). The former involved all organs or tissues, whereas the latter were limited only to major or target organs (lung, kidney, liver, spleen, heart and eyes). Although in the RESULTS section references are frequently made to statistically significant or insignificant differences between the treated groups and the controls, these data (the non-neoplastic findings) do not contain statistical analyses. The following numbers of animals were examined:

Time of death	Controls	Paraquat ion, ppm		
		12.5	37.5	100/125
Intercurrent				
Males	77-80	43-45	47	45-48
Females	83-87	37-41	44-46	49-52
Terminal Kill				
Males	39-40	15	13	12
Females	33	19	14	8

Kidney: The treatment-related changes in this organ involved tubular degeneration and/or dilatation, and hydropic degeneration. These changes were slightly more prevalent in males than in females and were largely restricted to the 100/125 ppm group, especially in the females. A comment is made on page 15 of this submission that the renal lesions were initially seen after the dose level had been raised from 100 to 125 ppm in week 36 of the study and consisted of mild hydropic degeneration in some proximal tubules and increased eosinophilia in others. Approximately three months after the dose level had been raised, dilatation and degeneration of tubules in the renal cortex occurred. There was also an increased incidence of degenerating cortical tubules in male mice receiving 37.5 ppm of paraquat. Data showing these and other frequently observed renal changes are summarized below.

Incidence* of renal changes observed at intercurrent deaths.

Paraquat ion (ppm)	0	12.5	37.5	100/125	0	12.5	37.5	100/125
Observation	Males				Females			
Tubular necrosis	14	20	40	65	7	7	9	50
Tubular dilatation	1	4	2	6	2	2	7	15
Hydropic degeneration			2	15				14
Nephropathy	16	26	17	8	18	15	22	10
Interstitial nephritis	39	31	28	21	25	32	37	23
Pelvic dilatation	29	42	51	50	9	10	4	15

* Percent of animals examined. Blank spaces denote the absence of pathological changes.

Incidence* of renal changes observed at terminal kill.

Paraquat ion (ppm)	0	12.5	37.5	100/125	0	12.5	37.5	100/125
Observation	Males				Females			
Tubular necrosis	8	13	38	67	6			63
Tubular dilatation		7	8	17	6	5	29	25
Interstitial nephritis	15	13	23	25	24	5		13
Pelvic dilatation	45	33	46	50	9	5	21	25

* Percent of animals examined. Blank spaces denote the absence of pathological changes.

Changes in the kidney such as nephropathy, interstitial nephritis and pelvic dilatation were considered as common findings in mice "of this age" and, therefore, not related to treatment, "although there were occasional statistically significant differences between treated groups and controls." As was already indicated, statistical analyses were not reported for these data. With regards to the intercurrent deaths, the term "of this age" must refer to the second year of the study when the death rate began to increase rapidly. The non-neoplastic findings were not reported for the individual animals or when each animal died.

Lung: Paraquat was essentially without effect on the lungs of mice. Only three findings in the 100/125 ppm group could probably be attributed to paraquat: a slight increase in the incidence of hypercellularity of alveolar walls in males and females; and a slight increase in the incidence of lung congestion and alveolar macrophages in the females. The most common findings in the lungs of animals that died before the termination of the study were as follows:

Paraquat ion (ppm)	0	12.5	37.5	100/125	0	12.5	37.5	100/125
Observation	Percent of animals examined							
	Males				Females			
Thickened alveolar walls	5	7	2	13	0	0	4	10
Perivascular inflammation	13	11	2	15	6	7	11	8
Congestion	25	33	19	35	24	27	28	37
Alveolar edema	26	35	23	31	41	34	24	40
Perivascular edema	14	9	11	13	13	12	11	15
Hemorrhage	5	4	4	6	3	2	11	8
Increased alveolar macrophages	8	9	4	6	5	7	2	13

Single instances of pneumonitis were observed in the control males, in each group of the paraquat-treated males, and in the 100/125 ppm group of the females. The control females had two instances of pneumonitis, and the remaining paraquat-treated groups had none.

There were no predominant findings in the lung at the terminal kill. Only single instances of some of the abnormalities reported for the intercurrent deaths were observed at the terminal kill in the controls and the paraquat-treated groups.

Liver, spleen and heart: The most frequent observations in these organs were as follows:

Paraquat ion (ppm)	0	12.5	37.5	100/125	0	12.5	37.5	100/125
Observation	Percent of animals examined							
	Males				Females			
Liver: necrosis	16	4	13	15	16	15	20*	8
fatty vacuolation	18	16	26*	21*	11	10	13	8
hepatitis	24	16	17	15	10	7	11	12
Spleen: hemopoiesis	27	16	23	11	30	28	22	27
Heart: myocarditis	9	20*	13*	10	6	7	11*	4

Except for the isolated dose-unrelated observations, marked with an asterisk, paraquat had no effect on the liver, spleen and heart.

Eye: There was a very high incidence of lenticular changes in male and female mice from the control and the paraquat-treated groups. These changes were observed in animals which died or had to be sacrificed before the termination of the study (intercurrent deaths) and at the terminal kill. The most frequent findings and their incidence are listed below.

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Paraquat ion (ppm)		0	12.5	37.5	100/125	0	12.5	37.5	100/125
Observation	Time of Death	Percent of animals examined							
		Males				Females			
All types of lenticular changes	I	81	86	83	87	83	88	93	86
	T	97	100	100	92	94	100	100	100
Basophilia with degeneration of posterior lens	I	24	37	34	38	25	43	34	16
	T	62	73	77	50	52	68	64	50
Lens fibers broken up into eosinophilic globules	I	26	42	38	36	29	35	39	33
	T	87	87	85	42	42	26	57	38
Vacuolation and liquefaction of lens	I	11	12	9	11	13	20	9	12
	T	38	13	46	17	27	26	36	0
Swelling of lens fibers	I	68	60	72	71	48	65	75	55
	T	97	100	100	92	91	100	100	100
Cataracts	I	5	2	2	0	8	10	5	8
	T	5	7	8	8	18	21	0	13

I: Intercurrent deaths. T: terminal sacrifice.

According to these data, some lenticular changes were more prevalent in the paraquat-treated animals than in the controls, but they were not dose-related. Data for the individual animals were not reported or how many animals had lenticular changes in both eyes or in one eye only.

The very high incidence of lenticular changes in the controls and in the paraquat-treated animals is somewhat surprising. According to Dr. Louis Kasza (Pathologist; HED/TB), special staining and fixation techniques should be used to distinguish true pathological changes from artifacts in the eye tissue. Furthermore, for the same reason, ocular tissues should really be examined by a pathologist with training and experience in eye pathology. Special staining and fixation techniques were not used in this study (p. 9 of this submission; EPA Accession No. 246504).

Other organs: Non-neoplastic findings from the terminal kill only were reported for the following organs/tissues: stomach intestinal tract, salivary gland, pancreas, bladder, ureters, testes, epididymis, prostate, seminal vesicles, preputial gland, ovary, uterus, cervix, vulva, mammary gland, endocrine glands, skin/subcutis, brain, spinal cord, sciatic nerve and the hemopoietic/lymphopoietic system. Findings which differed from controls are listed below:

	Paraquat ion (ppm)	0	12.5	37.5	100/125	0	12.5	37.5	100/125
	Observation	Percent of animals examined							
		Males				Females			
1	Testes: arrested spermatogenesis	70	80	85	75				
2	Epididymis: partial or complete reduction of sperm content	65	80	77	58				
3	Inflammation of preputial gland	35	33	46	58				
4	Preputial cysts	10	7	8	17				
5	Ovarian cysts					33	53	36	63
6	Ovarian atrophy					3	11	7	13
7	Uterus: hydrometra					36	42	43	25
8	Mammary gland: hyperplasia					6	16	21	12
9	Mammary gland: mastitis					3	5	14	12
10	Distended bladder	25	13	30	58	0	0	5	7
11	Thyroid: distended or cystic follicles	8	13	15	17	0	5	7	25
12	Degeneration of sciatic nerve	40	40	15	42	36	63	29	63
13	Hyperplasia of thymus	3	0	0	0	3	5	14	25

These data show that there were higher incidences of various abnormalities in the paraquat-treated mice than in the controls. By comparison with the controls, the increases were 2-33% and 3-30% in male and female mice, respectively. With the exception of two observations in the females (number 11 and 13 in the table above) and, possibly, three observations in the males (number 3, 10 and 11), the incidences were dose-unrelated.

7. Pathology: neoplastic findings

Paraquat, at all levels tested, had no effect on the incidence of benign and malignant neoplasms in both male and female mice. With the exception of lymphosarcomas, most of the tumors occurred during the test weeks 79-98 and at the terminal kill, and with similar frequency in the control and the paraquat-treated groups. The most prevalent benign neoplasms were lung adenomas and Harderian gland adenomas in the males and the females, pituitary adenomas in the females and liver nodules (Type A) in the males. The most prevalent malignant neoplasms were lymphosarcomas in the males and the females, and liver Type B nodules (undifferentiated sarcoma) in the males. Lymphosarcomas occurred earlier during the

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study than the other most prevailing tumors. Five lymphosarcomas (2 in the controls and 3 in the low- and the mid-dose paraquat-fed groups) were first observed during the test weeks 0-26. Although the incidence of lymphosarcomas increased considerably with time, the incidence generally was similar in the control and the paraquat-treated groups. Data concerned with the incidence of neoplastic findings are summarized below:

NEOPLASTIC FINDINGS: TOTAL

	Males					Females				
Paraquat ion (ppm)	0	0	12.5	37.5	100/125	0	0	12.5	37.5	100/125
Number of mice examined	60	60	60	60	60	60	60	60	60	60
Total tumors	56	70	71*	59	62*	63	68	74	46	67*
Benign tumors	27	37	39	29	33*	29	32	38	31	35*
Malignant tumors	28	31	32*	29	29	34	36	36	14	32
Indeterminate	1	2	0	1	0	0	0	0	1	0
Total tumor-bearing animals	39	41	41	36	42	42	47	44	33	45
Animals with multiple types of tumors	14	19	22	17	15	16	17	21	8	17

* Values obtained by the reviewer. Data in this table represent a portion of TABLE 27 of the submission (EPA Accession No. 246504). The remainder of TABLE 27 contains the listing of all neoplastic tumors in the following systems: alimentary, urogenital, endocrine, cardiovascular, integumentary, ocular, hemopoietic/lymphopoietic, nervous and mesenchymal. The incidence of neoplastic findings in these systems is also reported for test weeks 0-26, 27-52, 53-78, 79-98 and for the terminal kill (TABLES 28, 29, 30, 31 and 32 respectively). The listing of neoplastic tumors in each one of tables 28-32 is also preceded by a summary. However, there are discrepancies within TABLES 27, 30 and 31 and between TABLE 27 and TABLES 30 and 31. These discrepancies were corrected by the reviewer.

These data show that the incidence of tumors in the control groups and the paraquat-treated groups was about the same. Of the total number of tumors detected, about 50% was benign and 50% malignant. Data summarized below show that most of the tumors in the control and paraquat-treated groups occurred during the last quarter of the experimental period.

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TOTAL NUMBER OF NEOPLASTIC TUMORS IN PARAQUAT-FED MALE MICE: TIME OF OCCURRENCE OF TUMORS

Test Week	Paraquat ion (ppm)														
	0			0			12.5			37.5			100/125		
	M	B	I	M	B	I	M	B	I	M	B	I	M	B	I
0-26							1			1					
27-52	1			1	1		1			3	1		2		
53-78	7	4	1	4	8		10	7		8	9		12	5*	
79-98	9	8		11	6		10*	15*		8	10*	1	12	18*	
Terminal Kill	11	15		15	22	2	10	17		9	9		3	10	
Total	28	27	1	31	37	2	32	39		29	29	1	29	33	
Total tumors	56			70			71			59			62		

M = malignant B = benign I = indeterminate

* Values obtained by the reviewer. Details appear under NEOPLASTIC FINDINGS: TOTAL (above).

TOTAL NUMBER OF NEOPLASTIC TUMORS IN PARAQUAT-FED FEMALE MICE: TIME OF OCCURRENCE OF TUMORS

Test Week	Paraquat ion (ppm)														
	0			0			12.5			37.5			100/125		
	M	B	I	M	B	I	M	B	I	M	B	I	M	B	I
0-26	2									1	1				
27-52	3	1		7			5			2			6	2	
53-78	7	2		5	4		5	3		5	7		14	7	
79-98	20*	17*		12*	10*		13	11*		5	8*	1	10	21*	
Terminal Kill	2	9		12	18		13	24		1	15		2	5	
Total	34	29		36	32		36	38		14	30	1	32	35	
Total tumors	63			68			74			45			67		

M = malignant B = benign I = indeterminate

* Values obtained by the reviewer. Details appear under NEOPLASTIC FINDINGS: TOTAL (above).

Data summarized below (and on pp. 21 and 22) show the incidence of the most prevalent neoplasma, both benign and malignant.

DISTRIBUTION OF THE MOST FREQUENTLY OCCURRING TUMORS

Paraquat ion (ppm) ----->	0	0	12.5	37.5	100/125	0	0	12.5	37.5	100/125
	Percent of animals examined									
Benign tumors	Males					Females				
Lung adenoma[a]	13	18	12	15	18	13	8	8	7	17
Harderian gland adenoma[b]	8	12	19	12	13	3	3	14	7	7
Pituitary adenoma[c]	0	4	7	4	0	20	25	32	31	23
Liver nodules (Type A)[a]	12	17	15	13	5	3	0	2	3	2
Kidney adenoma[a]	3	3	3	2	10	0	0	0	0	0
Adrenal cortical adenoma[d]	2	3	5	2	2	0	0	0	0	0
Malignant tumors										
Lymphosarcoma[a]	27	18	27	22	27	40	38	37	17	38
Liver nodules (Type B)[a]	10	8	10	12	7	3	2	5	2	2
Lung adenocarcinoma[a]	0	3	2	0	5	2	0	3	0	0
Pituitary carcinoma[c]	0	0	0	0	0	2	3	2	0	0
Kidney carcinoma[a]	2	2	2	0	0	0	0	0	0	0
Mammary gland adenocarcinoma[a]	-	-	-	-	-	0	0	5	0	2

Numbers of mice examined in each group: a = 60; b = 58 or 59; c = 50-60; and d = 56-60.

These data show the following:

1. Lung adenomas, Harderian gland adenomas, liver nodules (Types A and B), and pulmonary adenocarcinomas occurred more frequently in the male mice than in the female mice, but the incidence was not dose-related.

2. Lymphosarcomas and pituitary adenomas were more prevalent in the females than in the males, especially pituitary adenomas. However, the incidence of pituitary adenomas in the females was similar in the control and the paraquat-fed groups.

3. Kidney adenomas and carcinomas and adrenal cortical adenomas were observed only in the males, whereas pituitary carcinomas were observed only in the females. The incidence of these tumors was low and was not dose-related.

4. At the 100/125 ppm level (highest tested), females had a higher incidence of lung adenomas and Harderian gland adenomas, and the males had a higher incidence of kidney adenomas and lung adenocarcinomas than did their controls, but these increases were statistically insignificant. There was also no evidence of a dose response.

5. At the 12.5 ppm level (lowest tested), males and females had a higher incidence of Harderian gland adenomas than their controls. This increase was statistically significant in the females ($p = 0.015$). However, as mentioned above, there was no evidence of a dose response.

MOST PREVALENT BENIGN TUMORS: TIME OF THEIR APPEARANCE

Paraquat ion (ppm)	0	0	12.5	37.5	100 / 125	0	0	12.5	37.5	100 / 125	0	0	12.5	37.5	100 / 125	0	0	12.5	37.5	100 / 125	0	0	12.5	37.5	100 / 125
Test Weeks	0-26					27-52					53-78					79-98					Terminal Kill				
Lung adenoma							1				1	3	3	4	1	3	2	2	3	7	4	5	2	2	3
				1								2		1	2	4	2	1	1	8	4	1	4	1	
Harderian gland adenoma									1		1	2	2	3	3	2		4	1		2	5	5	2	4
											1	1		1	1	1		3	1	3		1	5	2	1
Pituitary adenoma																		3	1			2	1	1	
						1				1	1		3	3	3	6	6	6	6	5	3	9	9	7	3
Liver nodules (Type A)											2	3	1	2	1	2	1	3	4	2	3	6	5	2	
														1		1				1	1		1	1	
Kidney adenoma																	1	2		4	2	1		1	2
Adrenal cortical adenoma																	1				1	1	3	1	1
Total tumors							1		1		4	8	6	9	5	7	5	14	9	13	12	20	16	9	10
				1		1				1	2	3	3	6	6	12	8	10	8	17	8	11	19	11	4

For each tumor listed in this table, the first line shows incidence in the male mice and the second line, in the female mice. These data show that nearly all benign tumors occurred during the second year of the study, especially during the test weeks 79-99 (terminal kill). For each test period listed, the overall incidence of tumors was similar in the control and the paraquat-treated groups.

MOST PREVALENT MALIGNANT TUMORS: TIME OF THEIR APPEARANCE

Paraquat ion (ppm)	0	0	12.5	37.5	100 / 125	0	0	12.5	37.5	100 / 125	0	0	12.5	37.5	100 / 125	0	0	12.5	37.5	100 / 125	0	0	12.5	37.5	100 / 125
Test Weeks	0-26					27-52					53-78					79-98					Terminal Kill				
Lymphosarcoma			1	1		1	1	1	3	2	5	2	6	5	6	6	1	4	3	5	4	7	4	1	3
	2			1		3	5	5	2	5	6	5	3	3	10	11	7	6	4	7	2	6	8	1	1
Liver nodules (Type B)											1		1			2	2	2	4	4	3	3	3	3	
															1	2	1	3	1						
Lung adeno-carcinoma												1			2		1	1		1					
																1		1					1		
Kidney carcinoma																					1	1	1		
Pituitary carcinoma																									
																1	1					1	1		
Mammary gland adenocarcinoma													1					1		1			1		
Total tumors			1	1		1	1	1	3	2	6	3	7	5	8	8	4	7	7	10	8	11	8	4	3
	2			1		3	5	5	2	5	6	5	4	3	11	15	9	11	5	8	2	7	11	1	1

For each tumor listed in this table, the first line shows incidence in the male mice and the second line, in the female mice. Although lymphosarcomas occurred earlier during the study and were more prevalent than the other malignant tumors, they occurred generally with the same frequency in the control and the paraquat-treated groups.

RESULTS FOR PART 2 OF THE STUDY (PARAQUAT LEVELS IN TISSUES AND PLASMA)

This part of the study was conducted concurrently with the oncogenic part (Part 1). Ten mice/dose/sex were used and the mice were sacrificed after 52 weeks of feeding. Although the major objective of this part of the study was to determine paraquat levels in tissues and plasma, other parameters were also to be examined. According to the experimental protocol, the animals were to be observed daily for clinical and behavioral abnormalities, and then they were to be examined grossly and histopathologically (abnormal tissues only) at the termination of the experiment. However, only data for paraquat levels in lung, kidney and plasma were reported, and no reference was made to the other data. Data concerned with tissue and plasma levels of paraquat are summarized below:

PARAQUAT ION CONCENTRATION IN LUNGS (ug/g) AFTER 52 WEEKS OF FEEDING TECHNICAL GRADE PARAQUAT

	Male Mice		Female mice	
Dosage (ppm)*	No. of animals	Paraquat level, mean and (SD)	No. of animals	Paraquat level, mean and (SD)
0		No results**		No results**
12.5	2	0.20 (0.09)		No results**
37.5		No results**	5	0.23 (0.17)
100/125	7	0.52 (0.25)	8	0.43 (0.11)

* Expressed as paraquat ion.
**No results = data were discontinued due to problems in analysis
The lower limit of detection was 0.1 ug/g.

These data indicate that the accumulation of paraquat in the lung appears to be dose-related. However, there were wide variations within some of the individual groups and too few values were reported for the 12.5 and 37.5 ppm groups. It is also unclear why so few animals were used in some determinations (unscheduled deaths were not reported).

PARAQUAT ION CONCENTRATION IN KIDNEYS (ug/g) AFTER 52 WEEKS OF FEEDING TECHNICAL GRADE PARAQUAT

	Male Mice		Female mice	
Dosage (ppm)*	No. of animals	Paraquat level, mean and (SD)	No. of animals	Paraquat level, mean and (SD)
0		No results**		No results**
12.5	1	0.19		No results**
37.5	1	0.74	8	0.70 (0.34)
100/125	8	1.17 (0.19)	8	1.61 (0.41)

* Expressed as paraquat ion.
**No results = data were discontinued due to problems in analysis
The lower limit of detection was 0.1 ug/g.

These data indicate that the accumulation of paraquat in the kidney appears to be dose-related, at least in the females. However, there were wide variations in the paraquat levels reported for the mid- and high-dose females (standard deviations are very high) and no data were reported for the females in the 12.5 ppm group. Inadequate data were reported for the male mice to conclude that the accumulation of paraquat in the kidneys of these animals was dose-related.

PARAQUAT ION CONCENTRATION IN PLASMA (ug/ml) AFTER 52 WEEKS OF FEEDING TECHNICAL GRADE PARAQUAT

Dosage (ppm)*	Male Mice		Female mice	
	No. of animals	Paraquat level, mean and (SD)	No. of animals	Paraquat level, mean and (SD)
0	7	Not detected	9	Not detected
12.5	8	0.014 (0.012)	10	0.039 (0.016)
37.5	4	0.024 (0.001)	8	0.025 (0.010)
100/125	8	0.051 (0.008)	9	0.056 (0.019)

* Expressed as paraquat ion.
The lower limit of detection was 0.006 ug/ml.

These data show that the concentration of paraquat in plasma appears to be dose-related, especially in the males.

In summary, small amounts of paraquat ion were detected in plasma, kidneys and lungs of male and female mice. The levels observed appeared to be dose-related.

DETERMINATIONS NOT PERFORMED

Hematological determinations were never performed in this study and organs were not weighed. Hematological tests are important in an oncogenic study because they detect leukemia which can occur early in a study. However, according to Dr. Louis Kasza, Pathologist (HED/TB), leukemia is extremely rare in a mouse. Although organs were not weighed, no reference was made to hypertrophy of organs in the detailed pathology report for this study. Considering also that paraquat was not oncogenic to the SPF Alderly Park mouse, the absence of hematological tests and organ weights is not regarded critical to the acceptance of this study as a valid oncogenic study.

CLASSIFICATION OF THIS STUDY: Core Minimum.

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